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The effect of additions of *Aegilops longissima* chromosomes on grain protein in common wheat*

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Summary. The effect of various chromosomes of *Aegilops longissima* when added to the common wheat cultivar 'Chinese Spring' was evaluated at two levels of nitrogen fertilization for absolute and relative amount of protein in the grain. All the added chromosomes of *Ae. longissima* increased protein percentage: protein increase by chromosomes D, C and A averaged 3.8% while that by chromosomes F, E, G and B averaged 1.7%. Addition lines F, D and C had a significantly higher protein weight per grain. On the other hand, lines A, E and G had reduced grain protein weight per grain as compared with that of 'Chinese Spring'. Line C carries the HMW glutenin and some of the gliadin subunits of *Ae. longissima.* The effect of this line, however, and obviously that of the other lines on protein content was through genes controlling the level of storage protein rather than through genes that code directly for these proteins. Nitrogen fertilization affected protein content and the relative amount of the various protein fractions in a similar manner in every addition line. When high levels of nitrogen fertilization were compared to low ones, the relative amount of the HMW glutenins remained constant while that of HMW gliadins increased and that of the LMW subunits decreased. In contrast to the nitrogen effect, increase in protein content by the addition of *longissima* chromosomes did not change the relative amounts of the various protein fractions.

Key words: *Aegilops longissima* chromosomes – Nitrogen fertilization – Protein content – Wheat

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Introduction

The nutritional quality of wheat is largely determined by the protein content of its grains (Kasarda et al. 1971; Olson and Sander 1975). No wonder, therefore, that this trait has been subjected to intensive studies. Johnson et al. (1973) reported that protein percentage, in a collection of 20,000 cultivated wheat varieties, varied from 7% to 22% but only 5% of this variation was genetically determined. This narrow range of genetic variability is one of the main constraints for the improvement of grain protein content in wheat.

This narrow genetic basis of cultivated varieties, resulting, among others, from modern breeding practices (Feldman and Sears 1981), has stimulated intensive research efforts to increase protein content through induced mutations. Results obtained so far by this approach have been relatively poor (Corpuz et al. 1983).

The wild relatives of wheat have a much wider range of genetic variability in grain protein percentage and, therefore, may serve as a potential source for genes contributing to high grain protein content. Avivi (1978, 1979) found that several Israeli collections of the wild tetraploid wheat *T. turgidum* var. 'dicoccoides', the immediate progenitor of most cultivated wheats, contained an exceptionally high percentage of grain protein, ranging from 14 to 29% when grown in its natural habitat, and up to 43% when grown under greenhouse conditions. Sharma et al. (1981) found in five accessions of another wild tetraploid wheat, *T. timopheevii* var. 'araraticum', a range of 23 to 30% for in grain protein in material grown in a nursery. High grain protein types were also discovered in species of *Aegilops.* Yamashita et al. (1957) analyzed the flour quality of *Ae. squarrosa, Ae. cylindrica* and *Ae. ventricosa* and reported a somewhat higher percentage of protein (15–16%) than that of cultivated wheats (12–14%). Mettin (1964) found a relatively high percentage of protein in the flour of *T. boeoticum* (25%), *Ae. speltoides* (26%) and *Ae. squarrosa* (23%). In a more recent study, Mettin et al. (1977) studied grain protein percentages in 23 species of *Aegilops* grown under nursery conditions and found an overall variation in protein percentage ranging from 21 to 31%.

The high grain protein percentage of wild wheat relatives may prove useful as a gene source in a quality breeding program. A useful approach for studying the genetics of high grain protein in wild wheats is through the use of a series of alien addition lines, each possessing the full chromosome complement of a common wheat cultivar and one pair of chromosomes from a wild relative. In such lines, the contribution of each alien chromosome pair to grain protein content can easily be evaluated. Moreover, any interaction of the protein genes from the alien species with those of the cultivated wheat as well as with other traits of the cultivated parent can also be studied. This paper reports on the effect of the different chromosomes of *Ae. longissima* on grain protein percentage and weight per grain, when added to common wheat.

