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Inheritance of Glutenin Protein Subunits of Wheat

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Summary. The inheritance of the high-molecular-weight (HMW) glutenin protein subunits in hexaploid wheat has been investigated by using sodium dodecyl sulphate-polyacrylamide gel electrophoresis to examine the segregation of these subunits in 496 test-cross seeds. The parents of the F_1 hybrid were chosen so that the test-cross seeds segregated for all the HMW glutenin bands. Two glutenin subunits from one parent, believed to be controlled by genes on chromosome 1D, segregated as alternatives to two glutenin subunits from the other parent, a result that supports the assumption that these subunits are controlled by allelic genes at each of two loci that are very closely linked. Similar results were obtained for glutenin subunits believed to be controlled by chromosome 1B, which suggests that these subunits are controlled also by allelic genes at each of two loci that are very closely linked. A single glutenin subunit band, believed to be controlled by chromosome 1A, segregated as an alternative to a single glutenin band from the other parent, except that one seed did not possess either band. It was concluded that these bands are controlled either by allelic genes or by non-allelic genes that are very closely linked.

Key words: Wheat – Seed proteins – Glutenin protein subunits

Introduction

The glutenin fraction of the seed proteins of hexaploid wheat (*Triticum aestivum*) makes up approximately 40 per cent of the total seed proteins and it is believed that this fraction plays an important role in determining the visco-elastic properties of wheat-flour doughs because it contains protein aggregates of high molecular weight (up to several million) formed by the association of a number of constituent polypeptide chains (Kasarda et al. 1976 for a review). These aggregates can be broken down into their component subunits by treatment with the detergent, sodium dodecyl sulphate (SDS) and 2-mercaptoethanol (2-ME). Following this treatment the component subunits can be separated by electrophoresis in polyacrylamide gels containing SDS (SDS-PAGE). With this procedure several subunits of high molecular weight (apparent molecular weight in the range 80,000 to 140,000) have been identified in hexaploid wheat and all the evidence presently available indicates that the genes that control these subunits are located on the long arms of chromosomes 1A, 1B and 1D (Bietz et al. 1975; Lawrence and Shepherd 1980; Payne et al. 1980; Lawrence and Shepherd 1981). In addition, extensive variation has been detected in the high-molecular-weight (HMW) glutenin subunits: tests of an international collection of 98 wheat cultivars showed that the number of bands present in each cultivar ranged from three to five and 34 different banding patterns were identified (Lawrence and Shepherd 1980). Examination of these patterns revealed that some bands, or band combinations, never occurred together in the same cultivar, i.e., some bands, or band combinations, behaved as alternatives to each other. Three groups of such bands were identified. In group 1, two alternative patterns occurred, each consisting of a pair of bands (controlled by chromosome 1D). In group 2, three variants were observed, two consisting of single bands of different mobility while the third is a null form (controlled by chromosome 1A). In group 3 nine patterns were recorded, four having different single bands and five having a pair of bands. Four of these nine patterns were shown to be controlled by genes on chromosome 1B, and it is likely that the other five patterns also are controlled by genes on this chromosome.

As noted previously (Lawrence and Shepherd 1980), the most likely explanation for glutenin subunit bands occurring as alternatives to each other is that they are controlled by alternative forms of the same gene (alleles). However, with the exception of a pair of bands controlled by chromosome 1D (Payne et al. 1980) the presumed

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allelism of the genes that control those bands that are mutually exclusive has not been tested by determining whether these genes behave as alternatives in an inheritance study. Such a study has now been undertaken, and the results are reported in this paper. This study also permitted an estimate to be made of the strength of the linkage between the two loci on the long arm of chromosome 1D that control HMW glutenin bands and likewise for the two loci on the long arm of chromosome 1B.

Materials and Methods

A test-cross procedure was used to examine the inheritance of the HMW glutenin subunits of wheat. Two wheat cultivars, 'Gluyas' (an old Australian cultivar) and 'UP.301' (of Indian origin), which have contrasting glutenin subunit banding patterns, were intercrossed to produce an F_1 hybrid. This hybrid was then crossed as female to a tester-line that had been specially bred so that it possessed only one HMW glutenin band instead of the usual three to five bands present in most cultivars of bread wheat. The proteins in each kernel derived from this cross were extracted by treatment with SDS and 2-ME and then electrophoresed in 8.3% acrylamide gels containing SDS (Lawrence and Shepherd 1980). Each test-cross seed was then scored for the presence or absence of particular glutenin bands of either 'Gluyas' or 'UP.301' origin.

