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ELD AND PROTEIN QUALITY OF MAIZE GRAIN AS AFFECTED BY FERTILIZER APPLICATION*

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Abstract

A field experiment with maize (*Zea mays* L.) under irrigated condition on a Sulfic Tropaquept soil at 9 rates of nitrogen applications with constant rates of P, K and 3 widely varying rates of P K and with constant N application showed a four fold increase in grain yield at 200 kg N ha^{-1} (3.73 t ha^{-1}) after which the yield decreased. The yield of crude protein as well as its concentration in grain however, increased up to an application of 350 kg N ha^{-1}. A combination of P and K of 75 and 225 kg ha^{-1} respectively at 300 kg N ha^{-1} gave about a ten fold increase in grain yield over no P and no K application. The crude protein yield was also the maximum at this combination although the concentration of CP was at its maximum for no P and no K application. Increase in grain CP was accompanied by a decrease in its quality as indicated by the amino acid content in grain protein. Lysine showed a significant decrease ($y=4.192-0.75$ N, $r=0.784^{*}$) with increasing crude protein content. The yield per hectare of the EAA as well as the non-EAA however increase with increasing dry matter yield as influenced by fertilizer application. Increasing the content of a particular amino acid in grain protein to raise the nutritional quality of it by applying plant nutrients in excess of what is needed to avoid nutrient deficiency and decrease in yields is possible only if such an application do not increase the CP or N content. However, production per unit area of individual amino acid always increases with increase in dry matter yield which can otherwise compensate the degraded protein quality.

Introduction

Judicious fertilizer applications to attain maximum economic yield particularly for cereal based agriculture has gained a tremendous popularity over the last decades.

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It is a general observation that increasing the rate of N fertilizer application is always accompanied by an increase in grain protein with concomitant decrease in the yield of cereals, particularly *Z. mays* (Verma and Singh, 1976; Rending and Broadbent, 1979). Application of higher amounts of either N or P or P and K in soils relatively deficient in one of these elements failed to give higher yield or yield increases is also reported (Kumaraswamy *et al.*, 1975; Fox *et al.*, 1976). This is consistent with the improvement of the effectiveness of N fertilizer use by crop in presence of a balanced supply of other nutrients, especially P and K as described by Arnon (1975) and Khattak *et al.* (1976).

Crop quality is an important aspect in the culture of it. Crop quality as it relates to its protein content is no less an important criterion as plants supply directly or indirectly the greater portion of human diet. According to an estimate of FAO, 25-30% of the population of some developing countries are suffering from protein deficiency (Chaudet, 1975). In Bangladesh, about 70% of the population that live below the subsistence level suffer from poor nutrition. Researches in the developed countries have directed their efforts towards increasing the protein contents of crop plants. Intensification of fertilizer use is one of the means used to arrive at this goal. However, it has been established that, for grains of several cereals including maize, increase in the protein content through N, P and K fertilization is often accompanied by a decline in quality, as shown by a decrease in the content of some essential amino acids (EAA) in protein (Sauberlich *et al.*, 1953; Keeny, 1970; Breteler, 1976; Eppendorfer, 1975; 1978; Rendig and Broadbent, 1979).

Keeping these facts in view, the present study was undertaken to investigate the effects of different levels of N, P and K fertilization on grain yield, grain protein content and grain amino acid composition of a local variety of maize in Thailand.

Experimental: The experiment was carried out on plots of 9 M^2 on a Sulfic Tropaquept soil situated in the premises of the Asian Institute of Technology, Bangkok, Thailand, during the dry season (January to May) of 1980 under irrigated conditions. The soil was clay loam having a pH 0.1 N $CaCl_2$ of 5.3, poor in N and P but rich in available K and Na supply. Commercial limestone applied at the rate of 7 t ha^{-1} raised the pH of the soil on an average to 6.8.

There were two sets of fertilizer treatments eg., (i) nine levels of N at the rates of 0, 25, 50, 100, 150, 200, 250, 350 and 450 kg ha^{-1} in triplicate with a basal dose of 225 kg P and 150 kg K ha^{-1}; (ii) "P K treatments" containing three levels of P and K, at the rates of 25, 75 and 225 kg ha^{-1} in all combinations in triplcates with a basal dose of 300 kg N ha^{-1}. Fifteen kg per ha of Mg were included in all the treatments. N,P, K and Mg fertilizers were supplied as ammonium sulphate, potassium chloride, triple super phosphate and magnesium carbonate respectively. The "N treatments" were arranged in a systematic and the "P+K treatments" in a factorial design. Three extra plots of the same size received 200 kg N ha^{-1} and 15 kg Mg ha^{-1} but no P and K fertilizers. The fertilizers were mixed together and were applied double bands. The doses of N (150 kg ha^{-1} and above) were split into 2 or 3 portions. The first dose was applied before sowing, the second between 18 and 21 days after germination of the seeds and the last portion at the tasseling stage of the crop.

