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Wsp-1, a set of genes controlling water-soluble proteins in wheat and related species

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Summary

Three water-soluble wheat endosperm proteins of the wheat variety Chinese Spring have been shown, by isoelectric focusing, to be the products of genes located on the long arms of chromosomes 7A, 7B and 7D. In the absence of any evidence of function these genes have been assigned the temporary symbol, *Wsp-1*.

Considerable intervarietal variation was found among a sample of 44 hexaploid wheat varieties. Five alleles at *Wsp-A1*, three at *Wsp-B1* and two at *Wsp-D1* were identified. Intrachromosomal mapping showed that *Wsp-B1* is located distally on the long arm of chromosome 7B.

Alien homoeoloci were identified on chromosomes $7H^{ch}$ of *Hordeum chilense*, 7H of *H. vulgare*, 7E of *Agropyron elongatum*, $7S^{l}$ of *Aegilops sharonensis* and 7V of *Dasypyrum villosum*. Some other loci encoding WSPs found in wheat and some alien species are also briefly described.

1. Introduction

In crop plants the value of genetic markers in genetic research and breeding practice is well documented. In hexaploid wheat (*Triticum aestivum*, $2n = 6x = 42$) more than 150 loci encoding enzymes and storage proteins have been identified and located to chromosome arms. The value of these loci is enhanced when they are mapped intrachromosomally, because they can be employed to add precision to cytogenetic manipulation both in wheat-alien introgression of chromatin carrying novel genes and in intervarietal selection systems (Ainsworth & Gale, 1987, Gale *et al.* 1989).

Among the possible sources for biochemical markers, mature grains are ideal in that protein level and type are usually not confounded by developmental effects. For this reason they have been the source of many enzyme or storage protein markers (see McIntosh (1988) for current lists). For use as genetic markers knowledge of the functions of gene products, while scientifically desirable, is not necessary, and as observed by Garcia-Olmedo *et al.* (1982), more systems can be resolved simply by analysis of total protein extracts.

This paper describes one such analysis. Isoelectric focusing (IEF) was applied to the water soluble fraction of proteins extracted from wheat grains. A subset of these proteins, referred to below as WSP-1, has proved to be very variable and thus potentially useful as a marker system for breeding purposes. Furthermore, by use of a restriction fragment length polymorphism (RFLP) genetic map already constructed for the critical chromosomes (Chao *et al.* 1989), one member of the *Wsp-1* gene set has been intrachromosomally mapped.

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2. Materials and Methods

(i) *Genetic stocks*

(*a*) *Aneuploid lines.* All the available compensating nullisomic-tetrasomic lines and ditelosomic lines of Chinese Spring (CS) developed by Sears (1954, 1966*a b*) were employed.

(*b*) *Varieties.* 41 hexaploid wheat varieties and two accessions representing *T. spelta* and *T. macha* and a synthetic hexaploid [McFadden & Sears (1964)] were surveyed for WSP-1 variation. A full list is given in Table 1.

(*c*) *Intervarietal chromosome substitution lines.* The group 7 intervarietal chromosome substitution lines of CS (Hope) developed by E. R. Sears (University of Missouri), Favorits (Carmen) by A. Giura (Research Institute for Cereal and Industrial Crops, Rumania) and CS (Synthetic) and Hobbit'S' (*T. Macha*) by

C. N. Law and A. J. Worland (Institute of Plant Science Research, Cambridge) were used to locate those WSP bands not present in CS.

(*d*) *Wheat–alien chromosome addition and substitution stocks*. The following wheat–alien sets of chromosome addition lines and their respective amphiploids were studied to reveal *Wsp-1* alien homoeoloci. CS/*Hordeum vulgare* cv. Betzes (Islam *et al.* 1975), CS/*Secale cereale* cvs. King II (Miller, 1973) and Imperial (Driscoll & Sears, 1971), CS/*Agropyron elongatum* (Dvorak & Knott, 1974), CS/*Aegilops umbellulata* (Kimber, 1967), CS/*Ae. sharonensis* (Miller, 1983) and CS/*Dasypyrum villosum* (Sears, E.R., unpublished; Montebove *et al.* 1987). The substitutions of *H. chilense* group 7 chromosomes into CS (Miller *et al.* 1985) were also employed.

(*e*) *Intrachromosomal mapping*. Forty random F_3 families from the cross Timgalen × RL4137 were used for intrachromosomal mapping of *Wsp-B1*.

