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α-Amylase and β-amylase homoeoloci in species related to wheat

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Summary

A study of α-amylase isozyme patterns from gibberellin-induced endosperms of wheat–alien genotypes (amphiploid, addition and substitution lines) resolved by flat-bed isoelectric focusing identified homoeoloci for *α-Amy-1* (malt α-AMY-1 genes) on chromosomes 6H of *Hordeum vulgare*, 6RL of *Secale cereale*, $6R^m$ of *S. montanum* and 6E of *Agropyron elongatum*. Homoeoloci for *α-Amy-2* (green α-AMY-2 genes) were identified on chromosomes $7H^{ch}L$ of *Hordeum chilense*, 7RL of *Secale cereale*, $7S^b$ of *Aegilops bicornis*, 7U of *Ae. umbellulata* and 7EL of *Agropyron elongatum*. Analysis of mature grain β-amylase identified *β-Amy-1* loci on chromosomes 4H of *H. vulgare*, $4H^{ch}$ of *H. chilense*, $4S^1$ of *Ae. sharonensis* and *Ae. longissima* and *β-Amy-2* loci on chromosomes 5RL of *S. cereale* and 5U of *Ae. umbellulata*. These gene locations provide further evidence for the homoeology of the alien chromosomes with wheat and for the conservation of gene synteny among wheat and its relatives.

1. Introduction

Approximately one hundred structural genes coding for particular enzymes have been located on specific chromosomes of hexaploid bread wheat, *Triticum aestivum* ($2n = 6x = 42$, AABBDD), and almost all of these are available as markers for alien genetic material in a wheat background. The genetics of α-amylase (EC 3.2.1.1) and β-amylase (EC 3.2.1.2) is well characterised in wheat. In this paper we describe the presence of similar loci in a number of related species.

In wheat, α-amylase production is controlled by two triplicate sets of genes; the *α-Amy-1* set, with loci on the long arms of the homoeologous group 6 chromosomes and the *α-Amy-2* set on the long arms of the group 7 chromosomes (Nishikawa & Noburhara, 1971; Gale *et al*, 1983). The 'malt' (α-AMY-1) amylase, comprising about 14 isozymes, is produced during germination. The 'green' α-AMY-2 enzyme is present in the pericarp during grain development and again at germination. However, the pericarp isozymes are only a subset of the 17 germination isozymes (Gale & Ainsworth, 1984). Although the genetics of α-amylase has been valuable in wheat evolutionary studies (Nishikawa *et al.* 1975, 1980), little attention has been paid to the identification of these enzymes in related species, except in cultivated barley (Brown & Jacobsen, 1982).

β-Amylase, assayed in mature grains, has been shown to be controlled by two series of homoeologous genes in hexaploid wheat: the *β-Amy-1* series with loci on chromosome arms 4Aα (*β-Amy-A1*) and 4DL (*β-Amy-D1*) (Joudrier & Cauderon, 1976; Joudrier, 1980; Ainsworth *et al.* 1983); and the *β-Amy-2* series with loci on 5AL (*β-Amy-A2*) and 5BL (*β-Amy-B2*) (Ainsworth *et al.* 1983; Dabrowska, 1983).

2. Materials and methods

(i) *Alien species, wheat–alien amphiploids, chromosome addition and substitution lines*

The following alien species obtained from the Plant Breeding Institute collection were examined: *Hordeum vulgare* cv. Betzes ($2n = 2x = 14$, HH); *H. chilense* ($2n = 2x = 14$, $H^{ch}H^{ch}$); *Secale cereale* cvs. King II and Imperial ($2n = 2x = 14$, RR); *S. montanum* ($2n = 2x = 14$, R^mR^m); *Aegilops bicornis* ($2n = 2x = 14$, S^bS^b); *Ae. sharonensis* and *Ae. longissima* ($2n = 2x = 14$, S^lS^l) *Ae. umbellulata* ($2n = 2x = 14$, UU); *Agropyron elongatum* (*Elytrigia elongata*), ($2n = 2x = 14$, EE).

The wheat–alien amphiploids, addition and substitution lines examined are shown in Table 1.

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Table 1. *Wheat–alien genotype analysed for α-amylase and β-amylase*

Alien chromosome donor species	Hexaploid wheat recipient cultivar	Wheat–alien genotypes	Original source of material
Hordeum vulgare cv. Betzes	Chinese Spring	Additions: A, B, C, D, E, F	Islam *et al.* (1975)
H. chilense	Chinese Spring	Amphiploid	Chapman & Miller (1978)
		Additions[a]. A [7H^{ch}], B, C, D, E, F	Miller *et al.* (1982*b*)
		Substitutions: (7A)7H^{ch}, (7B)7H^{ch}, (7D)7H^{ch} (7A)7H$^{ch}\alpha$, (7B)7H$^{ch}\beta$, (7D)7H$^{ch}\alpha$	Miller *et al.* (1985)
Secale cereale cv. King II	Holdfast	Amphiploid	Riley & Chapman (1958)
		Additions: 1R, 2R, 4R, 5R, 6R, 6RS, 6RL, 7R, 7RS, 7RL	
		Substitutions: (6A)6R, (6B)6R, (6D)6R (6A)6RL, (6B)6RL, (6D)6RL	Riley (1965)
S. cereale cv. King II	Chinese Spring	Amphiploid	Miller (1973)
		Additions: 1R, 2R, 3R, 4R, 5R, 5RS, 5RL, 6R, 7R	
		Substitution: (5A)5RL	
S. cereale cv. Imperial	Chinese Spring	Additions: 1R, 2R, 3R, 4R, 5R, 6R, 7R	Driscoll & Sears (1971)
S. montanum	Chinese Spring	Amphiploid	Miller (1973)
		Additions: 1R^{m}, 2R^{m}, 4R^{m}, 5R^{m}, 6R^{m}	
		Substitutions: (6A)6R^{m}, (6D)6R^{m}	Miller (1984)
Aegilops bicornis	Holdfast	Additions: 7S^{b}, 7S^{b} telo	Riley, Chapman & Miller unpublished
		Substitutions: (7A)7S^{b}, (7B)7S^{b}	
Ae. umbellulata	Chinese Spring	Amphiploid	Kimber (1967)
		Additions[a]: A, B, C [5U], D, F, G [7U]	Chapman *et al.* (1975);
		Substitutions: (5A)5U, (5D)5U, (7D)7U	Riley *et al.* (1972, 1973)
Ae. sharonensis	Chinese Spring	Amphiploid	
		Addition: 4S^{l}	Miller *et al.* (1982*a*)
		Substitutions: (4A)4S^{l}, (4B)4S^{l} (4D)4S^{l}	
Ae. longissima	Chinese Spring	Amphiploid	Feldman (1975)
		Additions: A, B, C, D, E, F, G	
Agropyron elongatum	Chinese Spring	Amphiploid	Dvořák & Knott (1974)
		Additions: I, II, III, IV, V, VI, VII	

[a] The homoeology to wheat chromosomes of the alien addition chromosomes employed in substitution lines is given in square brackets [].

