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Glutamate dehydrogenase and glutamine synthetase activities in maize under water and salt stress*

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Abstract. NADH-GDH activity from roots of maize plants subjected to polyethyleneglycol (PEG) and salt stress decreased rapidly after 12 h of treatment. GS activity did not diminish so markedly. In the first leaves, GS activity was surprisingly high when plants were subjected to both types of stress for 6 h. There were clear differences in the isoenzymatic patterns of GDH in extracts from stressed and control plants. The response of GDH activity to a secondary *in vitro* stress, induced by adding PEG to the extracts, was different in plants under water or salt stress.

Key words: *Zea mays*, glutamate dehydrogenase, glutamine synthetase, stress.

Abreviations: GDH, glutamate dehydrogenase; GS, glutamine synthetase; PEG, polyethyleneglycol.

During stress, plants tend to accumulate certain nitrogenous metabolites such as proline or glycinebetaine, presumably as a mechanism for preserving their metabolic functions (20). Nitrate assimilation is limited under adverse conditions, due to a decrease in nitrate reductase activity (e.g. 18), so a substantial portion of the nitrogen for these compounds presumably originates from protein turnover. It is in this context that the enzymes involved in the incorporation of ammonia to organic compounds which can act as precursors for different metabolites may have an important role in plant survival during stress.

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Given the fact that the environment presents deleterious characteristics, all proteins, and particularly key metabolic enzymes, must have or acquire certain characteristics to make them more suited to stressful conditions. Previous results (11) suggest that in the roots of maize plants under stress treatments ammonia assimilation could be accomplished by GDH since it is resistant to osmotic stress *in vitro*.

The aim of this report was to determine the response of two of the enzymes involved in ammonia incorporation to organic compounds, glutamate dehydrogenase (GDH; EC 1.4. 1.2) and glutamine synthetase (GS; EC 6.3.1.2), in maize plants under water or salt stress. We have also observed the effect of a secondary *in vitro* stress on GDH activity in extracts from control and treated plants.

MATERIALS & METHODS

Plant material. Maize seeds (*Zea mays* L., var. Chalqueño criollo) were sown in moist agrolite. After germination, the plantlets were watered daily for 15 days. They were kept in a growth chamber with a 12 h photoperiod and day/night temperature of 30/25°C. Water and salt stress treatments were imposed to 15-day old plants by transferring them to aqueous solutions of polyethyleneglycol (PEG-6000, J. T. Baker) or sodium chloride for 6 or 12 h during the light period. The concentration of PEG was 41.2% (equivalent to a water potential value of -2 MPa, 19) and that of NaCl was 150 mM (equivalent to a water potential value of -0.7 MPa). After the stress period was over, the plants were harvested; leaves and roots were cut and frozen immediately in liquid nitrogen.

Enzyme extracts. Frozen tissues from the control and stressed plants were ground to fine powder with mortar and pestle. The powder was then homogenized in a Polytron for 2 min with 2.5 volumes (w/v) of 50 mM Tris-HCI, pH 8.2, 1mM $CaCl_2$, 5 mM mercaptoethanol and 5% polyvinylpyrrolidone. The homogenate was filtered through cheesecloth and centrifuged at 14.000 x g for 30 min. All manipulations were performed at 4°C.

These extracts were used for the enzyme assays and for the secondary *in vitro* stress experiments. This stress treatment was carried out in a volume of 1 ml. PEG or PEG and proline were dissolved in 0.4 ml 50 mM Tris-HCI pH 6.5 prior to the addition of the crude extract. After adding the extracts, the tubes were vortexed and allowed to stand in ice for at least 45 min. The tubes were centrifuged at 3.000 x g for 15 min and the supernatants were used for enzyme activity determinations.