Aegilops longissima, a diploid species (2n = 14), whose genome is closely related to the B genome of common wheat (Feldman 1978), contains 20–26% protein in its grains (Avivi, unpublished). The availability of a series of *longissima* addition lines on the background of the common wheat cultivar 'Chinese Spring' (Feldman 1975) enabled us to evaluate the effect of individual *longissima* chromosomes on the grain protein level as well as on the relative amount of the various protein fractions in common wheat. These studies were carried out under two levels of nitrogen fertilization.

Materials and methods

The *longissima* addition lines produced at our laboratory (Feldman 1975) were grown in a net-house together with 'Chinese Spring' (the recipient variety) and *longissima* (the donor line) during the winter season of 1982/83. Plants were treated at two levels of fertilization: 8 g and 16 g of pure nitrogen per m^2. The seeds of the addition lines used in this experiment were obtained from plants cytologically checked at meiosis for 22 bivalents. Their alien addition nature was confirmed by the morphology of the plants during the experiment. Line B was composed of several genotypes (Table 2). Nevertheless, it was included in the experiment with the anticipation of finding the average performance of these types.

The experimental design consisted of four blocks with split plots; each block consisted of two main plots, one for each fertilization level. Each main-plot consisted of eight to nine rows: seven addition lines, 'Chinese Spring' and *Ae. longissima*. Because of poor germination, *Ae. longissima* was included only in one plot (a low nitrogen one). Each row represented a subplot and contained four plants of one genotype, spaced 10 cm apart. The distance between rows was 25 cm, and between plots 70 cm.

The parameters measured for each row were the average yield per plant, grain weight and grain protein percentage. Protein percentage was determined by infrared reflectance, using a neotec GQA. Protein weight per grain was estimated by multiplying the protein percentage by grain weight. The data were analyzed in a factorial analysis of variance. *Aegilops longissima* was not included in the statistical analysis.

The electrophoretic pattern of the total endosperm proteins of each genotype at the two fertilization levels was determined by a high resolution one-dimensional sodium-dodecyl

