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Isozyme variation and RFLPs at the β-amylase loci in wheat

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Summary. Forty-one hexaploid wheat genotypes have been examined for RFLPs detected by a β-amylase probe using three restriction enzymes, and for mature grain β-amylase isozyme polymorphism following IEF. The two homoeoallelic series assayed for RFLPs differed: little variation was found at group 2 chromosome homoeoloci, while the group 4/5 chromosome homoeoloci displayed considerable variation. Varieties that displayed a RFLP with one RE almost always did likewise with the other two REs, suggesting that most of the polymorphisms observed were due to large DNA rearrangements. Comparison of the variation in grain β-amylase isozymes with the RFLP results indicated strong associations between particular RFLP and isozyme alleles.

Key words: Hexaploid wheat – β-Amylase – Isozymes – RFLPs – IEF

Introduction

There is much interest in the use of various genetic markers in plant genetics research and practical crop breeding. It is envisaged that new marker techniques, particularly restriction fragment length polymorphisms (RFLPs), will allow the rapid construction of a detailed genetic map for any species and that such maps will be of value in crop breeding programmes through the identification of linkages between markers and genes of agronomic importance (Beckmann and Soller 1987; Edwards et al. 1987). To extend the number of available marker loci, both isozymes and RFLPs are being investigated in a number of crop species. These molecular markers have already been used to construct genetic maps of tomato (Bernatzky and Tanksley 1986), lettuce (Landry et al. 1987a, b) and maize (Helentjaris et al. 1986), and linkages between marker loci and loci controlling traits of agronomic importance have been found (Neinhuis et al. 1987; Osborn et al. 1987; Worland et al. 1988). In hexaploid bread wheat (*Triticum aestivum*), isozymes have been used extensively in genetic analysis (Ainsworth and Gale 1987), and so far approximately 115 isozyme and storage protein loci have been located to wheat chromosomes by various aneuploid analyses (Hart and Gale 1987), although only a few have been mapped intrachromosomally. In addition, there has been a small amount of genetic analysis based on RFLPs; variation in ribosomal cistrons has been documented and some of the variants have been mapped to particular chromosomes (Appels and Dvorak 1982; May and Appels 1987), and intrachromosomal mapping of variants on chromosome 1B has been reported (Snape et al. 1985). Variation at and near the sequences encoding HMW glutenin subunits has also been described (Harberd et al. 1986).

The genetics of mature grain β-amylase isozyme in wheat is well known (Ainsworth et al. 1983), and studies using a barley β-amylase cDNA as probe have enabled β-amylase encoding sequences to be located to chromosome arms using the aneuploids of 'Chinese Spring' (Sharp et al. 1988). There are two homoeoallelic series of loci: *β-Amy-2* on the group 2 chromosome short arms, and *β-Amy-1* on chromosomes 4Aβ, 4DL and 5AL. We report here on RFLPs detected with this probe in a range of hexaploid wheat varieties, and on the relationship between this variation and that identified by isoelectric focussing of the enzyme itself.

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Materials and methods

1 Genetic material

Forty-one genotypes of hexaploid wheat, listed in Table 2, were examined for both RFLP and isozyme phenotype. Various aneuploid stocks, identified at appropriate places in the text, were also used.

2 DNA procedures

Isolation of genomic DNA, restriction enzyme (RE) digestion, agarose gel electrophoresis, alkaline "Southern" blotting to nylon membranes and the hybridization methods have been described previously (Sharp et al. 1988). The 789 bp internal AccI fragment of barley β-amylase cDNA clone pcβc51 (Kreis et al. 1987) was used as the probe.

3 Isozyme analysis

Isozyme analysis was performed on selfed seed taken from plants used for DNA extraction. Modifications of the methods of Ainsworth et al. (1983) were used. Single endosperm halves of dry grain were milled in a microhammer mill, and the flour was mixed with 200 µl 12 m*M* DTT and incubated for approximately 1 h at room temperature. Following a brief 12,000 *g* centrifugation, the supernatants were used for isoelectric focussing. The β-amylase were separated by flat bed IEF on 0.25 mm thick, 125 mm wide polyacrylamide gels (5%C, 3%T) containing 2% w/v ampholyte (a 1:1:1 mixture of Pharmalyte 4.2–4.9 Pharmalyte 4.5–5.4 and Isolytes 4–6) and 10% glycerol. Electrode solutions were 0.1 *M* NaOH (cathode) and 0.4 *M* glutamic acid (anode). Gels were run at constant power (1 W cm^{-1}). Following prefocussing for 500 Vh, 25 µl of sample was applied by use of a silicone rubber tape with 7 mm wide wells laid about 1 cm from the cathode. IEF continued for 3500 Vh, and the gels were then stained for β-amylase activity as described by Ainsworth et al. (1983).