Enzyme assays. NAD-, NADH-GDH and GS assays were performed as described in Loyola-Vargas & Sánchez de Jiménez (9,10). The enzymatic activities were determined spectrophotometrically using the extracts from non-stressed plants and from those under

stress treatments for 6 and 12 h, and also in the tissue extracts subjected to the second *in vitro* stress. Aminative and deaminative GDH activities were defined as nanomoles of cofactor reduced or oxidized per minute per mg protein, respectively. GS activity was expressed as µmoles of glutamyl hydroxamate formed per min per mg protein.

Protein content was determined according to Peterson (14) using bovine serum albumin as standard.

Isoenzymatic determinations. Separation of isoenzymes was performed using a discontinuous electrophoretic system in polyacrylamide gels (2) and GDH activity was developed using the procedure described in Miranda-Ham & Loyola-Vargas (12).

RESULTS

NADH-GDH activity from roots of plants subjected to PEG of NaCl for 6 h did not differ significantly from that found in the control. However, after 12 h both PEG and NaCl-treated plants exhibited markedly reduced enzyme activity (Fig. 1A). In contrast, GS activity was not altered significantly and after 12 h, 70 and 105% of the initial activity was detected in PEG and NaCl treated plants, respectively (Fig. 1B).

The GDH and GS activities detected in the first leaves are shown in Fig. 2. NADH-GDH activity in stressed plants diminished with time, but to a lesser extent than in the control plants (Fig. 2A). There was a three-fold increase in GS activity in plants subjected to both types of stress for 6 h (Fig. 2B). Interestingly, after an additional 6 h of treatment, GS activities from both NaCl and PEG-stressed plants were again the same as those from control plants.

Since it has been suggested that NADH- and NAD-GDH activities are located in the same protein and that the isoenzymatic patterns can be modified under certain conditions, e.g. growth on different nitrogen sources (6), the changes that were found in the

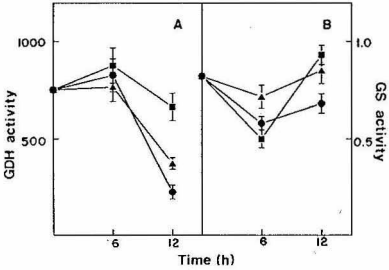

Fig. 1.— NADH-GDH (A) and GS (B) activities in roots from control (■), PEG- (●) and NaCl- (▲) treated maize plants. Each value is the mean ± SE of three independent experiments.

enzymatic activities might reflect changes in the isoenzymatic patterns for GDH in stressed and non-stressed plants. In order to study the different isoenzymatic patterns, a discontinuous electrophoretic system was employed, followed by *in situ* development of GDH activity.

The patterns obtained in zymograms of root extracts from plants under water and salt stress for 6 h were qualitatively similar to the control; however, there were marked differences in the activity ratios among peaks I, II and III in the control and in water and salt stressed plants (Fig. 3). The decrease in the activity in control plants after 12 h can be due to decreases in the amount of extant GDH

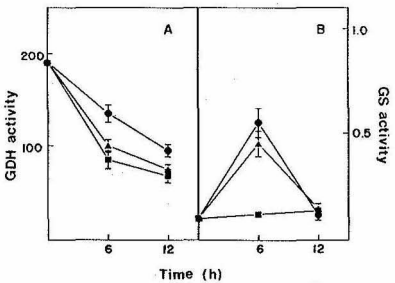

Fig. 2.— NADH-GDH (A) and GS (B) activities in the first leaves from control (■), PEG- (●) and NaCl- (▲) treated maize plants. Each value is the mean ± SE of three independent experiments.

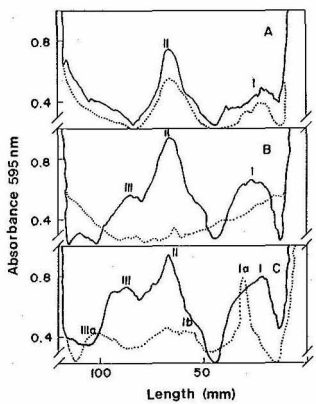

Fig. 3.— Scannings of zymograms for GDH activity from extracts of roots from control (A), PEG- (B) and NaCl- (C) treated plants. 6 h stress treatment, continuous line; 12 h treatment, dotted line.

isozymes; whereas in the case of the plants under the two stress treatments, there were decreases in the extant isozymes as well as the appearance of new GDH isoforms.

