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Allelic variation at α-Amylase loci in hexaploid wheat

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Summary. A study of α-amylase isozyme patterns from gibberellin-induced endosperms from more than 200 wheat genotypes has revealed allelic variation at five of the six *α-Amy-1* and *α-Amy-2* structural loci. These differences will find application as genetic markers and in varietal identification. The *α-Amy-B1* locus on chromosome 6B was most variable and displayed eight distinct allelic forms. The nature of the allelic phenotypes, observations of segregating populations and the number of in vivo translation products of mRNAs from the *α-Amy-1* and *α-Amy-2* loci indicated that the individual loci are multigenic, each consisting of tightly linked subunits which produce several different isoforms.

Key words: Hexaploid wheat – Endosperm – Allelic variation – α-Amylase – Isozymes – Isoelectric focusing

1 Introduction

The genes controlling isozyme phenotypes in wheat provide useful genetic markers for whole chromosomes or segments of chromosomes. They can be exploited in the production of intervarietal chromosome substitution lines and as markers for less easily scored genes of agronomic importance in selection programmes. Those enzyme structural genes which show considerable allelic variation also have applications in varietal identification and purity tests. Variation at enzyme marker loci is common among commercial wheat varieties and may therefore be assumed to have minimal detrimental effects on their agronomic performance. Such markers are preferable to morphological variants in breeding applications.

Protein structural genes are often found as triplicate sets in hexaploid wheat, *Triticum aestivum* (2n = 6x = 42), and several loci can be screened in a single electrophoretic separation. The redundancy afforded by the polyploid nature of *T. aestivum* also allows the accumulation of variation at single loci. Even the presence of null alleles is unlikely to result in any significant loss of function in the plant because of homoeoallelic compensation. Such variation might not be tolerated in a diploid.

α-Amylase is particularly suitable as a marker system in that two triplicate sets of loci are present in wheat on homoeologous chromosome groups 6 and 7 (Nishikawa and Nobuhara 1971) and the two types of gene products, α-AMY-1 and α-AMY-2, are distinct as visualised by isoelectric focusing (IEF). The *α-Amy-1* series of genes, on the long arms of chromosomes 6A, 6B and 6D, encodes the 'malt' enzyme produced in the germinating grain. The *α-Amy-2* series, on the long arms of chromosomes 7A, 7B and 7D, controls production of the 'green' enzyme produced both during early grain development and during the latter stages of germination (Gale and Ainsworth 1984). Two of the *α-Amy-1* (Nishikawa et al. 1981) and one of the *α-Amy-2* (Gale et al. 1983) loci have been mapped within their respective chromosomes and varietal and allelic differences in isozyme pattern have been noted.

However, previous analyses have considered individual isozymes separately. Below we describe a classification of allelic variation based on the several isozymes produced by each of the *α-Amy* loci. This allows varieties to be identified by a genetic, rather than simply phenotypic, system. Evidence is also provided to show that the complex allelic phenotypes are the products of single, albeit compound, loci.

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Table 1. Intervarietal chromosome substitution series employed in analysis of *α-Amy-1* and *α-Amy-2* allelic differences

Recipient variety	Donor variety	Origin
'Bersée'	'Hybride du Joncquois'	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Cappelle-Desprez'	'Mara'	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Cappelle- Desprez'	'Hybride du Joncquois'	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Chinese Spring'	'Cappelle-Desprez'	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Chinese Spring'	*Triticum spelta*	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Chinese Spring'	'Ciano 67'	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Chinese Spring'	'Lutescens 62'	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Chinese Spring'	'Synthetic'[a]	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Chinese Spring'	'Cheyenne'	R. Morris (Univ. Nebraska)
'Chinese Spring'	'Hope'	E. R. Sears (Univ. Missouri)
'Hobbit 'S''	'Bezostaya 1'	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Hobbit 'S''	*T. macha*	C. N. Law and A. J. Worland (Plant Breeding Institute)
'Hobbit 'S''	'VPM 1'	C. N. Law and A. J. Worland (Plant Breeding Institute)

[a] Synthetic hexaploid – amphidiploid of *T. dicoccum* × *Aegilops squarrosa* produced by E. R. Sears of University of Missouri, Columbia (McFadden and Sears 1946)

2 Materials and methods

2.1 Hexaploid varieties

Two hundred hexaploid wheat varieties and species, some of which will be mentioned in the text, were examined for α-amylase phenotype. A more extensive varietal list of α-amylase and other enzyme phenotypes will be presented elsewhere.