(ii) *Electrophoresis*

The method of induction of α-amylase isozymes in distal half-grains in response to gibberellic acid and the conditions for isoelectric focusing (IEF) are as described by Gale *et al.* (1983). α-AMY-1 and α-AMY-2 isozymes are resolved on pH 3·5–9·5 gels and for optimal resolution of α-AMY-2 isozymes, on pH 4–6·5 gels. β-Amylase was extracted from single grains and resolved by IEF as described previously by Ainsworth *et al.* (1983) (see Fig. 1).

3. Results and discussion

(i) *Barley* (Hordeum)

(a) *Chinese Spring* (*CS*)–Hordeum vulgare *cv. Betzes addition lines*. Examination of α-amylase isozymes from CS-Betzes addition lines confirmed the location of an *α-Amy-1* locus, *α-Amy-H1*, in addition C (Brown & Jacobsen, 1982). Addition C, which expressed the two major barley isozymes (Fig. 2*a*) is related to wheat homoeologous group 6 (Islam *et al.* 1981) and carries barley chromosome 6. Addition C also carries homoeologous aminopeptidase (*Amp-H1*) and glutamate oxaloacetate transaminase (*Got-H2*) loci (Hart *et al.* 1980).

The presence of an α-amylase gene in the *α-Amy-2* series on barley chromosome 1 carried by addition D (Brown & Jacobsen, 1982), which is related to wheat homoeologous group 7, could not be confirmed. The barley α-AMY-2 isozymes were both weak, and have similar isoelectric points (pI) to wheat isozymes.

The six barley β-amylase isozymes which do not co-focus with wheat isozymes were expressed by addition A which carries barley chromosome 4 (Fig.

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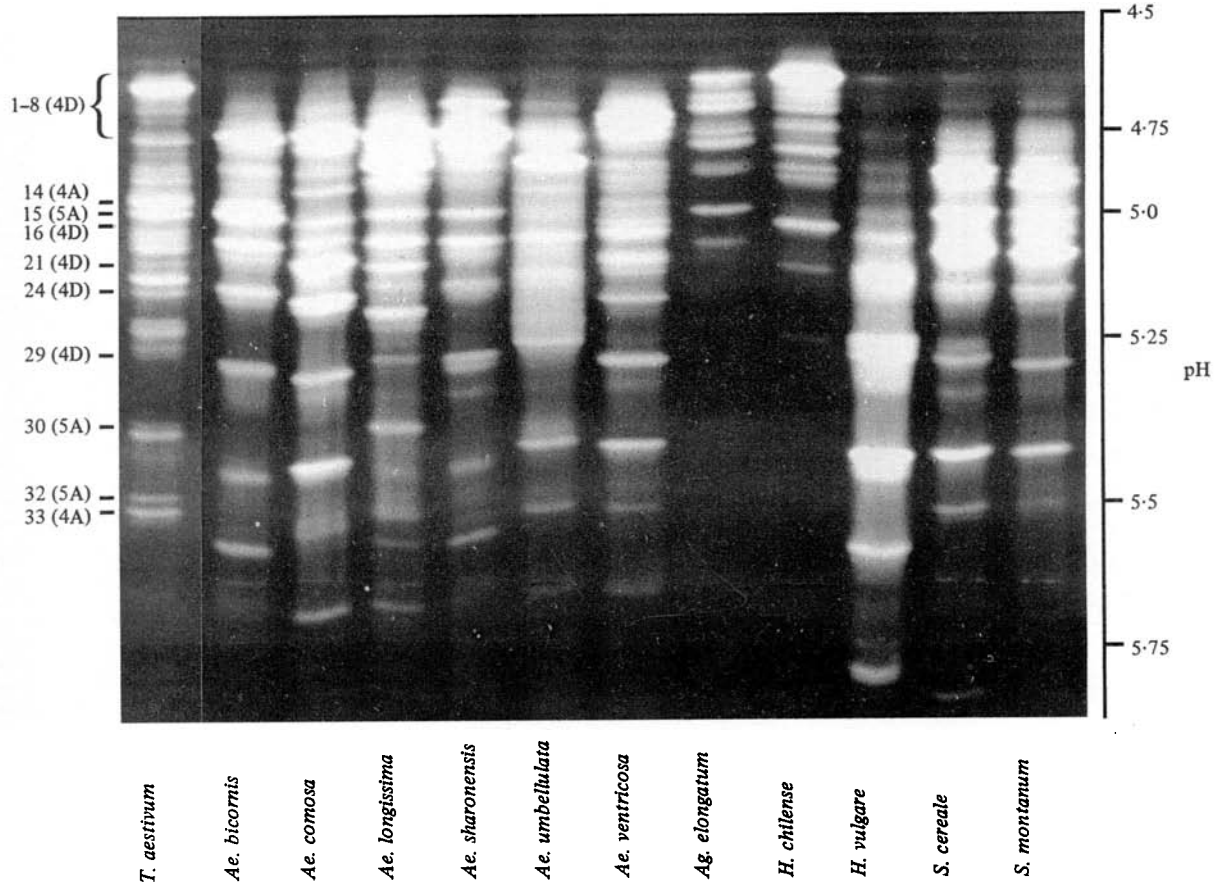

Fig. 1. β-Amylase phenotypes of wheat and related alien species. It is interesting to note that the β-amylase phenotypes of several of the alien species shown including *Secale cereale*, *S. montanum* and a number of *Aegilops* species, and particularly *Hordeum vulgare*, have a smeared appearance. Aqueous extracts from grains of these species also tended to be very viscous in comparison to extracts from wheat. The additional viscosity may well be caused by the higher levels of β-glucan, a major component of cell walls, found in grains of these species (R. J. Cooke, personal communication), and it is possible that the smearing may result from binding of β-amylase to β-glucan. Isozymes in Chinese Spring are numbered as described in Ainsworth *et al.* (1983).

