Glutamate Dehydrogenase Isomerization: A Simple Method for Diagnosing Nitrogen, Phosphorus, and Potassium Sufficiency in Maize (*Zea mays* L.)

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Improved nutrient-use efficiency in cropping systems is needed to increase farm income and to minimize possible nutrient emission to the environment. Plant glutamate dehydrogenase (GDH) offers a means for improved diagnosis of the nutrient status of crops. *Zea mays* L. DK-68 was fertilized with nutrient solutions containing different ratios of nitrogen, phosphorus, and potassium (N, P, K). Maize shoot GDH was fractionated to its population of isoenzyme by Rotoforation followed with native PAGE. The GDH of the control maize had the complete set of 28 isoenzymes. Fertilization of the maize with $N_1P_1K_1$ did not repress the 14 anodal but did repress the 14 cathodal isoenzymes. As the P content of the fertilizer increased in the sequence $N_0P_0K_0$, $N_1P_0K_1$, $N_0P_1K_0$, $N_1P_1K_1$, $N_0P_2K_0$, and $N_0P_4K_0$, the amination maximum velocity (V_{max}) of the enzyme decreased curvilinearly in the sequence 310, 246, 140, 130, 108, and 83 μmol min^{-1} mg^{-1}, respectively. The highest dry matter yield occurred when the fertilizer contained 1−3 mM P_i, which was thus the nutrient deficiency−sufficiency interphase. In the GDH integration of the signals from the N, P, and K so as to respond with a characteristic isoenzyme population pattern, the signal from N superseded that from K; in turn, the signal from P superseded those from N and K. Signal integration was therefore based on the dominance by the most nucleophilic nutrient. In this case, the decreasing order of nucleophilic dominance was $PO_4^{3-} > NH_4^+ > K^+$.

Keywords: *Zea mays; glutamate dehydrogenase; NPK signal integration*

INTRODUCTION

In crop production, optimal nutrient supply is usually achieved by the application of fertilizer (Marschner, 1995). Plant species and genotypes have specific nutrient requirements and different adaptation strategies to avoid the development of nutrient deficiencies (Vegh et al., 1997). However, the productivity gains achievable through fertilizer use have led to inefficiencies especially in fertilizer nitrogen management, which have now created serious problems of nutrient pollution in some areas (Follett et al., 1991). Emphasis is therefore shifting from maximizing production to minimizing nutrient emissions to the environment (Loneragan, 1997). In this regard, cultivation of nutrient efficient crop cultivars as a low-cost, low-input technology is one of the most effective approaches to minimizing nutrient emission to the environment.

Visual symptoms, supported by nutrient compositions of plant and soil samples, are the usual diagnostics for the nutrient status in cropping systems (Marschner, 1995). Normally, on the basis of nutrient composition data, some additional, and usually unknown, quantity of fertilizer is applied to the soil to compensate for inefficiencies in the nutrient management systems and nutrient losses from the system (Powers and Broadbent, 1989). This is one of the main sources of nutrient emission to the environment (Fletcher, 1991). Computer screening, research, and planning models have been developed to assist farmers in achieving maximum crop yields without polluting the environment with nutrients (Young et al., 1987; DeCoursey, 1990, 1991). These models are sometimes inefficient for the management of cropping systems on variable soil types (Ten Berge and Riethoven, 1997). For such cropping systems, field specific management is required (Cassman et al., 1996).

To ensure that available soil nutrients are utilized for optimal crop yields, low-input sustainable agricultural (LISA) practices are being widely encouraged (Madden, 1989). In practice, LISA requires that plants be grown at the narrow interphase between nutrient sufficiency and deficiency (Schaller, 1989). Soil and plant compositional data indicate the plant's nutrient contents, but they have very little biochemical basis, and they do not experimentally establish relationships between crop yield and crop nutrient element contents (Sinclair et al., 1997). On the other hand, despite the extensive studies on many plant enzymes including nitrate reductase, peroxidase (Bar-Akiva et al., 1970), pyruvate kinase (Besford, 1978), and phosphatase (Smyth and Chevalier, 1984), enzymatic test methods are not yet supplemental tools for diagnosing the nutrient status of plants (Marschner, 1995).