2.2 Intervarietal chromosome substitution lines

Intervarietal chromosome substitution lines for homoeologous groups 6 and 7 from several series (Table 1) were examined to identify chromosomal control of novel α-amylase isozymes.

2.3 Random lines

Random inbred lines developed from individual varietal hybrids were employed to investigate allelic relationships. Sixty F_6 'Hobbit 'S'' × 'Manella' and 100 F_6 'Sava' × 'Bilbo 'S'' lines developed by single seed descent were provided by Dr. J. W. Snape and 63 F_3 'Bilbo 'S'' × 'Bezostaya 1' lines were provided by Mr. A. J. Worland.

2.4 α-Amylase induction and isoelectric focusing

The method of induction of α-amylase production in distal half-grains of wheat in response to gibberellic acid and the conditions for isoelectric focusing are as described by Gale et al. (1982).

3 Results

3.1 Isozymes in 'Chinese Spring'

Isoelectric focusing of gibberellic acid (GA_3) induced half-grains of 'Chinese Spring' (CS) on pH 3.5–9.5 gels separates the isozymes of the α-AMY-1 ('malt' amylase, pI 6.6–7.5) and the α-AMY-2 ('green' amylase, pI 4.9–6.0) groups (Fig. 1).

Seventeen α-AMY-1 isozymes are resolved, 11 of which have been shown previously to be controlled by individual group 6 chromosomes (Fig. 1). The isozymes have been re-numbered from the system described by Gale et al. (1983) because a number of bands which were originally thought to be single have subsequently been shown to consist of two isozymes in some separations.

The 16 α-AMY-2 isozymes are better separated on pH 4.0–6.5 gels (Fig. 1). The original band designations of Gale et al. (1983) are retained. Control by individual chromosomes of group 7 has been assigned to 14 of the 16 isozymes.

3.2 Allelic variation at α-Amy-1 loci

3.2.1 α-Amy-A1. A single allelic variant relative to the standard *α-Amy-A1a* allele carried by CS and most

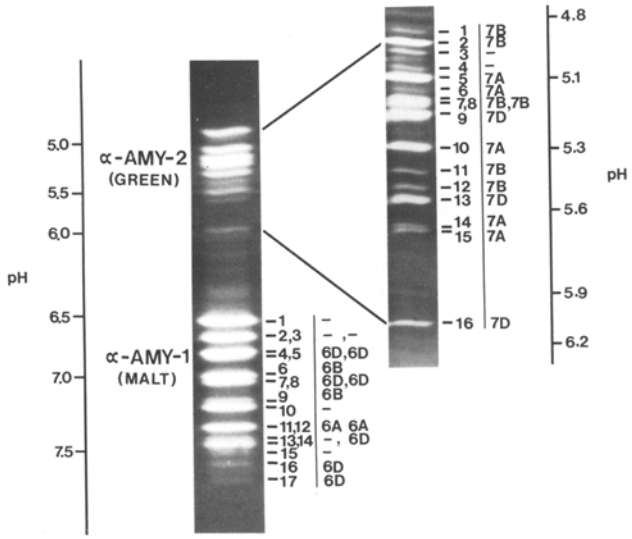

Fig. 1. The α-amylase phenotype of 'Chinese Spring' showing both 'malt' (α-AMY-1) and 'green' (α-AMY-2) isozymes. The chromosomal control of specific isozymes is indicated where known

