Effect of Nitrogen Level and Time of Application on the Protein Content and Amino Acid Composition of Irrigated Wheats

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The effect of increasing amounts of urea fertilizer (50, 100, 150 kg/ha) and time of its application on protein content and amino acid composition of seven wheat cultivars grown under irrigation in the tropics was studied in a factorial experiment. The protein increase that occurred with an increase in applied nitrogen was more marked when the lowest urea level was applied at the grain-filling stage. Compared with tropical grown millets and sorghums, the wheat cultivars had superior essential amino acid composition. Of all the amino acid analyzed, glutamic acid showed the most consistent increase with increase in applied nitrogen while lysine showed the most consistent decrease. The interactions between nitrogen treatments and stage of application were not significant for four amino acids—serine, proline, methionine, and phenylalanine. High negative correlations were found to exist between glutamic acid and certain amino acids that are not closely related to glutamic acid in the same proteins.

INTRODUCTION

In Nigeria, there is an increasing demand for wheat, most of which is imported. This is largely in response to the increasing demand for bread.

Wheat being a temperate crop thrives well only under low temperatures. Its cultivation in a tropical country like Nigeria is, therefore, presently restricted to latitudes 10–14 °N, mainly the Sudan Savanna and Sahel Zones. The local varieties are generally late maturing tall, low yielding, and are of poor quality. Research efforts are, therefore, being directed at identifying exotic varieties that are high yielding under Nigerian conditions and are of good quality.

Cereals including wheat contribute substantial amounts of energy and protein to the diets of many Nigerians whose intake of protein from animal sources is low. The content and quality of protein in these cereals may, therefore, determine the nutritional status of many Nigerians.

Protein can be increased by various cultural means including the application of nitrogenous fertilizers (Pushman and Bingham, 1976). Researches have shown that the protein content of the wheat grain frequently increases with increase in applied nitrogen (Pushman and Bingham, 1976; Syme et al., 1976). Also, the later the nitrogen application up to the bloom stage, the greater is the increase in grain protein (Terman et al., 1969).

In this paper, we report a study which was carried out to determine the effect of varying levels of nitrogen fertilizer on the protein and amino acid composition of seven wheat varieties which are suited to tropical conditions.

MATERIALS AND METHOD

Seven spring bread wheat (*Triticum aestivum* L.) varieties, Sonora 63 (V_1), Florence Aurore 8193 (V_2), Siete cerros (V_3), (Lee × 108) G8 56 (V_4), 12300 × LR 64A – 8156/Nor 67 (V_5), S311 × Norteno Jit 43 – 2L (V_6), and Tousson (V_7), which are all suited to Nigerian conditions, were grown under irrigation at Kadawa irrigation site (latitude 11° 39′N and longitude 8° 27′E), Kano State, Nigeria, in the 1978/79 and 1979/80 dry seasons.

The seeds of all the varieties were sown at the rate of 100 kg/ha on 15 and 10 November, 1978 and 1979, resepectively, in a randomized block design with three replications. Super phosphate fertilizer at 50 kg of P_2O_5/ha

was applied to all the plots at planting. At four weeks after planting (tillering stage) or at one week after flowering (early grain-filling stage) plots were supplied with urea fertilizer at rates of either 50 (N), 100 (N_2), or 150 (N_3) kg/ha.

The 1978 trial was irrigated 11 times and the 1979 trial 10 times, giving about 50 mm of water at each irrigation. Each trial was hand weeded twice. At maturity, on 15 and 13 March, 1979 and 1980, respectively, the trials were harvested, threshed, and bulked according to treatments. Two samples were then taken from each treatment for the determination of protein content and amino acid composition.

The grains were finely ground and stored at −4 °C in plastic screw-capped bottles. Before analysis, samples were dried for 24 h in a forced-drought oven at 80 °C to constant weight. Protein (N × 5.7) was determined by the microkjeldahl method. The samples were analyzed for amino acid content by hydrolyzing with 6 N HCl under reflux for 24 h. The acid was evaporated in vacuo in a rotary evaporator and the residue dissolved in pH 2.2 citrate-phosphate buffer and made up to a known volume. Aliquots of the hydrolysate were then run on a Technicon TSM – 1 amino acid analyzer equipped with an integrator and printer. Methionine values include that recovered as methionine sulfoxide.

The data were statistically analyzed on a main frame electronic computers. Main effects and interactions were tested for significance using the F test. Treatment means were compared by using least significant difference (lsd) at 5% whenever the F values were statistically significant (John, 1971).

