Pakistan J. Sci. Ind. Res., Vol. 20, No. 2, April 1977

EFFECT OF NITROGEN FERTILIZATION ON THE AMINO ACID CONTENT OF CORN GRAIN PROTEIN

MOHAMMAD SADIQ and MALIK HUSSAIN HAMID

Colorado State University, Fort Colins, Colorado, USA

I. HAQUE

Agricultural University, Lyallpur

(Received September 3, 1975; revised December 9, 1975)

Abstract. Corn plants were grown in the field, sampled and analyzed at various stages of growth. Amino acids pattern was studied in mature corn grains. Lysine decreased and tryptophan increased by 14% and 10%, respectively, when 150 kg nitrogen per hectare (N/ha) was applied. The maximum increase was observed in threonine and serine (about six and eight times, respectively). Histidine, glutamic acid, and glycine also increased significantly with nitrogen fertilizer application. Aspartic acid, argnine, methionine, isolcucine, phenyalanine and valine showed variable trends to nitrogen fertilization. A decrease with nitrogen application was observed in alanine, leucine, tyrosine and proline content. Total protein and grain yield were also increased with fertilization.

Corn ranks next to wheat and rice in grain production in Pakistan. It is relatively low in protein content and contains an unbalanced pattern of essential amino acids, particularly deficient in lysine and tryptophan. Improvements in basic corn protein composition would have far reaching consequences in solving ever increasing nutritional problems in Pakistan.

For the last two decades, one of the objectives of plant scientists has been to increase protein content genetically, but there are evidences indicating the improvements in protein content as well as in the pattern of various amino acids by the use of nitrogen fertilizers.

Dimova and Simeonova[3] showed that nitrogen fertilizers increased amino acids content of corn grain, particularly asparagine, glutamine and alanine. Beetsman and Stangle[1] studied the interaction between the nitrogen rates and free amino acids of corn silage and reported an increase in those required for rumen micro-organisms for maximum cellulose digestion and those that deaminate easily in the rumen. Five-fold increase was observed in glysine, serine, glutamic acid, proline and phenyl-alanine content of barley plants, with heavy rates of nitrogen in a pot experiments (Eppendorfer). Similar results were reported by Shaaban[4] and Dzanagov, *et al.*[12]. They observed 1.7 to 1.9 times more protein in corn grain when 120 kg N/ha was applied. Lysine, threonine, methionine, phenyl-alanine, tryptophan, leucine, and isoleucine were also increased in corn with nitrogen fertilization.

Mangel and Helal[10] investigated the effect of nitrogen supply on soluble amino acid fraction in spring wheat. They also reported that nitrogen increased the soluble amino acids especially glutamic acid, aspartic acid, alanine and serine.

In the present study, the amino acids response to nitrogen fertilization were investigated. The information so obtained may serve as a guide to develop sound fertilizer practices leading to maximum production of improved quality corn grain.

Materials and Methods

Corn variety "Neelam" (a variety grown in Pakistan) was grown for this study. The field was previously under non-experimental corn fodder, grown without the application of fertilizer to minimize residual effect, if any. The soil was sandy loam of medium fertility with pH 7.8. After preparing the seed bed, phosphorus (76 Kg P_2O_5 as TSP, per hectare) and potash (50 Kg K_2O as K_2SO_4 per hectare) were broadcast uniformly and plowed into the soil. Experimental plots of size 2.44 x 27.4 meters were prepared according to randomized block design and replicated 4 times. Corn seeds were sown with single row cotton drill; row to row distance was 0.6 meter. Ammonium sulphate, as a nitrogen source was placed 6 cm to the side and 6 cm below the seeds. The nitrogen rates were 0, 50, 100, 150 and 200 kg/hectare. Plants were thinned to 23 cm distance when they were about 31 cm tall.

The corn plants were sampled, separated into leaves and stalks and analyzed at 45 days, tassel formation and maturity for total nitrogen, protein, nonprotein non-amino acid nitrogen and free or soluble amino acids.

The mature grains were sampled, dried at 60° ground, seived and analysed for protein, total nitrogen, free amino acids and non-protein nitrogen. The spectrum of grain protein amino acids was prepared on ELL 193 amino acids analyzer, using 20 to 50 mg of grains in acid washed, heavy walled hydrolysis tube. Four ml of 6N HCl (ammonium free) was added. The air above the acid was replaced with nitrogen gas and tube was sealed immediately. Excess acid was evaporated at 40° ; after keeping the tube at 105° for 24 hr. The residue was dissolved in 2.2 pH buffer and analyzed to obtain the amino acids spectrum[4] (Sadiq). Tryptophan was determined with Spies and Dorris[16] method.

