RELATION OF VIABILITY AND STORAGE DETERIORATION TO GLUTAMIC ACID DECARBOXYLASE IN WHEAT[1]

PEKKA LINKO AND LARS SOGN[2]

ABSTRACT

Glutamic acid decarboxylase activity of 25 commercial wheat samples was highly correlated with percentage of germ-damaged or "sick" wheat ($r = -0.878^{***}$), germination percentage ($r = +0.921^{***}$), topographical tetrazolium test ($r = +0.912^{***}$), fat acidity ($r = -0.864^{***}$), and fluorescence ($r = -0.637^{***}$). The correlation involving germ damage is significantly higher (at 5% level) than that involving fluorescence. With 19 samples of new crop wheats of little germ damage and high germination percentage, the correlation between glutamic acid decarboxylase activity and viability was insignificant ($r = +0.185^{ns}$), largely owing to differences in decarboxylase activity of wheats from various locations and of different variety. It was concluded that though glutamic acid decarboxylase activity seemed to have little value in examining new crop wheats of high viability, either alone or together with other tests it may give a good picture of the storage background of wheat.

Wetting of wheat embryos causes a rapid decrease in the amount of free glutamic acid with simultaneous carbon dioxide evolution, and an increase in free gamma-aminobutyric acid (28,29). Though this was due to the immediate activation of glutamic acid decarboxylase in moistened wheat (7,27), glutamic acid decarboxylase activity decreases during prolonged storage, particularly at elevated moisture levels (7,37). Preliminary evidence indicated a possible correlation between decarboxylation of glutamate and viability, or storage deterioration, respectively.

Several enzyme reactions have been previously related with the viability of seeds. In 1922 Turesson (41) introduced a method using the enzymatic reduction of methylene blue as an indicator of viability. A few years later Russian workers used successfully indigo carmine for testing quick germinating seeds (cf. 36). Eidmann (14), Gadd and Kjaer (17), and Lakon (22) employed the reduction of selenium salts as germination indicator. Later Lakon (23,24) studied the enzymatic reduction of several tetrazolium derivatives in this respect, finding 2,3,5-triphenyl tetrazolium chloride the most suitable. Sorger-Domenigg *et al.* (40) developed a method for colorimetric estimation

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[2] Respectively, Assistant Professor and W. K. Kellogg Foundation Fellow, Kansas State University, Manhattan.

of the reduction product formazan. Amylase (16,32), catalase (12,13, 25), and peroxidase (5) activities have also been correlated with storage deterioration and viability.

In the present study various factors involved in the deterioration of wheat grain have been investigated, with special attention to biochemical methods in determining the degree of viability. The suitability of various methods in estimating storage deterioration of wheats of different background was studied, with emphasis on the relation of germ damage to the application of glutamic acid decarboxylase activity as an index of deterioration and viability.

Materials and Methods

A total of 49 wheat samples was investigated. Twenty-five of the samples were commercial mixtures of hard red winter wheats at various stages of deterioration (series 1). These samples had been stored at 4°C. for about a year before the analyses were made. Twenty samples were new crop wheats (eight hard red spring and twelve hard red winter) of relatively high viability (series 2), and four were frost-damaged wheats (series 3). Moisture content varied from 9.4 to 12.9%. All quantities used for various analyses, as well as results, are reported on a moisture-free basis. Unless otherwise mentioned, all experiments were performed in duplicate.

When whole grains were used for various determinations, they were ground in a Waring Blendor for 2 minutes. For experiments with wheat embryos, the germ ends of kernels were carefully cut off with a razor blade so that no germ remained in the endosperm end. The isolated germ ends were ground in a mortar.

Germ-Damaged or "Sick" Wheat. Germ damage was determined from 200-kernel samples by carefully removing the pericarp covering the embryo. Cream-colored or white embryos were considered alive and the wheat was classified sound. In all other cases wheat was judged as damaged ("sick").

Germination Test. One-hundred-kernel samples were surface-sterilized by soaking 2 minutes in 0.1% mercuric chloride solution, followed by a thorough rinsing in tapwater. The kernels were placed crease down on moist, sterile quartz sand in Petri dishes, covered with wet filter paper, and allowed to germinate in the dark at 24° to 26°C. for 7 days. Grains with normal sprouts were counted and removed every second day. Results were confirmed by the Seed Laboratory, Kansas State Board of Agriculture, Topeka.

