Rapid and Objective Methods for the Estimation of Pregermination and Viability in Barley[1]

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ABSTRACT

This article discusses developmental approaches in pursuit of rapid, objective methods for estimating pregermination and viability in barley and provides details of a method for each. The assay for pregermination consists of incubating an aliquot of barley extract with dilute starch and, after 30 min, testing for residual starch using iodine. Viability was estimated spectrophotometrically on propanol extracts of barley grists slurried with 1% 2,3,5-triphenyltetrazolium chloride. The methods provide reliable estimates of a barley's germination potential at the time of testing and predict what it will be after several months in storage. Accordingly, these methods could be useful in barley selection. Pregermination, viability, and storage time affect germination. Barleys that pregerminate at harvest could germinate again in the malthouse, but their ability to do so decreases as storage time increases. The rate of decrease appears to be related to viability and to the degree of pregermination. Canadian malting barley varieties (two- and six-rowed) are susceptible to pregermination and hence loss of germination in storage. The adverse effects of poor germination on malt quality were studied and results of a pilot-scale malting trial reported.

Keywords: Barley, Pregermination, Viability, Longevity, Selection techniques

Sound viable grains are required to ensure even germination in malting barley (5) and good baking properties of dough in bread wheats (6). In the absence of dormancy, cereals have a propensity to pregerminate in the field during extended periods of moisture at harvest. The adverse effects of pregermination on barley malts and breadmaking quality of wheat have long been recognized, and for years, selectors have visually selected for pregerminated grains. However, visual examination cannot detect incipient pregermination, and many techniques for detecting pregermination have been proposed. Similarly, germination has long been associated with seed vigor or viability, and techniques for estimating vigor have been developed.

Methods for estimating degree of pregermination are based on activity of enzymes (mainly α-amylase and lipase) that are synthesized when cereals pregerminate. Assays using a variety of principles detect α-amylase (2,7,12,18,20,21,23,28), including one that served as the standard method of the European Brewery Convention (EBC) and Institute of Brewing for a number of years (9,15). The two methods presently recommended by the EBC (10) are the Carlsberg fluorescein dibutyrate method (16,17). The former is also the recommended method of the ASBC (4) based on lipase activity and the Heineken methylene blue method (30) based on modification and dye penetration.

Methods for estimating seed vigor or viability in cereals and other species use one of several principles. Those traditionally applied to barley are based on the reduction of various tetrazolium salts, notably 2,3,5-triphenyltetrazolium chloride (TZ), to an insoluble red formazan by viable embryo cells (1,8,10,19,29). The automatic seed analyzer (ASAC-1000, Neogen Corp., Lansing, MI) operates on the conjecture that membranes of weak seeds in an aqueous environment allow ions to pass through, changing the conductivity of the medium. Similar approaches relate viability to pH changes of the solution (24) and to the ability of seeds to reduce solutions of resazurin (26). The rate of incorporation of labeled amino acids by embryo cells during protein synthesis has also been used as an index to estimate seed viability (27).

Canadian malting barley varieties are susceptible to pregermination in the field. Barley that pregerminates can regerminate in the malthouse after harvest, but its ability to do so decreases as storage time increases (1,17). Most selecting in Canada is done within a month or two after harvest, and even if germinations were done on the barleys at that time, the selector has no way of knowing how many of those selections would possess good germination several months later when the barley is called in. Testing preload samples for germination before authorization of loading ensures that only barleys with good germination are delivered. However, this procedure does not eliminate all problems. Selectors do not know how many selected barleys will lose germination in storage, which makes it difficult to know how much to select initially. Also, it is disappointing and costly to farmers advised in September that their barley was selected for malting to be informed several months later that it is no longer acceptable. Therefore, the present work was undertaken to investigate methods that selectors can use to identify barleys with potential germination problems and thus provide them with selection criteria.

None of the numerous methods proposed for this purpose is in widespread use today. They are either too subjective or time-consuming, or they do not adequately relate to the germination potential of a barley after several months of storage. This article discusses developmental approaches toward more suitable techniques and provides details of a rapid, objective method for pregermination and viability. The techniques were used to study the effects of pregermination and vigor on germination and malt quality; the results of these studies are presented.