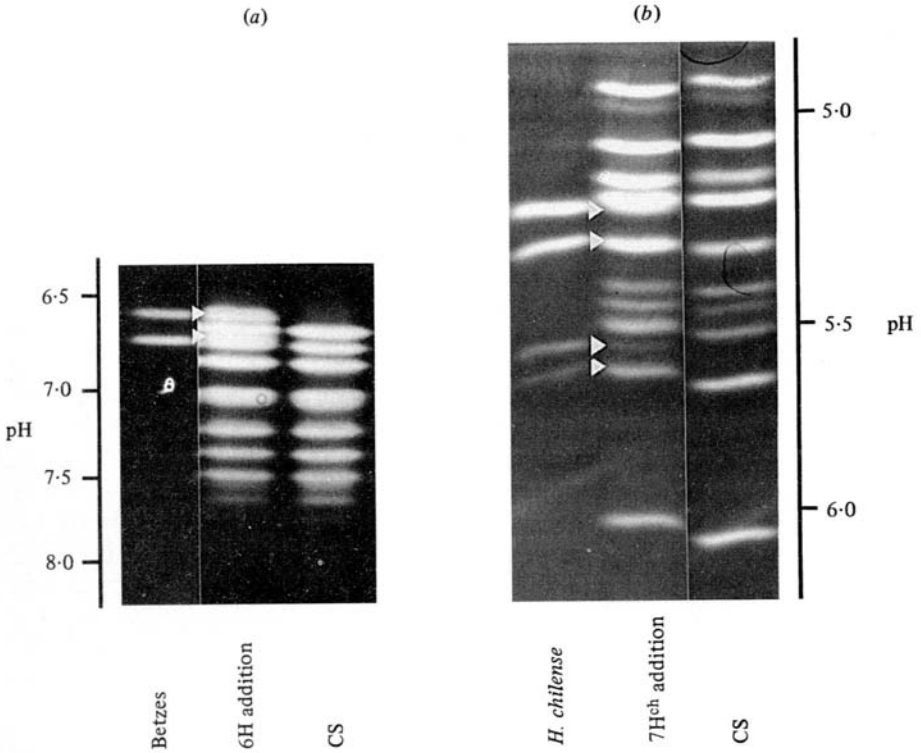

Fig. 2. α-Amylase phenotypes of wheat–*Hordeum* genotypes. (*a*) α-AMY-1 from Chinese Spring–*Hordeum vulgare* cv. Betzes (*b*) α-AMY-2 from Chinese-Spring–*H. chilense*. *Hordeum* isozymes expressed in wheat are arrowed.

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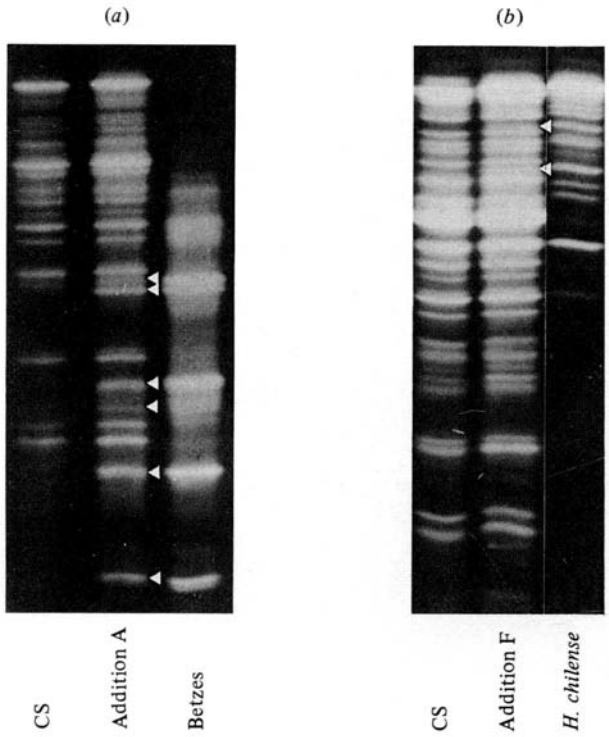

Fig. 3. β-Amylase phenotypes of wheat–*Hordeum* genotypes. (*a*) Chinese Spring–*Hordeum vulgare* cv. Betzes. (*b*) Chinese Spring–*H. chilense*. *Hordeum* isozymes expressed in wheat are arrowed.

3*a*). This concurs with previous evidence of Powling *et al.* (1981). The presence of a β-amylase locus on chromosome 4 is not in itself sufficient evidence for its homoeology with wheat group 4 because of the existence of β-amylase gene sets of loci, *β-Amy-1* and *β-Amy-2*, on the chromosomes of both wheat groups 4 and 5 respectively. The products of the *β-Amy-1* and *β-Amy-2* genes have overlapping pI ranges, and consequently it is not at present possible to ascertain with certainty whether the barley isozymes which are expressed in wheat are the products of loci related either to the wheat *β-Amy-1* or *β-Amy-2* sets. However, this chromosome also encodes for acid phosphatase (*Acph-H1*) (Powling *et al.* 1981), and alcohol dehydrogenase (*Adh-H1*) (Hart *et al.* 1980), two isozymes determined by homoeologous group 4 chromosomes in wheat. In view of this and the ability of chromosome 4 to substitute and compensate for the activities of chromosome 4D of wheat, it is almost certain that this chromosome is homoeologous with the wheat group 4 chromosomes and has been accordingly designated as 4H. It is therefore likely that the barley β-amylase locus present on 4H is *β-Amy-H1* rather than *β-Amy-H2*.

(b) *Chinese Spring*–H. chilense *addition and substitution lines. H. chilense* expressed two α-AMY-1 *isozymes* (Fig. 2*b*). However, these were not expressed in the α-AMY-1 phenotypes of any of the six CS–*H. chilense* addition lines examined, implying that none of the additions includes chromosome $6H^{ch}$ in its entirety.

The α-AMY-2 phenotype of *H. chilense* was expressed by addition A (Fig. 2*b*) and also in the telocentric addition line, which carries only the β arm. This chromosome has been designated as $7H^{ch}$ on the basis of its ability to substitute for wheat group 7 chromosomes and the presence of purple pigmentation of the culms (genes for the same phenotype in wheat are located on the short arms of 7B (Law, 1966)), in addition to the presence of the *α-Amy-*H^{ch}2 locus (Miller *et al.* 1985). The α-amylase phenotypes of the (7A)$7H^{ch}$, (7B)$7H^{ch}$ and (7D)$7H^{ch}$ substitutions were as expected, i.e. lacked the respective α-AMY-A2, α-AMY-B2, and α-AMY-D2 isozymes and showed the α-AMY-H^{ch}2 bands.

Two β-amylase isozymes from *H. chilense* are expressed in both the amphiploid and addition F (Fig. 3*b*). The remaining isozymes are not detectable in the wheat background because they co-focus with wheat isozymes. The presence of a β-amylase locus, designated *β-Amy-*H^{ch}*1*, on the addition F chromosome of *H. chilense* adds further weight to previous morphological and substitution evidence for its relationship with wheat homoeologous group 4 (Miller & Reader 1986).