4 Designation of RFLP loci and alleles

The current guidelines for the nomenclature of biochemical loci in wheat (Hart and Gale 1986) do not discuss RFLPs. Drawing on these guidelines, however, we suggest and use the following. The formal designation of a locus should be of the form: *XYZ36-3B* where 36 is the probe number within laboratory XYZ (a three latter code), and 3B is the chromosome involved. (There will usually be *XYZ36-3A* and *XYZ36-3D* loci, forming a *XYZ36-3* homoeoallelic series). In the text and on genetic maps, the locus name will often be shortened to *XYZ36* or just *36*. The number would be sequential within laboratory XYZ and may bear no relationship to the name of the clone used to produce the probe which detects the locus. With a known function probe, such as the one here, the function could be indicated after the locus name on genetic maps, either as a locus in the case of a gene already recognised by a protein product (e.g. *XYZ32*, *α-Amy-1*), or as a function where the precise protein has not been identified (e.g. *XYZ79* (arabinose isomerase)). Alleles, which will be specific to REs used, would be designated by RE, and a letter code with 'Chinese Spring' having the "a" allele by definition, as for isozyme variants (Hart and Gale 1986). Therefore, *XYZ36-3A-BamHI-a* would be the full formal description of the 'CS' allele on 3A using BamHI. However, in most text this would be shortened to *BamHI-a*, or to *a*.

Based on this, *PSR1*, together with the chromosome, will be used for RFLP loci found with probe 1, which is the 789 bp AccI fragment from clone pcβc51 (Kreis et al. 1987) from the Institute of Plant Science Research.

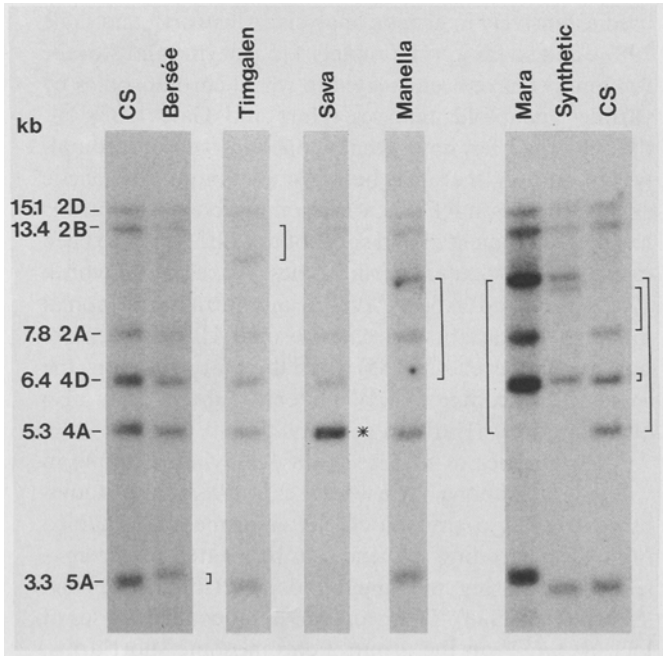

Fig. 1. RFLPs detected in BamHI digests. The size (kb) and chromosomal location of the 'CS' fragments are indicated on the *left*, and shifts in mobility are indicated by *brackets* between the position in 'CS' and the new position. 'CS' is also placed next to 'Synthetic' to show the small difference on chromosome 4D. * indicates the dosage increase in 'Sava'

Results

1 RFLP analysis

Three REs were used; BamHI (GGATCC), ApaI (GGGCCC) and DraI (TTTAAA). After digestion with BamHI, 'CS' gives a pattern of six bands (Sharp et al. 1988); their sizes and chromosomal location are given in Fig. 1. Analyses of DNA from the appropriate nullisomic-tetrasomic lines of 'CS' after digestion with both ApaI and DraI were performed to assign the chromosomal control of the observed hybridization bands (data not shown); ApaI gives four well-spaced fragments and a close triplet in 'CS' (Fig. 2), while DraI gives three widely-spaced fragments and a triplet of almost co-migrating fragments controlled by the three group 2 chromosomes (Fig. 3).

When analysing the other varieties, the absence of a 'CS' band and its replacement by a fragment of different length was taken as evidence that the chromosomal control of the resulting polymorphism resided on that chromosome on which the 'CS' band was located.

1.1 Polymorphisms of the *PSR1-2* homoeoloci

After BamHI digestion, only two varieties were found that displayed polymorphisms for group 2 fragments

