Theor. Appl. Genet. 59, 361-371 (1981)

TAG THEORETICAL AND APPLIED GENETICS

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Genetic Variation in Wheat Endosperm Proteins: an Analysis by Two-dimensional Electrophoresis Using Intervarietal Chromosomal Substitution Lines

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Summary. The major endosperm proteins in a range of genotypes of hexaploid wheat have been fractionated by two-dimensional electrophoresis. The genotypes included nine varieties and forty four intervarietal substitution lines in which chromosomes 1A, 1B, 1D, 6A, 6B or 6D from eight of the varieties have been introduced one at a time into a common genetic background. The appearance of different protein subunits was often correlated with a chromosome substitution. This showed that many of the genes for the high molecular weight protein subunits (molecular weight range 55,000 to 140,000 determined by SDS polyacrylamide gel electrophoresis) are specified by chromosomes 1A, 1B and 1D while many of the lower molecular weight subunits (molecular weight range 30,000 to 45,000) are specified by chromosomes 6A, 6B and 6D. The different protein subunits correlated with chromosome substitution could not always be recognised in the varietal source of the substituted chromosome. The different subunits specified by homologous chromosomes in different wheat varieties may differ in isoelectric point and/or molecular weight.

Key words: Wheat – Endosperm – Proteins – Electrophoresis

Introduction

Many of the storage protein subunits of hexaploid wheat *Triticum aestivum* are controlled by chromosomes of homoeologous groups 1 and 6 (Shepherd 1968; Wrigley and Shepherd 1973; Beitz et al. 1975; Brown et al. 1979; Brown and Flavell 1981). This has been established principally by electrophoretic analysis of endosperm extracts from aneuploid lines of the variety 'Chinese Spring' with different group 1 or group 6 chromosomes deleted. Wheat varieties show great variation in their electrophoretic storage protein pattern and hence in their protein composition (see Beitz et al. 1975; Autran and Bourdet 1975; Wrigley and Shepherd 1973; Baker and Bushuk 1978). Chromosome analysis of this variation can be conveniently carried out using intervarietal chromosome substitution lines. In such lines a pair of chromosomes of the recipient variety is substituted by the homologous pair from another variety. There are a few reports of detailed studies on protein subunit variation using chromosome substitution lines. Kasarda et al. (1976) used the set of lines in which the chromosomes of the variety 'Cheyenne' had been substituted into 'Chinese Spring' to show that chromosome 6A controlled the production of A-gliadin in 'Cheyenne' and other chromosomes of groups 1 and 6 controlled other endosperm proteins. The genetic control of certain proteins in the variety 'Thatcher' has also been deduced from studies on substitution lines (Solari and Favret 1970).

We have recently described a two dimensional electrophoretic system (Brown et al. 1979) that is useful for characterising wheat grain protein subunits, especially those with molecular weights greater than 40,000. This system has now been used to analyse the endosperm protein subunits in a large number of intervarietal substitution lines differing in chromosomes 1A, 1B, 1D, 6A, 6B or 6D. The results show that homologous chromosomes in different varieties may control endosperm protein subunits which differ not only in isoelectric point but also in molecular weight.

Materials and Methods

Genetic Stocks

Forty-four substitution lines from eight intervarietal chromosome substitution sets have been studied. These substitution sets involved the substitution into 'Chinese Spring' of pairs of chromosomes from the following varieties:

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*	'Cappelle-Desprez'	(French, winter)
**	'Cheyenne'	(North American, winter)
*	'Ciano 67'	(Mexican semi-dwarf, spring)
†	'Hope'	(North American, spring)
*	'Lutescens 62'	(Russian, spring)
*†	'Synthetic'	(amphiploid of *Ae. squarrosa* and *T. dicoccoides*)
†	'Timstein'	(North American, spring)
*	*T. spelta*	(ABD species)

* substitution sets developed by Dr. C.N. Law and Mr. A.J. Worland, Plant Breeding Institute, Cambridge.
** developed by Dr. R. Morris, University of Nebraska.
† developed by Dr. E.R. Sears, University of Missouri.
*† amphiploid developed by Dr. E.R. Sears, and substitution set by Dr. C.N. Law.

The forty-four substitution lines examined in this work involved the substitution of pairs of each of the chromosomes of homoeologous groups 1 and 6, from each of the eight varieties into 'Chinese Spring'. Of the 48 possible lines only CS ('Ciano' 6A) CS ('Hope' 6A) (6B) and (6D) were not used. All the intervarietal chromosome substitution sets had undergone at least five backcrosses except for the 'Timstein' set which had been backcrossed only three times.

Protein Extraction and Electrophoresis

Proteins from the ground endosperms of ten grains were extracted overnight in 1 ml of 2 M urea, 0.5 per cent SDS, and 0.6 per cent β-mercaptoethanol at 4°C. After centrifugation at 14,000 rpm for 30 minutes the protein contents of the supernatants were determined by the method of Bramhall et al. (1969). This extraction method extracts more than 80 per cent of the endosperm protein (Brown et al. 1979).

Proteins in the supernatants were separated first by polyacrylamide disc gel isoelectrofocussing, as described previously (Brown et al. 1979). The rod gels contained 2 M urea, 4 per cent acrylamide (bis: acrylamide ratio – 1:17.2) and 2 per cent carrier ampholytes (a 1:6 mixture of pH ranges 3 to 10 and 5 to 8). After pre-electrophoresis of the gels (Brown et al. 1979) a protein sample (about 100 μl containing 600 to 1,200 μg protein) was applied to the top of each gel, and the proteins isofocussed at 400 volts for 15 hours, followed by 800 volts for 1 hour.

The gel rods were then equilibrated for SDS electrophoresis (O'Farrell 1975; Brown et al. 1979) and loaded in pairs onto a slab gel containing 10 per cent acrylamide (bis: acrylamide ratio – 1:36.5) and 0.1 per cent SDS as described by Brown et al. (1979). Electrophoresis of the gel was carried out at 25 mA for 16 hours, after which the gel was stained by two different staining/destaining procedures (Brown et al. 1979). A critical evaluation of the reproduceability and limitations of this two dimensional electrophoretic technique for storage proteins are discussed in a previous paper (Brown et al. 1979).