EXPERIMENTAL

Barley and Malt Samples

Barley samples were obtained from the barley selection offices of Canada Malting Co. Ltd. in Winnipeg and Calgary and from loads taken off rail cars and trucks at the company's plants in Calgary and Thunder Bay. All samples represented distinct permits and locations. Composites were not considered suitable for this type of investigation. Malts were prepared either in the laboratory or in the plant following procedures previously reported (25).

Barley Germination Counts

Germination energy (GE) counts on the barleys were obtained by incubating 100 seeds at 14.5°C for 72 hr. The seeds were contained in a 90-mm petri dish lined with two filter papers (Whatman No. 1) wetted with 4 ml of demineralized water. The difference between this count and a second sample of 100 seeds similarly prepared (except that they were wetted with 8 ml of water) provided an estimate of water sensitivity. Counts of the 4-ml sample after 120 hr provided an estimate of germination capacity. For some samples, four-day, 6-ml determinations were conducted, alternatively or additionally. All determinations were made in duplicate.

Viability—Conductivity Approach

The conductivity principle for estimating seed viability was investigated in the following manner (an automatic seed analyzer was not available). Four barleys representing a range in

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germination were selected, and 2, 10, and 50 g of each barley was weighed into separate 250-ml beakers. To each was added 150 ml of demineralized water. The samples were allowed to stand overnight at room temperature. The samples were then stirred and filtered through filter paper (Whatman No. 41); conductivity readings of the filtrates taken.

Viability—Resazurin Approach

The dye resazurin has shown some capacity to predict and assess malt quality (13,22,31) and to estimate seed viability of peas, beans, and cucumbers (26). In the present study, the potential of this dye to estimate barley viability was investigated following basically the same procedure as that used to assess malt quality.

In triplicate, 10 g of barley and 20 ml of 0.0125% aqueous resazurin solution (pH adjusted to 7.0 with dilute HCl) were incubated for 17 hr in 35-ml stoppered test tubes at 40°C. The tubes were then inverted several times, and after an addition hour of incubation, color differences among the samples were visually assessed and assigned values of 1.0–7.0; purple samples received a 1.0 rating; colorless samples, 7.0 (Fig. 1). The value reported for each barley was the mean of the three samples.

An approach using a petri dish and filter paper was also investigated. Barley samples (10 g) were incubated for 18 hr at 37°C in a 9-mm petri dish fitted with a filter paper (Whatman No. 1) wetted with 4.0 ml of 0.0125% resazurin solution. After incubation the seeds were removed, and the percentage of the area of filter paper that had turned pink was estimated (Fig. 2). Each barley was tested in triplicate; the value reported for each barley was the mean of the three samples.

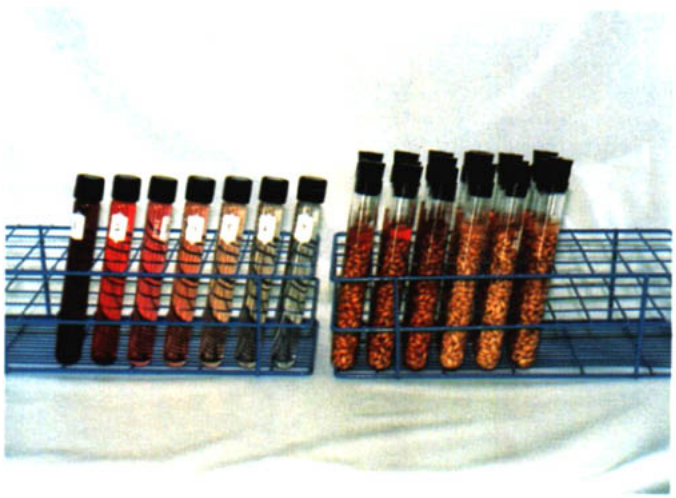

Fig. 1. Barley viability estimates by resazurin procedure. Resazurin standards (tubes 1–7) and typical color changes effected by different barleys.

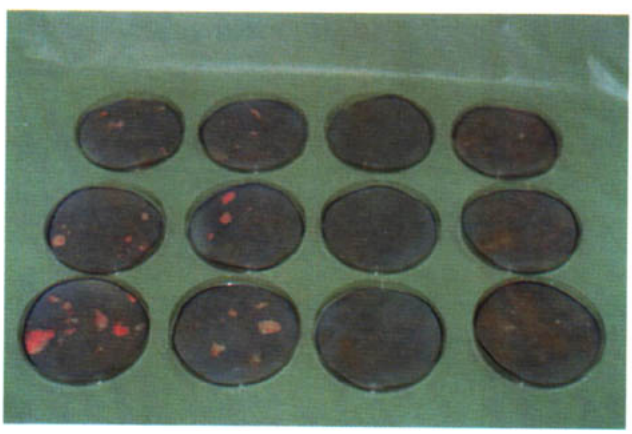

Fig. 2. Petri dish-filter paper resazurin approach to estimate seed viability (four barleys, three determinations); dishes after incubation, with seeds removed.

