Using the RNA synthetic activity of glutamate dehydrogenase to illuminate the natural role of the enzyme

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ABSTRACT
Glutamate Dehydrogenase (GDH; EC 1.4.1.2) catalyzes the reversible amination of α-ketoglutarate to glutamate, and the polymerization of nucleoside triphosphate(s) to RNA. But the natural role of the reversible amination reaction is the subject of an expanding conversation. The aim was to illuminate the natural role of GDH through its RNA synthetic activity. Stoichiometric combinations of mineral salts that targeted the GDH subunit compositions were applied to field-cultivated peanuts. GDH of seeds were made to synthesize RNA in the deamination and then in the amination direction. Free amino acids were analyzed by HPLC. Glutamate synthase (GOGAT) was assayed by photometry. Free amino acid yields increased from the control’s lowest (9.8 kg·ha⁻¹) and amination-deamination ratio (0.05) through 12.0 - 23.0 kg·ha⁻¹ in the K-, N+K+P+S-, Pi-, N+S-, S-treated peanuts with amination-deamination ratios between 0.6 and 10.0 until at the P+K-treated peanut which had the highest amino acid yield (52.4 kg·ha⁻¹) and the highest amination-deamination ratio (61). The Km and Vmax values of GOGAT were within the normal range. Yields of free amino acids resulting from GDH aminating activity increased from <1.0 kg·ha⁻¹ in the control, through 2.2 in the N+S-, 6.84 in the P+N-, 17.3 in the N-, to 42.6 kg·ha⁻¹ in the P+K-treated peanut. These results show that the natural role of the GDH amination activity is to assimilate escalating multiples of the quantities of NH₄⁺ ion as assimilated via the GS-GOGAT pathway. Peanut protein yields increased in parallel with GDH aminating activities and free amino acid yields such that the control peanut had the lowest protein (<26.0 kg·ha⁻¹) and the yields increased exponentially (500 - 600 kg·ha⁻¹) through the K-, P+K-, Pi-, N-treated to 910 kg·ha⁻¹ in the P+K-treated peanut with the highest aminating activity of GDH. The ability of GDH aminating activity to escalate protein yields of food crops could be employed to address protein-energy malnutrition syndrome of developing nations.

Keywords: Peanut; Stoichiometric Mineral Salt Combinations; GDH Amination-Deamination Ratio; Glutamate Synthase; Amino Acid and Protein Yields ha⁻¹

1. INTRODUCTION
Glutamate Dehydrogenase (GDH; EC 1.4.1.2) is an oxidoreductase that catalyzes the reversible reductive amination of α-ketoglutarate (α-KG) in the synthesis of glutamate. It also polymerizes nucleoside triphosphate(s) in the absence of template to produce RNA [1]. GDH is ubiquitous in all plants studied. Although the biochemical mechanisms of the oxidoreductase reaction have been studied in detail, and the function of the RNA is increasingly unfolding, the natural role of the reversible amination of α-KG is the subject of an expanding conversation [2-5]. The discovery of the glutamate synthase (GOGAT; EC 1.4.1.13), and glutamine synthetase (GS; EC6.3.1.2) cycle [6] with μM Km and μmoles·min⁻¹ Vmax values for NH₄⁺ ion compared with mM Km and mmoles·min⁻¹ Vmax values of GDH were interpreted to suggest that the GS-GOGAT cycle might be the only mechanism for NH₄⁺ ion assimilation and glutamate synthesis, while GDH might function in the deamination of glutamate [7]. But the Km value for the assimilation of NH₄⁺ ion by GDH has been demonstrated to be in the upper μM range and within the NH₄⁺ ion concentrations of the mitochondria [5,8]. The aim of this project was to illuminate the natural role of GDH through its RNA synthetic activity. Computer interpretation of NH₄⁺ ion and amino acid metabolism did not illuminate the biological role of the enzyme [9]. Some of the conversations also surround the inability to estimate the products of the oxidoreductase reaction over all the encompassing range of environmental conditions for plant growth. Some of the experimentations that have