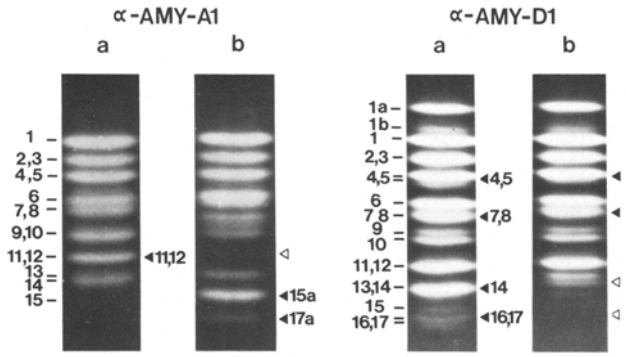

Fig. 2. Isozyme phenotypes of the allelic variants for the *α-Amy-A1* and *α-Amy-D1* loci. The bands encoded by each allele are *arrowed* (▷) and bands that are absent relative to the '*a*' allele indicated by ▶

other varieties, was detected. *α-Amy-A1b,* found only in a 'Bezostaya 1' and 'Kavkaz', two closely related varieties, encodes a novel phenotype in which bands 11 and 12 are absent and are replaced by two novel bands, 15a and 17a (Fig. 2).

Analysis of the chromosome group 6 substitution of 'Hobbit 'S'' ('Bezostaya 1') demonstrated clearly that this allelic difference is attributable to *α-Amy-A1* on chromosome 6A of 'Bezostaya 1'.

3.2.2 α-Amy-B1. Eight *α-Amy-B1* alleles were identified (Fig. 3). *α-Amy-B1a,* present in CS and a small number of other varieties, encodes, on the basis of nullisomic-tetrasomic analysis, two isozymes (bands 6 and 9).

Of the other seven alleles, two (*α-Amy-B1b* and *c*) are each characterised by the presence of two novel bands (1a and 1b) which have lower pIs than the α-AMY-1 isozymes of CS. One (*α-Amy-B1h*) is characterised by two novel bands with high pIs (17b, 17c) and four which involve variation exclusively concerning the isozymes in the α-AMY-1 mid-pI range (*α-Amy-B1d, e, f* and *g*).

α-Amy-B1b (bands 1a, 1b, 6, 9a), exemplified by 'Mara', was present in about 12% of varieties screened. Analysis of the chromosome group 6 substitutions of CS ('Ciano 67'), CS ('Lutescens 62'), CS (*T. spelta*), CS ('Cheyenne') and 'Bersée' ('Hybride du Joncquois') clearly demonstrates in each case that the substituted 6B chromosomes of the donor varieties carry *α-Amy-B1b.*

α-Amy-B1c (bands 1a, 1b, 6, 9) was observed only in a single variety, 'Sava'.

α-Amy-B1d (bands 6, 9a) was found only in 'Sicco'.

α-Amy-B1e (bands 6, 9a, 9b) was widespread, occurring in about 60% of varieties examined. A number of chromosome group 6 substitution lines were examined which showed that bands 9a and 9b were controlled by chromosome 6B. In CS ('Cappelle-Desprez 6B'), CS band 9 is replaced by bands 9a and 9b from 'Cappelle-Desprez'. In the 6B substitutions of 'Cappelle-Desprez' ('Hybride du Joncquois'), 'Cappelle-Desprez' ('Mara')

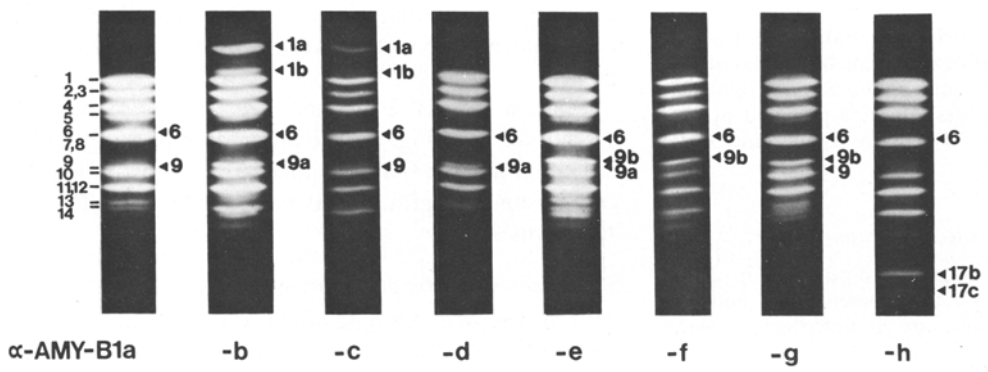

Fig. 3. Isozyme phenotypes of the allelic variants for the *α-Amy-B1* locus. The bands encoded by each allele are *arrowed* (▶)

