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INCORPORATION OF ^{15}N LABELLED UREA AND AMMONIUM INTO PROTEINS AND AMINO ACIDS OF *SORGHUM*

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Key Word Index—*Sorghum vulgare*; Gramineae; sorghum; protein and amino acid changes; grain development ^{15}N study.

Abstract—Protein fractionation studies in developing Sorghum kernel indicated a considerable decrease in the proportion of albumin and increase in prolamin, glutelin and residue proteins during grain development. The globulin fraction remained more or less constant. ^{15}N analysis indicated a turnover of albumin and globulin fractions. The nitrogen present in these fractions appeared into glutelin and residue proteins. At an early maturation stage ^{15}N from ammonium was detected in the residue fraction while that from urea was incorporated in both albumin and residue fractions. However, this difference disappeared as the grains matured. Incorporation of ^{15}N into basic amino acids was lower when compared to that in neutral and acidic amino acids at all stages of grain development.

INTRODUCTION

The factors responsible for the low biological value of Sorghum grain proteins have been identified as extreme deficiency of lysine and tryptophan [1], excessive content of leucine [2] and formation of tannin–protein complexes [3]. Application of nitrogenous fertilizers increases the protein content in the grain but the quality of the additional protein formed may be poor on account of the deposition of protein fractions which are deficient in lysine [4]. The nature of additional proteins synthesized as a result of nitrogen fertilization is not known with certainty.

In maize it has been shown that the albumin fraction decreases with a simultaneous increase in glutelin and prolamin during later stages of grain ripening [5–7]. Nitrogen fertilization may have a direct effect on the relative proportions of different protein fractions as well as on their amino acid compositions. Hence in the present investigation ^{15}N labelled urea and ammonium sulphate were used to trace the fate of applied nitrogen during grain development and its incorporation into different amino acids.

RESULTS

The distribution of ^{15}N atom% excess in protein nitrogen (TCA insoluble) is shown in Table 1. It was maximal at 7 days after injection and decreased during grain development. At all stages of grain development the enrichment was higher with ammonium than with urea. This indicated that ammonium was utilized more efficiently than urea. A total of 40% and 25% of the injected ^{15}N was recovered in grains at maturity from ammonium and urea respectively.

The incorporation of urea-^{15}N into different protein fractions is given in Table 2. Most of the ^{15}N injected was incorporated into residue and albumin fractions at 7 days after injection. The ^{15}N atom % excess decreased considerably in the globulin fraction as the grains matured, although the proportion of globulin did not change significantly. The ^{15}N atom % excess in albumin also decreased considerably. The decrease in ^{15}N atom % excess in these fractions indicated a turnover of these fractions. Most of the ^{15}N increase during development is accounted for by the residue protein followed by glutelin and prolamin. The proportion of albumin decreased while that of prolamin, glutelin and residue proteins increased during grain development. Globulin content on the other hand increased at 14 days after injection and decreased at maturity. Muravin and Kozhemyachko [8] reported that in wheat ^{15}N from urea and ammonium nitrate was incorporated in albumin and globulin fractions when applied during late growth stage. Nitrogen from foliar sprays at flowering was incorporated into glutelins also.

A similar trend in the incorporation of ^{15}N into different protein fractions was also observed when ammonium ^{15}N was injected (Table 3). At early maturation stage most of the ^{15}N from urea appeared in albumin and

Table 1. ^{15}N atom % excess distribution in TCA insoluble fraction

Days after injection	Treatment	% Protein	^{15}N atom % excess in TCA in soluble fraction
7	Ammonium sulphate	11.9	1.312
	Urea	11.9	0.552
14	Ammonium sulphate	11.6	0.537
	Urea	11.9	0.315
21	Ammonium sulphate	11.0	0.306
	Urea	11.3	0.178

Table 2. ^{15}N atom % excess and proportional distribution in various protein fraction from urea-^{15}N at different stages of grain development

Days after injection	^{15}N atom % excess				
	Albumin	Globulin	Prolamin	Glutelin	Residue
7	0.464	0.473	0.583	0.512	0.678
	(33.4)	(7.97)	(9.94)	(10.6)	(38.1)
% of total protein	39.2	9.10	9.78	10.2	30.6
14	0.266	0.265	0.335	0.326	0.379
	(16.4)	(10.7)	(13.6)	(15.2)	(44.2)
% of total protein	20.0	13.2	13.5	15.3	37.9
21	0.131	0.116	0.193	0.199	0.246
	(6.79)	(5.35)	(14.7)	(19.6)	(53.6)
% of total protein	10.5	9.42	15.6	20.0	44.5

Values in parenthesis represent the % distribution of ^{15}N.

residue fraction while most of the ^{15}N from ammonium appeared in the residue fractions. At maturity however, these differences were not observed. ^{15}N atom % excess decreased considerably in globulin fraction as the grains matured although the proportion of globulin did not change significantly. This indicated either a turnover of globulin synthesized earlier or its conversion into other fractions, mainly residue proteins. The decrease in the proportion of ^{15}N in albumin and globulin fractions and a corresponding increase in glutelin and residue fraction suggest that the nitrogen from these appears in glutelin and residue fractions. The overall decrease in ^{15}N atom % excess in globulin, prolamin, glutelin and residue proteins takes place on account of the increase in the amount of these protein fractions during grain development.

