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Biomass enhancement in maize and soybean in response to glutamate dehydrogenase isomerization

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Abstract

The relationship between nutrient composition, crop biomass, and glutamate dehydrogenase (GDH) isoenzyme pattern was investigated in soybean (*Glycine max*) and maize (*Zea mays*) by monitoring the nutrient induced isomerization of the enzyme from the seedling stage to the mature crop. GDH was extracted from the leaves of the plants, and the isoenzymes were fractionated by isoelectric focusing followed by native polyacrylamide gel electrophoresis. The isomerization V_{max} values for soybean GDH, similar to maize GDH increased curvilinearly from 200 - 400 μmol mg^{-1} min^{-1} as the inorganic phosphate nutrient applied to the soil decreased from 50 - 0 mM. In soybean, combinations of N and K, P, or S nutrients induced the acidic and neutral isoenzymes, and gave biomass increases 25 - 50 % higher than the control plant. GDH isoenzymes were suppressed in soybean that received nutrients without N, K, or P and accordingly the biomass was about 30 % lower than the control. Treatment of maize with NPK nutrients increased the GDH V_{max} values from 138.9 at the vegetative to 256.4 μmol mg^{-1} min^{-1} at the reproductive phase, and suppressed the basic isoenzymes, but induced both the acidic and neutral isoenzymes thereby inducing seed production (27.0 ± 1.4 g per plant); whereas both the acidic and basic isoenzymes were suppressed in the control maize, and seeds did not develop. Simultaneous induction of the acidic, neutral, and basic isoenzymes of GDH indicated the occurrence of senescence. Therefore in maize and soybean, the induction of the acidic and basic isoenzymes of GDH led to the enhancement of biomass.

Additional key words: GDH-induced biomass, *Glycine max*, isoelectric focusing, nitrogen, phosphorus, potassium, sulfur, *Zea mays*.

Introduction

Glutamate dehydrogenase [GDH, EC 1.4.1.2] isomerizes *in vitro* in response to α-ketoglutarate (α-KG) and NADH as substrates (Osuji *et al.* 2001), and also *in vivo* in response to the treatment of plants with nutrients (Osuji and Madu 1995, Hřib *et al.* 1997, Osuji and Madu 1997, Osuji *et al.* 1998, Osuji and Braithwaite 1999). Earlier biochemical and molecular studies had suggested the involvement of the amination activity of the enzyme in NH_4^+ salvage (King and Wu 1971, Duke *et al.* 1978, Jail and Srivastava 1981, Singh and Srivastava 1982, Jail and Shargool 1987, Lightfoot *et al.* 1988, Šukalović 1990, Magalhaes 1991, Melo-Oliveira *et al.* 1996, Turano *et al.* 1996). Demonstration of the non-proteolytic cleavage of the enzyme *in vitro* (Osuji *et al.* 2001) confirmed the chemical mechanism of the isomerization (Osuji *et al.* 1999). But the physiological function of the enzyme still remains unclear. Tobacco plants genetically transformed with the *gdh* gene produced more biomass than the control plants (Ameziane *et al.* 2000). The discovery of the biomass enhancement function and the description of the chemical basis of the isomerization have now set the stage for studies on the molecular mechanisms of the enzyme's function. Although the discovery of the biomass enhancement function moved the GDH controversy above and away from the discussions on the redox catalytic mechanism (Robinson *et al.* 1992, Osuji *et al.* 2001), the relationships between biomass production, GDH isoenzyme population distribution pattern, and plant nutrient supply have not been investigated. Neither has the isomerization of the enzyme from the seedling stage

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Abbreviations: NPKS - nitrogen, phosphorus, potassium, and sulfur nutrients; GDH - glutamate dehydrogenase ; α-KG - α-keto-glutarate; IEF - isoelectric focusing; PAGE - polyacrylamide gel electrophoresis; DAE - days after emergence.

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to the mature crop been investigated. However, GDH also isomerizes in response to plant senescence (Watanabe *et al.* 1997, 1989) arising from the re-mobilization of nitrogen derived from protein hydrolysis (Zimmerman 1960). Changes in GDH isoenzyme distribution patterns that are induced by the senescence (Loulakakis *et al.* 1994) of leaves have not been experimentally delineated from those induced by mineral nutrients so as to understand those isomerizations that define the biomass of plants. The results presented hereunder show that the induction of the acidic and neutral isoenzymes of GDH by treatment of crops with potassium, phosphorus, and nitrogen nutrients led to the enhancement of crop biomass. In the study, maize and soybeans were selected as the experimental plants because they have been used extensively in studies on the functions of the enzyme (Pryor 1990, Sakakibara *et al.* 1995, Stewart *et al.* 1995, Turano *et al.* 1996, Šukalović and Vuletić 2001) and in the physiology of nutrient uptake (Marschner 1998, Elliott *et al.* 1984, Jungk *et al.* 1990).