Viability—TZ Approach

Vitascope data for estimating barley viability using TZ (available from several suppliers, e.g., No. T-8877 from Sigma Chemical Co.) were generated following the EBC procedure (10). Experimental approaches aimed at imparting objectivity to the TZ test led to the method detailed below.

Fig. 3. Development of red formazan color applicable to barley grist-tetrazolium slurries of two barleys.

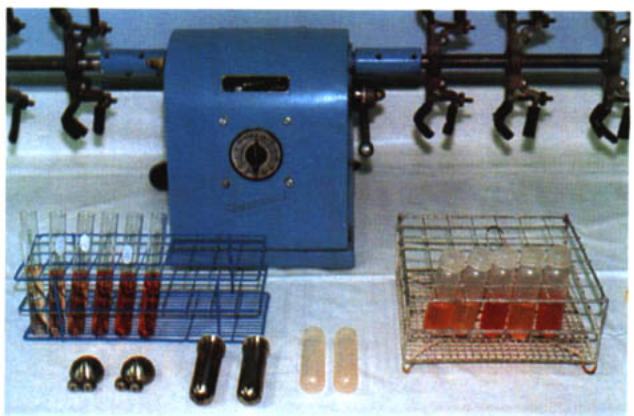

Fig. 4. Tetrazolium viability test; wrist shaker technique for red formazan color extraction.

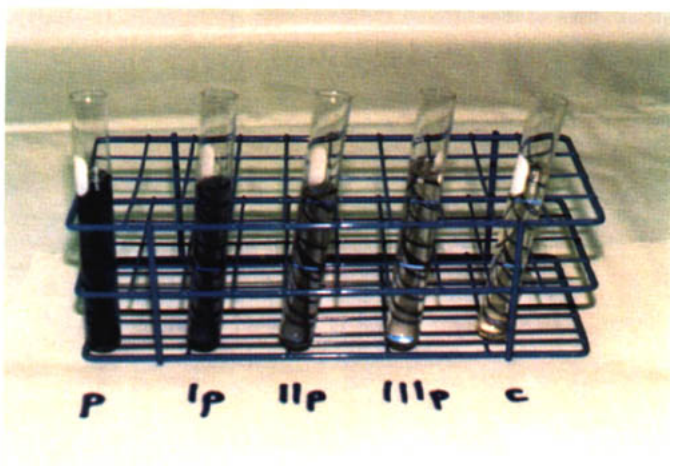

Fig. 5. Color range observed with starch-iodine α-amylase pregermination test. p = purple, l = light, c = clear.

A barley sample (7–10 g) was ground using a fine-grind mill setting according to ASBC method Malt-4 (3), and 3 g of the grist were weighed into a 50-ml polypropylene centrifuge tube. Forty samples were found to be a convenient size. Using a dispenser, 6 ml of 1% aqueous TZ was quickly added to each tube, completing the addition of reagent to the 40 samples in about 5 min. The bottoms of the tubes were examined for dry areas of grist, and if necessary, the contents were stirred with a fine glass rod. The tubes were capped and incubated in a 40°C water bath for 1 hr. Propanol (35 ml, 84%) was quickly added to each tube; the tubes were shaken vigorously and put back into the water bath for overnight extraction of the red formazan that formed during incubation (Fig. 3). The tubes were then shaken and centrifuged for 10 min at 10,000 rpm or filtered (Whatman No. 41). The supernatants or filtrates were attemperated at 40°C for about 5 min before absorbance at 480 nm was determined; the spectrophotometer was first zeroed with water. A technician following this procedure could conveniently analyze 80 samples per day.