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projected the GDH deamination role employed plant specimens that were subjected to carbon nutrient starvation, deprivation of photosynthesis, and/or of essential mineral nutrients [2,7]. The approach of the research project reported hereunder was different being environmentally broad in scope, and based on the analyses of mature seeds harvested from plants cultivated and fertilized under normal field plot conditions. Also, instead of analyzing NH4+ ion, glutamate, and NADH products of the reversible oxidoreductase reaction, the RNA products synthesized by the enzyme were analyzed. GDH has been synthesizing RNA since evolutionary time [10], the RNA functioning to permute and normalize the metabolic pathways at the mRNA level in response to wide variations of soil mineral ion concentrations.

Since GDH is a very important enzyme in plant carbon and nitrogen metabolism [2-5,7-10], its natural role may correlate positively with increases in protein, fatty acid, and carbohydrate yields. There is need to double and exponentially increase crop protein yields per unit area of land in order to minimize protein-energy malnutrition syndrome of developing countries. Could the natural role of GDH be associated with food crop yield doubling biotechnology? Peanut was chosen for this project because it is whole food/feed rich in proteins, fatty acids, carbohydrate etc, and consumed world-wide with considerable contribution to global economy; and its GDH is very active in RNA synthesis [11-13].

2. MATERIALS AND METHODS

Treatment of peanuts with mineral salt solutions: Peanut (Arachis hypogaea L. Cv. Virginia) seeds were planted in boxes 243.84 × 243.84 × 30.48 cm (width × length × depth) in the field plot, each box filled with Metro Mix 700 peat moss as described before [12]. About 100 - 110 seeds were planted per box. The applied mineral salt compositions mimicked and targeted the binomial sub-unit polypeptide compositions of the GDH isoenzymes [13]. The first box was left as the untreated control; the second box (N) was treated with 1 L of NH4Cl solution (25 mM), the third box (Pi) was treated with 1 L of Na2HPO4 solution (20 mM); the fourth box (S) was treated with 1 L of Na2SO4 solution (50 mM); the fifth box (K) was treated with 1 L KCl solution (4 mM); the sixth box (N + K) was treated with 1 L of combined NH4Cl (25 mM), Na2HPO4 (20 mM), Na2SO4 (50 mM), and KCl (4 mM) solution; the seventh box (P+K) was treated with 1 L of combined Na2HPO4 (20 mM) and KCl (4 mM) solution; the eighth box (N + S) was treated with 1L of combined NH4Cl (25 mM) and Na2SO4 (50 mM) solution; the ninth box (P + N) was treated with 1L of combined Na2HPO4 and Na2SO4 (50 mM) solution; the tenth box (P + S) was treated with 1L of combined Na2HPO4 (20 mM) and Na2SO4 (50 mM) solution as described before [12]. The boxes were watered every other day. Mineral salt solutions were applied sequentially, first at pre-flowering stage (2 weeks after seed germination), second at flowering, and third at post-flowering. When the leaves turned yellow (peanut maturity), pods and shoots were harvested, allowed to dry on the greenhouse floor for about 2 weeks, and weighed. Pods were shelled by hand, and the kernels (seeds) weighed. Seeds were stored at −30°C.

Purification and assay of GDH: GDH charge isomers were extracted from peanut seeds that were harvested from the control or mineral-treated boxes, and purified by electrophoresis as described before [11]. RNA synthetic activities of GDH isoenzymes were assayed in the deamination and amination directions in separate tubes.