Materials and methods

Isomerization of GDH: Maize (*Zea mays* L. hybrid KD-68) and soybean (*Glycine max* L. cv. Donegal) seeds were sowed in 10-dm^3 pots containing 9:1 mixture of sand:vermiculite, and watered with distilled water (control) or with solutions of N, P and K nutrients in single, and in combination at different ratios (5, 25, 50, 75, 100 mM NH_4Cl solutions; $N_1P_1K_1$ 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_1,K_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) as described previously (Osuji and Madu 1995, Osuji *et al.* 1998). Each nutrient treatment induced at least 95 % germination. About 3 to 5 weeks after germination (when the leaves of the control seedlings started to turn yellow), the entire shoots were harvested, immediately frozen with dry ice, ground to powder with pestle and mortar, and stored at -70 °C. GDH was extracted from the powdered tissues (25 - 30 g) as described before (Osuji and Madu 1995) with 50 cm^3 of extraction buffer (Loyola-Vargas and De Jimenez 1984). All the purification steps were carried out at 4 °C. Tissue debris were removed by centrifugation (4 000 *g*, 15 min). The cloudy supernatant was frozen at -20 °C, and was centrifuged (10 000 *g*, 15 min) after thawing. The crude extract was saturated to 50 % with solid $(NH_4)_2SO_4$ and the resultant precipitate was pelleted by centrifugation (10 000 *g*, 15 min). The pellet was dissolved in a minimum volume of 0.1 M Tris-HCl buffer, pH 8.5, and dialyzed against 3 changes of 10 mM Tris-HCl buffer (pH 8.5), each change being 4 dm^3. The volume of the GDH solution containing about 0.3 g protein was brought to ~50 cm^3 with ice-cold 10 mM Tris-HCl buffer (pH 8.5), and then subjected to Rotofor IEF (*Bio-Rad*, Hercules, CA, USA) (Osuji and Madu 1995). The 20 Rotofor fractions were collected, their pH values measured, and then dialyzed as described above.

GDH activities were determined by photometry at 340 nm (Osuji and Cuero 1992) with 0.4 - 35.0 mM α-KG, 2.0 -250.0 mM NH_4Cl, 0.16 mM NADH, 1.3 mM $CaCl_2$, and 0.2 cm^3 of GDH solution, in a final reaction volume of 3 cm^3 per assay. From the initial velocities of the reaction, double reciprocal plots were constructed (Osuji and Cuero 1992). The maximum velocity (V_{max}) was calculated from the replots (Segel 1975) of the 1/V-axis intercepts versus the reciprocals of the NH_4Cl concentrations. Protein concentrations were determined by the method of Lowry *et al.* (1951). Enzyme and protein assays were done in triplicate, and the average was calculated.

Equal volumes (0.5 cm^3) of the Rotofor fractions were concentrated 10-fold by vacuum centrifugation, followed by 7.5 % native PAGE at 4 °C (Osuji and Madu 1995). GDH charge isomer (isoenzyme population distribution) pattern was visualized by staining the electrophoresed gel in L-glutamate-NAD^+-phenazine methosulphate-tetrazolium blue solution (Cammaerts and Jacobs 1983).

Nutrient treatments: For the soybean crop, a 150 × 150 m field plot in the University farm, Waller County was plowed and subdivided into 10 ridges. The 2 border ridges were not used. Soybean seeds inoculated with *Bradyrhizobium japonicum* were sowed by hand with 4 cm spacing. The plot was watered immediately to 50 % field capacity, and twice per week. Seedlings were randomly harvested from the 8 ridges (20 seedlings per ridge) 2 weeks after planting, and used for the determination of GDH activity and isoenzyme pattern. The unharvested seedlings were allowed to continue to grow and 8 nutrient treatments were applied, one treatment per ridge. The stock solution of treatment 1 contained 1 mol dm^{-3} of each of NH_4Cl, KH_2PO_4 and Na_2SO_4; treatment 2 contained 1 mol dm^{-3} of each of NH_4Cl and Na_2HPO_4; treatment 3 contained 1 mol dm^{-3} of each of NH_4Cl and KCl, but 0.5 mol dm^{-3} of $Na_2S_2O_5$; treatment 4 contained 1 mol dm^{-3} of KH_2PO_4 and 2 mol dm^{-3} of NH_4Cl; treatment 5 contained 1 mol dm^{-3} of K_2HPO_4; treatment 6 contained 1 mol dm^{-3} of each of K_2HPO_4 and $Na_2S_2O_5$; and treatment 7 contained 1 mol dm^{-3} of each of $Na_2S_2O_5$ and NH_4Cl. The 8th treatment was the water control. The nutrient treatments and the water control were applied five times

at 4 d intervals by diluting 200 cm^3 of the stock nutrient solution to 8 dm^3 with distilled water, and deliver to the base of the soybean with a watering can. The first treatment was applied on the day 18 after planting.