(ii) *Methods*

(*a*) *Sample extraction and electrophoresis*. The endosperm half of a mature dry grain was crushed in a microhammer mill and incubated in 70 μl of distilled water at room temperature for 1 h. Flat bed isoelectric focusing was carried out on 0·25 mm thick, 17 cm wide polyacrylamide gels containing 2% (W/V) ampholyte (Isolyte 7-9, Isolyte 8-10 and Servalyte 9-11 in the ratio 3:3:1). 0·33 M citric acid and 1 M-NaOH were used for anolyte and catholyte, respectively. Constant power of 1 W/cm gel length with a maximum voltage of 2500 V was applied. Gels were prefocused for 1000 Vh and about 35 μl of each sample was applied upon the gel about 1 cm from the anode with 5 × 8 mm paper wicks. The wicks were removed at about 2500 Vh and focusing was terminated at 13500 Vh

(*b*) *Visualization*. Gels were placed in a solution of 58 g trichloroacetic acid, 250 mg brilliant blue R (dissolved in a small amount of distilled water), 180 ml methanol, 60 ml acetic acid and 770 ml distilled water for 8 h or overnight and destained with a solution of 300 ml ethanol, 100 ml acetic acid and 600 ml distilled water for 40 min. Occasionally the staining intensity was weak, and to obtain stronger staining gels were either restrained as above or stained for 2 h, destained for 10 min, restained for another 6 h and then destained until the background became clear. Interruption of the staining procedure in this way always increased the staining intensity of WSP bands.

(*c*) *Linkage estimation*. Six single F_4 grains from each of six different plants of each of 40 F_3 families were analysed to identify their F_2 genotype. This data was then combined with the results from the other biochemical and molecular markers on chromosome 7B in the same population and analysed as described by Chao *et al.* (1989).

3. Results

(ii) *Aneuploid analysis*

Nine major WSPs were resolved with isoelectric points (pIs) in the range pH 8·3–10·2 in extracts of CS. The results of nullisomic–tetrasomic and ditelosomic analyses showed that at least three of them, in the group indicated as II in Fig. 1, are controlled by genes on the long arms of the homoeologous group 7 chromosomes. The two proteins in group I could not be located to any chromosome. Outside of groups I and II another three proteins were shown to be controlled by genes on chromosome arms 2DS, 4DS (Fig. 1) and 7DS (Fig. 2). The results and discussion below are restricted to consideration of only the group of WSPs controlled by the long arms of the homoeologous group 7 chromosomes.

The group 7 nullisomic–tetrasomic and ditelosomic results are presented in Fig. 2. It can be seen that protein 1 was absent when chromosome 7A (CSN7A-T7B and CSN7A-T7D) or the long arm of chromosome 7A (CSDT7AS) was removed, and present when the short arm of chromosome 7A (CSDT7AL) was removed. Protein 3 was absent when chromosome 7B (CSN7B-T7A and CSN7B-T7D) or the long arm of chromosome 7B was removed (CSDT7BS), and present when only the short arm of chromosome 7B was absent (DT7BL). Similarly protein 4 was absent whenever the long arm of chromosome 7D was absent (CSN7D-T7A, CSN7D-T7B and CSDT7DS). The genes controlling these three proteins have been designated by the temporary symbols *Wsp-1*, *Wsp-B1* and *Wsp-D1*, respectively, pending further evidence of their function.

Protein 2 was not completely removed by removing any single chromosome. However, its intensity was reduced when chromosome 7A was absent. It has been proved that this band is not a single protein (see below).

(ii) *Intervarietal variation*

Among the 44 hexaploid wheat varieties screened for WSP-1 phenotypes ten different WSP-1 patterns were detected. An example of each pattern is shown in Fig. 3 and the classification of the varieties is given in Table 1.

It will be noted that, in Fig. 3, protein 2 in CS was resolved into two separate products, 2^+ and 2^-, for which varietal variation was also observable. This variation has been ignored in the following description of allelic variation because we were unable to consistently achieve the required resolution.

