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Genetic Variability and Improvement of Seed Proteins in Wheat

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Summary. Albumins, globulins, gliadins and glutenins presumably comprising 100 percent of the wheat seed proteins were sequentially extracted and electrophoresed on SDS-polyacrylamide gels. The SDS-electrophoretic patterns within each of the four fractions from *T. boeoticum, T. urartu, T. turgidum, T. timopheevii, T. aestivum, Ae. speltoides* and *Ae. squarrosa* were similar. They differed from one species to another only in a few minor components or density of certain components. Similarity in MW's of components, as indicated by the SDS-electrophoretic patterns, suggests that the wheats and *Aegilops* exhibit no variability for structural genes coding seed proteins. A minimum of 60 to 70 and a maximum of 360 to 420 structural genes with major or minor effects control the total seed protein in *T. aestivum*. Presumably, only one or the other homoeoallele was expressed in the polyploids. Different components of albumins and globulins presumably had distinct MW's and amino acid composition, while the components of gliadins and glutenins could be classified into a few groups each containing one or more components with the same MW and nearly identical amino acid composition. The genes for components with similar MW's and amino acid composition arose through multiplication of a single original gene and perhaps share the same regulatory mechanism. Seed protein content and quality in wheat might be improved through the incorporation of structural genes, coding for polypeptides with distinct MW's, from distantly related species, rather than by manipulation of the structural genes within the *Triticum-Aegilops* group. Regulatory mutants similar to opaque-2 of corn could be used to alter the proportion of gliadins in relation to albumins and globulins, to improve amino acid composition of wheat proteins.

Key words. *Triticum* - Albumins - Globulins - Gliadins - Glutenins

Introduction

The importance of protein content and quality of wheat grain in our nutrition is well recognized. The information on the genetics and regulation of the seed proteins, necessary for their improvement, however, is very limited. Halloran (1975) reported that the seed protein content in wheat was under the control of only a few major genes and a number of modifiers. Using intervarietal chromosome substitution lines, Kuspira and Unrau (1957) and Halloran (1976) postulated that many genes, each of small effect, controlled the seed protein content in wheat. Low protein content is reported to exhibit partial to complete dominance over high protein content (Johnson et al. 1973; Halloran 1975). The distinction between regulatory and structural genes controlling seed proteins appears to be confused in these genetic analyses and needs further clarification.

Among the four fractions of seed protein, i.e. albumins, globulins, gliadins and glutenins, the albumins and globulins are concentrated in the embryo and are rich in essential amino acids, while the gliadins and glutenins, constituting 75 to 90 % of the seed proteins, are present in the endosperm and are low in lysine. Chromosome assignment of genes controlling various components of gliadins has been attempted through one-dimensional electrophoresis (Solari and Favert 1967; Shepherd 1968; Boyd et al. 1969) and a combination of electrophoresis and isoelectric focusing (Wrighley and Shepherd 1973; Aragoncillo et al. 1975) using various aneuploid series of wheat. Recently, SDS-electrophoresis has been employed for chromosome localization of genes controlling glutenins (Orth and Bushuk 1974). Components of other protein extracts, such as albumins, purothionins and chloroform-methanol extracts, have been associated with different chromosomes. These analyses indicate that there are several genes coding for different fractions of seed proteins which are distributed throughout the seven homoeologous groups comprising A, B and D genome of *T. aestivum*. Information on divergence of homoeoalleles from the related genomes and their expression in the polyploids is unknown.

