Effect of Nitrogen Fertilizer Treatments on the Amino Acid Composition of Neepawa Wheat

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ABSTRACT

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The amino acid composition of Neepawa wheat grain from a 4 × 3 factorial experiment (granular fertilizer at 0, 100, 200, and 300 kg of N/ha applied to the soil and urea at 0, 50, and 100 kg of N/ha sprayed on foliage) was determined. Amino acid responses, when they occurred, were to the first increment of nitrogen in most cases. In general, glutamic acid, proline, phenylalanine, cystine, methionine, and tyrosine increased and lysine, histidine, arginine, aspartic acid, threonine, glycine, valine, and leucine decreased with either or both forms of nitrogen application. Cystine and glycine were affected by granular nitrogen applied to the soil but not by urea nitrogen applied to the foliage. Conversely, the percentages of valine, methioine, and tyrosine were affected by urea nitrogen but not by granular nitrogen. The interactions between nitrogen treatments were significant for 12 of the amino acids. Each amino acid was significantly correlated (+ or −) with one or more of the other amino acids.

Nitrogen fertilizer whether applied to the soil (Dubetz 1972, Fajerson 1961, Solsulski et al 1963) or to the crop (Finney et al 1957, Kolderup 1974) can be used to increase the protein content of wheat (*Triticum aestivum* L.), and changes in amino acid composition are associated with increased grain protein. Results from a greenhouse experiment (Larsen and Dissing Nielsen 1966) showed that increased applications of nitrogen resulted in increases in glutamic acid and proline and decreases in lysine and arginine, when expressed on a nitrogen basis. An application of nitrogen at 134 kg/ha (Abrol et al 1971) increased glutamic acid, proline, phenylalanine, and leucine appreciably and decreased lysine, valine, and threonine contents of wheat grain when calculated on a percentage basis. When the protein content of wheat increased after application of 46.5 kg/ha of nitrogen, glutamic acid, proline, and phenylalanine increased but most of the other amino acids, especially lysine, decreased (Kolderup 1974).

This study was done to determine whether relatively high levels of nitrogen fertilizer (to 300 kg/ha) applied to the soil or sprayed on the foliage at the flowering stage change the amino acid composition of wheat grain.

MATERIALS AND METHODS

A 4 × 3 factorial experiment consisting of four granular fertilizer treatments (0, 100, 200, and 300 kg of N/ha) and three solution treatments (0, 50, and 100 kg of N/ha) was conducted with Neepawa wheat at Vauxhall, Alberta, in 1974. The granular fertilizer (ammonium nitrate) was broadcast before seeding and the solution treatments (urea) were sprayed on the crop at the flowering stage. All plots received 100 kg of P/ha, and the experiment was replicated six times.

The plots were irrigated to field capacity whenever tensiometers placed at the 30-cm depth reached a soil moisture tension of 0.4 bar except during the flowering stage when a soil moisture stress of about 8 bar was imposed by withholding water. After the stress period, the plots were again irrigated to field capacity. During the growing season, the plots received four irrigations for a total of 220 mm. Soil analysis, yield, and protein content data were reported previously (Dubetz 1977) as was baking quality (Tipples et al 1977). Protein percentages (N × 7.5, on O.D. basis) ranged from 13.9 to 21.2%.

TABLE I
Effects of Four Levels of Granular Nitrogen (N) and Three Levels of Urea Nitrogen Applied as a Spray on the Main Effect Means of Amino Acids (g/100 g of Protein[a]) of Neepawa Wheat[b]

	Granular N (kg/ha)					Urea N (kg/ha)					
Amino Acid	0	100	200	300	Significance[c]	0	50	100	Significance	$\bar{X}$	Interaction
Lysine	3.73 a	3.30 b	3.14 b	3.24 b	**	3.54 a	3.31 b	3.21 b	**	3.35	**
Histidine	3.07 a	2.87 b	2.77 c	2.83 bc	**	3.01 a	2.86 b	2.79 b	**	2.89	**
Arginine	3.01 a	3.02 a	2.90 b	3.00 a	**	3.01 a	2.97 a	2.96 a	...	2.98	**
Aspartic acid	5.56 a	5.37 b	5.34 b	5.38 b	**	5.53 a	5.41 ab	5.29 b	**	5.41	*
Threonine	2.98 a	2.88 b	2.83 b	2.86 b	**	2.97 a	2.86 b	2.83 b	**	2.89	**
Serine	5.51 a	5.39 a	5.37 a	5.19 a	...	5.36 a	5.35 a	5.38 a	...	5.36	...
Glutamic acid	29.78 b	30.22 a	30.57 a	30.51 a	**	29.81 b	30.33 a	30.67 a	**	30.27	**
Proline	13.14 b	13.70 a	13.84 a	13.89 a	**	13.28 b	13.81 a	13.84 a	**	13.64	**
Cystine	1.18 b	1.35 a	1.23 ab	1.31 ab	**	1.27 a	1.26 a	1.26 a	...	1.27	**
Glycine	6.93 a	6.75 b	6.73 b	6.72 b	**	6.82 a	6.77 a	6.76 a	...	6.78	**
Alanine	4.34 a	4.39 a	4.50 a	4.31 a	...	4.38 a	4.38 a	4.38 a	...	4.38	...
Valine	4.52 a	4.49 a	4.60 a	4.27 a	...	4.69 a	4.48 ab	4.23 b	**	4.47	*
Methionine	0.67 a	0.65 a	0.55 a	0.59 a	...	0.61 b	0.53 b	0.70 a	**	0.61	...
Isoleucine	3.06 a	3.10 a	3.09 a	3.09 a	...	3.06 a	3.09 a	3.10 a	...	3.09	...
Leucine	6.69 a	6.64 ab	6.62 b	6.64 ab	*	6.70 a	6.63 b	6.61 b	*	6.65	**
Tyrosine	1.93 a	1.95 a	1.95 a	1.96 a	...	1.90 b	1.94 ab	1.99 a	**	1.95	...
Phenylalanine	3.88 b	3.94 ab	3.94 ab	4.02 a	*	3.87 b	3.98 a	3.99 a	**	3.95	**