In the scannings of the zymograms from first leaves extracts (Fig. 4), only peak II could be consistently detected in the control and in the stressed plants. When plants were subjected to a 6 h stress treatment, both peaks I and IV disappeared. In leaf extracts of PEG-stressed plants, a new peak of slower mobility (peak V) could be observed. When the period of treatment was prolonged for another 6 h, new peaks (Ia, III and IIIa) appeared in those plants under PEG-induced stress and only peak IV could be observed in the salt stressed plants (Fig. 4B and 4C).

In order to further explore the possibility that the changes in the peak ratios and the appearance of new bands of altered mobilities could be the result of differences in these enzymes at the molecular level, PEG was added to aliquots of the crude extracts of tissues from water and salt stressed plants to induce a secondary *in vitro* stress. On the other hand, since proline has shown to exert a "protective effect" to maintain the activity of different enzymes (11,13), this compound was added along with PEG to different aliquots of the crude extracts to observe if it has the same effect in this system. The concentration of proline employed for these experiments was 1 M, which is the maximal concentration of this metabolite found in plants under stress (7).

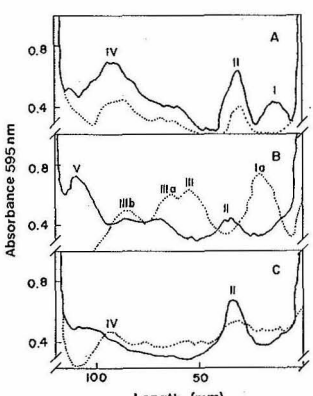

Fig. 4.— Scannings of zymograms for GDH activity from extracts of the first leaves from control plants (A), PEG- (B) and NaCl- (C) treated plants. 6 h treatment, continuous line; 12 h treatment, dotted line.

Figures 5 and 6 are only two examples of these sets of experiments in which the activity of GDH was assayed after the PEG-induced *in vitro* stress treatment. GDH activity of a root extract from a control plant exhibited a marked decrease when 30% PEG was added; whereas, in those from stressed plants, the decrease was 20% in the case of the salt treated and 35% in the water stressed ones (Fig. 5). The addition of 30% PEG to first leaves extracts showed a very different response in GDH activity; no deleterious effect could be observed in the case of the control plants whereas the ones that received water or salt treatments showed moderate decreases (Fig. 6).

When proline was added along with PEG, the response differed between the two tissues as well. In the root extracts, the level of GDH activity that could be detected after the secondary stress treatment was almost doubled in the control and in the water stress treatment

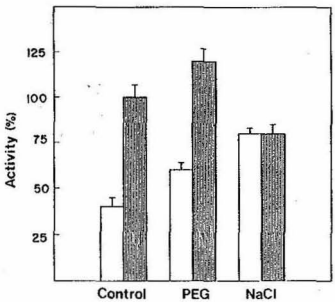

Fig. 5.— NADH-GDH activity in root extracts from control, PEG- and NaCl- treated plants for 12 h after they were subjected to a secondary *in vitro* stress treatment with 30% PEG (□) or 30% PEG + 1 M proline (|||). The tubes were incubated at 4°C for 45 min and centrifuged to separate the precipitated protein. GDH activity was assayed in the supernatant. 100% activity refers to that of the crude extract. Each value is the mean ± SE of three independent experiments.