Since phytohormones are regulated by nutrients (Salama and Wareing, 1979), the recent demonstration that the mitochondrial enzyme, glutamate dehydrogenase (GDH), isomerizes in response to phytohormones, and in doing so it regulates plant growth and differentiation (Osuji and Madu, 1997a), suggests that the enzyme could be sensitive to plant nutrient status. Although GDH responds to water and temperature stresses (Srivastava and Singh, 1987), toxic metals (Osuji et al., 1997), herbicides (Osuji, 1997), and phytohormones (Osuji and Madu, 1997a), its interaction with plant nutrients has not been studied. The impor-

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tance of its possible response to nutrients is that it may provide an opportunity for understanding nutrient-induced signaling and thus become an important method for the analysis of the nutrient sufficiency−deficiency status in cropping systems. Also, since the enzyme is induced by a complex variety of environmental factors, it is important to understand its mechanisms of signal discrimination and integration.

The amination kinetic constants (K_m and V_{max}) have shown that the enzyme has target sites for herbicides and heavy metals (Osuji, 1997). Although the amination reaction is active in vivo (Magalhaes, 1991; Fricke and Pahlich, 1992; Osuji and Madu, 1996, 1997a; Turano et al., 1996; Melo-Oliveira et al., 1996), its role in the redox and environmental stress monitoring in plants is still the subject of intensive research (Osuji, 1997; Osuji et al., 1997; Osuji and Madu, 1997b).

We present some of the basic principles of the isomerization of GDH in the integration of the signals from N, P, and K and the application of the reaction for diagnosing the nutrient sufficiency status in maize.

MATERIALS AND METHODS

Sand Culture Experiment. Sand was mixed in a 9:1 ratio with sphagnum peat moss to improve the water retention properties of the sand. Sand (6 kg) was placed in each of 60 plastic pots. The fertilizer treatments were as follows: control (no fertilizer) ($N_0P_0K_0$); $N_1P_1K_1$ solution containing 6 mM NH_4Cl, 2 mM KCl, and 2 mM KH_2PO_4; $N_1P_0K_1$ solution containing 2 mM NH_4Cl and 4 mM KCl; $N_0P_1K_0$ solution containing 3.5 mM Na_2HPO_4; $N_0P_2K_0$ solution containing 7 mM Na_2HPO_4; and $N_0P_4K_0$ solution containing 14 mM Na_2HPO_4. Each treatment was replicated 10 times. The initial nutrient treatments, including the control, were applied as 250 mL solutions to the potted sand, followed by thorough mixing. Pioneer brand hybrid maize (*Zea mays* L. DK-68) seeds were then sown in pots at the rate of five per pot. Seeds were allowed to germinate and to grow outside the greenhouse under local April−May temperature and light conditions. The fertilizer treatments (250 mL) were applied to the pots weekly. Total application in the 5 weeks of corn growth was 1 L of nutrient solution per pot. All of the pots were watered to ~50% field capacity daily.

Maize shoots were harvested at 5 weeks after sowing by cutting them off 1 cm above the sand surface. After the stem bases had been washed, the shoots were quickly frozen with dry ice. A maize shoot from each pot was used for dry matter determination. The remaining shoots per fertilizer treatment were ground to powder with a mortar and pestle in the presence of dry ice. The powders were stored at −70 °C.

Extraction of Maize GDH. GDH was extracted from 50 g of the powdered maize shoot as described by Loyola-Vargas and De Jimenez (1984) and partially purified by precipitation with solid $(NH_4)_2SO_4$. The protein, which precipitated between 40 and 55% $(NH_4)_2SO_4$ saturation, was collected by centrifugation (10000*g*, 20 min, 4 °C), dissolved in a minimum volume of GDH extraction buffer, and dialyzed at 4 °C (Osuji and Cuero, 1992; Osuji et al., 1997). The volume of the dialyzed enzyme was made up to ~60 mL with 10 mM Tris-HCl buffer (pH 8.2).

Rotoforation of GDH. Partially purified GDH (containing ~70 mg of protein) extracted from ~40 g of shoot was made 4 M with deionized urea and 2% with Bio-Lyte ampholyte (pH 3−10, 40% w/v). This solution (50 mL) was applied to the Rotofor cell and focused (Osuji and Madu, 1996). After isoelectric focusing, the 20 Rotofor fractions were collected and their pH values measured. The fractions were made 1 M with NaCl and then dialyzed against 10 mM Tris-HCl buffer (pH 8.2) to remove the ampholytes.

Native PAGE of GDH. Rotofor fractions (0.4 mL) were prepared and electrophoresed at 4 °C through 7.5% native PAGE. Electrophoresed gels were visualized for GDH activity by staining with L-glu-NAD^+-phenazine methosulfate-tetrazolium bromide reagent (Cammaerts and Jacobs, 1985).