For the maize experiment, 10 pots (20-dm^3) were filled with the sandy, silt, clay soil collected from the University farm. The pots were arranged on a grass lawn away from any shade. Maize seeds (*Zea mays* L. hybrid KD-68) were sowed in the pots, seven per pot, and watered three days per week. At 3 weeks after seedling emergence, the entire shoots of four seedlings per pot were harvested and combined to make a single sample. The maize GDH activity and isoenzyme population pattern were determined as described above. The remaining seedlings were allowed to grow in their pots. Five pots were left unfertilized as the control, whilst the remaining 5 were treated with 20 g of 13:13:13 granular NPK fertilizer per pot. The NPK fertilizer rate was within the range recommended for vegetable growth by the manufacturer (*Howard Johnson*, Milwaukee, WI, USA). The fertilizer was applied as top dressing to the pots 24 d after emergence. All pots including the controls were watered twice per week.

Monitoring the GDH response: Soybean leaf samples (~40 g) were collected from each of the 8 treatments 7 weeks after planting. On the other hand, maize leaf samples were randomly collected at 40, 60, 80, and 110 d after emergence, one leaf (leaf 4, 5, or 6) from a plant, leaves from replicate pots being combined to make one sample. All leaf samples were immediately frozen with dry ice, ground to powder, and stored at -70 °C for further use for GDH assay and isoenzyme analysis. At maturity, the maize seeds and soybean shoots were harvested, air dried, and weighed.

Results and discussion

GDH isoenzyme patterns in response to the treatment of soybean seedlings with nutrients of different NPKS combinations demonstrated a strict dependence on the concentrations of the nutrients (Fig. 1). This pattern was in agreement with previous pattern for the maize (Osuji and Madu 1995, Osuji *et al.* 1998). The GDH isoenzyme distribution pattern of a plant represents the total but not the average number of isoenzyme bands resulting from the treatment (Osuji and Madu 1995, 1997, Osuji *et al.* 1998, Osuji and Braithwaite 1999) because crop samples used for the extraction of GDH were not subjected to statistical selection. Therefore, in order to use the GDH isoenzyme patterns to deduce the nutrient status of a crop, the GDH isoenzymes patterns have to be calibrated against known concentrations and combinations of nutrients.

The GDH isoenzymes of the control soybeans (Fig. 1) were focused in Rotofor chambers 8 - 11. Treatment of the potted soybean seedlings with up to 25 mM NH_4Cl solution induced the formation of 28 isoenzymes in two rows of 14 isoenzymes per row. The pH values of the Rotofor fractions 4 - 18 were: 5.50, 5.96, 6.80, 7.40, 8.03, 8.43, 8.77, 9.10, 9.97, 10.22, 10.60, 11.10, 11.30, 11.40, and 12.00. Treatment with higher concentrations of NH_4Cl suppressed the GDH isoenzymes. With 50 mM NH_4Cl, there were only 5 isoenzymes and they focused in Rotofor chambers 9 - 13. Treatment of soybean with 5 mM Na_2SO_4 solution produced GDH isoenzymes in Rotofor chambers 6 - 14, but with 25 mM Na_2SO_4 28 isoenzymes were produced in two rows similar to the pattern induced by 25 mM NH_4Cl treatment. Higher concentrations of Na_2SO_4 suppressed the GDH isoenzymes. Treatment with up to 50 mM Na_2HPO_4 induced only about 10 isoenzymes, but higher concentrations of Na_2HPO_4 induced 28 isoenzymes in 2 rows similar to those induced by 25 mM NH_4Cl, and 25 mM Na_2SO_4 solutions. Therefore, the GDH isoenzymes induced by 25 mM NH_4Cl, 25 mM Na_2SO_4, and 100 mM Na_2HPO_4 treatments were similar because they fluctuated on the two rows. Treatment of soybean with low concentration (5 mM) of KCl solution alone suppressed the GDH isoenzymes thus indicating the sensitivity of the crop to the nutrient.

