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Genetic variation at storage protein-coding loci of common wheat (cv 'Chinese Spring') induced by nitrosoethylurea and by the cultivation of immature embryos in vitro

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Abstract Electrophoretic patterns of seed storage proteins, the high-molecular-weight glutenins and gliadins, were studied in 468 plants of the common wheat cultivar 'Chinese Spring' regenerated from callus culture of immature embryos, in 115 plants grown from seeds treated with nitrosoethylurea and in 260 control plants. From 5 to 21 single grains were analysed from each plant. In these three groups, the frequency of inherited mutations causing the loss of all proteins controlled by a locus (null-mutations, probably caused by a chromosomal deficiency) was 0.69%, 2.07%, and 0. 05% per locus (the differences were statistically significant), respectively, while that of mutations causing the loss of a single protein band was 0.11%, 0.33%, and 0.05%, respectively. The loss of all of the gliadins controlled by *Gli-B1* or *Gli-B2* (mutations were probably caused by a deletion of satellites of the corresponding chromosomes), was significantly higher than the loss of gliadins controlled by genomes A and D. Gene mutations altering the electrophoretic mobility of a single protein band in the pattern were found only in the second group of plants (0.44%). Therefore, chemical mutagenesis which produced not only more mutations than cultivation of immature wheat embryos in vitro, but also a higher ratio of mutations that altered DNA sequences, can be considered as an easier and comparatively more promising way for obtaining new improved variants of loci controlling biochemical characteristics in wheat. Somaclonal variation, on the other hand, was probably mainly caused by chromosomal abnormalities and could therefore hardly be considered as a useful tool in wheat breeding.

Key words Storage proteins · Induced mutagenesis
Somaclonal variation · Nitrosoethylurea
Common wheat (cv 'Chinese Spring')

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Introduction

The frequency and pattern of induced variation in loci controlling biochemical characters have not been well studied in wheat. However, the highly polymorphic proteins of the wheat grain, the high-molecular-weight (HMW) glutenins (Glu) and gliadins (Gli), could serve as a suitable model for such an analysis. In common wheat, these proteins are encoded by three Glu and six Gli loci mapped on the chromosomes of the first (*Glu-1* and *Gli-1*, long and short arms, respectively) and sixth (*Gli-2*) homoeological groups (Payne 1987). Each locus is complex and, as a rule, controls the synthesis of two or more jointly inherited proteins. Alleles at a locus differ by the presence/absence and/or electrophoretic mobility of the encoded proteins (Sozinov and Poperelya 1980; Payne and Lawrence 1983; Metakovsky 1991). Storage protein genes differ by the presence of deletions/insertions and single nucleotide substitutions (Kreis et al. 1985; Shewry and Tatham 1990). All of these data indicate that the variability at the Gli and Glu loci is not restricted a great deal by natural selection and that it can be easily traced by means of protein electrophoresis.

Somaclonal variation of regenerants obtained from wheat tissues cultivated in vitro was claimed by Larkin and Scowcroft (1981) to be a promising type of induced variation in wheat. However, contrasting values of the frequency of somaclonal variation in gliadin genes were found to range from only a few cases in several hundred regenerants (Maddock et al. 1985) to a mean value of 3.0 changes in the gliadin electrophoretic pattern of each regenerant plant (Larkin et al. 1984). This discrepancy was probably caused by mistakes in the interpretation of

gliadin electrophoregrams (Metakovsky et al. 1987). Finally, the means for studying somaclonal variation in wheat has been passed over without a definite answer having been obtained to the question about its frequency. It is expected, however, that the frequency of gene mutations in regenerants should be higher than spontaneous variability at the same loci (Ryan and Scowcroft 1987).

The aim of the investigation presented here was to analyse the frequency and types of inheritable changes induced at storage protein coding loci of common wheat through the cultivation of immature embryos and the use of the chemical supermutagen, nitrosoethylurea.

Materials and methods

As a control, gliadins of 260 plants of the common wheat cultivar 'Chinese Spring' and HMW glutenins of 205 of these plants were studied. Regenerants (468) were obtained from callus cultures of immature embryos (R1) as described by Galiba et al. (1985) and studied for both gliadins and HMW glutenins.

Gliadins and HMW glutenins were analysed in 115 and 113 plants (M1), respectively, grown from seeds exposed for 18 h to *N*-nitroso-*N*-ethylurea (2.5 ppm). The mutagen concentration used reduced seed germination by 50%. F_2 grains from the cross 'Chinese Spring' (M1 plant N40) × 'Kzul Bas' were also studied.

From 5 to 21 single grains were analysed from each plant. Gliadins were extracted with 70% alcohol and fractionated by acid polyacrylamide gel electrophoresis, pH 3.1 (APAGE), as described by Metakovsky and Novoselskaya (1991). The dry protein pellet obtained after this extraction was dissolved in TRIS-HCl buffer (pH 6.8) containing 3% mercaptoethanol and 1% sodium dodecyl sulfate (SDS) and used for SDS-electrophoresis (Laemmli 1970).