For demonstrating the arrays of RNA synthesized by GDH in the amination direction, the substrate solutions were prepared in 0.1 M Tris-HCl buffer (pH 8.0) containing the four NTPs (0.6 mM each), CaCl2 (3.5 mM), NH4Cl (0.875 mM), α-KG (10.0 mM), NADH (0.225 mM), 5 Units RNase inhibitor, 1 Unit DNase 1, and 5 µg of actinomycin D as described before [12]. Reaction was started by adding 0.2 mL of GDH charge isomers eluted per chamber of whole-gel eluter. The eluted GDH contained 3 - 9 µg protein per mL. Final volume of the reaction was brought to 0.4 mL with 0.1 M Tris-HCl buffer pH 8.0. In the deamination direction, the substrate solutions were prepared in 0.1 M Tris-HCl buffer (pH 8.6) containing the four NTPs (0.6 mM each), CaCl2 (3.5 mM), L-glu (3.23 µM), NAD+ (0.375 µM), NAD+ (0.375 µM), 5 Units RNase inhibitor, 1 Unit DNase 1, and 5 µg of actinomycin D as described before [11]. Reaction was started by adding 0.2 mL of GDH charge isomers eluted per chamber of the whole-gel eluter. The eluted GDH contained 3 - 9 µg protein per mL. Final volume of the reaction was brought to 0.4 mL with 0.1 M Tris-HCl buffer pH 8.6. Reactions were incubated at 16°C for 3 h and stopped by phenol-chloroform (pH 5.5) extraction of the enzyme. RNA was precipitated with ethanol, and dissolved in minimum volume of molecular biology quality water. RNA yield and quality were determined by agarose gel electrophoresis, and photometry. Assays were carried out in duplicate to verify the reproducibility of the results.

For determination of the yield of the product RNA in the amination direction, the substrate solutions were prepared in 0.1 M Tris-HCl buffer (pH 8.0) containing the four NTPs (0.6 mM each), CaCl2 (3.5 mM), NH4Cl (0.875 mM), α-KG (10.0 mM), NADH (0.225 mM), 5 Units RNase inhibitor, 1 Unit DNase 1, and 5 µg of actinomycin D as described above. Reaction was started by adding 0.2 mL of the pooled GDH charge isomers eluted in all the chambers of the whole-gel eluter. Final volume of the reaction was brought to 0.4 mL with 0.1 M
Tris-HCl buffer pH 8.0. In the deamination direction, the substrate solutions were prepared in 0.1 M Tris-HCl buffer (pH 8.6) containing the four NTPs (0.6 mM each), CaCl₂ (3.5 mM), L-glut (3.23 µM), NAD⁺ (0.375 µM), 5 Units RNase inhibitor, 1 Unit DNase 1, and 5 µg of actinomycin D as described above. Reaction was started by adding 0.2 mL of the pooled GDH charge isomers eluted in all the chambers of the whole-gel eluter. Final volume of the reaction was brought to 0.4 mL with 0.1 M Tris-HCl buffer pH 8.6. Reactions were incubated at 16°C for 3 h and stopped by phenol-chloroform 5:1 (pH 5.5) extraction of the enzyme. RNA was precipitated with ethanol, dissolved in 100 µL of molecular biology quality water, and chromatographed through Zymoclean Gel RNA spin column (Zymo Research, CA, USA). RNA yield was determined by photometry (NanoDrop spectrophotometer). Assays were repeated three times with different elutions of GDH, and the average RNA yields were calculated.

Extraction and assay of GOGAT: Glutamate synthase (EC 1.4.1.13) was extracted from 50 g of peanut seed by homogenizing with 100 mL of ice-cold 0.1 M potassium phosphate buffer (pH 8) containing 0.1 M KCl, 0.1% (v/v) Triton X-100, and 14 mM 2-mercaptoethanol [14], and partially purified as described before [15]. The activity was assayed by photometry within α-KG concentration range of 0.3 - 33.3 mM, and L-glutamine range of 0.3 - 66 mM. The concentration of NADH was fixed at 0.1 mM as described before [15]. Assays were repeated three times and the average was applied to calculate the activities as mmoles of NADH oxidized per minute per mg protein. Double reciprocal plots were constructed with the activities derived. Protein content was determined with the Folin-Ciocalteau reagent using lysozyme as the standard.

Proximate analyses: Free amino acids were custom extracted from peanut seeds and custom analyzed (UBE Analytical Laboratories, California, USA) by HPLC. Acid detergent fiber and total protein contents of peanut seeds were custom analyzed (Universal Testing, Illinois, USA) by standard gravimetry.