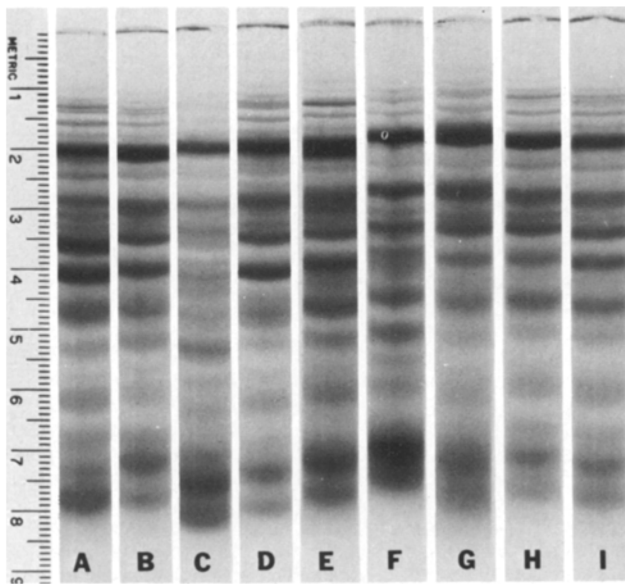

Fig. 1. SDS-electrophoretic patterns of albumins: A) *Triticum boeoticum;* B) *T. urartu;* C) *Ae. speltoides;* D) *T. turgidum;* E) *T. timopheevii;* F) *Ae. squarrosa;* G) *T. aestivum;* H) *boeoticum-urartu* amphidiploid; and I) Albumin mixture (1:1) from *T. boeoticum* and *T. urartu*

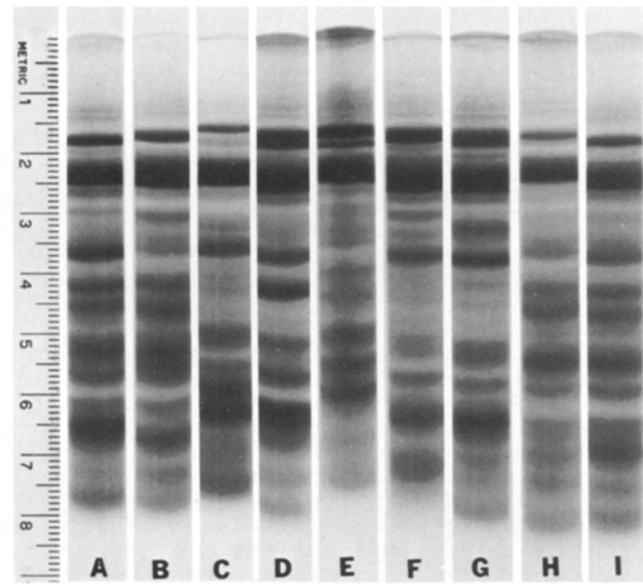

Fig. 2. SDS-electrophoretic profiles of globulins: A) *T. boeoticum;* B) *T. urartu;* C) *Ae. speltoides;* D) *T. turgidum;* E) *T. timopheevii;* F) *Ae. squarrosa;* G) *T. aestivum;* H) *boeoticum-urartu* amphidiploid; and I) Globulin mixture (1:1) from *T. boeoticum* and *T. urartu*

Most of the genetic studies on seed proteins have been done exclusively on *T. aestivum.* Due to limited genetic variability and high genotype × environment interaction for proteins in *T. aestivum* (Johnson et al. 1973) and sensitivity of expression of certain genes at different gene and genome dosages in the polyploids, unambiguous genetic analysis through a conventional genetic approach is virtually impossible. Among other approaches SDS-electrophoresis might be more suitable for such studies than electrophoresis and isoelectric focusing, since the latter reveal incomprehensible genetic variability among different genotypes as a result of minor amino acid substitutions. For a complete analysis of seed proteins, nutritionally important fractions-albumins and globulins-need to be investigated as thoroughly as possible. Studies on seed proteins in diploid and tetraploid wheats and diploid *Aegilops*, from which *T. aestivum* was derived, may throw more light on the nature of genetic variability and strategy for improvement of seed protein in the *Triticum-Aegilops* group.

This paper reports the results of SDS-electrophoresis of albumins, globulins, gliadins and glutenins from *T. boeoticum* (2n = 14), *T. urartu* (2n = 14), *T. turgidum* (2n = 28), *T. timopheevii* (2n = 28), *Ae. speltoides* (2n = 14), *Ae. squarrosa* (2n = 14), *T. aestivum* (2n = 42) and *boeoticum-urartu* amphidiploid. The SDS-electrophoretic patterns for various species within each of the fractions were similar suggesting a limited variability for the structural genes coding seed proteins. A hypothesis for the multiplication and expression of genes for gliadins and glutenins is discusssed.