The APAGE procedure routinely used enabled a gliadin pattern to be obtained for 'Chinese Spring' that consisted of 31 components (Akhmedov and Metakovsky 1987). However, some of these components could not be scored unambiguously because of their weakness and overlaps. In this work, we scored only 21 bands (Fig. 1): N6 for *Gli-A1*; N3, 4, 5, 8 for *Gli-B1*; N1, 2, 7, 10 for *Gli-D1*; N15, 16, 18, 20, 21 for *Gli-A2*; N9, 12, 13 for *Gli-B2*; and N11, 14, 17, 19 for *Gli-D2*.

In the SDS-electrophoretic pattern, HMW glutenin subunits were identified (Fig. 2) in accordance with Payne and Lawrence (1983). Only the *Glu-B1* and *Glu-D1* loci could be scored because of the absence of any subunit controlled by *Glu-A1* in 'Chinese Spring'.

Results

Variations in the patterns scored

It was suggested that a mutation may cause: (1) the loss of all the electrophoretic bands controlled by a locus (null-mutation, the first type of mutation); (2) the loss of only 1 band (the second type); or (3) a change in the mobility of a band (the third type). It was assumed that a mutation appeared first in a heterozygous state, manifesting itself by a relative decrease in the staining intensity of a band(s) (Metakovsky et al. 1993). Obviously, one could expect to find homozygotes as well as heterozygotes for a given mutation when analysing single grains from a mutant R1 or M1 plant.

An understanding of the genetic control of each component in the electrophoretic patterns of cv 'Chinese Spring' enabled us to explain each variation in the pattern and thereby to consider certain changes as not being caused by mutations. For example, 3 seeds of different R1 plants showed multiple changes in all zones of the SDS-pattern (Fig. 2, line 3), eventhough the APAGE pattern of these grains corresponded to the

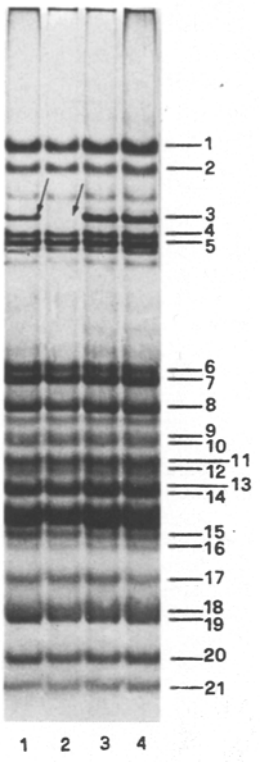

Fig. 1 Gliadin APAGE patterns of some single grains (*lanes 1–4*) from 1 plant (R1 N24) regenerated by callus culture of immature embryos of cv 'Chinese Spring'. Alterations in the pattern are indicated by *arrows*. Bands scored are *numbered*

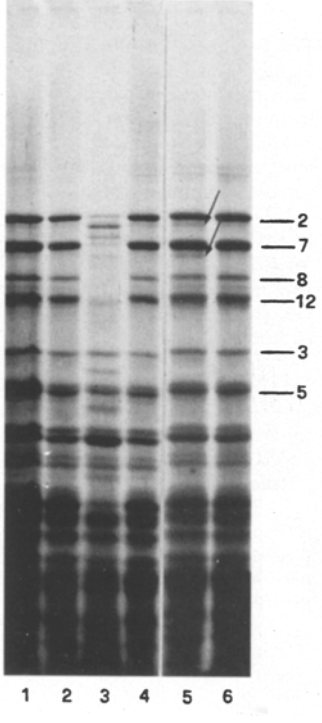

Fig. 2 SDS-electrophoretic patterns of grain storage proteins from single seeds (*lanes 1–6*) of cv 'Chinese Spring'. *3* Artefact changes in the pattern. *Arrows* show minor bands that were not scored. HMW glutenin subunits *2*, *7*, *8* and *12* are *numbered* in accordance to Payne and Lawrence (1983). Gliadins N3 and N5 are the same as on Fig. 1

standard. It was assumed that the simultaneous changes in proteins controlled by different loci were, in fact, artefacts occurring during the SDS-electrophoretic procedure. Overloaded lanes (Fig. 2, lane 1; Fig. 3, lane 16; Fig. 4, lane 4), the appearance of some additional minor bands in the HMW glutenin region of the SDS-pattern (Fig. 2, lane 5; Fig. 4, lane 17) and the relative intensity of bands 1 and 2 in the APAGE-pattern (which unpredictably varied in different runs) were also not assumed to be alterations.

