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Structural and Genetical Studies on the High-molecular-weight Subunits of Wheat Glutenin

Part 1: Allelic Variation in Subunits Amongst Varieties of Wheat (*Triticum aestivum*)

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Summary. The high-molecular-weight (HMW) subunits of glutenin from about 185 varieties were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). About 20 different, major subunits were distinguished by this technique although each variety contained, with only a few exceptions, between 3 and 5 subunits. Further inter-varietal substitution lines to those already described (Payne et al. 1980) were analysed and the results indicate that all the HMW subunits are controlled by the homoeologous group 1 chromosomes. All hexaploid varieties studied except 'NapHal' contained two major subunits controlled by chromosome 1D. Their genes were shown to be tightly linked genetically for only four different types of banding patterns were observed. The nominal molecular weights determined after fractionation in 10% polyacrylamide gels were between 110,000 and 115,000 for the larger of the two subunits and between 82,000 and 84,000 for the smaller. One quarter of the varieties contained only one major HMW subunit controlled by chromosome 1B whereas the rest had two. The chromosome 1B subunits were the most varied and nine different banding patterns were detected. All the subunits had mobilities which were intermediate between those of the two chromosome 1D-controlled subunits. Only two types of HMW subunit controlled by chromosome 1A were detected in all the varieties examined; a single variety never contained both of these subunits and 40% of varieties contained neither. The chromosome 1A-controlled subunits had slightly slower mobilities in 10% gels than the largest HMW subunit controlled by chromosome 1D. About 100 single grains were analysed from each of five different crosses of the type (F_1 of variety A × variety B) × variety C. The results indicate that the genes on chromosome 1B which control the synthesis of subunits 6, 7, 13, 14 and 17 are allelic, as are the genes of the chromosome 1A-controlled subunits, 1 and 2*.

Key words: Glutenin – *Triticum* – Genetics – SDS – Polyacrylamide-gel-electrophoresis

1 Introduction

The high-molecular-weight (HMW) subunits of glutenin constitute only a small proportion of the total glutenin complex of the wheat endosperm (Payne and Corfield 1979). In papers of this series, they have been singled out for detailed biochemical and genetical analysis because of their causal relationship with glutenin elasticity and bread-making quality (Payne and Corfield 1979; Huebner and Wall 1976; Arakawa et al. 1977; Burnouf and Bouriquet 1980; Payne et al. 1981). This introductory paper describes the genetic variation in HMW subunits in a large selection of hexaploid wheat varieties of *Triticum aestivum* L, as determined by one-dimensional polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE).

2 Materials and Methods

Plant Material

Most of the varieties used in this study were taken from the collection of wheats maintained at the Plant Breeding Institute, Cambridge, UK. A range of Australian and New Zealand varieties were kindly provided by Drs. J.R. Syme, C.R. Wrigley and R. Slack. The inter-varietal substitution line, 'Chinese Spring' ('Timstein' 1B) was developed by E.R. Sears and has been maintained at this Institute for several generations. The lines 'Capelle-Desprez' ('Vilmorin 27' 1D) and 'Chinese Spring' ('Ciano 67' 1D) were developed at this Institute.

SDS-PAGE

The proteins of flour samples were extracted and fractionated in 10% polyacrylamide gels by the method of Laemmli (1970) as modified by Payne et al. (1980). Gels were also made with polyacrylamide at a concentration of 5% and methylenebisacrylamide at 0.26%. Electrophoresis was overnight at 8 mA constant current and until the tracking dye, pyronin Y had reached the bottom of the gel. In some experiments where quantification of stained bands was required, the gel was cut into longitudinal strips approximately 1 cm wide so that each strip contained the fractionated products

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of one sample. The strip was scanned at 560 nm using a modified Hilger-Gilford spectrophotometer. Gels with proteins precipitated by 10% trichloracetic acid without stain were scanned at 280 nm.

