Glutamic Acid Decarboxylase Activity as a Measure of Percent Germination for Barley[1]

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ABSTRACT

A simple, rapid procedure was developed for determining barley glutamic acid decarboxylase activity. Activity was measured by nondispersive infrared analysis of the rate of carbon dioxide evolution from a buffered glutamic acid solution in which a ground barley sample was suspended. About 15 min was required for an individual measurement. Activity of the enzyme proved to be a useful index of viability, and the procedure might be employed to supplement the currently used germination tests. All the samples together, including both two-rowed and six-rowed barleys, showed a highly significant correlation between activity and percent germination ($r = 0.916^{***}$). In samples representing a single cultivar, the correlation was even better, with a correlation coefficient of 0.982^{***} obtained for the cultivar Larker. Correlation coefficients obtained in relating the logarithm of the enzyme activity to percent germination were about the same as those found using the activity alone. The procedure was not applicable to samples containing a significant number of sprouted kernels.

The enzyme glutamic acid decarboxylase (EC 4.1.1.15), also called glutamate decarboxylase, that catalyzes the decarboxylation of L-glutamic acid to yield carbon dioxide and γ-aminobutyric acid, is widely distributed in plants (Schales and Schales 1957). The enzyme from *Escherichia coli* has been isolated (Shukuya and Schwert 1960a, Strausbauch et al 1967), and has been more thoroughly studied than glutamic acid decarboxylase from any other source (Shukuya and Schwert 1960b, 1960c). The *E. coli* enzyme, which has a molecular weight of 310,000, is a hexamer composed of identical subunits of about 50,000 molecular weight (Strausbauch and Fischer 1970a). Each subunit has one molecule of the coenzyme pyridoxal 5'-phosphate attached to a lysine residue, and the sequence of nine amino acids at the binding site has been determined (Strausbauch and Fischer 1970b).

The presence of glutamic acid decarboxylase in cereal grains is easily detected, with barley showing activities higher than those exhibited by wheat, rye, and oats (Rohrlich and Siebert 1964). The enzyme appears to be important in germination (Inatomi and Slaughter 1971, Rohrlich 1957), and is one of the first to become activated when a dried wheat kernel is rehydrated (Linko 1960). Its importance is perhaps indicated by the existence of mutant spores of *Bacillus megaterium* with a requirement for γ-aminobutyric acid for germination and no detectable glutamic acid decarboxylase activity (Foerster and Foerster 1973). In barley, as in other cereal grains (Galleschi et al 1975, 1977; Linko and Milner 1959), the enzyme is present primarily in the embryo and not in the endosperm (Rohrlich 1964), although a small amount is also detectable in the aleurone layer (Duffus et al 1972). The barley enzyme has been partially purified and characterized (Beevers 1951).

Glutamic acid decarboxylase activity has been used as a quality index to measure the storage condition of wheat (Cheng 1959, Linko 1961, Linko and Sogn 1960). A highly significant correlation was found between activity of the enzyme and germination percentage. Similar correlations between activity and viability, as measured by percent germination, have been found for corn, oats, rice, and beans. Bautista and Linko (1962) used glutamic acid decarboxylase activity to measure damage in artificially dried and stored corn, and Bautista et al (1964) employed it as a viability index for artificially dried and stored rice. Grabe (1964) studied activity of the enzyme as a measure of seed deterioration and seedling vigor of corn and oats, and correlated activity with percent germination. With beans (*Phaseolus vulgaris* L.), James (1968) found a highly significant correlation between germination and activity for the cultivar Taylor; the correlation for Tendercrop was not significant. In soybeans, on the other hand, Abdul-Baki and Anderson (1973) did not find glutamic acid decarboxylase activity to be a suitable index for measuring seed vigor.

Because a high correlation has been found between activity of the enzyme and viability for all of the cereal grains investigated, the present detailed study was undertaken to examine the relationship for barley. In barley grown for malting, percent germination is the most important single characteristic for measuring quality, and any rapid procedure that might be used to supplement the currently employed germination tests, which require several days (ASBC 1976, USDA 1975), would be useful. In the present study, activity of the enzyme was determined by nondispersive infrared analysis of the rate of carbon dioxide production from a buffered glutamate solution in which a ground barley sample was suspended. This technique was employed to take advantage of the high sensitivity afforded and to avoid ambiguities due to factors such as respiration and swelling, which might arise in interpreting the results of a manometric procedure (Cheng et al 1960).

MATERIALS AND METHODS

Barley Samples

An attempt was made to obtain as many barley samples as possible with less than 90% germination. Samples were obtained from D. W. Fulk (Federal Grain Inspection Service, Grandview, MO), W. J. Olson (Fleishmann Malting Co., Minneapolis, MN), N. Prentice (Barley and Malt Laboratory, Madison, WI), R. Ruud (North Dakota State Seed Department, Fargo, ND), L. E. Weisner (Montana State University, Bozeman, MT), and D. M. Wesenberg (USDA, Aberdeen, ID).

