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RELATION OF RESPIRATORY AND ENZYMATIC ACTIVITY TO CORN SEED VIABILITY[1,2]

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Seed germination like any other growth process must depend on respiratory metabolism. Failure to germinate, therefore, may be due to breakdown in some part of the respiratory machinery. Perhaps the strongest evidence for this relationship is the success often reported (1, 2, 7, 9, 15) in estimating viability with the tetrazolium test which is known to involve dehydrogenase systems (8, 16). Otherwise there is little information on the changes in metabolic and enzymatic activity of seeds associated with loss of viability. The most extensive enzyme work so far has been with catalase and peroxidase and the results have not established any general correlation between activity and viability (6, 11, 13).

The plan of the present work was to explore the relationship between certain indices of respiratory metabolism and viability in corn. Alcohol and malic dehydrogenases were chosen because they were found previously (16) to be especially active in tetrazolium reduction in this seed. Cytochrome oxidase was included because there was evidence that it was the principal terminal oxidase in corn (12). Manometric measurements of overall respiratory capacity also were made as a test of the general hypothesis that failure of respiratory metabolism is an important cause of loss in viability and in the search for clues to other enzymatic systems limiting viability. It was hoped in this way to provide a more systematic basis for the development of quick tests for viability. A practical quick test of this kind would depend on the breakdown of respiratory or enzymatic activity at an early stage of the germination process. The 18-hour stage in corn was chosen because the embryo cells have then become fully turgid and this is approximately the stage at which most information about the tetrazolium test is available. Freezing and drying treatments were used to produce a range of viability because these approximate adverse conditions which are encountered in production and marketing.

MATERIALS AND METHODS

Hand harvested ears were collected from three open pollinated hybrids, B6 × W22, B6 × L289, and Ia. 4297 at moisture levels of 32 to 44 %. Husked ears were frozen and/or dried to 8 to 12 % moisture under various conditions (table I) to provide lots with a wide range of germination percentages. Seeds were not disinfected because of possible danger of the fungicide affecting the metabolic properties of the excised embryos. However, lots showing surface fungal growth during germination did not have significantly higher respiratory or enzymatic activities than others of equal germination which showed no sign of contamination. Only in the case of lot 24-51a, which had visible mold damage in the embryo, was there any evidence that mold activity affected the measurements reported.

Germination tests were made with 100 seed samples on moist blotters in an alternating temperature germinator (9 hr, 30° C, 15 hr, 20° C). Vigor ratings which estimated speed as well as extent of germination were calculated as follows. The number of normal seedlings per 100 seeds counted each day was multiplied by the reciprocal of the time in days in the germinator. The values for each day were then totalled when germination was complete in 7 or 8 days. The cold test was essentially a measure of germination under adverse conditions, principally low temperature and exposure to seed-invading fungi. The method, following that of Svien and Isely (18), was as follows. Samples of 100 seeds were planted in a substrate consisting of 1/3 Pythium-infected soil and 2/3 sand, which was maintained at 60 % saturation. After 1 week at 10° C the temperature was raised to 30° C and the seedlings counted after 5 days.

Tetrazolium tests were made on 100 seed samples by soaking for 18 hours at 30° C, bisecting longitudinally, and exposing cut side down in 0.05 % 2,3,5-triphenyltetrazolium chloride in the dark for 2 hours. To be classified as viable, normal staining of the

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plumule, scutellar node, and central area of the scutellum was required (9).

For enzymatic and respiratory measurements seeds were soaked in water for 18 hours at 30° C, embryo side down. At this stage of germination the seeds appeared to be fully imbibed but only rarely had the radicle emerged. Embryos of 50 seeds were excised with a small spoon-shaped spatula, bisected longitudinally, and cut into approximately 0.5 mm slices which were kept in an ice bath. The slices then were washed, mixed, and blotted dry, and approximately ¼ of the sample was weighed and used for enzymatic assay, and the remainder was used for respiratory measurements. For the former, the slices were ground cold in 0.02 M, pH 8 phosphate (Na_2-HPO_4-KH_2PO_4) in a glass homogenizer to make a 10 % homogenate on a fresh weight basis. This was centrifuged cold at $1000 \times g$ for 8 minutes to remove cellular debris, and part of the supernatant was used for dehydrogenase assay. Previous work (19) had shown that essentially all the malic and alcohol dehydrogenase activity was in this fraction. The remainder was centrifuged cold at $20{,}000 \times g$ for 1 hour to sediment the particle fraction containing cytochrome oxidase. The latter was suspended in 0.02 M, pH 7 phosphate and both enzyme fractions were stored in an ice bath until assayed.