(ii) *Rye* (Secale) *species*

(a) *Holdfast*–Secale cereale *cv. King II addition and substitution lines, Chinese Spring*–S. Cereale *cv. King II addition lines and Chinese Spring*–S. cereale *cv. Imperial addition lines.* An α-AMY-1 locus, *α-AMY-R1*, was located on chromosome 6R in all three addition sets (Figs. 4a, 5a, *b*). α-Amy-R1 is also located on the long arm of 6R because the phenotype of the CS–King II 6RS telocentric addition was identical to that of CS, whereas the 6RL telocentric addition shows the rye isozymes (not shown). In the 6R addition of King II to Chinese Spring, only the lowest pI isozyme is expressed, possibly indicating heterozygosity in the rye stock (Fig. 5*a*). This may also be the case in the CS–Imperial 6R addition which expressed three α-AMY-2 isozymes in addition to the CS complement, only two of which appear in the zymogram of the Imperial rye parent examined (Fig. 5*b*).

The α-AMY-1 zymograms of the (6A)6R, (6B)6R and (6D)6R and the (6A)6RL, (6B)6RL and (6D)6RL substitution lines lacked the respective α-AMY-A1, α-AMY-B1 and α-AMY-D1 isozymes, as expected (Fig. 4*b*).

The *α-Amy-2* locus in King II and Imperial rye, *α-Amy-R2*, was expressed in both 7R additions and CS–King II 7RL telocentric additions, but not the CS–King II 7RS additions (Fig. 4*c*), thereby confirming its location on the long arm of chromosome 7R.

In all three wheat–rye addition sets examined, a rye β-amylase structural locus was identified on chromosome 5R, and located on the long arm of this chromosome in the two King II addition sets. The

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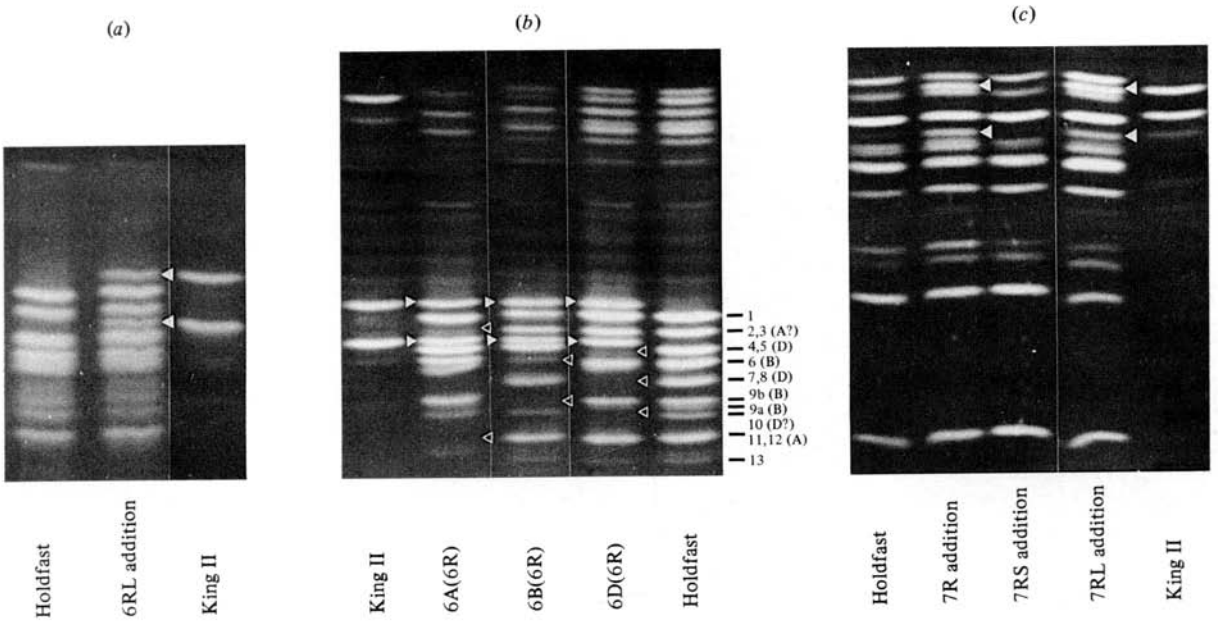

Fig. 4. α-Amylase phenotypes of Holdfast–*Secale cereale* cv. King II genotypes. (*a*) α-AMY-1 (additions). (*b*) α-AMY-1 (substitutions). (*c*) α-AMY-2 (additions). Rye isozymes expressed in wheat are arrowed (◀); the wheat isozymes encoded by the α-*Amy-1* genes on chromosomes 6A, 6B and 6D which are removed in the substitution phenotypes are arrowed (◁).

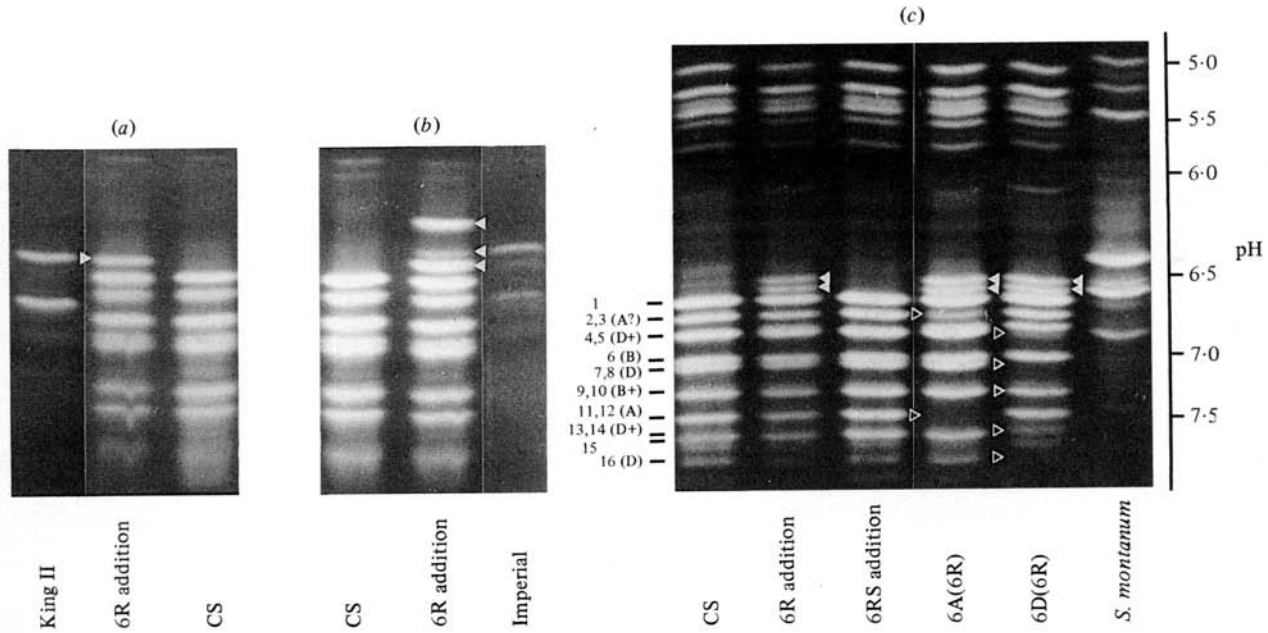

Fig. 5. α-Amylase phenotypes of wheat–rye genotoypes (*a*) α-AMY-1, Chinese Spring–*S. cereale* cv. King II. (*b*) α-AMY-1, Chinese Spring–*S. cereale* cv. Imperial. (*c*) α-AMY-2, Chinese Spring–*S. montanum*. Added and substituted isozymes (α-*Amy-1* for wheat group 6) identified as in Fig. 4.