Materials and Methods

Seed proteins comprising albumins, globulins, gliadins and glutenins were extracted from one or more accessions of each of *T. boeoticum* (G 1758, G 1916, G 1937, G 2609, G 3120), *T. urartu* (G 1545, G 1734, G 1754, G 1937, G 3135), *T. turgidum* (G 497, G 1392, G 2122, G 2137, G 3058, G 3100), *T. timopheevii* (G 383, G 993, G 1763, G 2608, G 2660), *T. aestivum* (G 357, G 524, G 529, G 531), *Ae. speltoides* (G 1273, G 1274), *Ae. squarrosa* (G 963) and *boeoticum-urartu* amphidiploid (G 1004 × G 1545, G 1004 × G 1754). These accessions were taken from the wheat collection maintained at the University of California, Riverside.

Albumins were extracted with distilled water from the ground seed (1:3 w/v) for 30 min with continuous stirring in an ice bath. The mixture was centrifuged at 10,000 × g for 15 min. The pallet was resuspended and extracted for the second time with three volumes of distilled water for 30 min and centrifuged. The pooled supernatant from the two extracts was dialysed against distilled water for 48 hrs at 4°C. The dialysate was centrifuged and freeze-dried.

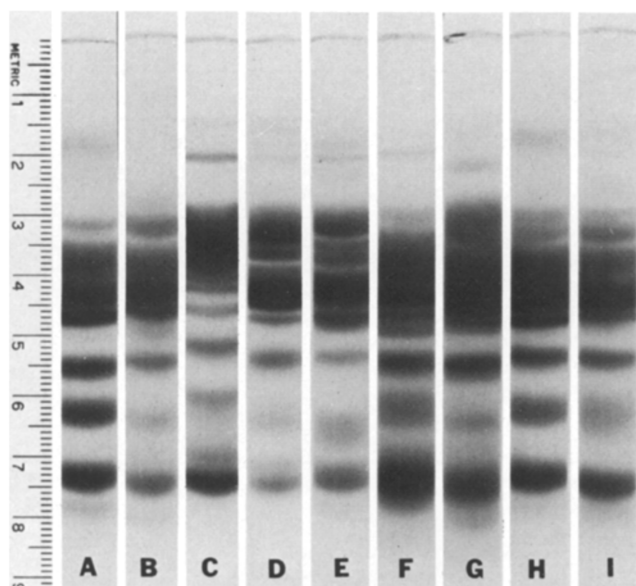

Fig.3. SDS-electrophoretic patterns of gliadins: A) *T. boeoticum;* B) *T. urartu;* C) *Ae. speltoides;* D) *T. turgidum;* E) *T. timopheevii;* F) *Ae. squarrosa;* G) *T. aestivum;* H) *boeoticum-urartu* amphidiploid; and I) Gliadin mixture (1:1) from *T. boeoticum* and *T. urartu*

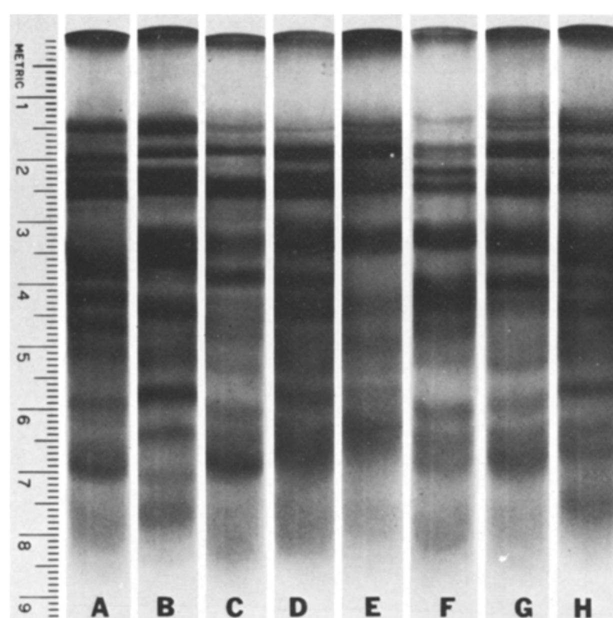

Fig.4. SDS-electrophoretic patterns of glutenins: A) *T. boeoticum;* B) *T. urartu;* C) *Ae. speltoides;* D) *T. turgidum;* E) *T. timopheevii;* F) *Ae. squarrosa;* G) *T. aestivum;* and H) *boeoticum-urartu* amphidiploid

To extract globulins the pallet, after extraction of albumin, was rinsed thoroughly with distilled water without resuspension and finally extracted twice each for 30 min in 5 % aqueous NaCl solution (1:3 w/v). The supernatant (10,000 × g for 15 min) was dialysed against distilled water for 48 hrs at 4°C. The globulins, being insoluble in distilled water, precipitate in the dialysis tubing. The globulin precipitates collected after centrifugation were dispersed in a large volume of distilled water and vigorously shaken to wash them free from albumins which are often extracted in salt solutions. After two washings, the precipitates were dispersed in a small volume of distilled water and freeze-dried.