GDH isomerization curve: The V_{max} values of the enzyme isomerization increased curvilinearly as the inorganic phosphate concentration decreased (Table 1, a non-competitive inhibition kinetics), similarly to the isomerization curve obtained for maize (Osuji *et al.* 1998). The curvilinear response suggested the restriction of crop metabolism and GDH activity under excess phosphate supply because in the soybean, a decrease of the applied phosphate from 50 to 0 mM increased the V_{max} values by 100 %, but a decrease from 100 - 50 mM merely increased the V_{max} values by 33 %. In maize also, a decrease in the applied phosphate from 7 to 0 mM increased the V_{max} value by 100 %, but a decrease from 14 to 7 mM merely increased the V_{max} values by 50 % (Osuji *et al.* 1998). The curvilinear response of the enzyme activity to the applied phosphate is analogous to and a reflection of the dependence of the enzyme activity on pH gradients (Osuji and Madu 1996) because the enzyme's V_{max} value is a function of its electrochemical potential. The limitation imposed on the crop metabolism by GDH may be due to increased fragmentation of the subunits of the enzyme (Osuji *et al.* 1999, 2001) at high

mineral ion (nucleophile) concentrations (Fig. 1). The impact of mineral nutrients on the GDH activities might have been exerted via the redox stress by the electrochemical potentials of the cells associated with nutrient transport. This result suggests that the function of GDH is dependent on the isoenzyme distribution pattern, the greater the fragmentation of the subunits, the lower were the V_{max} values. Because it covers the range of the responses of a crop's GDH to the spectrum of nutrient concentrations and combinations with inorganic phosphate that support the crop's normal metabolism

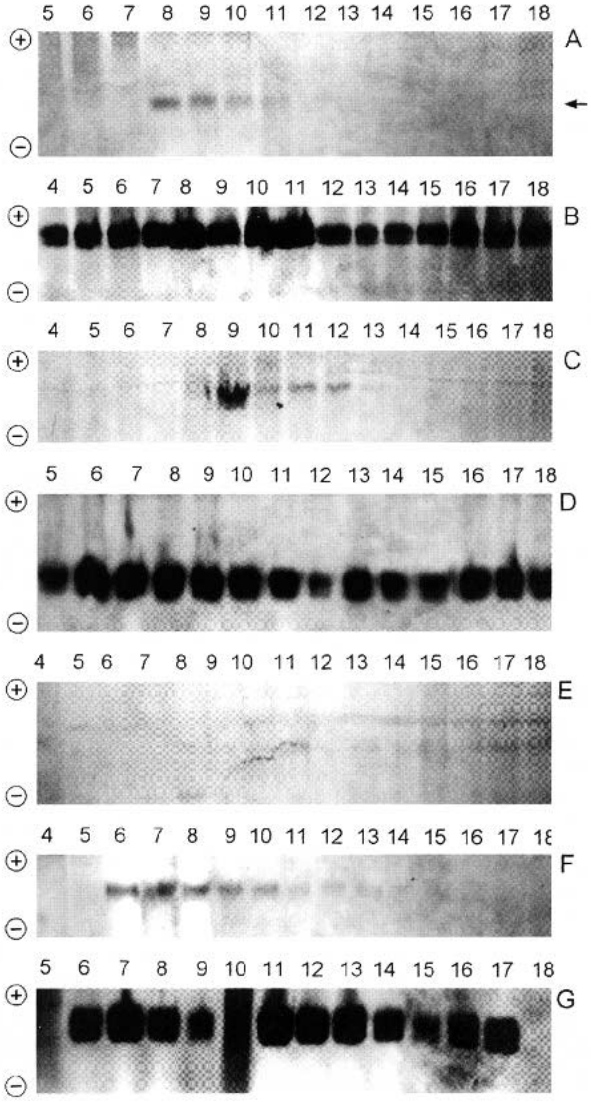

Fig. 1. GDH isoenzyme patterns in response to treatment of soybean with N, P, and S nutrients. The GDH extracted from soybean seedlings under each nutrient treatment was Rotoforated, followed with native PAGE. GDH isoenzyme distribution patterns were visualized by staining the electrophoresed gel with tetrazolium blue reagent. *A* - control, *B* - 25 mM NH_4Cl, *C* - 50 mM NH_4Cl, *D* - 25 mM Na_2SO_4, *E* - 50 mM Na_2SO_4, *F* - 50 mM Na_2HPO_4, *G* - 100 mM Na_2HPO_4.