Proteins from different genotypes were isofocussed on separate rod gels but two rod gels were inserted into each slab gel in opposite orientation, so that two sets of isofocussed proteins were fractionated side by side on the same slab gel by SDS electrophoresis (Brown et al. 1979). This enabled genotypes to be compared on the same SDS polyacrylamide gel. All conclusions presented in this paper are from such comparisons. However for presentation purposes photographs of different gels have been mounted together. Conclusions regarding the presence or absence of a subunit have been made only from gels with adequate resolution in the area in question.

Measurement of pH Gradients

Isoelectrofocussed rods were cut into 5 mm slices and placed in 2 ml of degassed distilled water. After half an hour of gentle shaking to elute the ampholines from the gel, the pH was measured.

Molecular Weight Calibration of SDS Gels

Molecular weights were inferred from protein mobilities using the following proteins as standards: cytochrome c (12,500), chymotrypsinogen A (25,000), hen egg albumin (45,000), bovine serum albumin (67,000), aldolase (4 subunits of 40,000), catalase (4 subunits of 60,000) and ferritin (450,000). Note that the molecular weights of endosperm storage proteins determined by SDS polyacrylamide gel electrophoresis are often overestimates owing to their unusual amino acid composition. (Hamauzu et al. 1974, 1975; Sexson et al. 1978).

Results

The Genetic Control of Protein Subunits in the Variety 'Chinese Spring'

The protein subunit pattern formed by the two dimensional electrophoresis of proteins extracted from endo-

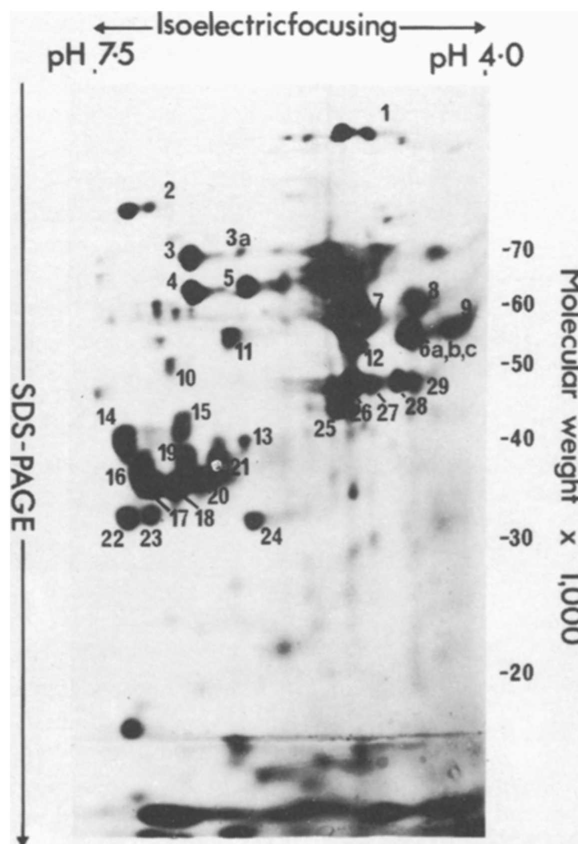

Fig. 1. Two-dimensional electrophoretic pattern of proteins from 'Chinese Spring'. This figure is reproduced from Brown et al. (1979)

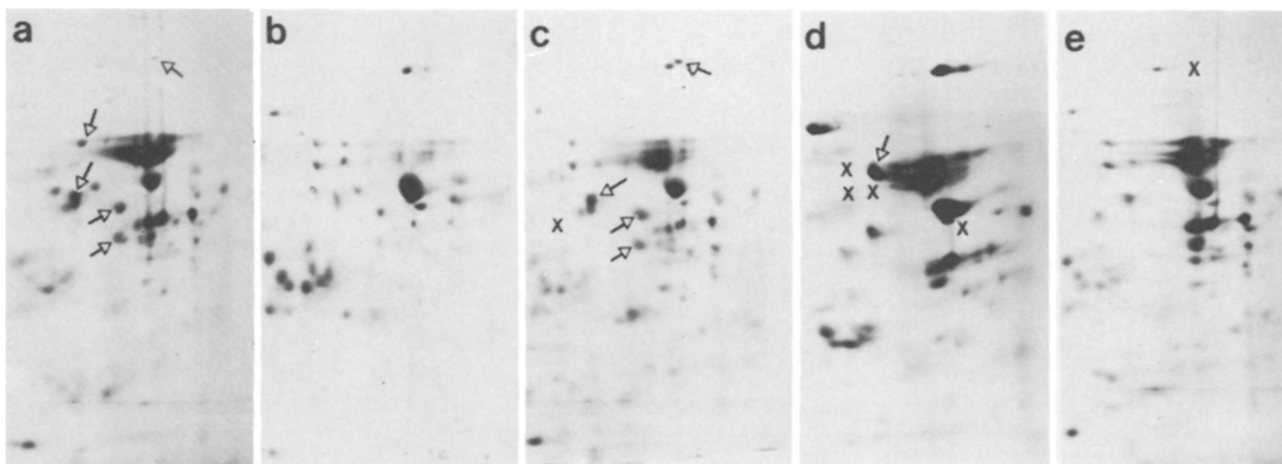

Fig. 2a-e. Two-dimensional electrophoretic patterns of proteins from Hope and CS (Hope) substitution lines. **a** 'Hope'. **b** Mixture of extracts of 'Hope' and 'Chinese Spring'. **c** CS ('Hope' 1A). **d** CS ('Hope' 1B). **e** CS ('Hope' 1D). X's show the locations of absent 'Chinese Spring' subunits. The open arrows in **a** indicate those subunits transferred with chromosome 1A (in **c**) and 1B (in **d**)

sperms of the variety 'Chinese Spring' has been described previously (Brown et al. 1979). It is reproduced here (Fig. 1) as a reference for the results reported in this paper. Studies on endosperms from aneuploid derivations of 'Chinese Spring' have shown that chromosome 1A controls the subunit numbered 10 in Figure 1; chromosome 1B controls subunits numbered 3, 4, 5, 6a and 12; chromosome 1D controls subunits numbered 1, 2, 6b 6c, 7, 8, 9 and 11 (Brown et al. 1979). Chromosome 6A controls subunits numbered 20 and 24; chromosome 6B controls subunit 14 and chromosome 6D controls subunits 17 and 18 (Brown and Flavell 1981).