Both oxidase and dehydrogenase activity were determined by a rapid colorimetric technic, the former based on that of Ginter and Smith (4) and the latter developed earlier in the present study (19). Conditions adopted for malic dehydrogenase assay were as follows: 0.032 M, pH 8 phosphate (Na_2–K), 0.04 M, pH 8 malate, 2.9×10^{-4} M diphosphopyridine nucleotide (Schwarz), 1.08×10^{-5} M oxidized dye (2,6-dichlorobenzeneoneindo-3′-chlorophenol, Eastman), 0.1 M, pH 8 cyanide, sufficient pig heart diaphorase (19) to saturate the system, and sufficient diluted enzyme solution and water to make a total volume of 2.5 ml. The conditions for alcohol dehydrogenase were the same except for 0.14 M alcohol and 0.08 M, pH 8 semicarbazide in place of malate and cyanide. The conditions for cytochrome oxidase assay were as follows: 0.04 M, pH 7 phosphate (Na_2–K) 7.2×10^{-5} M reduced dye (4), 1.4×10^{-5} M cytochrome c, sufficient catalase to destroy H_2O_2 in the dye, and enough enzyme suspension and water to make a total volume of 2.5 ml. The stock enzyme preparations were stable for several hours at 0° C, but after dilution at 30° C they were unstable and were used within 3 to 4 minutes and 1 to 2 minutes for the dehydrogenases and oxidase, respectively.

Reaction rates were determined at 30° C by optical density readings taken at 5 second intervals for a 1 to 2 minute period (17). Four concentrations of each enzyme preparation were used and proportionality curves were plotted to determine average rates and to correct for blanks, when necessary, by extrapolation to zero enzyme concentration. For comparison with the manometric data, rates were expressed as Q values, equivalent microliters oxygen uptake per mg dry weight per hour. Dry weight of slices corresponding to the two enzyme fractions was calculated from the fresh weight of slices homogenized and the water content of slices determined on equivalent material by oven-drying at 100° C.

Respiratory activity of slices suspended in 0.05 M, pH 6 phosphate (Na_2–K) was measured in 50-ml Warburg flasks, using an O_2 gas phase in the O_2 and aerobic CO_2 measurements (Q_{O_2} and Q_{CO_2}, respectively) and purified tank N_2 for anaerobic CO_2 measurements ($Q^N_{CO_2}$). Aerobic CO_2 evolution was by the two flask direct method. Gas exchange rates were linear for the one hour observation period and no significant change in bound CO_2 was observed. All indices of respiratory capacity are expressed as Q values, based on oven-dry weight of slices after removal from flasks.

It should be recognized that this conventional technic for determining tissue respiratory capacity measures neither the respiration of the embryo in the intact corn seed nor the maximum capacity of the tissues. Removal of the embryo from the kernel and reduction to slices increased respiratory rates several fold. Furthermore, slices on wet filter paper showed further increase in O_2 consumption and CO_2 evolution of 25 % and 70 %, respectively, as compared with slices submerged in buffer, even with an O_2 atmosphere. A full understanding of the significance of these physical factors on corn embryo respiratory capacity will require further study, but for the present study of relative respiratory capacities of various seed lots the technic described proved adequate.

Results and Conclusions

The treatments and viability analyses of the 31 lots are summarized in table I. Cold test results were lower than germination percentages only in those lots dried at elevated temperatures. The same lots did not have correspondingly lower vigor ratings; so the difference in their behavior under favorable and unfavorable conditions did not appear to be due to growth rate. The variation in vigor ratings among lots germinating 98 to 100 % indicated differences in speed of germination. For all lots, except those germinating close to 0 and 100 %, of course, the tetrazolium test gave higher values than the germination test. The treatments involved in these lots apparently caused more damage to metabolic mechanisms essential to germination than to those involved in tetrazolium reduction.

The respiratory capacity and enzymatic activity data presented are the means of two analyses based on 50-seed samples. With the exception of cytochrome oxidase the 3 corn varieties used showed no significant differences in respiratory or enzymatic activity, so the data have been pooled. An estimate of variability, due largely to sampling rather than analytical technic, is given in table II, along with means for lots of high germination to indicate the levels of activity for viable seeds in the corn varieties studied.

