The Protein Fractions in Chemically Induced High-Protein Wheat Seed [1]

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ABSTRACT

The protein content of Arthur winter wheat grain was increased by a foliar application of 70 g. per ha. terbacil (3-*tert*-butyl-5-chloro-6-methyluracil). The protein content of Inia spring wheat grain was increased by applications of both 250 g. per ha. simazine (2-chloro-4,6-*bis*-ethylamino *s*-triazine) and supplemental nitrogen. The proteins from the seed were separated into albumins, globulins, gliadins, and glutenins by appropriate solvent extractions. The higher protein seed, based on percent total nitrogen, obtained either by chemical or nitrogen applications, contained more nitrogen as gliadins than seed from the control plots for both varieties, whereas simazine reduced the percent of total meal nitrogen incorporated into the albumin fraction of Inia spring wheat. The gliadins from high- and low-protein seed differed electrophoretically.

It has been demonstrated that small quantities of triazines, notably simazine (2-chloro-4,6-*bis*-ethylamino *s*-triazine), will increase the protein content of food and forage crops (1–3). Terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) has also been shown to increase the protein content of wheat (2). These chemicals, which are usually used as herbicides, apparently act as growth regulators when applied at rates less than 1 kg. per ha. At these rates they may increase nitrate uptake in plants and thereby produce forage or seed with a high protein content (4).

Grain seed of the same genotype which contains more protein due to application of these chemicals or nitrogen fertilizer produces more vigorous plants and in some

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cases higher yields (2,5–7). The mole percentages of glutamic acid and proline in wheat seed hydrolysates have also been shown to be positively related to seedling vigor and yield (6).

High-protein seed of rice (8), corn (9,10), wheat (11,12), and barley (13) generally accumulate larger amounts of glutelin, zein, gliadin, and hordein, respectively, when the protein is increased by fertilizers or genetic modification.

The objective of this research was to compare the protein fractions of nitrogen or chemically induced high-protein seed with low-protein seed grown under the same environmental (field) conditions. Wheat meal was analyzed instead of the flour, because of the importance of the embryo and aleurone layer to the developing seedling. In addition, whole grain is consumed in developing countries where chemical induction of high-protein grain would be most relevant.

MATERIALS AND METHODS

Samples of Arthur winter and Inia spring wheat were obtained from tests harvested in Michigan in 1969 and Mexico in 1970, respectively. The Michigan seed was from adjacent field plots, and the seed from Mexico was a composite of four replicates from a field experiment previously reported (2). Seed of uniform weight was ground with mortar and pestle until the meal passed through a 40-mesh screen. All chemicals were standard reagent grade. Total nitrogen content was determined by a micro-Kjeldahl method (14).

Fractionation of Meal Proteins

The albumins and globulins were extracted from meal (5 g.) treated with *n*-butyl alcohol (15) to remove lipids. This defatted meal was extracted with water and the supernatant dialyzed against water and centrifuged. The precipitate of the original water extraction was re-extracted with a pH 6.5, 0.023M phosphate buffer made 0.5M in NaCl. The supernatant of this extraction was dialyzed against water and then centrifuged. The supernatants and precipitates of this final centrifugation were combined with the supernatants and precipitates of the original centrifugation and are hereafter referred to as albumins and globulins, respectively.

Gluten proteins were obtained from the wet meal residue after water and salt extraction by the procedure of Jones et al. (16), as modified by Woychik et al. (17). Glutenins were separated from gliadins by adjusting the pH of a 70% ethanolic-0.01M acetic acid solution of gluten proteins to pH 6.5 to 6.7 (17,18), allowing the solution to stand overnight at 4°C., and then centrifuging off the glutenins.

All data are expressed on a moisture-free basis. The extraction of the different protein fractions was replicated four times. The data were subjected to analysis of variance and differences noted where the F values were significant.

Gel Filtration

Gel-filtration chromatography of albumins was performed on Sephadex G-100 with sodium acetate buffer, pH 4.4, as described by Moureaux et al. (19). Albumins (2 mg. nitrogen) were dissolved in the column buffer and eluted with the same buffer at 4°C. at a flow rate of about 25 ml. per hr. Fractions of approximately 7 ml. were collected by means of a time-lapse fraction collector. The absorbance of each tube was measured at 280 nm. The contents of the tubes from a single

TABLE I. EFFECT OF TERBACIL APPLICATIONS ON THE NITROGEN CONTENT OF PROTEIN FRACTIONS OF ARTHUR WINTER WHEAT

Protein Fraction	Terbacil Application g./ha.	Nitrogen mg./g. meal
Albumins	0	1.9
	70	2.0
Globulins	0	1.7
	70	2.0
Gliadins	0	7.5**
	70	9.3
Glutenins	0	6.6
	70	8.9
Total meal N	0	22.0**
	70	25.1

**F-value for difference between means significant at 0.01 level.

absorption peak were combined, dialyzed against cold water, and freeze-dried.