The plots were manually seeded with a locally improved variety of maize known as "Suwan-I" which is resistant to downy mildew disease. It is a composite, flint-type variety, derived from random mating of 36 lines. The population density was 67,000 plants ha^{-1}.

Furrow irrigation was applied from 7 days after emergence of the seedlings and continued until 15 to 20 days before harvesting to maintain the water at the root zone depth approximately at field capacity.

Adequate plant protection measures were taken throughout the growing season. The harvesting at maturity took place 95 to 100 days after germination.

Analytical procedure : Dry matter content was determined by drying at 85°C for 48 h. For chemical analyses, sun-dried seeds were ground and passed through a 0.5 mm sieve. Total N was determined by the micro-Kjeldahl method and the crude protein was calculated by multi-playing with 6.25.

Amino acids determined on an LKB Amino Acid Analyser, Model 4104, using lithium buffer system after oxidizing and hydrolyzing the ground grains according to a slightly modified procedure of Masson *et al.* (1979).

Results and Discussion

Grain yield : The effect of N, P and K application on the yield of grain are shown in Fig. I. and Table I.

Fig. I shows that with increasing rates of application at constant doses of P and K, level of yield rose from 0.90 t ha^{-1} without N fertilizer to the maximum of 3.73 t ha^{-1} on an average with 200 kg N ha^{-1}. Beyond this dose the average yield diminished. Grain yields at 100 kg N ha^{-1} and above were significantly different from that obtained at zero N application ($F=6.39$). The ıcrease in yield from zero nitrogen application up to 200 kg N ha^{-1} was und to show linearity. A recent review of experimental results shows that trogen increases yields rapidly and more or less linearly upto a transition ›int beyond which yield may change little or may decrease slowly and linearly ,ooke, 1972).

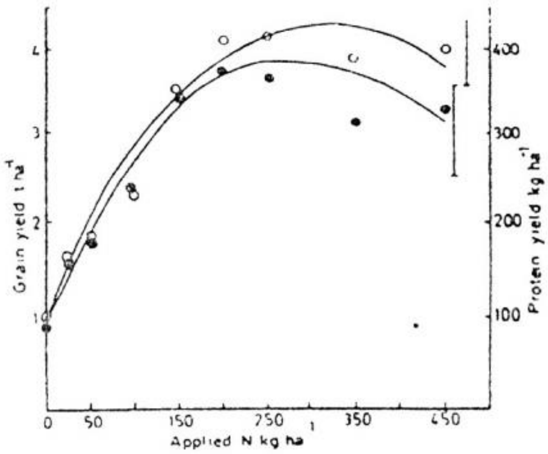

Fig. 1. Effect of N fertilizer application on the grain yield ● and protein yield O of maize. The rate of P and K applications were 225 and 150 kg ha^{-1} respectively. The vertical bars represent the LSDs.

From previous experiments, it was established that K has little influence on the yield of maize in the soil under study while P was the limiting factor. Taking this into consideration, a single treatment of zero P and zero K with a basal dose of 200 kg N ha^{-1} was added to see the absolute effect of P and K over the other combinations. This treatment was considered as the absolute zero or control for the "P+K treatment". Despite the addition of 200 kg N ha^{-1} the grain yield in this treatment was a bare 0.37 t ha^{-1}. When the applied rate of N was kept constant at 300 kg ha^{-1} an increase in average grain yield with increasing P rate at K application of 25 kg ha^{-1} was observed (Table 1). At P levels of 75 and 225 kg ha^{-1}, the yield increased in presence of 225 kg ha^{-1} over that of 25 kg ha^{-1}. At 225 kg ha^{-1}, about 47% higher yield was observed for P applications of more than 25 kg ha^{-1}. The analysis of variance indicated that there was no significant main effects either of P or of K, neither was there any significant P x K interaction on grain yield.

