GLUTAMIC-ASPARTIC TRANSAMINASE IN THE GERMINATING BARLEY EMBRYO[1]

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ABSTRACT

Selected barley varieties from irrigated land at Aberdeen, Idaho, were studied for possible relationships between (a) germination rate and embryo glutamic-aspartic transaminase (GAT) activity; (b) GAT activity and kernel nitrogen content; and (c) germination rate and kernel nitrogen content.

Six barley varieties known to differ in their germination rates revealed no consistent relationships between GAT and germination rate. Two-rowed varieties were consistently high in GAT and germination rate. GAT increases of 36 to 49% during the first 24 hours were exhibited by these six varieties, while their embryo proteins changed by −3 to 8%. This fact plus a decreased response of the embryo homogenates to added pyridoxamine-5-phosphate as germination progressed suggests that the early increases in GAT result from activation of existing apoenzyme by endogenous coenzyme rather than via apoenzyme synthesis or the slow hydration of existing holoenzyme.

The nitrogen contents of isogenic two-rowed and six-rowed barleys were varied by growing at different spacing intervals. These samples revealed only inconsistent increases in GAT content with increased nitrogen content. The samples with the higher nitrogen contents tended to germinate more slowly.

The existence of transaminases in ungerminated cereal embryos and in the embryos of both monocotyledonous and dicotyledonous plants at various times after moistening is well established. The relatively high transaminase activities of seed embryo homogenates (1,7,10) suggest a possible function in the early life of the seedling. Although there is no direct evidence to indicate that any one of the transaminases is involved in the initiation of germination, the fact that a number of alpha-keto acids are substrates for some of the transaminases and for respiration suggests that some of the transaminases

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may serve to prime the respiratory chain with the less stable alpha-keto acids when conditions are otherwise favorable for germination.

Leonard and Burris (7) reported that two varieties of barley which were higher in embryo glutamic-aspartic transaminase (GAT) activity and kernel nitrogen content germinated more rapidly under malting conditions than two other varieties which were lower in GAT activity and nitrogen content. On the other hand, Finlay (5) has observed that increased kernel nitrogen resulting from the use of fertilizer on replicated field plots has little effect on the germination rates of ten barley varieties known to possess different germination rates. The existing evidence thus appears to indicate that, whereas the nitrogen content of the kernel has little effect on the germinate rate, there may be some correlation between kernel nitrogen and GAT activity of the embryo, or between the latter and germination rate.

The present study was undertaken to examine these possible relationships and to determine whether an assay of GAT activity would be a useful index of germination characteristics of barley.

Materials and Methods

The varieties of barley selected for differences in germination rate were grown during the summers of 1957, 1958, and 1960 on irrigated field plots at Aberdeen, Idaho. They consisted of the six-rowed barleys, *Hordeum vulgare* L., varieties Ankober, Kindred, 50-N-15, and C. I. 5000, and the two-rowed barleys, *H. distichum* L., varieties Hannchen and Ymer. Ankober, Hannchen, and Ymer were found to be rapid germinators, Kindred tended to be intermediate in rate, and 50-N-15 and C. I. 5000 were characteristically slower. Samples used to study the effect of kernel nitrogen content upon GAT activity also were grown on irrigated land at Aberdeen, Idaho, during 1960. They consisted of the isogenic pair, 16-vv-20 (six-rowed) and 16-VV-20 (two-rowed), which were grown at spacings varying from ¼ to 8 in. in order to alter the supply of available nutrients.

The barley samples were stored in metal cans for at least 6 months at 25°C. to permit the loss of any post-harvest dormancy.

The term *germination* is used according to Evenari (4) whereby the protrusion of the root is used as an indication that the germination process is completed; subsequent development of the embryo constitutes normal growth. By this definition the faster-germinating varieties were almost completely germinated by 24 hours, and the slower varieties required from 48 to 60 hours (Table I). Germinations were carried out in the dark in Petri dishes containing two circles

(9-cm.) of Whatman[3] No. 1 filter paper, 4 g. of barley, and 6 ml. of distilled water at 16°C. and 95% r.h. in a pilot malting chamber. Although none of the barleys was water-sensitive according to the procedures of Essery *et al.* (3) even when as much as 10 ml. of water per dish was used, surface sterilants such as 0.1% mercuric chloride or ethanol-hypochlorite solutions for periods of 30 to 60 seconds lowered the germination rates of some of the barleys. In view of this and because germination times of relatively short duration were used, the barleys were not treated to reduce the number of microorganisms on the kernels.