(Osuji *et al.* 1998), the GDH isomerization curve is useful for identifying inadequate/toxic nutrient supply in plants. The GDH K_m values did not respond systematically to the nutrient treatments of the seedlings (Table 1) probably because the enzyme interacts with a multitude of nucleophiles as substrates of low specificity.

GDH isomerization during crop growth: The GDH pattern of the unfertilized soybean (Fig. 2*A*) was similar to those induced by 50 mM Na_2HPO_4, 50 mM NH_4Cl, and about 40 mM Na_2SO_4 treatments, the difference being that in the unfertilized seedlings, the acidic GDH

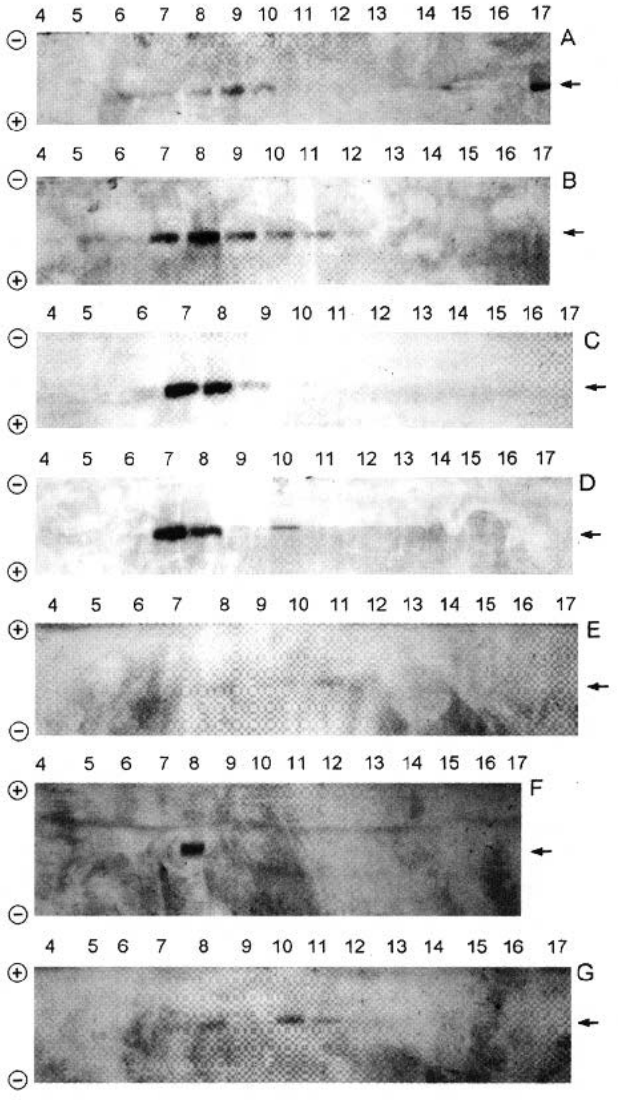

Fig. 2. Response of GDH to treatment of soybeans with nutrients of different N, P, K, and S compositions. The GDH extracted from the leaves of field grown soybean under each nutrient treatment was subjected to IEF, followed with native PAGE. GDH isoenzyme distribution patterns were visualized by staining the electrophoresed gel with tetrazolium blue reagent. *A* - control soybeans, *B* - soybeans treated with NKPS (treatment 1), *C* - NP (treatment 2), *D* - NKS (treatment 3), *E* - NKP (treatment 4), *F* - KP (treatment 5), and *G* - KPS (treatment 6).

isoenzymes (Rotofor fractions 5 - 7) were induced. Soybean is normally fertilized with PK nutrients.

The control (treatment 8) soybean GDH isoenzyme pattern at 7 weeks after planting (transition from the vegetative to the reproductive state) did not change from that of the control unfertilized seedlings (Fig. 2*A*). This suggested that the basal nutrient condition which included soil moisture, nutrient concentrations and composition that affected the GDH in the unfertilized seedlings did not change during 7 weeks after seed planting. Also the basic isoenzymes of GDH were suppressed by all the nutrient treatments in the field thus showing that the nutrient levels available to the crop were different from 25 mM NH_4Cl, 25 mM Na_2SO_4, and 100 mM Na_2HPO_4 (Fig. 1) that induced the complete set of soybean GDH isoenzymes. It was not possible to differentiate one soybean treatment from the other based on visual inspection because the vegetative growth of all the 8 experimental soybeans was equally good. The cv. Donegal is a vegetative-type of soybean because it flowered, but did not produce pods. Soybean treatments 1 - 4 induced the neutral and acidic GDH isoenzymes (Figs. 2*B-D*) similar to the control soybean seedlings although some of the isoenzyme bands were lower in intensity than the bands induced by treatment 1 (Fig. 2*B*). Treatment 5 without N and S (Fig. 2*F*), treatment 6 without N (Fig. 2G), and treatment 7 without P and K (Fig. not shown) suppressed virtually all the GDH isoenzymes, thereby supporting the results in Fig. 2*A-E*. In particular, treatments 1 - 4 with favourable NPKS ratios for soybean induced the acidic and neutral GDH isoenzymes.