Seven novel proteins were detected from these

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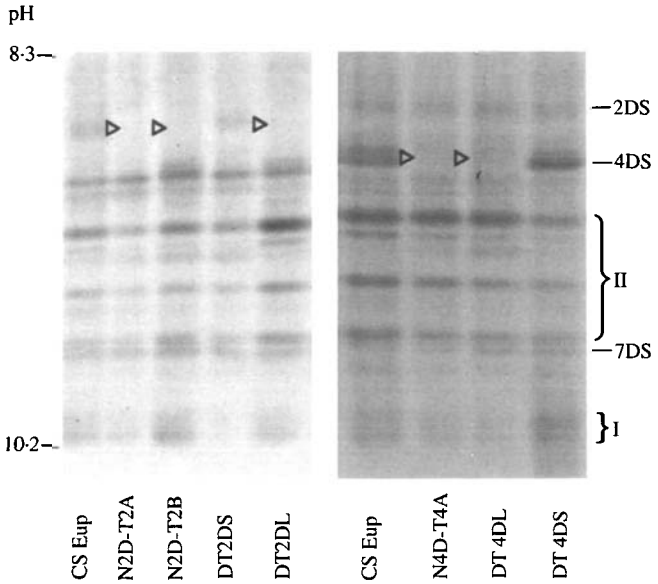

Fig. 1. WSP phenotypes of homoeologous groups 2 and 4 CS aneuploid lines, showing the relative position of WSP-1 proteins and proteins controlled by genes on chromosome arms 2DS and 4DS. Arrows indicate the absence of CS proteins.

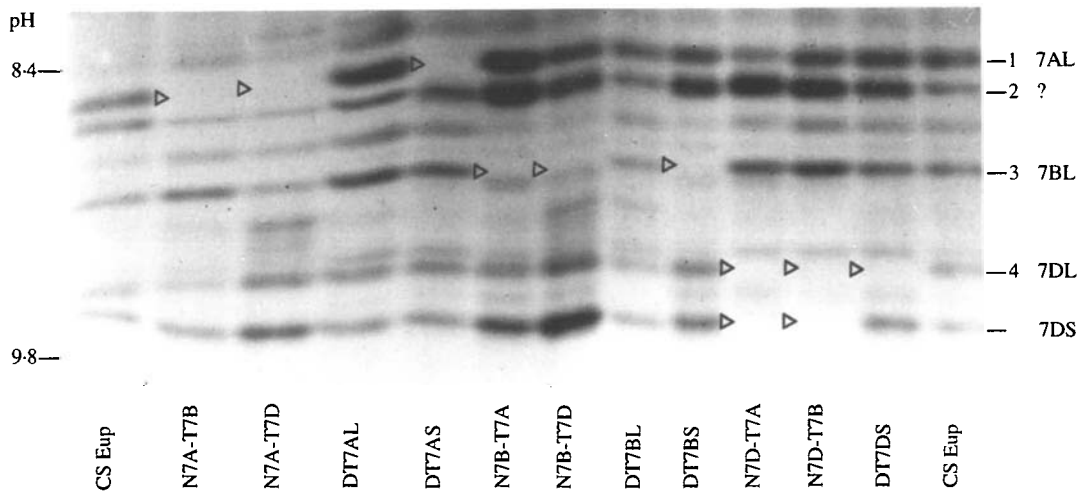

Fig. 2. WSP-1 phenotypes of homoeologous group 7 aneuploid lines of CS. Standard protein numbers and their chromosomal control are indicated at right. Arrows indicate the absence of CS proteins.

varieties (Fig. 3). Six of these focused in the same pH range as the CS *Wsp-1* products and one, designated 0a, had a lower pI. The gene controlling this latter protein was shown to be located on chromosome 7B in the Favorits (Carmen) substitution series (Fig. 4B). This protein was also expressed in *T. macha* and in the Hobbit'S' (*T. macha* 5BS.7BS) substitution but not in Hobbit'S' (*T. macha* 5BL.7BL) substitution (not shown), and thus is most likely to be controlled by a gene on the short arm of chromosome 7B.

Chromosomal location of the genes encoding four of the remaining six proteins was established by analysis of intervarietal chromosome substitution lines: thus proteins 1a and 3a were shown to be controlled by genes on chromosome 7A (Fig. 4A, B); protein 2b on 7B (Fig. 4A) and protein 4a on 7D (Fig. 4C).

These analyses allowed five alleles to be described at the *Wsp-A1* locus by reference to proteins 1, 1a and 3a: allele *a* encodes 1, allele *b* encodes 1a, allele *c* encodes 1a and 3a, allele *d* encodes 3a and *e* is a null allele which encodes none of these; three alleles at *Wsp-B1* locus by reference to proteins 3 and 2b; allele *a* encodes 3, allele *b* encodes 2b and a null allele *c*; and two alleles at *Wsp-D1* locus by reference to proteins 4 and 4a: allele *a* encodes 4 and allele *b* encodes 4a (Table 1).

Favorits (Carmen 7A) (Fig. 4B) expressed an unexpected WSP-1 pattern, for it did not express protein 1a, known to be controlled by chromosome 7A, which was present in the donor parent Carmen. This line is likely to be nullisomic for chromosome 7A. This will be discussed below. Two other duplicate Favorits (Carmen 7A) substitutions (not shown) were observed to have the expected phenotype.