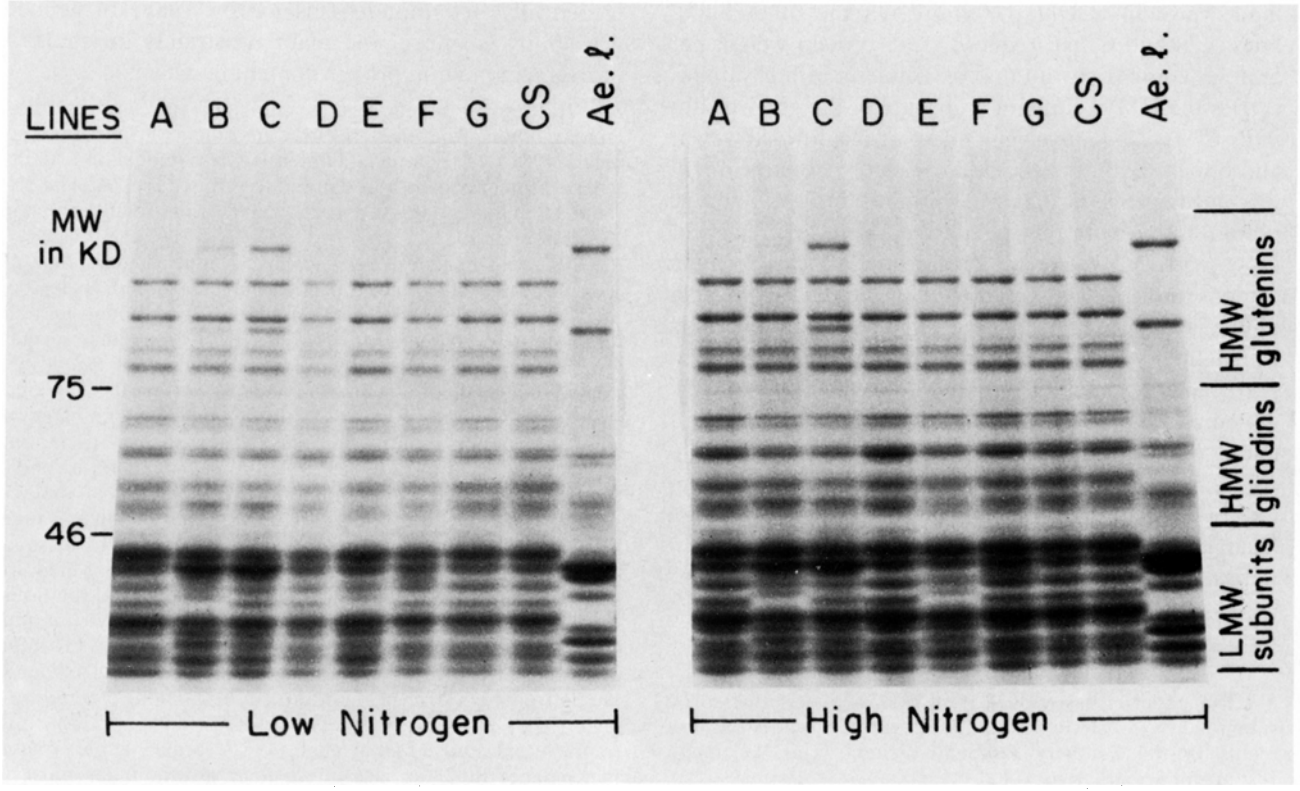

Fig. 1. SDS polyacrylamide gel electrophoresis patterns of seven *longissima* addition lines (*A–G*), 'Chinese Spring' (*CS*) and *Aegilops longissima* (*Ae. l.*) at two levels of nitrogen fertilization

sulphate (SDS) polyacrylamide gel electrophoresis, as described by Galili and Feldman (1983a). The conditions for protein extraction were similar for every combination: 10 mg flour were dissolved in 1 ml sample buffer solution (consisting of 10% glycerol, 3% SDS, 5% 2-mercaptoethanol, 66 mM Tris-HCl, pH 6.8) for 2 h at room temperature. The samples were then heated at 100 °C for 2 min and centrifuged in an Eppendorf centrifuge for 2 min. Sixteen ml of the supernatant were then loaded on each lane of the gel. The protein bands in each gel pattern were divided into three fractions according to their molecular weight (Galili and Feldman 1983a) (Fig. 1): the high molecular weight (HMW) glutenins (above 75 KD), the high molecular weight (HMW) gliadins (46–75 KD) and low molecular weight (LMW) subunits (under 46 KD). Quantitative analysis of the stained gel patterns was obtained from densitometer tracings according to Galili and Feldman (1983b). Coomassie brilliant blue R-250 (Sigma) stained gels were photographed and a 1 : 1 transparent sheet was prepared. Each lane from this sheet was scanned by a Gilford spectrophotometer scanning apparatus at 500 nm. Under the extraction conditions used, a linear relation was found between the volume of protein extract loaded on each lane and the area under the scan tracing of each fraction. The area of each fraction was expressed by its weight in mg, and the relative area of each fraction was expressed as percentage of the total area.

Results

Analysis of grain protein percentage and weight per grain

Data were collected for each genotype at the two levels of nitrogen fertilization. The analysis of variance for grain protein percentage and protein weight per grain is given in Table 1 for a split plot model with four blocks considering two factors: fertilization at two levels and genotypes with eight levels (seven addition lines and 'Chinese Spring'). No significant interaction was found between levels of fertilization and the genotype. The average increase in grain protein as a result of increased fertilization was significant for both protein percentage and protein weight per grain. The general mean of grain protein percentage of all the addition lines and 'Chinese Spring' was 15.2% at the low fertilization level vs. 18.8% at the high level; average protein weight per grain was 4.5 mg at the low fertilization level vs. 5.4 at the high one.

Since the interaction between fertilization and genotypes was not significant, the mean values representing

Table 1. Analysis of variance for grain protein percentage and protein weight per grain (mg)

Source of variation	df	Protein %			Protein weight per grain		
		Mean square	F	P (F)	Mean square	F	P (F)
Block	3	1.80			0.09		
Fertilization	1	198.88	80.10	0.004	11.26	36.00	0.0093
Error (a)	3	2.48			0.31		
Genotypes	7	15.39	15.27	0.0001	7.21	50.28	0.0001
Fertilization × genotype	7	0.43	0.43	0.875	0.23	1.62	0.1570
Error (b)	42	1.01			0.14		

Table 2. Means[a] of grain protein percentage, grain weight (mg), grain protein weight (mg) and grain yield per plant (g) of the different *longissima* addition lines