Except the control soybean and soybean treatments 1 - 4, the GDH isomerization V_{max} values (Table 1) for the other field soybeans were too low to be measured accurately by photometry thus confirming the virtual absence of the isoenzyme patterns from those soybeans. The soybean GDH activities (Table 1) were normal because they fell within the range reported by Turano *et al.* (1996).

Values of V_{max} (Table 1) that were below the isomerization curve indicated nutrient inadequacy, and/or imbalance because the isomerization curve covers the responses of the crop's GDH to the spectrum of nutrient concentrations and combinations that support the crop's normal metabolism. The importance of the curve is that it guides the proper interpretation of the GDH isoenzyme patterns.

The GDH isoenzyme pattern for the maize without nutrients (Fig. 3*A*) showed similarity to that induced by 7 mM Na_2HPO_4 (Osuji *et al.* 1998) treatment. Analysis of the maize leaf GDH isoenzyme patterns 40 DAE (transition from vegetative to the reproductive phase) (Fig. 3*B*) showed the induction of the row 4 acidic GDH

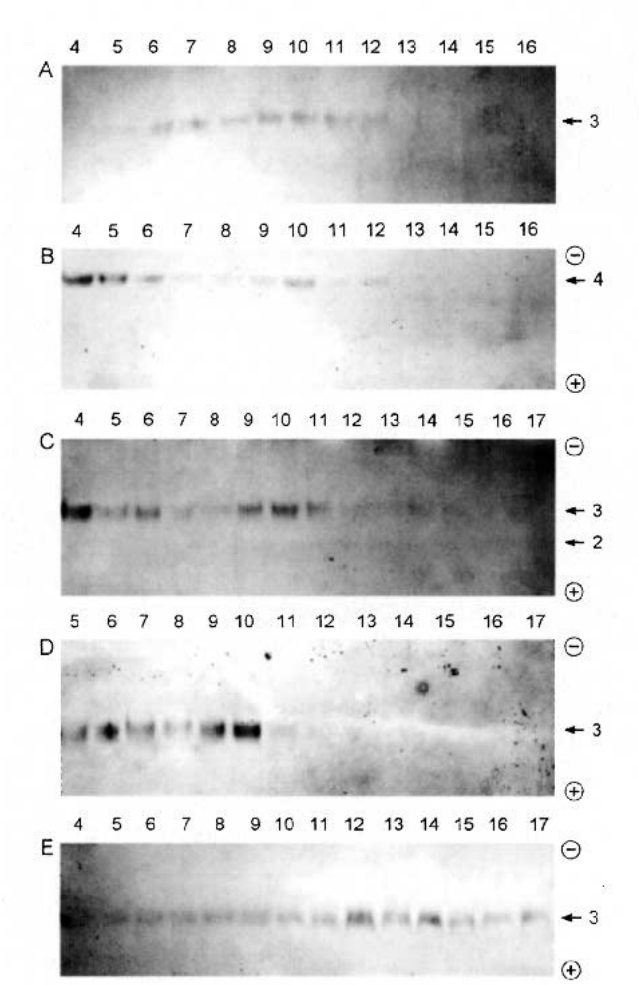

Fig. 3. Monitoring the response of GDH to nutrient changes during maize maturity. The GDH extracted from the leaves at each stage of maize growth was subjected to IEF, followed with native PAGE. GDH isoenzyme distribution pattern was visualized by staining the electrophoresed gel with tetrazolium blue reagent. *A* - 20 DAE, no fertilization, *B* - 40 DAE, *C* - 60 DAE, *D* - 80 DAE, *E* - 110 DAE.

isoenzymes but more suppression of the neutral and basic isoenzymes typical of the patterns induced by NK nutrient treatment (Osuji *et al.* 1998). But the GDH isoenzyme pattern for the control maize remained the same as it was at 20 DAE. The GDH isoenzyme pattern for the control maize was therefore the baseline control. The change in GDH isoenzyme pattern consequent upon the fertilization of the maize showed that the signals from the basal nutrient level were superceded by those from the applied nutrients. Analysis of the maize leaf GDH isoenzyme patterns 60 DAE showed pronounced induction of the row 3 acidic and neutral isoenzymes (Fig. 3*C*) for the fertilized maize, but the GDH pattern for the control maize remained the same as it was at 20 DAE. The GDH isoenzyme pattern (Fig. 3*C*) was typical of the calibrated GDH isoenzyme pattern induced by 50 - 75 mM NH_4Cl

(Osuji and Madu 1995). The isoenzyme pattern at 60 DAE therefore superceded that at 40 DAE. The change represented the nutrient remobilization occurring in the period during the development of maize seeds.