To facilitate removal of the embryos from the ungerminated samples, the kernels were soaked in distilled water for 2 hours at room temperature. Sufficient embryos (25 to 60) were homogenized in ice-cold 0.05*M* sodium phosphate buffer, pH 7.0, in 0.2*M* sucrose, or in the buffer without sucrose, with a Tenbroeck glass homogenizer to provide 10 ml. of 1.5% (fresh weight) homogenate for the ungerminated and 24-hour preparations. A 3% homogenate was used for the 48-hour samples. The homogenates were centrifuged at 4,000 × gravity for 5 minutes at 4°C. and the resulting supernatants used as enzyme after a 10- to 30-fold dilution with 0.05*M* *tris* (hydroxymethyl) aminomethane chloride (Tris) buffer, pH 8.0, in 0.2*M* sucrose solution.

The production of oxalacetate at 30°C. was followed for the assay of GAT activity by the method of Cammarata and Cohen (2) with a Beckman DU spectrophotometer equipped with thermospacers. The 1960 samples were examined by the same procedure except that a Gilford Instrument Laboratories Model 200 Optical Density Converter with a 10-in. strip chart recorder was employed. With this arrangement the enzyme activity was determined from readings made at intervals of about 27 seconds during the first 4 minutes of the reaction. The reaction mixture consisted of 1 ml. of 0.05*M* L-aspartate in 0.05*M* Tris buffer, pH 8.0, 1 ml. of suitably diluted enzyme to which was added, after a 10-minute equilibration period, 1 ml. of 0.05*M* alpha-ketoglutarate in 0.05*M* Tris buffer, pH 8.0. Reaction blanks consisted of 1 ml. of the diluted enzyme plus 2 ml. of 0.05*M* Tris buffer. Reactions from which the aspartate or alpha-ketoglutarate were omitted individually exhibited no significant increases in absorbance. When coenzyme was employed, 0.1 to 0.2 ml. of undiluted homogenate were incubated with 0.1 ml. of suitably diluted

[3] The mention in this publication of a trade product, equipment, or a commercial company does not imply its endorsement by the U.S. Department of Agriculture over similar products or companies not named.

coenzyme solution in 0.2*M* sucrose, pH 7.0, for 10 minutes at 30°C. The preincubated enzyme was then diluted with buffered sucrose as before and 1 ml. was added to the reaction mixture.

Transaminase results are expressed as Q_T (embryo), the micromoles of oxalacetate formed per hour per embryo, and as Q_T (protein), the micromoles of oxalacetate produced per hour per mg. protein.

Substrates and coenzymes were CfP or A grade obtained from the California Corporation for Biochemical Research.

Nitrogen was determined by a modification of the nesslerization procedure of Johnson (6). Protein was determined by the method of Lowry *et al.* (8) with purified barley albumin (9) as the protein standard.

Results

The percentage germination values and nitrogen contents of the barleys used for GAT determinations are summarized in Table I. The germination rates of the first three varieties were consistently in the 90 to 100% range at 24 hours, despite considerable differences in nitrogen contents of the samples. The three slower varieties showed appreciably more variability in germination rate from one year to another, even though their nitrogen contents varied from 2.05 to 2.23%. An examination of these data does not support the view that nitrogen content *per se* has an appreciable effect on germination rate of the barleys.

TABLE I
NITROGEN CONTENT AND PERCENTAGE GERMINATION OF BARLEYS USED FOR TRANSAMINASE ASSAY

VARIETY	1957			1958			1960		
	N[a]	Germination[b]		N	Germination		N	Germination	
		24 Hours	48 Hours		24 Hours	48 Hours		24 Hours	48 Hours
	%	%	%	%	%	%	%	%	%
Ankober	2.10	97	99	1.82	96	99	2.04	97	100
Hannchen	1.76	97	98	2.19	94	99	2.40	90	99
Ymer	2.51	95	100	2.00	94	100	2.12	93	100
Kindred	2.06	87	96	2.11	82	94	2.19	41	98
50-N-15	2.17	82	99	2.21	59	91	2.20	10	91
C.I. 5000	2.20	72	96	2.14	74	90	2.23	63	96

[a] Dry basis.
[b] Average of duplicate determinations.