Gel-filtration chromatography of gliadins was performed on Sephadex G-100 with 0.1M acetic acid, as described by Beckwith et al. (18). Gliadins (8 mg. nitrogen) were dissolved in the column buffer and eluted with the same buffer at room temperature using a flow rate of 35 to 40 ml. per hr. The elution was collected in 12-ml. fractions. Absorbance of individual tubes was measured at 280 nm. The contents of tubes from a single absorption peak were combined and freeze-dried.

Polyacrylamide Gel Electrophoresis

The procedure of Davis (20) was used to compare the polyacrylamide-gel-electrophoretic properties of the albumin and gliadin fractions obtained by gel filtration. The glutenins were reduced and alkylated by the procedures of Cavins and Friedman (21) and Paulis et al. (22). Solutions for electrophoresis were prepared by dissolving freeze-dried protein in 0.1M acetic acid solution made 4M in dimethylformamide (23). Bromphenol blue dye was used as the visual marker. Gels were stained with Coomassie blue in 12.5% trichloroacetic acid. Unabsorbed dye was removed by soaking the gel overnight in trichloroacetic acid solution. The gels were photographed on Kodak Ektapan Film using a Wratten G filter.

RESULTS AND DISCUSSION

The foliar application of 70 g. per ha. of terbacil at tillering increased the total nitrogen content of Arthur winter wheat grain 14% compared to the control, whereas the gliadins were increased 24% (Table I).

The application of 225 kg. N per ha. to Inia significantly increased the total nitrogen content of the meal as well as of the absolute amounts of the major protein fractions (Table II). Simazine applications of 250 g. per ha. increased the total nitrogen content and the gliadin content only when 75 kg. N per ha. was applied. This is indicated by the significances of the interactions of nitrogen level with simazine treatment. At a nitrogen rate of 75 kg. per ha., simazine increased the total protein 15% while the gliadin was increased 34% (Table II). Although similar

TABLE II. THE NITROGEN CONTENT (mg. N PER g. MEAL) OF PROTEIN FRACTIONS OF INIA SPRING WHEAT GROWN WITH DIFFERENT NITROGEN AND SIMAZINE APPLICATIONS

Nitrogen Fraction	Simazine Application g./ha.	Nitrogen Application (kg./ha.) 75 mg. N/g.	225 mg. N/g.	Mean
Albumins	0	2.4	2.9	2.6
	250	2.1	2.6	2.4
	Mean	2.2	2.8*	
Globulins	0	1.3	2.4	1.8**
	250	2.0	2.6	2.3
	Mean	1.6	2.5**	
Gliadins	0	3.5	5.2	4.4**
	250	4.7	5.3	5.0
	Mean	4.1	5.3**	
N X simazine interaction (gliadins)		**		
Glutenins	0	4.2	7.5	5.8
	250	5.6	7.4	6.5
	Mean	4.9	7.4**	
Total meal N	0	19.6	29.1	24.4**
	250	22.5	29.8	26.2
	Mean	21.1	26.4**	
N X simazine interaction (total meal N)		**		

*,**F-value for difference between means or of interaction significant at 0.05 and 0.01 levels, respectively.

TABLE III. PERCENTAGE OF TOTAL NITROGEN FOUND IN GLIADIN FRACTION FOR ARTHUR WINTER WHEAT

Terbacil Application g./ha.	Percentage
0	33.8*
70	36.8

*F-value for difference between means significant at 0.05 level.

differences for the glutenins occurred both with Arthur and Inia, they were not statistically significant.

Changes in individual protein fractions expressed as percent of the total nitrogen found in a given fraction were significant only for the gliadin fraction in Arthur (Table III), and the albumin and gliadin fractions in Inia (Table IV). The percent of the total nitrogen found in the gliadin fraction for Arthur was increased by terbacil, whereas the corresponding percentage for Inia could be increased by both nitrogen and simazine applications; but simazine-induced increases occurred only at the lower level (75 kg. per ha.) of nitrogen application. Apparently, simazine decreased the percent of total nitrogen found in the albumin fraction of Inia regardless of rate of nitrogen application.

The gel-filtration elution patterns for the albumins and subsequent

TABLE IV. PERCENTAGE OF TOTAL NITROGEN FOUND IN ALBUMIN AND GLIADIN FRACTIONS FOR INIA SPRING WHEAT

Nitrogen Fraction	Simazine Application g./ha.	Nitrogen (kg./ha.) 75	225	Mean
Albumin	0	12.4	9.9	11.2**
	250	9.2	8.6	8.9
	Mean	10.8	9.3	
Gliadin	0	18.1	18.2	18.2*
	250	20.9	17.8	19.4
	Mean	19.5	18.0*	
Nitrogen X simazine interaction		*		

*,**F-value for difference between means or of interaction significant at 0.05 and 0.01, respectively.

