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The genetics of β-amylase isozymes in wheat

1. Allelic variation among hexaploid varieties and intrachromosomal gene locations

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Summary. Thirty-three β-amylase isozymes were separated in 'Chinese Spring' by IEF and the structural genes encoding seventeen of these were located by nullisomic analysis. The locations of the previously reported *β-Amy-1* loci on chromosome arms 4Aβ (*β-Amy-A1*) and 4DL (*β-Amy-D1*) were confirmed and another set, *β-Amy-2*, was found on the group 5 chromosomes. A locus on 5AL (*β-Amy-A2*) was identified by nullisomic analysis and another on chromosome 5B (*β-Amy-B2*) was identified by analysis of inter-varietal chromosome substitution lines. The loci are complex, each coding for several isozymes, and allelic variation occurs at all four. Two alleles were identified at *β-Amy-A1*, five at *β-Amy-D1*, five at *β-Amy-A2* and two at *β-Amy-B2*. Eleven different β-AMY phenotypes were distinguished amongst the 46 wheat varieties screened. The *β-Amy-A2* locus was mapped by employing chromosome 5A recombinant lines and found to be closely linked and proximal to the awn inhibitor, *B1*, on the long arm. An attempt to map the *β-Amy-D1* locus on chromosome 4D showed the gene to be located in the distal region of the long arm.

Key words: Hexaploid wheat – β-amylase – Isozymes – Isoelectric focusing

1 Introduction

Relatively few marker genes are available in hexaploid bread wheat (*Triticum aestivum*, 2n = 6x = 42), particularly at loci with no known effects on agronomic characters. Marker genes are required for several reasons, e.g. for following chromosomes during genetic manipulations using aneuploid techniques; for the verification of inter-varietal chromosome substitution lines; for intrachromosomal assays for quantitative genetic variation; for the assessment of homoeologies of alien chromosomes in alien-wheat chromosome addition lines; for the verification of alien-wheat chromosome additions and substitutions; and for screening chromosome segments transferred in alien-wheat genetic transfers where marker genes are linked to genes of agronomic importance. Genetic variation at enzyme loci provides a particularly useful source of marker genes and more than 60 loci have been assigned to specific chromosome arms, mainly by forms of nullisomic analysis (Hart 1982).

Although many enzyme systems have been studied, there are few reports of allelic variation and still fewer loci have been mapped intrachromosomally. β-Amylase (α-1,4-glucan maltohydrolase, E.C. 3.2.1.2) which in mature wheat grains is present as many isozymes, provides a suitable system both for the identification of allelic variation and for intrachromosomal gene mapping. The genetics of the β-amylase system in wheat has been the subject of some investigation, mainly by Joudrier and co-workers. Loci have been identified on the β arm of chromosome 4A (Joudrier 1980) and the long arm of 4D (Joudrier and Bernard 1977).

Initial observations using flat-bed isoelectric focusing indicated that the β-amylase system in wheat may be more complex than has been described by previous workers. This paper presents the results of an investigation into the genetics of β-amylase in 46 varieties of hexaploid wheat and various aneuploid and chromosome substitution line derivatives.

2 Materials and methods

2.1 Electrophoretic procedures

2.1.1 Enzyme extraction. Single dry grains were milled in a microhammer mill (Paulis and Wall 1979), sonicated for 3 s in

extraction solution (1 mg flour: 3 µl 0.2% calcium chloride containing 0.2 M 2-mercaptoethanol) and allowed to stand overnight at 4 °C. The extracts were centrifuged at 12,000 × g for 10 min at 4 °C and the supernatant dialysed for 1 h against 0.2% calcium chloride.

2.1.2 Isoelectric focusing. Isoelectric focusing was performed with Multiphor electrofocusing apparatus (LKB) with a self-regulating power supply (LKB) on Ampholine PAG plates, pH 4.0–6.5 (LKB). A constant power of 1 W cm^{-1} width of gel was applied with cooling at 4 °C and the gel was prefocused for 30 min. Small filter papers (Whatman 3 MM, 5 × 10 mm) were soaked with 20 µl of extract and applied to the surface of the gel 2 cm from the cathode. The samples were removed after 30 min and electrofocusing was terminated after a further 2 h.

2.1.3 Enzyme visualisation. Staining was carried out using a modification of the method of Sargeant and Walker (1978). The gel was soaked in a 3% solution of starch (which had previously been boiled) for 10 min. The starch solution was poured off and, after an incubation period of 1 min, the gel was flooded with a solution of 1.5×10^{-3} M iodine, 3.5×10^{-3} M potassium iodide and 2% acetic acid. The positions of the β-amylase isozymes appear as colourless bands against a dark blue background. The pH gradient in the gel was determined prior to staining, by taking pH readings at 0.5 cm intervals across the gel with a surface pH electrode (Pye Unicam).

2.2 Genotypes

2.2.1 Aneuploid lines. The nullitetrasomic and ditelosomic genotypes of 'Chinese Spring' developed by Sears (1954, 1966a, 1966b) were employed. Of the 42 possible compensating nullisomic-tetrasomic combinations, all but 4 were available (CSN2A-T2B, CSN4A-T4B, CSN4D-T4B and CSN6B-T6D). Where nullisomic effects on zymograms were observed, the relevant ditelosomics were analysed (CSDT4Aα, CSDT4Aβ, CSDT4DS, CSDT4DL and CSDT5AL). CSDT5AS was not available.

2.2.2 Hexaploid wheat varieties. Forty-six hexaploid wheat varieties were examined: 'Atlas 66', 'Azteca 67', 'Bersée', 'Bezostaya I', 'Bounty', 'Brigand', 'C306', 'Capitole', 'Cappelle-Desprez', 'Champlein', 'Cheyenne', 'Chinese Spring' (CS), 'Ciano 67', 'Desprez 80', 'Fenman', 'Glennson', 'Highbury', 'Hobbit', 'Hobbit 'S'', 'Holdfast', 'Hope', 'Koga II', 'Little Joss', 'Lutescens 62', 'Manella', 'Mara', 'Maris Huntsman', 'Maris Ranger', 'Maris Sportsman', 'Minister Dwarf', 'Poros', 'Sappo', 'Sava', 'Sicco', 'Spica', 'Synthetic' (an amphiploid of *Triticum dicoccum* × *Aegilops squarrosa* produced by Professor E. R. Sears, of University of Missouri, Columbia (McFadden and Sears 1946)), 'SD1', 'SD2', 'Timmo', 'Timstein', 'Tom Thumb', *T. macha*, *T. spelta*, 'Vilmorin 27', 'VPM' and 'Wembley'.