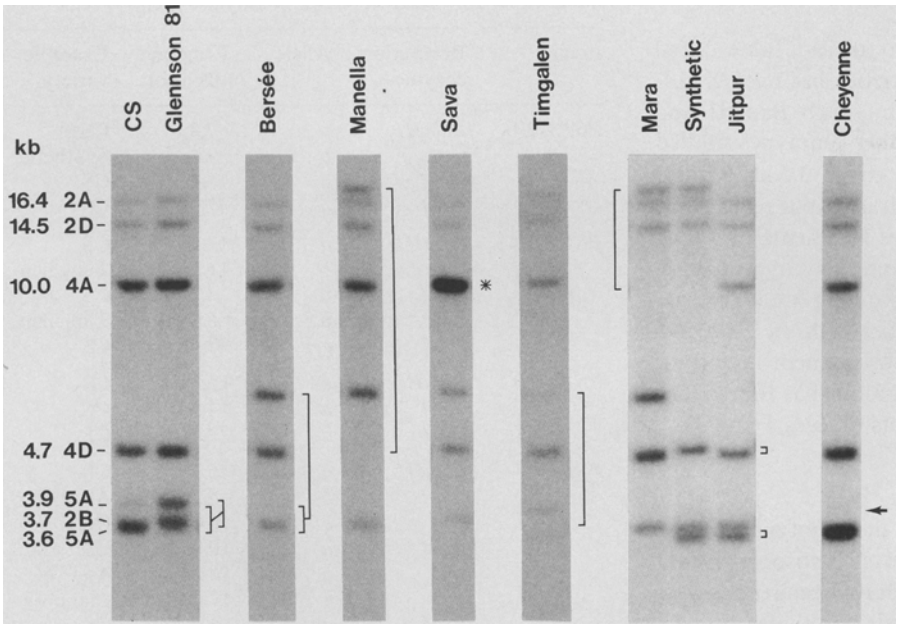

Fig. 2. RFLPs detected in ApaI digests. The size (kb) and chromosomal location of the 'CS' fragments are indicated on the *left*, and shifts in mobility are indicated by *brackets* between the position in 'CS' and the new position. * indicates the dosage increase in 'Sava'. The *arrow* indicates the position of the 5A fragment missing in 'Cheyenne'

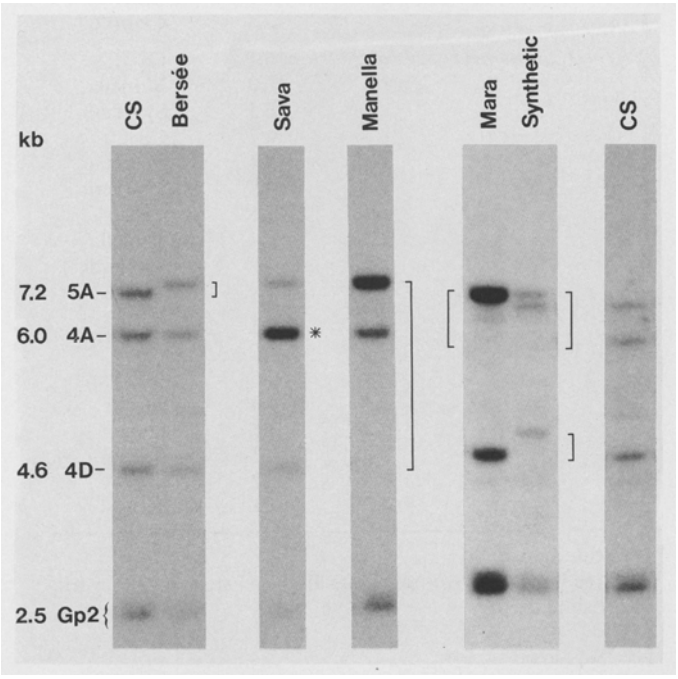

Fig. 3. RFLPs detected in DraI digests. The size (kb) and chromosomal location of the 'CS' fragments are indicated on the *left*, and shifts in mobility are indicated by *brackets* between the position in 'CS' and the new position. * indicates the dosage increase in 'Sava'

(Fig. 1): 'Timgalen' has the *PSR1-2B-BamHI-b* allele giving a smaller fragment (11.2 kb) than the *a* allele, while 'Synthetic' has a 9.4 kb fragment instead of the 7.8 kb fragment in 'CS', so has the *PSR1-2A-BamHI-b* allele. 'Timgalen' also has the *PSR1-2B-ApaI-b* allele which replaces the 3.7 kb fragment in 'CS' with one of 6.0 kb (Fig. 2). No polymorphism was found using DraI; all varieties examined had the same close triplet of fragments.

1.2 *PSR1-4A* polymorphisms

With each of the REs used, three alleles were found (Table 1 and Figs. 1–3). 'CS' and most of the varieties studied had *PSR1-4A-BamHI-a, ApaI-a* and *DraI-a*. 'Atlas 66' and 'Sava' each have a fragment of the same size for all three REs, but the fragments have an increased dose (scans of autoradiographs suggest that the signal is two to three times as intense as in 'CS' – data not shown), so these varieties have the *BamHI-b, ApaI-b*, and *DraI-b* alleles. Four genotypes ('Ciano 67', 'Mara'. 'Synthetic' and *T. macha*) have the *BamHI-c, ApaI-c* and *DraI-c* alleles, with fragments of 10.0, 17.4 and 7.4 kb with BamHI, ApaI and DraI, respectively (Table 2).

1.3 *PSR1-4D* polymorphisms

Three alleles were found with each enzyme (Tables 1 and 2, Figs. 1–3). Most varieties had the *BamHI-a, ApaI-a* and *DraI-a* alleles, giving 6.4 kb BamHI, 4.7 kb ApaI and 4.6 kb DraI bands. 'Manella' and 'Pané 247' had *b* alleles with each enzyme, with the *a* fragments being replaced by 10 kb BamHI, 17.4 kb ApaI and 7.4 kb DraI fragments. In each of these cases, the new 4D fragments appear very similar to the fragments resulting from the *PSR1-4A-BamHI-c, ApaI-c* and *DraI-c* alleles (Figs. 1–3). 'Synthetic' alone has *PSR1-4D-BamHI-c, ApaI-c,* and *DraI-c* alleles, with BamHI and ApaI giving fragments just larger than the *a* alleles, while the *DraI-c* allele gives a fragment considerably large than the *DraI-a* allele –4.9 kb compared to 4.6 kb (Figs. 1–3).