Fluorescence. Fluorescence was determined according to a slightly modified procedure of Cole and Milner (10). One gram of ground

kernels or 250 mg. of ground germ ends were weighed into Erlenmeyer flasks each containing 25 ml. of 0.2*M* hydrochloric acid. The mixtures were shaken by hand at certain time intervals, and allowed to stand overnight at 25°C. After filtering through Whatman No. 5 paper, the clear solutions were diluted, if necessary, and used for fluorescence determinations. Measurements were made with a Coleman Photoelectric fluorometer with B_1-S and PC-1 filters; the instrument was standardized to read 60 with 0.1 p.p.m. sodium fluorescein solution.

Fat Acidity. Fat acidity determination was based on the procedure described in *Cereal Laboratory Methods* (1). Ten grams of freshly ground material were extracted in a Goldfisch extractor with 50 ml. of petroleum ether (Skelly Solve B; b.p. 63° to 69°C.) for 6 hours. After removal of the solvent, the extract was dissolved in 25 ml. of isopropyl alcohol-benzene-water mixture (48:50:2, by volume) containing 0.2% of phenolphthalein, and titrated to a distinct pink color with 0.0129*N* potassium hydroxide solution. Fat acidity was reported as mg. of potassium hydroxide required to neutralize the free fatty acids in 100 g. of ground material.

Dehydrogenases. Dehydrogenase activity was determined by two different methods, both based on the enzymatic reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) to formazan. The first method was a modification of Lakon's (23) topographical test. The kernels were soaked overnight in distilled water at 4°C., cut lengthwise, and again soaked 12 hours in darkness in 1% TTC-solution (M/15 phosphate buffer, pH 7.3) at 25°C. The percentage of colored embryos in 200 kernels was then estimated by visual examination. Only kernels with completely stained embryos and scutellums were judged viable.

The second method was a modification of that proposed by Sorger-Domenigg *et al.* (40). Five milliliters of 1% TTC-solution were added to a test tube containing 1 g. of ground kernels or 250 mg. of ground germ ends. After two vacuum infiltrations the test tubes were incubated 1 hour at 38°C. The formazan produced was extracted with 25 ml. of acetone, and the extinction was measured at 520 mμ with a Beckman Model DU spectrophotometer.

Decarboxylases. Glutamic and pyruvic acid decarboxylase activities were determined manometrically as previously described by Cheng *et al.* (8). The activities were reported as microliters of carbon dioxide produced by 500 mg. of ground kernels or 100 mg. of ground germ ends during 30 minutes from 1 ml. of 0.1*M* substrate solution (M/15 phosphate buffer, pH 5.8). Determinations were performed in triplicate. The standard deviation of decarboxylase activity determinations was $\pm 2\%$. The reproducibility was also determined by calculating

tion between fat acidity and germ damage percentage ($r = +0.457^{***}$). On the other hand, the correlation coefficients $r = +0.81^{***}$ (2) and $r = +0.847^{***}$ (3) obtained by Baker and co-workers between fat acidity and germ damage are virtually the same as found in this study ($r = +0.837^{***}$ in series 1; $r = +0.825^{***}$ in series 2).

Zeleny and Coleman (42) emphasized the importance of three types of free acidic compounds in cereal grains in relation to the degree of deterioration, namely, fatty acids, acid phosphates, and amino acids. According to them phosphates and amino acids increase significantly only in wheat samples that have undergone a considerable degree of deterioration, whereas a highly significant increase in fat acidity may appear at very early stages of deterioration. Linko and Milner (29) have shown, however, that rapid changes in the composition of free amino acids may occur. Glass and Geddes (18) reported recently that deteriorating wheat exhibits an increase in inorganic phosphorus, apparently due to the action of phytase on phytic acid. Although they observed a greater increase in fat acidity than in inorganic phosphorus, the latter increased more rapidly at the early stages of deterioration. The results from the present study as compared with those previously obtained clearly suggest that increased fat acidity is an indication of lowered viability, though it does not have to be primarily associated with any of the known deteriorative processes involved.