Assay of GDH Activity. The Rotofor fractions containing the GDH isoenzymes were pooled in equal volumes and used for the photometric assays at 340 nm (Osuji and Cuero, 1992). For the kinetic analysis, GDH (EC 1.4.1.2) amination activity was assayed with 0.4−35.0 mM α-ketoglutarate, 2.0−250.0 mM NH_4Cl, 0.16 mM NADH, 1.3 mM $CaCl_2$, and 0.2 mL of GDH solution. Substrates were prepared in 0.1 M Tris-HCl buffer (pH 8.0). The final volume of each assay was 3 mL.

From the initial velocities of the amination activity, double-reciprocal plots were constructed (Osuji and Cuero, 1992). The values of the true kinetic constants, V_{max} and K_m, were calculated from the replots (Segel, 1975) of the 1/*V*-axis intercepts versus the reciprocals of the NH_4Cl concentrations.

Protein concentrations were determined according to the method of Lowry et al. (1951). Enzyme and protein assays were done in triplicate; the results reported are the averages ± standard deviations of the assays.

RESULTS AND DISCUSSION

NPK-Induced Isomerization of Maize GDH. Figure 1 shows the GDH isoenzyme population distribution patterns induced by the different fertilizer treatments. In response to the control sand culture ($N_0P_0K_0$), the maize GDH displayed the maximum number of 28 isoenzymes (Figure 1A). However, the population distribution pattern deviated from the typical binomial distribution because the second row of isoenzymes had 14 isoenzymes instead of 3−5 (Osuji and Madu, 1995). The charge isomers also focused in Rotofor chambers 5−18, encompasing the wide pH range from 5.3 to 9.5. Normally, maize GDH focuses in Rotofor chambers 6−13, encompasing only the pH 5.5−7.5 range (Osuji and Madu, 1995). GDH isomerism is due to the binomial assembly of the 3 subunits to form 28 hexameric isoenzymes that are divided into 7 charge isomers (Osuji and Madu, 1995, 1997b). The presence of additional basic charge isomers (Rotofor chambers 14−18) suggests that the isomerization involved changes in subunit composition in addition to increases in the isoelectric points (p*I*) of some of the isoenzymes. The induction of basic isoenzymes as part of the response reaction further expands the capacity of the enzyme to respond to diverse environmental factors.

Fertilization of the maize with $N_1P_1K_1$ solution suppressed nearly all of the cathodal isoenzymes (rows 3 and 4), but the 14 anodal isoenzymes (row 2) persisted. The combination of two nutrients ($N_1P_0K_1$) induced the expression of 14 isoenzymes in row 4 but suppressed all the more anodal isoenzymes (Figure 1C). Therefore, the presence of N and K suppressed all of the isoenzymes in rows 1−3 (compared with Figure 1A). The isoenzyme pattern induced by $N_1P_0K_1$ (Figure 1C) was similar to that induced by ≥75 mM NH_4^+ (Osuji and Madu, 1995, 1996). This shows that under the sand culture of corn, the 4 mM K^+ enhanced the effect of the 2 mM NH_4^+ compared to that of 75 mM NH_4^+. Panels B and C of Figure 1 also showed distinct divisions of the isoenzyme populations into basic (Rotofor chambers 13−18) and acidic groups (Rotofor chambers 4−10) in further support of the induction of basic isoenzymes in response to nutrients.

Fertilization of the corn with P_i ($N_0P_1K_0$) enhanced the expression of the row 1 and 2 isoenzymes but suppressed all the more cathodal isoenzymes (Figure 1D). The responses to $N_1P_1K_1$ and $N_0P_1K_0$ treatments appeared similar, but the isoenzymes in row 1 were