1.4 *PSR1-5A* polymorphisms

BamHI and DraI both reveal two alleles (Table 2 and Figs. 1–3). 'CS' and 16 other varieties have *PSR1-5A-BamH1-a* and *DraI-a* alleles with 3.3 kb BamHI and 7.2 kb DraI fragments, while the other genotypes studied have *BamHI-b* and *DraI-b* alleles giving 3.5 kb BamHI and 7.4 kb DraI fragments. In contrast, ApaI reveals five alleles (Table 2 and Fig. 2): *a* gives fragments of 3.6 kb and 3.9 kb (the larger being less intensely hybridizing); *b* gives a 3.6 kb fragment alone; *c* giving a 6.0 kb fragment, is possessed by all the varieties with the *BamHI-b* and *DraI-b* alleles; *d* gives a 3.5 kb fragment in 'Jitpur' and 'Synthetic', and 'Glennson 81' alone has the *e* allele, with 3.7 and 4.0 kb ApaI fragments (Tables 1 and 2).

2 Isozyme studies

The allelic composition of most of the genotypes studied here at the RFLP level at the loci on chromosome 4A, 4D and 5A controlling seed β-amylase have been reported by Ainsworth et al. (1983). Re-analysis for the most part confirmed these designations; the allelic phenotypes are identified in Fig. 5. The experiments resulting in the few reclassifications and additional alleles detected by the ultrathin IEF gels used here will be detailed elsewhere (Gale et al. in preparation).

Analysis of 'CS' nullisomic-tetrasomic and ditelosomic stocks (Fig. 4) shows, as concluded by Ainsworth et al. (1983), that only chromosomes 4A, 4D and 5A are associated with the production of specific grain β-amylase isozymes. Of particular relevance is the fact that no group 2 chromosomes, which carry sequences homologous to the β-amylase cDNA probe, show any such activity. In addition, chromosome 5B, identified by Ainsworth et al. (1983) as having a β-amylase gene in 'Synthetic', shows no activity. A careful re-examination of the entire 'CS' ('Synthetic') intervarietal chromosome substitution series has established that the novel phenotype, described previously as shown by the CS(Synthetic 5B) substitution line, was in fact due to an allele carried by *β-Amy-D1* on chromosome 4D.

This finding clearly indicates, as does the RFLP analysis, that the three loci comprise a single set of β-amylase genes, even though they are not located on homoeologous chromosome arms. For this reason, and considering the evidence presented previously (Sharp et al. 1988), the symbol of the chromosome 5A locus has been changed from *β-Amy-A2* to *β-Amy-B1*. The *β-Amy-1* alleles at the three loci are listed with the RFLP alleles for all the genotypes in Table 2.

3 Relationship between RFLP and isozyme alleles

Examination of Table 2 reveals that these is considerable correspondence between the RFLP and isozyme alleles; these have been summarized in Table 3. At the loci on chromosomes 4A and 4D, the RFLP alleles usually pick out rare isozyme alleles, but the *β-Amy-D1b* allele and *β-Amy-A1d* allele are not associated with an RFLP difference detectable with the three REs used here (Table 3).

At the chromosome 5A locus, where the *ApaI-a, b* and *d* alleles are subclassifications of the *BamHI-a* and *DraI-a* alleles, and the *ApaI-c* and *e* alleles are subclassifications of the *BamHI-b* and *DraI-b* alleles, the correspondence is less clear. However, all but one of the 25 varieties carrying the *BamHI-b* and *DraI-b* alleles also have the isozyme *β-Amy-B1d* allele. The exception, 'Glennson 81' (isozyme *a* allele), is itself unusual as it has

Table 1. RFLPs detected at the β-amylase loci

Locus	Restriction enzyme	Allele	Fragment size (kb)	Example variety
PSR1-2A	*BamHI*	*a*	7.8	CS
		b	9.4	Synthetic
	ApaI	*a*	16.4	CS
	DraI	*a*	(2.5)	CS
PSR1-2B	*BamHI*	*a*	13.4	CS
		b	11.2	Timgalen
	ApaI	*a*	3.7	CS
		b	6.0	Timgalen
	DraI	*a*	(2.5)	CS
PSR1-2D	*BamHI*	*a*	15.1	CS
	ApaI	*a*	14.5	CS
	DraI	*a*	(2.5)	CS
PSR1-4A	*BamHI*	*a*	5.3	CS
		b	5.3*	Atlas 66
		c	10.0	Ciano 67
	ApaI	*a*	10.0	CS
		b	10.0*	Atlas 66
		c	17.4	Ciano 67
	DraI	*a*	6.0	CS
		b	6.0*	Atlas 66
		c	7.4	Ciano 67
PSR1-4D	*BamHI*	*a*	6.4	CS
		b	10.0	Manella
		c	6.5	Synthetic
	ApaI	*a*	4.7	CS
		b	17.4	Manella
		c	4.8	Synthetic
	DraI	*a*	4.6	CS
		b	7.4	Manella
		c	4.9	Synthetic
PSR1-5A	*BamHI*	*a*	3.3	CS
		b	3.5	Bersée
	ApaI	*a*	3.6+3.9	CS
		b	3.6	Cheyenne
		c	6.0	Bersée
		d	3.5	Jitpur
		e	3.7+4.0	Glennson 81
	DraI	*a*	7.2	CS
		b	7.4	Bersée