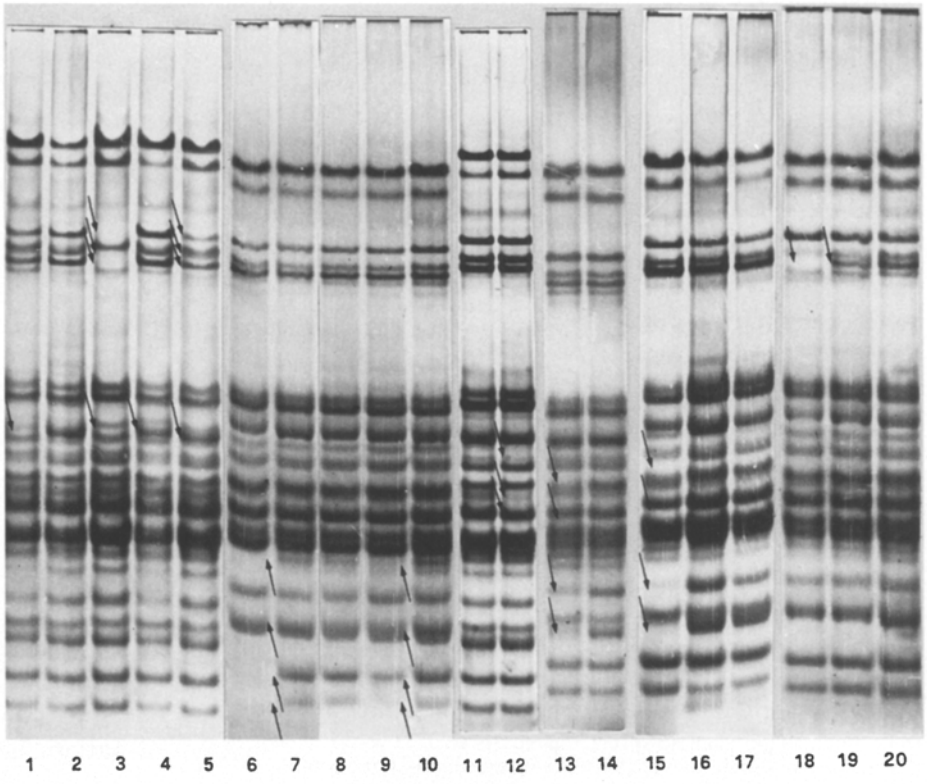

Fig. 3 Alterations in the gliadin APAGE pattern in single grains from plants of cv 'Chinese Spring' regenerated from callus culture. *Lane 1* Loss of band N8 (plant R1 N77); *2, 7, 8, 10, 11, 14, 16, 17, 20* normal pattern; *3, 6, 15* null-mutants for *Gli-B1-*, *Gli-A2-*, *Gli-D2*-controlled bands, respectively; *4* decrease in intensity of band N8; *5, 9, 12, 13* decrease in intensity of all *Gli-B1-*, *Gli-A2-*, *Gli-B2-*, *Gli-D2*-encoded bands, respectively; *18* loss of band N5 (plant R1 N143); *19* decrease in intensity of band N5. Changes in the patterns are indicated by *arrows*