When more immediate estimates of viability were required, overnight extraction was replaced with extraction by homogenizer or wrist shaker. When a homogenizer was used, 20 rather than 35 ml of 84% propanol was added, and the mixture was homogenized for 1 min with a homogenizer (model PT10/35, Polytron) at setting 5.0. Color extraction by wrist shaker was done with stainless steel centrifuge tubes (custom-made, with screw cap) (Fig. 4). Grist (2 g) and TZ solution (4 ml) were incubated in the tubes. Two stainless steel ball bearings (diameter, 1 cm) were then placed into the tube; 20 ml of 84% propanol was added, and the tubes were shaken at maximum setting for 15 min. The contents of the tube were then centrifuged in regular tubes and the absorbance of the supernatants read at 480 nm.

Pregermination—Falling-Number Approach

Falling-number data were generated on an AB unit (Stockholm, Sweden) following the ICC procedure (14).

Pregermination—Starch-Iodine Approach

Existing tests for determining α-amylase based on the starch-iodine reaction were not considered suitable for use by commercial barley selectors; thus, the following simplified procedure was developed.

Working with a batch size of 60 samples, 12–14 g of barley was finely ground in a Udy mill fitted with a 1-mm screen. The grist (5 g) was weighed into a 125-ml Erlenmeyer flask, and 50 ml of water was added. The flask was stoppered and shaken vigorously by hand. After 1 hr, the flask was shaken again and allowed to stand for 16 hr (overnight) at room temperature. The next morning, the flask was shaken and the contents filtered through a filter paper (Whatman No. 4), returning the first few milliliters. One milliliter of the filtrate was transferred into a 35-ml test tube. After sampling the 60th sample, the rack was placed into a 40°C water bath. Dilute starch (10 ml)—50 ml of reagent b starch solution according to ASBC method Malt-7 (3) diluted to 1 L with water—was added to the first sample at 0 sec and to each additional tube at 30-sec intervals thereafter, completing the addition to the 60th sample at 1,770 sec. After each addition, the contents of the tube were mixed by inverting the tube twice. At 1,800 sec, 1 ml of iodine solution—50 ml of 0.02*N* iodine solution prepared according to ASBC method Malt-4 (3) diluted to 500 ml with 2.0*N* H_2SO_4, stored in an amber bottle—was added to the first sample and to each additional tube at 30-sec intervals thereafter. Immediately after each addition, the contents of the tube were mixed by inverting the tube; the color was visually assessed and rated as purple (p), light purple (lp), llp, lllp, or clear (c) (Fig. 5).

If a more objective color assessment was required, absorbance was determined at 570 nm after zeroing the spectrophotometer with water. The spectrophotometric readings must be taken at a standardized time after addition of the iodine. In the present work, one sample was read every 30 sec, beginning immediately after the iodine was added and visually assessing the color of the last (60th) sample (at 3,610 sec).

RESULTS AND DISCUSSION

Table I presents germination data on four series of barleys representing two- and six-rowed varieties from the 1987–89 crop years. Although the mean germination counts after approximately one year in storage were still over 90% in the case of the 1987 and 1988 crops, many barleys of both crop years experienced significant loss of germination potential during storage. All the barleys of both series were the two-rowed Harrington variety. However, the 1989 barleys, which were tested about two months after harvest, consisted of 238 two-rowed barleys, mostly Harrington, and 135 six-rowed barleys of the varieties Bonanza and Argyle. Of the 238 two-rowed barleys, 41 had germinations of less than 95% (mean of the 41 was 83%). Of the 135 six-rowed barleys, 18 had germinations of less than 95% (mean of the 18 was 86%). These and other data generated by the Canada Malting Co. in the past eight years show that Canadian commercial barleys, both two- and six-rowed, are susceptible to germination loss during storage.

The primary objective of this work was to identify a method (or methods) that could be used in the selection process to provide a rapid estimate of a barley's germination potential and longevity. The success of the methods described in the previous section in achieving this objective is discussed below.

TABLE I
Germination Data Applicable to Four Series of Barley from the 1987–89 Crop Years Generated at Various Times After Harvest

Barley Series	Months After Harvest	Germination, % Mean	Range	Mean of the Lowest 20% of Samples
1987 (n = 100, Harrington)	2	95	70–100	87
	12	92	37–100	73
	22	87	5–100	57
1988 (n = 100, Harrington)	4	97	64–100	89
	12	96	55–100	85
1989 (n = 238, two-rowed)[a]	2	96	36–100	86
1989 (n = 135, six-rowed)[b]	2	97	62–100	89

[a]220 Harrington, 10 Ellice, 8 Klages.
[b]62 Bonanza, 73 Argyle.