Table 1. Grain yield (t ha^{-1}) and protein yield (kg ha^{-1}) as affected by different levels of P and K fertilizers at a constant N application of 300 kg ha^{-1} (average of three replicates)

P+K treatments[a]	P_{25}[b]	P_{75}	P_{225}
K_{25}[b]	2.12(246.25)	2.93(318.17)	3.21(335.95)
K_{75}	3.01(328.90)	2.65(292.69)	3.06(332.15)
K_{225}	2.36(260.57)	3.46(377.37)	3.42(371.42)
L. S. D. at 5% level	1.18(90 85)		

[a] In the absence of P and K fertilizers with 200 kg N ha^{-1} : 0.37 t ha^{-1} as yield and 43.98 kg ha^{-1} as protein yield.

[b] Subscripts denote kg of element ha^{-1}

The figures in parentheses represent the grain protein yield.

The 1000 grain weight (Table 2) appeared virtually to be independent of any studied treatment except when maize received no N or no K and P or a dose of 25 kg N ha^{-1}. Consequently, the yield differences as pointed out earlier must be attributed to the difference in the number of grains per unit area.

Table 2. Values for 1000 grain weight of maize obtained for differrnt N, P and K treatments (average of three replicates)

Applied N[a] (kg ha^{-1})	1000 grain weight (g)	P+K treatments[b]	1000 grain weight (g)
0	166	P_0K_0	128
25	172	$P_{25}K_{25}$	189
50	186	$P_{25}K_{75}$	189
100	195	$P_{25}K_{225}$	180
150	202	$P_{75}K_{25}$	193
200	187	$P_{75}K_{75}$	187
250	191	$P_{75}K_{225}$	192
350	188	$P_{225}K_{25}$	188
450	205	$P_{225}K_{75}$	199
		$P_{225}K_{225}$	192
L. S. D. at 0.95	16.9	—	18.4

a P and K applications were 225 and 150 kg ha^{-1} respectively

b the subscripts denote the applied P or K in kg ha^{-1}. N applied were 200 kg for P_0K_0 and 300 kg for other treatments in kg ha^{-1}.

So, it is observed that the maximum and the minimum yields of grain dry matter were obtained for the same dose of 200 kg N ha^{-1}. This emphasizes the need for an adequate supply of P and K in the better and efficient utilization of applied N by the crop. This was also proveen in the "P+K treatment" where 300 kg N ha^{-1} application failed to give higher yield at low P and K application. Likewise, Fox *et al.* (1976) have reported the absence of any positive effect on grain yield for higher N and P application in a soil, poor in available P. However, the present result and others (Keeney, 1970; Rendig and Broadbend, 1979; Grove *et al.*, 1980) suggested that the maximum yield of dry matter is obtained for a dose of 200 kg N ha^{-1}, irrespective of the variety of maize or cultural practices.

Protein content and yield: The grain N content and hence the crude protein (C.P.) content was found to be significantly affected (F significant at 0.95) by the applied N levels. The CP in grain varied between 9.86 and 12.15 %. The minimum CP content was found at 100-150 kg N ha^{-1} and the maximum at 350-450 kg N ha^{-1}. However, the CP content at P_0K_0 was also similar to that observed at 350-450 kg N ha^{-1}. For other P and K treatments the CP content varied little among the individual treatment (Table 3).

Table 3. Grain crude protein (C.P) content (%) of maize as affected by various levels of N, P, and K fertilizers

Applied N[a] kg ha^{-1}	C.P. (%)	P+K treatments[b]	C.P (%)
0	10.93	P_0K_0	12.15
25	10.44	$P_{25}K_{25}$	11.63
50	10.04	$P_{25}K_{75}$	10.95
100	9.86	$P_{25}K_{225}$	11.11
150	10.11	$P_{75}K_{25}$	10.88
200	10.97	$P_{75}K_{75}$	11.03
250	11.33	$P_{75}K_{225}$	10.89
350	12.15	$P_{225}K_{25}$	10.57
450	12.09	$P_{225}K_{75}$	10.94
		$P_{225}K_{225}$	10.87

a P and K applications were 225 and 150 kg ha^{-1} respectively

b the subscripts denote the applied P or K in kg ha^{-1}. N applied were 200 kg for P_0K_0 and 300 kg for others in kg ha^{-1}.

The CP yield in grain followed the pattern of grain yield for the "N treatments". A maximum of 322% yield increase in crude protein was obtained for N application of 250 kg N ha^{-1} (Fig. 1). The CP yield in the grain was found to be linear function of N application (y = 171.67 + 0.69 N, 'r' = 0.721***). Although the CP content in the grains for "P+K treatments" did not vary appreciably. Its production however, was dictated by the grain yield at different treatments of P and K (Table 1). There was a difference of 758% higher yield between P_0K_0 and the yield obtained at P_2K_3. Analysis of variance showed that P main effect dominated over K main effect in regulating the CP yield in grain.