3 Results

3.1 Variation in HMW Subunits Amongst Varieties

A typical example of one of the many SDS-PAGE separations of varieties is shown in Fig. 1. In all, 14 major HMW subunits of different mobility are distinguishable in this gel out of the 20 found in the complete survey of 185 varieties named in Table 1 and each has been assigned a different number. The numbering system is consistent with that described in our previous communications (Payne et al. 1980; Payne et al. 1981) except for 2 bands (see legend of Fig. 1). The nominal molecular weights of the HMW subunits as determined in 10% gels range from 82,000 to 125,000 although equilibrium-sedimentation studies (Hamauzu et al. 1975) suggest these values are overestimates. With a few exceptions, each contains only between three and five major HMW subunits of glutenin (cf. Fig. 1). How-

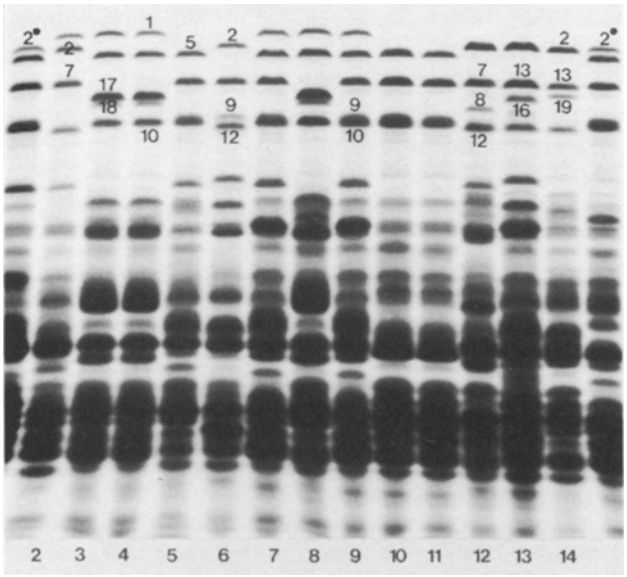

Fig. 1. SDS-PAGE using a 10% gel of flour samples from fifteen varieties of bread-wheat. Subunits present are: slot one, 2*, 5, 7, 9 and 10, slot two, 1, 2, 7 and 12; slots three and four, 1, 5, 17, 18, and 10; slot five, 5, 7, 9 and 10; slot six, 2, 7, 9 and 12; slot seven, 1, 5, 7, 9 and 10; slot eight, 1, 5, 17, 18 and 10; slots nine to eleven, 1 (slot nine only), 5, 7, 9 and 10; slot twelve, 2, 7, 8 and 12; slot thirteen, 2, 13, 16, and 12; slot fourteen, 2, 13, 19 and 12; slot fifteen, 2*, 5, 7, 9 and 10. A slight change was made in the numbering system of subunit bands described previously (Payne et al. 1980) which was based on only 7 varieties. When many more varieties were analysed, it became clear that the allocation of three 1 Dy subunits (10, 11 and 12) was too complicated so subunit 11 was withdrawn

Table 1. Varieties analysed by SDS-PAGE

Variety	Country of origin
Adam	Austria
Alcedo	Germany
Aobakomugi	Japan
Aquila	UK
Arawa	New Zealand
Arjun	India
Armada	UK
Arminda	The Netherlands
Atlas 66	USA
Atou	France
Avalon	UK
Axel	France
Azteca 67	Mexico
Bajio 67	Mexico
Banks	Australia
Bersée	France
Bezostaya I	USSR
Bilbo	UK
Blue Boy	USA
Bonanza	USA
Bounty	UK
Bouquet	France
Brigand	UK
Broom	UK
Budifen	Chile
Cama	Belgium
Cappelle-Desprez	France
Chalk	UK
Champlein	France
Chenab 70	Pakistan
Cheyenne	USA
Chinese Spring	China
Ciano 67	Mexico
Cledor	France
Clement	The Netherlands
Cook	Australia
Courtot	France
Condor	Australia
Copain	France
Disponent	Germany
Dragon	France
Durin	UK
Eloi	France
Era	USA
Eucarp	Germany
Federation	Australia
Flanders	France
Flicker	New Zealand
Flinor	France
Florence Aurore	France
Fournil	France
Frondoso	France
Frontier	UK
Gabo	Australia
Gamin	France

Table 1. (Continued)