Results and Discussions

To understand the behavior of nitrogen metabolism during growth period, corn plant leaves were analyzed at various stages of growth. Total nitrogen content and various other nitrogen fractions increased with nitrogen fertilization. Different nitrogen forms accumulated till tassel formation but onwards decreased significantly. Fig. 1, Fig. 2, Fig. 3.

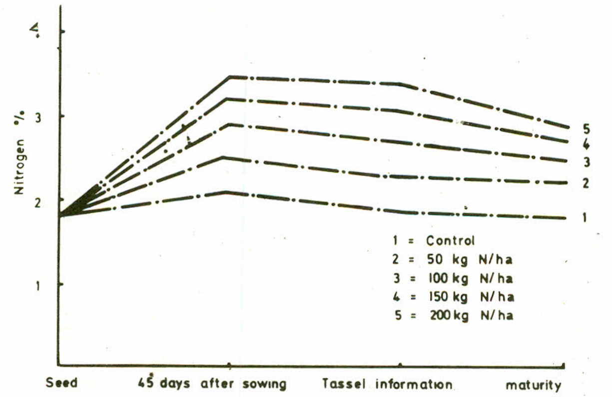

Fig. 1. Effect of nitrogen fertilization on total nitrogen content of corn leaves at various stages of growth (% dry matter).

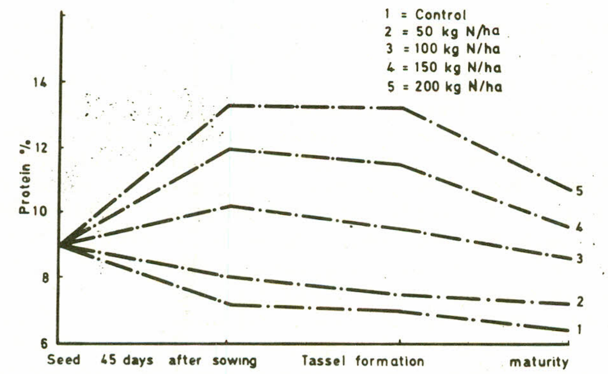

Fig. 2. Effect of nitrogen fertilization on protein content of corn leaves at various stages of growth (% dry matter).

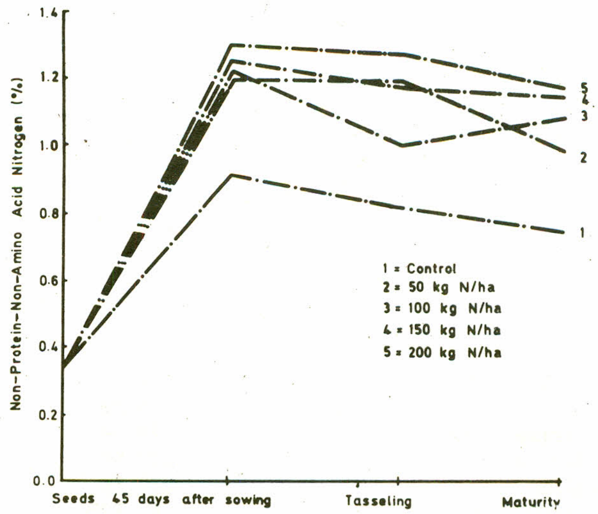

Fig. 3. Effect of nitrogen fertilization on non-protein non-amino acid – N in corn leaves at various stages of growth (% dry matter basis).

The decrease in leaf protein, total nitrogen and non-protein non-amino acid nitrogen at tassel formation was small, which, possibly, may be due to growth dilution effect[13] (Sadiq, *et al.*) Translocation of nitrogenous fractions from leaves to grains or roots could be the major cause for the decrease in these fractions at maturity[1] (Murneek).

The chemical composition of corn grains and ear (cob plus grain) yield per plot is shown in Table 1. Total nitrogen, protein, free amino acids, non-protein nitrogen and yield of mature grains increased significantly with nitrogen fertilization.