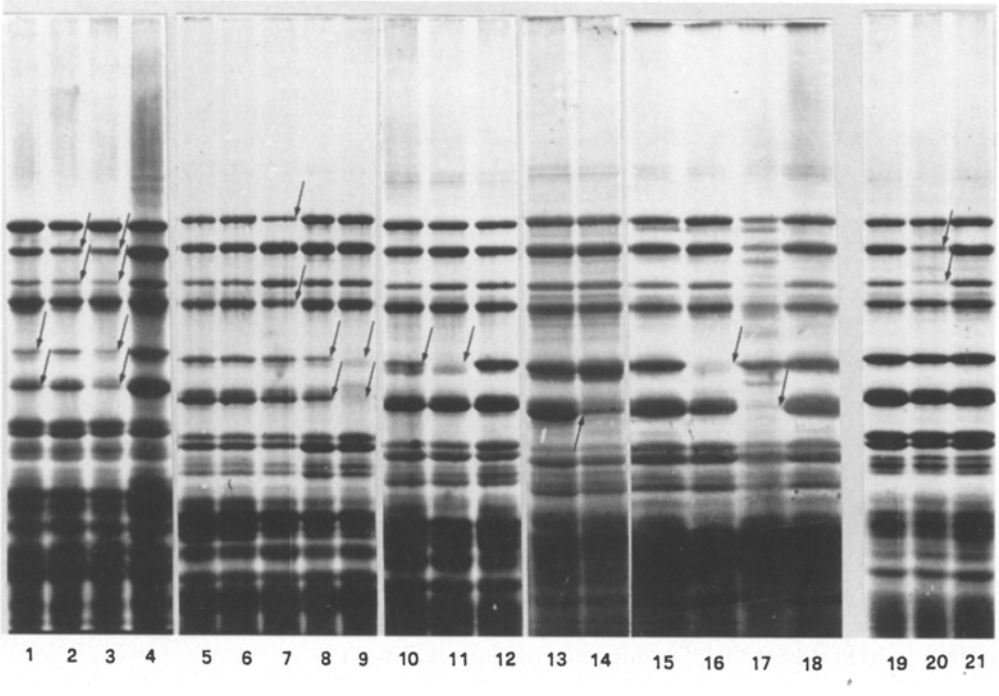

Fig. 4 Changes in the 'Chinese Spring' SDS-electrophoretic pattern found in single grains from regenerant plants (*lanes 1–18*) and from plants grown from seeds treated with nitrosoethylurea (*lanes 19–21*). *1, 8* Decrease in intensity of bands N3 and N5 (heterozygotes for *Gli-B1*-null); *2* (and *20*), *7* decrease in intensity of *Glu-B1-*, *Glu-D1*-encoded HMW subunits, respectively; *3* decrease in intensity of *Glu-B1*-encoded HMW subunits and bands N3 and N5 (R1 N272, monosomic for the chromosome 1B); *4, 5, 6, 12, 13, 15, 18, 19, 21* standard pattern; *9* loss of bands N3 and N5 (Gli-B1-null); *10, 14* decrease in intensity of bands N3 and N5, respectively; *11* (and *16*), *17* loss of bands N3 (R1 N24) and N5 (R1 N143), respectively. Alterations in the patterns are indicated by *arrows*

Control plants

Two cases of changes in the electrophoretic patterns of control grains could be explained as being caused by mutations at storage protein coding loci.

First, a decrease in the intensity of 1 *Gli-B1*-controlled band (N3) was found in both the APAGE and SDS-electrophoretic patterns in 1 of the 10 grains studied in 1 of the control plants (the pattern was identical to that shown on Fig. 1, line 1 and Fig. 4, line 10). We assumed that this effect was caused by a mutation of the second type in the heterozygous state.

Second, an obvious null-mutation for *Glu-D1*, in the heterozygous state, was found in 1 of the 7 grains studied from the other plant (the pattern was similar to that shown on Fig. 4, line 7). The effect was not caused by monosomy for chromosome 1D because the APAGE pattern of this grain was identical to the standard.

Both of the mutations found in the control had to be newly arisen since they appeared in the heterozygous state in a single grain per plant (Metakovsky et al. 1993). Therefore, these mutations could not be transferred into the material that gave rise to R1 or M1 plants. The frequency of spontaneous mutagenesis at *Glu-1* and Gli of 'Chinese Spring' calculated on the basis of these results is about 1×10^{-3} per locus, per generation.

Regenerants

Twenty-eight R1 plants carried 32 different changes in their electrophoretic patterns (Table 1). Null-mutations

Table 1 Mutations at storage protein coding loci found in regenerants of the common wheat cultivar 'Chinese Spring' (*Homo* Homozygote · *Het* heterozygote)

Number of the plant	Locus	Number of		Homo/Het[a]	Type of mutation[b]	Figure/line[c]	
		Grains studied	Mutant grains			APAGE	SDS
24	*Gli-B1*	5	2	Het (1)	2	1/1	4/10
				Homo (1)		1/2	4/11
25	*Gli-B2*	5	1	Het (1)	1	3/12	
29	*Gli-B2*	15	5	Het (4)	1		
				Homo (1)		Not shown	
32	*Gli-B2*	20	2	Het (2)	1		
48	*Gli-B2*	15	1	Het (1)	1		
59	*Gli-B2*	15	3	Het (3)	1		
77	*Gli-B1*	10	2	Het (2)	2	3/4	
84	*Gli-B2*	10	1	Het (1)	1		
88	*Gli-B2*	5	1	Het (1)	1		
106	*Gli-D2*	13	1	Het (1)	1	3/13	
109	*Gli-B2*	10	2	Het (2)	1		
121	*Gli-B1*	5	2	Het (1)	1	3/5	4/8
				Homo (1)		3/3	4/9
143	*Gli-B1*	7	2	Het (1)	2	3/19	4/14
				Homo (1)		3/18	4/17
147	*Gli-B1*	10	3	Het (2)	1		
				Homo (1)			
151	*Gli-B1*	10	2	Het (2)	1		
199	*Gli-B2*	10	2	Het (2)	1		
214	*Glu-D1*	10	3	Het (3)	1		4/7
219	*Glu-B1*	5	1	Het (1)	1		
220	*Gli-B1*	7	2	Het (2)	1		
232	*Gli-A2*	10	2	Het (1)	1	3/9	
				Homo (1)		3/6	
272	*Gli-B1*	7	2	Het (2)	1		4/3
	Glu-B1	7	2	Het (2)	1		4/3
302	*Glu-B1*	10	2	Het (2)	1		
313	*Gli-B1*	14	2	Het (2)	1		
314	*Gli-B1*	6	3	Het (2)	2		
				Homo (1)			
327	*Glu-B1*	5	1	Het (1)	1		
387	*Gli-D2*	5	3	Het (3)	1		
402	*Gli-B1*	20	4	Het (4)	1		
	Gli-D2	20	4	Het (4)	1		
423	*Gli-A1*	20	2	Het (1)	1	Not shown	
				Homo (1)		Not shown	
	Gli-B1	20	3	Het (3)	1		
	Gli-D1	20	4	Het (4)	1	Not shown	