Variety	Country of origin
GK-Protein	Hungary
Glenlea	Canada
Goya	France
Granta	UK
Hackman Komugi	Japan
Hauser	USA
Hedgehog	UK
Heima-Desprez	France
Heines Kolben	Germany
Highbury	UK
Hilgendorf	New Zealand
Hira	India
Hobbit	UK
Holdfast	UK
Hope	USA
Hunters	New Zealand
Hustler	UK
Hybrid 46	UK
Insignia	Australia
Jana	Poland
Janak	India
Joss Cambier	France
Kador	France
Karamu I	New Zealand
Kavkaz	USSR
Kharkov	USSR
Kinsman	UK
Kite	Australia
Kleiber	Germany
Koga II	Germany
Kolibri	Germany
Kopara	New Zealand
Lancota	USA
Little Joss	UK
Lovrin 24	Rumania
Magdelena	France
Manella	The Netherlands
Manitou	Canada
Mara	Italy
Mardler	UK
Maris Beacon	UK
Maris Butler	UK
Maris Dove	UK
Maris Freeman	UK
Maris Fundin	UK
Maris Huntsman	UK
Maris Nimrod	UK
Maris Ranger	UK
Maris Widgeon	UK
Marksman	UK
Markus	Germany
Martonvasari-4	Hungary
Marquis	Canada
Mayo 64	Mexico

Table 1. (Continued)

Variety	Country of origin
Mega	UK
Mexifen	Chile
Mexique 50	Mexico
Millewa	Australia
Mironovskaya 808	USSR
Monopol	Germany
Moti	India
Narbada 4	India
Narbada 112	India
Nautica	The Netherlands
Nong Da	China
Norin	Japan
Norman	UK
Odessa 4	USSR
Olympic	Australia
Opel	Germany
Oroua	New Zealand
Partizanka	Yugoslavia
Pataka	New Zealand
Payne	USA
Peko	France
Pembina	Canada
Peragis	Germany
Ploughman	UK
Ponca	USA
Poncheau	France
Poros	Germany
Pratap	India
Prelude	Canada
Priboy 1	USSR
Prince	Belgium
Purcari Hostianum	France
Pusa Lerma	India
Ralle	France
Rannie 12	France
Rannyaya	Hungary
Red Fife	Canada
Redman	Canada
Red River 68	USA
Reward	Canada
Rex	France
Rongotea	New Zealand
Sage	USA
Sappo	Sweden
Saratovskaya-29	USSR
Sava	Yugoslavia
Scout 66	USA
Selkirk	Canada
Sentry	UK
Shabarti Sonora	India
Shortim	Australia
Sicco	The Netherlands
Sirius	Germany
Slavia	Czechoslovakia
Solar	USA

Table 1. (Continued)

Variety	Country of origin
Spartacus	UK
Spica	Australia
Sportsman	UK
Stormguard	France
Stuart	UK
Svenno	Sweden
Takahe	New Zealand
Thatcher	Canada
Timmo	Sweden
Timstein	USA
Val	Belgium
Valmy	France
Viking	Denmark
Villein	UK
Vilmorin 27	France
Vuka	Germany
Waggoner	UK
West Desprez	France
Wizard	UK
Yeoman	UK

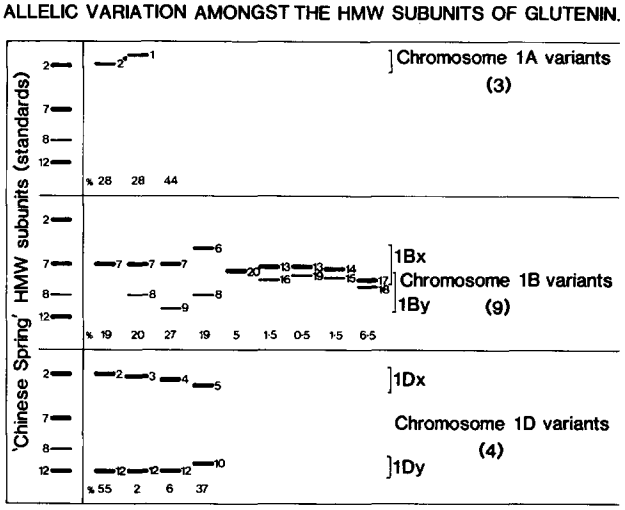

Fig. 2. Variation in the banding patterns of HMW subunits of glutenin found in 185 varieties. The subunits have been split up into three groups according to whether they are coded for by chromosome 1A, 1B or 1D. On the left-hand side of each of the groups are the HMW subunits of 'Chinese Spring'. This, standard, subunit composition enables the relative positions of variants from each of the three groups to be superimposed. A variety will have any combination of one variant type from each of the three groups. The frequency of each of the variants in varieties is expressed as a percentage of the total for each group 1 chromosome