[a]Protein = N × 5.7.
[b]Means followed by different letters differ significantly at $P < 0.05$.
[c]* $P = 0.05$; ** $P = 0.01$.

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TABLE II

Interaction of Granular Nitrogen (N) and Urea Nitrogen Applied as a Spray at Heading on the Amino Acid Composition (g/100 g of Protein[a]) of Neepawa Wheat[b]

	Granular N/Urea N												Std.
Amino Acid	**0/0**	**0/50**	**0/100**	**100/0**	**100/50**	**100/100**	**200/0**	**200/50**	**200/100**	**300/0**	**300/50**	**300/100**	**Error**
Lysine	4.34 a	3.59 b	3.25 bcd	3.45 bc	3.30 bcd	3.16 cd	3.09 d	3.14 cd	3.20 cd	3.28 bcd	3.24 cd	3.21 cd	0.078
Histidine	3.45 a	2.98 b	2.79 cd	2.95 bc	2.89 bcd	2.78 cd	2.76 d	2.75 d	2.80 cd	2.87 bcd	2.83 bcd	2.79 cd	0.042
Arginine	3.13 a	2.98 bc	2.92 bc	3.03 ab	3.06 ab	2.97 bc	2.86 c	2.87 c	2.97 bc	3.01 abc	3.00 abc	3.00 abc	0.034
Aspartic acid	5.91 a	5.45 b	5.32 b	5.43 b	5.39 b	5.30 b	5.38 b	5.39 b	5.26 b	5.42 b	5.42 b	5.30 b	0.079
Threonine	3.20 a	2.91 a	2.82 a	2.96 a	2.87 a	2.81 a	2.85 a	2.81 a	2.83 a	2.89 a	2.84 a	2.84 a	0.156
Glutamic acid	28.36 d	29.88 bc	31.10 a	29.77 c	30.38 abc	30.50 abc	30.79 a	30.39 abc	30.52 abc	30.33 abc	30.66 ab	30.55 abc	0.193
Proline	11.89 b	13.70 a	13.82 a	13.51 a	13.72 a	13.87 a	13.70 a	14.00 a	13.82 a	14.03 a	13.81 a	13.84 a	0.175
Cystine	1.02 c	1.31 ab	1.20 abc	1.40 a	1.33 ab	1.31 ab	1.32 ab	1.13 bc	1.25 abc	1.33 ab	1.29 ab	1.31 ab	0.057
Glycine	7.16 a	6.91 b	6.72 b	6.71 b	6.81 b	6.75 b	6.72 b	6.69 b	6.78 b	6.69 b	6.69 b	6.78 b	0.060
Valine	4.99 a	3.40 b	4.16 b	4.69 ab	4.26 b	4.53 ab	4.65 ab	5.03 a	4.11 b	4.44 ab	4.22 b	4.14 b	0.183
Leucine	6.93 a	6.64 b	6.51 b	6.64 b	6.66 b	6.62 b	6.61 b	6.61 b	6.65 b	6.63 b	6.63 b	6.65 b	0.034
Phenylalanine	3.66 b	3.93 a	4.07 a	3.84 ab	3.99 a	4.00 a	4.00 a	3.99 a	3.82 ab	4.00 a	4.01 a	4.06 a	0.056

[a]Protein = N × 5.7.

[b]Means followed by different letters differ ($P < 0.05$).