β-amylase phenotype of the (5A)5RL substitution lacked the three isozymes (bands 15, 30, 32) previously shown to be controlled by the locus *β-Amy-A2* on chromosome 5A of Chinese Spring (Ainsworth *et al.* 1983). Previous workers have also implicated 5R as carrying the β-amylase locus in rye, in the cultivars Petkus (Bernard *et al.* 1977), Dakold, Imperial and King II (Artemova, 1982).

A number of β-amylase isozymes expressed by *S. cereale* cv. King II, are not observed in the Holdfast–King II amphiploid (Fig. 6*a*). The difference between the rye bands present in King II and in the amphiploid probably occurs because of heterogeneity within the outbreeding cultivar King II, so that the stock now available is no longer identical to that orginally used by Riley & Chapman (1958). A similar situation has already been noted for variation at *Est-R5* in the same material (Ainsworth *et al.* 1986).

This suggests that the β-amylase locus on 5RL of rye is homoeoallelic with the *β-Amy-2* set. Although

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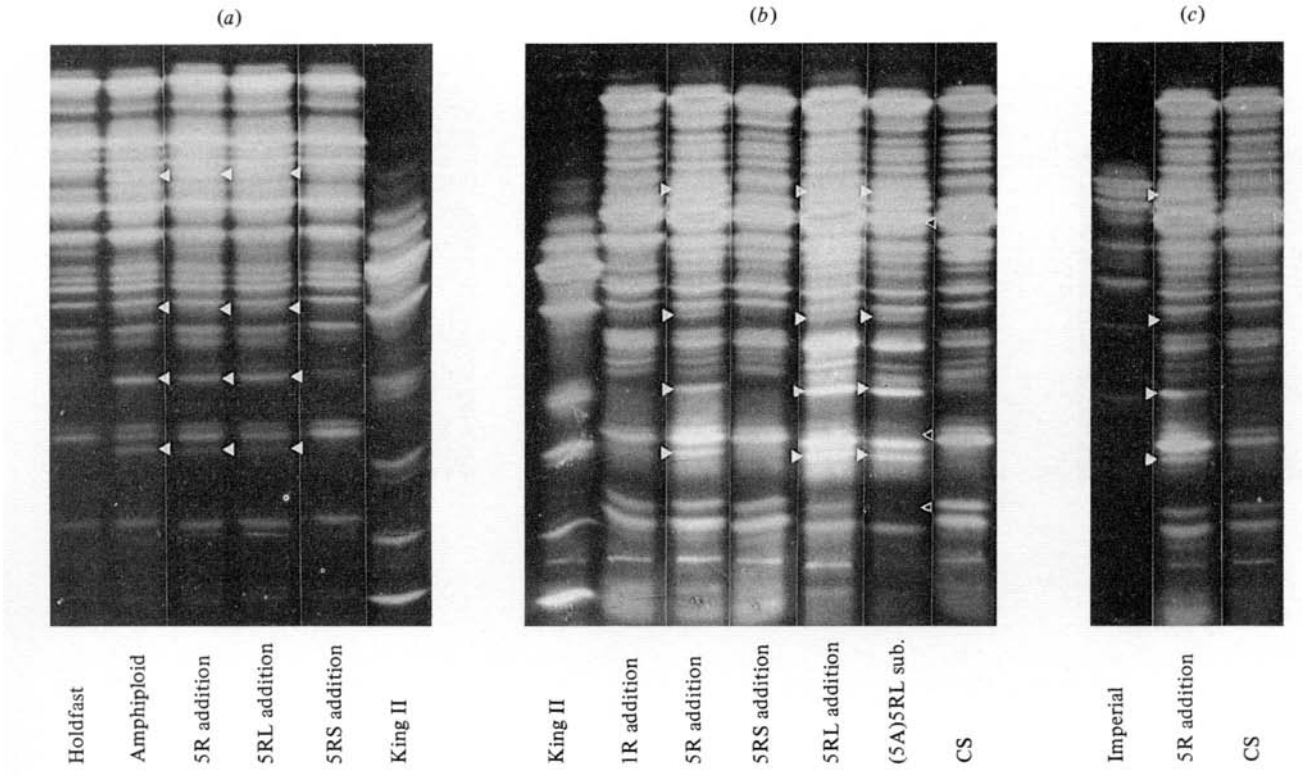

Fig. 6. β-Amylase phenotypes of wheat–rye genotypes. (*b*) Chinese Spring–*S. cereale* cv. King II. (*c*) Chinese Spring–*S. cereale* cv. Imperial. Arrows as in Fig. 4.

there is some homology between chromosome 5R and wheat group 4 (Miller, 1984; Zeller & Hsam, 1984) the bulk of the evidence supports homoeology between 5R and wheat group 5. This includes the presence of homoeologous triosephosphate isomerase (*Tpi-R1*) and shikimate dehydrogenase (*Skdh-R1*) loci, which are located on 5R in rye and group 5 in wheat (Pietro & Hart; 1985; Koebner & Shepherd, 1983), and the chromosome substitution evidence of Bielig & Driscoll (1970). The evidence favours the designation of the rye β-amylase locus as *β-Amy-R2*.

(b) *Chinese Spring*–S. montanum *addition and substitution lines*. The presence of *α-Amy-R1* was demonstrated on chromosome $6R^m$ of *S. montanum*. Of the five α-AMY-1 isozymes in the phenotype of *S. montanum*, two were expressed in the CS–*S. montanum* $6R^m$ addition (Fig. 5*c*). The two highest pI isozymes have similar pIs to CS isozymes and would not be detected, but the strong low pI rye isozyme is clearly not present in the phenotype of the $6R^m$ addition possibly indicating heterogeneity in the rye parent. Analysis of the (6A)$6R^m$ and (6D)$6R^m$ substitutions gave similar results to those from the equivalent Holdfast–King II substitutions.