All samples were from the 1973-1980 crops. Eighty-eight samples were examined, including 28 cultivars and representing 75 six-rowed and 13 two-rowed barleys. Twenty-seven of the samples were Larker, a commonly grown six-rowed malting barley. One additional Larker sample, which was found to have sprouted, was also examined, but was not included in the statistical analysis. Upon receipt, samples were stored in sealed jars at 4°C until needed. Dockage was removed before analysis by shaking 30 times in a Tyler no. 8 screen, and broken kernels were removed manually.

Apparatus and Reagents

Reagent grade monosodium and disodium phosphates used to

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prepare buffers were obtained from Sigma Chemical Co., and L-glutamic acid was a Sigma grade chemical from the same source. Benzoxazolone from ICN Pharmaceuticals was used as a preservative in buffers stored for several days before use.

Rate of carbon dioxide production was measured with a Beckman Instruments model 865 nondispersive infrared analyzer equipped with a 0–10 mV Linear Instruments Corp. model 282 recorder. All measurements were made in a 1 × ½ in. i.d. flow-through cell, which provided a sensitivity of 0–25,000 ppm (v/v) with a repeatability of ±50 ppm on the most sensitive setting. Although it was not required in the sensitivity range employed, the optical system contained a filter to eliminate interference from water vapor. Gas used for calibration of the instrument was certified standard carbon dioxide in nitrogen obtained from Matheson. Prepurified grade nitrogen was employed as a purge gas. Gas in the system was circulated with a Lab-Line model 5312 peristaltic pump with the flow controlled by means of two four-way valves (no. B-43YF2, Whitey Co., Cleveland, OH). All samples to be analyzed were ground in a Udy Cyclone Sample Mill (Udy Corp., Boulder, CO) having a screen with holes 1.0 mm in diameter.

Glutamic Acid Decarboxylase Activity

Glutamic acid decarboxylase activity was measured with the apparatus shown in Fig. 1. A 5.0000-g ground barley sample was placed in the 250-ml Erlenmeyer flask, and the system was flushed with nitrogen with the four-way valves in the positions shown. The position of each valve was then changed, and with the tube between the flask and the peristaltic pump clamped shut, 35.00 ml of substrate solution was added from the buret. The position of valve B (Fig. 1) was then changed to form a closed loop, and, with the clamp removed, gas in the system was circulated at approximately 100 ml/min with the peristaltic pump. Throughout the reaction, the slurry resulting from the addition of the substrate solution was stirred vigorously by means of the magnetic stirrer. Temperature of the water bath was maintained at 30.0° C, and the precaution was taken of equilibrating the substrate at 30.0° C before pouring it into the buret. Substrate solution was composed of 0.100 *M* L-glutamic acid in 0.500 *M* sodium phosphate buffer, pH 5.20. To prevent the growth of fungi, 0.10% (w/v) benzoxazolone was added as a preservative. This allowed the solution to be kept for at least two weeks without deterioration.

Rate of carbon dioxide production, as determined by the nondispersive infrared analyzer, served as a measure of glutamic acid decarboxylase activity. After an initial lag period of a few minutes, required for initiation of the decarboxylation reaction, the rate of carbon dioxide production became linear and then gradually decreased with time. The linear portion of the carbon dioxide vs time curve was used to determine activity of the enzyme, which was calculated as ppm carbon dioxide per minute per 5.0000 g of dry barley, with a correction made in each case for the moisture content of the barley. Triplicate analyses were made on all samples.

Percent Germination

Germination tests were performed by the Kansas State Seed Laboratory, Topeka, according to procedures outlined by the U.S Department of Agriculture (1975). As a precaution, each sample was subjected to the prechill procedure described in the above publication for breaking dormancy before germination. Percent germination was determined after four days of germination between blotters at 20° C, and again after seven days. Except where specified otherwise, percent germination means percent germination after seven days.

Laboratory Germination

To study the effect of germination on glutamic acid decarboxylase activity, barley was germinated in the laboratory in a Cleland model 500 germinator from Burrows Equipment Co., Evanston, IL. Samples of approximately 35 g were germinated between blotters for one to seven days in the dark at 20° C and 100% relative humidity. Blotters were moistened each day with deionized