and 'Bersée' ('Hybride du Joncquois') band 9b is replaced by bands 1a and 1b. In 'Hobbit "S"' ('Bezostaya 1' 6B), band 9 is replaced by 9a.

α-Amy-B1f (bands 6, 9b) was rare and found only in 'Sappo'.

α-Amy-B1g (bands 6, 9, 9b) was relatively rare and was identified in only five varieties including Cheyenne and was demonstrable in the CS ('Cheyenne 6B') substitution lines.

α-Amy-B1h (bands 6, 17b, 17c) is distinctive in producing a phenotype which includes two novel bands 17b and 17c with pIs higher than the normal range of the α-AMY-1 isozymes and lacks band 9. This allele was confined to *T. macha* and was confirmed as a 6B allele in the 'Hobbit "S"' (*T. macha* 6B) substitution line.

3.2.3 α-Amy-D1. In addition to the CS allele *α-Amy-D1a* which encodes 6D bands 4, 5, 7, 8, 14, 16 and 17, about 50% of varieties examined carried a variant allele, *α-Amy-D1b*. *α-Amy-D1b* produces a phenotype in which bands 16 and 17 are absent and 14 is present as a weaker band (Fig. 2). *α-Amy-D1b* is not always easy to identify and concentrated extracts are used in order to resolve all the bands, consequently causing a loss in clarity among the more acidic α-AMY-1 isozymes. A number of substitution series confirmed the nature of this allelic difference; CS ('Cappelle-Desprez'), CS ('Cheyenne') and CS ('Synthetic'), i.e. all *α-Amy-D1a* (*–b*) and the 6D substitution of 'Cappelle-Desprez' ('Hybride du Joncquois'), 'Hobbit "S"' ('Bezostaya 1') and 'Berśee' ('Hybride du Joncquois'), i.e. all *α-Amy-D1b* (*–a*).

3.3 Allele variation at α-Amy-2 loci

3.3.1 α-Amy-B2. 'Chinese Spring' and 'Chinese White' differ from all other varieties examined in that they alone carry the *'a'* allele at the *α-Amy-B2* locus. All other varieties carry the variant allele *α-Amy-B2b* which produces a phenotype in which band 11 is absent and is replaced by a novel band, 3a, close to band 3 (Fig. 4). Band 3a was previously referred to as B1 by Gale et al. (1983). The absence of band 3a and the presence of band 11 was demonstrated in the zymograms of all seven of the 7B substitutions with 'Chinese Spring' as the recipient parent.

3.3.2 α-Amy-D2. The allele *α-Amy-D2a* encodes three bands (9, 13 and 16) and is carried by CS and most other varieties. Three varieties, 'VPM 1', 'Synthetic' and 'Largo' carry the variant allele *α-Amy-D2b*, which has previously been described by Gale et al. (1984). *α-Amy-D2b* differs from all other α-amylase allelic variants in that the complete set of three *α-Amy-D2a* bands are absent and are replaced by two novel bands 8a and 12a

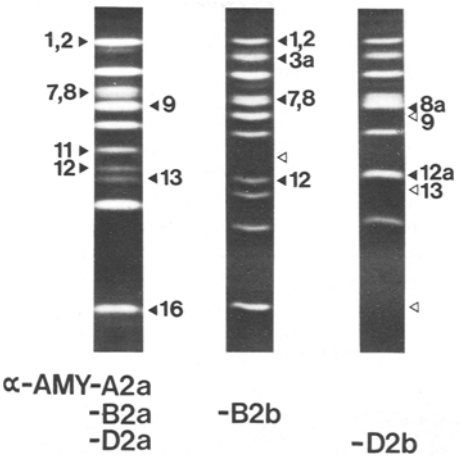

Fig. 4. Isozyme phenotypes of the allelic variants at the *α-Amy-B2* and *α-Amy-D2* loci (symbols as in Fig. 2)