Lines in which these chromosomes have been replaced by their homologues from other hexaploid varieties have now been studied. The two-dimensional electrophoretic patterns of endosperm proteins from these substitution lines and the donor varieties of the substituted chromosomes are described below. Only those substitution lines which differ from 'Chinese Spring' are described individually. Differences could not be reliably discerned in the other lines and therefore it is assumed that the chromosomes introduced into 'Chinese Spring' specify proteins indistinguishable from those specified by the homologous 'Chinese Spring' chromosomes.

Subunit Variation in Substitution Lines Involving Chromosomes of Homoeologous Groups 1 and 6

a) Substitution Lines of 'Hope'

In the two-dimensional subunit pattern of CS ('Hope' 1A) (Fig. 2c) subunit 10 of 'Chinese Spring' (Fig. 1) is absent and four new subunits of molecular weights approximately 130,000; 55,000; 52,000 and 44,000 are present. All four subunits are present in the electrophoretic pattern of 'Hope' (Fig. 2a).

CS ('Hope' 1B) (Fig. 2d) does not produce the subunits controlled by 'Chinese Spring' chromosome 1B viz: 3, 4, 5, and 12 (the effect on subunit 6 cannot be resolved), but produces a new subunit of molecular weight approximately 68,000. The variety 'Hope' contains this subunit but not subunits 3, 4, 5, or 12. (Fig. 2a).

There are no differences between CS ('Hope' 1D) (Fig. 2e) and 'Chinese Spring' (Fig. 1) except for the absence of the more acidic protein spots in the subunit group numbered 1 (Fig. 1). These subunits are not in 'Hope' (Fig. 2a). This and the other conclusions is supported by the electrophoretic pattern of a 1:1 mixture of extracts from 'Chinese Spring' and 'Hope' (Fig. 2b).

The subunit pattern of 'Hope' (Fig. 2a) also has two subunits of molecular weight approximately 57,000 – not seen in any of the group 1 or group 6 'Chinese Spring' ('Hope') substitution lines.

b) Substitution Lines of 'Timstein'

In the two-dimensional protein subunit pattern of CS ('Timstein' 1A) (Fig. 3b) at least four subunits are found which are not in 'Chinese Spring'. All are present in 'Timstein' (Fig. 3a). Their approximate molecular weights are 54,000; 45,000; and 43,000. Subunit 10, controlled by chromosome 1A of 'Chinese Spring' (Fig. 1) is not present in CS ('Timstein' 1A).

The protein subunit pattern of CS ('Timstein' 1B) (Fig. 3c) does not contain subunit 3 and only traces of subunits 4 and 5 (Fig. 1), controlled by chromosome 1B of 'Chinese Spring'. Two protein subunits having a molecular weight of approximately 68,000 – similar to subunit

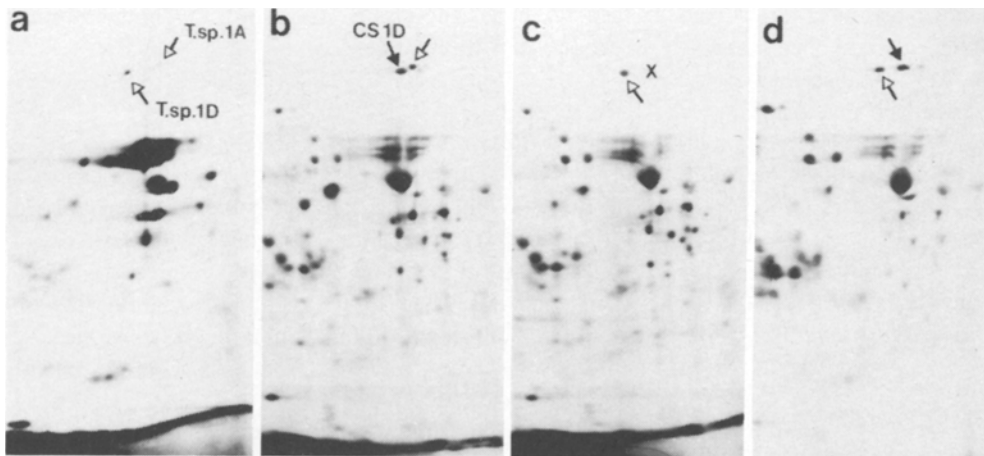

Fig. 3a-d. Two-dimensional electrophoretic patterns of proteins from 'Timstein' and CS ('Timstein') substitution lines. **a** 'Timstein'. **b** CS ('Timstein' 1A). **c** CS ('Timstein' 1B). **d** CS ('Timstein' 6D). X's show the locations of absent 'Chinese Spring' subunits. The arrows in b and c show the subunits not present in 'Chinese Spring' and the arrows in **a** show these subunits in 'Timstein'

3, are present in the pattern of 'Timstein' (Fig. 3a) but probably not 'Chinese Spring' (Fig. 1), and, possibly, both are produced in the CS ('Timstein' 1B) line. The protein subunit pattern of CS ('Timstein' 6D) (Fig. 3d) does not contain subunit 17, controlled by chromosome 6D of 'Chinese Spring'.

c) Substitution Lines of Triticum spelta

A high molecular weight subunit, approximately 130,000 – controlled by chromosome 1A of *T. spelta* (Fig. 4b), is labelled in the electrophoretic pattern of *T. spelta* (Fig. 4a). All the 'Chinese Spring' chromosome 1D-controlled subunits, except for the more acidic protein spots of subunit group 1 (Fig. 1), are in CS (*T. spelta* 1D) (Fig. 4c). This is confirmed in Figure 4d which shows the pattern of a mixture of extracts of 'Chinese Spring' and CS (*T. spelta* 1D). In the subunit pattern of *T. spelta* there are at least two subunits not observed in the substitution lines. Also subunits 3, 4, and 5 of 'Chinese Spring' (Fig. 1) are absent from *T. spelta* but present in CS (*T. spelta* 1B) (gel not shown).

d) Substitution Lines of 'Ciano 67'

CS ('Ciano' 1B) (Fig. 5c) shows a number of differences from 'Chinese Spring'. Of the subunits 3, 4, 5, and 12, controlled by chromosome 1B of 'Chinese Spring' (Fig.