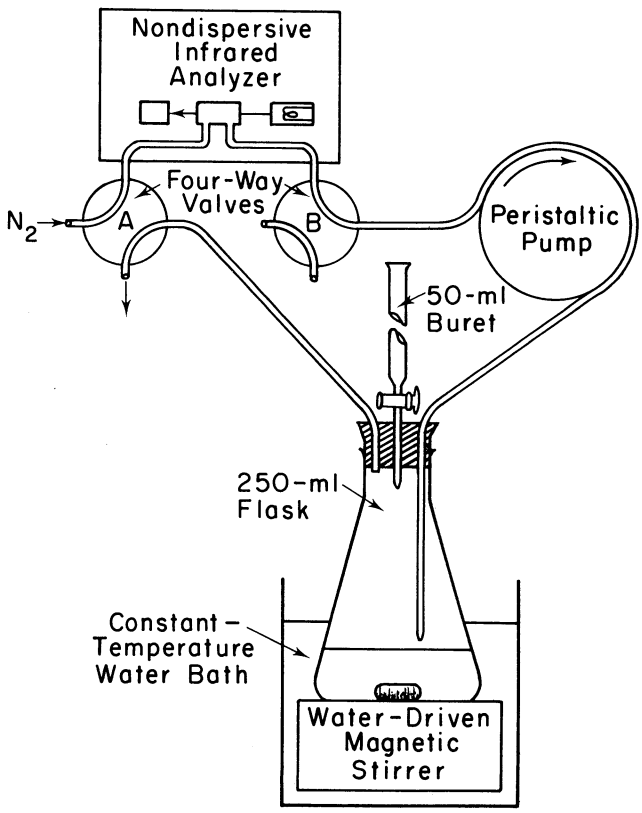

Fig. 1. Apparatus used for measuring barley glutamic acid decarboxylase activity.

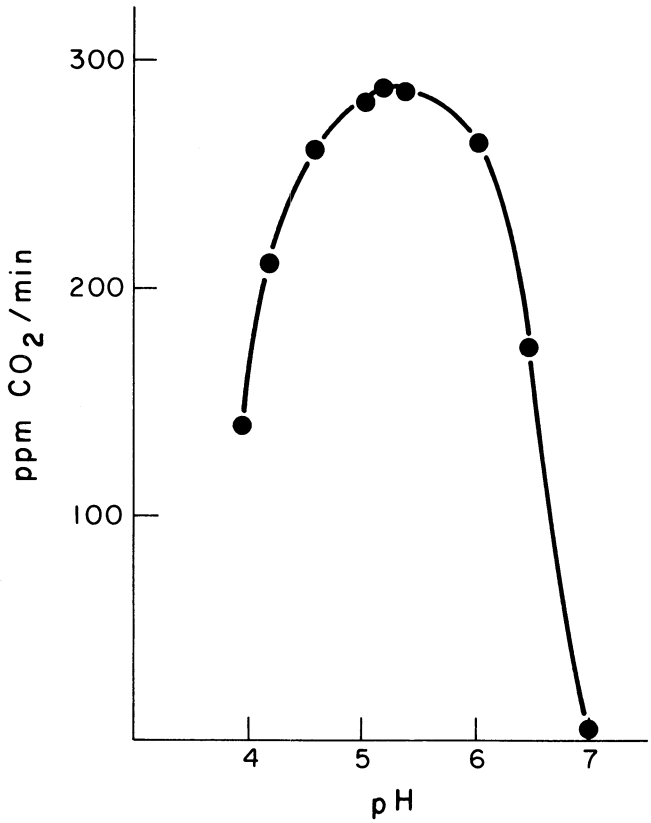

Fig. 2. Glutamic acid decarboxylase activity of barley as a function of pH. Each point represents the activity, expressed as ppm CO_2/min, of 5.0000 g of Klages barley in 0.500 *M* sodium phosphate containing 0.100 *M* L-glutamate.

water to ensure an adequate supply of moisture for germination. Upon removal from the germinator, samples were frozen immediately and dried by lyophilization.

RESULTS AND DISCUSSION

Reaction Conditions

Substrate solution employed for measuring barley glutamic acid decarboxylase activity was 0.100*M* L-glutamic acid in 0.500*M* sodium phosphate. When activity of the barley enzyme in this buffer was examined as a function of pH, the pH optimum was found to be 5.2 (Fig. 2). At this pH, the glutamic acid makes a major contribution to the buffering capacity of the solution, and the possibility was considered that depletion of the acid by the enzymatic reaction might cause a change in pH. The pH of the solution, however, was found to be quite stable, and changed only a few hundredths of a unit during the approximately 15 min required for making an activity measurement.

Addition of substrate solution to a ground barley sample produces a paste, and accessibility of the enzyme to the substrate appears to be restricted when an insufficient amount of substrate solution is added. An experiment was therefore conducted to determine the optimal ratio of barley to substrate solution. With a 5.0000-g barley sample, maximum activity was obtained when 35 ml of substrate solution was added (Fig. 3). Subsequent experiments showed that 5 g of barley gave the correct response for the range of activities of the samples included in the study. Unlike the wheat enzyme (Linko 1961), the activity of barley glutamic acid decarboxylase increases to a maximum and then decreases as the concentration of L-glutamate in the substrate solution is increased (Fig. 4). The lowered activity at the higher concentrations of glutamate indicates an inhibition of the enzyme by excess substrate. Despite the altered kinetics, however, carbon dioxide production by the barley enzyme was found to be an approximately linear function of the amount of barley present over the range of 0–5 g (Fig. 5).

Glutamic acid decarboxylase from both *E. coli* (Shukuya and Schwert 1960c) and wheat (Kott 1973) was found to be inactivated at temperatures below 25° C, and the enzyme from wheat was shown to have maximum activity at 30° C (Cheng et al 1960). In barley, the enzyme is rapidly inactivated at temperatures above 50° C, and to a lesser degree at temperatures above 30° C (Beevers 1951). A temperature of 30° C was therefore selected as suitable for measuring activity of the barley enzyme. In addition to being nearly optimal for stability of the enzyme, this is only slightly above room temperature and can easily be maintained with high accuracy by means of a water bath.