TABLE II
Relationship Between Germination and Conductivity Readings for Four Barleys (Three Extract Types per Barley)

Barley Sample	Germination, % GE[a]	WS	GC	Conductivity[b] Barley-to-Water Ratio (g:ml)[c] 2:150	10:150	50:150
1	100	5	100	84	319	1,890
2	89	24	92	68	258	1,260
3	52	8	64	44	190	866
4	0	N/A	0	70	318	1,430

[a]GE = germination energy, WS = water sensitivity, GC = germination capacity.
[b]Conductivity readings were taken on a radiometer (CDM83, Copenhagen) conductivity meter at 24°C; conductivity of demineralized water was 1.07 microsiemens.
[c]Filtrate was prepared after soaking each weight (2, 10, and 50 g) of each barley in 150 ml of water overnight at room temperature.

Conductivity

Preliminary results relating conductivity readings of seed exudate to germination potential are presented in Table II. Good ranking agreement of the four barleys by the three barley-water ratios was obtained, but the readings did not line up with the germination values. In accordance with the theory of operation of the technique, a negative correlation between conductivity and germination was expected.

However, the good ranking agreement of the barleys by the three barley-water ratios was encouraging and led to the testing of the 100 Harrington barleys from the 1987 crop referred to in Table I, using a barley-water ratio of 25 g:150 ml. Testing was done about four months after harvest. The mean for the 100 samples was 526 microsiemens (range, 295–741). When correlated with regular germination counts generated on the barleys about two months after harvest and again about a year after harvest, correlation coefficients were +0.29 and +0.39, respectively.

The 100 barleys were also malted. The highest correlations between the conductivity readings and malt quality parameters were −0.43 for average acrospire length, −0.29 for wort color, 0.27 for wort pH, and 0.25 for fine-grind extract. Of several barley quality parameters tested, the conductivity readings correlated highest (−0.43) with barley β-glucan, as measured by a slight modification of the McCleary method (11). The correlation coefficients between the conductivity readings and germination counts or other barley and malt quality parameters were not considered high enough to warrant further investigation of the technique, and it was abandoned. However, the technique may have some future application because it showed wide differences among the samples, and the results were repeatable.

Resazurin

In studies showing that this agent has some capacity to assess malt quality, good malt quality was equated with its ability to reduce a solutuion of this dye to a colorless state (13,22). At pH 7.0, a dilute solution of resazurin sodium salt is deep purple. When soaked in a solution, good malts changed the color to a pinkish, then orangish, and finally clear color. In the present work, it was anticipated that sound vigorous barley seeds would bring about similar color changes and that weak seeds would induce no color change. Positive correlations with germination were therefore expected.

When the procedure was applied to the 1987 crop Harrington barleys in Table I, negative correlations were obtained between the resazurin readings and germination counts on the barley. The readings gave correlations of −0.40 and −0.32, respectively, with GE values generated on the barleys about two months arter harvest and about 12 months after harvest. A possible explanation for the negative correlations was recognized when subsequent work on pregermination revealed that pregerminated barleys were the ones most prone to render the resazurin solutuion colorless. Not only were α-amylase and lipase synthesized on pregermination, but also enzymes capable of rendering solutions of resazurin colorless (assuming that neither α-amylase nor lipase is capable). As shown later, pregermination leads to loss of vigor and germination capacity in storage, consistent with the observations and negative correlations obtained here.

However, the relationship between a barley's propensity to render a resazurin solution colorless and its germination potential was considered too weak and erratic to form the basis of a reliable selection technique. Efforts to improve the sensitivity and repeatability of the test included investigation of 1) more dilute resazurin solutions (0.00125–0.000125%), 2) different ratios of barley to solution, 3) ground barley instead of whole kernels, 4) different incubation temperatures, 5) different reaction vessels (beakers, test tubes, Erlenmeyer flasks), 6) various buffer solutions as media for dissolving the resazurin, 7) spectrophotometric color assessments at various wavelengths, and 8) agitation of the samples during incubation. None of these trials offered improvement. It seemed that the poor repeatability among triplicates was due to one or two nonrepresentative seeds in the sample. The approach using petri dish and filter paper was only partially successful at localizing the effects of such seeds and was too subjective (Fig. 2). In addition, results were adversely affected by the stacking of the dishes during incubation; dishes at the bottom of stacks consistently showed less color change. As a result of these failures, no further work with resazurin was done.