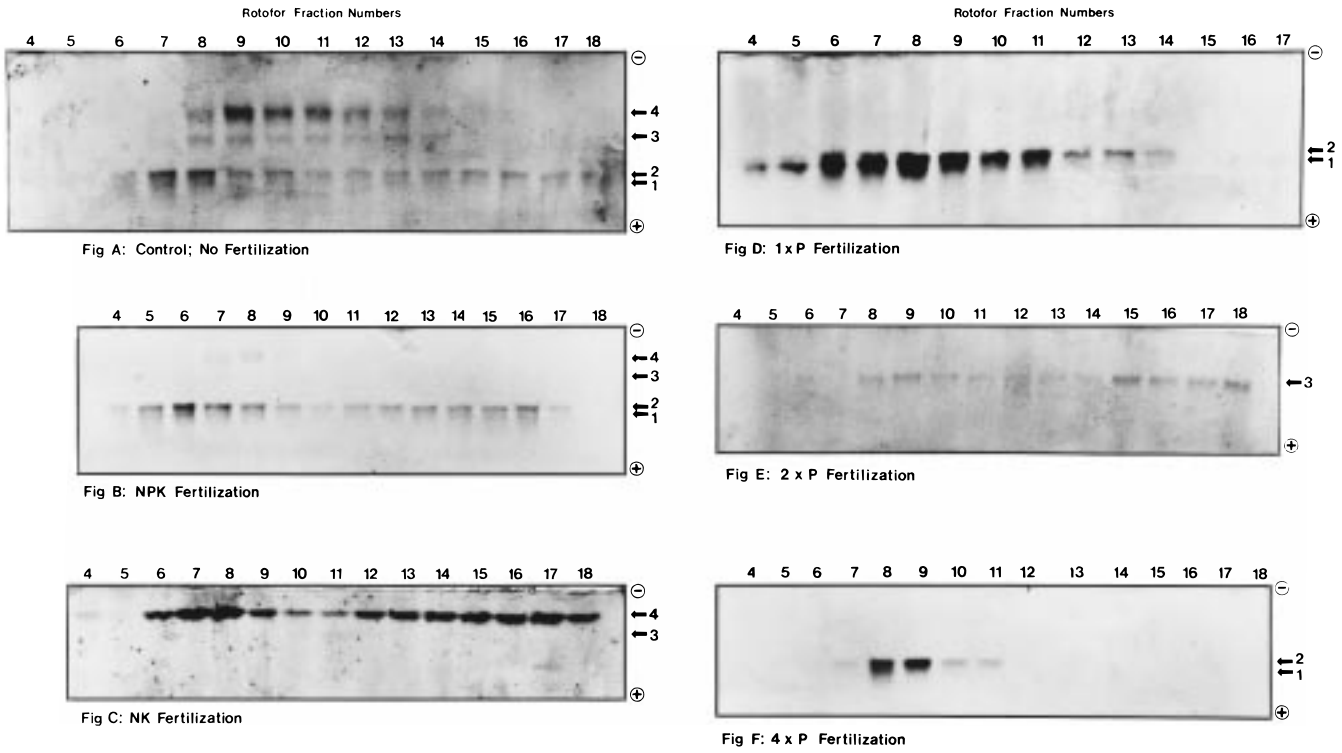

Figure 1. GDH diagnosis of the N, P, and K nutrient status in maize. The GDH extracted from maize shoots under each fertilizer treatment was Rotoforated, followed with native PAGE. GDH isoenzyme population distribution was visualized by staining the electrophoresed PAG with tetrazolium blue reagent. The rows of GDH isoenzymes were numbered from the most anodal as 1.

more abundant in the $N_0P_1K_0$ than in the $N_1P_1K_1$ treatment. Conversely, the row 2 isoenzymes were more abundant than the row 1 isoenzymes in the $N_1P_1K_1$ treatment. Therefore, the $N_1P_1K_1$ and $N_0P_1K_0$ treatments suppressed the cathodal isoenzymes and induced fluctuations in the isoenzyme distribution patterns in rows 1 and 2. These results suggest that the $N_0P_1K_0$ (Figure 1D) and $N_1P_1K_1$ (Figure 1B) interactions with GDH were at the nutrient deficiency−sufficiency interphase in maize.

A comparison of panels A−D of Figure 1 shows the effects of N, P, and K nutrition on the enzyme. The isoenzyme pattern in Figure 1A was in response to the basal condition, which was composed of the cellular nutrient contents, water, and insignificant nutrients available from the sand and peat moss. External supply of $N_1P_1K_1$, $N_1P_0K_1$, and $N_0P_1K_0$ converted the isoenzyme patterns to those in panels B, C, and D of Figure 1, respectively. Therefore, the effect of the cellular nutrient contents (Figure 1A) on GDH was quickly overridden by the effects of the external supplies of N, P, and K. The weekly supply of nutrients minimized accumulation of excess nutrients in the soil and ensured that, once absorbed by the maize, they were not stored in the organs but utilized readily for metabolism. These are thus the ideal conditions for utilizing GDH isomerization to diagnose crop nutrient status.