2.2.3 Inter-varietal chromosome substitution lines. Where β-amylase isozyme variation was detected, inter-varietal chromosome substitution lines were employed to identify the chromosomes responsible. The following substitution series developed by C. N. Law and A. J. Worland at the Plant Breeding Institute were examined: 'Bersée' ('Koga II'), 'CS' ('Cappelle-Desprez'), 'CS' ('Synthetic') and 'Koga II' ('Bersée'). identify the chromosomes responsible for additional allelic variation where substitution lines were not available.

2.2.4 Random lines. Three sets of homozygous lines developed by J. W. Snape at the Plant Breeding Institute from the crosses 'Manella' × 'Hobbit 'S'', 'Maris Sportsman' × 'Highbury' and 'CS' × 'Hobbit 'S'' (*T. spelta* 5A) were screened in order to

2.2.5 Segregating lines for gene mapping. A β-amylase locus on chromosome 4D was mapped against the dwarfing gene *Rht2* (Gale et al. 1975) using F_2 grain from the cross 'Maris Huntsman' × 'Hobbit'. *Rht2/Rht2*, *Rht2/rht2* and *rht2/rht2* genotypes were distinguished using the seedling gibberellin insensitivity test (Gale and Gregory 1977).

A locus on chromosome 5A was mapped using chromosome 5A long arm recombinant lines derived from 4 crosses made by C. N. Law and J. W. Snape using the method of Law (1966): CSDT5AL × 'CS' ('Cappelle-Desprez' 5A), 'CS' monosomic 5A × 'CS' ('Cappelle-Desprez' 5A), CSDT5AL × 'CS' (*T. spelta* 5A) and 'CS' monosomic 5A × CS (*T. spelta* 5A).

3 Results

3.1 β-Amylase isozymes in 'Chinese Spring'

In extracts of single mature grains of 'CS', 33 isozymes can be distinguished which have pIs ranging from pH 4.6–5.5 (Fig. 1). Analysis of the available compensating nullisomic-tetrasomic and ditelosomic combinations confirmed the involvement of chromosome arms 4Aβ and 4DL (Fig. 2) as previously described by Joudrier and Cauderon (1976). Chromosome 4A controls the production of isozyme bands 14 and 33 whilst chromosome 4D controls the production of 12 isozymes (bands 1–8, 16, 21, 24 and 29). In addition to chromosomes 4A and 4D, chromosome 5A was also shown to be responsible for the synthesis of β-amylase isozymes, and encodes bands 15, 30 and 32 (Fig. 2). Although CSDT5AS was not available, these bands were present in the zymogram of CSDT5AL, thus implicating the long arm of chromosome 5A.

No loci were identified on chromosome 5B or 5D or on any of the other remaining 5 chromosome groups. However CSN5B-T5D lacks all isozymes associated with chromosome 5A. The loss of the 5A gene(s) is likely to have been caused by homoeologous pairing induced by the removal of the *Ph* locus on chromosome 5B (Riley and Chapman 1958). Sixteen isozymes were not located by nullisomic analysis, probably because of co-focusing of isozymes encoded by more than one gene on different homoeologues.

3.2 Varietal variation

Considerable variation in β-amylase isozymes was found among the varieties which were classified into 11 main phenotypic groups (Types A–K, Table 1 and Fig. 3). The two largest classes (E and F) each contain 12 varieties. Of the remaining classes, C, G, H, J and K are represented by only a single variety.

The variation in β-amylase isozymes is complex in that all variant phenotypes (relative to CS) differ both by lacking isozymes and having 'novel' isozymes i.e.

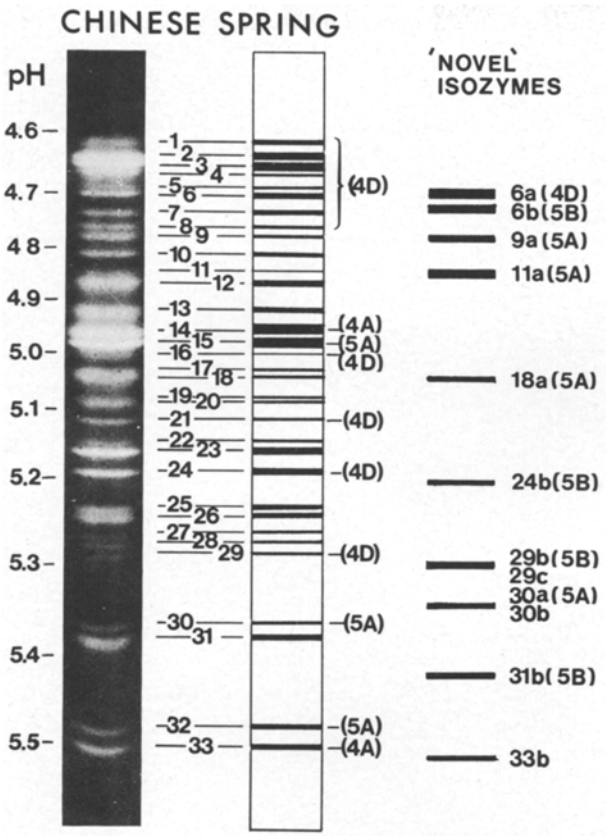

Fig. 1. 'Chinese Spring' euploid β-amylase zymogram and diagram showing isozymes of known chromosomal control. The 'novel' isozymes shown on the *right* are a summary of all the extra bands found in other varieties and their chromosomal control. See sections 3.2 and 3.3

bands not present in 'CS'. No variety was found which simply lacked isozymes found in 'CS' or had novel isozymes. The variant isozymes together with those of 'CS' are shown in Fig. 1.

Type A

The 'CS' type, exhibited by seven varieties.

Type B

Five varieties had a β-amylase phenotype which lacked isozyme bands 15, 30 and 32, shown by nullisomic analysis to be controlled by chromosome 5A, and had an extra band, band 9a, in the acidic part of the zymogram.

Type C

T. spelta had an identical β-amylase zymotype to Type B varieties, but with an additional band (33b) close to and on the cathodal side of band 33.