### Table 1. Stoichiometric combinations of mineral nutrients.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N (25 mM)</th>
<th>S (50 mM)</th>
<th>K (4 mM)</th>
<th>P (20 mM)</th>
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<tr>
<td>20 mM Na₃PO₄</td>
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<td>P+S</td>
<td>P+K</td>
<td>P+P</td>
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<td>K+S</td>
<td>K+K</td>
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<tr>
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<td>N+S</td>
<td>S+S</td>
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<td>25 mM NH₄Cl</td>
<td>N+N</td>
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</table>

Mineral treatments: N was 1 L of 25 mM NH₄Cl. S was 1 L of 50 mM Na₂SO₄. K was 1 L of 4 mM KCl. P was 1 L of 20 mM Na₂HPO₄. P+N was 1 L of combined 20 mM Na₂HPO₄ and 25 mM NH₄Cl. P+S was 1 L of combined 20 mM Na₂HPO₄ and 50 mM Na₂SO₄. P+K was 1 L of combined 20 mM Na₂HPO₄ and 4 mM KCl. N+S was 1 L of combined 25 mM NH₄Cl and 50 mM Na₂SO₄.

3. RESULTS

Stoichiometric combinations of mineral salts mimicking the GDH isoenzyme ratios: The stoichiometric combinations of mineral salts (Table 1) mimicked the binomial subunit assembly and distribution of the GDH isoenzymes. Furthermore, to target the GDH subunits directly, the stoichiometric combinations of mineral salts (Table 1) were applied at the µM instead of the M level typical of inorganic fertilizers; and at two or more times in the life of the crop so that at all times the GDH subunit compositions did not fluctuate widely. In addition to the NH₄⁺, PO₄³⁻, K⁺, and SO₄²⁻ (N, P, K, S) added, there were trace micronutrients (Ca²⁺, Borate, Mg²⁺, Mn²⁺, Fe³⁺, and Silicate) in the peat moss. GDH is composed of three subunit polypeptides assembled binomially in hexameric molecules to give 28 isoenzymes [16]. The stoichiometric combinations (Table 1) made for internal repeats in the treatments thus limiting stochastic variability in the treatments, imposed firm control on the number of experimental repeats, and consolidated the biochemical comprehensiveness of the project design. The growth conditions made for unrestricted photosynthesis, water supply, and environment-wide supply of mineral salts.

GDH isomerization in response to the mineral ions: The full complement of the GDH isoenzymes was present in the control peanut. But the anionic isoenzymes declined quickly with increasing electromagnetic activity of the mineral ions until at the P+P-, Pi-, P+N-, and P+K-treated peanuts when the cationic GDH isoenzymes were predominant [12]. There have been suggestions that the anionic isoenzymes of GDH are deaminating [17]. Therefore, the stoichiometric mineral ion combinations produced diverse environment-wide biochemical conditions that induced the full spectrum of the isomerization of GDH. The biochemical design of the project was thus comprehensive. This was important for the balanced judgment of the free amino acids, protein, and cellulose yields in relation to the GDH deamination and amination activities.

The peanut growth conditions induced and synchro-
nized the isomerization of the GDH and synthesis of RNA by the GDH [12]. GDH isomerization and synthesis of RNA as the target sites of mineral salt action are due to the binomial distribution of its three subunits in the hexameric isoenzymes, on the basis of the twin nonallelic GDH\(^1\) and GDH\(^2\) gene structure, with the gene (GDH\(^1\)) encoding the more acidic subunits (\(\alpha\) and \(\alpha\)) being heterozygous and co-dominant, whereas the other gene (GDH\(^2\)) encoding the less acidic subunit (\(\beta\)) is homozygous [18].