Comparison of GAT Activity of Embryos from Barleys Possessing Different Germination Rates. The results of the GAT determinations with the embryo homogenates from 1957, 1958, and 1960 samples are

presented in Figs. 1 and 2. Although expressing enzyme activity on the basis of the protein or nitrogen present in a given preparation is a preferred procedure in many situations, the use of either of these values by itself can produce a misleading picture of the development of an enzyme in systems experiencing rapid and relatively large changes in protein or other nitrogenous substances. Because cereal embryos are an example of such a system, the present data are expressed on the basis of the activity per embryo, as well as the activity per mg. protein of the homogenate.

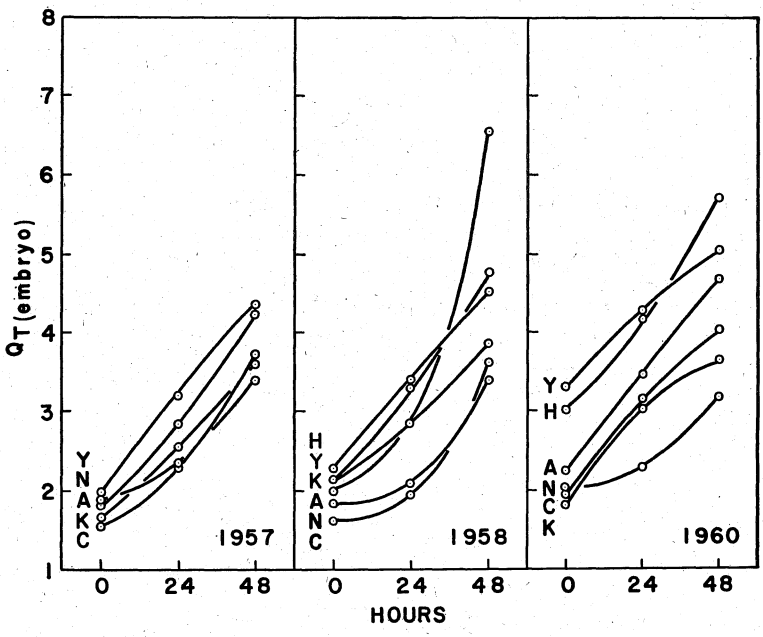

Fig. 1. GAT activity of barley embryo homogenates during the first 48 hours after moistening, expressed on a per-embryo basis. A, Ankober; H, Hannchen; Y, Ymer; K, Kindred; N, 50-N-15; and C, C.I. 5000.

In general the Q_T (embryo) values in Fig. 1 tend to increase in a nearly linear fashion during the first 48 hours after moistening, although there are some notable exceptions, such as 50-N-15, in which the activity increases in an exponential manner with time for all three of the years, and the 1958 Ankober samples, which exhibited an unusually large increase after 24 hours. The varieties Hannchen and Ymer are consistently high with respect to the Q_T (embryo) value. These are two-rowed varieties and although they tend to have somewhat larger embryos than the varieties C.I. 5000, 50-N-15, and Kin-