Sensitivity and Precision

With the 5-g samples employed, sensitivity of the nondispersive infrared analyzer proved to be well in excess of that required for measurement of barley glutamic acid decarboxylase activity. Accuracy of the instrument was determined primarily by the accuracy with which the calibration gas was prepared. Because the same calibration gas was used throughout the investigation, this was of lesser importance than precision. For all 88 samples

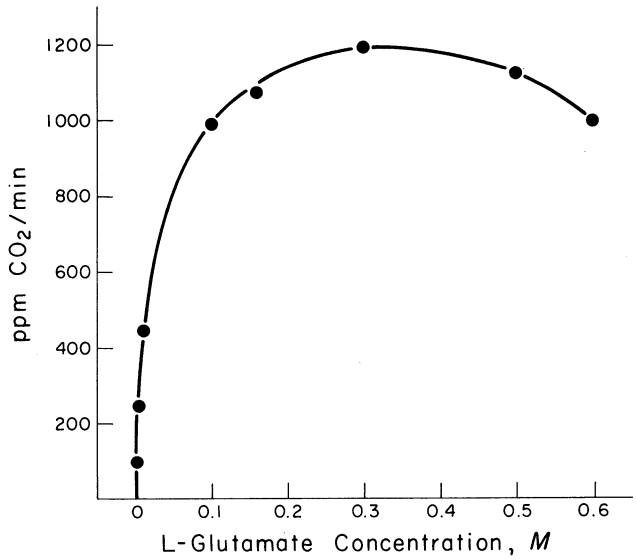

Fig. 4. Glutamic acid decarboxylase activity of barley as a function of L-glutamate concentration. Each point represents the activity, expressed as ppm CO_2/min, of 5.0000 g of Klages barley in 0.500*M* sodium phosphate containing the indicated concentration of L-glutamate, pH 5.20.

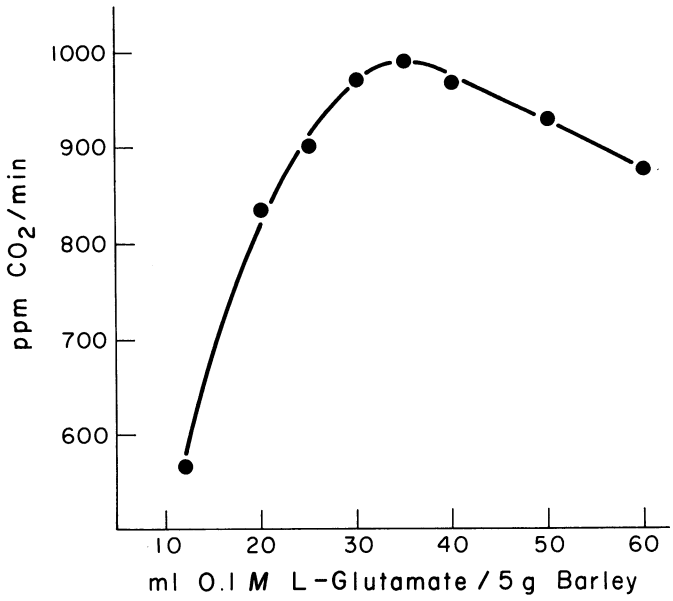

Fig. 3. Glutamic acid decarboxylase activity of barley as a function of substrate volume. Each point represents the activity, expressed as ppm CO_2/min, of 5.0000 g of Klages barley in the indicated volume of 0.500*M* sodium phosphate containing 0.100*M* L-glutamate, pH 5.20.

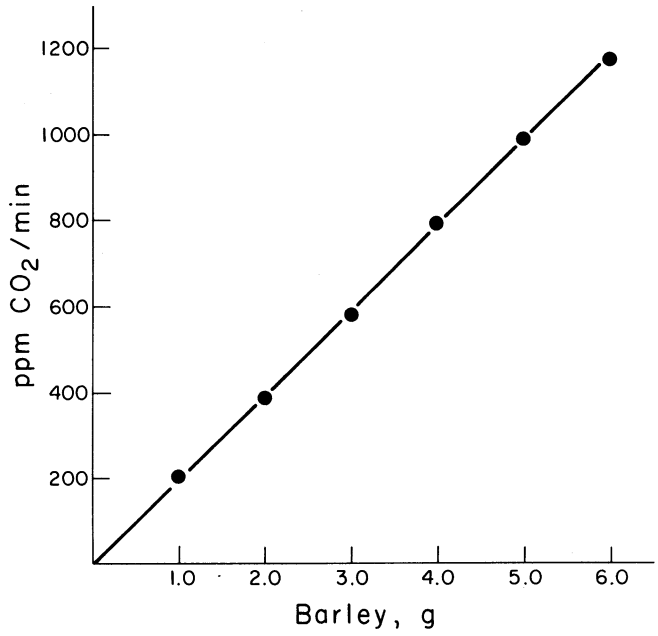

Fig. 5. Carbon dioxide production by barley glutamic acid decarboxylase as a function of the amount of barley present. Measurements were made with the indicated weights of Klages barley in 0.500*M* sodium phosphate containing 0.100*M* L-glutamate, pH 5.20.

included in the study, average standard deviation for an individual measurement was ±18.2 ppm CO_2/min or, expressed as coefficient of variation, 2.57%. For the samples of greatest interest—those having 90% germination or above—the standard deviation became ±19.8 ppm CO_2/min, and the coefficient of variation 1.93%.