Deaminating and aminating activities of GDH; RNA synthesis by GDH in the deamination direction (Figure 1) shows that the GDHs of several of the treated peanuts were active in the deamination direction. The deaminating activities of the GDH of N\(^+\)P\(^+\)K\(^-\)S\(^-\) (Figure 1(b)), NH\(_4\)Cl\(^-\) (similar to Figure 1(b)), P+N-treated (Figure 1(d)) peanuts were characterized by high molecular weight RNAs (>10 kb) synthesized by GDH. The low deaminating activities of the GDH of P+K\(^-\) (Figure 1(f)) and K-treated (similar to Figure 1(f)) peanuts were characterized by low molecular weights (~100 bases long) of GDH-synthesized RNAs. RNA synthesis by GDH in the amination direction (Figure 2) showed that the GDHs of several of the treated peanuts were also active in the amination direction. The low aminating activities of the GDH of control (Figure 2(a)), and P+S-treated (Figure 2(b)) peanuts were characterized by low molecular weights (~100 bases long) of GDH-synthesized RNAs. Similarly, the high aminating activities of the GDH of Pi- (Figure 2(d)), P+N- (Figure 2(e)), and P + K-treated (Figure 2(h)) peanuts were characterized by high molecular weight RNAs (>10 kb) synthesized by the enzyme. The deamination and amination product RNAs (Figures 1 and 2) confirmed that the stoichiometric mineral ion treatments induced the full spectrum of isomerization of peanut GDH and differentiated deamination from the amination activity.

Amination-Deamination ratio of GDH; Figures 1 and 2 visually demonstrated the differentiation of the deaminating from the aminating activities of GDH by the

![Whole Gel Elution Chambers](image1)

**Figure 1.** Deamination activity of peanut GDH; RNA arrays synthesized by whole gel-purified GDH isoenzymes of the seeds harvested from control or mineral salts-treated peanuts. In addition to the four NTPs, CaCl\(_2\), RNase inhibitor, DNase 1, and actinomycin D, the deamination reaction cocktail contained NAD\(^+\) (0.375 \(\mu\)M), glutamate (3.23 \(\mu\)M) and 0.2 mL of GDH isoenzymes eluted per chamber of the whole-gel eluter. Reactions were incubated at 16\(^{\circ}\)C for 3 h, stopped by phenol-chloroform (pH 5.5) extraction of the enzyme, and product RNA was precipitated with ethanol. RNAs were electrophoresed on 2% agarose gels. The RNA profiles per treated peanut were reproducible in replicate agarose gels. MM is RNA molecular weight marker; total RNA marker was isolated from corresponding peanut seeds by the method of [38].

Figure 2. Amination activity of peanut GDH: RNA arrays synthesized by whole gel-purified GDH isoenzymes of the seeds harvested from control or mineral salts-treated peanuts. In addition to the four NTPs, CaCl$_2$, RNase inhibitor, DNase 1, and actinomycin D the amination reaction cocktail contained NH$_4$Cl (0.875 mM), α-KG (10.0 mM), NADH (0.225 mM), and 0.2 mL of GDH isoenzymes eluted per chamber of whole-gel eluter. Reactions were incubated at 16˚C for 3 h, stopped by phenol-chloroform (pH 5.5) extraction of the enzyme, product RNA was precipitated with ethanol. RNAs were electrophoresed on 2% agarose gels. The RNA profiles per treated peanut were reproducible in replicate agarose gels. MM is RNA molecular weight marker; total RNA marker was isolated from corresponding peanut seeds by the method of [38].

stoichiometric mineral salt combinations. The aminating capacity (amination-deamination ratio) varied widely. Compared with the control, treatment of the peanut with P+K increased the aminating capacity by >1000 folds (Table 2) thus showing that the stoichiometric mineral ions functioned to displace the equilibrium position of the GDH oxidoreductase activity. This permitted the aminating activity to function with minimal interference from the deaminating activity and vice versa per hexameric GDH molecule and per landscape of crop. Also, yields of the GDH-synthesized RNAs per treated crop (Table 2) showed that whereas the amination activity rose up to ~40 µg RNA µg$^{-1}$ GDH, the deamination activity was limited to a lower maximum of ~19 µg RNA µg$^{-1}$ GDH. Therefore, the peanut GDH was much more active in the amination than in the deamination direction. Yields of product RNAs in the two reaction directions were not always reciprocally related thus further suggesting that the amination was uncoupled from the deamination reaction by the electromagnetic activity of the mineral ions. The untreated Virginia peanut and several of the mineral-treated peanuts (N+P+K+S, NH$_4$Cl,
Table 2. GDH deamination and amination activities, GOGAT activities, free amino acids profiles, and total protein contents of peanut seeds harvested from control and mineral salts-treated plots.