Gliadins were extracted twice with 70 % ehtanol (1:3 w/v) for three hours with continuous shaking from the pallet saved after globulin extraction. The extraction mixture was centrifuged at 10,000 × g for 15 min and the supernatant was dialysed against distilled water for 48 hrs at 4°C. The dialysate was centrifuged in order ot get rid of milky suspension and the resultant clear supernatant was freeze-dried.

For extraction of pure glutenins, free from contamination with gliadins, the method of Bietz, Shepherd and Wall (1975) was followed. The pallet after gliadin extraction was extracted with 0.7 % aqueous solution of acetic acid for 2 hrs (1:20 w/v). The mixture was brought to a concentration containing 70 % ethanol by the additions of a calculated amount of 95 % ethanol and was shaken for 20 min. The pH of the mixture was adjusted to 6.8 by the addition of 2N NaOH. The gliadins being soluble in 70 % ethanol stay in solution while the glutenins precipitate. The container was placed overnight in a refrigerator to allow the glutenin precipitate to settle. The supernatant containing soluble gliadins was discarded. The residue including precipitates was lyophilized. The dry residue was incubated with 0.01 M sodium phosphate buffer containing 0.4 % SDS and 1 % 2-mercaptoethanol at 40°C for 16 hrs and centrifuged. The supernatant containing SDS-glutenin complex was fractionated like the other extracts.

The SDS-polyacrylamide gel electrophoresis was performed according to the method of Weber and Osborn (1969) in 10 % polyacrylamide gels using sodium phosphate buffer (pH 7.0) containing 0.1 % SDS. The SDS-protein complexes were prepared by dissolving one mg of dried protein extracts in 0.5 ml of 0.01 M sodium phosphate buffer containing 0.4 % SDS and 1 % 2-mercaptoethanol. The mixture was incubated at 40°C for two hours. The samples for the gel columns were prepared by mixing 150 µl of the incubated mixture, 25 µl of 2-mercaptoethanol and 10 µl of 0.05 % bromophenol blue. Only 100 µl of the mixture was applied to each column. The electrophoresis was performed at one ma/column for 15 min and then at 8 ma/column for 2 1/2 hrs. The gels were fixed and stained simultaneously according to Koenig et al. (1970). The solution consisted of 1 part of 1 % Coomassie brilliant blue and 40 parts of 6 % trichloroacetic acid in acetic acid-methanol-water (1:20:80, v/v/v). Destaining was done with three changes of acetic acid-methanol-water (20:120:280, v/v/v). In cases of poor staining the gels were stained and destained for a second time. The gels were stored in 7 1/2 % acetic acid until photographed.

Results

Representative SDS-electrophoretic patterns of albumins, globulins, gliadins and glutenins from various

Table 1. Number of major and minor components in SDS-electrophoretic and isoelectric focusing (IEF) profiles of different seed protein extracts of *Triticum* and *Aegilops* species

	Number of components						
	SDS-electrophoresis					Isoelectric focusing	
Species	albumins	globulins	gliadins	glutenins	Total	Gliadins	Alubmins
T. boeoticum	21-23	19	9-10	17	64-67	16-18	33-37
T. urartu	17-22	19	9-11	18	63-70	21-25	34-43
T. turgidum *	22-24	19	10-12	16	67-71	20-24	34-35
T. timopheevii **	20-23	18	9-10	14	61-65	19-30	34-35
T. aestivum	22-25	18	11-12	16	67-71	25-26	34-43
Ae. speltoides	20-21	15	11-12	17	64-66	23	47
Ae. squarrosa	22-24	18	10	16	66-68	23	34
boeoticum-urartu amphidiploid	22	21	11	17	71	21-26	29-30

* includes *T. dicoccoides*, *T. durum* and *T. dicoccum*
** includes *T. araraticum*

Triticum and *Aegilops* species are shown in Figs. 1-4, respectively. The migration of the SDS-protein complexes in the gels in a given time was slower in gels containing more proteins. Because protein concentrations per column differed from one species to another, the fastest but apparently homologous bands in some gels did not migrate the same distance (Figs. 1 and 3).