Activity as a Function of Viability

Figure 6 shows glutamic acid decarboxylase activity plotted as a function of percent germination for all 88 barley samples included in the study. The dashed lines indicate a deviation of one standard error of estimate (115.9 ppm CO_2/min per 5 g) from the solid regression line (y = 10.49x + 43.87), calculated by the method of least squares.

Correlation between glutamic acid decarboxylase activity and percent germination was highly significant (r = 0.916***). In calculating the regression line, four samples that had activities more than three times the standard error of estimate above or below the regression line were not included. Even with these samples, however, the correlation coefficient dropped only to 0.860***, which still indicates a highly significant correlation. It is perhaps interesting that all four had low germination (27–81%) and were from the 1980 North Dakota crop, which showed a high incidence of frost damage. The presence of one sample with low activity (<200 ppm CO_2/min per 5 g) and relatively high germination (72%) indicates that any germination requirement for glutamic acid decarboxylase is satisfied by a relatively low level of activity.

Logarithm of Glutamic Acid Decarboxylase Activity

If denaturation of barley glutamic acid decarboxylase is a unimolecular process that obeys first-order kinetics, then the logarithm of the concentration of active enzyme would decrease by a constant times the time, minus an integration constant. Since viability decreases linearly with time, one might expect a better correlation of percent germination with the logarithm of the activity than with the activity. In Fig. 7, the logarithm of the activity is plotted as a function of percent germination for all 88 samples, with the calculated regression line (log y = 0.00722x + 2.34) shown by the solid line and the dashed lines defining an area within one standard error of estimate (0.0617) of the regression line. The correlation coefficient calculated using only those points within three times the standard error of estimate of the regression line was 0.922***. This shows a highly significant correlation and is about the same as the correlation coefficient found when the activity was not plotted on a logarithmic scale. When all the samples were included in the calculation, the correlation coefficient became 0.859***, which again indicates a highly significant correlation and is almost identical to the value obtained with the unaltered activity. Clearly, at least for the samples included in the study, the anticipated improvement in the correlation with percent germination was not realized when the enzyme activity was plotted logarithmically.

It is perhaps significant that with the logarithm of the activity, nine samples gave points that deviated from the regression line by more than three times the standard error of estimate, as opposed to only four with the activity alone. The nine samples that did not fit were again of low germination (1–81%), and, with the exception of the sample having 1% germination, were from the frost-damaged 1980 North Dakota crop. Seven of the nine samples had activities below the regression line, suggesting that the conditions to which they were subjected, probably including low temperatures, had caused activity of the enzyme to be lost more rapidly than viability. The one exception was the sample with 1% germination, which had low activity, probably because it was composed almost entirely of "dead" kernels that had continued to lose activity after they would no longer germinate.

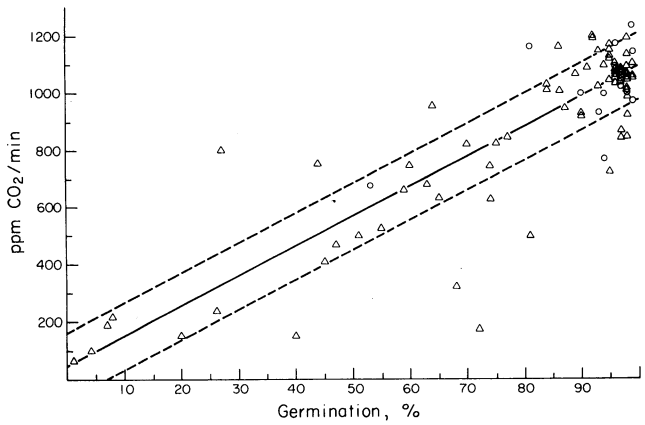

Fig. 6. Glutamic acid decarboxylase activities, expressed as ppm CO_2/min, of 5.0000-g samples of two-rowed (o) and six-rowed (Δ) barleys as a function of percent germination.

Six-Rowed Barleys

Point distribution was not sufficiently good to allow the two-rowed barleys to be examined separately, but for the six-rowed barleys the correlation between glutamic acid decarboxylase activity and percent germination was about the same as for the combined six-rowed and two-rowed barleys. The regression line was changed little; the coefficient of correlation was slightly higher (0.929*** vs 0.916***); and the standard error of estimate (113.2 ppm CO_2/min per 5 g) was close to the value obtained for the combined sampling. Distribution of activities for the two-rowed barleys around the regression line for the six-rowed barleys was very even, with seven points above the line and six below it.