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*GDH deamination or amination is µg RNA µg⁻¹ GDH under the assay conditions described. Each is the average of 3 determinations from 3 different purifications of GDH. The error values were under 10% of the averages. *Amino acids are mg/100g of seeds. †GOGAT apparent km value for α-KG is µM. ‡GOGAT Vmax is µmoles min⁻¹ mg⁻¹ protein. *Seed lignocelluloses Kg·ha⁻¹; GDH-a Kg amino acid aminated via GDH ha⁻¹; GDH-d Kg amino acids deaminated by GDH ha⁻¹; kg seed protein ha⁻¹.

N+S) induced GDH amination-deamination ratios of <1.0 (Table 2). This was also similar to the response of Valencia peanuts [13]. These suggest that in marginal and harsh environments (irregular rain fall, soil erosion, low soil organic carbon content, extreme temperatures, inadequate solar radiation, limited fertilizer supply etc) in which a few crop species are made to grow, plant GDHs could demonstrate some deamination molecular reaction mode [2,7,19]. GDH aminating capacity was very low in the control peanut (Table 2). The GDH aminating capacity of the N+K+S-treated peanut (the one closest to the control) was ten times higher than the control (Table 2). Was the GDH molecular aminating activity virtually silent in the control peanut? Several of the treated crops (P+S, P+N, Pi, K etc) were intermediate with their GDH amination capacity between 1.0 and 12.34.
The proximate free amino acid yields were low (<26.0 kg·ha⁻¹). Peanut free amino acid yields increased (12.0 - 23.0 kg·ha⁻¹) through the K-, N+K+P+S-, Pi-, N+P+K-treated peanuts with aminating capacities between 1.0 and 2.0 until at the P+K-treated peanut which had a very high aminating capacity of 61 and a high amino acid yield of 52.4 kg·ha⁻¹. Peanut free amino acids followed the trends of seed and shoot lignocellulose yields [24], fatty acid and seed yields [10]. Assimilation of NH₄⁺ ion and α-KG by GDH results to glutamate synthesis, the gate-way to the metabolism of most of the other amino acids. The correspondence of the trends of the amino acid yields, GDH aminating capacity and cellulose yields suggest that the natural role of GDH was the assimilation of NH₄⁺ ion and α-KG, but not depletion of the glutamate pool by deaminating it to α-KG in the treated peanuts. If GDH had been deaminating [7], the amination relationship with free amino acid yields would have been inverted.

**Protein yield:** In the untreated peanut, N + P + K + S-, N + S-treated etc peanuts (Table 2) where the GDH aminating-deamination ratios were very low (<1.0), and the free amino acid yields were low (<26.0 kg·ha⁻¹), the protein yields were also low (~500.0 kg·ha⁻¹). Then the protein yields increased (500 - 600 kg·ha⁻¹) through the K-, P+S-, Pi-, N-treated etc peanuts with aminating capacities between 1.0 and 10.0 until at the P+K-treated peanut which had the highest aminating capacity and the highest protein yield of 910 kg·ha⁻¹. The trend of peanut protein yields followed similar trends as the seed lignocellulose yields, shoot lignocellulose yields [24], fatty acid and seed yields [10]. If the enzyme had a deaminating natural role [7], the relationships between GDH aminating capacity, cellulose and protein yields would have been inverted. Peanut protein contents are from 17% - 25% [26], therefore, the control peanut’s protein content was normal without protein catabolism that could have induced major deamination of glutamate to α-KG.