Viability—TZ Approach

The vitascope and seed-fixation methods that use TZ to estimate barley viability are subjective and cannot meet the need of selectors to handle large numbers of samples each day. In these methods, half-seeds are longitudinally sectioned by blade or abrasion and treated with a solution of TZ. Viability is estimated visually, based

TABLE III
Estimates of Tetrazolium Viability for Six Barleys by Three Formazan Color-Extraction Procedures

Barley	Absorbance, 480 nm for Extraction Procedure[a]: Dissolution Overnight (at 40°C)	Homogenizing (for 1 min)	Wrist Shaker (for 15 min)
1	0.17	0.30	0.23
2	0.17	0.29	0.27
3	0.30	0.45	0.43
4	0.31	0.47	0.53
5	0.50	0.60	0.75
6	0.58	0.73	0.90

[a] Values represent means of two determinations.

TABLE IV
Repeatability Data and Effect of Some Variables on Two Barleys Analyzed by the Tetrazolium (TZ) Viability Test[a]

	Absorbance, 480 nm	
	Barley 1	Barley 2
Repeatability, n = 10		
Mean	0.21	0.52
Range	0.21–0.22	0.51–0.55
SD	0.005	0.012
cv	2.3%	2.3%
Concentration of TZ, %		
0.2	0.20	0.32
0.5	0.22	0.42
1.0	0.22	0.50
2.0	0.22	0.54
Age of TZ		
2 hr	0.24	0.54
7 mo	0.22	0.52
30 mo	0.22	0.52
Incubation temperature, °C for 60 min		
25	0.16	0.28
40	0.23	0.51
60	0.16	0.27
Incubation time, min at 40°C		
10	0.11	0.19
30	0.17	0.37
60	0.21	0.48
120	0.22	0.59
Grist grind mill		
Udy (1-mm screen)	0.13	0.24
ASBC fine	0.21	0.50
ASBC coarse	0.27	0.55

[a] All data were generated by the standard procedure described in the text (overnight extraction) except the variable under investigation.

on the number of embryos that develop a red color and on the degree of redness.

In the present work, when barley grists were slurried and incubated with a dilute TZ solution, the slurry took on a red-speckled appearance (Fig. 3), with intensity in line with that of the vitascope or seed-fixation method. It was further recognized that if a solvent was found that would dissolve the water-insoluble red formazan, viability could be estimated spectrophotometrically.

Several acidic, basic, organic, and inorganic solvents found were investigated on a trial-and-error basis, including ethanol, which has shown some capacity to solubilize formazan from plant tissue cultures (29). The two most effective solvents were dichloromethane (DCM) and propanol. Although not as effective as DCM, propanol was adopted for routine application because the former presented handling difficulties. DCM is immiscible in water and layered at the bottom of the tube. It also reacted with polypropylene centrifuge tubes.

Typical data obtained by overnight extraction and by more immediate extraction using homogenizer and wrist shaker are presented in Table III. Repeatability data generated by overnight extraction are presented in Table IV. The repeatability performance of the test on any given day was very acceptable. Coefficients of variation of 2.3% were obtained for both barleys in Table IV. Day-to-day repeatability was not as good. Coefficients of variation for 12 barleys tested on six different days over a four-week period ranged from 3.0 to 7.5%.

Ruggedness data applicable to several variables are included in Table IV. TZ of 2% concentration typically showed the widest differences among barleys, although for routine work, 1% was considered adequate. Solutions were stable for at least two years when stored in an amber bottle at 4°C. Incubation temperature and time significantly affected color development and to minimize day-to-day variation. It was necessary to closely adhere to the specified conditions. ASBC Malt-4 (3) coarse-grind grists developed the most color, but the ASBC Malt-4 fine grind was adopted for routine work because (as discussed in the next section) it was also suitable for α-amylase extraction. The Udy grind (1-mm screen) was the least suitable of the three in terms of differentiating barleys. It was important to attemperate the supernatants at 40° C to remove opaqueness before determining absorbance. The supernatants, however, were stable for at least 24 hr.

Other variables investigated were 1) temperature of the TZ solution when added to the grist, 2) tightly capped versus loosely capped tubes during incubation, 3) light versus darkness during incubation, 4) freshly prepared versus week-old dilute propanol, 5) concentration of propanol, 6) different suppliers of the TZ reagent, and 7) attemperation temperature. Of these variables, the concentration of propanol had the most significant affect on the repeatability performance of the test. However, the slight differences in concentration that would be expected among preparations would not be significant enough to account for the higher day-to-day variation compared with within-day variation.