Fluorescence. Previous work in this laboratory (10,33) has shown that extracts of damaged wheat embryos exhibit an increase in fluorescence, which precedes the respiratory increase indicative of mold growth. On the other hand, fluorescence of sound wheat samples of widely different source, variety, and class has been found low and virtually uniform. In the present study the fluorescence value was determined both with whole kernels and with isolated germ ends. The correlation between these two measurements is very high ($r = +0.920^{***}$). There was generally little change in fluorescence in samples of 20 to 100% germination (Fig. 1). The correlation between fluorescence and germination percentage in all of the 25 samples of series 1 was $r = -0.758^{***}$. This value agrees well with results obtained by Cole and Milner (10; $r = -0.775^{***}$), and Sorger-Domenigg *et al.* (40; $r = -0.663^{***}$). However, when correlation coefficients were calculated, neglecting four extreme samples of very high percentage of germ damage, a poor correlation was obtained ($r = -0.479^{**}$). Similar results were obtained with series 2, as shown by Table III. In this case the over-all correlation between fluorescence and germination percentage ($r = -0.957^{***}$) was significantly higher

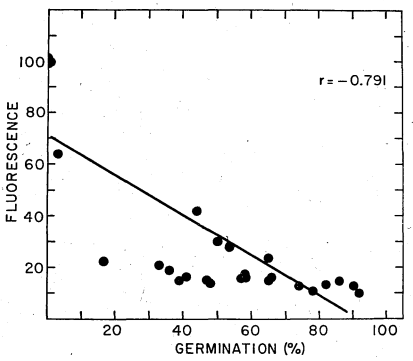

Fig. 1. Relation between fluorescence and germination percentage in series 1.

(at 0.1% level) than the same correlation after neglecting the extreme sample No. 20 ($r = -0.412^{**}$), indicating that sample No. 20 greatly influences the correlation. Figure 1 suggests that at very early stages of deterioration little increase in fluorescence appears to take place. With advancing deterioration fluorescence increases, accompanied by an increase in distribution of values obtained with wheat samples of the same viability. In particular, unexpectedly high fluorescence values may be encountered in samples of little or no viability, supporting the theory (26) that primary browning products formed during the early stages of deterioration are not fluorescent; viability may be lost before any browning of the embryo can be visually detected, and browning in turn precedes any marked increase in fluorescence. After viability is lost, nonenzymatic browning advances, leading to highly fluorescent compounds.

Glutamic Acid Decarboxylase Activity. Although Cheng *et al.* (9) showed that the marked immediate carbon dioxide evolution from wetted wheat germ is due to enzymatic decarboxylation of free glutamic acid, evidence for a negative correlation between glutamic acid decarboxylase activity and germ damage or fluorescence, respectively, was obtained during storage of moist wheat (7). Király and Farkas (21) found a markedly decreased glutamic acid decarboxylase activity in wheat infected by stem rust *(Puccinia graminis).* As can be seen from Table I and Fig. 2, different samples of wheat in series 1 exhibited a relatively large variation in the decarboxylation of glutamate. Glutamic acid decarboxylase activity in series 1 was highly correlated with germ damage ($r = -0.878^{***}$), viability ($r = +0.921^{***}$), topographical TTC-test ($r = +0.912^{***}$), fat acidity ($r = -0.864^{***}$), and fluorescence ($r = -0.637^{***}$). Though the simple linear correlations between germination percentage and glutamic acid decarboxy-

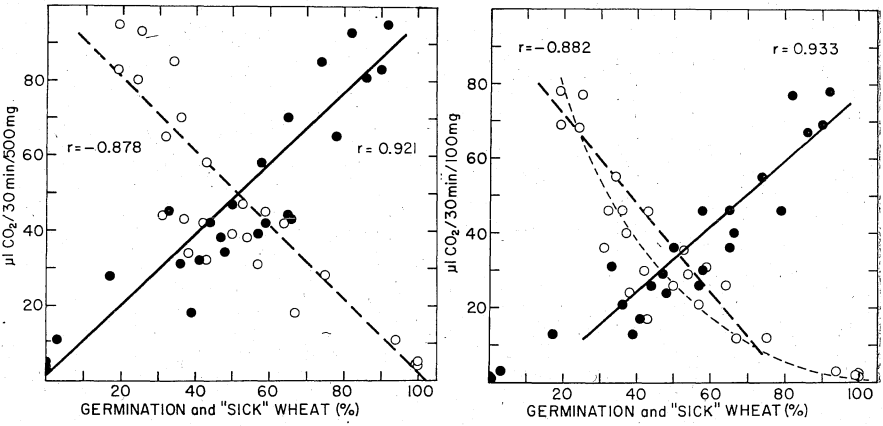

Fig. 2. Relation between glutamic acid decarboxylase activity and germination percentage (black circles), or "sick" wheat (germ damage) percentage (white circles) in series 1. Left: determinations with whole kernels; right: determinations with isolated germ ends.

lase activity are high and significant (Table II), it appeared likely that the enzyme activity decreases relatively faster at the early stages of deterioration, when only a few kernels have totally lost their viability.