ever, the variety 'NapHal', in agreement with other work (Bietz et al. 1975; Lawrence and Shepherd 1980), is unique amongst the wheats tested in possessing only two subunits. Flour samples of a few variety stocks contained more than five different HMW subunits. When single grains were analysed by SDS-PAGE at least two different banding patterns emerged for each stock, each pattern consisting of between three and five bands. These flour samples must therefore either consist of two varieties accidentally mixed or be a single variety which is heterogeneous and consequently they were not included in the results of this survey.

The variation in the composition of HMW subunits as detected with 10% polyacrylamide gels has been split into three groups in Fig. 2 according to whether the genes with control the synthesis of the subunits are located on chromosome 1A, chromosome 1B or chromosome 1D (Payne et al. 1980). All the subunits were clearly distinguished in this system except for 2 and 2*. Flours thought to contain either or both of these subunits were also fractionated in 5% polyacrylamide gels. In this system the two subunits were fully resolved, subunit 2* having the greater electrophoretic mobility (Payne and Holt unpublished).

Only two HMW subunits are controlled by chromosome 1A (1 and 2*) and in 10% gels, they have the slowest mobilities of all the subunits. About half the varieties analysed do not contain a chromosome 1A subunit, a quarter contain subunit 1 and a quarter subunit 2* but none contains both subunits (Fig. 2). In contrast, six HMW subunits are controlled by chromosome 1D (Fig. 2): five of them were assigned in a previous study (Payne et al. 1980) and the other, subunit 4, by the analysis of the intervarietal chromosome substitution line 'Capelle-Desprez' ('Vilmorin 27' 1D). In this line, subunit 4 (from 'Vilmorin 27') has replaced the 1D-controlled subunit 2 of 'Capelle-Desprez' (results not shown). The six subunits fall into two distinct groups with respect to their electrophoretic mobility, 1Dx (subunits 2-5) and 1Dy (subunits 10, 12). All varieties contain one subunit each of these two groups except for 'NapHal', which contains neither. However, only four of the eight possible combinations of 1D HMW subunits were detected in the 185 varieties studied. For instance, subunit 5 was always associated with subunit 10 and never with 12 (Figs. 1, 2 and Lawrence and Shepherd 1980).

Twelve different HMW subunits, more than half the total number, were assigned to genes on chromosome 1B: four on the basis of a previous genetical study (Payne et al. 1980); subunits 17 and 18 after the analysis of the intervarietal chromosome substitution lines 'Chinese Spring' ('Timstein' 1B) and 'Chinese Spring' ('Ciano 67' 1B) (results not shown); and six from indirect evidence. Varieties containing the latter subunits had either one or no subunits designated for control by chromosome 1B, they had their full complement of chromosome 1D subunits and

crossed to a third variety which had a different chromosome 1B-controlled subunit from either of the other two parents. The parental types and the possible recombinants are equivalent to the ('Cheyenne' × 'Holdfast') × 'Spica' progeny in Fig. 4 except all progeny will additionally have 1 dose of the chromosome 1B subunit from the third parent.