Larker

One would expect genetic differences among barley cultivars to

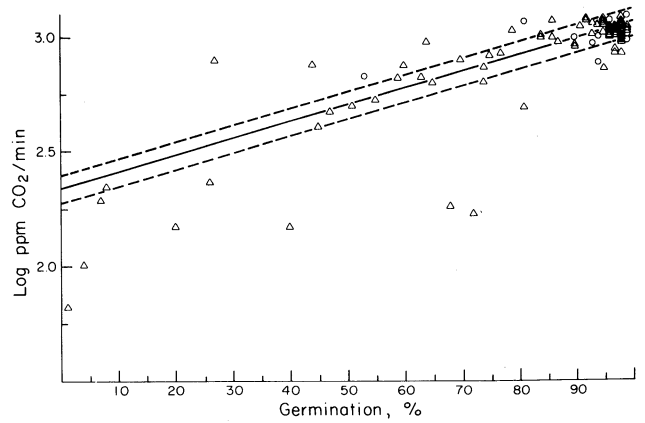

Fig. 7. Glutamic acid decarboxylase activities, expressed as ppm CO_2/min, of 5.0000-g samples of two-rowed (o) and six-rowed (Δ) barleys plotted logarithmically as a function of percent germination.

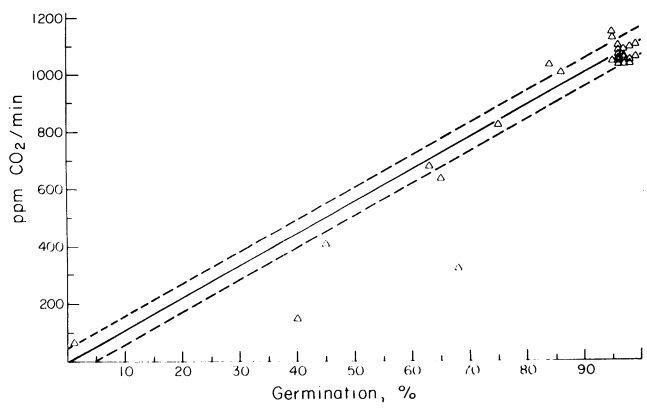

Fig. 8. Glutamic acid decarboxylase activities, expressed as ppm CO_2/min, of 5.0000-g samples of Larker barley as a function of percent germination.

be reflected in different glutamic acid decarboxylase activities. For example, the three Klages samples examined all had activities more than one standard error of estimate above the regression line, indicating high activity for this cultivar. There was, however, an insufficient number of samples of any one cultivar with high or low activity to allow any firm conclusions to be drawn about differences between cultivars. The 27 samples of Larker were an exception, but, in this case, the activities were neither unusually high nor unusually low compared to the other samples in the study.

If the apparent differences in activities between cultivars are real, greater consistency should be found in a group of samples composed of a single cultivar compared to a random sampling including many cultivars. To test this hypothesis, glutamic acid decarboxylase activities of the 27 Larker samples were plotted against percent germination (Fig. 8). As expected, the correlation between activity and percent germination was significantly better than that obtained for all the samples together. Coefficient of correlation was 0.982***, which indicated an excellent correlation, and the standard error of estimate of 49.8 ppm CO_2/ min per 5 g was less than half that for the combined sampling. When two samples that gave points more than three times the standard error of estimate below the regression line were included in the calculations, the coefficient of correlation became 0.943***, which was still considerably better than the value calculated for all the samples together. Significantly, perhaps, the two samples that did not fit gave points well below the regression line (> six times the standard error of estimate). Both samples were again low-germination (40 and 68%) barleys from the frost-damaged 1980 North Dakota crop.

When the glutamic acid decarboxylase activities were plotted on a logarithmic scale for the Larker samples (Fig. 9), the correlation between activity and percent germination was still highly significant. The correlation coefficient decreased slightly, to 0.963***, but, on the other hand, the points were more closely clustered around the regression line than when the activity was not plotted logarithmically. Even when three samples were included that gave points more than three times (> six times in this case) the standard error of estimate below the regression line, the coefficient of correlation dropped only to 0.950***.

What one would like to do, of course, is to use the glutamic acid decarboxylase activity to predict percent germination. When activity of the enzyme was considered to be the independent variable and percent germination the dependent variable, the standard error of estimate for the Larker barleys was calculated to be 4.37% germination, which decreased to 3.87% when the activity was replaced by its logarithm. If the regression line was used to calculate percent germination for the Larker samples having 90% germination or above, which are the ones of primary interest, the average deviation from the percentage found by the germination test was ±2.4% germination. When the activity was replaced by its logarithm, the average deviation was ±2.0%. Replicate germination tests on the same group of barleys showed that the average deviation for an individual test was ±0.6% germination from the mean for that sample.