Line	Homoeologous group[b]	Protein %	Grain weight (mg)	Grain protein weight (mg)	Grain yield per plant (g)
A	$2S^l$	18.3	22.7	4.14	1.29
B	see note[c]	16.1	30.0	4.84	2.41
C	$1S^l$	18.6	30.7	5.73	2.02
D	$7S^lL/4S^lS$	19.0	31.8	6.07	1.77
E	$2S^l+6S^l(-6B)$	16.8	24.1	4.06	1.32
F	$5S^l$	16.9	37.8	6.38	2.07
G	$3S^l$	16.4	23.9	3.88	1.21
CS		14.8	32.0	4.75	4.58
SE		0.36	0.66	0.13	0.22

[a] The mean values represent the average of the values obtained at the two levels of N fertilization

[b] The assignment of *longissima* chromosomes to the homoelogous groups indicate their genetic relationship to the homoeologous groups of the common wheat cultivar 'Chinese Spring'. This assignment was based on Feldman (unpublished); Netzle and Zeller (1984); Hart and Tuleen (1983)

[c] $6S^l(-6b)+$(t″ $2S^lL$ or tl″ $2S^lL+2S^lL/1S^lL$ or rarely 1″ $2S^lL/1S^lL$)

the average of the values obtained with the two fertilization levels were used to test the differences between the different genotypes using the Duncan test at 5% level of significance (Table 2).

The results indicated that every chromosome of *Ae. longissima*, when added to Chinese Spring, increased protein percentage. The experimental conditions permitted a clear differentiation between two levels of influences: that of chromosomes D, C and A which increased protein percentage by about 3.8% and that of chromosomes F, E, G and B which had a weaker but significant effect of increasing protein percentage by about 1.7%.

Since grain protein percentage represents the ratio between the protein components and the total dry matter, an increase in protein percentage may reflect either an increase in the crude protein component or a decrease in the carbohydrates, or both. While lines F, D and C had a significantly higher protein weight per grain than 'Chinese Spring', lines A, E and G had reduced protein weight per grain (Table 2). Hence, in the latter lines, the high protein percentage resulted from a smaller amount of carbohydrates per grain rather than from an increase in the protein components.

Interestingly, chromosome F had a strong positive effect on grain weight (37.8 mg vs. 32.0 mg of 'Chinese Spring'). All the addition lines exhibited a lower grain yield per plant than 'Chinese Spring'.

The grain protein percentage of *Ae. longissima* under conditions of low fertilization, was 20.2.

Qualitative analysis of the gels

The electrophoretic pattern of the addition lines was similar to that of 'Chinese Spring', except for the following: line C, whose *longissima* chromosome is related to homoeologous group 1, had two additional HMW glutenin subunits and, at least two gliadin subunits; lines B and E, which contain a *Longissima* chromosome related to homoeologous group 6, had two additional gliadin subunits but lacked the gliadin bands which are controlled by chromosome 6 B of 'Chinese Spring' (Fig. 1, in the LMW subunits). In line B an interesting phenomenon occurred: a pattern similar to line C could be observed in many gels but mainly at a low level of fertilization. Our explanation for this phenomenon is tentatively the following: as can be seen from Table 2, line B is comprised of several genotypes, some with the long arm of chromosome $1S^l$ ($1S^l L$), which carries the genes for HMW glutenins. The transmission of this chromosomal arm was apparently dependent on the level of fertilization: a higher level resulted in a reduced transmission of this arm. This phenomenon is under further study.

Quantitative analysis of the gel patterns

From the densitometer tracings of the gel patterns, a two factorial analysis of variance was done for the area under each protein fraction: HMW glutenins, HMW gliadins and LMW subunits. The factors considered were fertilization and genotype. Gels were considered as statistical blocks (Table 3). In each fraction, the interaction between fertilization and genotype was not significant for both expressions of the area, meaning that the absolute and relative response of each fraction to the level of fertilization was similar for all genotypes.