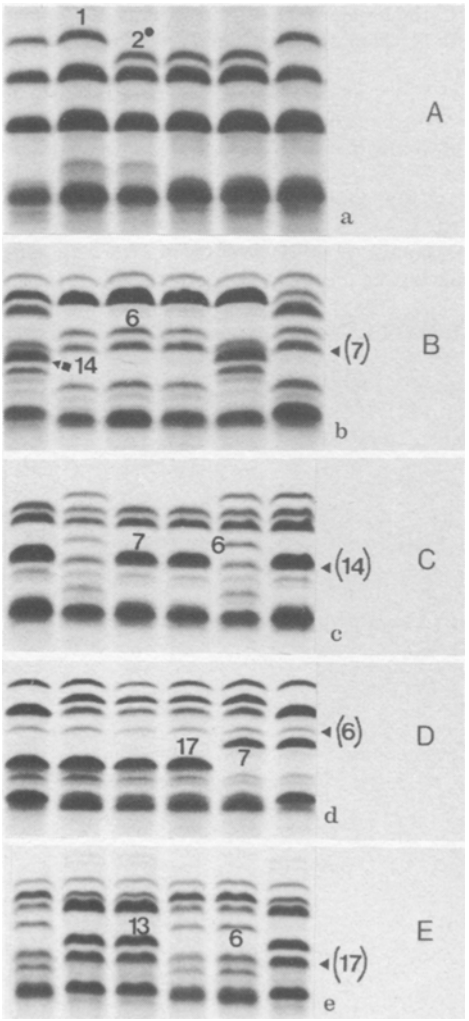

Fig. 5. Single grain analysis of progeny from a ('Cheyenne' × 'Holdfast') × 'Spica', b ('Sappo' × 'Flanders') × 'Freeman', c ('Sicco' × 'Flanders') × 'Sappo', d ('Freeman' × 'Sonora 64') × 'Flanders' and e ('Lancota' × 'Flanders') × 'Sonora 64'. The subunit numbered in brackets is the control subunit belonging to the third parent.

b) Experimental results

About 100 half grains were analysed by SDS-PAGE from each of 5 crosses. The various banding patterns obtained from each cross are shown in Fig. 5 and the results of the analysis are summarised in Table 2. For the ('Cheyenne' × 'Holdfast') × 'Spica' cross, 76 grains contained subunit 1 but not subunit 2* and 82 grains contained 2*. There were no grains which contained both subunits and no grains which contained neither. Therefore, no recombinants were detected in the sample analysed and as expected, the parental types occurred with a frequency ratio close to 1:1 (X^2 {1} = 0.23, PO.9-0.8). The structural genes for these two subunits must either be allelic on chromosome 1A or less than 0.190 cM ($P > 0.05$) apart. Similarly, no recombinants were detected in progenies of the four crosses set up to determine the proximity of the HMW glutenin genes on chromosome 1B (Fig. 5b-e, Table 2), indicating that the genes for 1Bx subunits (6, 7, 13, 14 and 17) are also either allelic or very close together. One grain of the ('Lancota' × 'Flanders') × 'Sonora 64' cross lacked both subunits 13 and 6 but it was also the only grain to lack a principal ω-gliadin known to be controlled by genes on the short arm of chromosome 1B (results not shown). Thus, rather than being a recombinant, the grain must be monosomic for the 1B chromosome of 'Sonora 64' having arisen, most probably, by the non-disjunction of a chromosome pair during meiosis in the F_1 parent.

4 Discussion

The variation in the patterns of HMW glutenin subunits amongst different varieties of hexaploid wheat described here is similar to that published recently by Lawrence and Shepherd (1980) though there are some discrepancies. For instance, both research groups obtained the same number of chromosome 1B subunit patterns although a few were different. In addition, we detected four different types of

Table 2. Segregation frequencies of HMW subunits

Cross	Subunits tested		Chromosome control by:	Single grain analysis				
	A	B		No. analysed	A	B	A + B	–
a) (Cheyenne × Holdfast) × Spica	1	2*	1A	158	76	82	0	0
b) (Sappo × Flanders) × Freeman	14	6	1B	104	54	50	0	0
c) (Sicco × Flanders) × Sappo	6	7	1B	99	52	47	0	0
d) (Freeman × Sonora 64) × Flanders	7	17	1B	104	48	56	0	0
e) (Lancota × Flanders) × Sonora 64	13	6	1B	100	48	51	0	1

banding pattern for the chromosome 1D-controlled HMW subunits as opposed to their two. The detection of different banding patterns by each research group can probably be accounted for by the different wheat varieties used in the two studies although the precise electrophoretic conditions, particularly the concentrations of acrylamide and methylenebisacrylamide used to make up the gel, can significantly affect the patterns of the HMW subunits (Payne and Holt unpublished).

In spite of the marked variation in subunit patterns it is probable that its extent is underestimated by one-dimensional SDS-PAGE. On certain occasions when proteins are fractionated particularly well, slight differences in mobility between bands of HMW subunits from different varieties are evident. This is particularly so for subunit 7. Of the variation that is detected, it is clear that most of it is due to subunits whose genes are controlled by chromosome 1B. This may give support to the hypothesis that the origin of the B genome in wheat was polyphyletic (Kimber and Athwal 1972).