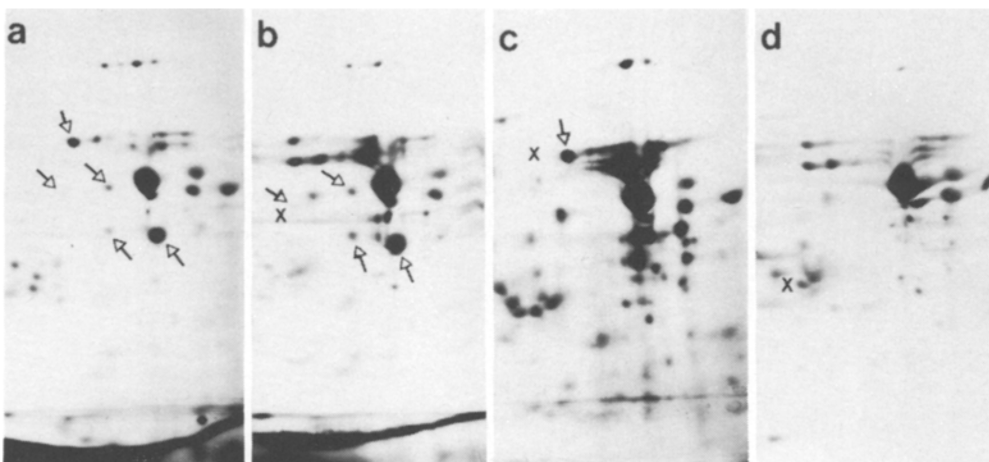

Fig. 4a-d. Two-dimensional electrophoretic patterns of proteins from *Triticum spelta* and CS (*T. spelta*) substitution lines. **a** *Triticum spelta*. **b** CS (*T. spelta* 1A). **c** CS (*T. spelta* 1D). **d** A mixture of extracts of CS (*T. spelta* 1D) and 'Chinese Spring'. The open arrows point to subunits which appear to be controlled by chromosomes 1D or 1A of *T. spelta* while the solid arrows indicate subunits controlled by chromosome 1D in 'Chinese Spring'

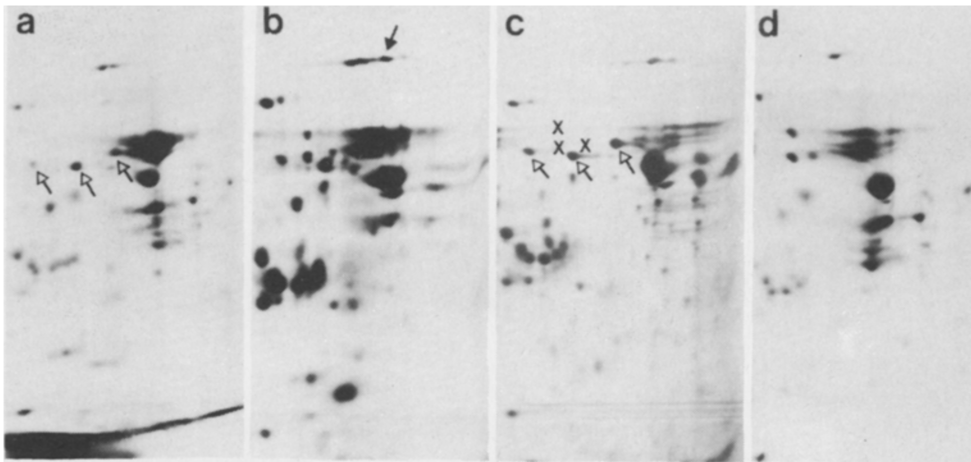

Fig. 5a-d. Two-dimensional electrophoretic patterns of proteins from 'Ciano' and CS ('Ciano') substitution lines. a 'Ciano'. b Mixture of extracts of 'Ciano' and 'Chinese Spring'. c CS ('Ciano' 1B). d CS ('Ciano' 1D). X's show the position of deleted 'Chinese Spring' subunits. The open arrows in a and c show the positions of the 'Ciano' subunits which appear to be controlled by chromosome 1B. The closed arrow in b indicates the more acidic subunits of 'Chinese Spring' group 1 absent from 'Ciano' a and from CS ('Ciano' 1D) d

1), only subunit 12 is found in CS ('Ciano' 1B). Three subunits of molecular weights approximately 64,000, 62,000, and 60,000 are absent from 'Chinese Spring'. These subunits are present in both 'Ciano' (Fig. 5a) and a mixture of 'Ciano' and 'Chinese Spring' (Fig. 5b).

CS ('Ciano' 1D) (Fig. 5d) contains only the subunits of 'Chinese Spring' group 1 with more basic PI's. The difference in pI from the group I subunits of 'Chinese Spring' is clearly shown in the electrophoretic patterns of a mixture of 'Ciano' and 'Chinese Spring' extracts (Fig. 5b).

CS ('Ciano' 1D) also produces subunits 2, 7, 8, 9, 11, and at least two components of subunit 6 (Fig. 1) all of which are produced by 'Ciano' itself.

e) Substitution Lines of 'Lutescens'

Subunits 3, 4, and 5 of 'Chinese Spring' (Fig. 1) are not produced in CS ('Lutescens' 1B) (Fig. 6b) or 'Lutescens' (Fig. 6a). One subunit of approximately 68,000, observed in 'Lutescens' but not 'Chinese Spring', is present in CS ('Lutescens' 1B).

f) Substitution Lines of 'Synthetic'

The only group 1 chromosome substitution line of 'Synthetic', showing differences from 'Chinese Spring' is CS ('Synthetic' 1D) (Fig. 7c). In the pattern of this line, 'Chinese Spring' subunits 7, 8, 9, 11, and at least one component of 6 (Fig. 1) are absent. These subunits are also absent from the pattern of 'Synthetic' itself (Fig. 7a). 'Chinese Spring' subunit group 1 is present in CS ('Synthetic' 1D) but subunit 2 has a more acidic pI than that of 'Chinese Spring' (Fig. 7c). These more acidic group 2 subunits can also be seen in the pattern of 'Synthetic' (Fig. 7a). In addition, two subunits are produced in 'Synthetic' (Fig. 7a) and CS ('Synthetic' 1D) (Fig. 7c) but not 'Chinese Spring' (Fig. 1) with molecular weights 64,000 and 54,000. Although the 1A and 1B chromosome substitution line patterns are identical to that of 'Chinese Spring', 'Synthetic' itself (Fig. 7a) produces only subunits 4 and 5, but not 3, 10 or 12 (Fig. 1).