The tester-line with the single HMW glutenin band was selected from amongst F_2 progeny derived from intercrossing the cultivars 'Nap Hal' and 'Gabo'. 'Nap Hal', in common with about 25 percent of cultivars tested so far, does not possess a glutenin subunit band controlled by chromosome 1A (Lawrence and Shepherd 1980) and, unlike all other cultivars tested, it does not possess a pair of HMW glutenin subunit bands controlled by chromosome 1D (Bietz et al. 1975). The tester line has the null condition for both chromosomes 1A and 1D inherited from 'Nap Hal' with the single band that it possesses being derived from the 'Gabo' parent. It is presumed that this band is controlled by chromosome 1B.

Results and Discussion

The banding patterns of the total seed proteins of the three parent cultivars 'Gluyas' (P1), 'UP.301' (P2) and the test-cross parent (P3) are shown in Fig. 1. There are at least four HMW glutenin bands in the 'Gluyas' pattern, five in the 'UP.301' pattern but only one in the test-cross parent and each of these 10 bands has a different electrophoretic mobility. The F_1 seeds derived from intercrossing 'Gluyas' and 'UP.301' possessed the sum of the parental HMW glutenin subunit bands but some of these bands were often quite faint (Fig. 1). As expected, all of the test-cross seeds possessed the single HMW glutenin band of the test-cross parent (P3) and there was segregation for HMW glutenin bands characteristic of either 'Gluyas' or 'UP.301' (e.g. samples T1 to T7 in Fig. 1). Because the parental glutenin bands all have different mobilities, it was possible to score the test-cross seeds unambiguously for the presence or absence of all these bands. The test-cross seeds were scored also for the presence or absence of another band derived from 'UP.301' that was not present in either 'Gluyas' or the test-cross parent: this band is thought to be a prolamin protein (Fig. 1).

As mentioned previously, an examination of the HMW

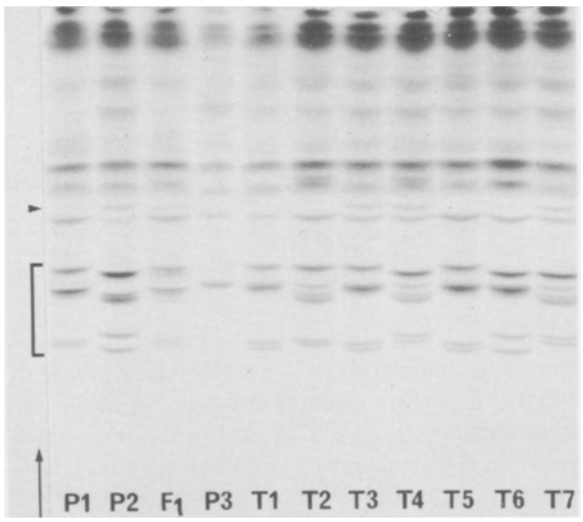

Fig. 1. SDS-PAGE patterns of total seed proteins of test-cross parents and progeny: P1 = wheat cultivar 'Gluyas', P2 = wheat cultivar 'UP.301', F_1 = the hybrid between 'Gluyas' and 'UP.301', P3 = test-cross parent with a single HMW glutenin subunit band, T1 to T7 = different test-cross progeny obtained after crossing the F_1 hybrid to parent P3. The bands that lie within the region designated by the square bracket are HMW glutenin subunits. The arrow head indicates the position of the prolamin band in 'UP.301' (P2) that was present in some test-cross progeny but not others. The vertical arrow indicates the direction of protein movement

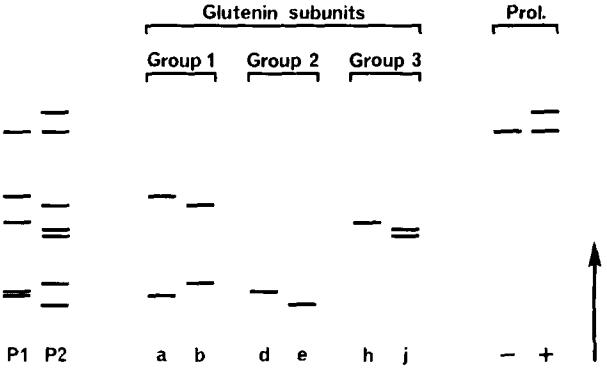

Fig. 2. Diagram of the SDS-PAGE patterns of the HMW glutenin subunit bands and the slowest-moving prolamin (Prol.) bands of 'Gluyas' (P1) and 'UP.301' (P2). The HMW glutenin subunit patterns of these two cultivars are shown partitioned into three groups, where the bands or band combinations within each group are those that were found in a survey of an international collection of wheat cultivars to be alternatives to each other. The letters used to denote these component bands or band combinations – a b d e h j – are those that have been used previously for these particular band combinations (Lawrence and Shepherd 1980). The (+) sign is used to denote the presence of the additional prolamin band present in 'UP.301' and the (–) sign indicates its absence. The arrow indicates the direction of protein movement