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Table 1. *WSP-1 phenotypes and genotypes in 44 wheat accessions*

		Protein										Genotype		
Type	Variety	1	1a	1b	2	2a	2b	3	3a	4	4a	*A1*	*B1*	*D1*
A	CS, Cheyenne, Highbury, Atlas 66, Bezostaya 1, Cappele-Desprez, Bersee, Desprez 80, Pavon, P168, Lutescens, Chris, Purple Pericarp, Karcag, Champlein, Vilmorin 27, Sportsman, Courtot	+	−	−	+	−	−	+	−	+	−	*a*	*a*	*a*
B	Hope, Spica	−	+	−	−	−	+	−	+	+	−	*c*	*b*	*a*
C	Carmen, RL4137, H93–70, Poros, Holdfast, Koga II, Rendevous, Huntsman, Luna, Grana, Hobbit'S' *T. spelta* (IPSR1220017)	−	+	−	+	−	−	+	−	+	−	*b*	*a*	*a*
D	Condor, *T. macha* (IPSR124001)	−	−	−	+	−	−	−	−	+	−	*e*	*c*	*a*
E	Favorits, Fiorello, Ciano 67, Moulin, Timgalen	+	−	−	+	−	−	−	−	+	−	*a*	*c*	*a*
F	C591	+	−	+	+	+	−	+	−	+	−	*a*	*a*	*a*
G	Sicco	−	−	−	−	−	−	−	+	+	−	*d*	*c*	*a*
H	Synthetic (IPSR1190903)	+	−	−	+	+	−	+	−	+	+	*a*	*a*	*b*
I	Thatcher	+	−	−	+	+	−	+	−	+	−	*a*	*a*	*a*
J	Perziven	−	+	−	+	+	−	+	−	+	−	*b*	*b*	*a*
Controlled by chromosome 7		A	A	?	?	?	B	B	A	D	D			

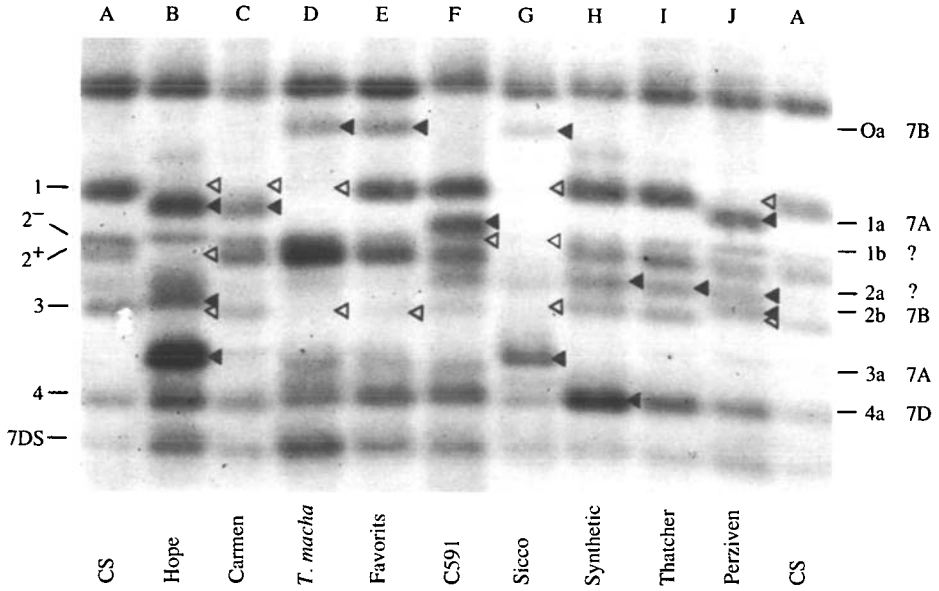

Fig. 3. Ten WSP-1 phenotypes found in a sample of 44 hexaploid genotypes. Novel (▶) or absent (▷) CS proteins are arrowed. Novel proteins and their chromosomal control are marked at right.