No $7R^m$ addition is available and consequently no *α-Amy-R2*^{m} locus could be demonstrated.

The α-amylase analyses of the *S. cereale* and *S. montanum* additions described provide good evidence for the locations of *αAmy-R1* and *α-Amy-R2* on chromosome arms 6RL and 7RL respectively. This adds to previous evidence for the maintenance of gene synteny between 6R and wheat group 6 provided by the presence of homoeologous aminopeptidase (*Amp-R1*) and glutamate oxaloacetate transaminase (*Got-R2*) loci (Tang & Hart, 1975) notwithstanding the evidence for a 2RS/6RS translocation in *S. cereale* (Van Heemert & Sybenga, 1972) carrying the *Gli-R2* locus (Lawrence & Shepherd, 1981). Chromosome 7R shares partial homology with wheat group 4 on the basis of an acid phosphatase (*Acph-R1*) loci (Tang & Hart, 1975; Hart, 1978) and group 7, on the basis of *α-Amy-R2*, and is known to represent a chromosome 7/4 translocation, relative to wheat (Koller & Zeller, 1976).

(iii) Aegilops *species*

(a) *Holdfast*–Aegilops bicornis *addition and substitution lines*. *Ae. bicornis* α-AMY-2 isozymes were expressed in the $7S^b$ addition and in both (7A)$7S^b$ and (7B)$7S^b$ substitution lines, indicating the presence of *α-Amy-S*^{b}*2* on this chromosome (Fig. 7*a*). No *Ae. bicornis* α-AMY-2 isozymes were evident in the phenotype of the $7S^b$ telocentric addition suggesting that this telocentric chromosome is homoeologous to the short arms of the wheat group 7 chromosomes. Removal of chromosomes 7A and 7B in the (7A)$7S^b$ and (7B)$7S^b$ substitutions resulted in the loss of the respective α-AMY-A2 and α-AMY-B2 isozymes, as expected.

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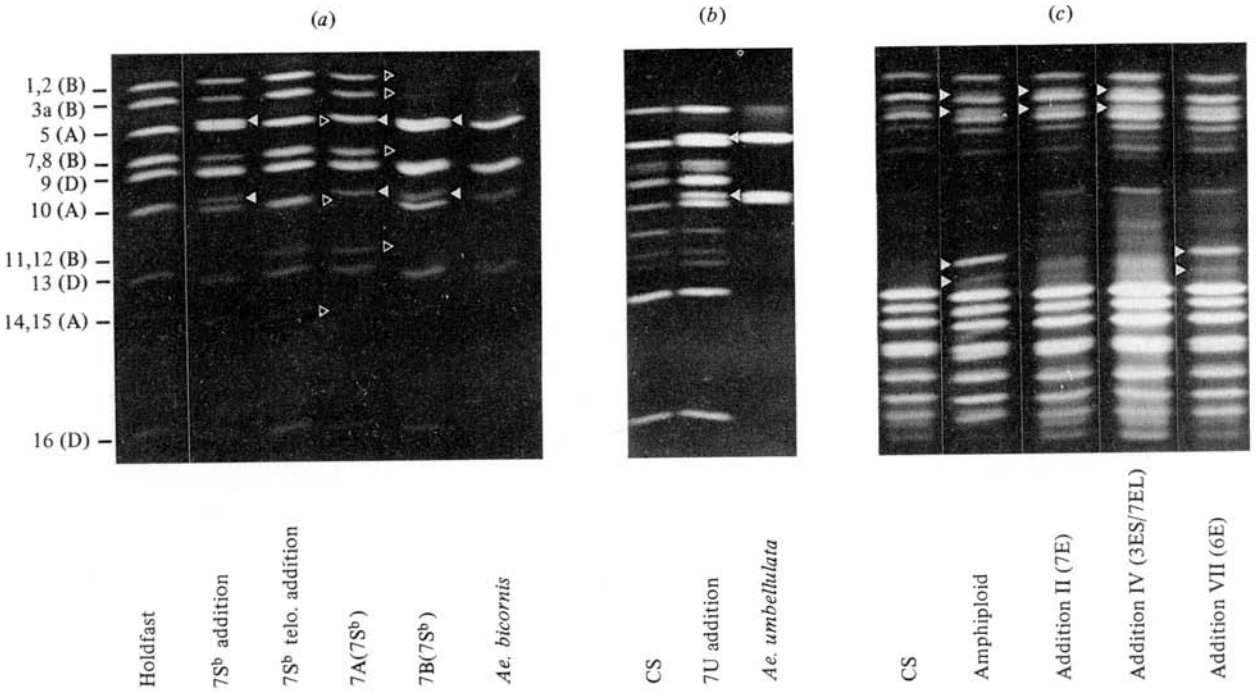

Fig. 7. α-Amylase phenotypes from wheat–*Aegilops* spp. and wheat–*Agropyron elongatum* genotypes. (*a*) α-AMY-2, Holdfast–*Ae. bicornis*. (*b*) α-AMY-2, Chinese Spring–*Ae. umbellulata*. (*c*) α-AMY-1 and α-AMY-2, Chinese Spring–*Ag. elongatum*.

The identification of the locus *α-Amy-S^{b}2* on chromosome 7S^b of *Ae. bicornis* adds further support to its homoeologous designation, in addition to its ability to substitute for the wheat group 7 chromosomes.

(b) *Chinese Spring*–Ae. umbellulata *addition and substitution lines.* CS–*Ae. umbellulata* addition G, which has been designated 7U, expressed both of the major α-AMY-2 isozymes from *Ae. umbellulata*, indicating the presence of the homoeolocus *α-Amy-U2* (Fig. 7*b*). These isozymes were also evident in the phenotype of the (7D)7U substitution, in which the wheat 7D isozymes encoded by *α-Amy-D2* were replaced by the two isozymes from *Ae. umbellulata*.

No *α-Amy-1* locus was demonstrated on any of the six additions even though there is some evidence that the chromosome in addition A is homoeologous with wheat group 6. Chromosome A, which encodes prolamins (Shepherd, 1973), will substitute for the chromosomes of wheat group 6 but provides little compensation (Kimber, 1968) and has been observed to pair at meiosis with wheat telocentrics 6BL and 6DS (Athwall & Kimber, 1973). The *Ae. umbellulata* chromosomes present in additions B, C, D and G have been designated 1U, 2U and 7U on evidence from substitution (Chapman *et al.* 1975) and biochemical markers (Lawrence & Shepherd, 1981; Koebner & Shepherd 1983). The lack of an *α-Amy-U1* locus may indicate that either a substantial part of the long arm of 6U is absent from addition A or that the locus is located on the chromosome of *Ae. umbellulata* which has yet to be isolated.