It is first of interest to examine the changes in

TABLE I

TREATMENTS AND VIABILITY INDICES

LOT	TREATMENT	GERM %	COLD TEST % **	TETR TEST % †	VIGOR RATING *
16-51a	R. D. (room dried)	100	100	...	25.4
23-51b	R. D.	100	100	100	19.4
11-52	R. D.	100	98.5	100	27.8
7-51a	R. D.	100	97	99	26.9
12-51a	65 hrs, 40° C	100	68	100	27.3
3-51b	R. D.	99.5	100	100	24.9
7-52	18 hrs, 2° C; R. D.	99.5	99.5	100	23.2
4-51a	R. D.	99.5	98	100	21.5
15-51b	R. D.	99	99	100	20.8
8-51b	R. D.	99	96	99	22.8
12-52	68 hrs, 50° C	98.5	91	100	27.4
6-52	R. D.	98	100	99	23.1
14-51a	65 hrs, 40° C	97.5	21	99	22.3
11-51b	68 hrs, 40° C	95.5	42	100	17.8
13-51b	65 hrs, 40° C	90.5	31	100	16.2
1-52	4 hrs, −7° C; R. D.	89	88.5	95	19.7
5-52	72 hrs, 50° C	72	2.5	98	17.2
24-51a	R. D.	55	45	75	15.0
1-51a	Frozen in field; R. D.	51.5	55	62	10.7
14-52	8 hrs, −7° C; 100 hrs, 50° C	44.5	25.5	60	11.3
3-52	12 hrs, −7° C; R. D.	43	38	60	11.3
2-51b	Frozen in field; R. D.	39.5	41	54	8.6
9-52	16 hrs, −7° C; R. D.	39	44.5	67	11.5
10-52	16 hrs, −7° C; 100 hrs, 45° C	30	7.5	60	7.5
8-52	18 hrs, 2° C; 110 hrs, 50° C	29	7	50	5.2
13-52	8 hrs, −7° C; R. D.	25.5	26	33	7.8
4-52	12 hrs, −7° C; 114 hrs, 50° C	18	1.5	45	3.2
2-52	4 hrs, −7° C; 114 hrs, 50° C	9.5	0	40	1.7
5-51b	16 hrs, −7° C; R. D.	1	0	0	0.2
10-51b	16 hrs, −7° C; 74 hrs, 40° C	1	0	0	0.1
18-51b	65 hrs, 40° C	0	0	0	0

* Two 100-seed samples.
** One 100-seed sample for "51" collections, two for "52" collections.
† One 100-seed sample.

respiratory and enzymatic activity in the early stages of germination, during which the standard 18-hour sample was taken. Table III summarizes such data for a typical high germinating lot of corn. It should be pointed out that the "0 hour" sample actually represents slices from dry embryos suspended in aqueous medium for about one hour before measurements began. During this period the cells probably became fully imbibed and respiration was rapidly activated. It is assumed that "0 hour" activity was the potential respiratory capacity of the dormant seed since no further increase in respiratory rate occurred during the subsequent 2 hours in the Warburg flasks. The small increases in dry weight per embryo during the 27-hour period were of doubtful significance because of incomplete excision of scutellar tissue, especially at "0 hour" and possibly at 9 hours. Because of the lower activity of scutellar tissues, however, it could be estimated that this loss did not affect the Q values by more than 5 %, even with "0 hour" seeds. The protein nitrogen content of slices also remained essentially constant during the 27-hour period.

TABLE II

RESPIRATORY AND ENZYMATIC ACTIVITIES OF CORN EMBRYOS

	MICROLITERS PER HR PER MG DRY WT	
	A *	B *
Q_{O_2}	3.83 ± 0.42	0.27
Q_{CO_2}	2.14 ± 0.31	0.19
$Q^{N}_{CO_2}$	1.79 ± 0.20	0.15
Malic dehyd	21.1 ± 2.4	1.9
Alcohol dehyd	44.7 ± 6.3	6.5
Cytochrome oxidase	0.59 ± 0.22	0.19
RQ **	0.58 ± 0.06	
F/R **	0.86 ± 0.13	

* A. Mean ± stand. dev. for 13 lots, germ. 98 to 100 %.
B. Stand. dev. of mean diff. for duplicate samples of all lots.
** C. Mean ± stand. dev. for 27 lots, germ. above 10 %.