RESULTS AND DISCUSSION

The grain yield and protein content of wheat varieties studied are given in Table I. Although the small differences in protein content among the varieties were not statistically significant, the protein yield calculated from yield and protein content showed greater variation. Variety 12300 LR 64 A - 3156 Nor 67 (V_5) in addition to having the highest protein yield had the highest grain yield per hectre. Apart from Sonora 63 (V_1), Florence Aurore (V_2) had significantly lower protein yield than each of the remaining five varieties.

The effect of increasing nitrogen and varying the time of its application on the protein content of wheat grain is shown in Table II. When nitrogen was applied at the tillering stage of growth, protein content was signficantly

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Table I. Yield and Protein in Seven Wheat Varieties Averaged over Three Levels of Urea Nitrogen and Two Stages of Application (Mean of Two Years)[a]

| | varieties | | | | | | | | |
	V_1	V_2	V_3	V_4	V_5	V_6	V_7	SE	LSD (0.05)
yield, kg/ha	3540[d]	3121[e]	4398[a]	3734[c]	4523[a]	3645[cd]	4185[b]	55.8	172
protein, g/100 g	11.2	12.0	9.9	11.9	10.9	12.2	11.3	0.43	
protein yield, kg/ha	396[bc]	373[c]	442[ab]	447[ab]	496[a]	445[ab]	475[a]	18.4	57

[a] V_1 = Sonora 63, V_2 = Florence Aurore 8193, V_3 = Siete Cerros, V_4 = (Lee × 108) G8 55) G8 56, V_5 = 12300 LR64 A − 3156 Nor 67, V_6 = 5311 × Norteno, V_7 = Tousson. Means followed by different letter differ significantly ($P < 0.05$).

Table II. Interaction of Urea Nitrogen and Stage of Application on the Yield and Protein Content of Seven Wheat Varieties (Mean of Two Years)[a]

| | 50 kg N/ha | | 100 kg N/ha | | 150 kg N/ha | | | |
	early[b]	late[c]	early	late	early	late	SE	LSD (0.05)
yield, kg/ha	3682[d]	3484[e]	4086[b]	3838[cd]	4267[a]	3910[c]	51.7	159
protein, g/100 g	9.6[b]	11.5[a]	12.0[a]	11.1[a]	12.2[a]	11.7[a]	0.40	1.2
protein yield, kg/ha	354[e]	398[de]	479[ab]	425[cd]	521[a]	457[bc]	17.0	52

[a] Means followed by different letters differ significantly ($P < 0.05$). [b] Early = tillering stage. [c] Late = early grain-filling stage.

Table III. Amino Acids Expressed as Percent of Recovered Amino Acid in Seven Wheat Varieties Averaged over Three Levels of Urea Nitrogen and Two Stages of Application (Mean of Two Years)

| | varieties | | | | | | | |
	V_1	V_2	V_3	V_4	V_5	V_6	V_7	SE
Lys	4.06	4.01	4.49	4.38	4.48	4.20	4.34	0.077
His	3.46	3.92	3.95	4.14	3.87	3.96	3.84	0.053
Arg	5.90	5.70	5.97	5.97	5.85	5.68	5.30	0.041
Asp	6.77	5.70	6.19	6.18	6.15	6.13	5.86	0.082
Thr	3.17	3.28	3.48	3.43	3.51	3.37	3.45	0.058
Ser	4.68	4.92	4.97	4.83	5.16	5.02	4.89	0.040
Glu	29.36	29.32	28.60	28.69	18.10	28.82	28.93	0.133
Pro	8.89	9.67	9.16	9.15	9.07	9.34	9.71	0.136
Gly	4.86	4.79	5.13	5.08	4.69	4.54	4.43	0.044
Ala	4.14	4.56	4.18	3.97	4.01	3.96	4.07	0.054
Val	4.11	4.21	4.29	4.09	4.40	4.44	4.51	0.048
Met	1.27	1.03	1.11	1.38	1.28	1.38	1.16	0.035
Ileu	3.93	3.76	3.33	3.26	3.77	3.72	3.87	0.051
Leu	7.23	7.24	7.36	7.43	7.79	7.75	7.81	0.061
Tyr	3.08	3.19	3.14	3.21	3.13	2.94	3.00	0.055
Phe	5.09	4.70	4.64	4.82	4.73	4.76	4.73	0.072

