Theor Appl Genet (1983) 65: 41–46

Increasing grain protein content of hard red winter wheat (*Triticum aestivum* L.) by mutation breeding*

L. M. Corpuz, E. G. Heyne and G. M. Paulsen
Department of Agronomy, Kansas State University, Throckmorton Hall, Manhattan, KS 66506, USA

Received December 29, 1982
Communicated by D. von Wettstein

Summary. Poor adaptability or functional quality of much germplasm used for breeding high-protein hard red winter wheats prompted mutagenesis as an alternative means of increasing grain protein content. Four hard red winter wheat genotypes – KS644 ('Triumph// Concho/Triumph'), 'Kaw', 'Parker', and 'Shawnee' – were treated with 0.40 M ethyl methanesulfonate (EMS). Advanced lines (M_8–M_{10}) were selected that had a 3-year mean grain protein advantage of 0.7% to 2.0% over controls. Increased grain protein content was generally associated with decreased grain yield and kernel weight, but some high-protein mutant lines had yields or kernel weights similar to those of original genotypes. Changes in height and lodging induced by EMS were generally favorable, most mutants being shorter and lodging less than controls, but blooming date was generally delayed, a deleterious change. One line also changed from resistant to segregating for wheat soil-borne mosaic virus. Mutant lines might be utilized in cross-breeding programs, particularly if negative pleiotropic effects and linkages are absent.

Key words: *Triticum aestivum* – Wheat – Protein – Mutation

Introduction

Grain yield and quality are usually the primary objectives of hard wheat improvement, but increased grain protein content is receiving more attention because of its relation to nutrition and baking properties. Hard winter wheat should contain 11.7 to 12.3% protein for leavened bread, but that level is not attained by many modern hard red winter wheat cultivars (Jackel 1979).

Most efforts to increase wheat grain protein content use existing genetic sources of variation (Johnson and Lay 1974; Johnson and Mattern 1975; Konzak 1977). Induced mutations, however, also can improve the trait in adapted cereal cultivars. The entire range of protein variability in cultivated rice was reproduced in a single cultivar by treating seeds with ethyl methanesulfonate (EMS) (Swaminathan 1969) or gamma rays (Tanaka and Takigi 1970). Gamma irradiation of 'Sonora 64' wheat produced 1 to 2% higher grain protein content in mutants, one of which became the commercial cultivar 'Sharbati Sonora' in India (Swaminathan 1969). The grain protein content of another gamma ray-induced mutant from cultivar 'Lerma Rojo', 'Pusa Lerma', was average at normal nitrogen fertilizer levels but increased markedly at high nitrogen fertilizer levels (Swaminathan 1972).

We initiated a long-term program to develop high-protein mutant lines from three wheat cultivars and one advanced breeding line adapted to the major U.S. wheat area to complement the limited amount of germplasm that is presently available. This report covers the final evaluation of advanced-generation mutant lines for protein content and agronomic characteristics. Outstanding mutants might be used directly as new cultivars or as parents for developing high-protein cultivars.

Materials and methods

Seeds of four hard red winter wheat genotypes – KS644 ('Triumph//Concho/Triumph'), 'Kaw', 'Parker' and 'Shawnee' – were treated with 0.40 M EMS and high-protein variants, which we considered high-protein mutants, were obtained from an original population of about 16,000 M_1 spaced plants

* Contribution no. 82-517-J, Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA

(Miezan 1976). After six generations of selection for grain protein content and desirable plant type, 23 M_7 mutant lines were retained from the four genotypes. Mixing time of flour from the 23 lines was determined by the U.S.D.A. Grain Marketing Research Laboratory.

The 23 M_7 EMS mutants included five 'Kaw' lines, eight 'KS644' lines, four 'Parker' lines, and six 'Shawnee' lines. The original genotypes were included in all tests. Reselections were made from spikes which were bulked to grow 25 entries of each M_7 mutant line and control in 0.9-m-long single-row plots at Manhattan, Kansas, in 1977. All mutant lines and control genotypes were replanted in 0.9-m-long single-row plots with four replications at Manhattan and Hutchinson, Kansas, in 1978. Checks for each genotype were planted every fifth row. Reaction to wheat soil-borne mosaic virus was noted at early seedling stage. Data on 100-kernel weight and grain protein content were collected after harvest. Protein percentage was determined by infrared reflectance on 10-g ground whole-grain samples and reported at 14% moisture.