Type D

Three varieties lacked bands 15 and 32 but had an additional band (9a).

Type E

Twelve varieties gave a β-amylase zymotype which lacked the 3 isozymes controlled by chromosome 5A (15, 30 and 32) but which had 3 novel bands (11a, 18a and 30a). Band 11a is a strong band located in the acidic region in the position of band 11, band 30a is located on the anodal side of band 30, and band 18a is a strong band located in the position of band 18.

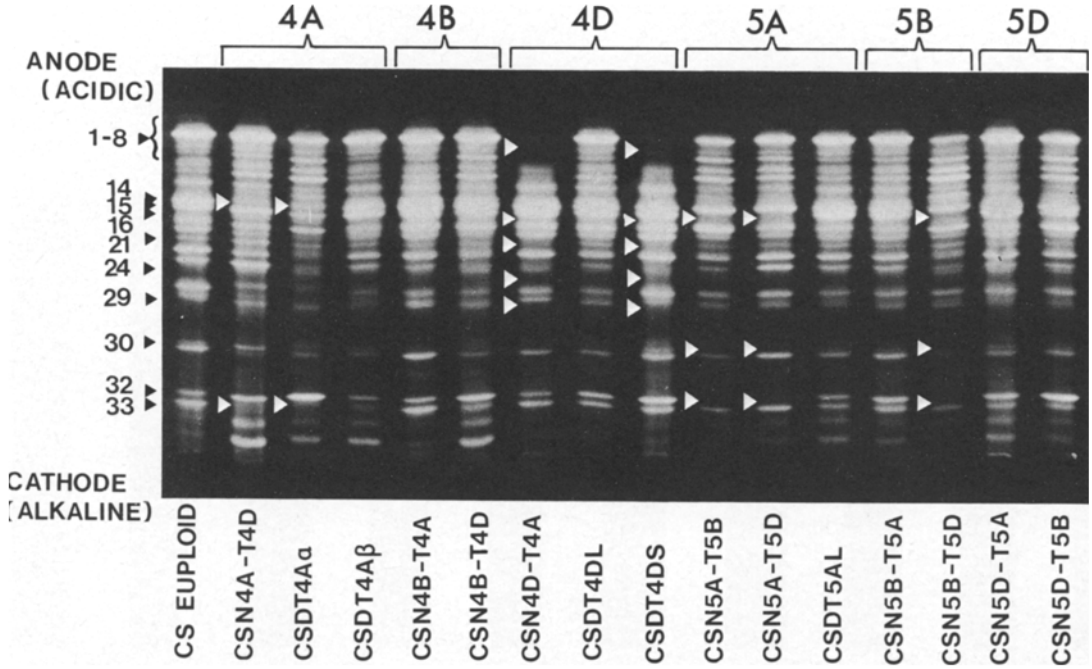

Fig. 2. β-Amylase zymograms of chromosome group 4 and 5 nullisomic-tetrasomic and ditelosomic aneuploid genotypes in 'Chinese Spring'. *Arrows* indicate the absence of 'CS' bands.

Table 1. β-Amylase isozyme variation in 46 varieties of hexaploid wheat showing the phenotypes and alleles at the four loci responsible for their production

Type	Variety	Genotype														
		β-Amy-A1		*β-Amy-D1*					*β-Amy-A2*					*β-Amy-B2*		
		a	b	a	b	c	d	e	a	b	c	d	e	a	b	
A	'Chinese Spring', Glennson, Highbury, Lutescens 62, Wembley, C306, SD2	+		+					+					+		
B	Atlas 66, Cheyenne, Koga II, Timmo, Timstein	+		+						+				+		
C	*Triticum spelta*	+		+						+				+		
D	Holdfast, Sappo, SD1	+		+								+		+		
E	Bezostaya I, Brigand, Capitole, Desprez 80, Fenman, Hobbit, Hope, Little Joss, Poros, Sava Sicco, Spica	+		+									+	+		
F	Bersée, Bounty, Cappelle-Desprez, Champlein, Hobbit 'S', Maris Huntsman, Maris Ranger, Maris Sportsman, Minister Dwarf, Tom Thumb, Vilmorin 27, VPM	+			+								+	+		
G	Manella	+						+					+	+		
H	Synthetic hexaploid		+			+				+						+
I	Azteca, Ciano 67		+				+			+				+		
J	*Triticum macha*		+				+				+			+		
K	Mara		+					+					+	+		

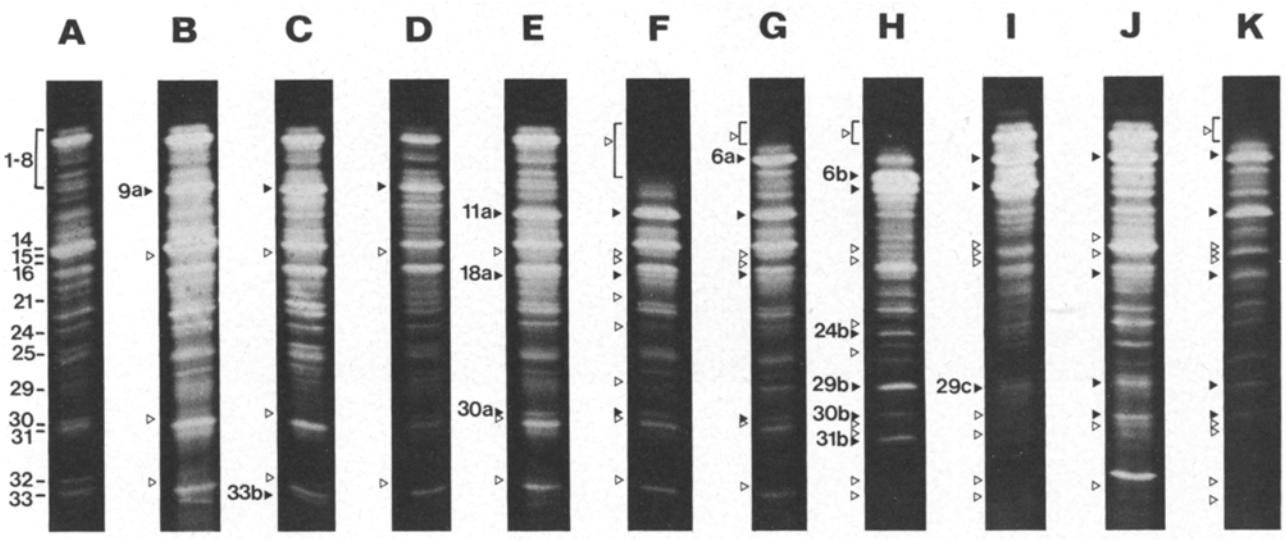

Fig. 3. Variation in β-amylase phenotype (*Types A–K*) showing novel isozymes relative to 'Chinese Spring' (▶) and absent 'CS' isozymes (▷)

Type F

Twelve varieties had a similar β-amylase phenotype to type E varieties but the 12 isozymes shown by nullisomic analysis to be controlled by chromosome 4D (bands 1–8, 16, 21, 24 and 29) were also absent.