(Fig. 4). *α-Amy-D2b* is derived, in all three genotypes carrying it, from alien sources. 'VPM 1' (Doussinault and Dosba 1977) carries a large segment of, or possibly the entire chromosome 7D from *Aegilops ventricosa* (Gale et al. 1984). The D genomes of both 'Synthetic' and 'Largo', and hence the *α-Amy-D2* alleles, are derived directly from *Ae. squarrosa*. 'Synthetic' is an amphidiploid of *T. dicoccum*×*Ae. squarrosa* (McFadden and Sears 1946), while 'Largo' is derived from a cross between *T. turgidum* and *Ae. squarrosa* (Joppa and Williams 1982).

Analysis of the group 7 chromosome substitutions of CS ('Synthetic' 7D) and 'Hobbit "S"' ('VPM 1' 7D) demonstrated the association of *α-Amy-D2b* with chromosome 7D.

3.4 Segregation at α-amylase loci

The 'allele' descriptions above assume that the several bands controlled by factors on a single chromosome arm (up to seven in the case of *α-Amy-D1*) are the products of a 'single' locus. A report describing two *α-Amy-1* loci quite widely separated on the long arm of chromosome 6B (Nishikawa et al. 1981) has led us to examine the segregation of *α-Amy-B1* 'alleles' more closely.

The phenotypes of 60 F_6 homozygous random lines from the cross 'Manella' (*α-Amy-B2b*)×'Hobbit "S"' (*α-Amy-B2g*) were examined for segregation at *α-Amy-B2*. The two alleles involved differ by the presence of bands 1a, 1b and 9a versus the presence of the strong band, 9b, in the mid-pI range, in addition to CS band 9.

A sample of the segregants is shown in Fig. 5. Although any non-parental types would be easily identified (as patterns including both 1a, 1b *and* 9b or lacking all three bands), none were observed.

Table 2. Analysis of allelic differences at *α-Amy* loci in segregating random lines

Locus	Alleles	Cross	Family	No. lines	Non-parental recombinants
α-Amy-A1	*'a'×'b'*	'Bilbo 'S''×'Bezostaya 1'	F_3	63	0
α-Amy-B1	*'g'×'b'*	'Hobbit 'S''×'Manella'	F_6	60	0
	'c'×'a'	'Sava'×'Bilbo 'S''	F_6	100	0
α-Amy-D2[a]	*'a'×'b'*	'Fundin'×'VPM 1'	F_3	28	0

[a] Data from Gale et al. (1984)

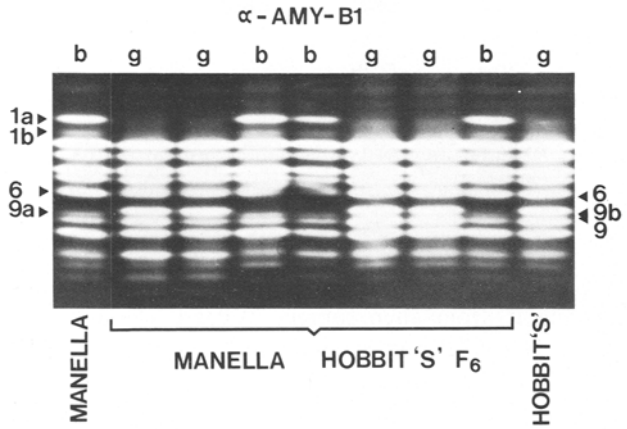

Fig. 5. α-AMY-1 phenotypes of a sample of F_6 homozygous lines from the cross 'Hobbit 'S'' (*α-Amy-B2b*) × 'Manella' *α-Amy-B2g*) showing the allelic segregation of isozymes encoded by the *α-Amy-B2* locus

Analysis of 100 F_6 lines from the cross 'Sava' (*α-Amy-B1c*)×'Bilbo 'S'' (*α-Amy-B1a*) gave the same result as did random lines from crosses in which differences at the *α-Amy-A1*, and *α-Amy-D2* loci were segregating (Table 2).