The response to single nutrient concentrations was further studied with P nutrition. Doubling the P concentration ($N_0P_2K_0$) abolished the GDH isoenzymes in rows 1, 2, and 4 but enhanced those in row 3 from 6 in the control maize (Figure 1A) to 11 (Figure 1E). Quadrupling of the P concentration ($N_0P_4K_0$) suppressed virtually all of the isoenzymes, leaving only 4 in the row 2 isoenzymes. The P levels used in the experiments ensured increasing saturation of the phosphate transporters, the K_m values of which range from 5 to 50 μM phosphate (Dunlop et al., 1997; Epstein, 1976). The stress responses of GDH to the P and $N_1P_0K_1$ treatments could also be due to nutrient imbalances. Soil stresses induced by nutrient imbalances affect corn growth and grain yield (Kovacevic et al., 1997).

The results in Figure 1 show that GDH responded systematically to the N, P, and K nutrient status in maize, each isoenzyme population distribution pattern being specific to a defined nutrient status. This conclusion is in agreement with previous results because the responses of maize GDH to nitrogen nutrient (Osuji and Madu, 1995) were different from those to Pb^{2+} (Osuji et al., 1997), which were in turn different from those to N, P, and K (Figure 1).

Phosphorus and maize were used in this study because the high P fixation by most soils (ICRISAT, 1995; Grant and Robertson, 1997) requires the addition of P fertilizer to meet the nutrient sufficiency required, especially in maize cropping systems (Fageria and Baligar, 1997). Currently, much of the cultivated land throughout the world is deficient in available P_i for crop growth, and this is one of the main factors that limits food production in many countries (Wasaki et al., 1997; Pandey et al., 1994).

Kinetics of the Response of GDH to NPK. The isomerization was quantitated by determination of the V_{max} values of the amination reaction. For each GDH response, a replot of the $1/V$-axis intercepts versus the reciprocals of the NH_4Cl concentrations was constructed. All of the replots (Figure 2) showed a noncompetitive inhibition relationship. The control GDH had the highest V_{max} value, 310.0 ± 14.3 μmol min^{-1} mg^{-1}, and the V_{max} values decreased with increasing P content of the fertilizer. Phosphate is therefore the primary effector of the enzyme. This suggests that the enzyme has a target site for P, and this site has a noncompetitive inhibition relationship with the NH_4^+

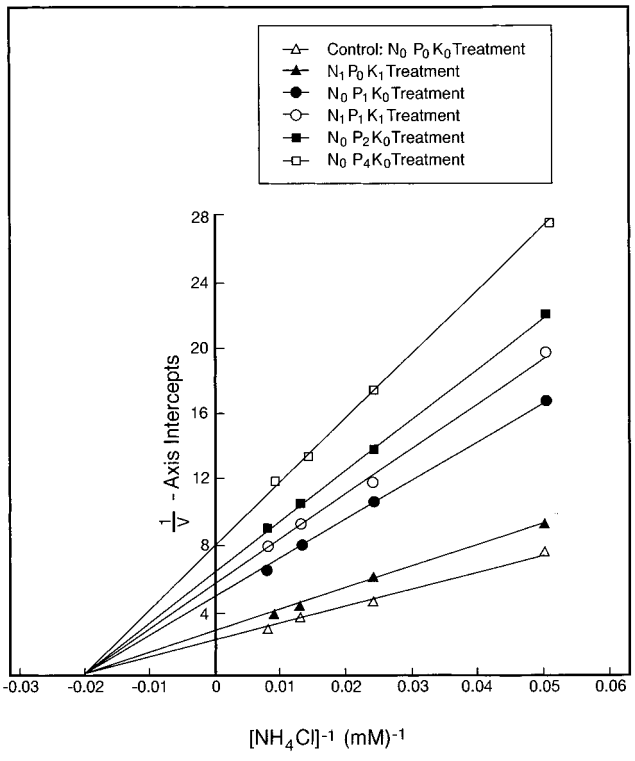

Figure 2. Replots of the data from double-reciprocal plots for the determination of the true V_{max} values for maize GDH. To determine the initial velocities, Rotoforated GDH (0.2 mL, 1.4 ± 0.05 mg of protein/mL) was assayed at varied α-ketoglutarate concentrations in the presence of a constant (0.16 mM) NADH concentration with NH_4Cl held at varied fixed levels, in a total volume of 3 mL per assay.

site of the enzyme. GDH possesses a target site for organonitrogen herbicides (Osuji, 1997). The interaction of the enzyme with the herbicides was due to the nucleophilicity of the herbicides. By the same mechanism, the phosphoryl group is also a reactive nucleophilic center in enzyme catalysis (Fersht, 1985). The inhibition relationships between GDH and phosphate, lead, and herbicides suggest that the regulation could be at the transcriptional, translational, and/or post-translational steps of GDH gene expression.