Table IV. Comparison of Essential Amino Acids in Wheat with Those of Sorghum, Millet, and FAO Provisional (g/100 g of Protein)

amino acid	wheat (mean of 7 var.)	sorghum[a] (mean of 16 var.)	millet[b] (mean of 7 var.)	FAO
Lys	4.11 (3.88–4.34)	1.92 (1.5–2.6)	2.67 (2.45–2.91)	4.3
Thr	3.19 (3.05–3.37)	3.42 (3.1–3.9)	4.13 (3.90–4.35)	3.3
Val	4.09 (3.84–4.46)	4.81 (4.3–5.3)	5.76 (5.21–6.17)	2.8
Ile	3.46 (2.98–3.78)	4.05 (3.4–4.6)	4.63 (4.37–4.94)	4.3
Meth	1.18 (1.00–1.32)		1.68 (1.39–2.05)	
Leu	7.10 (6.55–7.55)	13.92 (12.4–14.7)	11.67 (11.18–12.63)	4.9
Tyr	2.92 (2.90–3.13)	4.05 (3.7–4.4)	3.42 (3.19–3.68)	2.5
Phe	4.54 (4.13–4.92)	5.47 (4.9–5.8)	5.54 (5.02–6.05)	2.9

[a] Okoh et al., 1982. [b] I.A.R. Cropping Scheme Meeting, 1983.

increased by the first 50 kg/ha increment of nitrogen (N_2) but the second 50 kg/ha increment (N_3) resulted only in a nonsignificant increase. In contrast, when nitrogen was applied at the grain-filling stage, significant increases in protein content did not occur with an increase in applied nitrogen. Furthermore, it is important to note that the wheat cultivars had higher (statistically significant) grain protein content when the lowest level of nitrogen was applied at the grain-filling stage compared with the same level of application at the tillering stage (Table II). The calculated grain protein yield (Table II) was also higher (although not significant) when the lowest level of nitrogen was applied at the grain-filling stage. This suggests that in a developing country like Nigeria, where only limited quantity of fertilizer may be available to farmers, maximum benefit from wheat, in terms of protein yield, may

be obtained by paying particular attention to the time of application of nitrogen at low levels.

Table III gives the amino acid composition of seven wheat varieties expressed as percentages of recovered amino acid. This gives a common base for useful comparison of amino acids present in different wheat varieties with varying levels of protein. In all the amino acids, there were only slight quantitative differences among some of the varieties and these were generally not statistically significant.

The major cereals grown in Nigeria that constitute the main sources of dietary protein are sorghum, millet, and maize. The nutritional quality of the tropically grown wheat studied here was evaluated by comparing the essential amino acid composition with that of sorghum, millet, and FAO provisional pattern (Table IV). Except

Table V. Interaction of Urea Nitrogen and Stage of Application on Percentage Amino Acid Content of Seven Wheat Varieties (Mean of Two Years)[a]

	50 kg/ha		100 kg/ha		150 kg/ha		SE	LSD (0.05)
	early	late	early	late	early	late		
Lys	4.40[a]	4.44[a]	4.22[ab]	4.24[ab]	4.16[b]	4.33[ab]	0.072	0.22
His	3.72[c]	3.93[ab]	4.04[a]	3.97[ab]	3.84[bc]	3.76[c]	0.049	0.15
Arg	6.79[b]	7.00[a]	6.83[b]	6.80[b]	6.72[b]	6.46[c]	0.038	0.12
Asp	6.27[b]	6.31[b]	6.11[b]	6.26[b]	6.88[a]	6.99[a]	0.076	0.23
Thr	3.45[ab]	3.43[b]	3.23[c]	3.61[a]	3.35[bc]	3.30[bc]	0.054	0.17
Ser	4.95[a]	4.97[a]	4.89[a]	4.98[a]	4.87[a]	4.96[a]	0.037	0.11
Glu	28.33[c]	27.68[d]	29.24[b]	28.47[c]	29.88[a]	29.38[b]	0.123	0.38
Pro	9.28[a]	9.49[a]	9.26[a]	9.21[a]	9.33[a]	9.17[a]	0.126	0.39
Gly	4.80[b]	4.96[a]	4.68[b]	4.81[b]	4.75[b]	4.73[b]	0.041	0.13
Ala	4.12[ab]	4.20[a]	4.00[b]	4.18[a]	4.08[ab]	4.18[a]	0.050	0.15
Val	4.36[ab]	4.24[ab]	4.22[b]	4.31[ab]	4.38[a]	4.24[ab]	0.045	0.14
Met	1.22[a]	1.27[a]	1.19[a]	1.22[a]	1.24[a]	1.24[a]	0.104	0.32
Ileu	3.79[a]	3.60[bc]	3.57[bc]	3.71[ab]	3.54[bc]	3.75[a]	0.047	0.14
Leu	7.60[a]	7.54[a]	7.59[a]	7.45[ab]	7.34[b]	7.57[a]	0.056	0.17
Tyr	3.07[bc]	3.09[bc]	3.13[abc]	3.12[abc]	2.93[c]	3.26[a]	0.051	0.16
Phe	4.78[a]	4.89[a]	4.79[a]	4.75[a]	4.70[a]	4.77[a]	0.067	0.21