Type G

'Manella' showed a β-amylase phenotype which was missing bands 15, 30 and 32 and has the novel bands 11 a, 18 a and 30 a as in Types E and F above. In addition, 5 of the isozymes controlled by chromosome 4D were absent (bands 1–4 and 16) and there was a strong novel band (6 a) in the position of band 6.

Type H

'Synthetic' gave a very different β-amylase phenotype from the 'CS' type. The 2 isozymes controlled by chromosome 4A (bands 14 and 33), 5 isozymes controlled by 4D (bands 1–4 and 24) and the 3 isozymes controlled by 5A (bands 15, 30 and 32) were all absent, together with bands 25 and 31. Six novel bands were evident: band 9 a (as seen in Types B, C and D) band 6 b (on the cathodal side of band 6), bands 24 b (on the cathodal side of band 24), band 29 b (on the cathodal side of band 24), band 30 b (on the anodal side of band 30) and band 31 b (on the cathodal side of band 31).

Type I

'Azteca' and 'Ciano 67' had a zymotype which lacked bands 14 and 33 (chromosome 4A), band 16 (4D), band 31 and bands 15, 30 and 32 (5A) and had 3 novel bands, 6 a, 9 a and 29 c, this latter band being located on the cathodal side of band 29.

Type J

Triticum macha showed a β-amylase phenotype in which bands 14 and 33 (4A), 16 (4D) and 30 (5A) were absent and 4 novel bands were present (6 a, 18 a, 30 a and 29 c).

Type K

A single variety, 'Mara', gave a zymotype which lacked bands 14 and 33 (4A), 1–4 and 16 (4D), bands 15, 30 and 32 (5A) and band 31. Five additional bands were present (6A, 11 a, 18 a, 24 c and 30 a).

The 11 different β-amylase phenotypes thus differ in having different combinations of 12 novel isozymes (6 a, 6 b, 9 a, 11 a, 18 a, 24 b, 29 b, 29 c, 30 a, 30 b, 31 b and 33 b) together with combinations of 19 missing isozymes (1–8, 14–16, 21, 24, 25, 29, 30, 31, 32 and 33). The remaining 14 isozymes are invariant (9–13, 17–20, 22, 23 and 26–28). However, it is difficult to make definitive statements about 4 isozymes (6, 9, 11 and 18) which may or may not be present in phenotypes which include the much stronger novel bands 6 a, 9 a, 11 a and 18 a in the same positions.

3.3 Identification of chromosomal control of β-amylase isozymes not present in 'Chinese Spring'

Intervarietal chromosome substitution lines were employed to assign chromosomal control of the observed variation in β-amylase phenotpyes. In addition, these genotypes can be used to verify the chromosomal control of isozymes which has been previously established by nullisomic analysis.

3.3.1 Chromosome 4D substitutions. The 12 Type F varieties lacked all the bands shown by nullisomic analysis to be controlled by chromosome 4D. Analysis of the chromosome 4D substitutions of 'Koga II' (Type B) into 'Bersée' (Type F) showed that all the above 12 bands are expressed in the 'Bersée' ('Koga' 4D) substitution (Fig. 4). Similarly, where 'Bersée' 4D was substituted for 'Koga' 4D, these bands were not expressed (not shown). A number of other substitution series including Type F varieties and other varieties were examined with similar results. In all 4D substitutions examined the 4D isozymes were either all expressed or were all absent; there were no intermediate cases.

3.3.2 Chromosome 5A substitutions. Seven isozymes were established as being under the control of chromosome 5A, 3 of which are present in 'CS' (15, 30 and 32) and 4 novel isozymes (9 a, 11 a, 18 a and 30 a).

Analysis of the group 5 substitutions of 'Koga II' into 'Bersée' enabled the novel isozyme bands 9 a, 11 a,

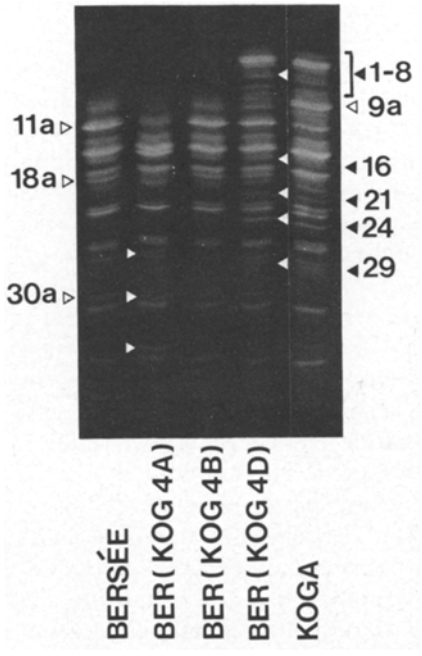

Fig. 4. β-Amylase zymograms of chromosome group 4 'Bersée' ('Koga') chromosome substitution lines. Isozyme bands controlled by chromosome 4D (▶) and 5A (▷) are *arrowed.* Note: 'Bersée' ('Koga' 4A) has the 'Bersée' phenotype except for 3 additional bands derived from 'CS', presumably retained in the production of the 'Bersée' monosomic used to make the substitution