The V_{max} values of the GDH isomerization in response to the $N_0P_0K_0$, $N_1P_1K_1$, $N_1P_0K_1$, $N_0P_1K_0$, $N_0P_2K_0$, and $N_0P_4K_0$ fertilizer treatments were 310.0 ± 14.3, 130.0 ± 5.1, 246.4 ± 9.7, 140.6 ± 8.5, 108.2 ± 4.4, and 83.3 ± 3.8 μmol min^{-1} mg^{-1}, respectively. These data show that the response of the isomerization to the P doses was curvilinear, the curvature being between 1 and 3 mM P_i concentration. This curvature is important in the diagnosis of the nutrient supply status of maize. Because P is required for optimal crop growth (Marschner, 1995; Fageria and Baligar, 1997), the curvilinear response of GDH isomerization to P established a quantitative basis for interpreting crop yield in terms of crop nutrient composition data.

Response of Maize Dry Matter to the GDH Isomerization. The nucleophilic interaction between GDH and phosphate permitted further discussion of the response of maize dry matter to the isomerization reaction. The maize dry matter yields in the $N_0P_0K_0$, $N_1P_1K_1$, $N_1P_0K_1$, $N_0P_1K_0$, $N_0P_2K_0$, and $N_0P_4K_0$ fertilizer treatments were 2.5 ± 0.2, 9.7 ± 0.6, 4.2 ± 0.3, 2.4 ± 0.2, 2.2 ± 0.2, and 2.5 ± 0.2 g per maize shoot, respectively. Therefore, the greatest dry matter yield was induced by the row 2 isoenzymes (Figure 1B), and it was followed by the dry matter yield induced by the row 4 isoenzymes (Figure 1C). All of the other isoenzyme population distribution patterns of GDH induced virtually the same dry matter yield.

The responses of the maize dry matter to the P dosess and to the GDH amination V_{max} values also showed that there was a threshold dry matter level, of ~2.4 g, which remained constant despite changes in the V_{max} values. The GDH- and P-insensitive dry matter threshold was presumably dependent on the water-use efficiency and photosynthesis by maize (Curry et al., 1990; Reddy et al., 1995). However, $N_1P_0K_1$ fertilization doubled, while $N_1P_1K_1$ quadrupled, the yield. Also, although GDH isomerization, and the P doses did not change the threshold dry matter yield, optimal dry matter yield was not attained when P was absent. Therefore, $N_1P_0K_1$ was inadequate for inducing optimal maize yield in the sand culture.

A consideration of the response of the dry matter yield to GDH isomerization showed that the optimal dry matter yield occurred at the curvature in the curvilinear response of GDH to the P doses. Therefore, the curvature between 1 and 3 mM PO_4^{3-} concentration defined the nutrient deficiency−sufficiency interphase of maize. This result explains why the GDH isoenzyme population patterns induced by the $N_1P_1K_1$ and $N_0P_1K_0$ fertilization fluctuated between rows 1 and 2 (Figure 1B,D). The importance of the GDH method is that it experimentally establishes a quantitative relationship between dry matter yield and the externally applied nutrient concentration. This biochemical approach is readily applicable for the nutrient efficiency screening of other crop species. The insensitivity of the maize dry matter yield to the P doses means that the traditional dry matter response to P (Mallarino, 1995; Fageria and Baligar, 1997) would be an inadequate criterion for diagnosing the nutrient sufficiency status of the DK-68 hybrid maize genotype.

The GDH isoenzyme pattern induced by $N_1P_0K_1$ was not diagnostic of the sufficient nutrient status because the maize would need to be fertilized with $N_1P_0K_0$ containing >75 mM nitrogen to enable the GDH isoenzymes to assume the population pattern (fluctuate) as in Figure 1C. This is, however, the high level of nitrogen fertilizer that is usually applied to corn (Hooker et al., 1983) to achieve above average grain yields (Cardwell, 1982), which also carries a possible risk of pollution of the environment with nitrate (Keeney and Follett 1991).

Figure 3 shows that all of the experimental maize plants displayed vivid symptoms of mineral nutrient deficiency, the yellow coloration being common to all treatments. Especially, visual symptoms could not distinguish between the maize responses to the high doses of P ($N_0P_1K_0$, $N_0P_2K_0$, and $N_0P_4K_0$) and the response to the nutrient deficiency ($N_0P_0K_0$). A comparison of the visual symptom (Figure 3), the fertilizer treatment, and the corresponding dry matter yield shows that the GDH isoenzyme population distribution pattern (Figure 1) was the most adequate prognostic response of the maize to N, P, and K.