Table 4 shows the means of the absolute and relative area of each fraction under the scan tracing at the two levels of fertilization. The means values represent the average values obtained in the various genotypes. A Duncan test (at 5% level of significance) was performed for the two fertilization levels. In all genotypes the area under each fraction increased as a result of fertilization (the level of significance for those increases are in-

Table 3. Mean squares from the analysis of variance for the area under the scan tracing (expressed in mg) and the relative area[a] of each protein fraction

Source of variation	df	HMW glutenins		HMW gliadins		LMW subunits	
		Area	Relative area	Area	Relative area	Area	Relative area
Gel	1	46,208**	220.00**	6,641	1.67	2,096	260.66**
Fertilization	1	12,012**	1.96	94,503**	108.5**	67,988**	139.68**
Genotype	7	9,131**	9.58**	7,108*	15.61*	48,477**	9.38
Fertilization × genotype	7	1,537	2.02	6,933	3.06	14,087	5.19
Error	15	881	2.26	2,760	4.64	5,407	5.21

*,** Significant at 5% and 1% level of probability, respectively

[a] Calculated by the area of the relevant fraction divided by the whole area under the scan tracing of the gel pattern

Table 4. Means of the area under the scan tracing (expressed in mg) and of the relative area[a] of each fraction at the two levels of N fertilization. (Means with different letters designate significant differences at the 5% level)

	HMW glutenins		HMW gliadins		LMW subunits	
	Area (mg)	Relative area (%)	Area (mg)	Relative area (%)	Area (mg)	Relative area (%)
High fertilization	196 a	14.4 a	367 a	27.2 a	783 a	58.4 b
Low fertilization	157 b	13.9 a	258 b	23.5 b	690 b	62.6 a

[a] Calculated by the area of the relevant fraction divided by the whole area under the scan tracing of the gel pattern

Table 5. Means[a] of the area under the scan tracing (expressed in mg) and of the relative area[b] of each fraction in the various addition lines

	HMW glutenins		HMW gliadins		LMW subunits	
	Area (mg)	Relative area (%)	Area (mg)	Relative area (%)	Area (mg)	Relative area (%)
A	177	13.9	326	25.5	759	60.6
B	168	14.1	277	23.4	728	62.5
C	291	17.6	368	22.2	977	60.1
D	170	13.0	383	28.5	760	58.5
E	158	13.8	285	25.4	659	60.8
F	150	13.0	287	24.5	724	62.5
G	158	14.6	286	26.9	617	58.4
CS	139	12.9	284	26.1	664	61.0
SE	14.8	0.75	26.2	1.07	36.7	1.14

[a] The mean values represent the average of the values obtained in the two levels of N fertilization
[b] Calculated by the area of the relevant fraction divided by the whole area under the scan tracing of the gel pattern

dicated in Table 3). However, the relative size of each fraction depended upon the level of fertilization: the HMW glutenins remained at a constant level (about 14% of the total area) under high and low fertilization, while that of the HMW gliadins increased significantly and that of the LMW subunits decreased significantly at the high fertilization level.

The means of the area under the scan tracing and of the relative area of each fraction in the various addition lines are presented in Table 5. The mean values represent the average of the values obtained under the two levels of nitrogen fertilization. As can be seen from the table, small but significant differences exist among genotypes for the relative amounts of the three protein fractions. It is interesting to note that 'Chinese Spring' had the lowest percentage of HMW glutenins. Line C had the highest percentage of HMW glutenins, presumably due to its two additional bands, but a low percentage of HMW gliadins.

Discussion

Genetic control of protein content (percentage and weight per grain)

Each chromosome of *Ae. longissima,* when added to the full gene complement of 'Chinese Spring', caused an increase in grain protein percentage. Some chromosomes had a major effect (lines D, C and A) while others had a relatively minor one (lines B, E, F and G). A minor increase in grain protein percentage was also reported in several other alien addition lines.

Law et al. (1978) investigated the effect of alien chromosomes, homoeologous with the group 2 of common wheat, on protein percentage in grains of substitution lines of *Aegilops comosa* (2M), *Ae. umbellulata* ($2C^u$) and *Secale montanum* ($2R^m$) having the background of 'Chinese Spring': all these chromosomes had a positive effect on protein percentage. In both addition and substitution lines of *Agropyron elongatum* Dvorak and Sosulski (1974) found that five out of the seven chromosome of *A. elongatum* increased protein percentage. However, in all these instances the increase was relatively minor-

amounting to about 2%. Differences in protein percentage between aneuploid lines and their euploid control have been frequently considered as the physiological result of their lower fertility, and statistical techniques were used to compare different lines on a same yield level (Law and Brown 1978). Such techniques used such unwarranted assumptions as linear relationships between protein percentage and yield, and the possibility of extrapolation.