The GDH isoenzyme pattern for the fertilized maize (Fig. 3*D*) was virtually the same at 80 DAE as at 60 DAE, while the GDH pattern for the control maize remained the same as that at 20 DAE. At 110 DAE, the fertilized maize exhibited a GDH pattern (Fig. 3*E*) in which all the row 3 acidic and basic isoenzymes were present typical of the standard maize GDH induced by 100 mM NH_4Cl (Osuji and Madu 1995). At 110 DAE, the maize seeds had matured. Therefore, there was an increase in the leaf N nutrient content from 75 to 100 mM after seed development. The increased N could have resulted from senescence-related hydrolytic processes (Watanabe *et al.* 1989) taking place in the leaves. Therefore as in the case of avocado fruit ripening (Loulakakis *et al.* 1994), changes in maize GDH isoenzyme pattern associated with plant metabolism at the vegetative and reproductive stages were different from those resulting from the senescence period. The GDH isoenzyme bands were undetectable in the control maize at 110 DAE probably because the maize leaves had become yellow at that stage.

The maize GDH V_{max} values (Table 2) fitted into the inorganic phosphate concentration range of the isomerization curve unlike the GDH V_{max} values of soybean that fell outside the curve. The values of the maize kinetic constants were normal because they fell within the range reported earlier (Osuji and Madu 1996). The baseline V_{max} value was 138.9 μmol mg^{-1} min^{-1} at 20 DAE. The V_{max} increased from the baseline value to 256.4 μmol mg^{-1} min^{-1} at 40 DAE showing that the signals from the basal nutrient concentration were overridden by those from the applied nutrients. The basal nutrient concentration included the residual nutrient combination and concentration in the soil from previous seasons, and soil water content in relation to soil physical properties. Similar increases in the GDH activity of maize treated with NH_4NO_3 have been reported (Ribaudo *et al.* 2001). GDH integrates all the multitude of signals in the soil to give one signal expressed as the isomerization V_{max} value and the isoenzyme pattern (Osuji *et al.* 1998, Osuji and Braithwaite 1999). Where nutrients were not applied, the V_{max} remained low throughout crop maturity and there was no seed development.

The pre-harvest GDH V_{max} values (Tables 1 and 2) demonstrated the importance of the unfertilized control crop in the proper interpretation of the GDH isoenzyme patterns. GDH responds to the ambient environmental

Table 1. GDH kinetic constants and biomass of soybeans treated with nutrients of different N, P, K, and S compositions.

Treatments	V_{max} [mmol g^{-1} min^{-1}]	K_m [mM]	Biomass [kg(d.m.) $plant^{-1}$]
Control Seedlings	310.0 ± 25.1	25.0 ± 1.3	-
100 mM Na_2HPO_4 (seedlings)	117.0 ± 9.8	66.7 ± 4.4	-
75 mM Na_2HPO_4 (seedlings)	126.1 ± 6.3	74.8 ± 6.1	-
50 mM Na_2HPO_4 (seedlings)	154.0 ± 11.2	83.3 ± 5.9	-
35 mM Na_2HPO_4 (seedlings)	204.0 ± 6.6	78.5 ± 5.4	-
25 mM Na_2HPO_4 (seedlings)	236.0 ± 12.3	122.0 ± 8.8	-
10 mM Na_2HPO_4 (seedlings)	280.0 ± 9.4	67.9 ± 7.3	-
50 mM NH_4Cl (seedlings)	400.0 ± 31.8	500.0 ± 25.0	-
25 mM NH_4Cl (seedlings)	182.0 ± 13.7	125.0 ± 8.4	-
5 mM NH_4Cl (seedlings)	400.4 ± 9.3	45.0 ± 4.8	-
Before nutrient application	145.6 ± 11.2	78.4 ± 6.9	-
Field reatment (control)	118.0 ± 8.1	25.0 ± 1.2	2.02
Field treatment (NPKS)	130.0 ± 8.6	20.8 ± 1.5	2.53
Field treatment (NP)	56.0 ± 3.0	25.0 ± 1.7	2.88
Field treatment (NKS)	22.0 ± 1.8	11.1 ± 0.5	2.49
Field treatment (NPK)	23.9 ± 2.1	12.5 ± 1.8	3.03

Table 2. GDH V_{max} [mmol g^{-1} min^{-1}] values at different DAE and seed biomass [g $plant^{-1}$] of maize as affected by NPK (nutrient treatments were not applied at 20 DAE).