In series 2 the correlation between glutamic acid decarboxylase activity and viability ($r = +0.744^{***}$) was significantly lower (at 5% level) than in series 1. Again, the neglect of sample No. 20 in statistical calculations resulted in an insignificant correlation (Table III). Wheats in series 2 were obtained from widely different sources, differing as to variety and growth conditions. All were from the 1959 crop. It has been shown previously that marked differences in the activity of glutamic acid decarboxylase occur in different varieties of wheat, as well as in wheats from various locations. It is, therefore, most likely that the varietal differences in new crop wheats of high viability are about the same order of magnitude as differences due to the beginning deteriorative processes. This led to the conclusion that though glutamic acid decarboxylase activity seemed to have little value in examining new crop wheats, it alone or together with other tests may give a good picture of the storage background of wheat.

Carboxylase Activity. A preliminary study (7) had indicated that there were virtually no varietal differences in regard to pyruvic acid decarboxylase activity in wheat grains. As can be seen from Table IV, the correlation coefficient in series 1 between germ damage and carboxylase activity is significantly higher (at 5% level) than that involving glutamic acid decarboxylase activity, but no significant differ-

TABLE IV

COMPARISON OF PYRUVIC AND GLUTAMIC ACID DECARBOXYLASE ACTIVITIES IN RELATION TO GERM DAMAGE AND VIABILITY. CORRELATION COEFFICIENTS

	Series 1		Series 2	
	Pyruvate	Glutamate	Pyruvate	Glutamate
Percent germ damage	−0.940**	−0.759*		
Percent germination	+0.973**	+0.985***	$+0.494^{ns}$	-0.088^{ns}

ence was found in regard to viability. The five samples examined for their carboxylase activity in series 2 were chosen so that there was no correlation between glutamic acid decarboxylase activity and germination percentage. The correlation involving carboxylase was numerically greater, but still insignificant, evidently owing to the small number of samples investigated.

Tetrazolium Tests. Though the topographical TTC-test has been widely accepted as a means of detecting viability of seeds (11,20,36, 38,39), difficulties in evaluation of the results have been also reported (4,19,30,31). In the present study the topographical TTC-test generally gave the best correlation with both germination and germ damage percentages (Tables II, III, and V). However, the differences between correlations involving TTC-test and fat acidity, respectively, in series 2 were not significantly different at the 5% level. The correlations between TTC-test and viability were significantly higher (at 0.1% level) than correlations involving glutamic acid decarboxylase activity and viability. Even when sample No. 20 in series 2 was neglected in statistical calculations (Table III), a high positive correlation between TTC-test and germination percentage was obtained ($r = +0.849***$). The correlation between the topographical TTC-test and germination percentage ($r = +0.990***$) in series 1 was significantly higher (at 0.1% level) than that involving the colorimetric TTC-test ($r = +0.902***$). This agrees with the relatively low correlation obtained by Sorger-Domenigg *et al.* (40; $r = +0.602***$). It was interesting that a high positive correlation exists between the topographical TTC-test and glutamic acid decarboxylase activity in series 1 ($r = +0.912***$ with whole kernels).

Frost Damage. Germination percentage was found to decrease and

TABLE V

SIMPLE CORRELATIONS WITH FROST-DAMAGED WHEAT SAMPLES

	Percent Germ Damage	Glutamic Acid Decarboxylase Activity	Fluorescence	Topographical TTC-Test	Fat Acidity
Percent germination	−0.982**	-0.608^{ns}	−0.926*	+0.996***	−0.933*

germ damage, fat acidity, and fluorescence to increase with increasing relative amount of frost damage (Table V). This was contrary to the observations of Baker *et al.* (3), who found low fat acidity values regardless of the degree and amount of frost damage. They suggested that freezing of wheat does not cause hydrolytic deterioration of fats. However, it seems quite likely that during prolonged storage deteriorative processes may be even faster in grains initially affected by frost. No significant correlation between germination percentage and glutamic acid decarboxylase activity was found, but the correlation involving topographical TTC-test was high ($r = +0.996^{***}$).

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