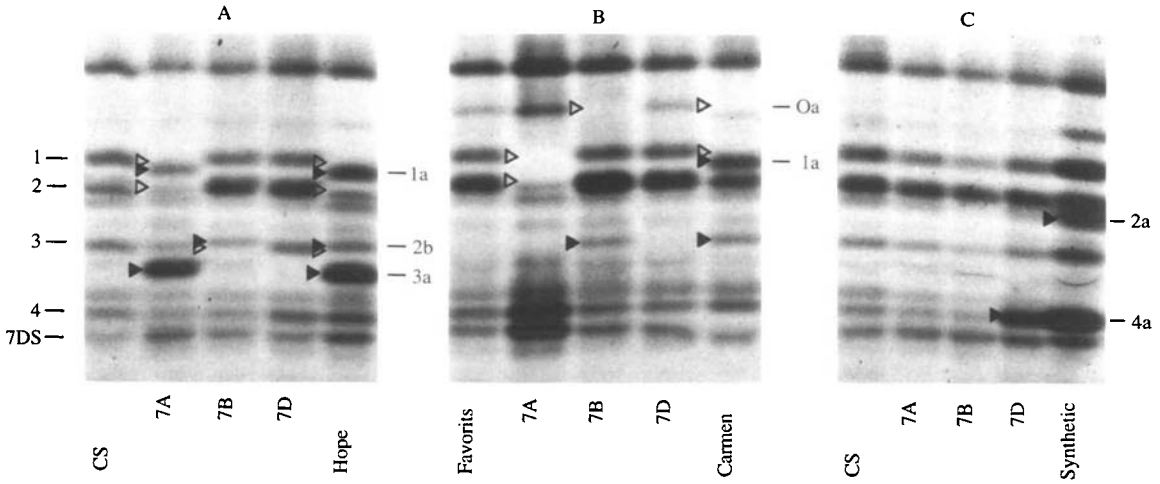

Fig. 4. WSP-1 phenotypes of the homoeologous group 7 intervarietal chromosome substitution lines of A, CS (Hope); B, Favorits (Carmen) and C, CS (Synthetic). Differences between the donor and recipient parents are marked as in Fig. 3.

(iii) *Intrachromosomal location of* Wsp-B1

The analysis of F_4 grains of 40 F_3 families derived from the cross Timgalen (*Wsp-B1c*) × RL4137 (*Wsp-B1a*) allowed segregation at *Wsp-B1* to be tested against segregation at several other RFLP and enzyme loci located on the relatively densely mapped chromosome arm 7BL (Chao *et al.* 1989).

The analysis showed linkage of *Wsp-B1* only with endopeptidase, *Ep-B1*, and the RFLP locus, *Xpsr121*, both of which are located in the distal region of 7BL. The linkage values obtained were: *Wsp-B1-Xpsr121*, 16·6 ± 6·9, and *Wsp-B1-Ep-B1*, 31·6 ± 12·1, establishing the gene order from the centromere as *Ep-B1*, *Xpsr121* and *Wsp-B1* (Fig. 5).

(iv) *Chromosomal location of WSP genes in species related to wheat*

(*a*) *Hordeum chilense*. The WSP patterns of the three group 7 substitution lines of CS/*H. chilense*, $7H^{ch}$ (7A), $7H^{ch}$ (7B) and $7H^{ch}$ (7D), were analysed and, as expected, each lacked the respective wheat WSP-1 protein while expressing a single protein derived from *H. chilense* (Fig. 6). The gene controlling this protein was designated *Wsp-H*^{ch}*1*.

(*b*) *Hordeum vulgare*. Barley var. Betzes showed six major WSP proteins. One lay within the WSP-1 region and was expressed by the 7H (barley chromosome 1) addition of CS/Betzes (Fig. 7A). The gene controlling this protein was designated *Wsp-H1*.

Addition 2H (barley chromosome 2) also expressed one barley protein, which was located on the acidic side of the four wheat WSP-1 proteins.

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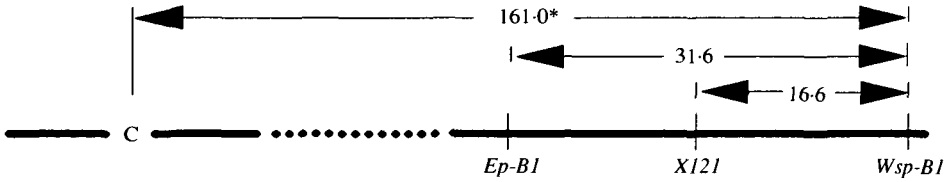

Fig. 5. The location of *Wsp-B1* on the long arm of chromosome 7B. *Calculated from Chao *et al.* (1989).