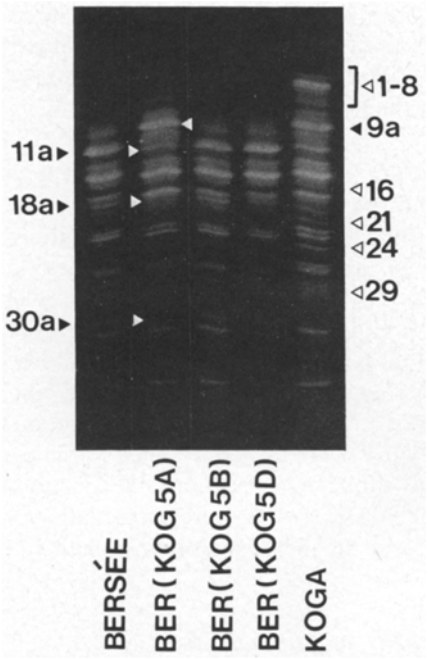

Fig. 5. β-Amylase zymograms of chromosome group 'Bersée' ('Koga') chromosome substitution lines. Isozyme bands controlled by chromosomes 5A (▶) and 4D (▷) are *arrowed*

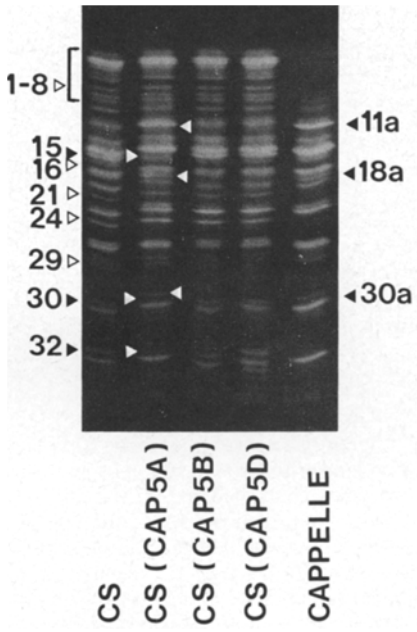

Fig. 6. β-Amylase zymograms of chromosome group 5 'Chinese Spring' ('Cappelle-Desprez') chromosome substitution lines. Isozyme bands controlled by chromosome 5A (▶) and 4D (▷) are *arrowed*

18 a and 30 a to be shown to be controlled by chromosome 5A (Fig. 5). In the 'Bersée' ('Koga' 5A) substitution, band 9 a from 'Koga' is expressed while 'Bersée' bands 11 a, 18 a and 30 a are absent.

The 3 isozymes shown by nullisomic analysis to be controlled by chromosome 5A (bands 15, 30 and 32) have been confirmed as such by analysis of the group 5 substitutions of 'Cappelle-Deprez' into 'Chinese Spring' (Fig. 6). In the substitution 'CS' ('Cappelle' 5A), 'CS' bands 15, 30 and 32 are removed and bands 11 a, 18 a and 30 a are added to the zymogram.

3.3.3 Chromosome 5B substitution. The group 5 substitutions of 'Synthetic' (Syn) into 'Chinese Spring' show the chromosomal control of β-amylase isozymes by both 5A and 5B (Fig. 7). In the 'CS' ('Syn' 5A) substitution, band 9 a from 'Synthetic' replaces bands 15, 30 and 32. In the 'CS' ('Syn' 5B) substitution, 4 novel bands from 'Synthetic' are expressed in the 'CS' background (bands 6 b, 24 b, 29 b and 31 b), thus implicating chromosome 5B. However, the replacement of 'CS' chromosome 5B does not result in the removal of any 'CS' isozymes, thus confirming the nullisomic result that 'CS' carries a null allele(s) on 5B.

3.3.4 Random lines. The novel isozyme band 6 a (Types G, I, J and K) was found to be controlled by chromosome 4D by analysis of segregating F_6 homozygous lines from the cross 'Manella' × 'Hobbit 'S''. Only

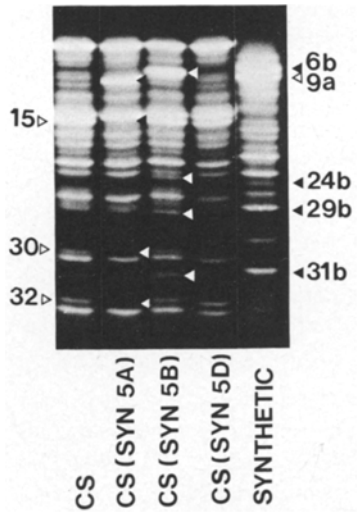

Fig. 7. β-Amylase zymograms of chromosome group 5 'Chinese Spring' (Synthetic) substitution lines. Isozyme bands controlled by chromosomes 5A (▷) and 5B (▶) are *arrowed*

parental β-amylase phenotypes were observed and the novel band 6 a segregated with the other isozymes controlled by 4D (Fig. 8).

3.3.5 Isozymes of unknown chromosomal control. Substitution lines were not available which identified the chromosomal control of the novel bands 29 c, (Type I), 30 b (Type H; 'Synthetic') and 33 b (Type C; *T. spelta*). Band 29 c may be the same as band 29 b in 'Synthetic',

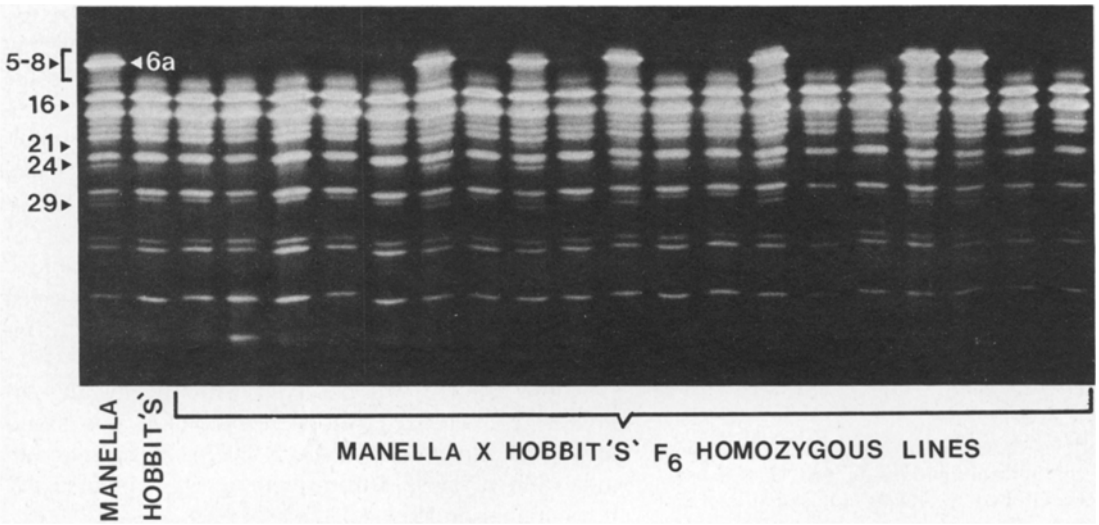

Fig. 8. β-Amylase phenotypes of F_6 homozygous lines from the cross 'Manella' × 'Hobbit 'S'' showing the segregation of isozyme bands controlled by chromosome 4D

controlled by 5B. Similarly, the chromosome responsible for the absence of band 31 (Types H, I and K) was not identified. In 'Synthetic', the chromosomal control of the absence of band 25 could not be established.