With the exception of difference in density of the albumin components at 3.6 and 4.0 cm in *Ae. speltoides* (Fig. 1c), most of the major and minor components were essentially identical in *T. boeoticum*, *T. urartu*, *T. turgidum*, *T. timopheevii*, *T. aestivum*, *Ae. speltoides*, *Ae. squarrosa* and *boeoticum-urartu* amphidiploid (Fig. 1). The total number of major and minor components of albumins varied from 17 to 25 (Table 1) over all the *Triticum* and *Aegilops* accession observed. The profile of *boeoticum-urartu* amphidiploid (Fig. 1H) was similar to that of the albumin mixture (1:1) of *T. boeoticum* and *T. urartu* (Fig. 1I).

Similarly in the case of globulins (Fig. 2), with the exception of the density difference for components at 3.6 and 4.3 cm, different species of *Triticum* and *Aegilops* had similar patterns. The total number of major and minor components of globulins varied from 15 to 21 among different accessions of the *Triticum* and *Aegilops* species. As for the albumin patterns, the globulin pattern of the *boeoticum-urartu* amphidiploid (Fig. 2H) was the same as that of the globulin mixture (1:1) of *T. boeoticum* and *T. urartu* (Fig. 2I) and represented the addition of its parental patterns (Fig. 2A, 2B). Although the albumin and globulin spectra were very similar with respect to MW distribution, they comprised components with altogether different solubility characteristics.

The gliadin patterns (Fig. 3) of different *Triticum* and *Aegilops* species were also quite similar. They differed from each other only in concentration of a group of massive bands between 3.5 and 4.5 cm. Proteins extracted with 70% ethanol, although frequently referred to as gliadins, also contain albumins and low MW glutenins (Nielson et al. 1968). Most of the gliadin bands are presumably between 3.5 and 4.6 cm of the profiles, while the faster moving bands with low MW's comprise albumins and the slow-moving faintly staining bands consists of low MW glutenins. Once again, the gliadin spectra of the *boeoticum-urartu* amphidiploid (Fig. 3H) and gliadin mixture (1:1) of *T. boeoticum* and *T. urartu* (Fig. 3I) were identical. The total number of major and minor components of gliadins varied from 9 to 12 among different accessions tested. The MW distribution of the gliadin components was narrower than that of the albumins and globulins.

Like the other three fractions, the glutenin profiles (Fig. 4) were also very similar from one species to another, especially in migration of various components. As for other fractions, there were the usual concentration differences of components at 1.5 and 5.9 cm (Fig. 4) among different species. The number of glutenin components varied from 14 in *T. timopheevii* (Fig. 4E) to 18 in *T. urartu* (Fig. 4B). The glutenin pattern of the *boeoticum-urartu* amphidiploid

(Fig.4H) represented a simple addition of the parental spectra (Fig.4A, 4B) for MW and density of different components. The general appearance of the glutenin spectrum was more like that of the albumin and globulin spectra than that of its closely related gliadins.

Discussion

Number of genes coding seed proteins

The protein extraction procedure followed here is expected to extract 100 percent of the seed proteins, although the absence of proteinous nitrogen in the residue pallet was not tested. Assuming that (i) all the polypeptides, the product of the structural genes coding seed proteins, are represented either as a major or as a minor component in one, and only one, fraction of the seed protein extracts, i.e. albumins, globulins, gliadins and glutenins, and (ii) there is no post translation modification, the total number of major and minor components of the four fractions of seed proteins should correspond to the number of genes controlling the seed proteins. Most of the above requirements are adequately fulfilled. The four fractions were very distinct with respect to their solubility. Sufficient precautions were taken to avoid extraction of the same components in two different extracts. Albumins were extracted twice before the salt extraction, which is otherwise known to extract albumins besides globulins. The globulin precipitates were washed to get rid of any contaminant albumin.