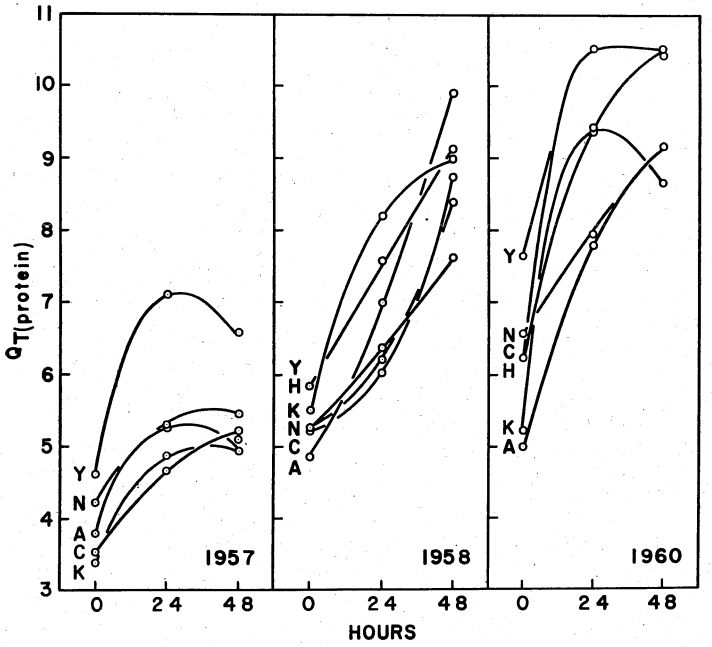

Fig. 2. GAT activity of barley embryo homogenates during first 48 hours after moistening, expressed on the basis of the protein present. SD = 0.25. Letter symbols are the same as for Fig. 1.

dred, it does not appear that embryo size is the only determining factor because Ankober also has relatively large embryos and it is not consistently high in its Q_T (embryo) values.

The S.D. of ± 0.25 was found for the Q_T (protein) values obtained with the 1957 and 1958 samples. At the statistical 95% confidence level a difference of approximately twice the standard deviation, or 0.50 in the Q_T (protein) values, is required. The only instance where such a large difference occurs between the rapid-germinating varieties Hannchen, Ankober, and Ymer and the other varieties is at 24 hours with the 1958 samples. Numerical ranking of the varieties of barley used in these experiments in decreasing order of their Q_T (protein) values for the 0- and 24-hour samplings, the period during which the highest germination rates occur and in which the influx of nitrogenous materials to the embryo is lowest, reveals the following order, with the average numerical rank in parentheses: Ymer (1.2); Hannchen (2.3); 50-N-15 (3.2); Kindred and C.I. 5000 (4.0); and Ankober (4.3). The two-rowed varieties Hannchen and Ymer are more active in embryonic GAT activity on the basis of Q_T (protein), as well as on the basis of Q_T (embryo). Since these are the only two-rowed barleys

TABLE II

EMBRYO PROTEIN CONTENT DURING GERMINATION

VARIETY	PROTEIN (PER EMBRYO)		
	0 Hours	24 Hours	48 Hours
	γ	γ	γ
1957			
Hannchen	... ...	... ...	... ...
Ankober	487 ± 2	548 ± 2	840 ± 95
Ymer	440 ± 4	458 ± 4	738 ± 23
50-N-15	444 ± 16	447 ± 30	626 ± 32
Kindred	494 ± 7	530 ± 9	733 ± 9
C.I. 5000	440 ± 19	500 ± 40	715 ± 10
1958			
Hannchen	415	418	503
Ankober	413	411	558
Ymer	372	437	522
50-N-15	348	338	406
Kindred	406	444	510
C.I. 5000	309	328	412
1960			
Hannchen	444	442	543
Ankober	449	447	509
Ymer	430	408	484
50-N-15	311	300	347
Kindred	346	323	424
C.I. 5000	311	303	382

in the group studied, it appeared the level of GAT activity might be associated with barley type; however, as data obtained with isogenic two- and six-row barleys will illustrate, exceptions to this thesis also were found.

It is evident from the curves in Figs. 1 and 2 that basing the activity on the amount of protein present markedly alters the patterns obtained. After the first 24 hours the Q_T (protein) values tend to level off or to actually decrease as a result of the influx of nitrogenous substances from the endosperm and the attendant synthesis of embryo protein. The protein contents of the embryos during this period are summarized in Table II. A comparison of these values indicates that there is more variation in the time required for a net synthesis of protein to occur than there is for the increases in GAT activity.