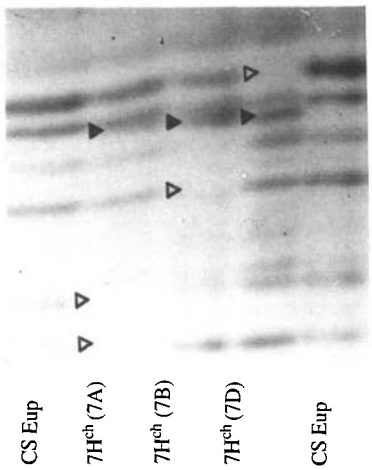

Fig. 6. WSP-1 phenotypes of the homoeologous group 7 substitution lines of CS/*Hordeum chilense*. Novel and absent CS proteins are marked as in Fig. 3.

All six addition lines lack the *Wsp-A1a* protein controlled by chromosome 7A in CS. The same phenomenon was also observed in some of the CS/*Secale cereale* additions (see below) and the CS/*Ae. sharonensis* addition lines (not shown), and is discussed below.

(*c*) *Aegilops sharonensis*. The amphiploid and three addition lines, 2S^{l}, 4S^{l} and 7S^{l}, of CS/*Ae. sharonensis* were examined. Addition 7S^{l} expressed the two alien proteins expressed by the amphiploid. One of these focused between proteins 3 and 4 and the other had a lower pI than the *Wsp-A1a* product (Fig. 7B). As with the CS/*Ag. elongatum* additions, it is not obvious whether both these two proteins are controlled by a single locus, but the protein with the higher pI is likely to be a product of *Wsp-S^{l}1*.

(*d*) *Dasypyrum villosum*. Of the five available CS/*D. villosum* addition lines (1V, 2V, 4V, 6V and 7V), all, except 7V, gave the same WSP-1 pattern as that of CS. Addition 7V expressed one novel WSP protein (Fig. 7C), encoded by the homoeolocus *Wsp-V1*.

(*e*) *Secale cereale*. The CS/*S. cereale* var. King II and Imperial amphiploids showed very similar WSP-1 patterns. The CS/King II amphiploid and 4R addition, which is a 4RL.7RS translocation relative to wheat (Koller & Zeller, 1976), expressed a novel protein with lower pI than the *Wsp-1* products (Fig. 7D). It is probable therefore that the gene encoding this protein is located on 7RS.

(*f*) *Aegilops umbellulata*. Novel WSPs were observed in the addition lines carrying 1U, 2U, 5U and 6U, but not 7U. The amphiploid expressed the whole set of alien WSPs (Fig. 8A). Based on the pIs of these proteins, it is likely that the locus controlling the protein expressed in the addition 1U is a homoeolocus to that controlling the protein in addition I of *Ag. elongatum* and the locus controlling the protein in 2U is a homoeolocus to that controlling the protein in addition 2H of CS/*Hordeum vulgare*. WSPs controlled by group 1 and 2 chromosomes in hexaploid wheat were also observed in these regions. However no WSPs have yet been observed to be controlled by either the homoeologous group 5 or 6 chromosomes in hexaploid wheat.

(*g*) *Agropyron elongatum*. Analysis of the seven CS/*Ag. elongatum* addition lines revealed that two additions, II and IV, expressed the three proteins which were not present in CS. One of these three proteins focuses in the WSP-1 pH range while the other two have lower pIs (Fig. 8B). Though both lines contain the long arm of *Ag. elongatum* 7E (Hart & Tuleen, 1983), it is not yet clear whether these three proteins are encoded by a single locus on this chromosome arm. But one, the one with the highest pI, is likely to be encoded by a WSP-1 homoeolocus, designated *Wsp-E1*.

Addition I, which carries a chromosome homoeologous to the group 1 chromosomes of wheat (Dvorak, 1980; Hart & Tuleen, 1983), also expressed an *Ag. elongatum* WSP protein at the acidic end of the gel (Fig. 8B). Wheat WSP proteins controlled by the homoeologous group 1 chromosomes were also observed in this region.

4. Discussion

Apart from the fact that WSP-1 are water soluble protein products found in mature wheat grains, we have no further information regarding their function. However, they do appear to be an, as yet, unreported group of proteins. The protein name and gene symbol, *Wsp-1*, of these new markers can only be regarded as temporary, pending further information as to their identity.