Four-Day Germination

In examining the relationship between glutamic acid decarboxylase activity and viability, percent germination after seven days was used as a measure of viability. If the seven-day germination values were replaced by percent germination after four days, the correlation between activity and percent germination was virtually unchanged. For example, the coefficient of correlation for all the samples together was 0.905*** when the germination after four days was used, compared to 0.916*** with the seven-day germination values. For the Larker samples, the coefficient of correlation changed only from 0.982*** for the germination after seven days to 0.970***, and, with the logarithm of the activity, the correlation coefficient was 0.966*** vs 0.963*** for the seven-day germination values. Similar results were obtained for the six-rowed barley samples.

Germ-Damaged Samples

Some of the samples examined contained kernels that showed evidence of germ damage, indicated by a brown coloration at the germ ends. One such sample having 47% germination was subdivided into two samples, composed, respectively, of kernels with and without visible evidence of germ damage. As anticipated, the germ-damaged sample had a glutamic acid decarboxylase activity 14.4% lower than the one composed of apparently healthy kernels. As activity of the enzyme is found primarily in the embryo, this result was not unexpected. A separate experiment in our own laboratory, in which the kernels of a sample were divided into germ and endosperm ends by slicing them with a razor blade, showed approximately 80% of the activity to be in the germ end.

Sprouted Samples

One Larker barley from the 1974 crop was composed entirely of "dead" kernels. Although the sample had 0% germination, its glutamic acid decarboxylase activity was 716 ppm CO_2/ min per 5 g, which would be nearly correct for a sample having 65% germination. Upon examination under a microscope, the sample was found to have sprouted. When barley was allowed to germinate for seven days under laboratory conditions as described, activity of the enzyme increased steadily with germination time after a slight initial decline (Fig. 10). Because a sprouted kernel can no longer germinate, it would be expected to be nonviable while, at the same

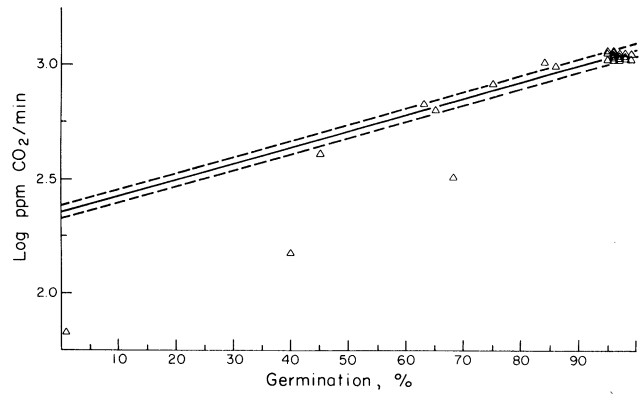

Fig. 9. Glutamic acid decarboxylase activities, expressed as ppm CO_2/ min, of 5.0000-g samples of Larker barley plotted logarithmically as a function of percent germination.

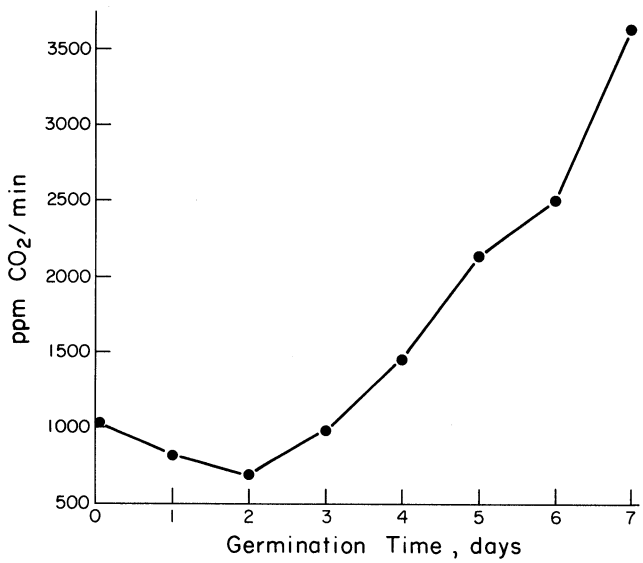

Fig. 10. Glutamic acid decarboxylase activity of barley as a function of germination time. Activities, expressed as ppm CO_2/ min, were measured on 5.0000-g samples of Bonanza barley germinated under laboratory conditions for the times indicated.

time, having high activity. Use of glutamic acid decarboxylase activity as a measure of viability would, thus, not be applicable to a sample containing a significant number of sprouted kernels. This would not appear, however, to be a major disadvantage of the procedure, because sprouting is relatively easy to detect by visual inspection or by α-amylase activity (Mathewson et al 1982).

LITERATURE CITED

ABDUL-BAKI, A. A., and ANDERSON, J. D. 1973. Relationship between decarboxylation of glutamic acid and vigor in soybean seed. Crop Sci. 13:227.

AMERICAN SOCIETY OF BREWING CHEMISTS. 1976. Methods of Analysis. Barley-3. The Society, St. Paul, MN.

BAUTISTA, G. M., and LINKO, P. 1962. Glutamic acid decarboxylase activity as a measure of damage in artificially dried and stored corn. Cereal Chem. 39:455.