The application of the GDH isomerization method for the diagnosis of the nutrient supply status of a cropping system essentially involves the determination of the nutrient compositions that induce the GDH isoenzyme population distribution patterns to fluctuate at the

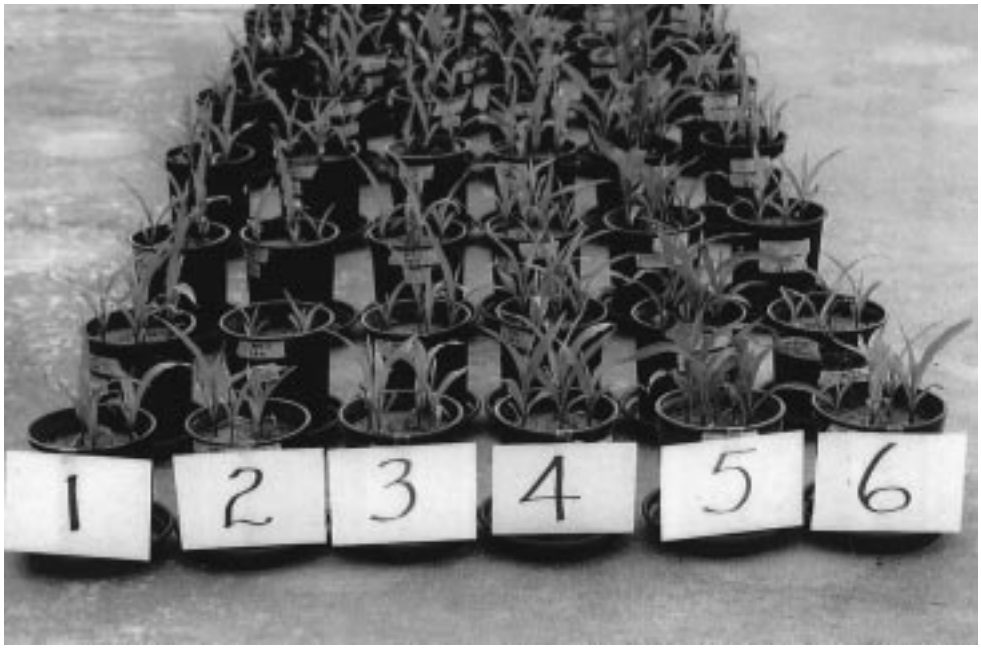

Figure 3. Visual diagnosis of nutrient status in the experimental maize plants, 3 weeks after planting. Maize rows 1, 2, 3, 4, 5, and 6 refer to the $N_0P_1K_0$, $N_0P_2K_0$, $N_0P_4K_0$, $N_1P_0K_1$, $N_1P_1K_1$, and $N_0P_0K_0$ fertilizer treatments, respectively.

environmentally sustainable optimal dry matter yield. Because the method utilizes the relationship between GDH isomerization and crop yield to interpret nutrient composition data, it guides the farmer to set the correct yield goals based on the response of his crop's GDH to the nutrients and other inputs that are remaining in the soil from previous cropping seasons. Setting of accurate yield goals helps to prevent excess application of fertilizer and possible subsequent damage to the environment (Peterson and Frye, 1989).

The curvature in the GDH response to P doses enables the farmer to decide whether to leave the soil nutrient status as it is or to add more fertilizer to meet the optimal yields sustainable by his soil types. Also, during the crop's vegetative growth phase, the GDH isomerization enables the farmer to check whether his crop's current GDH isoenzyme population distribution patterns are adequate for the preset yield goals. If the isoenzyme patterns have deviated from those that lead to the optimal yield, then corrective fertilizer applications may be made. Whereas previous methods (Young et al., 1987; Follett, 1989) only simulated yield, the GDH method helps the farmer to identify nutrient imbalance and to verify the capacity of his crops to attain the set yield goals.

Advance verification of a crop's yield performance is vital to the farmer because it removes the element of uncertainty from nutrient management operations, thereby discouraging excessive fertilization. Therefore, the GDH method is the farmer's perfect guide for a more efficient nutrient management of his cropping system because it simplifies his translation of the plant and soil nutrient analysis data so as to achieve the environmentally sustainable optimal crop yield. Nutrient deficiency−sufficiency ranges deduced from plant compositional data (Olson and Kurtz, 1982) have been of limited application in the nutrient management of cropping systems (Peterson and Frye, 1989). The GDH diagnostic method is more useful because the isomerization is in response to the nutrients that are available to the plant for promoting its growth (Burns, 1992).