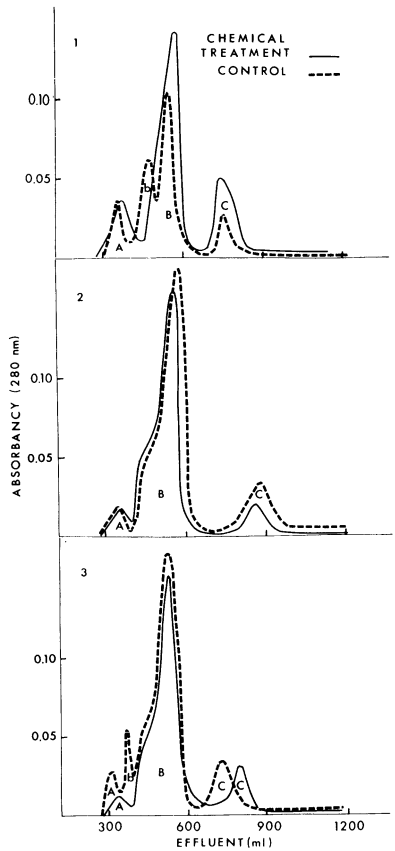

Fig. 1. Comparison of elution patterns from Sephadex G-100 column (4.0 X 110 cm.) of gliadins from wheat seed grown under different nitrogen and chemical treatments. 1: Arthur wheat, 30 kg. N per ha.; chemical treatment: 70 g. terbacil per ha.; 2: Inia wheat, 75 kg. N per ha.; chemical treatment: 250 g. simazine per ha.; 3: Inia wheat, 225 kg. N per ha.; chemical treatment: 250 g. simazine per ha.

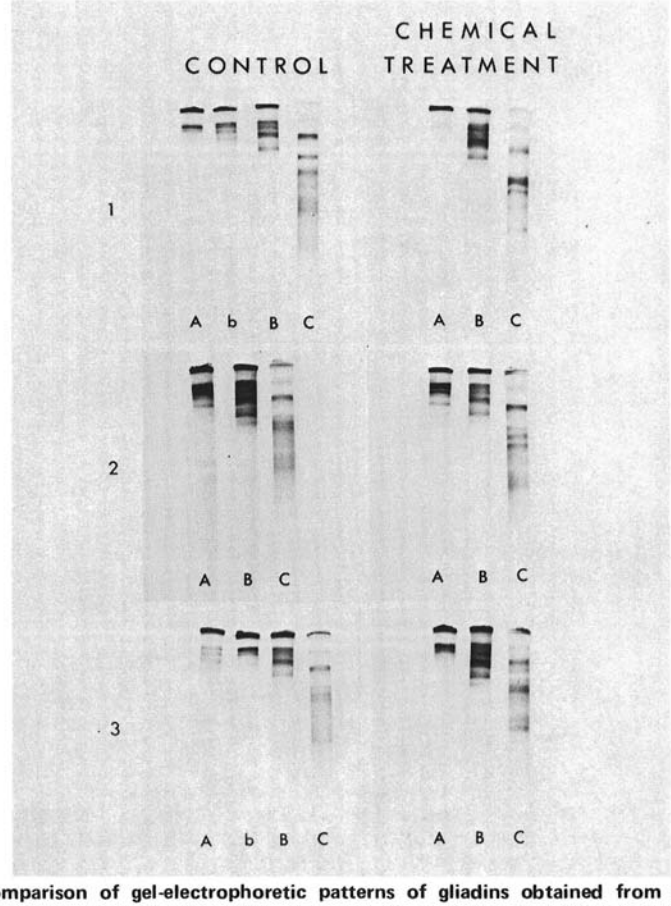

Fig. 2. Comparison of gel-electrophoretic patterns of gliadins obtained from the different Sephadex G-100 column fractions (Fig. 1). Sets 1, 2, and 3 correspond to graphs 1, 2 and 3 of Fig. 1, respectively. Letters denoting the different electrophoretic patterns designate the fraction in Fig. 1 from which the electrophoretic sample was taken.

polyacrylamide-gel-electrophoretic patterns from each gel-filtration peak were essentially the same for a given variety regardless of treatment.

The globulins were not sufficiently soluble in buffers at concentrations required for chromatography and electrophoresis.

Gel filtration of gliadins (Fig. 1) from Arthur seed resulted in three peaks from high-protein (chemical treatment) and four peaks from low-protein meal with the additional peak (b) preceding the major peak (B). The electrophoretic patterns for Inia of gliadin gel-filtration peaks B and C showed the same major bands while minor differences occur for the patterns of peak A (2,3 in Fig. 2). The significance of the small peaks (b) preceding the major peak (B) remains unclear. When peaks B and b were combined and resubjected to gel-filtration chromatography, only a single peak corresponding to the major peak (B) was seen. Whether this is an artifact of the chromatographic system or indicates peak b is being lost, perhaps by conversion to one of the components in peak B, remains uncertain.

Electrophoretic patterns of reduced and alkylated glutenins from Arthur and Inia seeds were essentially identical, although the bands from high-protein seed (chemical treatment) were more intense than bands from the low-protein (control) seed.

This study has clearly established that high-protein seed, whether it is produced by applications of supplemental nitrogen or applications of nontoxic levels of herbicides, contains more gluten proteins, probably as gliadins, than low-protein seed. Based on percent total meal nitrogen, simazine appears to decrease the albumin content. These changes may be of interest as they relate to seedling vigor.

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