BAUTISTA, G. M., LUGAY, J. C., CRUZ, L. J., and JULIANO, B. O. 1964. Glutamic acid decarboxylase activity as a viability index of artificially dried and stored rice. Cereal Chem. 41:188.

BEEVERS, H. 1951. An L-glutamic acid decarboxylase from barley. Biochem. J. 48:132.

CHENG, Y.-Y. 1959. The kinetics and occurrence of wheat glutamic acid decarboxylase. M.S. thesis, Kansas State University, Manhattan.

CHENG, Y.-Y., LINKO, P., and MILNER, M. 1960. On the nature of glutamic acid decarboxylase in wheat embryos. Plant Physiol. 35:68.

DUFFUS, C. M., DUFFUS, J. H., and SLAUGHTER, J. C. 1972. Glutamate decarboxylase in barley aleurone and its relationship to α-amylase development during germination. Experientia 28:635.

FOERSTER, C. W., and FOERSTER, H. F. 1973. Glutamic acid decarboxylase in spores of *Bacillus megaterium* and its possible involvement in spore germination. J. Bacteriol. 114:1090.

GALLESCHI, L., FLORIS, C., and COZZANI, I. 1977. Variation of glutamate decarboxylase activity and γ-amino butyric acid content of wheat embryos during ripening of seeds. Experientia 33:1575.

GALLESCHI, L., FLORIS, C., MELETTI, P., and COZZANI, I. 1975. On the location of glutamate decarboxylase in the caryopsis of hard wheat (*Triticum durum*) and its activity during early germination. Experientia 31:28.

GRABE, D. F. 1964. Glutamic acid decarboxylase activity as a measure of seedling vigor. Proc. Assoc. Off. Seed Anal. 54:100.

INATOMI, K., and SLAUGHTER, J. C. 1971. The role of glutamate decarboxylase and γ-aminobutyric acid in germinating barley. J. Exp. Bot. 22:561.

JAMES, E. 1968. Limitations of glutamic acid decarboxylase activity for estimating viability in beans (*Phaseolus vulgaris* L.). Crop Sci. 8:403.

KOTT, H. 1973. Effect of wetting temperature on glutamic acid decarboxylase activity in wheat embryos. Cereal Chem. 50:1.

LINKO, P. 1960. Water content and metabolism of wheat during short storage and germination. Ann. Acad. Sci. Fenn. Ser. AII 98.

LINKO, P. 1961. Simple and rapid manometric method for determining glutamic acid decarboxylase activity as quality index of wheat. J. Agric. Food Chem. 9:310.

LINKO, P., and MILNER, M. 1959. Enzyme activation in wheat grains in relation to water content. Glutamic acid-alanine transaminase, and glutamic acid decarboxylase. Plant Physiol. 34:392.

LINKO, P., and SOGN, L. 1960. Relation of viability and storage deterioration to glutamic acid decarboxylase in wheat. Cereal Chem. 37:489.

MATHEWSON, P. R., FAHRENHOLZ, C. H., BOOTH, G. D., POMERANZ, Y., and MILLER, B. S. 1982. Results of collaborative testing using a simplified, rapid colorimetric alpha-amylase assay for evaluation of sprouted wheat. Cereal Chem. 59:108.

ROHRLICH, M. 1957. Glutaminsäuredecarboxylase im Getreide. Getreide Mehl 7:89.

ROHRLICH, M., and SIEBERT, K. 1964. Glutaminsäuredecarboxylase im keimenden, reifenden und lagernden Getreide. Dtsch. Lebensm. Rundsch. 60:369.

SCHALES, O., and SCHALES, S. S. 1957. Decarboxylations and transaminations in extracts of higher plants. Arch. Biochem. Biophys. 69:378.

SHUKUYA, R., and SCHWERT, G. W. 1960a. Glutamic acid decarboxylase. I. Isolation procedures and properties of the enzyme. J. Biol. Chem. 235:1649.

SHUKUYA, R., and SCHWERT, G. W. 1960b. Glutamic acid decarboxylase. II. The spectrum of the enzyme. J. Biol. Chem. 235:1653.

SHUKUYA, R., and SCHWERT, G. W. 1960c. Glutamic acid decarboxylase. III. The inactivation of the enzyme at low temperatures. J. Biol. Chem. 235:1658.

STRAUSBAUCH, P. H., and FISCHER, E. H. 1970a. Chemical and physical properties of *Escherichia coli* glutamate decarboxylase. Biochemistry 9:226.

STRAUSBAUCH, P. H., and FISCHER, E. H. 1970b. Structure of the binding site of pyridoxal 5′-phosphate to *Escherichia coli* glutamate decarboxylase. Biochemistry 9:233.

STRAUSBAUCH, P. H., FISCHER, E. H., CUNNINGHAM, C., and HAGEN, L. P. 1967. Crystallization and properties of glutamate decarboxylase from *Escherichia coli* strain w^+. Biochem. Biophys. Res. Commun. 28:525.

USDA. 1975. Part 201, Federal Seed Act Regulations. Seed Testing Regulations. U.S. Dept. Agric., Washington, DC.

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