The glutenins are frequently contamined with gliadins. The method for glutenin extraction followd here reduces their contamination with gliadins. This is evident from the glutenin spectrum (Fig.4) which is very different from that of the gliadins (Fig.3). Furthermore, similarity of the electrophoretic patterns among different accessions of a given species and among different species for each of the fractions suggests the absence of any random modification of polypeptides of the storage proteins. Specific modification, mostly comprising inter- and intra-molecular disulfide bridges, is nullified on complexing of proteins with SDS in the presence of 2-mercaptoethanol.

Fractionation of the polypeptides in the SDS-electrophoretic system is purely a function of their MW's and not the native charge (Reynolds and Tanford 1970). Therefore, more than one polypeptide corresponding to different structural genes but with the same MW would migrate as a single band in the SDS-electrophoretic pattern. With the present approach, therefore, one can only estimate a minimum number of genes coding seed proteins in wheat. By pooling the number of major and minor components of SDS-electrophoretic patterns of the four fractions in individual species, the minimum number of genes coding seed proteins ranged from 60 to 70 in every *Triticum* and *Aegilops* species tested (Table 1). Isoelectric focusing of the seed proteins or two-dimensional fractionation combining electrophoresis and isoelectric focusing reveals more components and hence genes coding seed proteins (Wrighley and Shepherd 1973). The number of components in the IEF pattern of the albumin extract (B.S. Gill and H.S. Dhaliwal, unpublished) was close to that of their SDS-pattern (Table 1) but the number of components in the IEF-pattern of the gliadins was as high as 30 against 9 to 12 in the SDS-electrophoretic spectra (Table 1), suggesting that there are more genes coding gliadins than indicated by the SDS-electrophoretic system. By combining electrophoresis and isoelectric focusing, Aragoncillo et al. (1975) resolved the non-gliadin component of 70 % ethanol extract, presumably comprising 2 to 3 bands in the SDS-pattern between 5 and 8.0 cm (Fig.3), into 21 components. Assuming that the globulins behave like albumins and glutenins like gliadins, we can add about 60 to 70 more genes coding the seed proteins in addition to the 60 to 70 genes already estimated from the SDS-electrophoretic patterns. Thus, there are 120 to 140 genes coding for seed proteins in each of the diploid *Triticum* and *Aegilops* species. Tetraploid and hexaploid wheats, combining two and three genomes from different diploid *Triticum-Aegilops* species, respectively, must possess two and three times as many structural genes for the seed proteins as in the diploid species. *Triticum aestivum* (AABBDD), therefore, possesses about 360 to 420 genes coding for its seed proteins. This estimate of the number of genes is obviously too high to be realized by any biometrical approach or manipulated by any breeding method.

Variability and expression of structural genes for proteins

SDS-electrophoretic patterns of each of the four fractions from different *Triticum* and *Aegilops* species

were similar. They differed from one species to another occasionally, more in the concentration of certain components than qualitatively. This suggests that among different species of *Triticum* and *Aegilops* the structural genes for the seed proteins have not diverged from one species to another. This may be expected because the *Triticum* and *Aegilops* species investigated here are closely related to each other by descent either through convergent or divergent evolution.

Fractionation in IEF is the function of native charge of polypeptides. But the IEF patterns of tetraploid and hexaploid wheats for each of the fractions did not show more bands when compared with those of the diploid wheats and *Aegilops* (Table 1), especially when the polyploids contain genomes from two and three distinct diploid species, respectively. This may be due to one or both of the following reasons: (i) The homoeoalleles from different species code for polypeptides which, besides their homogeneity for MW's, have also not diverged in their amino acid composition. (ii) In polyploids only one or the other homoeoallele is expressed.

During attempts to locate certain protein components on chromosomes, some of the components in two-dimensional fractionation were never absent when each of the three homoeologues was missing at a time (Aragoncillo et al. 1975), suggesting that some of the polypeptides coded by homoeoalleles from different genomes of hexaploid wheats had not diverged in their MW's and amino acid composition. But this may be true only for a small fraction of homoeoalleles. Mostly, the homoeologous chromosomes from A, B and D genomes had distinct components (Wrighley and Shepherd 1973; Aragoncillo et al. 1975) presumably representing diverged homoeoalleles.