Nitrogen taken up by the plants, generally as nitrate, increased with nitrogen application (Kamrath and Nunez, 1967). This increased nitrate supply might have enhanced the nitrate reductase enzyme activity[7] (Hageman and Flesher) resulting in higher incorporation of nitrogen in organic combinations, mostly amino acids and protein.

The amino acid content of corn grain is shown in Table 2. Nitrogen fertilization affected amino acids variably.

TABLE 1. EFFECT OF APPLIED NITROGEN ON CORN GRAIN NITROGENOUS FRACTIONS AND YIELD.

Nitrogenous fractions	Percent	Control	50 kg N/ha	100 kg N/ha	150 kg N/ha	200 kg N/ha	LSD 5% level of significance
Protein	Grain dry wt	8.91a	10.42b	11.76c	13.50d	14.59e	0.92
	Total nitrogen	80.2	82.1	83.3	86.1	78.8	—
Free A. A.	Grain dry wt	0.006a	0.007a	0.008b	0.01c	0.011d	0.001
	Total nitrogen	0.36	0.31	0.33	0.38	0.35	—
Non-P	Grain dry wt	0.34a	0.49c	0.49c	0.38b	0.62d	0.046
Non-A.A.	Total nitrogen	19.44	17.59	16.37	13.52	20.85	—
Non-P	Grain dry wt	0.3459	0.4971	0.4948	0.3949	0.6328	—
Total-N	Grain dry wt	1.77a	2.16b	2.38c	2.52d	2.97c	0.186
Yield (kg of ears/plot)		24.8a	32.1b	39.3c	43.7d	45.8d	3.6

P, protien; A.A., amino acid; N, nitrogen; LSD, least significant difference.

TABLE 2. EFFECT OF NITROGEN FERTILIZATION ON THE AMINO ACID OF CORN GRAIN (mg OF AMINO ACID PER GRAM OF PROTEIN NITROGEN). *

Amino acid	Control	50 kg N/ha	100 kg N/ha	150 kg N/ha	LSD (5%LS)
Lysine	123.8c	108.4b	101.1a	106.9b	3.4
Tryptophan	30.8b	28.7a	32.45c	33.8d	1.7
Histidine	21.7	41.9	—	—	—
Argnine	18.9bc	17.9b	21.8c	10.19a	3.6
Aspartic acid	476.9d	359.3c	262.2a	322.7b	24.3
Threonine	27.3a	47.9b	147.3c	167.0d	17.9
Serine	39.9a	35.9a	177.1b	346.3c	10.9
Glutamic acid	920.3a	1211.38b	1352.13c	1379.6c	42.2
Proline	58.0d	48.5c	25.00b	17.6a	2.9
Glycine	258.74a	275.45a	327.54b	437.9c	21.8
Alanine	545.5d	474.3c	422.9b	365.3a	19.5
Valine	227.3b	262.3d	234.0c	192.6a	6.3
Mathionine	82.52b	159.9d	51.1a	107.9c	8.9
Isoleucine	161.54b	185.0c	160.6b	142.6a	5.8
Leucine	827.9c	826.4c	751.6b	665.74a	14.5
Tyrosine	324.48d	242.5b	269.15c	242.6a	6.7
Phenylalanine	296.5	262.9	275.5		—

*A average of 4 repeats; a, b, c, and d indicate significant difference; LSD, least significant difference.

Lysine in the control was 123.8 mg which decreased to 106.9 mg when 150 kg N/ha was applied. Although tryptophan increased from 30.8 mg in the control to 33.8 mg in the grains receiving 150 kg N/ha, this increase was quite small. Threonin, serine, glutamic acid, glycin and histidine increased significantly with nitrogen fertilization.

Aspartic acid, argnine, methionine, isoleucine, valine, and phenyl-alanine followed no definite trend. Aspartic acid in the control was 476.9 mg which decreased to 359.2 mg and 262.2 mg with 50 and 100 kg N/ha, respectively ; and then increased to 322.7 mg when 150 kg N/ha was applied.

Methionine, more or less, followed the similar trend. Non-significant decrease was observed in argnine content with 50 kg N/ha but increased significantly when 100 kg N/ha was applied. The application of 150 kg N/ha decreased argnine content to almost half of the control. Isoleucine and valine increased with low nitrogen application (50 kg N/ha) but higher rates (100 and 150 kg N/ha) had adverse effect.

Nitrogen fertilization decreased proline, alanine, leucine, and tyronine.