Thus, we have no evidence that any of the α-amylase structural genes are duplicated on their respective chromosome arms. Indeed, all of the differences analysed behave, for mapping purposes, as alternative alleles at single loci.

4 Discussion

Considerable variation can be found in a range of wheat varieties relative to the 17 α-AMY-1 and 16 α-AMY-2 isozymes identified in 'Chinese Spring'. The variation appears in the form of multiple alleles at five out of six compound loci which make up the *α-Amy-1* and *α-Amy-2* triplicate sets of genes on the long arms of the group 6 and group 7 chromosomes, respectively. In our sample of 200 varieties and hexaploid species, which included many closely related genotypes, a total of 18 different phenotypes was recorded. Thus, among the other protein structural genes which show variation of value in varietal identification, α-amylase genes rank similarly to the storage protein loci, *Glu-1*, and *Gli-1* (Payne and Lawrence 1983), and the β-amylase loci, *β-Amy-1* and *β-Amy-2* (Ainsworth et al. 1983). Among the other enzyme loci which display polymorphisms, *α-Amy-1* and *α-Amy-2* display more allelic variation than has been found for grain esterase, *Est-5* (Ainsworth et al. 1984), glucose phosphate isomerase, *Gpi-1* (Chojecki and Gale 1982), leaf peroxidase *Per-1* (Ainsworth et al. 1984), mature grain peroxidase (Benito et al. 1980), hexokinase, *Hk-1* (Ainsworth 1983) and alcohol dehydrogenase, *Adh-1* (Suseelan et al. 1983). A classification of the variation at these α-amylase and other enzyme loci in more than 150 varieties including current British wheat and their ancestors will be provided in Ainsworth et al. (1986).

Although many enzyme structural loci have been identified in wheat over the past two decades (Hart 1984), very little allelic variation at the individual loci has been recognised until recently. Much of this variation has been identified with the relatively recent use of IEF: iso-electric focusing separations always show more bands than are found using conventional electrophoresis. This raises the question as to whether the multiple bands all represent different structural gene products, or whether some are products of the post-translational modification of fewer isozymes, or whether some are simply artefacts of the separation system. Previously, we have provided evidence that not all of the differences among the five major bands associated with *α-Amy-B2* are post-translational products or non-genetic artifacts (Gale et al. 1983). This conclusion is reinforced by the series of alleles found at the apparently most variable locus, *α-Amy-B1*. Alleles *'a'* to *'h'* show various combinations of bands 1a with 1b, 9b, 9a, 9 and 17b with 17c (Fig. 3), indicating that at least five of the nine bands associated with the locus are independent products of a compound locus. Although no intra-locus recombination, which would be observed as a non-parental segregant, was found among the total of 251 progenies from crosses involving variation at *α-Amy-B1*, it must be probable that the multiple allelic system arose in this way. A similar series of related alleles has