In the developed countries, agricultural productivity gains achieved through fertilizer use have led to excessive fertilizer application, which have now created serious problems of nutrient pollution in some areas. In view of this, new approaches are needed for minimizing nutrient emissions to the environment (Loneragan, 1997). The results presented herein show that emission of plant nutrients to the environment may be minimized by cultivating nutrient efficient cultivars, selected on the basis of their GDH-dependent nutrient deficiency−sufficiency status.

Signaling and Signal Integration by GDH. It has been demonstrated that GDH isomerizes in response to cellular redox changes (Osuji and Madu, 1996, 1997a; Osuji et al., 1997; Osuji, 1997) and, in so doing, it alerts the citric acid cycle about the imminent changes in the environment. A natural substrate of the enzyme is α-ketoglutarate, a member of the citric acid cycle. Therefore, when the enzyme responds to N, P, and K by changing its isoenzyme population pattern, the citric acid cycle and the energy metabolism of the cell are affected accordingly. The ability of the GDH isoenzymes to assume different net charges covering a wide pH range indicates the wide range of possible structural changes the enzyme can undergo upon reception of environmental signals. The isomerization of the maize GDH (Figure 1) in response to changes in the soil nutrients is a visual demonstration of the signaling reaction of the enzyme. Many signaling proteins undergo similar substantial structural changes in their energy transduction reactions (Schindelin et al., 1997). The changes in the subunit composition of the isoenzymes and the accompanying energy transductions were substantial (Figure 1), judging from the magnitude of the changes in the V_{max} values.

The decreases in the velocity of the isomerization reaction with increasing P doses, without significant changes in the maize dry matter yields, illustrate the GDH advance warning system (Osuji and Madu, 1997a) because it had received the redox signals when the other metabolic reactions had not received them. The GDH

signaling system is therefore upstream (Osuji, 1997) in the cascade of mechanisms by which plants respond to their environment. The noncompetitive inhibition interference in the GDH catalytic mechanism by soil P_i concentrations (Figure 2) suggests that the enzyme interacts with the nutrients. Compared with other signal transduction reactions in plants (Aducci, 1997; Lee, 1998; Roberts et al., 1992), the GDH signaling system is unique, especially in its localization in the mitochondrial matrix rather than in the plasma membrane.

From the results in Figure 1A−D, GDH has the ability to receive signals from soil nutrients, to integrate them, and to synthesize a single response reaction to them. The corn GDH response to the combination of two nutrients ($N_1P_0K_1$) was similar to the response to 75 mM NH_4Cl (Osuji and Madu, 1995). The response to the combination of three nutrients ($N_1P_1K_1$) was different from the response to the two-nutrient combination ($N_1P_0K_1$) but similar to the response to 3.5 mM P_i (Figure 1D). Therefore, in the GDH integration of the N, P, and K signals to synthesize a single response reaction, the signal from P superseded those from K and N. However, the presence of N and K was manifested in other forms because the first row of isoenzymes was more suppressed in the integrated response to N, P, and K than in the response to 3.5 mM P_i. The responses to K doses will be presented in subsequent publications, but Figure 1B−D and the response of the enzyme to high NH_4Cl concentrations (Osuji and Madu, 1995) show that the signal from N superseded that from K, whereas that from P superseded that from N. These illustrate the signal discrimination−integration mechanisms of the enzyme.

The sequence of the signal dominance by P, N, and K is a reflection of the sequence of their relative nucleophilicities. In this regard, the dominant nucleophile in the control treatment ($N_0P_0K_0$) was a water molecule, which is less nucleophilic than K, N, and P. This explains why the GDH isoenzyme patterns induced by N, P, and K (Figure 1B−F) were dominant over that induced by water (Figure 1a). Therefore, the decreasing order of signal dominance during the signal integration by GDH is P > N > K > O, that is, $PO_4^{3-} > NH_4^+ > K^+ > H_2O$. It also implies that the cellular P and K concentrations of the control corn were so dilute that their signals were overridden by those of H_2O. These are some of the basic principles of the isomerization and signaling reactions of GDH. In view of the numerous inducers (pesticides, heavy metals, pathogens, phytohormones, plant nutrients, etc.) of the GDH isomerization, the signal integration on the basis of the inducers' relative nucleophilic dominance brings orderliness to the induction mechanism and simplicity to the application of the isomerization as a diagnostic tool in metabolism.

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