The hybrid varieties of maize in respect to inbred lines have in general higher yield potential with lower or similar N or CP content. Comparison of present data with those of Russel and Pier (1980) shows that the variety "Suwan-1" has the same agronomic performances (CP content and grain yield) as those of some inbred lines although it is a crossbred. The absence of heterosis is probably due to the fact that the different varieties which constitute the hybrid "Suwan-1" are very closely related. This might also explain the relatively constant values for N content in the grains irrespective of the "P+K treatment".

Amino acid composition of grain : To determine the protein quality of maize grains, samples from some of the treatments with varying N contents in grain were selected for amino acid analysis. Eight samples, 4 from each of the fertilizer treatments were selected among the 57 replicates. The N content, dry matter yield and amino acid composition (as g amino acid 16 g N^{-1} or as percent protein and as % dry matter) of the selected grains are presented in Table 4. The correlation coefficient values obtained when regression equations were calculated to see the relationship between the amino acids as function of protein and of dry matter are presented in Table 5.

The contents of lysine, arginine and glycine in protein decreased significantly with increasing grain N(or CP) content while those of the other amino acids did not show appreciable variation. When considered as % of dry matter, all the amino acids showed significant incrcrease with increasing grain N content indicating that its yield per ha has increased with dry matter yield upto an N application of 200 kg ha^{-1}. The levels of P or K applications affected the yield of any amino acid per ha through their effect on dry matter yield.

The present results are consistent with those of other workers (Keeney 1970; Todorov *et al.*, 1976; Rendig and Broadbent, 1979) who observed that lysine and some other EAAs decreased in protein with increasing grain

Table 4. Effects of N, P and K fertilizers on the content of amino acids expressed as g amino acid in g N^{-1} (% protein) and as % dry matter (values in parentheses)

Treat.[1]	N_{50}	N_{150}	N_{200}	N_{450}	P_0K_0	$P_{25}K_{225}$	$P_{225}K_{25}$	$P_{225}K_{225}$
Cyst	1.9 (0.2)	2.1 (0.2)	2.0 (0.2)	2.0 (0.2)	2.2 (0.3)	2.3 (0.2)	2.2 (0.2)	2.0 (0.2)
Asx	6.5 (0.7)	6.6 (0.6)	6.4 (0.7)	6.5 (0.8)	7.4 (0.9)	7.2 (0.8)	7.3 (0.7)	6.7 (0.7)
Met	2.2 (0.3)	2.4 (0.2)	2.3 (0.3)	2.3 (0.3)	3.1 (0.4)	3.0 (0.3)	2.4 (0.2)	2.4 (0.2)
Thr	3.4 (0.4)	3.6 (0.3)	3.5 (0.4)	3.6 (0.4)	3.8 (0.5)	3.9 (0.4)	3.8 (0.4)	3.6 (0.4)
Ser	4.8 (0.5)	5.1 (0.4)	5.1 (0.6)	5.3 (0.6)	5.4 (0.6)	5.2 (0.6)	5.5 (0.5)	5.2 (0.5)
Glx	18.7 (1.9)	19.3 (1.7)	20.3 (2.5)	21.2 (2.6)	21.4 (2.7)	21.6 (2.3)	21.6 (2.1)	20.9 (2.2)
Gly	3.8 (0.4)	4.1 (0.4)	3.7 (0.4)	3.6 (0.4)	3.8 (0.5)	4.1 (0.4)	4.1 (0.4)	4.1 (0.4)
Ala	7.3 (0.7)	7.4 (0.7)	7.7 (0.9)	8.2 (1.0)	8.3 (1.0)	8.2 (0.9)	8.1 (0.8)	7.9 (0.8)
Val	5.1 (0.5)	5.4 (0.5)	5.2 (0.6)	5.3 (0.6)	5.4 (0.6)	5.6 (0.6)	5.4 (0.5)	5.2 (0.5)
Ile	3.4 (0.3)	3.5 (0.3)	3.6 (0.4)	3.8 (0.4)	3.8 (0.5)	3.8 (0.4)	3.8 (0.4)	3.7 (0.4)
Leu	11.6 (1.2)	11.8 (1.1)	12.8 (1.5)	13.9 (1.7)	13.8 (1.7)	13.6 (1.5)	13.4 (1.3)	13.6 (1.4)
Tyr	4.1 (0.4)	4.2 (0.3)	4.4 (0.4)	4.5 (0.5)	4.7 (0.5)	4.8 (0.4)	4.5 (0.4)	4.8 (0.4)
Phe	4.5 (0.5)	4.6 (0.4)	4.8 (0.6)	5.1 (0.6)	5.1 (0.6)	5.1 (0.5)	5.2 (0.5)	5.1 (0.5)
Lys	2.9 (0.3)	3.1 (0.3)	2.7 (0.3)	2.6 (0.3)	2.8 (0.4)	3.0 (0.3)	3.0 (0.3)	2.8 (0.3)
His	2.9 (0.3)	3.1 (0.3)	2.9 (0.3)	2.8 (0.4)	2.9 (0.4)	3.2 (0.3)	3.2 (0.3)	2.9 (0.3)
Arg	4.9 (0.5)	5.2 (0.5)	4.9 (0.6)	4.7 (0.6)	4.7 (0.6)	5.2 (0.6)	5.2 (0.5)	4.9 (0.5)
Pro	8.8 (0.9)	9.2 (0.8)	9.2 (1.0)	9.3 (1.2)	9.9 (1.2)	10.9 (1.2)	9.9 (1.0)	9.5 (1.0)
% N	1.61	1.42	1.82	1.99	1.99	1.71	1.57	1.66
Yield* ($t\ ha^{-1}$)	1.76	2.45	4.68	2.26	0.12	2.07	3.62	4.63