3.4 The nature of the β-amylase structural genes

The analyses of the 'CS' NT lines, and various random lines, has implicated four chromosomes in the control of the production of β-amylase isozymes in wheat: chromosomes 4A (2 isozymes), 4D (13), 5A (7) and 5B (4). This raises the question as to whether the several bands per chromosome are controlled by 'single' loci or by several loci per chromosome. A 'single' locus could embrace either a single gene with post-translational modification to several products or a compound locus with closely linked subunits producing the different isozymes.

To address this question a number of random lines were examined in which these multi-band differences were segregating. A total of 230 lines from crosses of 'Maris Huntsman' × 'Hobbit 'S'' (F_2), 'Manella' × 'Hobbit 'S'' (homozygous F_6) and 'CS' × 'Hobbit 'S'' (*T. spelta* 5A) (homozygous F_6) were investigated. These lines were segregating for 4D and 5A phenotypes. A sample of the 'Manella' × 'Hobbit 'S'' lines are shown in Fig. 8, showing segregation of 8 chromosome 4D bands. This sample is typical of all the chromosome 4D or 5A phenotypes scored in that only parental band patterns are observed, indicating that a single or compound locus is involved on each chromosome. By combining the data for the loci on chromosomes 4D and 5A, a maximum size in terms of recombination units, can be assigned by calculating the upper 95% confidence limit on the observed 'within gene' recombination estimate of zero. This figure is 0.003 (0.3%), indicating that for mapping purposes the loci on 4D or 5A may be considered as single units.

The loci are subsequently referred to as members of 2 homoeologous sets. *β-Amy-A1* and *β-Amy-D1* are members of the first set reported on chromosomes 4A and 4D (*β-Amy-1*)[1]. *β-Amy-A2* and *β-Amy-B2* are the members of the newly described set on chromosomes 5A and 5B (*β-Amy-2*).

3.5 Allelic variation in β-amylase isozymes

Having established that the β-amylase isozymes which co-segregate are the products of a single and complex locus on each chromosome, the different segregating combinations have been assigned allele symbols.

Alleles are named by sufixing the locus symbol with letters. 'Chinese Spring' is always assigned the 'a' allele and variants are identified by 'b', 'c' etc. For example, th allele at the locus *β-Amy-D1* carried by CS is *β-Amy-D1a* and the 4 allelic variants described below are identified as *β-Amy-D1b* to *e.* (Table 1).

β-Amy-A1

Two alleles for this locus on chromosome 4A are identified: *β-Amy-A1a* (41 varieties), encoding for isozyme bands 14 and 33 and null allele *β-Amy-A1b* (5 varieties, Types H–K).

1 The gene symbol *β-Amy-1* was assigned by Hart (1982) as no symbols were given by Joudrier and Cauderon (1976) or Joudrier (1980)

Table 2. The alleles of the *β-Amy-D1* locus showing the isozyme phenotypes

Alleles	Isozyme bands												
	1	2	3	4	5	6	7	8	6a	16	21	24	29
β-Amy-D1a	+	+	+	+	+	+	+	+		+	+	+	+
β-Amy-D1b													
β-Amy-D1c					+	+	+	+		+	+		
β-Amy-D1d	+	+	+	+	+	+	+	+	+		+	+	+
β-Amy-D1e					+	+	+	+	+		+	+	+

β-Amy-D1

The 5 allelic variants at the 4D locus produced phenotypes which were made up of combinations of the 12 'CS' isozymes shown to be controlled by 4D by nullisomic analysis and the novel isozyme 6a (Table 2).

β-Amy-A2

The 5 alleles of the *β-Amy-A2* locus encode for different combinations of 7 isozymes: 3 are found in 'CS' (15, 30 and 32) together with 4 novel isozymes (9a, 11a, 18a and 30a) (Table 3).

β-Amy-B2

All varieties examined, with the exception of 'Synthetic', carry the 'a' allele which shows a null phenotype for the four 5B isozymes encoded by the 'b' allele of 'Synthetic' (bands 6b, 24b, 29b and 31b).

The 46 wheat varieties investigated which have 11 different β-amylase phenotypes can now be classified with respect to the alleles at the 4 loci which have been recognised (Table 1). Types B and C both have the genotype *β-Amy-A1a, -D1a, -A2b, -B2a* but are distinct since *T. spelta* (Type C) has the novel band 33b, for which chromosomal control has not been identified.

3.6 Intrachromosomal gene mapping

3.6.1 β-Amy-A2. Intrachromosomal recombinant lines have been produced to map the long arm of chromosome 5A with respect to the centromere, the awning inhibitor locus (*B1/b1*), the speltoid ear type locus (*Q/q*) and the vernalisation locus (*Vrn1/vrn1*). Recombinant lines are the products of a single round of recombination fixed as homozygotes. A subset of the chromosome 5A lines, involving crosses of 'CS' genotypes with the intervarietal substitutions of chromosome 5A from 'Cappelle-Desprez' (Cap) and *T. spelta* in 'CS', were segregating at the *β-Amy-A2* locus. These lines, 43 from CSDT5AL and 'CS' euploid×'CS' ('Cap' 5A) and 29 from CSDT5AL and 'CS' euploid× 'CS' (*T. spelta* 5A), allowed *β-Amy-A2* to be mapped against the centromere, *B1*, and *Vrn1*, and the centromere, *Q* and *Vrn1* respectively.

Close linkage was observed with the awning inhibitor, *B1*, where only one recombinant was found among 43 gametes ($P = 0.023 \pm 0.023$). In addition, an indication of loose linkage with *Q* was obtained (11 recombinants in 29, $P = 0.37$).