The two-dimensional protein subunit pattern of CS ('Synthetic' 6A) (Fig. 7d) includes a protein subunit ab-

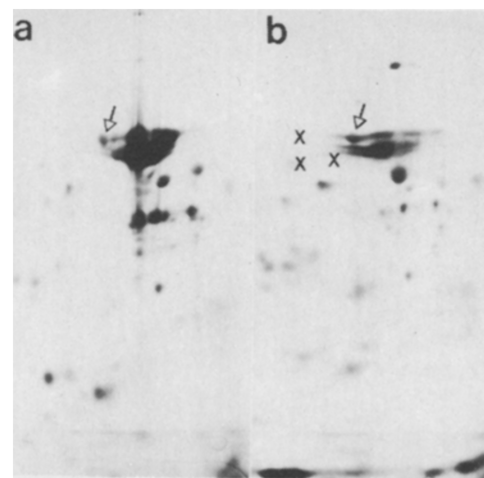

Fig. 6a and b. Two-dimensional electrophoretic patterns of proteins from 'Lutescens' and CS ('Lutescens' 1B) substitution lines. a 'Lutescens'. b CS ('Lutescens' 1B). X's show the positions of deleted 'Chinese Spring' subunits. The arrows indicate a subunit not found in 'Chinese Spring'

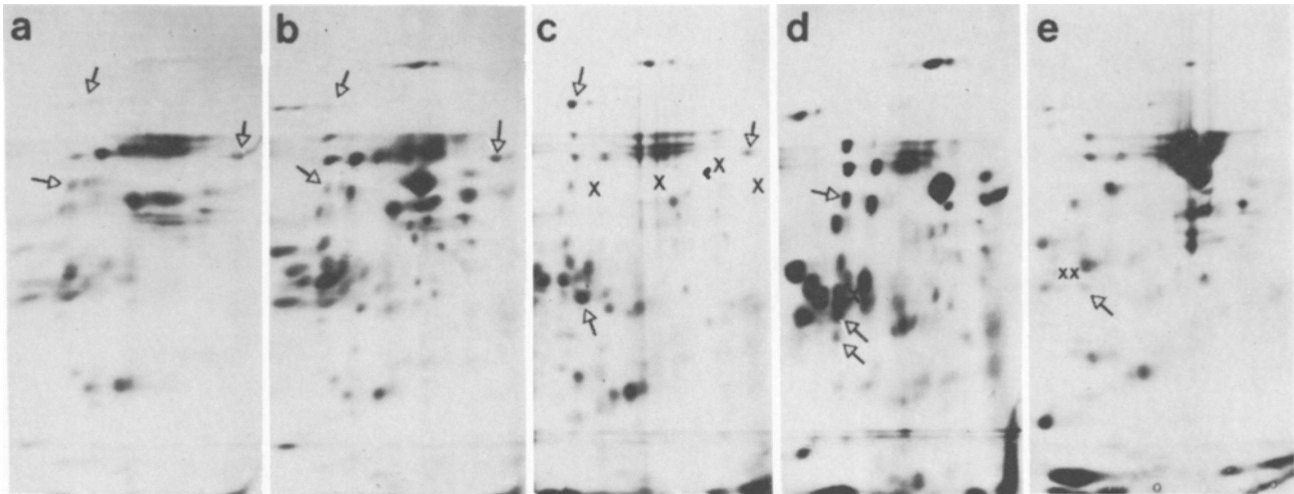

Fig. 7a-e. Two-dimensional electrophoretic patterns of proteins from 'Synthetic' and CS ('Synthetic') substitution lines. **a** 'Synthetic'. **b** A mixture of 'Synthetic' and 'Chinese Spring' extracts. **c** CS ('Synthetic' 1D). **d** CS ('Synthetic' 6A). **e** CS ('Synthetic' 6D). X's mark the locations of deleted 'Chinese Spring' subunits. The arrows in **a** show the 'Synthetic' subunits found in CS ('Synthetic' 1D) **c** or CS ('Synthetic' 6A) **d**. The arrows pointing to lower molecular weight subunits in **c**, **d** and **e**, mark subunits not seen in 'Chinese Spring' (Fig. 1)

sent from 'Chinese Spring' with a pI between subunits 10 and 11, and with a molecular weight of 55,000.

It also appears that subunit 20 is deleted from this line, but the absence or presence of subunit 24, also controlled by chromosome 6A of 'Chinese Spring' cannot be clearly distinguished. The subunit of 55,000 daltons is seen in the pattern of 'Synthetic' (Fig. 7a) and also in that of the mixture of protein extracts of 'Chinese Spring' and 'Synthetic' (Fig. 7b).