[a] In brackets, number of seeds of a given type found

[b] 1, Loss of all components controlled by a locus; 2, loss of a single band; 3, change in mobility of a band

[c] Reference to the photograph; the number of the figure and lane in this Fig. are indicated. Only one (first in the Table) of all cases of an identical change in the pattern is shown on the photograph

were found at all eight storage protein-coding loci scored and were obviously responsible for 28 of these 32 different changes.

In particular, the R1 plant N423 carried grains with null-mutations at all three *Gli-1* loci (Table 1). No one grain of this plant had mutations at two or three Gli loci simultaneously or had a mutation at any Glu locus. Therefore, the mutant grains were not monosomics for chromosomes 1B or 1D. Grains with a null-mutation at *Gli-B1* were also observed in the 7 other regenerant plants (Table 1), 2 of which also had grains homozygous for the mutation (Fig. 3, line 3). In plant N272, 2 grains were obvious monosomics for chromosome 1B because they had a decreased intensity not only of *Gli-B1*-encoded gliadins, but also of *Glu-B1*-controlled HMW glutenin subunits (Fig. 4, line 3).

A null-mutation at *Gli-B2* was the most frequently observed mutation, occurring in 9 R1 plants (Fig. 3, line 12, Table 1). Only 1 seed was found, however, to be probably carrying this mutation in the homozygous state (data not shown). Some grains of R1 plant N214 carried a heterozygous null-mutation at *Glu-D1* that was accompanied by an increase in the staining intensity of both *Glu-B1*-controlled subunits (Fig. 4, line 7).

A mutation of the second type causing the loss of band N3 was found in 2 plants (N24 and N 314), both of which carried grains heterozygous (Fig. 1, line 1 and Fig. 4, line 10) and homozygous (lines 2 and 11), respectively, for the mutation. In R1 N143, an analogous mutation caused the loss of band N5 (heterozygotes, Fig. 3, line 19 and Fig. 4, line 14; homozygotes, Fig. 3, line 18 and Fig. 4, line 17). A decrease in the staining of band N8 accompanied by a higher intensity of other gliadins controlled by *Gli-B1* (bands N3, 4, 5) was observed in 2 grains in R1 N77 (Fig. 3, line 4).

No R1 plant carried a mutation of the third type. An identical number of mutations was found for the *Gli-1* and *Gli-2* loci (Table 1).

In all cases but one, identical mutations were found in plants originating from different calli. In R1 N147 and N313, originating from the same callus, however, the mutation may have arisen from one event, and this possibility was accepted for the calculation of the frequency of mutagenesis. The appearance of the monosomic (N272) was also considered to be a consequence of one mutational event. A total of 30 independent cases of mutation at storage protein-coding loci was found in the 468 R1 plants studied. This gives a frequency of about 8.0×10^{-3} per locus, which is statistically higher ($F = 14.06$***) than that found for the control plants. For the Gli and *Glu-1* loci, the frequency was 9.3×10^{-3} and 5.3×10^{-3}, respectively.

Most of the mutations, 20 out of 26 for Gli and 4 out of 5 for *Glu-1*, were found at loci on chromosomes 1B and 6B. The difference in the frequency of mutations between *Gli-B1* and either *Gli-A1* or *Gli-D1*, and between *Gli-B2* and *Gli-A2* were statistically significant ($F = 10.76$**, and $F = 8.19$**, respectively).

Chemical mutagenesis

Null-mutants at *Gli-A1*, *Gli-D1* and *Glu-D1*, which were rare in R1 plants, were not found at all in M1 plants. The loss of all *Gli-B1*- and *Gli-B2*-controlled components, however, was found in 9 and 7 M1 plants, respectively (Table 2). Moreover, 3 plants (N88, N96, N101) carried both these mutations, and 1 grain was even found to be apparently heterozygous for both these mutations simultaneously (Fig. 5, lines 2, 7).

Mutations of the second type, all at *Gli-2* loci, were found in M1 plants N51, N56 and N75 (Table 2; Fig. 5, lines 6, 9 and 11).