The analysis of the entire set of recombinant lines indicates that the gene order from the centromere is *B1, Q, Vrn1* (J. W. Snape, personal communication). The single recombinant between *β-Amy-A2* and *B1* had the 'Cap' 5A centromere and β-amylase phenotype and the 'CS' alleles at the *B1, Q* and *Vern1* loci, indicating the *β-Amy-A2* is located proximal to *B1*.

The figure of 37% for *β-Amy-A2* to *Q* is within the range of values (30–41%) obtained for recombination between *B1* and *Q* (Chin 1944; Philiptschenko 1930; Watkins 1927), thus confirming the close linkage of *β-Amy-A2* with *B1*. These results are summarised in Fig. 9.

3.6.2 β-Amy-1. The 2 *β-Amy-1* loci probably comprise part of a homoeoallelic series across the long arms of the group 4 chromosomes. The dwarfing genes *Rht1* and *Rht2* comprise similar series on the homoeologous α and S arms of chromosomes 4A and 4D respectively

Table 3. The alleles of the *β-Amy-A2* locus showing the isozyme phenotypes

Alleles	Isozyme bands						
	9a	11a	15	18a	30a	30	32
β-Amy-A2a			+			+	+
β-Amy-A2b	+						
β-Amy-A2c			+	+	+		+
β-Amy-A2d	+					+	
β-Amy-A2e		+		+	+		

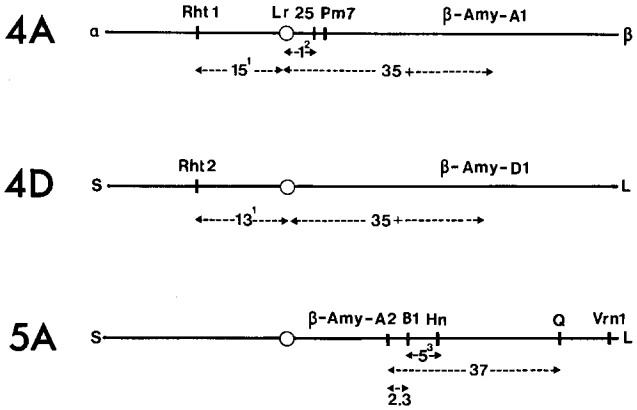

Fig. 9. Genetic maps of chromosomes *4A, 4D* and *5A* showing the proposed map positions of *β-Amy-A1, β-Amy-D1* and *β-Amy-A2*. Note: [1] McVittie et al. (1978), [2] Driscoll and Bielig (1968), [3] Sears (1975). For sources of the positions of other loci see McIntosh (1974, 1979)

and are linked to the centromeres by the about 15% (McVittie et al. 1978). Therefore, a test of linkage between a *β-Amy-1* locus and a dwarfing gene should scan the proximal regions of the long arms.

Such a test was carried out using 78 F_2 grains from the cross 'Hobbit' (*Rht2, β-Amy-D1a*)×'Maris Huntsman' (*rht2, β-Amy-D1b*). Individual grains were halved and the seedlings raised from the proximal halves were assayed for gibberellin sensitivity. This allowed complete classification of the heterozygous and two homozygous classes at the *Rht2/rht2* locus. The distal halves were assayed for *β-Amy-D1* phenotype and classified as *β-Amy-D1b/b* homozygotes or *-a/a* and *-a/b* which were indistinguishable.

Segregation at the *β-Amy-D1* locus fitted a 3 : 1 ratio (62 : 16, $P > 0.05$) and the *Rht2* locus showed a 1 : 2 : 1 ratio (20 : 38 : 20; $P > 0.05$), indicating that transmission was normal. However, the distribution of alleles at the two loci displayed independent assortment ($\chi^2_{(5)} = 1.53$, $P > 0.05$), indicating that the genes are unlinked. Thus although the precise location of *β-Amy-D1* has not been ascertained, it clearly lies in the distal region of the long arm of chromosome 4D (> 35% recombination from the centromere).

4 Discussion

The chromosomal location of β-amylase structural genes in wheat has previously been investigated using polyacrylamide gel electrophoresis by Joudrier and co-workers who identified three β-amylase components in 'CS'. Analysis of nullitetrasomic and ditelosomic lines showed components C_1 and C_2 to be controlled by linked genes on chromosome arm 4Aβ and C_3 to be controlled by chromosome arm 4DL (Joudrier 1980; Joudrier and Cauderon 1976).

The use of isoelectric focusing has enabled the β-amylase isozymes of 'Chinese Spring' to be resolved into 33 bands in contrast to the 3 components described by Joudrier and Cauderon (1976). This number itself may be an underestimate as sixteen bands are not removed by nullisomic analysis and therefore each may consist of more than one isozyme with the same pI encoded by more than one structural gene, on different homoeologous chromosomes. A similar situation was found by Brewer et al. (1969), for a number of different enzymes where nullisomic analysis proved ineffective.

There has in the past, been much confusion concerning the various forms of β-amylase found in cereal grains. However, it is now generally accepted that there are two main forms of the enzyme, a free form extracted by using water or a saline solution and a bound or latent form extracted by addition of a reducing agent or proteolytic enzyme (Daussant and Skakoun 1981). Latent β-amylase in wheat seeds is thought to be bound to the glutenin fraction by disulphide bridges (Rowsell and Goad 1962; Ewart 1977) and can thus be released by a reducing agent or by protease treatment. During germination the increase in β-amylase activity is attributed to the conversion of bound β-amylase to the free form through the action of endogenous reducing agents or proteases (Harris 1962; Kruger 1979).

In this study, total β-amylase (free plus bound) has been investigated, the bound enzyme being liberated by the addition of the reducing agent 2-mercaptoethanol. When samples without mercaptoethanol were electrofocused the resultant β-amylase zymograms were blurred, particularly at the alkaline end, and extended further into the alkaline region. This phenomenon has been reported by Niku-Paavola et al. (1973) for barley β-amylase and it was suggested that the alkaline components consisted of β-amylase bound to other constituents and polymers of the smaller monomeric forms. It therefore appears that the distinction between 'free' and 'bound' β-amylase is of little consequence in a genetic context in the wheat system but that the addition of a reducing agent to the samples prior to electrophoresis is essential if spurious results are to be avoided.