glutenin subunits of an international collection of wheat cultivars showed that some bands, or band combinations, are mutually exclusive and that they could be assigned to three groups. In Figure 2 the bands that make up the HMW glutenin patterns of 'Gluyas' (sample P1 in Figs. 1, 2) and 'UP.301' (sample P2 in Figs. 1, 2) have been divided into three groups where the bands or band combinations within each group are those that were found in the international collection to be alternatives to each other. Thus the HMW glutenin subunit banding pattern of 'Gluyas' is made up of pattern a in group 1, pattern d in group 2 and pattern h in group 3, while the pattern of 'UP.301' is made up of patterns b, e and j. In addition to these HMW glutenins, two faster-moving bands of 'UP.301' and one of 'Gluyas', thought to be prolamins because of their mobility in the gels, are included in Fig. 2. These prolamin banding patterns have been designated (+) and (−) respectively, with the (+) sign indicating the presence of the additional band in 'UP.301'. Glutenin band number 3 (counting from the origin) in 'Gluyas' (P1 in Fig. 1) usually occurs as a broad band, but it resolves into a strongly staining band and a weak band of slightly slower mobility when the gels are run for a longer period than was used in this study. Because these two bands were not clearly separated in the present study, they are shown as a single band in Fig. 2 (P1 and pattern h).

Protein extracts from 497 test-cross seeds were analysed electrophoretically and the banding pattern of each seed was classified according to the scheme illustrated in Fig. 2. Thus the progeny T1, T2 and T4 in Fig. 1 were scored as aeh−, adj− and bdj+, respectively. The single HMW glutenin band characteristic of the test-cross parent (P3, Fig. 1) was present in all but one of the 497 seeds, indicating that 496 of these seeds had come from test-crosses. The exceptional seed, besides lacking the band from P3, possessed both band patterns a and b, unlike all other seeds. It is clear that this seed came from accidental self-fertilization of the F_1 plant rather than from the test-cross and it was excluded from further analysis. The segregation ratios for the various glutenin subunit and prolamin band patterns among the remaining 496 test-cross progeny are summarized in Table 1.

The group 1 glutenin subunits present in parents P1 and P2 (patterns a and b, respectively, Fig. 2) segregated as strict alternatives in the test-cross progeny consistent with a 1:1 ratio (Table 1). The absence of any individual with a non-parental band combination permits two conclusions to be drawn. First, this result is strong support for the assumption that allelic genes control these alternatives. Thus, assuming that the two bands of pattern a are controlled by genes at two different loci, then it is likely that the genes controlling the pair of bands in pattern b occur at the same two loci. The second conclusion that can be drawn is that the two loci involved are closely linked, for no recombinant individuals were observed in this study, i.e. no test-cross seeds were found that possessed either the slow-moving band of pattern a associated with the fast-moving band of pattern b, or the slow-moving band of pattern b associated with the fast-moving band of pattern a. The absence of any recombinant individuals amongst 496 test-cross progeny sets an upper limit for the recombination fraction between the loci of 0.74% at the 5% level of significance.

Table 1. Segregation of HMW glutenin subunit bands, and one prolamin band, among 496 test-cross progeny

Seed protein[a]	No. of progeny with indicated seed protein phenotype			χ^2 (P1:P2 = 1:1)	P
	Parental		Non-parental		
	P1	P2			
Glutenins					
Group 1	256(a)	240(b)	0	0.52	0.30-0.50
Group 2	256(d)	239(e)	1	0.58	0.30-0.50
Group 3	255(h)	240(j)	1	0.45	0.50-0.70
Prolamins	254(−)	242(+)	0	0.29	0.50-0.70

[a]The groups 1, 2 and 3 and patterns a, b, d etc. correspond to those shown in Fig. 2

The group 3 glutenin subunits (patterns h and j, Fig. 2) also segregated as alternatives in the test-cross progeny consistent with a 1:1 ratio, except that one seed had neither parental pattern (Table 1). However, when the embryo from this seed was grown, it was found that pollen mother cells (PMC's) at metaphase I of meiosis possessed 20 bivalents plus an unpaired univalent chromosome, instead of the usual 21 bivalents. It is probable that this plant was derived from a female gamete of the F_1 parent that possessed only 20 chromosomes instead of the usual 21 and the missing chromosome was the one that carries the genes that control patterns h and j. It is likely that the missing chromosome is chromosome 1B, since patterns h and j are believed to be controlled by this chromosome (Lawrence and Shepherd 1980). This assumption is supported by the observation that the unpaired univalent chromosome possessed a satellite on its short arm, as expected if it was chromosome 1B. Thus the null phenotype of this individual can be accounted for by aneuploidy. The absence of any individual possessing a non-parental band combination for patterns h and j arising from rare recombination indicates, once again, that allelic genes probably control the bands in these alternatives and that the genes that control the two bands of patterns j and h (which, as noted above, actually consists of two bands of very similar mobility) are very closely-linked.