TABLE III

Correlation Coefficient[a] Between Amino Acids of Neepawa Wheat (Means of Four Replicates, Four Granular Nitrogen Treatments, and Three Urea Nitrogen Treatments)

	Lysine	Histi-dine	Argi-nine	Aspartic Acid	Threo-nine	Serine	Gluta-mic Acid	Pro-line	Cyst-ine	Gly-cine	Ala-nine	Valine	Methio-nine	Iso-leu-cine	Leu-cine	Tyro-sine	Phenyl-alanine
Lysine	1.00																
Histidine	0.97	1.00															
Arginine	0.67	0.67	1.00														
Aspartic acid	0.70	0.71	0.46	1.00													
Threonine	0.91	0.92	0.64	0.83	1.00												
Serine	0.26	0.25	0.11	0.39	0.34	1.00											
Glutamic acid	−0.85	−0.82	−0.64	−0.60	−0.80	−0.15	1.00										
Proline	−0.77	−0.78	−0.47	−0.69	−0.80	−0.29	0.74	1.00									
Cystine	−0.35	−0.29	−0.12	−0.28	−0.29	−0.20	0.25	0.45	1.00								
Glycine	0.64	0.61	0.45	0.57	0.68	0.23	−0.64	−0.77	−0.44	1.00							
Alanine	0.03	0.00	−0.08	0.02	0.06	0.05	−0.27	−0.42	−0.16	0.40	1.00						
Valine	0.17	0.15	−0.08	0.19	0.18	0.00	−0.47	−0.41	−0.37	0.20	0.24	1.00					
Methionine	0.30	0.28	0.26	−0.28	0.11	−0.11	−0.38	−0.21	0.11	0.10	0.21	−0.08	1.00				
Isoleucine	0.05	0.05	0.31	−0.01	−0.02	−0.07	−0.22	−0.31	−0.28	0.20	0.34	0.10	0.24	1.00			
Leucine	0.67	0.70	0.58	0.56	0.69	0.10	−0.70	−0.70	−0.43	0.67	0.08	0.25	0.16	0.52	1.00		
Tyrosine	−0.51	−0.50	−0.20	−0.60	−0.54	−0.24	0.45	0.49	0.38	−0.27	0.06	−0.56	0.35	0.08	−0.40	1.00	
Phenylalanine	−0.62	−0.60	−0.50	−0.50	−0.65	0.21	0.75	0.59	−0.23	−0.47	−0.39	−0.20	−0.35	−0.33	−0.52	0.16	1.00

[a]r = 0.369 ($P \leqslant 0.01$) and r = 0.25 ($P \leqslant 0.05$).

Representative samples of whole wheat from each treatment were ground (40 mesh), hydrolyzed without oxidation at 110° C for 22 hr, and analyzed for all amino acids with a 121 M Beckman amino acid analyzer. The amino acids were expressed as grams of amino acid per 100 g of protein. Data for each amino acid were analyzed statistically by the analysis of variance method and means were compared by Duncan's multiple range test (Duncan 1955). Simple correlation coefficients between amino acids also were calculated.

RESULTS AND DISCUSSION

The main effects of granular and urea nitrogen on amino acid composition are shown in Table I. In most cases, when amino acid responses occurred, they were to the first increment of nitrogen. In general, glutamic acid, proline, phenylalanine, cystine, methionine, and tyrosine increased, and lysine, histidine, arginine, aspartic acid, threonine, glycine, valine, and leucine decreased after one or both forms of nitrogen application. Cystine and glycine percentages were affected by granular nitrogen but not by urea nitrogen. Conversely, the percentages of valine, methionine, and tyrosine were affected by urea nitrogen but not by granular nitrogen. This suggests that when nitrogen was made available to wheat at the flowering stage of growth, there was preferential synthesis of some of the amino acids. Serine, alanine, and isoleucine were not affected by nitrogen.

Table I also shows the level of significance of the interactions of granular and urea nitrogen on the amino acids. The 12 amino acids for which there was a significant interaction between the nitrogen treatments appear in Table II. The significant interaction for most of the amino acids was due mainly to the large response from the first increment of urea or granular nitrogen in the absence of the other form of nitrogen. For glumatic acid, proline, cystine, valine, and phenylalanine, the percentages increased, and for lysine, histidine, arginine, aspartic acid, threonine, glycine, and leucine, they decreased.

Because of the apparent close relationship among amino acids, correlation coefficients were calculated (Table III). Each amino acid was significantly correlated with one or more of the other amino acids. For example, lysine was positively correlated ($P \leqslant 0.05$) with histidine, arginine, aspartic acid, threonine, glycine, methionine, and leucine and negatively correlated with glutamic acid, proline, cystine, tyrosine, and phenylalanine. Serine, alanine, and valine had the least correlations with other amino acids.

Changes in amino acid composition of wheat grain protein that occurred as a result of nitrogen fertilization generally agree with other studies (Abrol et al 1971, Kolderup 1974, Larsen and Dissing Nielsen 1966) in that proline, glutamic acid, and phenylalanine increased and lysine, valine, and threonine decreased. Kolderup (1974) implied that only three amino acids increased, but we found six. Also, Abrol et al (1971) reported an increase in leucine with increased nitrogen fertilizer, but we found a decrease.

Our results suggest that the stage of growth at the time of nitrogen application can influence the percentage of certain amino acids in the grain, ie, glycine.

High positive correlations between amino acids suggest that these amino acids are closely related in common proteins. Kolderup (1974) suggested, and we showed (Dubetz et al 1979), that nitrogen fertilization changed the proportions of the soluble protein fractions of wheat grain but did not change the proportions of the amino acids in the individual fractions.

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