Subunits 17 and 18 (Fig. 1), controlled by chromosome 6D of 'Chinese Spring', are not detectable in the subunit pattern of CS ('Synthetic' 6D) (Fig. 7e), and one additional subunit MW 33,000 not found in 'Chinese Spring' is produced in this line. The patterns of CS ('Synthetic' 6A) and (6D) show subunits not found in 'Chinese Spring' arrowed in Figure 7c, d and e. These subunits cannot be clearly distinguished in the patterns of either 'Synthetic' or the mixture of 'Chinese Spring' and 'Synthetic' extracts (Figs. 7a, b).

g) Substitution Lines of 'Cappelle-Desprez'

Protein subunit 10 (Fig. 1) controlled by chromosome 1A of 'Chinese Spring' is present in CS ('Cappelle-Desprez' 1A) (Fig. 8b) and two subunits, not in 'Chinese Spring' but present in 'Cappelle-Desprez', are also in this substitution line. These subunits have a molecular weight of approximately 60,000 and they have similar pI's to

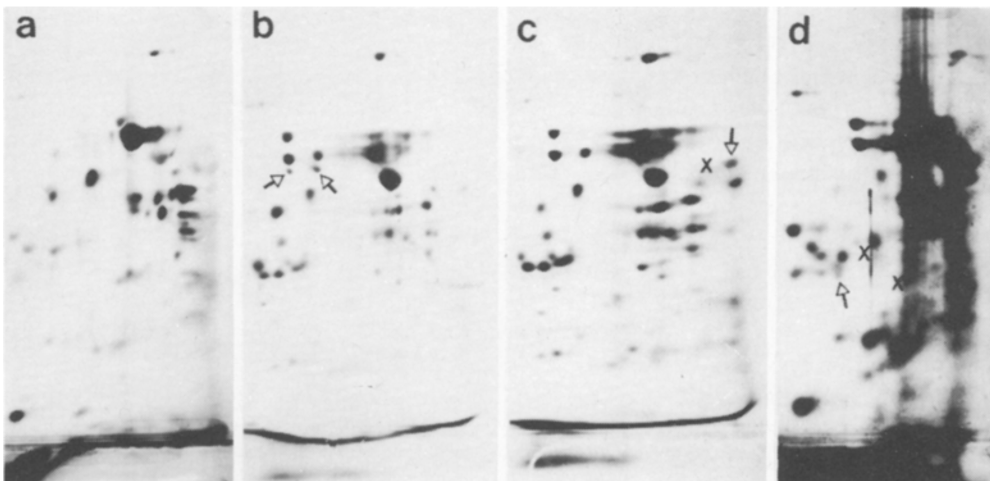

Fig. 8a-d. Two-dimensional electrophoretic patterns of proteins from 'Cappelle-Desprez' and CS ('Cappelle-Desprez') substitution lines. **a** 'Cappelle-Desprez'. **b** CS ('Cappelle-Desprez' 1A). **c** CS ('Cappelle-Desprez' 1D), and **d** CS ('Cappelle-Desprez' 6A). X's show the positions of deleted 'Chinese Spring' subunits and arrows show new subunits produced in these lines

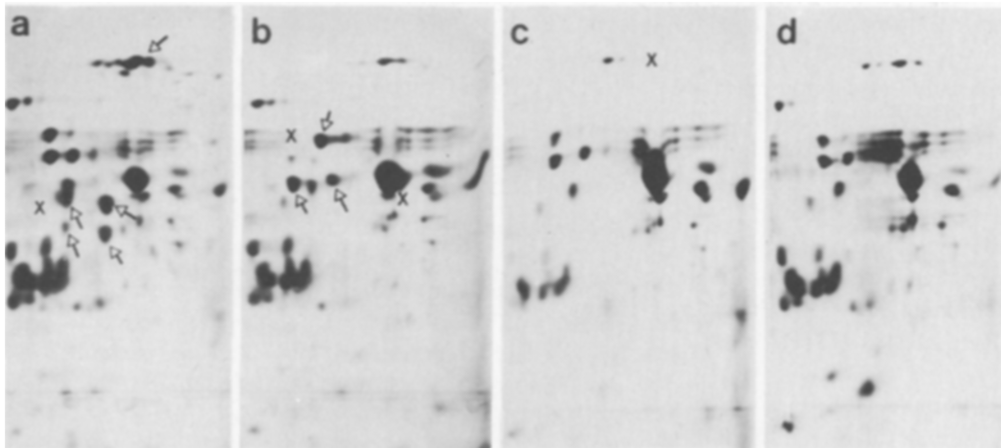

Fig. 9a-d. Two-dimensional electrophoretic patterns of proteins from 'Cheyenne' and CS ('Cheyenne') substitution lines. **a** CS ('Cheyenne' 1A). **b** CS ('Cheyenne' 1B). **c** CS ('Cheyenne' 1D). **d** Mixture of extracts of CS ('Cheyenne' 1D) and CS euploid. X's show the locations of deleted 'Chinese Spring' subunits, and arrows show the presence of subunits not found in 'Chinese Spring'

subunits 4 and 5 of 'Chinese Spring' (Fig. 1). Subunit 8 (Fig. 1) is absent from the pattern of CS ('Cappelle-Desprez' 1D) (Fig. 8c), and a new subunit of the same molecular weight is produced.

In CS ('Cappelle-Desprez' 6A) (Fig. 8d) protein subunits 20, and possibly 24 of 'Chinese Spring' (Fig. 1) are absent. The absence of these proteins is probably due to the removal of 'Chinese Spring' 6A. A new protein subunit is observed directly below subunit 18 of molecular weight 33,000 daltons.

h) Substitution Lines of 'Cheyenne'

When compared with 'Chinese Spring' euploid (Fig. 1) the two-dimensional protein pattern of CS ('Cheyenne' 1A) (Fig. 9a) shows a number of major differences. Firstly, subunit 10, controlled by 'Chinese Spring' 1A is not produced but five new subunits are present with molecular weights of 130,000+; 52,000; 50,000; 45,000; and 44,000. However, these subunits (except the one with the highest molecular weight) are not seen in the two-dimensional subunit pattern of 'Cheyenne' (not illustrated). The 52,000 subunit has a very similar molecular weight and slightly more acidic pI than subunit 10 of 'Chinese Spring'.

'Chinese Spring' (Fig. 1) and CS ('Cheyenne' 1B) (Fig. 9b) also have a number of major differences. Subunits 3 and 12, controlled by chromosome 1B of 'Chinese Spring', are not produced in CS ('Cheyenne' 1B). The concentration of subunits 4 and 5 is extremely reduced when compared with CS ('Cheyenne' 1A) (Fig. 9c) (extracts electrophoresed on the same gel). Three new subunits are produced with molecular weights of 67,000; 56,000; and 55,000. The first of these is produced in the same area of the gel as subunits 3, 4 and 5 of 'Chinese Spring' (Fig. 1). This subunit is seen in the two-dimensional patterns of 'Cheyenne' itself. The other two subunits (56,000 and 55,000) are similar in molecular weight to subunit 11 and have pI's either side of it. These subunits are not present in the protein subunit pattern of 'Cheyenne'.