There are some anomalies between the analysis presented here and that of Joudrier and co-workers. These authors find three qualitatively similar but electrophoretically distinct β-amylase components. Components C_1 and C_2 are encoded by genes on chromosome 4A between which no recombination has been observed (Joudrier and Cauderon 1976). C_3 is encoded by a gene on chromosome 4D (Joudrier and Bernard 1977). Considering C_1 and C_2 as a single unit, 3 phenotypic variants were observed by Joudrier and Cauderon (1976). Here, the isozymes controlled by chromosome 4A comprise only a small part of the total enzyme extracted and only the 'Chinese Spring' type and a null variant were found. The C_3 component, however, is probably identical to the most acidic group of isozymes seen here to be encoded by the *β-Amy-D1* locus on chromosome 4D. It seems likely that the C_1 and C_2 components are encoded by the *β-Amy-A1* locus described here.

The *β-Amy-2* set of genes, located on homoeologous Group 5, has not previously been identified. The reasons for this are probably two-fold. Firstly, and more importantly, the resolving power of IEF allowed the detection of the nullisomic effect on the 5A isozymes 15, 30 and 32 of 'CS' which might not be observed in conventional electrophoretic separations. Secondly, intervarietal substitution lines have not, to our knowledge, been analysed by previous workes. This strategy allowed the identification of the chromosomal locations of a number of the *β-Amy-1* and *β-Amy-2* isozymes which are not present in 'Chinese Spring' and thus for which nullisomic analysis was not appropriate.

Precise map locations are available to date for only four enzyme structural genes: *α-Amy-1* (Nishikawa et al. 1981), *α-Amy-B2* (Gale et al. 1983), *Got-E3* (Hart et al. 1976) and

Gpi-D1 (Chojecki et al., in press). A precise map location is described here for *β-Amy-A2* which was found to be closely linked and proximal to the awning inhibitor, *B1* and *β-Amy-D1* located distally on the long arm of chromosome 4D, and was found to be unlinked to *Rht2*.

There is extensive variation in β-amylase phenotypes amongst the hexaploid wheats investigated and allelic variation occurs at all 4 loci described. In total, 11 different phenotypes were observed in the sample of 46 verieties. This is a much higher frequency of allelic variation than was reported by Joudrier and Bernard (1977), who found 6 phenotypes in a sample of 280 varieties.

There is currently much interest in the identification of cereal varieties by electrophoretic methods. β-amylase would appear to be an ideal enzyme for this purpose because of the existence of considerable allelic variation. To date, the most comprehensive attempt at the classification of wheat varieties using isozymes is by Salinas et al. (1982), who studied 5 enzyme systems, none of which was represented by more than 4 phenotypes.

Further analysis of a larger sample of wheat varieties is likely to reveal different β-amylase phenotypes. With the 4 loci and the number of alleles so far identified, 100 combinations are possible, of which only 10 have been recorded in the sample of 46 varieties. However, many of these varieties have similar pedigrees. It is unlikely that all 100 genotypes are in fact possible and some combinations may be selected against, such as those with several null alleles. It is interesting to note that there is a tendency for varieties which lack β-amylase isozymes at one locus to compensate for the loss with additional isozymes at other loci. The varieties 'Azteca' and 'Ciano' (both *β-Amy-A1b, -D1d, -A2b, -B2a*), 'Synthetic (*β-Amy-A1b, D1c, A2b, -B2b*), *T. macha* (*β-Amy-A1b, -D1d, -A2c, -B2a*) and 'Mara' (*β-Amy-A1b, D1e, -A2e, B2a*) are all null for the *β-Amy-A1* locus on chromosome 4A but have alleles with additional bands at 4D ('Azteca', 'Ciano', *T. macha* and 'Mara') or 5B ('Synthetic') loci.

The β-amylase loci *β-Amy-D1* and *β-Amy-A2* each appear to be complex, with several subunits close together and therefore can be considered for mapping purposes, as single loci. *β-Amy-D1* encodes 12 isozymes in varieties carrying the allele *β-Amy-D1a*. In crosses between varieties carrying *β-Amy-D1a* and those with the null allele *β-Amy-D1b* all 12 bands segregate as a single block. This is also the case with the *β-Amy-A2* alleles although fewer isozymes are involved. It may be that many enzyme structural genes are compound, as has previously been described for enzymes such as alcohol dehydrogenase in maize (Schwartz and Endo 1966), esterase in maize (MacDonald and Brewbaker 1970) and α-amylase in a number of organisms. In wheat, the *β-Amy-B2* locus on chromosome 7B (Gale et al. 1983) and esterase loci (Nakai 1979) have been shown to be complex.

The number of β-amylase isozymes present in the wheat grain seems particularly high. A possible reason is that gene duplication followed by mutation and differentiation of the resultant gene subunits has occurred. Mechanisms for tandem duplication leading to a compound locus could be unequal exchange between sister chromatids and homologous chromosomes and regional redundant duplication of DNA (Ohno 1970). Once a complex gene locus has been established, recombination between the differentiated subunits within the locus will give different combinations of the subunits. This is likely to be the origin of the different alleles of the complex locus *β-Amy-A2* on chromosome 5A, all of which comprise different combinations of the same seven isozymes (Table 3).

In wheat, α-amylase structural gene loci are complex and more than 30 isozymes are encoded by the 6 structural genes (Gale et al. 1983). There is evidence that, at the molecular level, α-amylase duplicated gene subunits are not present as tandem arrays but have spacer regions between them (D. C. Baulcombe, personal communication). This arrangement might allow subunit differentiation more readily than might occur with tandem arrays where mutants would tend to be eliminated.

An alternative explanation for some of the heterogeneity of β-amylase isozymes is that the loci are not complex and single gene products are subject to post-translational modification, such as phosphorylation and methylation, which could be responsible for diverse changes in isozyme isoelectric points. Immunochemical methods have indicated that differences in wheat β-amylase isozymes seen during germination are due in part to modification of pre-existing β-amylase (Daussant and Corvazier 1970). Post-translocational modification has also been demonstrated in the α-amylase isozymes of a number of cereals. In wheat, trimethylation of lysyl residues has been shown (Motojima and Jakagucki 1982) and in barley, α-amylase was found to be partially glycosylated (Rodway 1978). However, in rice, separate mRNAs have been implicated for the different isozymes rather than glycosylation (Miyata and Akazawa 1982). It seems likely that the heterogeneity of wheat β-amylase isozymes may be due in part to both the presence of complex genes and to post-translational modification.

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