Waines (1973) noted a locus in chromosome 7D controlling one protein band in CS. Later this locus

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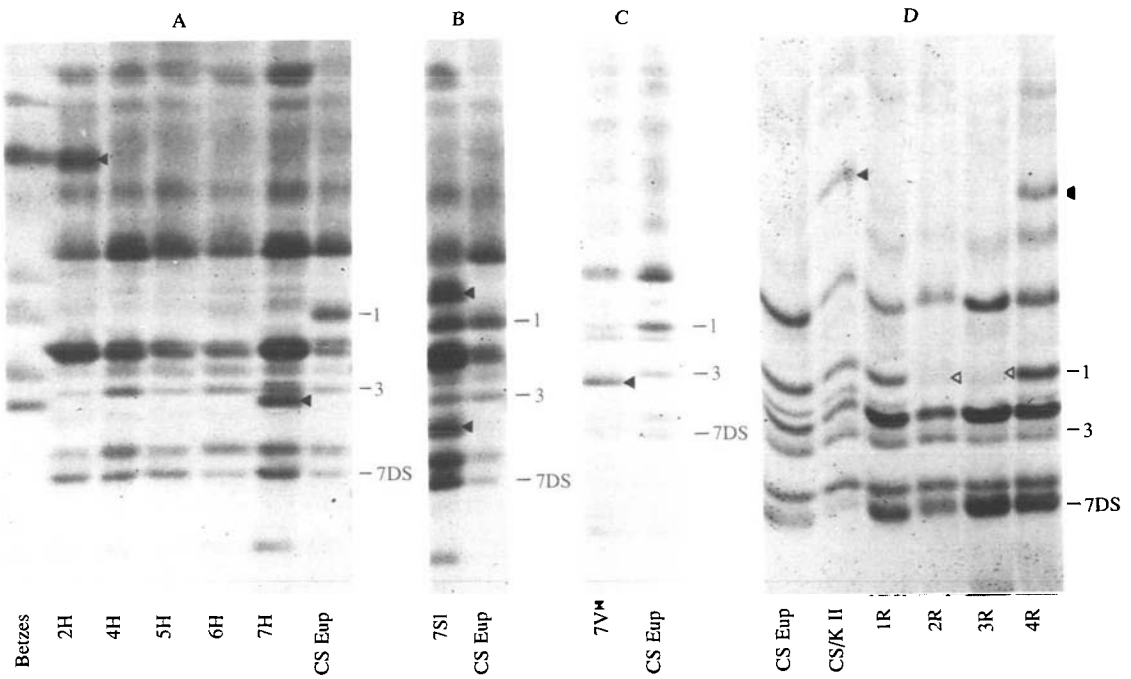

Fig. 7. WSP-1 phenotypes of A, CS/*H. vulgare* var. Betzes; B, CS/*Ae. sharonensis*; C, CS/*S. cereale* var. King II. Novel and absent CS proteins are marked as in Fig. 3.