**NADH-GOGAT activity:** The Km and Vmax values (Table 2) of GOGAT were within the normal range [14] thus showing that the stoichiometric mineral salt combinations neither inhibited nor activated the enzyme. Therefore, the GS-GOGAT pathway functioned normally in the wide concentration variations (Table 1) of mineral ions as in the untreated control. This permitted the exclusion of methionine sulfoximine treatment [27] from the project. Only the GOGAT of the Pi-treated peanut had a high Km value (5.0 mM αKG) suggesting very low activity of the enzyme. This could be related to the down-regulation of the mRNA encoding the enzyme by the GDH-synthesized RNA in the Pi-treated crop [12].

The natural role of GDH: The free amino acid yields and the GDH amination capacity enabled estimations of...
the quantities of NH₄⁺ ion assimilation and of glutamate deamination by GDH. The normal GOGAT activity, taken together with the high deamination reaction mode of the GDH of the control peanut suggest that GS-GOGAT cycle played a major role in the assimilation of NH₄⁺ ion and α-KG in the control peanut (Table 2). Using the free amino acid yield as the index for the total NH₄⁺ ion assimilated, the free amino acid yield of 9.8 kg·ha⁻¹ for the control peanut could be attributed to the activity of the GS-GOGAT cycle since the GDH amination activity was virtually silent in the treatment and could not make a substantial contribution to the production of glutamate. Furthermore, the high deamination activity of the GDH of the control peanut could have eroded some of the glutamate by deaminating it to α-KG although aspartate aminotransferase and glutamate decarboxylase also degrade glutamate even more efficiently [3]. Therefore, the adjusted total free amino acid should be slightly higher than 9.8, but for the purpose of estimating the quantity of amination it was assumed that GS-GOGAT accounted for all the NH₄⁺ ion assimilation of the control peanut. Mineral treatment of the peanut increased the GDH amination activity but decreased the deamination activity with corresponding increases in the free amino acid yields (Table 2). Since GOGAT remained constant throughout the peanuts, the mineral nutrient-induced increases in free amino yields were attributable to GDH amination activity, the higher the GDH amination activity the lower the quantity of glutamate lost via GDH deamination function etc.

In the N+P+K+S-treated peanut with 14.7 kg·ha⁻¹ of free amino acids (Table 2), the quantity of amino acids resulting from amination by GDH was 4.9 kg·ha⁻¹ by subtraction of the free amino acids (9.8 kg·ha⁻¹) due from amination by GS-GOGAT cycle. The amination capacity (0.6) of the GDH of the N+K+P+S-treated peanut permits the calculation of the possible quantity of free amino acids (8.17 kg·ha⁻¹) lost as NH₄⁺ ion due to the deamination function of GDH etc. In the P+K-treated peanut with 52.39 kg·ha⁻¹ of free amino acid yield (Table 2), the quantity of amino acids resulting from amination by GDH was 42.59 kg·ha⁻¹ by subtraction of the free amino acids (9.8 kg·ha⁻¹) due from amination by GS-GOGAT cycle. The amination capacity (61) of the GDH of the P+K-treated peanut permits the calculation of the quantity of amino acids (0.75 kg·ha⁻¹) lost as NH₄⁺ ion due to the possible deamination function of GDH. Similarly, the quantities of amino acids lost as NH₄⁺ ion from the other peanuts as a result of the possible deamination function of GDH was estimated (Table 2). In the literature, previous estimates of the GDH deamination role were expressed superficially as activity per unit weight of protein. The RNA synthetic activity of GDH was different because it advanced far beyond the proximate level to permit the estimation of the possible quantities of amino acids eroded by the enzyme’s deaminating activity per unit area of land. The results show that the natural role of the GDH deamination activity is to erode a fraction (Table 2) of the NH₄⁺ ion assimilated by the enzyme; and the natural role of the GDH amination activity is to assimilate exponential multiples of the quantities of NH₄⁺ ion as assimilated via the GS-GOGAT cycle. Therefore, the amination capacity of GDH is the quantitative statement of the amination efficiency of the enzyme. Stability of GOGAT over wide variations of mineral ion concentrations and compositions (Table 1) is evidence for the role of the GS-GOGAT cycle as the housekeeping machine for the salvage of NH₄⁺ ion and α-KG. On the other hand, because GDH amination capacity increased in response to differentiating concentrations and compositions of mineral ions, it is the responsive mechanism for large-scale assimilation of NH₄⁺ ion and α-KG (amination) that kicks into action under favorable growth conditions for crops. The coincidence in parallel of the trends of GDH amination-deamination ratios, free amino acids, total protein, fatty acids and peanut yields are strong evidence that the crop yield doubling biotechnology [12] is the collateral chemical support on which the aminating role of GDH stands. Labeling of NH₄⁺ ion or glutamate and computational simulations [9] of amino acid flux did not illuminate the natural role of GDH probably because among other kinetics considerations, the rapidly reversible oxidoreductase reaction embodies a substantial incompleteness in the relative quantities of reaction products estimated. The assay of the RNA products instead is more complete because the RNA synthesized is stable, not hydrolyzed by GDH.