Respiratory capacity increased steadily throughout the 27-hour period with no marked change in RQ. In the beginning this increase was probably due primarily to activation of existing enzymes. Some cell enlargement probably occurred before 18 hours and cell division began shortly afterward (14). Though no net protein synthesis was found, it is likely that considerable enzyme formation occurred between 18 and 27 hours. With the two dehydrogenases the only significant increase in activity came in this period. Cytochrome oxidase activity, on the other hand, increased most in the first 9-hour period.

TABLE III

EFFECT OF TIME OF SOAKING ON THE RESPIRATORY AND ENZYMATIC ACTIVITY OF EMBRYO SLICES

HRS SOAKED	DRY WT PER EMBRYO * (MG)	Q_{O_2} **	Q_{CO_2}	RQ	MALIC DEHYD **	ALCOHOL DEHYD †	CYT OXIDASE †
0	23.7	1.44	0.93	0.65	15.9	35.4	0.41
9	25.5	2.30	1.34	0.58	16.2	34.2	0.71
18	26.9	3.90	2.26	0.58	16.6	38.9	0.75
27	27.9	5.32	2.85	0.54	24.7	47.4	0.81

* Dry wt are averages of 15 determinations except at "0 hour" which is 5.
** Respiratory rates are μl gas exchange per mg dry wt per hour, aver. of 3 determinations.
† Enzymatic activities are equivalent μl O_2 uptake per mg dry wt per hr, aver. of 3 determinations.

As far as the present data go, it appears that the 18-hour sample occurred at a time when activation of existing enzymes was complete and new enzyme synthesis was underway. It is also evident that substantial amounts of respiratory enzymes were already present in the dry seed.

The relationship among the metabolic and viability indices must be examined in terms of sample means of the various lots, and this is most conveniently done by the following graphical comparisons. Ultimately, it would be desirable to know the relationship between the metabolic activity of individual seeds and their ability to germinate, and some inference in this regard also may be possible. This was attempted by comparing the variation in metabolic indices among the lots with germination with what would be expected if all live seeds retained maximum metabolic activity and all dead seeds had none. This would result in a linear regression line from 0 % germination and 0 metabolic activity to 100 % germination and maximum metabolic activity, referred to hereafter as the " all or none " relationship.

Figure 1 shows that oxygen consumption of the various lots at 18 hours was closely related to the germination percentage. The major deviations from a linear correlation were with the mold damaged sample 24-51a and 3 lots, 3-52, 9-52, and 13-52, which contained a large number of seeds which initiated growth but failed to produce normal seedlings and were not counted as germinated. These seeds probably had nearly normal respiratory activity at 18 hours. With these exceptions, and on the " all or none " basis, the data indicate that individual seeds would not germinate if their respiration was much below normal at 18 hours and furthermore that nonviable seeds in most cases had little or no respiratory activity at that stage. The respiratory quotients and F/R ratios ($Q^{N}_{CO_2}/Q_{CO_2}$) varied little with germination percentage (table II) down to below 10 % where analytical errors became serious. This indicated that both aerobic and anaerobic CO_2 evolution varied with germination like O_2 consumption; so that there was no evidence of differential inhibition of CO_2 evolution and O_2 consumption mechanisms.

Figure 2 shows that malic dehydrogenase activity decreased with germination percentage in a roughly linear fashion, again with the exception of the 4 lots cited above. However, enzyme activity did not decrease to the same extent as germination percentage. This is particularly evident in the lots which had considerable enzyme activity remaining but contained few viable seeds. It appeared that individual seeds could not have lost appreciable malic dehydrogenase activity and remained viable, but at the same time nonviable seeds may have retained considerable activity.

A somewhat similar relationship between alcohol dehydrogenase activity and germination percentage is shown in figure 3, with two noteworthy differences. First, there was less evidence of enzyme activity in nonviable seeds, though some lots in the 10 to 40 % germination range had somewhat higher enzyme activity than would be expected from the germination percentage (" all or none " basis). Also, as in the case of malic dehydrogenase, the 3 lots with abnormal seeds had high alcohol dehydrogenase activity. Second, 3 lots in the region of 50 % germination had such low enzyme activity that some seeds must have suffered very severe reduction in activity without losing viability. On the whole, alcohol dehydrogenase seemed to be more sensitive than malic dehydrogenase to the conditions causing lowered viability, but there was less likelihood that it was the limiting factor.