Effect of Kernel Nitrogen Content upon GAT Activity of the Embryo. Isogenic two-rowed and six-rowed barleys which had been grown at space intervals of from ¼ to 8 in. were selected for study on the basis of their nitrogen contents. Such samples presumably differed in respects other than nitrogen content, but many environmental factors affecting growth were similar. Some of the pertinent characteristics of these barleys are presented in Table III. The larger spacing intervals were conducive to higher nitrogen contents and

kernel weights in both types of barley, but the germination rates of such samples tended to be lower. The lowered germination rates may be an effect of the degree of ripeness at the time of harvest, since the sample of two-rowed barley grown at the 8-in. interval had some (4.6%) kernels which possessed a green cast; however, the two-rowed sample grown at the 4-in. interval did not appear to contain such unripe kernels and it also germinated at a relatively low rate. The germination rate of the two-rowed samples is consistently greater than that of the six-rowed material.

TABLE III

NITROGEN CONTENTS AND GERMINATION RATES OF ISOGENIC TWO-ROWED AND SIX-ROWED BARLEYS

BARLEY	SPACING DURING GROWTH	N[a]	KERNEL WEIGHT	GERMINATION	
				24 Hours	48 Hours
	in.	%	*mg.*	%	%
16-vv-20 (six-rowed)	0.5	1.73	28.0	51 ± 4	99 ± 0
	4	2.01	33.8	69 ± 2	99 ± 0
	8	2.07	33.9	41 ± 3	97 ± 0
16-VV-20 (two-rowed)	0.25	2.18	46.3	92 ± 2	99 ± 0
	1	2.31	47.8	89 ± 2	99 ± 2
	4	2.60	47.9	77 ± 1	96 ± 0
	8	2.76	49.8	78 ± 4	97 ± 1

[a] Dry basis.

The results of assays for GAT with embryo homogenates prepared in 0.2*M* sucrose in 0.05*M* phosphate, pH 7.0, are summarized in Fig. 3. The Q_T (protein) values for the ungerminated six-rowed barleys exhibit a nearly linear relationship between kernel nitrogen and embryo GAT activity; however, this pattern does not exist at 24 or 48 hours. The activities of the two-rowed samples tend to follow a different pattern: there is no correlation between Q_T (protein) and nitrogen content among the ungerminated samples, but the 24- and 48-hour samples showed gradual increases in activity as the nitrogen level increased.

A comparison of the 24-hour germination readings (Table III) with the respective Q_T (protein) values of Fig. 3 reveals some differences between the two-rowed and six-rowed barley types. There is relatively better germination of the six-rowed barley possessing the highest Q_T (protein) value within its group, but of the two-rowed samples, those possessing the highest Q_T (protein) values at 24 hours are somewhat slower to germinate. It is also evident that while the two-rowed samples as a group have a higher germination rate than the six-rowed samples, they are lower in GAT activity as measured by

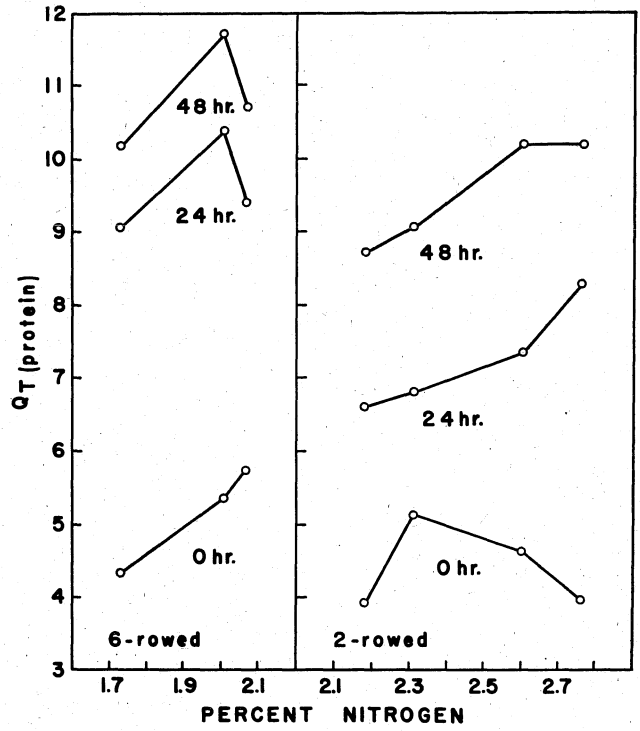

Fig. 3. GAT activity of embryos from isogenic six-rowed and two-rowed barleys containing different amounts of nitrogen during the first 48 hours after moistening.