* Increased dose
The three Group 2 *DraI* fragments are bracketed since they are of very similar size

Table 2. Allelic composition of the wheats studied at the RFLP and β-amylase isozyme loci on chromosomes 4A, 4D and 5A

Variety	Locus											
	PSR1-4A				*PSR1-4D*				*PSR1-5A*			
	BamHI	*ApaI*	*DraI*	*β-Amy-A1*	*BamHI*	*ApaI*	*DraI*	*β-Amy-D1*	*BamHI*	*ApaI*	*DraI*	*β-Amy-B1*
CS	a	a	a	a	a	a	a	a	a	a	a	a
Atlas 66	b	b	b	.	.	.	.	.	.	b	.	b
Avalon	.	.	.	.	.	.	.	.	b	c	b	d
Bersée	.	.	.	.	.	.	.	b	b	c	b	d
Bezostaya 1	.	.	.	.	.	.	.	.	b	c	b	d
C306	.	.	.	.	.	.	.	.	.	b	.	.
Cappelle-Desprez	.	.	.	.	.	.	.	b	b	c	b	d
Champlein	.	.	.	.	.	.	.	b	b	c	b	d
Cheyenne	.	.	.	.	.	.	.	.	.	b	.	b
Ciano 67	c	c	c	c	.	.	.	.	.	b	.	b
Desprez 80	.	.	.	.	.	.	.	.	b	c	b	d
Fenman	.	.	.	.	.	.	.	.	b	c	b	d
Glennson 81	.	.	.	.	.	.	.	.	b	e	b	.
Grana	.	.	.	.	.	.	.	.	b	c	b	d
Highbury	.	.	.	.	.	.	.	.	.	.	.	.
Hobbit	.	.	.	.	.	.	.	.	b	c	b	d
Hobbit "S"	.	.	.	.	.	.	.	b	b	c	b	d
Jitpur	.	.	.	.	.	.	.	.	.	d	.	b
Lutescens 62	.	.	.	.	.	.	.	.	.	.	.	.
Manella	.	.	.	d	b	b	b	b	b	c	b	d
Mara	c	c	c	c	.	.	.	b	b	c	b	d
Maris Huntsman	.	.	.	.	.	.	.	b	b	c	b	d
Maris Ranger	.	.	.	.	.	.	.	b	b	c	b	d
Maris Sportsman	.	.	.	.	.	.	.	b	b	c	b	d
Minister Dwarf	.	.	.	.	.	.	.	b	b	c	b	d
Moulin	.	.	.	.	.	.	.	.	b	c	b	d
Pané 247	.	.	.	d	b	b	b	b	b	c	b	d
Poros	.	.	.	.	.	.	.	.	b	c	b	d
Rendezvous	.	.	.	.	.	.	.	.	b	c	b	d
RL4137	.	.	.	.	.	.	.	.	.	b	.	c
Sava	b	b	b	.	.	.	.	.	b	c	b	d
Sicco	.	.	.	.	.	.	.	.	.	b	.	d
Synthetic	c	c	c	b	c	c	c	c	.	d	.	b
SD1	.	.	.	.	.	.	.	.	.	b	.	c
SD2	.	.	.	.	.	.	.	.	.	.	.	.
Timgalen	.	.	.	.	.	.	.	.	.	.	.	b
Timstein	.	.	.	.	.	.	.	.	.	b	.	b
T. macha	c	c	c	c	.	.	.	.	.	b	.	.
T. spelta	.	.	.	.	.	.	.	.	b	c	b	d
Vilmorin 27	.	.	.	.	.	.	.	b	b	c	b	d
VPM	.	.	.	.	.	.	.	b	b	c	b	d

. = *a* allele

the *ApaI-e* allele. In addition, one *β-Amy-B1d* variety, 'Sicco', does not have *BamHI-b* or *DraI-b* alleles. The two *ApaI-d* variants among the group with *BamHI-a* and *DraI-a* show no correspondence to isozyme phenotype, while the *ApaI-a* and *ApaI-b* possessing group show limited correspondence (Table 3).

Discussion

The results presented here represent a first assay of the extent of RFLP variation at a low copy-number sequence in a large number of varieties of wheat, and also allow a very direct comparison of the level of, and relationships between, variation revealed by RFLP and isozyme analyses since the clone used is the transcribed region of a gene encoding an enzyme detectable after IEF. RFLPs and isozyme polymorphisms as detected by IEF appear to be approximately as frequent, since the number of alleles detected at each of the loci are very similar (Table 2). However, a more extensive search for RFLPs with more REs might be expected to reveal more variation in DNA sequences around the genes.