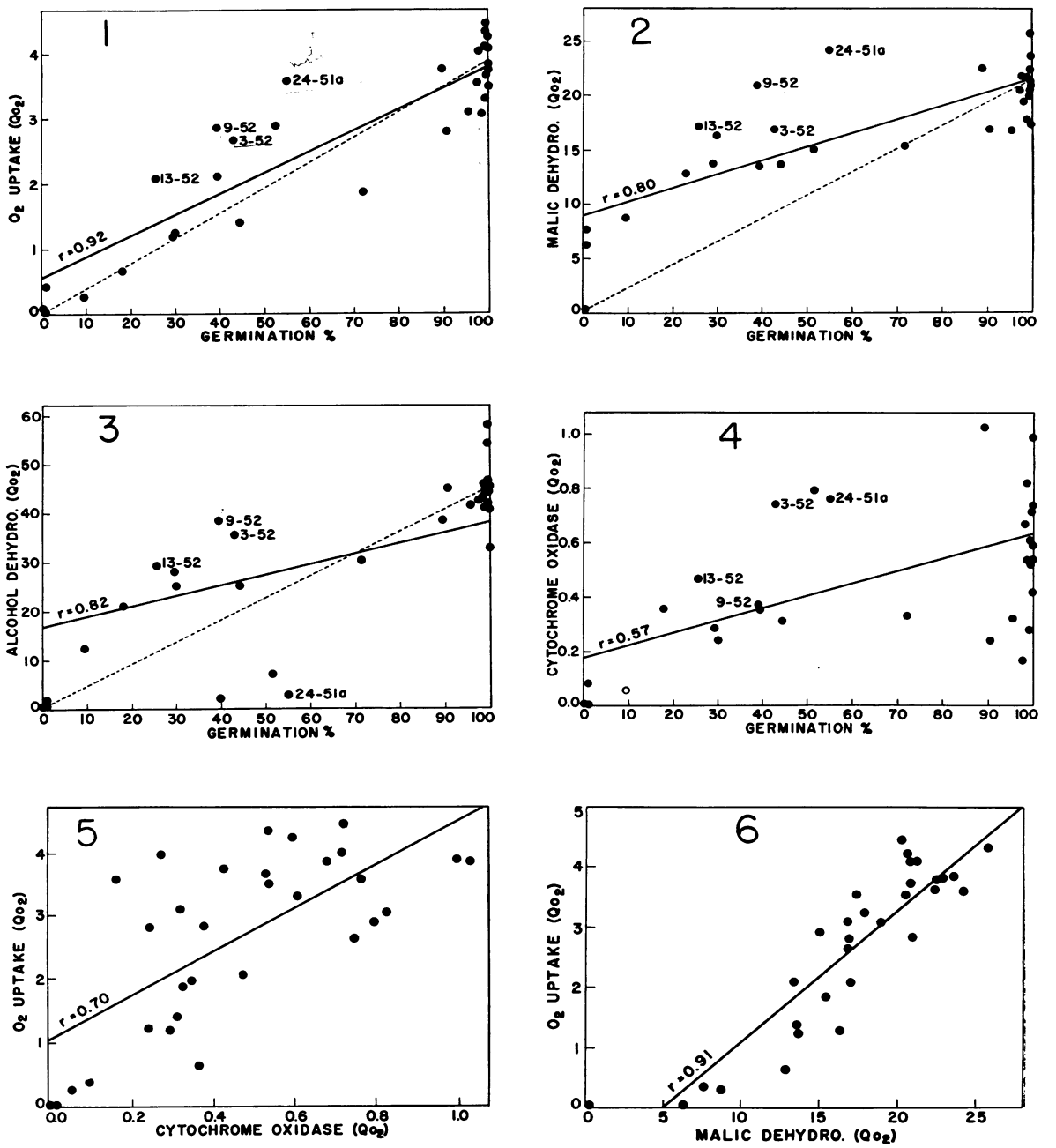
The greater variability of cytochrome oxidase in relation to germination is evident in figure 4 as well as in table II. This was partly due to varietal differences, but the data also indicated that some seeds may have had much reduced oxidase activity and still remained viable.