In contrast to the situation observed in the regenerants, 4 different mutations of the third type were found in the M1. An interesting case was M1 N50. Out of the 10 first grains studied, 2 were apparently heterozygous for the mutation that caused an increase in the APAGE mobility of band N5 (Fig. 5, line 13) while 2 others were heterozygotes for *Gli-B2*-null. Out of 11 plants grown from other M1 N50 seeds, 1 was unexpectedly found to be homozygous for the mutation decreasing the APAGE mobility of the other band, N4 (Fig. 6, line 2). Therefore, it could well be that genes controlling bands N5 and N4 were both affected in the M1 plant N50. In addition, an increase in the APAGE mobility of band N20 was encountered in plant N81 (Fig. 5, line 14) and of *Gli-D2*-controlling fast-moving β-gliadin (this band was not scored for mutations of the first and second types because of its overlapping with other bands) in plant N99 (Fig. 5, line 17).

The absence of null-mutations at *Gli-B1* or *Gli-B2* in the control indicated their induction by the mutagen. If all cases of alterations in the protein patterns found in M1 were consequences of independent mutational events, the frequency of mutations induced by the mutagen was as high as 2.8×10^{-2} per storage protein-coding locus, significantly higher ($F = 9.47$**) than that observed in R1 plants. For the Gli and *Glu-1* loci, the frequency was 3.6×10^{-2} and 4.4×10^{-3}, respectively.

In the M1, a significantly higher frequency of induced mutations was shown for loci located on chromosomes 1B and 6B, similar to that found for R1 plants (Table 2). Induced mutations at these loci is probably peculiarity of the 'Chinese Spring' genotype.

Inheritance of changes in storage protein electrophoretic patterns

Sixteen half-seeds from different R1 and M1 plants were grown, and 8 fertile plants were obtained. It was found that all of the grains from the 3 R2 plants grown from homozygous half-seeds (R1 plants N121, N143 and N147) carried a particular mutation in the homozygous state, thereby confirming its inheritance.

Also, 5 R2 plants were obtained from R1 half-seeds identified as being heterozygotes for a mutation. Grains from these plants gave a genetic segregation for a muta-

Table 2 Mutations at storage protein coding loci found in plants grown from grains treated with nitrosoethylurea

Number of M1 plant	Locus	Number of		Homo/Het[a]	Type of mutation[a]	Figure/line[a]	
		Grains studied	Mutants found			APAGE	SDS
1	*Gli-B1*	10	1	Het (1)	1		
3	*Gli-B1*	10	1	Het (1)	1		
5	*Gli-B2*	10	2	Het (2)	1		
8	*Gli-B2*	15	6	Het (6)	1		
11	*Gli-B2*	15	2	Het (2)	1		
28	*Gli-D2*	10	1	Het (1)	1		
44	*Gli-B1*	10	2	Het (2)	1		
50	*Gli-B1*	21	2	Het (2)	3	5/13	
	Gli-B1	21	1	Homo (1)	3	6/2	
	Gli-B2	21	3	Het (3)	1		
51	*Gli-A2*	20	4	Het (2)	2	Not shown	
				Homo (2)		5/6	
54	*Glu-B1*	10	2	Het (2)	1		4/20
56	*Gli-D2*	20	3	Het (3)	2	5/9	
75	*Gli-D2*	20	2	Het (1)	2	Not shown	
				Homo (1)		5/11	
77	*Gli-B1*	10	1	Het (1)	1		
81	*Gli-A2*	20	3	Het (2)	3	Not shown	
				Homo (1)		5/14	
88	*Gli-B1*	15	1	Het (1)	1		
	Gli-B2	15	1	Het (1)	1		
93	*Gli-B1*	10	1	Het (1)	1		
96	*Gli-B1*	10	1	Het (1)	1		
	Gli-B2	10	3	Het (3)	1		
99	*Gli-D2*	5	1	Het (1)	3	5/17	
101	*Gli-B1*	10	2	Het (1)	1		
				Homo (1)			
	Gli-B2	10	4	Het (1)	1		
102	*Gli-B2*	20	8	Het (7)	1		
				Homo (1)			
109	*Gli-B1*	10	2	Het (1)	1		
				Homo (1)			

[a] See Table 1 for explanations

tion (Table 3), but correspondence to the 1:2:1 ratio was confirmed only for R2 N220. A plant grown from a heterozygous half-seed of R1 N77 produced only 4 grains that were homozygous for the mutation out of 57 tested (Fig. 3, line 1). Moreover, only 2 out of 132 grains were homozygous for the null-mutation at *Gli-B2* in the 3 R2 plants heterozygous for this mutation (Table 3). This finding indicates a low viability of either gametes carrying the mutant chromosomes (Payne et al. 1984) or of genotypes homozygous for this mutation, or both.