4. DISCUSSION

Synthesis of RNA by hexameric GDH isoenzymes is an integral chemical property of the enzyme [28]. The functions of GDH-synthesized RNA have illuminated several hitherto inexplicable biological phenomena including the detoxification of xenobiotics by plants [1], permutation of the primary metabolic pathways at the mRNA level [12], the evolutionary adaptation of metabolism to wide variations of the concentrations and compositions of the mineral nutrient environment [10], regulation of fatty acid contents by lipoxygenase [29], regulation of the oleic acid/linoleic acid ratios, and elimination of allergenic arachins [10,12,24]. Therefore, RNA synthesis is appropriate biochemical reaction for evaluating the activity of GDH [11,30]. Results in Figures 1 and 2, [10,12] are the first time that large quantities of non-genomic RNAs are being subjected side-by-side with genomic RNA to rigorous molecular biology pro-
of fatty acids in peanut [10]. Messenger RNAs encoding the regulatory enzymes listed above are fully or partially down-regulated via spatial permutation by their homologous GDH-synthesized RNAs. Therefore, these results together with the normal steady activity of GO-GAT (Table 2) suggest that the supply of α-KG for Krebs cycle metabolism is not via protein degradation and the GDH deamination of glutamate [35]. In view of the miniscule quantities of α-KG possibly produced by GDH deamination activity, the deamination mode of GDH may be a kinetic metabolic state that enables the enzyme to synthesize RNAs (Figure 1) for down-regulating and coordinating the mRNAs encoding phosphate translocator, GBSS, PGM, GARS/GART etc to assure that carbon and nitrogen metabolism are not shut down simultaneously [10,12] when the crop is growing in deficit environments. The parallel coincidence of the trends of GDH amination-deamination ratios, free amino acids yields, cellulose, fatty acids and total protein yields with peanut yield are strong evidence that the crop yield doubling permutation of the metabolic pathways at the mRNA level by GDH-synthesized RNA [12,13,24] is the collateral chemical support for the aminating role of GDH.

The economic importance of the GDH aminating activity (Table 2) in crop production may be deduced by comparing the total protein, and pod yields of the untreated, N+P+K+S (similar to commercial fertilizer), and P+K-treated peanuts. The protein and pod yields of the P+K-treated peanut were at least double those of the N+P+K+S, and untreated peanuts which were the normal yields from farmers’ plots [25]. The doubling of protein yield by maximizing the GDH aminating activity is a positive litmus test in support of the natural role of the enzyme. There is need to maximize and double crop protein yields per unit area of land in order to feed a malnourished population of infants, mothers, and children in some of the developing zones of the world that are deficient in animal/fish protein-rich diets [36,37]. If staple food crops were to double and escalate their protein yields simply by maximizing their GDH aminating activity, without increasing the cultivated land area, without increasing fertilizer and water applied, without increasing man-hour input, substantial plant proteins to meet part of the protein turnover needs of kwashiorkor children could be locally obtained by the developing nations.

REFERENCES