The segregation studies described in this and the previous study (Payne et al. 1980) suggest that the variation in HMW subunits amongst varieties is due to allelic genes which occur at five loci: two on the long arm of chromosome 1D (Bietz et al. 1975) controlling the 1Dx and 1Dy subunits; two on the long arm of chromosome 1B (Bietz et al. 1975) controlling the 1Bx and 1By subunits, but only one on the long arm of chromosome 1A (Lawrence and Shepherd 1981). The apparent dissimilarity with respect to the number of HMW subunits controlled by chromosome 1A and its homoeologues was studied further by an analysis of various diploid species having the genomic constitution AA (results not shown). Most of them (*Triticum aegilopoides, T. sinskajae, T. thaoudar* and *T. monococcum*) actually contained two HMW subunits; one of similar mobility to either subunit 1 or subunit 2* and the other, weaker, subunit of similar mobility to the 1By subunits. However, one of the diploids tested, *T. urartu*, only contained one subunit identical in electrophoretic mobility to subunit 2*. From this work, subunits 1 and 2* of breadwheat can be regarded as 1Ax types, their structural genes being homoeoallelic to the genes for the 1Bx and 1Dx subunits. Likewise, the additional subunits found in some of the A genome diploids are probably 1Ay types produced by a gene (or genes) homoeoallelic to genes responsible for the 1By and 1Dy subunits.

The finding that some varieties of breadwheat do not synthesize 1By and 1A subunits whereas others do, can be explained if the structural genes for these subunits are missing. Alternatively the genes are either permanently repressed or their base sequences have been changed through mutation. For instance, an alteration of an initiator sequence would prevent protein transcription and translation whereas an insertion of a terminator sequence would produce a low-molecular-weight protein which would pass undetected in our electrophoretic systems. Presumably, evolutionary changes of this kind to the structural genes would not be selected against but would be perpetuated because these proteins only form a very small proportion of the total storage protein in the wheat endosperm (Payne and Corfield 1979).

The actual position of the HMW genes on the long arms of the group 1 chromosome is not known although crosses are currently being set up to determine this. It is clear from segregation studies however that the genes for the 1Dx and 1Dy subunits are tightly linked, as are those of the 1Bx and 1By subunits. For instance, subunits 6 and 8 (1Bx and 1By subunits respectively) remained linked and never segregated in 417 F_2 progenies of several crosses (Payne et al. 1981, and more recent work). This tight genetic linkage would explain why only a restricted number of different combinations of 1Bx and 1By subunits and 1Dx and 1Dy subunits were detected in the analysis of varieties (Fig. 2).

One of the 1Bx subunits, subunit 6, is most unusual in that it stained much less strongly with Coomassie Blue R than any of its allelic counterparts (Fig. 3). Although Coomassie Blue is known to complex with proteins in varying amounts depending upon their amino acid composition (Van Kley and Hale 1977) the decreased dye binding to subunit 6 is most likely to be caused by a lower concentration of protein because allelic proteins must have somewhat similar biochemical structures. Supporting this argument are two other pieces of evidence: (1), the stained subunit 6 band is of similar intensity to complementary bands of allelic subunits but is much narrower and is not the same thickness, and (2) subunit 6 is also produced a weak, opaque band after precipitation in the gel with 10% trichloracetic acid, a method which is claimed to give a closer relationship between band intensity and protein amount.

An explanation for the weak staining of subunit 6 may relate to the number of structural genes present. Although it has already been postulated that the variation in HMW glutenin subunits is controlled by five active loci in breadwheat, it is possible that each of them is complex and composed of numbers of reiterated genes. Such reiteration has already been established in maize for zein storage proteins (Viotti et al. 1979). Given a similar situation in wheat, combined with variation in numbers of structural genes at each locus, then correlated variations in protein amount are likely. Indeed, supporting evidence for a correlation between protein amount and gene dosage comes from (1), chromosome 1B-monosomic grains which only have one out of the three 1B chromosomes present in the endosperm of euploid grain, produce only one third the amount of the HMW subunits controlled by that chromosome (Payne et al. 1980); (2), reciprocal F_1 progeny of crosses used to