Treatments	20 DAE	40 DAE	60 DAE	80 DAE	110 DAE	Seed mass
NPK	-	256.4 ± 10.3	161.3 ± 7.2	156.3 ± 3.3	151.5 ± 2.4	27.0 ± 1.4
Control	138.9 ± 4.4	128.2 ± 6.7	71.4 ± 3.7	0	0	no seeds

conditions of plants (Barash *et al.* 1975, Lauriere *et al.* 1981, Hartmann 1982, Yamaya *et al.* 1984, Srivastava and Singh 1987, Magalhaes 1991, Osuji 1997). The control unfertilized crops did not differentiate the effects of the environment from those of the applied nutrients because it is not possible to subtract the GDH isoenzyme patterns and the values of the GDH kinetic constants of the control crop from those of the fertilized crops. Rather, it was the GDH kinetics and isoenzyme pattern of the unfertilized control that delineated the effects of the applied nutrients from the effects of the environment. Therefore, the unfertilized plant was just like any treatment of the plants, the main difference being that no additional nutrients were applied.

GDH isomerization and biomass enhancement: Biomass increases in the soybean were expressed on the basis of shoot dry mass, but seed dry mass was used in the case of maize. The dry mass yields of soybean shoots per treatment were 2.02, 2.53, 2.88, 2.49, 3.05, 1.41, 1.89, and 1.80 kg for the control and treatments 1 to 7, respectively. Treatments 1 - 4 gave yields 25 - 50 % (average of 2.74 ± 0.27 kg per ridge) higher than the control. They were the soybeans that gave only the acidic and neutral GDH isoenzymes (Fig. 2). Nutrient treatments 1- 4 were also the tests for the adequacy of the conventional soybean fertilizer composition, and they also gave the highest yield. The biomasses produced from treatments 5 - 7 were lower (1.70 ± 0.26 kg per ridge) than those from treatments 1 - 4, and were about 30 % lower than that of the control. Treatments 5 - 7 also suppressed the soybean GDH isoenzymes. Therefore, GDH isomerization preceded biomass enhancement because the soybean biomass increased after the acidic and neutral GDH isoenzyme had been induced, but decreased after they were suppressed.

The control maize tasseled, but the ears failed to bear any seeds. Therefore, the residual nutrients available prior to nutrient application was insufficient for sustaining the reproductive phase of maize. On the other hand, each maize plant treated with NPK (13:13:13) nutrient developed one ear, with a yield of 27.0 ± 1.4 g(seeds) $plant^{-1}$. Therefore as in the soybean, the maize yield increased after the acidic and neutral GDH isoenzymes had been induced by nutrients. The failure of the control maize to develop seeds was probably due to the inability of the 3rd row neutral GDH isoenzyme pattern at 20 DAE to change to the 4th row acidic and neutral isoenzyme pattern at 40 DAE. The interphase between the vegetative and reproductive stages in maize is around 40 DAE. The acidic and neutral isoenzymes being the biomass-related isoenzymes were simultaneously induced at the beginning of that interphase. This again shows that biomass was enhanced in response to the induction of specific GDH isoenzyme population pattern.

The physiological function of GDH may therefore reside in the differential charge gradients of its isoenzymes. In the soybean, all the GDH isoenzyme patterns that corresponded to the increased biomass possessed the neutral and acidic isoenzymes, but lacked the basic isoenzymes (Fig. 2). Also the treatment of maize with fertilizer induced the neutral and acidic isoenzymes of GDH while suppressing the basic isoenzymes (Fig. 3), with concomitant production of seeds. Therefore, the biomass enhancement associated with GDH (Ameziane *et al.* 2000, Osuji and Madu 1997) could be a function performed by the cooperation of its neutral and acidic isoenzymes. The relationship between GDH isomerization V_{max} and phosphate concentration on one hand, and between biomass enhancement and the acidic and neutral isoenzymes on the other hand suggests that the enzyme could be a useful molecular marker for pre-harvest monitoring of the biomass response of a plant to the applied fertilizer.

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