It was of interest to compare the indices of metabolic activity with the cold tests, vigor ratings, and tetrazolium tests of the various lots. No corresponding decreases in metabolic activity were found which might explain those cold test results which were lower than the germination percentages. Similarly, no evidence was found that any of the metabolic indices were more closely correlated with vigor rating (speed of germination) than with extent of germination. In the case of the tetrazolium test, however, lots which had high tests in comparison with the germination percentage also had relatively high dehydrogenase activities. This would be expected if tetrazolium reduction were limited by dehydrogenase activity.

Graphical comparison of enzymatic activities and respiratory capacities also provided some evidence on the extent to which the enzymes measured may have limited respiration as well as germination. The wide variation in oxidase activity in lots of high Q_{O_2} values (fig 5) shows that cytochrome oxidase probably did not limit O_2 consumption except perhaps in lots of very low O_2 consumption. On the whole, the oxidase activity found was sufficient to support less than half the observed O_2 consumption, which indicated that it may not be the principal terminal oxidase in corn embryos.

Figure 6 shows that the correlation of malic dehydrogenase activity with O_2 consumption was much higher than in the case of the oxidase, though it is clear that considerable activity must have remained in nonrespiring seeds. For this reason and because the level of malic dehydrogenase activity was many times greater than that necessary to account for the observed O_2 consumption, it seems probable that this dehydrogenase was not limiting respiration but was merely highly correlated with another enzyme or other enzymes which did.

A similar comparison of alcohol dehydrogenase activity and anaerobic CO_2 evolution in figure 7 shows a rough proportionality, with the exception of the 3 lots previously mentioned which were very low in enzyme activity but in which about half the seeds were viable. As with malic dehydrogenase, the al-



FIGS. 1–7. Each point represents mean of 2 determinations. Solid line is a regression line; broken line represents "all-or-none" relationships (see text).

FIG. 1. Relationship of O_2 consumption rate to % germination.

FIG. 2. Relationship of malic dehydrogenase activity to % germination.

FIG. 3. Relationship of alcohol dehydrogenase activity to % germination.

FIG. 4. Relationship of cytochrome oxidase activity to % germination.

FIG. 5. Relationship of cytochrome oxidase activity to O_2 consumption.

FIG. 6. Relationship of malic dehydrogenase activity to O_2 consumption.

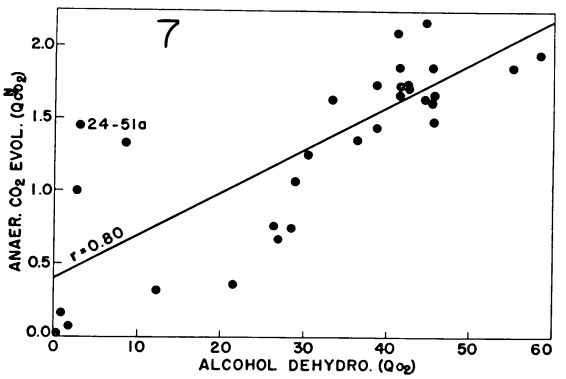


FIG. 7. Relationship of alcohol dehydrogenase activity to anaerobic CO_2 evolution.

cohol dehydrogenase activity found in almost all lots was much higher than necessary to account for anaerobic respiration, even if it were purely alcoholic. It is improbable, therefore, that this dehydrogenase was limiting.

DISCUSSION

Within the limitations of analyses using multiple seed samples, the evidence presented indicates that most seeds which failed to germinate had negligible respiratory activity at the 18-hour stage of germination. However, 3 lots contained a considerable number of seeds which apparently had nearly normal respiratory activity at this stage but did not germinate normally. The occurrence of such seeds, of course, would limit the accuracy of any quick viability test based on respiratory activity of the whole embryo at an early stage of germination.

Of the 3 respiratory enzymes measured, malic dehydrogenase activity was most closely correlated with both O_2 consumption and germination percentage. However, the high level of activity found and the pattern of variation among lots with respect to both respiration and germination made it unlikely that this enzyme limited viability in most seeds. The pattern of variation in alcohol dehydrogenase with respiration and germination was somewhat different, but again there was little evidence that this enzyme played a limiting role in metabolic processes controlling growth. The very low level of cytochrome oxidase activity and the great variability with respect to both O_2 consumption and germination indicated that the oxidase had little significance in limiting variability in the corn samples involved in this study. Not only was the oxidase activity low in comparison with O_2 consumption but it was only about 1/3 that found by Maxwell (12) by a manometric method at about the same stage of germination. The present colorimetric method had previously yielded activities equal to or greater than the manometric method on corn root tips (4) and rat organs (17) so the disagreement with Maxwell's report is probably due to varietal differences.