The cause of the higher day-to-day variation was not identified but seemed to be associated with the red color (formazan) extraction. Extraction by wrist shaker not only showed wider differences among barleys (Table III) but also gave better day-to-day repeatability. However, the technique is not as conducive to handling large numbers of samples each day as is the overnight extraction procedure.

TABLE V
Relationship Between Germination and Pregermination As Determined by Falling Number (Four Barleys and Four Barley Grists: Wheat Flour Mixtures)[a]

Barley Sample	Barley GE	FN Value, sec[b] Ratio of Barley Grist to Wheat Flour, g 7:0	5:2	3:4	1:6	Mean
1	60	74	125	198	289	172
2	70	53	72	146	241	128
3	85	147	138	252	362	225
4	100	208	284	428	540	365

[a] Data generated by R. B. Campbell, Agriculture Canada Research Station, Winnipeg.

[b] Falling number (FN) and germination energy (GE) data generated approximately one year after harvest; FN on the sound wheat flour was 460 sec.

Pregermination—α-Amylase by Falling Number

Four barleys representing a range in germination of 60–100% were ground using a fine-grind setting, according to ASBC method Malt-4 (3). Falling-number values were generated on the grists and on mixtures of each grist with a sound wheat flour according to the grist-flour ratios shown in Table V.

Good agreement between GE and falling-number values was obtained for all four barleys and grist-flour ratios. The barleys were about one year old at time of testing. Falling-number data were not generated on these barleys immediately after harvest, but based on α-amylase estimates obtained by a starch-iodine procedure (discussed below), the falling number could be used as a selection tool at harvest to identify barleys with potential germination problems if volume were not a major consideration. In the present work, however, the approach was abandoned in favor of the starch-iodine procedure.

Pregermination—α-Amylase by Starch-Iodine Test

The blue color reaction traditionally associated with starch and iodine has formed the basis of numerous α-amylase assays. Its simplicity encouraged investigation of the approach in the present work and led to the assay described in the materials and methods section.

The visual color-assessment procedure used in the assay is rapid and adequate for screening purposes. When the iodine was added, sound barleys that contained no α-amylase became purple, whereas barleys that had severely pregerminated and were high in α-amylase remained clear (they actually turned yellowish). Samples in these groups were rated p and c, respectively, and samples between these two extremes were rated light purple, llp, or lllp (Fig. 5). For some barley series, where more objective assessments were desired, the colors were measured spectrophotometrically at 570 nm (as described in the materials and methods section). The assay seems readily adaptable to automation by flow-injection analysis, which would essentially eliminate the tedium associated with the manual procedure and would be desirable for selectors, who encounter large numbers of samples.

Some repeatability statistics applicable to the test are presented in Table VI. Analytical variables investigated included different grist types, extractants, extraction-separation procedures, and various infusion parameters, such as the ratio of enzyme extract to starch substrate and incubation temperature and time. Essentially all variables examined affected the repeatability performance of the test, and as with most analytical assays, it was necessary to standardize conditions and adhere to them. The conditions presented were suitable for barleys of the 1987-89 crop years in western Canada and are proposed as the standard procedure. The sensitivity of the test, however, could be altered as needed by varying some of the parameters. For example, when some wheat samples were tested, it was necessary to increase the concentration of starch solutuion. Instead of 50 ml, 500 ml of reagent b (ASBC method Malt-7 [3]) starch solution were diluted to 1 L.

Udy grind and ASBC Malt-4 fine-grind grists produced comparable extracts, whereas extracts produced from ASBC Malt-4 coarse-grind grists were not satisfactory. The Udy grind was adopted over the ASBC Malt-4 fine grind because selectors use it to screen for protein by near-infrared reflectance

spectroscopy. Therefore, one grist preparation would serve both purposes in the selection process. Depending on a selector's in-house requirements, however, the ASBC Malt-4 fine-grind grist may preferable because it is suitable for the TZ test, whereas the Udy was not suitable (Table IV).

As an extractant, water was as effective as were dilute solutions of sodium chloride. Overnight extraction at room temperature was adopted for convenience, although results were comparable with 1-hr extractions with an orbital shaker at 40°C or with standing at room temperature for 1 hr with frequent shaking by hand.