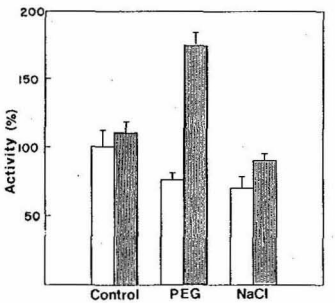

Fig. 6.— NADH-GDH activity in first leaf extracts from control, PEG- and NaCl-treated plants for 12 h after they were subjected to a secondary *in vitro* stress treatment with 30% PEG (□) or 30% PEG + 1 M proline (|||). The tubes were incubated at4°C for 45 min and centrifuged to separate the precipitated protein. GDH activity was assayed in the supernatant. 100% activity refers to that of the crude extract. Each value is the mean ± SE of three independent experiments.

by the addition of proline. In contrast, proline had no effect in the salt-stressed plants (Fig. 5). For the leaf extracts, proline increased GDH activity significantly only in the PEG treated plants.

DISCUSSION

The present study has shown that GDH activity in roots from control and stressed plants is maintained at similar levels during the first 6 h of treatment (Fig. 1A). After 12 h of treatment, GDH activity is relatively low in water and salt stressed plants, while GS activity is detected at similar levels as in the control (Fig. 1B).

In the first leaves, there is a continuous loss of GDH activity during the stress treatment and in the controls. However, a significant increase of leaf GS activity could be observed in the first 6 h in stressed samples which was not observed in the controls. This increase concurs with the enhancement of GS activity observed in *Suaeda* leaves (1) and in salt resistant bean and maize plants (15).

The activities of GDH and GS differ significantly depending on both the length of the stress treatment and on the tissue. Note that the important characteristic of the response is the length and not the type of the stress treatment in contrast with the findings in *Canavalia ensiformis* (12).

The data on GDH and GS activities assayed in extracts from plants subjected to water and salt stress contrast markedly with our previous studies dealing with *in vitro* stress treatments (11). When stress was administered under *in vitro* conditions, root GDH seemed to be more resistant than root GS; whereas when whole plants were used, it was just the opposite case. These data caution against uncritical extrapolation of data obtained in *in vitro* studies to the whole plant level and may be a reminder that other considerations, such as subcellular localization, availability and concentration of substrates and effectors also influence the actual *in vivo* activity.

The levels of GDH activity found in root and leaf extracts from control and stressed plants correlated well with those found in the zymograms. In roots, although the GDH activity levels were similar in the three extracts from plants treated for 6 h, there were clear differences in the ratios of peak I, II and III. In contrast, only one common peak (peak II) was found in the leaf extracts from control and stressed plants at 6 h. The appearance and disappearance of new bands of higher and lower mobilities in the zymograms could be due to a number of factors: *de novo* synthesis of new isoenzymes, conformational rearrangement, or modification of pre-existing enzymes, such as phosphorylation or binding of polyamines (3), that lead to alterations in their catalytic and regulatory properties allowing them to function under these conditions. The latter two alternatives

can be substantiated in terms of a favorable thermodynamic interaction of water molecules with proteins that allows the maintenance of a certain degree of hydration and thus conserve their functionality (4, 8, 11, 13). This aspect is currently being further examined.

Significant differences in GDH activity in extracts from control and stressed plants could be observed when these extracts were subjected to a second *in vitro* stress. The response depended on the tissue which originated the extract and on the type of stress treatment given to the plant. When proline was added to the three types of extracts (Figs. 5 and 6), the response to this metabolite was also differential. In root and leaf extracts from water stressed plants, the inclusion of proline resulted in twice as much GDH activity compared to those extracts with PEG alone, whereas in extracts of salt-stressed roots or leaves, no such response was observed. It is clear that the response to stress with regard to GDH is dependent on both the tissue and the type of stress.

Recent studies suggest that GDH plays a minor role in ammonia assimilation (16). Several lines of evidence suggest that the main function of GDH is that of glutamate catabolism (17). If this is so, then the stress-induced decreases in GDH activities in maize roots may serve to curtail glutamate turnover, making it available for continued operation of the glutamate synthetase cycle as well as for stress-related proline synthesis (5). The validity of this interpretation requires further investigation.

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