The joint inheritance of all components in the modified (mutant) variant of the *Gli-B1a* allele was confirmed by an analysis of 120 F_2 grains of the cross 'Chinese Spring' (M1 N40) × 'Kzul-Bas'. In this cross, a *G1i-Blq*-controlled band of 'Kzul-Bas' (1*, Fig. 6) had an electrophoretic mobility similar to that of the mutant band N4 of 'Chinese Spring' in both APAGE and SDS-electrophoresis. However, in the patterns of all 32 F_2 grains homozygous for the absence of *Gli-B1q*, a strong band (apparently mutant band N4) was present (for example, Fig. 6, line 3), showing an allelism of genes controlling the synthesis of this band and band 1*.

Discussion

In this work, three types of changes in the electrophoretic patterns of the seed storage proteins of 'Chinese Spring' were found, the same number as those caused by spontaneous mutagenesis in the set of 180 common wheat cultivars (Metakovsky et al. 1993).

The first type of change, loss of all components controlled by a locus, was presumably caused by a chromosomal deficiency (Payne et al. 1984; Pogna et al. 1985; D'Ovidio et al. 1991; Sabelli et al. 1992), although the presence of regulatory mutations were also suggested in a few cases (Lafiandra et al. 1987). The level of spontaneous variability of this type in our investigation was about 0.05%, which is similar to that which has been found at Gli loci in many common wheat cultivars; ranging from 0.08% – 0.23% per locus, per generation (Metakovsky et al. 1993). In R1 and M1, however, the frequency was as high as 0.69% and 2.07%, respectively. Earlier, Davies et al. (1986) found that more than 3% of regenerants had changes in their alcohol dehydrogenase-1 electrophoretic pattern, with most of these mutants being aneuploids. Out of 149 regenerants

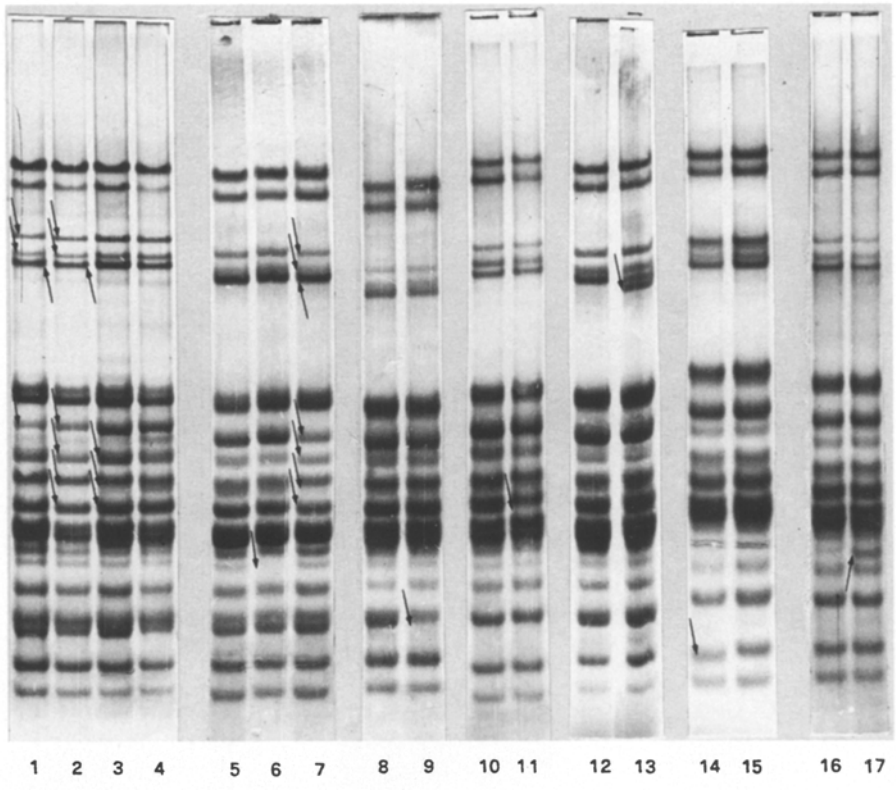

Fig. 5 Alterations in gliadin APAGE pattern found in single grains of plants grown from seeds treated with nitrosoethylurea. *Lane 1* decrease in intensity of all *Gli-B1*-controlled bands; *2*, *7* decrease in intensity of all *Gli-B1*- and *Gli-B2* bands simultaneously; *3*, *9*, *11* decrease in intensity of all *Gli-B2*- encoded bands, of band N19 (plant M1 N56) and a loss of band N14 (M1 N75), respectively; *4*, *5*, *8*, *10*, *12*, *15*, *16* standard pattern; *6* loss of band N16 (M1 N51); *13*, *14*, *17* increase in mobility of bands N5 (M1 N50), N20 (M1 N81) and of a *Gli-D2*-controlled β-gliadin (M1 N99), respectively. Changes in the patterns are indicated by *arrows*