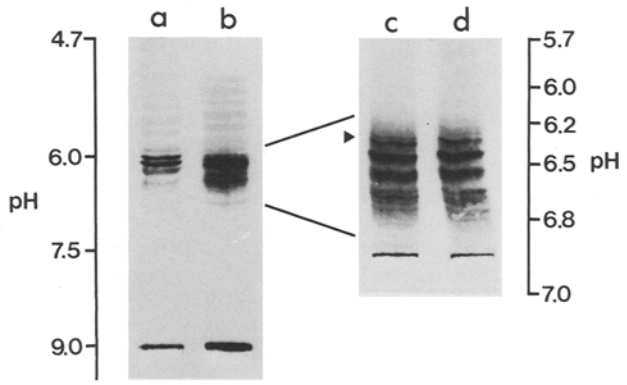

Fig. 6. Immunoprecipitated mRNA translation products of the *α-Amy-1* genes. *Tracks a* and *b* show, respectively, in vitro and in vivo translation products of mRNAs extracted from GA-treated aleurones of 'Chinese Spring', precipitated with *α-Amy-1* specific antiserum and resolved on a pH 3 – 10 gel. *Tracks c* and *d* show the same in vivo translation products from 'Hybride du Joncquois' (*α-Amy-B1b, -D1a*) and 'Bezostaya 1' (*α-Amy-B1e, -D1b*) resolved on a pH 5 – 7.5 gel. Band differences are indicated by *arrows*. For the method of extraction and resolution see Baulcombe and Buffard (1983)

been described in wheat at the *β-Amy-A2* locus (Ainsworth et al. 1983).

Evidence from molecular studies also indicates that the individual *α-Amy* loci are 'multigenes'. Baulcombe (1983) has shown that there are at least six in vitro translation isoform products of the *α-Amy-1* series though this is regarded as a low estimate of the number of genes in the three families. Our analysis, using narrower pH range gels allowing higher resolution, has provided confirmation of this (Fig. 6).

Immunoprecipitation and electrophoresis, both of the products of the *α-Amy-1* genes in vivo, i.e. from aleurone cells, and in vitro translation products from mRNAs isolated from the same cells (Fig. 6, tracks a and b) indicates that more RNA species than one per locus are produced in both cases. With increased resolution of similarly precipitated in vivo translation products of mRNAs from the varieties 'Hybride du Joncquois' and 'Bezostaya 1', at least nine isoforms are apparent (Fig. 6, tracks c and d). This estimate of the number of genes in the *α-Amy-1* series is of the same order as that obtained by Baulcombe et al. (1984) using restriction enzyme digests and hybridisation studies. In addition, it is interesting to note that the in vivo translation products from 'Hybride du Joncquois' (*α-Amy-B1b, α-Amy-D1a*) and 'Bezostaya 1' (*α-Amy-B1e, α-Amy-D1b*) vary in a manner similar to the isozyme variation associated with the variant alleles *α-Amy-B1b* and *α-Amy-D1b*.

Clearly, while such molecular studies do not provide evidence that all of the isozyme bands described are separate gene products, it is equally clear that each of the *α-Amy-1* loci does produce several mRNA species. Similar evidence is also available for the *α-Amy-2* gene series (Baulcombe et al. 1984).

The isozyme analyses described above require more than nine multigene family members to account for the 17 α-Amy-1 isozymes in 'Chinese Spring' and the nine other bands found among the other varieties screened. Post-translational modification by glycosylation (Rodway 1978) and methylation (Motojima and Sakaguchi 1982) has been described for cereal α-amylases and could be operating between isoforms which appear to be present always as pairs, e.g. the *α-Amy-B1* products, 1a and 1b. An analysis of the α-amylase isozymes produced by the nullisomic-tetrasomic genotypes, lacking in turn, the 15 wheat chromosomes in homoeologous groups 1–5 did not identify the location of any such modifying genes (Gale et al. 1983). However, modifying genes which were triplicated across the A, B and D genomes would consequently not be exposed in such a deletion analysis.

Previous genetic analyses on the wheat α-amylase system are confined to those of Gale et al. (1983) and Nishikawa and co-workers (Nishikawa and Nobuhara 1971; Nishikawa et al. 1981). The latter authors identified ten critical isozymes, each of which was assigned a structural locus symbol, of which nine were identified as products of genes on the chromosomes of homoeologous groups 6 and 7. This system therefore considers the genetic control of individual isozymes, rather than the more complex segregating patterns described above, and implies that there is more than one structural locus on several of the group 6 and 7 chromosomes (6B, 6D and 7B).

With one important exception, the results presented here accommodate those presented by Nishikawa et al., whose several loci and attendant allelic variants are explicable as variation at the *α-Amy-1* and *α-Amy-2* compound loci.