Four entries with the highest grain protein content relative to the nearest check from each mutant line and control were retained. Those entries were grown in 0.9-m-long 3-row plots in a randomized complete block design with four replications each at Manhattan and Hutchinson in 1979. Nitrogen fertilizer was applied at blooming time at the rate of 100 kg N/ha. Maturity as days to anthesis from January 1 was recorded at Manhattan and plant height from ground level to top of the spike after anthesis was measured at both locations. Protein percentage and 100-kernel weight were determined following harvest. Two 'Kaw' lines, two 'Parker' lines, three 'Shawnee' lines, and four 'KS644' lines were selected on the basis of grain protein content and desirable kernel appearance. The 11 final selections and respective controls were grown in a yield trial using 4.6-m-long, 3-row plots at Manhattan and Hutchinson in 1980. The design was a randomized complete block in six replications at both locations. Nitrogen fertilizer at 100 kg/ha N was applied at blooming time. Data were collected on plant height, maturity, lodging, grain yield, test weight, 100-kernel weight, and protein content.

Temperature and moisture conditions were near normal during the 1978 growing season. The 1979 season was characterized by a dry autumn, record cold and snow cover during winter, and excellent precipitation during spring. The 1980 season was dry during autumn and mild during winter. High temperatures during early June caused early cessation of grain development.

Results

Mean grain protein percentages of the 23 final EMS mutant selections and controls in 1978 and 1979 are presented in Table 1. Spearman's coefficients of rank correlation of the lines by protein content between years were positive and significant in all mutant groups ($r = 0.46$, 'Kaw'; 0.57, 'KS644'; 0.59, 'Parker'; and 0.78, 'Shawnee').

F ratios showed that mean protein at the two locations significantly differed between mutant lines and corresponding controls during 1979 (Table 2). 'Kaw' mutant lines KS 76663 through KS 76665, 'KS644' lines KS 76667 through KS 76674, 'Parker' line KS 76678 and 'Shawnee' lines KS 76682 and KS 76684 through KS 76687 were especially higher, exceeding the respective controls by 0.4 to 1.7 percentage points (Table 1). Despite a significant location x line interaction for protein content in the KS644 mutant lines, mean protein values were consistently higher in the high-protein variants than in the controls at both locations.

Lines with high protein content usually had low kernel weight (Table 1). The highly negative Spearman's correlation coefficients between protein and kernel weight in 'Kaw', 'Parker' and 'Shawnee' lines in 1978 supported that trend (Table 3). The lack of correlation between protein and kernel weight in those lines and the positive correlation in the 'KS644' lines in 1979 (Table 3) was associated with less variation in both traits that year than in 1978.

Some lines were significantly taller or shorter than the control in all groups (Tables 1 and 2). Lines that flowered late in Manhattan generally had high protein content (Table 1) as indicated by highly significant correlations between protein content and days to anthesis in 'Kaw' and 'Shawnee' lines (Table 3). Parents and mutant selections reacted similarly to wheat soil-borne mosaic virus ('Kaw' and 'Parker', susceptible; 'KS644' and 'Shawnee', resistant) except for one segregating 'KS644' line (Table 1).

Some of the final 1980 selections traced back to the same line (Table 4). Mean grain protein content over both locations significantly differed among lines and controls in all groups (Table 5). A significant location x line interaction was again observed in 'KS644' lines, but mean protein content was substantially higher in all high-protein variants in that group than in the control at both locations. All 'Kaw', 'Parker', and 'Shawnee' selections except 'Parker' selection KS 76678-1 had significantly higher protein content than the controls (Table 4). Mixing times of mutant lines were usually shorter than or equal to those of controls.

Grain yield was significantly lower in all mutant selections except KS 76663-1, KS 76663-2, and KS 76673 than in controls (Tables 4 and 5). The magnitude of yield reduction among 'Shawnee' lines varied between locations, resulting in a significant location x line interaction.

Kernel weight was significantly lower in most mutant selections than in controls, but was similar in selections and controls in a few instances. Kernel weight of three selections (KS 76671-1, KS 76671-2, and KS 76685) substantially exceeded that of the control. The inverse association of grain protein content with grain yield and kernel weight was supported by significant negative Spearman's correlations between protein content and yield in all mutant groups and between protein content and kernel weight in 'Kaw', 'KS644' and 'Parker' lines (Table 6).