The relationship observed between the 2 dehydrogenase activities and germination percentages suggested a possible explanation for the higher values in the tetrazolium test than in the germination test. Dehydrogenase activity in many lots was higher than would be expected from the germination data on the "all or none" basis. In other words, dehydrogenase activity was not reduced as much, or in as many seeds, as was the activity of another enzyme (or enzymes) which did limit growth. If tetrazolium reduction, then, was controlled by dehydrogenase activity, particularly malic or alcohol dehydrogenase, the tetrazolium test would be expected to overestimate viability. Similar high tetrazolium results have been reported by Goodsel (5) and Bennett (3) with frozen corn. While such observations do not disprove the general value of the tetrazolium method, they do emphasize the caution necessary in its use where seeds may have been subjected to freezing and artificial drying.

Since cold tests measure ability of corn to germinate under adverse conditions, lots having low cold tests might be less vigorous than lots with high cold tests but with similar germination percentages. Furthermore, the former might be expected to show reduced respiratory capacity. Our tests, however, gave no evidence of relationship of cold test with vigor rating or with respiratory activity. Other factors affecting growth or susceptibility to soil fungi must have been involved. It is interesting to note that the cold test results were lower than germination percentages only in those lots which were oven dried at elevated temperatures. This is in agreement with earlier reports of Livingston (11) and others that artificial drying of seed corn caused greater susceptibility to soil organisms.

While the present results support the hypothesis that breakdown in the respiratory machinery may be an important cause of the loss of viability, it is clear that more extensive study and probably more refined technics will be necessary to determine the extent to which this is true and to locate the site of breakdown. The multiple seed samples necessary in the present technic did not allow an adequate evaluation of variation among individual seeds. It may be necessary, therefore, to employ a method for comparing enzymatic and metabolic activities with ability to grow on individual seeds. In fact, from the topographic nature of the tetrazolium test it may be desirable to confine the metabolic measurements to critical parts of the embryo. Scaling down of the present manometric and colorimetric technics appears feasible, especially with corn seeds, so that metabolic analyses might be performed on half seeds, but there is still insufficient evidence as to whether a valid growth determination could be made on the other half. Such technics, however, would be very useful in investigating the metabolic factors controlling tetrazolium reduction in embryo parts.

SUMMARY

Changes in respiratory capacity and in the activity of malic and alcohol dehydrogenase and cytochrome

oxidase of corn embryos were measured during the early stages of germination. These metabolic indices at the 18-hour stage were compared with the germination percentage, tetrazolium test, vigor rating, and cold test of 31 lots of corn of varying viability. Loss of viability appeared to be closely associated with respiratory failure in most seeds. Malic dehydrogenase activity was more closely correlated with germination percentage and respiratory capacity than that of the other 2 enzymes, although considerable malic activity was retained in nonviable seeds. It is doubtful whether inactivation of these 3 enzymes was a major cause of loss of viability, but it appeared likely that dehydrogenase activity was more closely correlated with tetrazolium reduction than with germination percentage. Variations in respiratory metabolism did not explain the differences between germination percentage and cold test or vigor rating.

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GLUCOSE DISSIMILATION IN THE HIGHER PLANT. EFFECT OF AGE OF TISSUE[1,2]

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We have recently described a series of experiments (4) from which it was concluded that a sequence of glucose breakdown other than that of glycolysis by the Embden-Meyerhof-Parnas (E.M.P.) pathway plays a part in the respiration of several plant tissues. There were indications from this work that an alternative method of glucose dissimilation (Warburg-Dickens, Direct Oxidation pathway) was particularly prominent in the older aerial parts of plants and by contrast the respiration of a highly meristematic tissue (corn root tip) was shown to occur exclusively by the classical glycolysis sequence (5). In this paper we will present evidence, obtained from experiments with a wide variety of plant parts of different ages that in juvenile and undifferentiated tissues generally, the E.M.P. sequence is of major importance, but that as the tissue ages, the Direct Oxidation pathway comes to play an increasingly important role.

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