Accordingly, the variant allele, 'Amy 6A1^{m}', reported for the 'Amy 6A1' locus on chromosome 6A may well be part of the phenotype of *α-Amy-A1b* described above. Of the two loci reported on chromosome 6D, 'Amy 6D1' has three alleles while 'Amy 6D2' is invariant. 'Amy 6D2' (which has also been referred to as *α-Amy 1* by Hart 1984) controls 'band 7', which is equivalent to bands 4 and 5 described here and which also are invariant. 'Amy 6D1' and 'Amy 6D2' are clearly components of the compound locus *α-Amy-D1*. Of the two 'Amy 6D1' allelic variants, it is likely that 'Amy 6D1^{o}' is the same as *α-Amy-D1b*.

'Amy 7A1', the locus on chromosome 7A, was invariant, which was also the case for *α-Amy-A2* described here.

'Amy 7B1' and 'Amy 7B2' are considered by Nishikawa and co-workers to be separate loci on chromosome 7B. 'Amy 7B2' (also referred to as *α-Amy3* by Hart 1984) has an allelic variant 'Amy 7B2^{m}' in which 'band 14' is diminished. 'Band 14' is equivalent to band 3a here which is absent from the phenotype encoded by the variant allele *α-Amy-B2b*. 'Amy 7B2^{m}' and *α-Amy-B2b* are therefore probably equivalent, although the latter allele also involves further variation (band 11).

Nishikawa and Nobuhara (1971) describe an allelic variant for the 'Amy 7D1' locus on chromosome 7D, 'Amy 7D1^{o}', which refers to the loss of the highest pI band ('band 11'). This band is equivalent to band 16 here, which is one of those affected in the phenotype produced by *α-Amy-D2b*. Thus, these two alleles appear to be the same.

The major difference between the results presented here and those of Nishikawa et al. (1981) concerns their finding of two separate *α-Amy-B1* loci, 'Amy 6B1' and 'Amy 6B2', 20.6 recombination units apart, on chromosome 6B. They showed that two bands, one clearly

equivalent to 1a and the other probably equivalent to 9b, segregated in a non-allelic but linked manner in F_2 progeny from the cross 'Chinese Spring' × *T. spelta* var. 'duhamelianum'. The 60 homozygous recombinant lines examined here from the *α-Amy-B1b/g* hybrid would, on the same hypothesis, have been expected to yield about 12 lines with recombinant band patterns; however, none were observed.

This difference notwithstanding, the allelic differences specificed by the variant 'Amy 6B1' and 'Amy 6B2' alleles coincide with some of those specified by the *α-Amy-B1* alleles described here. For example, 'Amy 6B2' refers to the presence of 'band 10' (the highest pI *α-Amy-1* band) equivalent to bands 1a and 1b which are components of the *α-Amy-B1b* and *c* phenotypes described here. 'Amy $6B2^{0}$' refers to the absence of bands 1a and 1b which describes part of the patterns associated with the remaining six alleles, *α-Amy-Ba, d, e, f, g* and *h*.

The unmapped locus 'Amy 4' encoding 'band 4' with its allelic variant 'Amy 4^{m}' (where 'band 4' is reduced in intensity) is shown here to be a further component of the 'α-Amy-6B' locus. 'Band 4' is equivalent to band 10 which cannot be removed entirely by nullisomy for any group 6 chromosome. However, observation of the phenotypes of the *α-Amy-B1* alleles shows that this band correlates in intensity with bands 9, 9a and 9b controlled by *α-Amy-B1* (Fig. 3); band 10 is strong when band 9 is present, but reduced in intensity when 9a or 9a and 9b are present. Band 10 must therefore include two isozymes, one of which is a product of *α-Amy-B1*. The compound locus *α-Amy-B1*, therefore includes the genes previously described as 'Amy 6B1', 'Amy 6B2' and 'Amy 4'.

The precise nature of the structural control of the multiple band patterns is likely only to emerge from the further study of isolated and cloned wheat α-amylase genes. However, it is clear that for the varieties examined here, the complex isozyme patterns seen on IEF gels can be explained in terms of allelic differences. These can be used for varietal identification to supplement the storage protein analyses currently used for wheat and barley varieties.

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