CS ('Cheyenne' 1D) (Fig. 9c) has only one major difference from 'Chinese Spring' (Fig. 1). The group of subunits, numbered 1 (Fig. 1) often comprises five subunits of very similar molecular weight and slightly different pI's. Chromosome 1D of 'Cheyenne' appears to produce only the two subunits with the more basic pI's and not the spots with more acidic pI's. This is clearly shown by comparing CS ('Cheyenne' 1D) (Fig. 9c) and a mixture of the extracts of CS ('Cheyenne' 1D) and 'Chinese Spring' (Fig. 9d). The subunits with more acidic pI's are also absent from 'Cheyenne'. CS ('Cheyenne' 1D) produces the other subunits controlled by chromosome 1D of 'Chinese Spring' viz 2, 7, 8, 9, 11 and at least two of subunits 6a, b and c (Fig. 1). However, of these subunits only numbers 2, 11 and possibly one component of subunit 6 ((Fig. 1) appear to be produced in 'Cheyenne'.

Discussion

The results presented in this paper provide further examples of variation of wheat storage proteins in different varieties. They also provide new evidence for varieties other than 'Chinese Spring' (Brown and Flavell 1981) that many subunits with molecular weights greater than 50,000 are controlled by chromosomes of homoeologous group 1 and many subunits with molecular weights between 30,000 and 45,000 are controlled by chromosomes of homoeologous group 6. A summary of the subunits controlled by chromosomes 1A, 1B, 1D, 6A, 6B and 6D in the varieties studied is provided in Tables 1, 2, 3 and 4.

Subunits Controlled by Group 1 Chromosomes

Among the subunits controlled by 1A chromosomes are some with molecular weights greater than 130,000. This is noteworthy because until recently only chromosomes 1B and 1D of 'Chinese Spring' (Bietz et al. 1975; Brown et al. 1979) and 1D of 'Cheyenne' (Kasarda et al. 1976) have been reported to control high molecular weight subunits. These high molecular weight subunits are thought to play a major role in determining important characteristics of dough structure (Bietz et al. 1973; Payne et al. 1979).

The finding that 1A chromosomes in certain varieties control the production of high molecular weight subunits (see also Payne et al. 1980) discounts the conclusion held previously that it was the D genome that contributed the highest molecular weight endosperm protein subunits into hexaploid wheat. The presence of high molecular weight subunits in the endosperm of many diploid and tetraploid *Aegilops* and *Triticum* species, not known to contain the D genome of hexaploid wheat (Brown 1979) also argues against the high molecular weight subunits being contributed only with the D genome. The group of high molecular weight subunits controlled by chromosome 1D in 'Chinese Spring' (subunit group 1, Fig. 1) consists of at least five subunits separable by isoelectricfocussing. It is possible that these are isoelectricfocussing variants of a single gene, the products of five genes or of fewer genes with some charge variants. Only the varieties 'Cappelle-Desprez', 'Lutescens', 'Synthetic' and 'Timstein' appear to produce subunits in group 1 similar to 'Chinese Spring'. The varieties 'Ciano', 'Cheyenne', 'Hope' and *T. spelta* produce only the more basic types of subunit in the 'Chinese Spring' group. This suggests either that the subunits in group 1 are controlled by more than one gene or that different varieties have post-translational modification activities which affect different subunits.

The different subunits controlled by the 1B chromosomes of different varieties differ in charge but show only relatively small variations in molecular weight.

Subunits Controlled by Group 6 Chromosomes

The electrophoretic systems described in this paper do not resolve well the subunits with molecular weights between 30,000 and 40,000 many of which are controlled by group 6 chromosomes (Wrigley and Shepherd 1973; Brown and Flavell 1981). Nevertheless careful inspection of the gel patterns for the substitution lines suggests that most varieties have similar subunits to those in 'Chinese Spring'. This needs to be examined more fully by other electrophoretic systems. Some substitution lines do show clear differences in these subunits, however, as indicated in Table 4.

Variation in the Control of Subunit Expression?

The conclusions indicated in Tables 1-4 regarding which subunits are controlled by which chromosomes of the varieties studied are based upon the assumption that in each substitution line the genetic background is identical to that of 'Chinese Spring' and the substituted chromosome is identical with its homologue in the donor variety. In most cases, this assumption is supported by the results because the different subunits introduced into 'Chinese Spring' in each substitution line are found in the variety from which the substituted chromosome was extracted. The examples fitting this pattern are summarised in Table 5.

Table 1. Variant protein subunits in chromosome 1A substitution lines

Source of Chromosome 1A	'Chinese Spring' subunit 10	No. of new subunits	Approximate molecular weights
'Chinese Spring'	√		50,000
'Cappelle-Desprez'	√	2	60,000 (Both)
'Cheyenne'	X	5	130,000+; 52,000; 50,000 45,000; 44,000
'Ciano'	√	0	
'Hope'	X	4	130,000+; 55,000; 53,000 44,000
'Lutescens'	√	0	
'Synthetic'	√	0	
'Timstein'	X	4	54,000; 50,000; 45,000 43,000
T. spelta	√	1	130,000+

√ = present; X = absent

Table 2. Variant protein subunits in chromosome 1B substitution lines

Source of Chromosome 1B	'Chinese Spring' subunits 3 4 5 12	No. of new subunits	Approximate molecular weights
'Chinese Spring'	√ √ √ √		70,000; 63,000; 64,000; 52,000
'Cappelle-Desprez'	√ √ √ √	0	
'Cheyenne'	X √ √ X	3	68,000; 56,000; 55,000
'Ciano'	X X X √	3	64,000; 62,000; 60,000
'Hope'	X X X X	1	68,000
'Lutescens'	X X X √	1	68,000
'Synthetic'	√ √ √ √	0	
'Timstein'	X √ √ √	1	68,000
T. spelta	√ √ √ √	0	