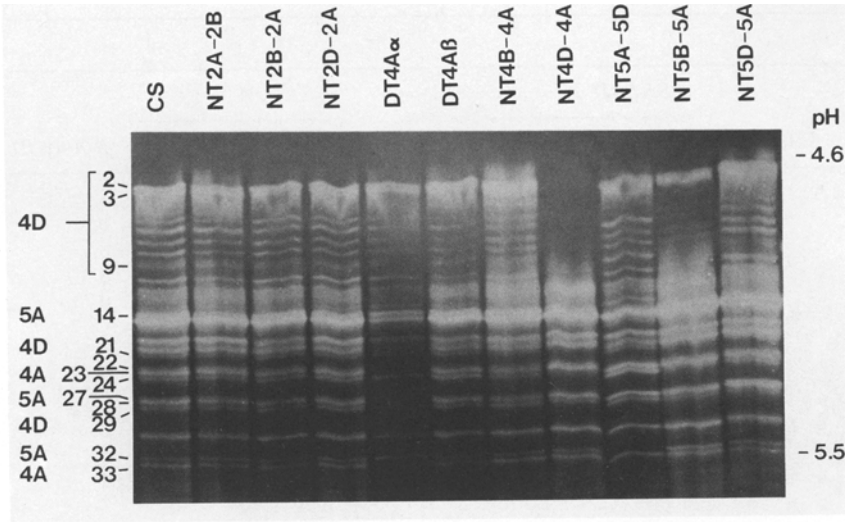

Fig. 4. β-Amylase zymograms after IEF of various chromosome group 2, 4 and 5 nullisomic-tetrasomic and ditelosomic aneuploid genotypes of 'Chinese Spring'. The chromosomal control of various isozymes, and their designation from Ainsworth et al. (1983) is indicated on the *left*

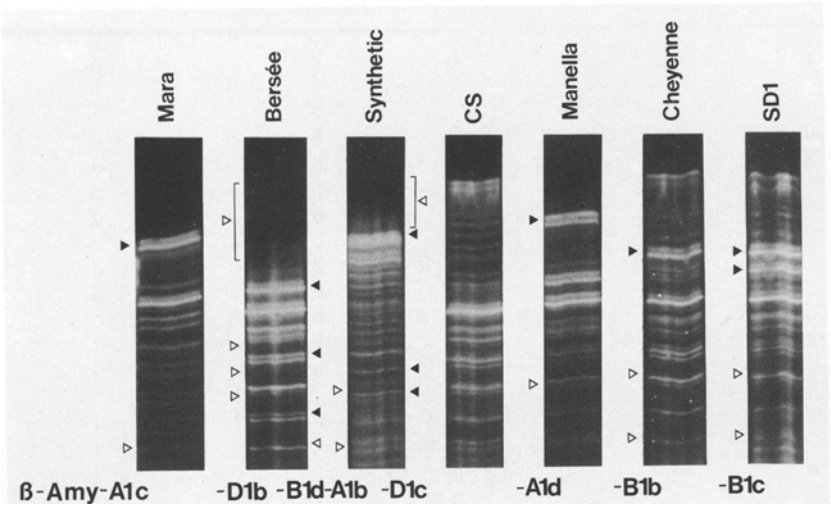

Fig. 5. β-Amylase zymograms after IEF of genotypes displaying the different alleles at the three *β-Amy-1* loci. *Above* each allele designation, the isozymes ▷ missing and ▶ gained in comparison to 'Chinese Spring' are indicated

Table 3. Relationship between RFLP and isozyme alleles

Chromosome	RFLP *PSR1* allele				Isozyme *β-Amy* allele
	BamHI	*ApaI*	*DraI*		
4A	*a*	*a*	*a*	(35)	*a*(35), *d*(2)
	b	*b*	*b*	(2)	
	c	*c*	*c*	(4)	*b*(1), *c*(3)
4D	*a*	*a*	*a*	(38)	*a*(27), *b*(11)
	b	*b*	*b*	(2)	*b*(2)
	c	*c*	*c*	(1)	*c*(1)

	RFLP *PSR1* allele			Isozyme *β-Amy* allele	RFLP *PSR1* allele *ApaI*	Isozyme *β-Amy* allele
	BamHI	*DraI*				
5A	*a*	*a*	(16)	*a*(6), *b*(7), *d*(2), *e*(1)	*a*(5)	*a*(4), *b*(1)
					b(9)	*a*(2), *b*(4), *c*(2), *d*(1)
					d(2)	*b*(2)
	b	*b*	(25)	*d*(24)	*c*(24)	*d*(24)
				a(1)	*e*(1)	*a*(1)

The numbers in brackets are the number of varieties carrying the particular allele

1 RFLPs

The genotypes of each variety examined with respect to the β-amylase RFLP and isozyme loci are listed in Table 2.

1.1 *PSR1-2* loci

Little variation at the loci on group 2 chromosome was found with the three REs used. Indeed, in both of the only two varieties which did show variation, the alleles probably derive from alien sources; 'Synthetic' (*PSR1-2A-BamHI-b*), being a man-made amphiploid (McFadden and Sears 1946, Sears 1976), carries chromosome 2A from the *T. dicoccum* parent used, while 'Timgalen' (*PSR1-2B-BamHI-b* and *ApaI-b*) is known to carry a segment from *T. timopheevi* on 2BS containing the *Sr36* gene (McIntosh 1983).