The differences in protein percentage between the different *longissima* addition lines could not be accounted for in terms of variation in yield per plant or grain weight, as can be deduced from Table 2: certain lines (C and D) has a relatively high yield per plant, high grain weight and high protein percentage while others (F and B) had high yield, high grain weight and a relatively lower protein percentage. The correlation between protein percentage and yield or grain weight is not necessarily negative; a similar observation has been reported elsewhere (Johnson et al. 1969). Also, a drastic reduction in spike size caused only a small compensation in protein percentage (Levy, unpublished). We therefore concluded that the genetic factors of *Ae. longissima*, which increase grain protein percentage, do not necessarily depend on grain weight or on plant yield. Although their yields per plant differed significantly, no adjustment should therefore be made, as Law and Brown (1978) did, when comparing the protein percentage of the addition lines with that of 'Chinese Spring' at a similar yield level.

Thus, while the increase in grain protein percentage in lines C and D, which had a similar grain size to 'Chinese Spring', was due to higher production of protein, this increase in line A, which had a smaller grain, resulted from a reduction in carbohydrate production. On the other hand, line F had a higher protein weight per grain and a higher protein percentage than 'Chinese Spring' despite the increased production of carbohydrates (about 20% larger grains). Therefore, the effects on grain protein should be evaluated in terms of protein weight per grain, which takes into account protein percentage and grain size and is not affected by the amount of carbohydrates. Protein weight per grain has been shown to be an interesting parameter for breeding (Loffler and Busch 1982; Levy et al., unpublished) since it was positively correlated with protein percentage and grain weight. At the same time, no correlation was found between protein weight per grain and plant yield (Loffler and Busch 1982). Lines F, D and C, which exhibited both a high protein percentage and high protein weight per grain, may have valuable genes for increasing protein content without affecting yield.

The increase of protein percentage without reducing carbohydrate production by at least three major genes (on chromosomes C, D and F) is in agreement with our previous reports showing segregation of several genes for high protein in F_2 populations from crosses between *durum* wheat and its wild relative *T. dicoccoides* (Avivi et al. 1983) as well as with other studies dealing with genetic control of seed protein in cultivated wheats (McNeal 1970; Jastra et al. 1978).

Most storage proteins in wheat are coded by genes located on chromosomes of homoeologous groups 1 and 6 (Wrigley and Shepherd 1973; Payne et al. 1980; Galili and Feldman 1983 a, b). It appears from Table 2 that the *longissima* addition lines carrying chromosomes such as F and D increased protein synthesis though they lacked genes for HMW glutenin and gliadin subunits, it is concluded that they act through providing conditions which promote synthesis of storage proteins rather than through direct coding for these proteins. Similarly, the increase in protein percentage in line C might also be due to genes that affect the level of protein synthesis.

Comparison between environmental and genetic effects on grain protein content and composition

Our report indicated that the environmental factors (fertilizer) affected protein content and the relative amounts of the various protein fractions in a different manner than the genetic ones. Fertilization increased protein percentage and protein weight per grain irrespectively of the genotype: that is, all the addition lines, as well as 'Chinese Spring', responded similarly. On the other hand, the increase in protein percentage caused by the genetic factors depended on the added chromosomes: lines C, D and A had a higher protein percentage than lines B, E, F and G, and the effect of the added chromosomes on protein weight per grain was an increase in the case of lines F, D and C, or a decrease for lines G, E and A while line B had no influence.

Protein composition of all genotypes was affected by fertilization in a similar manner. At a high level of fertilization, resulting in elevated protein percentages, relative levels of the HMW glutenins remained constant while those of the HMW gliadins increased and those of the lower MW subunits decreased. These results are in good agreement with those of Fullington et al. (1983) who found, in different wheat varieties, that the increase in total protein percentage due to fertilization was accompanied by a proportional decrease in albumin and globulin (the low MW subunits). However, unlike the environmental effect, the increase in protein percentage by additional chromosomes did not have a general trend of influencing protein composition: there were unpredictable differences between lines in the proportion of the various fractions.

It is therefore concluded that genetic and environmental effects on the protein composition and content should be considered separately. The differential effect

of these two factors may have a different significance relating to nutritive value and baking quality.

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