Flowering occurred two or more days later in some mutant selections than in the controls (Table 4); high

Table 1. Mean protein content, kernel weight, height, days to anthesis, and reaction to wheat soil-borne mosaic virus (WSBM) of 23 EMS mutant lines and respective controls grown at Manhattan and Hutchinson, Kansas, during 1978 and 1979

Selection number	1978			1979			
	Protein %	Kernel weight g/100	WSBM reaction[a]	Protein %	Kernel weight g/100	Height cm	Days to anthesis[b]
'Kaw' lines							
KS76661	13.9	2.05	S	11.3	3.22	112	141
KS76662	13.4	2.21	S	12.1	2.89	117	144
KS76663	14.4	1.84	S	12.5	2.79	112	142
KS76664	14.1	2.29	S	12.5	2.75	111	144
KS76665	14.6	2.01	S	12.2	3.01	119	144
Kaw (CI 12871)	13.3	2.53	S	11.5	3.18	115	142
LSD (0.05)	–	–	–	0.6	0.06	2	1
'KS644' lines							
KS76667	14.2	3.35	R	13.1	3.43	109	139
KS76668	13.4	2.58	R	12.4	2.95	118	144
KS76669	12.8	3.16	R	12.4	3.24	111	140
KS76670	12.7	3.23	R	12.6	3.45	106	139
KS76671	12.9	3.35	R	12.8	3.48	104	139
KS76672	13.4	3.00	R	12.5	3.25	104	141
KS76673	13.3	2.76	Seg	12.6	3.24	108	141
KS76674	12.9	2.96	R	12.5	3.20	109	139
KS644	12.6	3.03	R	12.0	3.44	109	139
LSD (0.05)	–	–	–	0.3	0.07	2	1
'Parker' lines							
KS76677	14.3	1.48	S	11.7	2.96	107	143
KS76678	15.0	1.64	S	12.4	2.76	102	142
KS76679	13.9	1.62	S	11.8	2.96	105	142
KS76680	13.5	2.16	S	11.8	2.98	109	142
Parker (CI 13285)	13.2	1.84	S	11.6	2.87	107	141
LSD (0.05)	–	–	–	0.3	0.13	1	1
'Shawnee' lines							
KS76682	14.4	2.11	R	12.4	2.18	110	146
KS76683	14.4	2.20	R	11.6	2.51	115	145
KS76684	15.6	1.54	R	12.6	1.98	111	146
KS76685	15.6	2.00	R	12.9	2.41	112	146
KS76686	16.1	1.29	R	13.2	1.80	108	146
KS76687	14.6	2.02	R	12.0	2.25	112	146
Shawnee (CI 14157)	13.2	2.33	R	11.5	2.50	120	145
LSD (0.05)	–	–	–	0.4	0.10	2	NS

[a] R = resistant; Seg = segregating; S = susceptible
[b] Manhattan data only

protein content was positively correlated with days to anthesis (Table 6).

Lodging ratings in Manhattan are shown in Table 4. 'Parker' and 'Shawnee' mutants and controls were excellent in lodging resistance, whereas 'Kaw' and 'KS644' mutants and controls were intermediate to poor.

Discussion

Most efforts to increase grain protein content used high-protein parents of other classes (Konzak 1977). Those parents frequently complicated the problem because of their poor adaptation, inadequate grain functional (milling and baking) quality, or other defects that must be eliminated by extensive selection or backcrossing to desirable types. The present results demonstrated that EMS induces high-protein mutants that should be as useful as less adapted germplasm for breeding high-protein cultivars. The EMS-induced mutants, however, appeared less useful for direct use as high-protein cultivars. Rabson (1976) also concluded that all induced and spontaneous mutants were unsatisfactory without some modification.

Table 2. F ratios for grain protein content, kernel weight, height, and days to anthesis of EMS mutant lines grown at Manhattan and Hutchinson, Kansas, during 1979

Source	Protein	Kernel weight	Height	Days to anthesis[a]
	F ratio			
'Kaw' lines				
Location	179.54**	206.19**	1708.41**	–
Line	6.50**	96.97**	32.63**	4.67**
Replication	2.98*	5.89**	14.33**	0.90
Location × line	1.57	4.99**	0.76	–
'KS644' lines				
Location	1702.55**	56.38**	2729.81**	–
Line	14.69**	41.20**	52.36**	19.48**
Replication	9.99**	3.09*	6.26**	1.95
Location × line	2.51*	2.90**	1.69	–
'Parker' lines				
Location	897.92**	8.83**	2364.94**	–
Line	14.79**	4.40**	35.65**	5.84**
Replication	4.89**	1.77	13.72**	5.47*
Location × line	2.21	1.25	0.67	–
'Shawnee' lines				
Location	215.78**	12.54**	2137.78**	–
Line	21.08**	63.17**	51.40**	2.68
Replication	4.67**	0.90	7.37**	1.10
Location × line	1.14	1.03	1.51	–