√ = present; X = absent

Table 3. Variant protein subunits in Chromosome 1D substitution lines

Source of Chromosome 1D	'Chinese Spring' subunits 1 2, 7 8 9 11	No. of new subunits	Approximate molecular weights
'Chinese Spring'	all present		125,000; 88,000; 60,000; 62,000; 56,000; 53,000
'Cappelle-Desprez'	all present except no. 8	1	60,000
'Cheyenne'	all present[a]	0	
'Ciano'	all present[a]	0	
'Hope'	all present[a]	0	
'Lutescens'	all present	0	
'Synthetic'	only subunit 1 present	3	88,000; 64,000 54,000
'Timstein'	all present	0	
T. spelta	all present[a]	0	

[a] The more acidic protein subunits of Group 1 are absent

Not all substitution lines show this additive pattern, however. Some substitution lines have subunits which should have been deleted with the removal of the 'Chinese Spring' chromosome and not reintroduced with the chromosome substitution because these subunits are not found in the donor variety. These lines are listed in Table 6. In other substitution lines, subunits were found which are not present in 'Chinese Spring' or the donor variety. These are listed in Table 7.

To interpret these results, it is necessary to consider whether the substitution lines have the correct genotype. It is possible through error in stock construction for recombination to have occurred between the 'Chinese Spring' and substituted homologues. This could produce results such as those in Table 6. Alternatively, the 'Chinese Spring' chromosome could be maintained through error instead of being substituted.

If there was heterogeneity within a seed stock used in the production of a substitution line, it would be possible for subunits not in 'Chinese Spring', or the donor variety, to be introduced into the substitution line. This problem could produce the results listed in Table 7. These various possibilities are not easy to test rapidly.

An alternative hypothesis is that the expression of some storage protein genes is regulated by other genes. On this hypothesis for example, 'Cappelle-Desprez', 'Cheyenne', Synthetic' and *T. spelta* could possess some allele in common with 'Chinese Spring' for subunits controlled by group 1 chromosomes but these would not be expressed except in the 'Chinese Spring' genetic background (Table 6). The possibility that there is an allele-specific regulatory system should not be ignored, in spite of the inability of the substitution line results presently available to prove it.

Although there is difficulty in relating some of the pro-

Table 4. Variant protein subunits in Group 6 substitution lines[b]

Chromosome	'Chinese Spring' subunits 20	24	No. of new subunits	Molecular weights
'Chinese Spring' 6A	√	√		35,000; 32,000
'Cappelle-Desprez' 6A	×	?[a]	1	33,000
'Cheyenne' 6A	×	×	1	33,000
'Synthetic' 6A	×	?[a]	1	55,000
'Timstein' 6A	√	√	0	
T. spelta 6A	√	√	0	
'Chinese Spring' 6B	14			40,000
'Cappelle-Desprez' 6B	√		0	
'Cheyenne' 6B	×		3	48,000; 45,000; 30,000
'Ciano' 6B	√		0	
'Synthetic' 6B	√		0	
'Timstein' 6B	√		0	
T. spelta 6B	√		0	
'Chinese Spring' 6D	17	18		35,000 (both)
'Cappelle-Desprez' 6D	√	√	0	
'Cheyenne' 6D	√	√	0	
'Ciano' 6D	√	√	0	
'Lutescens' 6D	√	√	0	
'Synthetic' 6D	×	×	1	33,000
'Timstein' 6D	×	√	0	
T. spelta 6D	√	√	0	

√ = present; × = absent
[a] subunit could not be clearly distinguished
[b] the genetic control of subunits numbered 14, 17, 18, 20 and 24 in 'Chinese Spring' by Group 6 chromosomes is described in Brown and Flavell 1981

Table 5. Substituted chromosomes specifying subunits not found in 'Chinese Spring' but recognised in the donor variety

Chromosome	No. of subunits	Chromosome	No. of subunits
'Cappelle-Desprez' 1A	2	'Synthetic' 1D	3
'Cappelle-Desprez' 6A	1	'Synthetic' 6A	1
'Cheyenne' 1B	1	'Synthetic' 6D	1
'Cheyenne' 6A	1	'Timstein' 1A	4
'Cheyenne' 6B	3	'Timstein' 1B	1
'Ciano' 1B	3	*T. spelta* 1A	1
'Hope' 1A	4	'Capelle-Desprez' 1D	1
'Hope' 1B	1		

Table 6. Group 1 chromosomes producing typical 'Chinese Spring' subunits, not present in the donor variety

Chromosome	'Chinese Spring' subunits produced (Fig. 1)
'Cappelle-Desprez' 1B	3, 4, 5
'Cheyenne' 1D	7, 8, 9
'Synthetic' 1B	3, 12
T. spelta 1B	3, 4, 5, 12

Table 7. Chromosomes carrying alleles only expressed in the 'Chinese Spring' genetic background

Chromosome	No. of alleles
'Cheyenne' 1A	4
'Cheyenne' 1B	2

tein subunits to their counterparts in 'Chinese Spring', especially in the area of the gel containing the gliadin subunits 13-24 (Fig. 1), four varieties ('Hope', 'Lutescens', 'Synthetic' and *T. spelta*) produce some major protein subunits which are not detectable in the patterns of any of their group 1 and 6 chromosome substitution lines studied. This would suggest that (a) the genes controlling these proteins are on other chromosomes, or (b) the genes coding for them have been lost in the production of the substitution lines, or (c) the genes controlling them are not expressed in the 'Chinese Spring' background.

The range of variant subunits specified by homologous chromosomes is particularly interesting. Some 1A chromosomes carry genes for high molecular weight subunits, others do not. In varieties which do not show these high molecular weight subunits, are the genes absent or repressed? Different 1B chromosomes appear to specify subunits not only with different isoelectric points, which could be the result of base substitutions etc., but also with different molecular weights.

It is possible therefore that there is 'allelic' variation in the length of the nucleotide sequences specifying storage proteins. Alternatively the mRNAs or proteins could be processed differently in different varieties.

Acknowledgement

This work was supported in part by grant no. 1667/RI/RB from the International Atomic Energy Agency, Vienna.

We thank P.I. Payne for critically reading an earlier version of the manuscript.

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Received December 15, 1980
Communicated by R. Riley

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