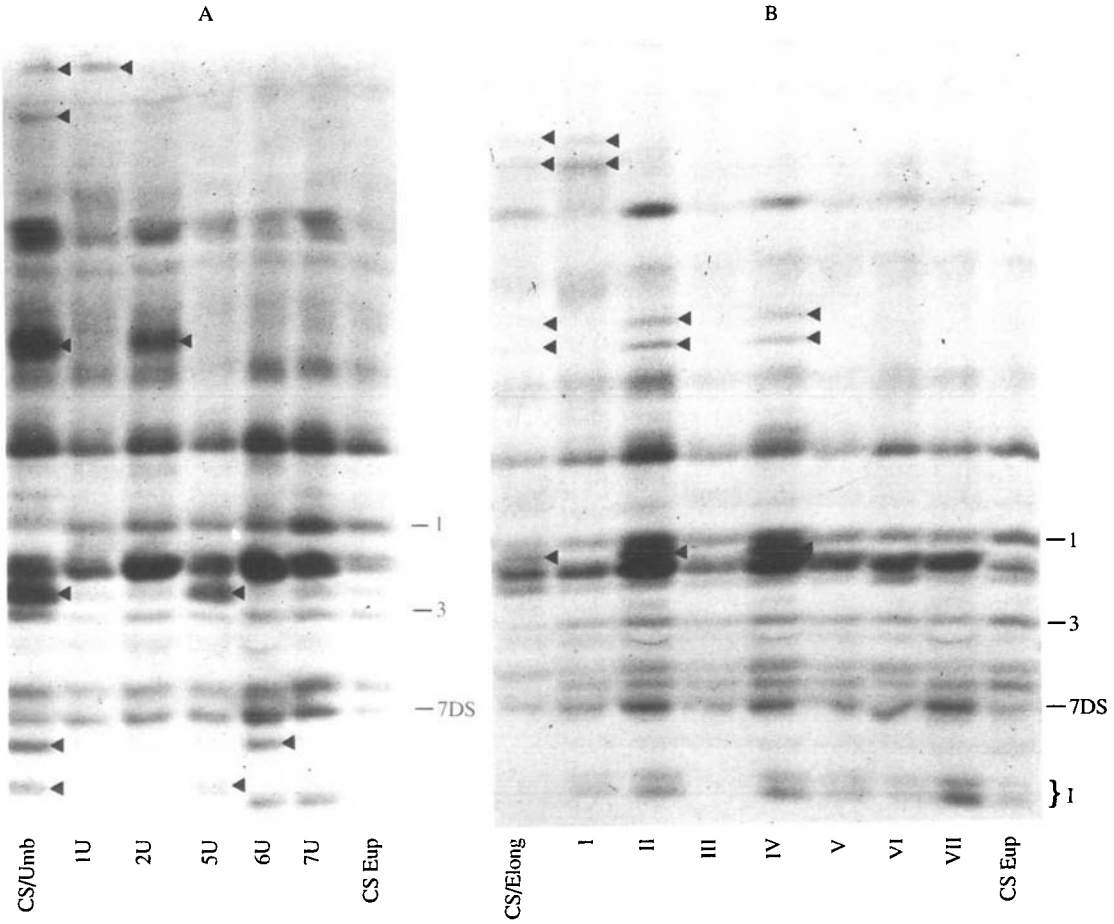

Fig. 8. WSP-1 phenotypes of A, CS/*Ae. umbellulata* and B, CS/*Ag. elongatum*. Novel CS proteins are marked as in Fig. 3.

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was located by Aragoncillo *et al.* (1975) to the short arm of this chromosome along with another locus on chromosome arm 7BS. Salcedo *et al.* (1980) identified loci controlling the production of low molecular weight gliadins on chromosomes 7A and 7D. These proteins are, however, insoluble in water (Salcedo *et al.* 1979) and thus not identical to WSP-1.

In a study of aqueous-alcohol soluble prolamin storage proteins of *H. chilense*, Payne *et al.* (1987) identified proteins by two-dimensional electrophoresis which were controlled by genes located on chromosome $7H^{ch}$. It is not clear whether these gene products were water soluble, however it is clearly possible that the proteins identified here as WSP-$H^{ch}1$ are the same as one or more of the proteins described in the previous study.

The *Wsp-1* set of loci displays more allelic variation than many of the other protein loci already catalogued in wheat, and should find application in both intra-specific and inter-specific chromosome manipulations. When used for varietal verification, WSPs located outside of the pI range of WSP-1 can be analysed along with WSP-1 on the same IEF gels and thus allow many more phenotypes be detected. In the survey conducted here some 20 phenotypes were observed among 44 hexaploid wheat varieties (not shown).

The WSP protein 1, controlled by chromosome 7A, was missing in one of the Favorits (Carmen 7A) substitution lines. This line also lacks *Per-A4* (Liu & Gale, unpublished), a locus on the short arm of chromosome 7A. In both cases, the intensity of those proteins controlled by chromosome 7D is enhanced, so it is likely that this line is nullisomic for 7A and tetrasomic for 7D.

The same WSP-1 protein 1 was also absent in a number of CS based wheat-alien addition lines. These included all the six available CS/*H. vulgare* var. Betzes addition lines, the amphiploid and $4S^{l}$ addition of CS/*Ae. sharonensis* and the 2R, 3R and 6R additions of CS/*S. cereale* var. King II. The source of this variation is not yet known, however it is likely to be due to a null allele at *Wsp-A1* or, more likely, a deletion of part of the chromosome including the *Wsp-A1* locus. All of these stocks have been assayed for *Ep-A1* (Koebner *et al.* 1988) and the latter has always been found to be present. Thus a small terminal deletion is consistent with the intra-chromosomal map location of *Wsp-1*. The fact that the variants have been found in lines produced over three decades, in locations as far apart as UK and Australia, suggest that the standard CS stock may contain a mixture of biotypes. It seems unlikely that the same mutation/deletion event could have occurred independently so many times. It should be noted that this difference does not concur with that found at *Gpi-D1* by Chojecki & Gale (1982) used to describe a further biotype among CS stocks. A complete analysis of CS-based genetic stocks is under way to more precisely identify the source of the variation.

The simple and rapid intrachromosomal location of *Wsp-B1* to the distal region of chromosome arm 7BL is the first demonstration of the power and value of the RFLP mapping project at the Institute of Plant Science Research, Cambridge. It is probable that many more protein marker genes will be mapped in this way to enhance the already rapidly growing genetic map of hexaploid wheat.

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