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

for lysine, adequate amounts of the essential amino acids were present in the wheat varieties. However, when compared to sorghum and millet (Table IV), wheat has a much higher amount of lysine. It is known that the utilization of lysine and isoleucine in protein is affected by the amount of leucine present (Haper et al., 1955; Doesthale et al., 1970, Monteiro et al., 1982). A lysine to leucine ratio greater than 4.6 has been reported to hinder the utilization of lysine (Doesthale et al., 1970). The wheat varieties have a mean lysine to leucine ratio of 1.73. Sorghum and millets grown in Nigeria have lysine to leucine ratios of 7.25 and 4.37, respectively (Table IV). These higher ratios in sorghum and millets will no doubt further make them nutritionally inferior to the cultivated wheats.

The variation in the individual amino acids with level and stage of application of nitrogen is shown in Table V. Increasing the applied nitrogen and varying its stage of application resulted in variation in the concentration of a number of amino acids. The interaction among nitrogen treatments and stage of application was not significant for only four amino acids—serine, proline, methionine, and phenylalanine. A number of workers (Gunthardt and McGinnis, 1957; Voiker, 1960; Sosulski et al., 1963) have shown that increasing application of nitrogen to wheat caused increases in the content of glutamic acid and proline in the grain proteins. Our studies with seven wheat cultivars showed that glutamic acid generally increased (significantly) with an increase in applied nitrogen (Table V). Proline was however not afffected either by the level of nitrogen or by the stage of application. In contrast to the findings of several workers (Larson and Nielsen, 1966; Abroil et al., 1971; Kolderup, 1974; Dubetz and Gardiner, 1979) that valine decreased with an increase in nitrogen, our results showed that this amino acid was generally not affected by an increase in nitrogen except for a significant increase observed when the highest level of nitrogen was applied at the tillering stage (Table V). This is in agreement with the recent research report of Bushuk et al. (1978) who showed that valine was fairly constant in irrigated Neepawa wheat grown under high rates of nitrogen. When individual wheat varieties were considered, however, it was observed that in two varieties (Sonora 63 and (Lee × 108) GB 55) valine significantly decreased with increases in applied nitrogen while in each of the remaining five varieties there was either a nonsignificant increase or no change at all. It is, therefore, likely that the decrease in the content of valine with increase in applied nitrogen may not apply to all wheat varieties under all growing condi-

tions. Lysine generally showed a decrease with increase in applied nitrogen, but this was only statistically significant when the highest level of nitrogen was applied at the tillering stage.

Correlation coefficient matrix for protein content and individual amino acids showed that lysine had the highest negative correlation with protein ($p < 0.01$). This observation is in agreement with the research report of several workers (Larsen and Nielsen, 1966; Abroil, 1971; Dubetz and Gardiner, 1979). Of all the amino acids, glutamic acid had the highest negative correlation with a number of other amino acids especially threonine, arginine, and lysine. It is interesting to note that glutamic acid is the major amino acid in gluten, the main storage protein of wheat (Lasen and Nielsen, 1966). Gluten is low in a nunber of essential amino acids particularly arginine (Larsen and Nielsen, 1966) which are present in high amounts in the albumin globulin proteins. The negative correlation between glutamic acid and certain amino acids (threonine, arginine, and lysine) is, therefore, in agreement with the fact that these amino acids are not closely related to glutamic acid in same proteins.