Available evidence suggests that some of the homoeoalleles may not be expressed in the polyploids. Orth and Bushuk (1974) reported suppression of synthesis of the subunits of glutenins coded by genes on A and B genomes in lines tetrasomic for chromosomes 2B, 3B and 6B. Similarly, component 2 of the non-gliadin fraction of 70 % ethanol extract, coded by a gene on chromosome 6B, was suppressed in tetrasomic 7B (Aragoncillo et al. 1975). Because of the extra dosages of homoeologues in the polyploids, the phenomenon of suppression may be operating more universally for various components of all the protein fractions, so that only one of the homoeoalleles is expressed in the polyploids.

There is a progressive decrease in the seed protein content from diploid to polyploid wheats in spite of obvious increases in the number of structural genes for seed proteins in the polyploids. The decrease in protein content in the polyploids is mainly attributed to their increased seed size, as there is a negative correlation between seed size and protein content. The precise mechanism for decrease in protein content with increase in seed size, however, is unknown. It may be due to: (i) a limit to total seed protein content per seed imposed by a general feedback inhibition irrespective of the seed size: (ii) expression of one or more homoeoalleles in the polyploids in such a way that their cumulative product is constant: or (iii) a limited storage space for proteins in the seed which may be localized peripherally so that the storage space decreases with per unit increase in seed volume. Evidence reported here indicates that only a limited number of homoeoalleles are expressed in the polyploids. Furthermore, the aleurone layer is rich in proteins and is distributed peripherally, but it accounts for only a small proportion of the total seed proteins. Gliadins and glutenins, constituting 80 to 90 % of the storage protein in wheat, are very similar to zein of corn in amino acid composition. Zein is reported to be synthesized primarily by polyribosomes (Brian et al. 1976) which are bound to the membranes of protein bodies, the sites of zein deposition (Burr and Burr 1976). The protein content in wheat may be regulated through the number, size, distribution or mRNA's specificity of protein bodies in wheat endosperm.

Evolution of structural genes for seed proteins: a hypothesis

Different components of each of the four fractions exhibit a wide spectrum of MW's ranging from 12,000 to 150,000. Our knowledge of a relationship among different components of a particular extract, or components from different extracts, with respect to their amino acid composition/sequence is very limited. Purification and characterisation have been done for only a few components of gliadins (Bietz, Huebner and Rothfus 1970) and glutenins (Bietz and Rothfus 1970). Components of different extracts with the same MW's usually

have a different amino acid composition, while the components of a particular extract with different MW's have a similar amino acid composition.

The number of components in IEF and SDS-electrophoretic spectra of albumins are approximately the same (Fig. 1; Table 1), while the number of components in IEF spectrum (Table 1) or two-dimensional fractionation (Wrigley and Shepherd 1973) of gliadins is 3 to 5 times more than that of their SDS-pattern. In this respect, gliadin is very similar to zein, its analogous protein from corn. Zein gives 4 to 5 bands in SDS-electrophoresis but 24 to 30 on IEF (Soave et al. 1976). Zein is also extracted with 70 % ethanol. It constitutes 50 to 55 % of the total seed protein and, like gliadins, is low in lysine. The amino acid composition of different IEF components of zein is very similar (Gianazza et al. 1971). Also different components of gliadins and glutenins show considerable similarity in amino acid sequences (Bietz et al. 1970; Bietz and Rothfus 1971). The majority of the gliadin components have been assigned to homoeologous groups 1 and 6 of *T. aestivum* (Wrighley and Shepherd 1973). Similar chromosomal assignment has not been possible for different components of zein. It is highly likely that different components of zein, gliadins and glutenins with the same MW, but slightly different amino acid compositon, are coded by genes evolved as a result of multiplication of a single original gene. Different replicates of a single gene accrued independent amino acid substitutions during the course of evolution which can be resolved as distinct components on IEF. The wide range in MW's of different components of a given extract with similar amino acid composition and common sequences (Bietz et al. 1970) might have been accomplished as a result of unequal crossover between sister chromatids (Smith 1976) in the structural genes for seed proteins.

All the structural genes which originated through multiplication of a single gene presumably comprise one transcription unit (polycistronic mRNA) or at least share the same or a common regulatory system. Brian et al. (1976) have reported membrane-bound polysomes, that synthesize zein, of large enough size to synthesize more than one Z23 polypeptides. The opaque-2 mutant of corn strongly represses the Z23 chains with only a little effect on other chains of zein (Soave et al. 1976) suggesting that different Z23 polypeptides presumably haven polycistronic mRNA. Other endosperm mutants of corn, o7, f12, alter amino acod composition by repressing uniformly all the zein polypeptides without adding or deleting any polypeptide. This suggests that a group or all the structural genes for zein share the same regulatory mechanism. The same may be true for other storage protein fractions of corn and various fractions of wheat.