Keto acids are present in variable amounts in plants. Those that occur abundantly and are in activated form would yield much of their corresponding amino acids, provided ammonia is not a limiting factor. This variability of keto acids availability may be responsible for heterogenous increase in amino acids[6] (Gustafson).

In addition to the direct ammination of corresponding keto acids, aminoacids are also formed by inter conversion. For example, glycine, through a number of reactions, is converted to serine and the cystine and methionine[2] (Davies).

Glycine —→ Serine —→ Cystine—→ Methionine

Any increase in glycine content would increase the serine, cystine, or methionine irrespective of corresponding keto acids. This may be one of the reasons of differential behavior of amino acids.

A third possibility could arise from the amino acids biosynthesis control system. Investigations of the feed back mechanism reveal that the presence of certain amino acids above a definite limit inhibit the synthesis of other amino acids. In accordance with the very complicated and variable mechanisms involved in amino acids synthesis, biosynthesis control on amino acids is not fully understood. Therefore, nothing can be said for sure, except that this system prevails. Biosynthesis control could be responsible for the observed decrease in proline, alanine, leueine, and tyronine[15] (Sims, *et al.*).

The tremendous increase in methionine could be the result of sulphur[5] (Eppendorfer) present in nitrogenous and potash fertilizers.

Conclusion

Nitrogen fertilization has increased total nitrogen, protein and free amino acids of corn fodder which, in turn could improve the digestibility of fodder[1] (Beetsman and Stangle). Increase in grain production can minimize the gap between food supply and consumption.

The results of this study suggest that grain protein, along with yield can be increased with nitrogen fertilization. The increase in tryptophan is quantitatively small but decrease in lysine content could degrade corn protein quality. The significance of the increase or the decrease of other amino acids to food or feed is rather difficult to assess. Further studies, especially on nutritional aspect of amino acid alterations are needed.

References

1. G. B. Beestman and P. J. Stangle, Soils and Fertilizers, Commonwealth Bur. Soil Sci., **29,** 1112 (1966).
2. D. D. Davies, *Recent Aspects of Nitrogen Metabolism in Plants* by Hewitt and Cutting (Academic Press, London, 1968), pp. 125-135
3. R. Dimova and I. Simeonova, Prochv. Agrokhim, **1,** 63-72, Soils and Fertilizer Commonwealth in Bur. Soil Sci., **29,** 3529 (1966).
4. Kh. B. Dzanagov, S. Kh. Dzanagov and T. K. Teziev, Agrokhim **4,** 14-17, Soils and Fertilizers Commonwealth Bur. Soil Sci., 32, 4005 (1969).
5. W. Eppendorfer, Plant and Soil, **29,** 425-438 (1968).
6. F. G. Gustafson, Plant Physiol., **21,** 49-62 (1946).
7. R. H. Hageman and D. Flesher, Plant Physiol., **35,** 700-708 (1960).
8. Philip B. Hawk, Bernard L. Oser and William H. Summerson, *Practical Physiological Chemistry* (P. Blaciston's Co. Inc, Philadelphia, 19€2).
9. M. L. Jackson, *Soil Chemical Analysis* (Cantable and Co. Ltd., London, 19€0).
10. K. Mangel and M. Helal, Soils and Fertilizers Commonwealth Bur. Soil Sci., **34,** 1674 (1971).
11. A. E. Murneek, Plant Physiol , **7,** 79-90 (1932).
12. S. Shaaban, Acker-U-Pf1 Baul, **28,** 221-238, Soils and Fertilizers Bur. Soil Sci., **32,** 730 (1969).
13. M. Sadiq and M. H. Hamid, Muslim Scientist, **4,** 22-27 (1975).
14. M. Sadiq, Effect of Nitrogen Fertilization on Nitrogen Metabolism in Maize, M.Sc. (Hons.) Soil Sci. Thesis, Univ. of Agriculture, Lyallpur (1972).
15. A. P. Sims, B. F. Folkes and A. H. Buffey, *Recent Aspects of Nitrogen Metabolism in Plants* by Hewitt and Cutting (Academic Press, London, 1968), pp. 91-114
16. J. R. Spies and C. Chamber Dorris, Anal. Chem., **21,** 1249-1268 (1949).
17. U. S. Salanity Lab. Staff, Diagnosis and Improvements of Saline and Alkali Soils, U.S.D.A. Handbook No. 60, 1-160 (1954).