The results in Table 4 show the ^{15}N atom % excess in basic, neutral and acidic amino acids when urea or ammonium were injected during grain development. At early stages of development the ^{15}N enrichment of neutral amino acids was higher in both the cases. Even the basic amino acids had a higher proportion of ^{15}N atom % excess compared to aspartic acid. Glutamic acid had a higher enrichment compared to the basic amino acids during grain development. The atom % excess decreased in all amino acids, which was mainly due to the increase in the total amino acid content as a result of higher protein synthesis. It was found that proportionately more label appeared in glutamic and aspartic acids when ammonium was used than when urea was used.

DISCUSSION

It is known that ammonia is incorporated into glutamate by the glutamic dehydrogenase pathway [9] or by the glutamine synthetase-glutamate pathway [10, 11]. The α-amino group of glutamic acid is rapidly used for the synthesis of other amino acids by transamination. Glutamic acid accounts for the major portion of grain protein nitrogen. The decrease in the proportion of ^{15}N in glutamic acid during grain development is due to its interconversion and fresh synthesis of unlabelled glutamic acid. Incorporation of ^{15}N into aspartic acid was quite low. Considering the fact that the total aspartic acid content is much lower than glutamic acid, the very low ^{15}N content might be due to its conversion to lysine or other amino acids. During maturation, the ratio of ^{15}N in aspartic to ^{15}N in glutamic acid increased. This was mainly due to a relative decrease in the ^{15}N content of glutamic acid with maturity.

The present results indicate that ^{15}N atom % excess decreased considerably in the globulin fraction as the grain matured, although the proportion of globulin in total protein did not change significantly. This indicated either a turnover of globulin synthesized earlier, or its incorporation into other fractions, mainly residual proteins. The decrease in the proportion of ^{15}N in albumin fractions and its corresponding appearance in glutelin and residue fractions suggest that the N from albumin also appeared in glutelin and residue fraction. The proportion of ^{15}N from ammonium incorporated into the prolamin fraction remained more or less constant during

Table 3. Distribution of ^{15}N atom % excess in various protein fraction from ammonium sulphate-^{15}N at different stages of grain development

Days after injection	^{15}N atom % excess				
	Albumin	Globulin	Prolamin	Glutelin	Residue
7	0.272	1.61	2.15	1.99	2.45
	(7.21)	(10.1)	(15.0)	(19.8)	(47.9)
% of total protein	37.9	9.00	10.0	14.3	28.8
14	0.646	0.686	0.931	0.906	1.19
	(13.6)	(9.23)	(14.2)	(14.9)	(48.1)
% of total protein	19.5	13.4	14.5	15.5	37.1
21	0.358	0.324	0.440	0.527	0.709
	(7.82)	(5.72)	(13.3)	(18.7)	(54.6)
% of total protein	12.0	9.63	16.5	19.3	42.7

Values in parenthesis represent the % distribution of ^{15}N.

Table 4. ^{15}N atom % excess in amino acids at different stages of grain development

Amino acids	^{15}N source	Days after injection 7 Enrichment (atom % excess)	14 Enrichment (atom % excess)	21 Enrichment (atom % excess)
Basic (Lysine, Arginine and Histidine)	Urea	0.139	0.117	0.063
	Ammonium sulphate	0.249	0.263	0.247
	Urea	0.405	0.240	0.193
Neutral				
	Ammonium sulphate	1.396	0.743	0.516
	Urea	0.161	0.105	0.107
Glutamic				
	Ammonium sulphate	0.510	0.243	0.223
	Urea	0.066	0.062	0.047
Aspartic				
	Ammonium sulphate	0.158	0.134	0.133
	Urea	0.409	0.590	0.439
Ratio Aspartic/Glutamic	Ammonium sulphate	0.309	0.550	0.596

grain development but when urea was used it increased from 10% in immature grain to 15% in the mature grain. The results obtained in this investigation suggest a turnover of albumin and globulin fraction synthesized during early stages of grain formation and the incorporation of the N thus released in to glutelin and residue proteins. Alternatively, albumin and globulin may combine with other protein fractions deficient in lysine and rich in leucine to form glutelin and residue proteins.

EXPERIMENTAL

Sorghum variety CSH-2 was grown on the Institute farm. At 10 days after ear emergence, 10 mg of N in the form of either urea-^{15}N or $(NH_4)_2SO_4$-^{15}N containing 94.6 and 95.6 atom % excess respectively was injected in to the shank portion immediately below the ear at two diametrically opposite points. The wounds were sealed immediately with Quickfix. Grain samples were collected at weekly intervals after injection up to 3 weeks, when the grains were fully mature. At each stage, kernels were dehusked and lyophilized.

Protein-fractionation. Proteins were extracted by the modified Mendel–Osborne solvent extraction method as described in ref [12]. The supernatant from the last extraction was checked for completeness of extraction. N was estimated by macrokjeldahl method [13].

^{15}N atom % excess. The method of ref [14] was followed for the determination of ^{15}N atom % excess by MS. Protein and non-protein fractions were separated by precipitation with 2.5% TCA as described in ref [15]. The amino acids (basic, neutral and acidic) were separated by high voltage electrophoresis method [16] after conventional acid hydrolysis. ^{15}N atom % excess was estimated in each fraction. The values reported are averages of at least duplicates which did not vary more than 2%.

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