1 subscripts denote the applied rate of N, P or K in kg ha^{-1}
* Values are of the selected samples from individual replicates

N while their content in grain dry matter always increased. This is because of the fact that zein—the major protein of maize grain—which is very poor in lysine, always increases with increasing crude protein (Mitchell *et al.* 1952; Rendig and Broadbent, 1979). Similar trends were also reported for oat and wheat by Eppendorfer (1978). Lysine and tryptophane are the first limiting amino acids in maize grain (Eppendorfer, 1975). Maize grain contains disproportionately higher amounts of leucine (Mifflin, 1975). This may cause amino acid imbalance thereby affecting the protein quality of maize grain. This has been clearly depicted in the present findings. The average leucine content of the grain was 13.02 g 16g N^{-1} as against 3.50 g 16 g N^{-1} for isoleucine. Such an imbalance can cause poor utilization of the maize grain protein. N application to maize and other cereals thus usually increases the content of crude protein, but its nutritional value is reduced due to the proportionate increase in the non-essential aminoacids.

Table 5. Correlation coefficients (r_1 and r_2) between the content of amino acids either in the protein (r_1) or in the dry matter (r_2) and grain nitrogen content

Amino acids	r_1	r_2
Cys	-0.10	0.86
Asx	0.04	0.89
Met	0.33	0.76
Thr	0.12	0.94
Ser	0.33	0.95
Glx	0.49	0.95
Gly	-0.73	0.91
Ala	0.63	0.97
Val	0.10	0.97
Ile	0.47	0.96
Leu	0.69	0.96
Tyr	0.57	0.96
Phe	0.43	0.95
Lys	-0.77	0.83
His	-0.52	0.92
Arg	-0.79	0.92
Pro	0.10	0.88

Significance level of r_1 and r_2: 0.71 (P=0.05) ; 0.83 (P=0.01) ; 0.92 (P=0.001)

So, it is observed that the increasing N concentration ie, increasing protein in grain significantly reduced the lysine concentration in it thereby degrading its quality. The increasing crude protein in grain was the outcome of increased N fertilization to obtain higher yield of grain. It could aptly be said from the present observation that the concentration of grain nitrogen is the main factor that determines the amino acid composition and ultimately the quality of maize grains. P and K influence the yield of dry matter, crude protein and amino acid composition of grain protein only indirectly by influencing the nitrogen utilization.

It is suggested that the idea of increasing the content in grain protein of a particular amino acid by applying plant nutrients in excess of what is needed to avoid nutrient deficiency and decrease in yields is not acceptable except only when fertilizers or changes in the environmental factors, decrease the grain N content. However, production per area of individual amino acids always increases in dry matter yield as evident in the present case also. This can some how compensate the decrease in grain protein quality. Moreover higher N contents in grain are normally associated with increase in true digestibility of the protein which may partly or completely compensate for a decreased protein quality. Whatever may be the case, it is clear that, only fertilizer application cannot improve the grain protein quality in terms of its amino acid composition. The possible breakthrough in this field can only be achieved or expected through genetic improvement of the maize.

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