With the group 2 glutenin subunits (patterns d and e, Fig. 2) the test-cross progeny were found to possess either

pattern d or e in approximately equal numbers except that one seed had neither parental pattern (Table 1). In this case, the null phenotype of the exceptional seed cannot be ascribed to aneuploidy, since the plant grown from this seed exhibited 21 bivalents at meiosis in many PMC's. Testing of the progeny of this plant verified that it lacked both bands d and e. There are several mechanisms that could account for the null phenotype. Possibly a small deletion or a mutation resulted in the loss of either band d or band e, but such events are expected to occur only very rarely. If bands d and e are controlled by allelic genes that contain a repeated DNA sequence or sequences, then unequal crossing over could result in the loss of most of the gene. An alternative possibility is that bands d and e are controlled by genes at separate, but closely-linked loci, each of which possesses a null allele. In this case rare crossing over between the two loci could produce a chromosome carrying the two null alleles. The possibility that chromosome 1A possesses two closely-linked loci controlling glutenin subunits cannot be considered unlikely, because chromosome 1A is almost certainly derived from the same ancestral chromosome as chromosomes 1B and 1D, and both of these chromosomes apparently possess two closely-linked loci controlling glutenin subunits. At present there is no evidence available to help choose between the various possibilities outlined above that might account for the null phenotype. From the segregation of the group 2 glutenin subunits it can be concluded that bands d and e are controlled either by allelic genes or by genes at separate, but closely-linked, loci.

The test-cross seeds also segregated in approximately equal numbers for the presence or absence of the additional prolamin band present in 'UP.301' (Table 1). The segregation of this prolamin band and the segregations of the group 1, group 2 and group 3 glutenin subunits were found to be independent of each other (Table 2). Although the chromosomal locations of the genes controlling the various glutenin subunit bands in 'Gluyas' and 'UP.301' have not been determined, from the results of studies of other cultivars it is likely that patterns a and b are controlled by genes on the long arm of chromosome 1D, patterns d and e by a gene(s) on the long arm of 1A and patterns h and j by genes on the long arm of 1B (Bietz et al. 1975; Lawrence and Shepherd 1980; Payne et al. 1980; Lawrence and Shepherd 1981). On this basis it is expected that the three groups of glutenin subunits would segregate independently of each other as was observed.

The chromosomal location of the gene controlling the extra prolamin band in 'UP.301' is not known. However, bands of similar mobility in the cultivars 'Chinese Spring', 'Hope', 'Cheyenne' and 'Timstein' are known to be controlled by genes located on chromosome 1B and, in 'Chinese Spring', it has been shown that these genes are located on the short arm of this chromosome (Lawrence and Shepherd 1980 and unpublished). Thus it is likely that the extra prolamin band in 'UP.301' is controlled also by a gene on the short arm of chromosome 1B. Given this, and that patterns h and j are controlled by genes on the long arm of chromosome 1B, then it is of interest to determine whether these genes are linked. However, no evidence of genetic linkage was revealed in the analysis of their joint segregation (Table 2).

Table 2. Joint segregation of the HMW glutenins subunit patterns and one prolamin band among 494[a] test-cross progeny and chi-square values for tests of independent segregation

		Glutenins					
Seed protein		Group 1		Group 2		Group 3	
		a	b	d	e	h	j
Glutenins							
Group 2	d	127	128				
	e	128	111				
Group 3	h	131	124	127	128		
	j	124	115	128	111		
Prolamins							
	–	123	129	123	129	133	119
	+	132	110	132	110	122	120

Joint segregation	χ^2[b]	P
a or b with d or e	0.55	0.30-0.50
a or b with h or j	0.00	1.00
a or b with – or +	1.40	0.20-0.30
d or e with h or j	0.55	0.30-0.50
d or e with – or +	1.40	0.20-0.30
h or j with – or +	0.19	0.50-0.70

[a] The two individuals with non-parental phenotype (see Table 1) have been omitted from the analysis

[b] The chi-square values were calculated using the 2 x 2 contingency table chi-square test and employing Yates' continuity correction

In summary, therefore, the results of the present study support the assumption that the different glutenin subunit bands in patterns a and b (group 1) and in patterns h and j (group 3) are controlled by allelic genes, and that bands d and e (group 2) are controlled either by allelic genes or by non-allelic genes that are very closely linked. The results also indicate that the two loci that code the group 1 glutenin subunits are very closely-linked as are the two loci that code the group 3 glutenin subunits. The close-linkage of these loci indicated by the results of this study agrees with the findings of Payne et al. (1981) who, in an analysis of lines derived from F_2 plants, also did not observe any individuals that were recombinant for these loci.

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