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Techniques for Intrinsically Labeling Wheat with ^{65}Zn

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Several techniques of intrinsically labeling wheat with ^{65}Zn were compared: stem injection of ^{65}Zn, stem injection of ^{65}Zn + $ZnSO_4$, foliar application of ^{65}Zn, and the addition of ^{65}Zn to a hydroponic solution. Incorporation levels of ^{65}Zn into the grain were 62.6% stem injected, 45.2% stem-injected ^{65}Zn + $ZnSO_4$, 57.5% foliar application, and 2.3% hydroponic solution. Four protein fractions were extracted from fat-free whole wheat flour. Distribution of ^{65}Zn into the protein fractions for all treatments, was 8.5–20.3% in albumins and globulins, 47.4–60.3% in glutenins, 1–2.6% in gliadins, and 9.8–28.3% in the remaining proteins. Separation of the fractions by gel chromatography showed that protein and Zn distributions were similar among the treatments and when compared to the controls. Zinc-65 distribution was similar to the natural Zn distribution. These data illustrate that stem-injected ^{65}Zn is incorporated in the same manner and ratios as Zn naturally utilized by wheat.

INTRODUCTION

The increased emphasis on determining the bioavailibility of trace elements from major food stuffs of the world as outlined in the USDA/ARS 6-year implementation plan 1984–1990 has placed new emphasis on developing a simple, cost-effective, rapid method of intrinsically labeling foods with isotopes.

Traditionally, hydroponic systems have been used to label plants intrinsically with radioisotopes. Bergh (1950) labeled sweet peas with radiozinc by adding 0.2 mCi of ^{65}Zn to a Hoagland's solution. After 24 h, the activity in plant material was determined. Bergh noted that most of the radiozinc remained in the roots.

In recent years several investigators have used hydroponics to label plants intrinsically with ^{65}Zn (Meyer et al., 1983; Levine et al., 1982; Ketelsen et al., 1984; Schmitt and Weaver, 1984). This technique, although the most physiologically natural, requires relatively large amounts of isotopes and produces large volumes of radioactive liquid waste. Also, the limited upward mobility of Zn and Fe in plants produced a low ratio of applied to incorporated isotopes (Noggle and Fritz, 1976). Garcia et al. (1977) endogenously labeled corn plants with ^{65}Zn in a hydroponic system. After tassel emergence the plants were placed in nutrient solution containing ^{65}Zn. After harvesting, only 27.6% of the ^{65}Zn was incorporated into the corn kernels. Similar incorporation, 21.3–27.6% of ^{65}Zn administered hydroponically to soybean plants, was reported by Janghorbani et al. (1983). In addition, the absorption via the root system is dependent upon a multiplicity of factors, pH, ionic concentrations, age of plant, ion chelation, etc., and these may vary for each plant species.

One method of fertilizing plants, foliar application, may be applicable to intrinsic labeling. Foliar application of phosphates, iron, and zinc has been used in agricultural systems to correct mineral deficiencies and to avoid possible soil interactions that would render the applied nutrient unavailable to the plant. The use of foliar appli-

cation for labeling plants has been very limited and, to our knowledge, our use of this method is currently unique.

A third technique is stem injection. Roach (1938) used a modification of this technique to determine mineral deficiencies in fruit trees. Since this method was used on trees, for which hydroponic culturing is not applicable, most advantages listed by Levy (1939) are not applicable. Only the fact that one could be sure the particular substance supplied would actually enter the plant system is pertinent to this study.

The only previous work concerning the absorption and translocation of stem-injected mineral isotopes is that of Zeind (1967). He concluded that in stem injection, the isotope distribution within corn seeds approached equilibration and that it was the most efficient method for intrinsically labeling plants.

We have been using a modification of Zeind's method (1967) to intrinsically label wheat, soy, and oats for the past 3 years. Criticism has been raised that although this technique provides good incorporation of the injected isotope into the seed, the "unnatural" method may result in deposition of Zn at higher than natural levels or in different forms than the physiological natural form.

This study was initiated to compare hydroponic, foliar, and stem-injection applications of ^{65}Zn to wheat and to characterize the proteins and their Zn content to determine which method produced the best incorporation without altering the seeds' natural protein content or Zn distribution.

MATERIALS AND METHODS

Wheat, *Triticum aestivum* var. Waldron, was grown in a greenhouse with supplemental lighting provided by 400-W high-pressure sodium lamps (Energy Technics, York, PA) to produce a 16-h light:8-h dark cycle. Plants were grown in 8-in. plastic pots, 7 plants per pot in soil with 30% perlite, and watered as needed. All plants except the ones grown in the hydroponic system were grown in the soil mixture.

The hydroponic system was a "closed aggregate" system with 70% perlite and 30% vermiculite. Modified Hoagland's solution plus the A–Z micronutrient solution were used (Hoagland and Arnon, 1950). Iron was added as

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