Partial to complete dominance of low protein content over high protein content in wheat and other cereals is presumably the expression of a dominance relationship between regulatory genes rather than between structural genes for seed proteins. Electrophoretic spectra of cultivars and mutants differing in protein content normally do not differ qualitatively, suggesting that the structural genes do not exhibit dominance-recessiveness relationships. Furthermore, among many structural genes for storage proteins, failure of expression of a few cannot produce detectable differences in total protein content. Regulatory genes are few in number and control the quantitative expression of several structural genes.

Improvement of seed proteins

Similarity of the SDS-electrophoretic patterns (Figs. 1-4) of each of the four fractions within and among different *Triticum* and *Aegilops* species suggests that there is practically no variability for structural genes coding for seed proteins. However, electrophoretic and IEF spectra reported so far revealed considerable variation for the structural genes, which is mostly attributable to minor amino acid substitutions. Manipulation of genes for polypeptides differing in only a few amino acids would hardly change amino acid composition of the total seed proteins. Comparing the SDS- and IEF-patterns of diploid, tetraploid and hexaploid wheats, it appears that the polypeptides with slightly different amino acid composition but the same MW are regulated in such a way that their cumulative product is independent of their number, since the hexaploid wheat with three homoeoalleles instead of one in the diploids gave the same intensity of components of different seed protein fractions. Manipulation of this seemingly tremendous but practically elusive variability within the *Triticum-Aegilops* group does not offer

a good prospect for improvement of proteins. This is corroborated by the results of protein analysis of 17,000 *durum* and common wheat cultivars (Johnson and Lay 1974), where the genetic component of the total grain protein variation was negligible, in spite of 7 to 22 % variation for protein content. The same was true for lysine percentage. Their claim of a transgressive segregate for high protein content from a cross of 'Atlas 66' × 'Nap Hal' was most probably due to different structural genes for proteins in the parental lines.

Seed protein content in wheat can probably be improved by incorporating structural genes, from related genera such as *Secale* and *Agropyron*, which code for components with MW's different from these of the corresponding fractions of *Triticum* or *Aegilops*. SDS-electrophoresis can be used for screening such genes and to subsequently follow their incorporation in wheat during segregating generations. It is likely that such genes would not be homoeoallelic to those of the *Triticum* or *Aegilops* genomes and would probably exhibit different sensitivity to feedback inhibition and gene or genome-dosage dependent expression. Fortunately, wheat can be crossed easily with other related genera, and with the help of aneuploid series available in wheat alien-substitution or addition lines can be produced to test the effect of specific structural genes from other genera on protein content. Reduced chromosome pairing between distantly related genomes, however, would interfere with the transfer of specific genes. With the development of advanced techniques for genetic alteration, such as cloning the desired genes and their integration to other genomes through suitable vectors, it would be possible to exchange structural genes for proteins among species which normally to not hybridize or whose genomes show no meiotic pairing.

The quality as well as quantity of wheat proteins can also be improved by the use of regulatory mutants similar to opaque-2 of corn (Mertz et al. 1964). Gliadins of wheat being similar to zein of corn would presumably respond to such a mutant in the same fashion. Mutants which would decrease the amount of gliadins in relation to albumins and globulins would perhaps be more suitable, as glutenin of wheat is very similar to gliadins (Bietz and Rothfus 1971) and is not rich in lysine like glutelin of corn. Differences in density of certain components in the SDS-patterns of various fractions suggest variation within and among different species for the regulatory genes. 'Atlas 66', a cultivar of common wheat, presumably possesses a regulatory gene which increases the protein percentage by 2 to 3 % in any background (Johnson et al. 1973). Similarly the variety 'Nap Hal' possesses a regulatory gene which suppresses certain glutenin components (Bietz, Shepherd and Wall 1975) and gives high lysine and high protein content. Such regulatory mutants can be easily induced (Bhatia et al. 1970). With thorough understanding of evolutionary and linkage relationships among genes controlling major seed proteins, and the mechanisms of their regulation by endosperm mutants, it would be possible to alter objectively the content and quality of wheat proteins.

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