[a] Manhattan data only
*, ** Significant at 5% and 1% levels, respectively

Table 3. Spearman's coefficients of rank correlation of grain protein content with kernel weight and days to anthesis of EMS mutant lines grown at Manhattan and Hutchinson, Kansas, during 1978 and 1979

Character	Protein content			
	'Kaw' lines	'KS644' lines	'Parker' lines	'Shawnee' lines
	r			
1978				
Kernel weight	−0.62**	−0.08	−0.63**	−0.80**
1979				
Kernel weight	0.09	0.26*	0.18	−0.24
Days to anthesis	0.47**	0.03	0.33	0.62**

*, ** Significant at 5% and 1% levels, respectively

At least one mutant, 'Shawnee' selection KS 76685, offered promise as germplasm in cross-breeding programs. That mutant had a 3-year (1978–1980) mean protein advantage of 2% and better lodging resistance than the parent cultivar. Adaptation, blooming date, and mixing time were similar for the mutant and the parent. Moreover, the increased 100-kernel weight of 0.25 g of selection KS 76685 over the parent indicated that it was not a morphological aberrant, a frequent occurrence with high-protein mutants (Kamra 1972).

All eleven high-protein variants selected for evaluation at the M_{10} generation from 16,000 M_1 plants suffered one defect or another, but no single defect was common to all eleven lines. The general association of increased protein content with decreased yield and kernel weight (Nagl 1973), for instance, was apparent in the

Table 4. Mean grain quality and plant agronomic characteristics of final selections of EMS-treated lines grown at Manhattan and Hutchinson, Kansas, during 1980

Selection number	Protein	Mixing time[a]	Yield	Kernel weight	Height	Days to anthesis	Lodging[b]
	%	min	kg/ha	g/100	cm		
'Kaw' lines							
KS76663-1	16.2	3	1277	2.36	110	147	3
KS76663-2	15.9	3	1470	2.39	112	146	3
Kaw (CI 12871)	14.6	3½	1666	2.60	114	145	4
LSD (0.05)	0.4	–	NS	0.14	2	1	–
'KS644' lines							
KS76668	15.5	⅞	1200	2.01	114	151	3
KS76671-1	14.3	1	1705	3.29	97	142	2
KS76671-2	14.5	1	1692	3.22	97	142	3
KS76673	14.2	1¾	1901	3.08	99	141	3
KS644	13.5	1¼	2209	3.02	101	142	4
LSD (0.05)	0.5	–	355	0.16	3	1	–
'Parker' lines							
KS76678-1	14.9	1½	1596	2.32	96	145	1
KS76678-2	15.7	1½	1627	2.20	93	146	1
Parker (CI 13285)	14.6	2¾	2241	2.47	103	146	1
LSD (0.05)	0.5	–	315	0.18	5	NS	–
'Shawnee' lines							
KS76682	16.2	3	1175	1.97	97	149	1
KS76684	16.3	3⅝	1103	1.69	95	149	1
KS76685	17.4	3½	1037	3.07	112	147	1
Shawnee (CI 14157)	15.1	3½	1700	1.89	112	147	2
LSD (0.05)	0.4	–	213	0.12	2	1	–

[a] 1976 data
[b] Lodging: 1 = no lodging to 5 = severe lodging. Manhattan data only

Table 5. F ratios for grain protein content and agronomic traits of final selections of EMS-treated lines grown at Manhattan and Hutchinson, Kansas, in 1980

Source	Protein	Yield	Kernel weight	Height	Days to anthesis
	F ratio				
'Kaw' lines					
Location	85.56**	39.44**	179.78**	34.32**	311.14**
Line	43.56**	3.49	9.08**	14.18**	14.00**
Replication	2.67	1.62	2.96	16.54**	4.00*
Location × line	0.04	0.04	4.45	15.25**	2.57
'KS644' lines					
Location	105.29**	114.20**	231.87**	15.46**	564.85**
Line	25.43**	7.47**	79.10**	45.80**	227.18**
Replication	0.82	1.21	1.24	2.39	2.61
Location × line	8.14**	1.29	2.27	2.86*	0.31
'Parker' lines					
Location	144.45**	113.21**	93.87**	0.96	44.18**
Line	10.82**	14.23**	6.40**	14.01**	1.64
Replication	0.87	1.47	0.71	2.32	1.45
Location × line	1.43	0.02	0.33	0.98	0.18
'Shawnee' lines					
Location	299.36**	256.91**	123.05**	12.12**	124.45**
Line	47.75**	19.19**	250.63**	194.93**	11.48**
Replication	3.25	3.20	4.32*	19.82	2.82
Location × line	1.90	9.67**	1.51	2.60	2.52