Q_T (protein). This contrasts with the data obtained with the barley varieties known to possess different germination rates in which the two-rowed varieties were consistently high in GAT activity and rate of germination.

Discussion

The increases in GAT activity observed in the barley embryos during the first 24 hours after moistening occur during a period in which the germination process is proceeding at a rather high rate. However, relatively small, and in some instances no detectable, increases in embryo protein were observed during this period. For example, the average Q_T (embryo) values for the six varieties of barley listed in Fig. 1 increased from 36 to 49% during the first 24 hours, while the average change in embryo protein was found to vary from –3 to 8%, using a method which was found to be reproducible to within less than 3% variation from the mean for duplicate 0-hour homogenates and to within 4% for duplicate 24-hour homogenates

(Table II). It is apparent that increases in GAT activity occur simultaneously with an increase in germination and that these events precede the period of active growth and protein synthesis within the barley embryo. Consequently, it is not illogical to assume that the GAT is in some way concerned with the germination process. However, the attempts to correlate germination *rate* with the initial (0-hour) transaminase activity or the rate of increase in GAT during the first 24 hours resulted in no consistent relationship, principally because of the low GAT activity of the variety Ankober, a rapid-germinating barley, and the variable levels of GAT in the embryos of the variety 50-N-15, which is a slow-germinating variety. If GAT is indeed required for germination, apparently a level of the enzymes well below that found in the varieties examined is sufficient and the data obtained reflect superoptimal rather than limiting levels of the enzyme. Thus GAT activity is not a reliable index of the germination characteristics of the barleys studied.

The apparent differences between the present data and those of Leonard and Burris (7) appear to result from differences in methods and timing. Their samples were germinated under pilot malting conditions, which involved a steeping period that may vary from as little as 24 hours to as much as 50 hours. As a result, some of their samples had germinated before the initial samplings were made. Since the Q_T (N), or the transamination quotient based on the amount of nitrogen present in the homogenates, was used as their basis for comparison, it appears that some of their lower Q_T (N) values may have resulted from large amounts of nitrogenous materials entering the embryo rather than from low levels of GAT. A third possible difference is that of the growing environment of the barleys. Our experience with samples from several geographical locations indicates that the effect of growing environment upon the barley kernel can be of prime importance as far as germination behavior and GAT activity are concerned.

The means by which the initial increase in GAT activity occurs is not evident from the present data, but some indication that the activation of existing apoenzyme by coenzyme may be involved was obtained when saturating levels (33 γ/ml.) of pyridoxamine-5-phosphate were added to embryo homogenates. Duplicate determinations with the five 1957 samples revealed that the 0-hour homogenates were stimulated by an average of 44% over the untreated control homogenates, and that the 24-hour preparations increased by only 27% and the 48-hour preparations by 20%. This suggests that both

apoenzyme and holoenzyme exist during the first few hours after moistening and that, as the embryo becomes fully activated, less of the apoenzyme as such is present because of the increased availability of endogenous coenzyme.

The results of the experiments with the isogenic two-rowed and six-rowed barleys of different nitrogen content do not follow any consistent pattern; the ungerminated six-rowed and the 24- and 48-hour two-rowed samples reveal nearly linear relationships between kernel nitrogen and embryo GAT activity, but the rest of the samples do not follow any recognizable relationship. The two-rowed samples tended to germinate more rapidly than the six-rowed samples, a fact in agreement with data on most of the samples listed in Table I, but the isogenic six-rowed materials were much higher in Q_T (protein) than the two-rowed samples at 24 and 48 hours. This further substantiates the conclusion that GAT activity varies quite independently of the ability of a barley to exhibit rapid or uniform germination. The nitrogen content of the isogenic two-rowed and six-rowed barleys appears to have no marked or consistent effect upon the GAT activity of the embryo; however, the limited number of suitable samples which were available and the relatively high levels of nitrogen in these samples limits interpretation of these data.

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