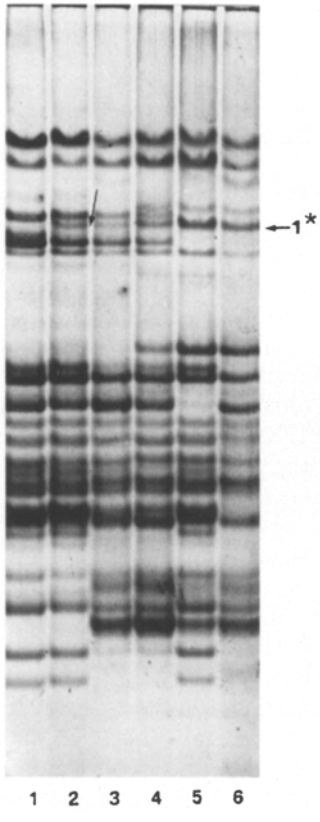

Fig. 6 Gliadin APAGE patterns of single F_2 grains from the cross 'Chinese Spring' (M1 N50) × 'Kzul Bas'. *Lane 1* Standard pattern of 'Chinese Spring'; *2* decrease in mobility of the band N4 (plant N50); *3–5* some F_2 hybrid grains of the cross; cv 'Kzul Bas'. Gliadin band of 'Kzul Bas' that has a APAGE mobility similar to a mutant variant of band N4 is indicated (*1**)

studied by Ryan and Scowcroft (1987), 22 were monosomics for 4A or 4D, lacking some β-amylase electrophoretic bands. Indeed, the frequency of aneuploidy in regenerants of common wheat can reach 29% (Karp and Maddock 1984). Monosomic genotypes (R1 N272) were identified in our work by analysing the biochemical loci mapped on the different arms of the same chromosome. Also, the frequent loss of all *Gli-B1*- or *Gli-B2*-controlled bands in 'Chinese Spring' could have been caused, in most cases, by a deletion of satellites of chromosomes 1B and 6B, respectively.

The repeated, probably genotype-specific, appearance of some spontaneous mutations in common wheat has been described earlier (Metakovsky et al. 1993), but the pattern of the repeated mutations differed between this and the present study. For example, a null-mutation at *Gli-B2* was rare in the set of 180 common wheat cultivars, but was induced (plants R1 and M1) quite frequently in 'Chinese Spring'.

Gene mutations (microdeletions or other alterations in DNA regulatory or coding sequences) may be responsible for the loss of a single band and for a change in the electrophoretic mobility of a band. The joint frequency of these second and third types of spontaneous mutations was about 0.01% per locus, per generation in common wheat (Metakovsky et al. 1993). In our work, these mutations occurred at a frequency of 0.05% in the control, 0.11% in R1 and 0.76% in M1 plants. Gene

Table 3 Segregation in grains of R2 plants grown from half-seeds assumed to be heterozygous for a mutation (*Homo CS* homozygote for the absence of a mutation · *Het* heterozygote · *Homo mut* homozygote for the mutation)

Number of R1 plant	Locus	Number of seeds studied	Number of grains in the three classes				
			Homo CS	Het	Homo mut	χ^2	P
59	*Gli-B2*	44	18	24	2	11.8	<0.01
77	*Gli-B1*	57	31	22	4	28.9	<0.001
88	*Gli-B2*	40	26	14	0	37.4	<0.001
199	*Gli-B2*	48	29	19	0	22.2	<0.001
220	*Gli-B1*	40	11	19	10	0.28	>0.80

mutations at the alcohol dehydrogenase-1 loci were not found in study of 551 regenerants of the cultivar 'Millewa' (Davies et al. 1986).

There have been some hopes of using somaclonal variation for wheat improvement (Larkin and Scowcroft 1981). For example, it is known that allelic variation at the storage protein-coding loci may influence wheat dough quality (Sozinov and Poperelya 1980; Payne 1987). One could expect to obtain new improved allelic variants of these loci as somaclonal variants. However, only negative variation has been found for dough quality and yield characteristics in many specially selected lines developed from regenerant plants (Ryan et al. 1987). The probable reason for the ineffectiveness of this approach is the low frequency of mutations of the third type, in which the DNA sequences are changed in the regenerant plants, while the variation caused by chromosomal abnormalities could hardly be considered to be useful for breeding purposes. It is interesting to note in this context that chemical mutagenesis produced a higher ratio (in our work, 0.44%) of mutations of the third type than the cultivation of immature wheat embryos in vitro. Therefore, the latter approach can be considered as a more promising and easier (although ecologically less pure) tool for obtaining new improved variants of loci controlling biochemical characteristics in wheat.

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