The β-amylase phenotype of the CS–*Ae. umbellulata* amphiploid included three isozymes from *Ae. umbellulata* with pIs differing from those of their wheat counterparts. These isozymes were also expressed in addition C (5U) (Fig. 8*c*). The (5A)5U substitution showed the expected loss of the three isozymes controlled by *α-Amy-A2* on chromosome 5A, in addition to the presence of the isozymes from *Ae. umbellulata* (Fig. 8*c*). However, another isozyme, CS band 25, of unknown chromosomal control was also absent. The β-amylase phenotype of the (5D)5U substitution was identical to that of the 5U addition (Fig. 8*c*). This is as expected, there being no detectable active *β-Amy-2* locus on CS chromosome 5D (Ainsworth *et al.* 1983).

There is good evidence for the designation of the *Ae. umbellulata* addition C as 5U, both from the ability to substitute for the chromosomes of wheat group 5 (Chapman & Riley, 1970) and from the presence of homoeoloci for *Tpi-U2* (Pietro & Hart, 1985) and *Skdh-U1* (Koebner & Shepherd, 1983). It is, therefore, likely that the β-amylase locus on 5U is homoeologous with the *β-Amy-2* set in wheat, and it is consequently designated *β-Amy-U2*.

(a) *Chinese Spring*–Aegilops sharonesis *addition and substitution lines.* β-Amylase isozymes from *Ae. sharonensis* are expressed in the CS–*Ae. sharonensis* amphiploid (Fig. 8*a*). These isozymes were present in the zymograms of all CS–*Ae. sharonensis* genotypes which contain chromosome 4S^1. These included the 4S^1 addition, the only addition available because of the preferential transmission of this chromosome

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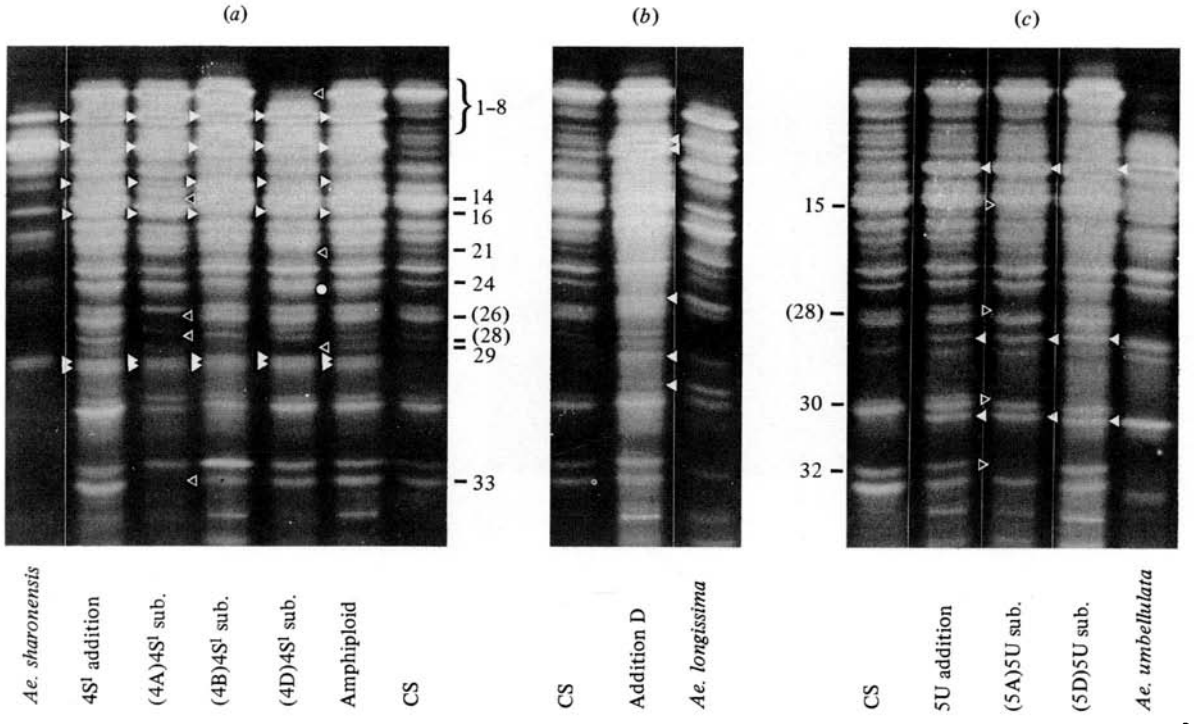

Fig. 8. β-Amylase phenotypes of wheat–*Aegilops* genotypes. (*a*) Chinese Spring–*Ae. sharonensis*. (*b*) Chinese Spring–*Ae. longissima*. (*c*) Chinese Spring–*Ae. umbellulata*. Arrows indicate the expression of *Aegilops* isozymes in wheat (◀) and the absence of wheat isozymes (numbered, ◁) encoded by *β-Amy-A1* or *β-Amy-D1* (Fig. 4*a*) and *β-Amy-A2* (Fig. 4*c*).

(Miller *et al.* 1982*a*), and the three group 4 substitutions, (4A)4S¹, (4B)4S¹ and (4D)4S¹. Removal of chromosomes 4A and 4D in the (4A)4S¹ and (4D)4S¹ substitutions, respectively, results in the loss of β-AMY-A1 and β-AMY-D1 isozymes (Ainsworth *et al.* 1983). Removal of chromosome 4B in the (4B)4S¹ substitution has no effect on the wheat β-amylase phenotype because no *β-Amy-1* locus on chromosome 4B has yet been demonstrated. Both comparisons of plant morphology of the addition line and the ability of this chromosome to be substituted for wheat chromosomes 4A, 4B and 4D provide strong evidence in favour of homoeology with wheat group 4 (Miller *et al.* 1982*a*; Miller, 1983). The homoeologous β-amylase locus in *Ae. sharonesis* is accordingly designated *β-Amy-S¹1*.

(b) *Chinese Spring*–Ae. longissima *addition lines*. Both CS–*Ae. longissima* addition D and the amphiploid expressed isozymes from *Ae. longissima* in addition to the CS isozymes (Fig. 8*b*).