1.2 *PSR1-4A* locus

At this locus, each of the REs reveals three alleles, and consideration of the data in Table 2 indicates that the same polymorphisms are being detected by each of the three REs. Almost all the varieties studied have the *a* alleles with all three REs, with only 'Atlas 66' and 'Sava' having the *b* alleles (increased dose of the same fragment), and only 'Ciano 67' and 'Mara' having *c* alleles. Examination of the available predigrees (Zeven and Zeven-Hissink 1976) does not suggest a recent common origin of the *b* allele as 'Atlas 66' (USA) and 'Sava' (Yugoslavia) do not have a parent in common after tracing their pedigrees back 4–5 "generations" (crosses), but it does suggest that the *c* alleles of 'Ciano 67' (CIMMYT) and 'Mara' (Italy) may have come from 'Mentana' (Italy) since this variety is present in the ancestry of both these varieties.

1.3 *PSR1-4D* locus

Each RE reveals three alleles at this locus, and as at the *PSR1-4A* locus, Table 3 shows that the allelic typings with each enzyme are in complete agreement. Almost all of the studied varieties have the *a* alleles, while the *c* alleles present in 'Synthetic' must represent an alien allele derived from the *Aegilops squarrosa* parent used to produce this line. 'Manella' (The Nederlands) and 'Pané 247' (Spain) have the *b* alleles. Both varieties have the French line 'Hybride a courte paille' in their parentage which may be the source of the allele; 'Pané 247' comes from the cross 'Mentana' × 'Hybrid a courte paille' while 'Manella' is derived from the cross 'Alba' × 'Heine VII', with 'Heine VII' coming from 'Hybride a courte paille' × 'Kronen' (Zeven and Zeven-Hissink 1976).

1.4 *PSR1-5A* locus

Here the three REs give slightly different allele distribution (Table 3). BamHI and DraI reveal two alleles which are both common in the varieties studied. There is complete agreement between the typings of these alleles; all varieties with *BamHI-a* have *DraI-a*, and all those with *BamHI-b* have *DraI-b*. In contrast, ApaI detects five alleles, but despite this there is still a considerable degree of correspondence between the different allelic types (Table 3). Except for 'Glennson 81' (*ApaI-e* allele), all varieties possessing *BamHI-b* and *DraI-b* have the *ApaI-c* allele, and the varieties with *BamHI-a* and *DraI-a* are split into three groups having the *ApaI-a, b,* or *d* alleles (Table 3). Neither the five varieties with the *ApaI-a* allele, nor the nine carrying *ApaI-b* appear to be closely related, while the *d* allele genotypes 'Jitpur' and 'Synthetic', are obviously not related. The source of the *ApaI-e* allele of 'Glennson 81', a CIMMYT wheat, is unknown as none of its parents have been examined.

1.5 The nature of the RFLPs

The correspondence (in many cases complete) between the alleles detected with three different REs in this range of varieties suggests that a large proportion of the RFLPs detected are due to reasonably large insertions or deletions of DNA segments near the β-amylase coding sequences. This type of change in plant DNA has been well documented (Flavell 1986), and means that a polymorphism detected between varieties with one RE is also likely to be detected by another RE. For example, the common polymorphism described here between varieties with the *PSR1-5A-BamHI-a* and *b* alleles is also detected by digests with ApaI and DraI (results presented here), and on a smaller sample of varieties is also detected after digestion with EcoRI, EcoRV, HindIII, PvuII, and SstI (results not shown).

Examination of Tables 1 and 2 and Figs. 1–3 reveals a remarkable coincidence of fragment sizes; both the chromosome 4A *c* alleles in 'Ciano 67', 'Mara', 'Synthetic' and *T. macha* and the chromosome 4D *b* alleles of 'Manella' and 'Pané 247' are defined by fragments of 10.0, 17.4 and 7.4 kb with BamHI, ApaI and DraI, respectively. It seems unlikely that these fragments in 'Manella' and Pané 247' are actually from 4A *c* alleles, as this would require these varieties to have unique 4D alleles giving fragments of the same length as the 4A *a* alleles. In either case, there would be identical fragment sizes between some alleles at the chromosome 4A and 4D loci. (It should be noted that all five varieties discussed here have non-*a* alleles at the *β-Amy-A1* locus identified by the presence of isoenzymes at similar isoelectric points (Fig. 5), and the possibility that the phenotype in 'Manella' and 'Pané 247' is actually a *β-Amy-D1* allele has been ruled out by extensive segregation tests). Perhaps the homoeology of the two regions involved means that they are subject to similar rearrangements. Support for this comes from the *5A-DraI-b* allele also being 7.4 kb, like the *4A-DraI-c* and *4D-DraI-b* alleles.

The *PSR1-4A* copy number variants of 'Atlas 66' and 'Sava' described here would also represent another type of insertional event, as would the complete absence of a sequence in a particular variety, as Landry et al. (1987) have described in lettuce. The rare RFLP differences that are detected with only one enzyme, such as the *PSR1-5A-ApaI-e* allele of 'Glennson 81' may be either a small insertion/deletion difference not affecting other RE sites at the level of resolution used here, or be examples of RFLPs due to single base changes.