*, ** Significant at 5% and 1% levels, respectively

Table 6. Spearman's coefficients of rank correlation among grain protein, yield, kernel weight, and days to anthesis of final EMS mutant selections grown at Manhattan and Hutchinson, Kansas, in 1980

Character	Protein	Yield	Kernel weight
	r		
'Kaw' lines			
Yield	−0.72**		
Kernel weight	−0.83**	0.83**	
Days to anthesis	0.79**	−0.86**	−0.92**
'KS644' lines			
Yield	−0.76**		
Kernel weight	−0.58**	0.76**	
Days to anthesis	0.51**	−0.79**	−0.90**
'Parker' lines			
Yield	−0.89**		
Kernel weight	−0.95**	0.93**	
Days to anthesis	0.84**	−0.75**	−0.85**
'Shawnee' lines			
Yield	−0.89**		
Kernel weight	−0.13	0.45*	
Days to anthesis	0.66**	−0.81**	−0.66**

*, **Significant at 5% and 1% levels, respectively

mutant lines studied. The exceptions to those adverse relationships – the lines whose yields and kernel weights were similar to the controls – probably would be useful as parental lines for high-protein cultivars. Those exceptions include lines KS 76663-1, KS 76663-2, and KS 76673 for grain yield and lines KS 76671-1 and KS 76671-2 for kernel weight.

Variability in mixing time, height, lodging, and blooming date were also apparently induced by EMS treatment. Segregation for wheat soil-borne mosaic virus reaction in one line from a resistant parental control (KS 76673) might also have been induced by EMS. Changes in height and lodging were generally favorable, but those in blooming date were deleterious. Mutants in which anthesis was altered all flowered later than the controls, a disadvantage under Great Plains conditions.

Genetically high-protein germplasm is widely used in wheat breeding programs (Johnson and Lay 1974; Konzak 1977). Induced mutations, as by EMS, can complement that high-protein germplasm, particularly to extend the range of genotypes having a more favorable protein content. The success of using high-protein mutant lines in crosses, however, depends on the absence of negative pleiotropic effects or tight linkages and, therefore, the ability to obtain desirable combinations of traits by recombination.

References

Jackel SS (1979) The baker's view. In: Wheat protein conference, Manhattan, Kansas. USDA, SEA ARM-NC-9, pp 1–10

Johnson VA, Lay CL (1974) Genetic improvement of plant proteins. J Agr Food Chem 22:558–566

Johnson VA, Mattern PJ (1975) Improvement of nutritional quality of wheat protein through increased protein content and improved amino acid balance. In: Report of research findings, Jan 1, 1973 – March 31, 1975. Contracts AID/Csd-1208 and AID/ta-C-1093. Agency for International Development, Department of State, Washington, DC

Kamra OP (1972) Breeding and screening techniques for seed protein improvement in crop plants. Induced mutations and plant improvement. In: Proc Study Group Meeting of IAEA-FAO, Buenos Aires 1970. IAEA, Vienna, pp 303–315

Konzak CF (1977) Genetic control of proteins in wheat. Adv Genet 19:407–582

Miezan K (1976) Genetic and environmental factors influencing protein content in wheat. MS Thesis, Kansas State University, Manhattan

Nagl K (1973) Mutation breeding for improved protein in durum wheat. Nuclear techniques for seed protein improvement. In: Proc Research Co-ordination Meeting of IAEA-FAO and the Gesellschaft für Strahlen- und Umweltforschung, Neuherberg 1972, IAEA, Vienna, pp 181–192

Rabson R (1976) Considerations on the use of protein mutants in cross-breeding. Induced mutations in cross-breeding. In: Proc Advisory Group of IAEA-FAO, Vienna 1975. IAEA, Vienna, pp 113–117

Swaminathan MS (1969) Role of mutation breeding in a changing agriculture. Induced mutations in plants. In: Proc Symposium of IAEA-FAO, Pullman 1969. IAEA, Vienna, pp 719–734

Swaminathan MS (1972) Mutational reconstruction of crop ideotypes. Induced mutations and plant improvement. In: Proc Study Group Meeting of IAEA-FAO, Buenos Aires 1970. IAEA, Vienna, pp 155–171

Tanaka S, Takigi Y (1970) Protein content of rice mutants. Improving plant protein by nuclear techniques. In: Proc Symposium of IAEA-FAO, Vienna 1970. IAEA, Vienna, pp 55–61