The homoeology of the addition D chromosome with wheat is not clear in that it carries loci which are homoeologous to wheat loci on three separate chromosome groups; *Adh-S¹1* which is homoallelic with the *Adh-1* set on group 4, lipoxygenase (*Lpx-S¹2*), homoeoallelic with the *Lpx-2* set on group 5 and endopeptidase (*Ep-S¹1*), homoeoallelic to the *Ep-1* set on group 7 (Hart & Tuleen, 1984). This complex homoeology suggests substantial interchange differences between the *Ae. longissima* chromosomes and those of wheat. Cytological studies show that *Ae. longissima* chromosome 4S¹ pairs with the 4S¹ chromosome of *Ae. sharonensis* which has been shown to have homoeology with the group 4 chromosome of wheat, indicating that the homoeology of 4S¹ with wheat group 4 is stronger than that with groups 5 and 7 (Netzle & Zeller, 1984). In view of this and the fact that *Ae. longissima* is regarded as conspecific with *Ae. sharonensis* (Tanaka, 1955, Miller, 1981), then β-amylase locus in *Ae. longissima* can probably be designated *β-Amy-S¹1*.

(c) *Chinese Spring*–Agropyron elongatum *addition lines*. The exact genotype of *Agropyron elongatum* used to make the wheat *Ag. elongatum* hybrid was not available. However, the amphiploid expresses two additional α-AMY-1 and two additional α-AMY-2 isozymes, presumably encoded by the homoeoloci *α-Amy-E1* and *α-Amy-E2* in *Ag. elongatum* (Fig. 8*c*). Examination of addition lines I–VII showed that *α-Amy-E1* was expressed in addition VII, and *α-Amy-E2* in two additions, II and IV (Fig. 8*c*).

These findings are in accordance with previous analyses of the homoeology of the *Ag. elongatum* addition chromosomes. Chromosome VII is homoeologous to wheat group 6 and is designated 6E (Dvořák, 1980). Chromosome 6E carries several homoeoloci present on wheat group 6 including aromatic alcohol dehydrogenase (*Aadh-E2*), aminopeptidase (*Amp-E1*) and glutamate oxaloacetate transaminase (*Got-E2*) (Hart & Tuleen, 1983).

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Table 2. *Summary of homoeoallelic-* α-Amy-1, α-Amy-2, β-Amy-1 *and* β-Amy-2 *loci in alien species*

	α-Amy-1		*α-Amy-2*		*β-Amy*	
Species	Locus	Chromosome	Locus	Chromosome	Locus	Chromosome
Hordeum vulgare cv. Betzes	*α-Amy-H1*	6H	—		*β-Amy-H1*	4H
H. chilense	—		*α-Amy-H^{ch}2*	7H^{ch}	*β-Amy-H^{ch}1*	4H^{ch}
Secale cereale cv. King II	*α-Amy-R1*	6RL	*α-Amy-R2*	7RL	*β-Amy-R2*	5RL
cv. Imperial	*α-Amy-R1*	6R	*α-Amy-R2*	7R	*β-Amy-R2*	5R
S. montanum	*α-Amy-R^{m}1*	6R^{m}L	—		—	
Aegilops bicornis	—		*α-Amy-S^{b}2*	7S^{b}	—	
Ae. sharonensis	—		—		*β-Amy-S^{l}1*	4S^{l}
Ae. longissima	—		—		*β-Amy-S^{l}1*	D
Ae. umbellulata	—		*α-Amy-U2*	7U	*β-Amy-U2*	5U
Agropyron elongatum	*α-Amy-E1*	6E	*α-Amy-E2*	7EL	—	

Chromosome II is homoeologous to wheat group 7 and is designated 7E (Dvořák, 1980); it carries the homoeolocus *Ep-E1* (Hart & Tuleen, 1983). The comprehensive analysis of this addition series by Hart and Tuleen (1983) provides evidence that chromosome IV is an interchange comprised of 3ES and 7EL; 7EL was shown to carry the endopeptidase locus *Ep-E1* and this is expected to also carry *α-Amy-E2*. The presence of both *Ep-E1* and *α-Amy-E2* on 7EL provides further evidence of gene synteny between this arm and the long arms of wheat group 7.

No β-amylase locus could be demonstrated; all CS–*Ag. elongatum* addition lines examined displayed β-amylase phenotypes identical to that of CS. However, the majority of bands in the phenotype of the accession of *Ag. elongatum* examined co-focus with identified CS bands (Fig. 1).

4. Conclusions

As marker genes in wheat, biochemical loci have an important advantage over other loci affecting the gross phenotype since both the products of the individual loci within a triplicate gene set, and allelic variants (including null alleles) at these loci, are distinguishable. Consequently, it is often possible to screen for the products of three individual enzyme marker loci, i.e. those making up a triplicate homoeoallelic gene series, in a single electrophoretic separation. In contrast, for genes for which the observed phenotype is far removed from the actual gene products, such as the loci controlling most morphological characters, recessive or null phenotypes at one locus are often masked by genetic compensation by homoeoallelic loci in the remaining two genomes.

The wheat α-amylase genes lend themselves still further to exploitation as markers because the products of six individual loci, three *α-Amy-1* and three *α-Amy-2*, can be resolved simultaneously. This is, of course, only possible because the products of the two gene sets have non-overlapping ranges of pI. The β-amylase genes, which also comprise two homoeoallelic sets, but have products with overlapping pI ranges are less valuable.

The α-amylase loci identified in this paper are summarised along with their homoeology to the wheat loci in Table 2. For β-amylase, the homoeologous relationships of alien genes to those in wheat are not as straightforward. However, the evidence presented here for the homoeologies of the chromosomes of barley, rye, *Ae. umbellulata*, *Ae. sharonensis* and *Ae. longissima* to wheat groups 4 and 5 is sufficiently strong to assign their various β-amylase genes to the *β-Amy-1* and *β-Amy-2* sets, respectively (Table 2).

It appears that, in general, only a single β-amylase locus is operative in most *Triticeae* genomes. The only contrary evidence derives from hexaploid wheat itself. The D and A genomes fit with this hypothesis. The D genome, from *Ae. squarrosa*, has so far been shown to carry a single *β-Amy-D1* locus, in the same way that only single *β-Amy-1* or *β-Amy-2* loci have emerged from the present study of the H, H^{ch}, R, R^{m}, U and S^{l} genomes. In the A genome of hexaploid wheat it is now proposed that the chromosome designated 4A is actually 4B (Dvořák, 1983), leaving only *β-Amy-A2* carried on 5A.

Two loci must therefore be assigned to the B genome: the *β-Amy-1* locus on '4A' and a *β-Amy-B2* locus on 5B (Ainsworth *et al.* 1983). The possibility remains, of course, that the B genome, the precise identity of which has not been ascertained, is of composite origin (Zohary & Feldman, 1961).

The exact relationships between β-amylase loci in alien species to those in their wheat counterparts will only become clear if the products of the *β-Amy-1* and *β-Amy-2* genes can be unequivocally distinguished. This is likely only by a demonstration that the two products are immunochemically or antigenically distinct as is the case with the products of the two α-amylase triplicate series of loci, *α-Amy-1* and *α-Amy-2* (Daussant & Renard, 1972; 1976).

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