There is a considerable difference in the variability of the two series of homoeoloci. The group 2 chromosome loci are monomorphic on the sample of *T. aestivum* varieties itself (except where known 'alien' chromosome segments are present), whereas the group 4/5 chromosome loci display considerable polymorphism (Table 2). The reason for this difference between the two series is unknown, but this finding may suggest that certain regions of the wheat genome are either more tolerant of change, or have a higher frequency of change.

The two non-*aestivum* hexaploids studied, *T. spelta* and *T. macha*, do not carry alleles that are rare or absent from the *T. aestivum* varieties examined (Table 3), suggesting that the genetic divergence of the hexaploid wheats from each other is not large. This would agree with a single origin of hexaploid wheat as recently as some 9,000 years age (Miller 1987).

'Synthetic' (*T. dicoccum* × *Ae. squarrosa*), on the other hand, has many rare or unique alleles; rare alleles at the *PSR1-4A* and *PSR1-5A* loci and unique alleles at the *PSR1-2A* and *PSR1-4D* loci (Table 3), indicating that the use of RFLPs will certainly facilitate the manipulation of alien segments in wheat (Gale and Miller 1987).

2 Relationship between RFLP and isozyme variation

The molecular evidence from the RFLP analysis suggests that each of the loci controlling β-amylases contains a single copy of a coding sequence in the wheat genome. The presence of a single hybridizing DNA fragment in 'CS' for all but one locus/RE combination and the maintenance of, or conversion to, a single fragment in all the polymorphisms detected among the varieties studied provides substantial, but not conclusive, evidence for this.

The *β-Amy-D1b* allele, which appears to be a 'null' allele in that it is characterized by the absence of isozymes present in 'CS', but no novel isozymes are present (Fig. 5, Ainsworth et al. 1983), is not characterized by the absence of the chromosome 4D fragments. This demonstrates the absence of a large or complete deletion of the coding region as the cause of the 'null' phenotype, and suggests that the gene has been inactivated by a small event, as has been shown to be the case with a "null" allele at a wheat storage protein locus (Payne et al. 1981; Forde et al. 1985).

The associations observed here between the RFLP and isozyme variation are unlikely to be due to the RFLP (i.e. the restriction enzyme) detecting the same base change(s) which give rise to different isozyme types, particularly as both the single nature of the fragments, and their size, indicate that the REs are not generally cutting at sites internal to the transcribed regions. Rather, the associations will be due to an ancient variation in sequence outside the genes which is close enough to the gene to be maintained in linkage disequilibrium through long pedigrees. This process would seem to be especially strong in wheat, a self-pollinating crop, where almost all crossing is controlled by man, and most breeding is by the pedigree method, so that homozygosity is attained relatively quickly. There would therefore appear to be relatively little chance of recombination compared with outcrossing species within the short chromosomal regions considered here where even the largest fragments represent approximately 3×10^{-3} cM (17 kb fragment in 1.7×10^{6} kb of wheat genome extending for 3,000 cM).

Such is the case at the chromosome 4A loci where the RFLP c alleles faithfully identify those wheat genotypes with the rare c isozyme allele. Of coure, some differences are not associated with detectable isozyme differences, such as the copy number variant detected at *PSR1-4A* with all three REs, and the *PSR1-4A-ApaI-e* variant of 'Glennson 81'. The chromosome 5A RFLP analyses indicate that recombination may indeed occur between the *β-Amy* gene and the restriction sites delineating the corresponding fragment. The *DraI-b* and *BamHI-b* alleles are possessed by 24 of the 25 varieties with the isozyme *d* allele which is restricted, in our sample, to European varieties. In just one genotype, the Dutch variety 'Sicco', The correspondence has been broken. Recombination is the most likely cause, however, the same result could arise from a second mutational event in the parentage of 'Sicco' which reconstitutes both the *DraI-b* and *BamHI-b* alleles.

If the association between RFLP and isozyme alleles found here for β-amylase is general at other loci in wheat, then RFLPs detected with 'anonymous' cDNA probes are likely to be associated with different protein products. It may be that regions of the wheat genome able to vary within protein coding regions also display more RFLP variation, at least as revealed by cDNA rather than genomic probes. Nevertheless, if the association does hold across loci, then anonymous cDNAs may be more than just a convenient supply of genetic markers, as they may in many cases also be detecting (by association) genetic variation at the protein level; one step closer to the whole plant phenotype.

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Note added in proof

The 7th Int. Wheat Genet. Symp. has agreed nomenclature for RFLP loci, and has reversed the names of chromosomes 4A and 4B. Therefore, the loci designated here as *PSR1-2A, PSR1-2B, PSR1-2D, PSR1-4A, PSR1-4D, PSR1-5A, β-Amy-A1, β-Amy-D1* and *β-Amy-B1,* should be designated *Xβ-Amy-2A, Xβ-Amy-2B, Xβ-Amy-2D, Xβ-Amy-4B, Xβ-Amy-4D, Xβ-Amy-5A, β-Amy-B1, β-Amy-D1* and *β-Amy-A1,* respectively.