Separation by filtration was more effective than by centrifugation or settling and would be the required procedure if the assay were adapted to flow-injection analysis. However, filtration is a somewhat time-consuming step and preferably excluded from assays with the large volume demands applicable here. Separation by settling allowed cursory estimates to be obtained, eliminating the need for filtration or centrifugation. The 1-ml aliquot of enzyme extract was sampled from the clear supernatant in the flask.

All reactants were stable throughout the periods of investigation. Grists were stable for at least two days at room temperature, dilute starch for at least one week when stored in a refrigerator at 4°C, and iodine solution for at least three months when stored in an amber bottle at room temperature.

The starch-iodine approach for estimating pregermination offers considerable flexibility. A standardized set of conditions is proposed, but selectors may choose to deviate from them to accommodate workload or in-house requirements. Sampling the extract from the supernatant (thus eliminating separation by filtration or centrifugation) and visual color assessment allow for greater speed and analysis of more samples per day, but at the expense of some precision. Also, brief studies indicate that the 30-sec interval between samples in the infusion and color-development steps could be omitted with minimal loss of precision. After sampling the 1-ml aliquot of extract for all 60 samples, the dilute starch (10 ml) could be added to all samples as quickly as possible (less than 5 min). Incubation could proceed for 25 min before the iodine is added to the samples in less than 5 min and in the same sequence used when the starch was added. An aqueous starch solution (0.05%, w/v) might replace the diluted preparation of reagent b ASBC Malt-7 (3), but this was not investigated in the present work. Appropriate comparisons would have to be conducted to ensure that the two preparations give comparable results. If successful, the former preparation would eliminate the need for buffer preparation and inclusion of β-amylase.

Effect of TZ Viability and Pregermination on Changes in Germination Potential That Occur During Storage

The effects of viability and pregermination as measured by the TZ and starch-iodine tests, respectively, on changes in germination that occurred during storage were studied in barleys from the 1987–89 crop years. Included in the 1987 barleys were the 100 Harrington barleys referred to in Table I. When tested for pregermination and viability about four months after harvest, 53 of the 100 barleys tested negative for pregermination, 24 tested slightly positive, and 23 tested highly positive. The mean TZ absorbance readings for the samples in each of these three groups was 0.56, 0.55, and 0.50, respectively. Germinations were generated on all 100 barleys at three time intervals after harvest. These data are shown in Table VII.

The 53 barleys that tested negative for pregermination still had a mean germination of 97% after 22 months in storage, 47 of them with germinations of 95% or greater. When tested for TZ viability four months after harvest, these 47 barleys had a mean TZ absorbance of 0.57, whereas the mean for the other six barleys was 0.45. At the other extreme, the 23 barleys that tested highly positive for pregermination had a mean germination of only 66% 22 months after harvest—21 with germinations less than 95% and two with germinations greater than 94%. The mean TZ absorbance for the 21 barleys when tested four months after harvest was 0.48, and the mean of the two barleys in the latter group was 0.66. Similar trends were found for the 100 Harrington barleys of the 1988 crop referred to in Table I.

Table VIII presents germination and pregermination data generated on 1989 two- and six-rowed barleys about two months after harvest. Again, the relationship between pregermination and germination was apparent. Germinations were not generated on the barleys after longer storage periods, but the trend seen in Table VII would be expected to apply. Note that both two- and six-rowed barleys were susceptible to pregermination and loss of germination during storage. Within barley type, no varietal differences were apparent.

TABLE VI
Repeatability Statistics for Three Barleys Analyzed 10 Times on the Same Day by the Starch-Iodine Pregermination Test

	Absorbance, 570 nm of Barley Sample		
Statistic	1	2	3
n	10	10	10
Mean	0.58	0.32	0.10
Minimum	0.47	0.26	0.07
Maximum	0.61	0.35	0.12
SD	0.039	0.027	0.014
cv	6.8%	8.4%	14.6%

TABLE VII
Effect of Pregermination and Viability on Changes in Germination That Occurred During Storage in 100 Harrington Barleys (1987 Crop)

	Pregermination[a]		
	Negative	Slightly Positive	Highly Positive
n	53	24	23
Mean germination, months after harvest			
2	98	96	88
12	98	92	76
22	97	85	66
Mean tetrazolium[a] viability (absorbance)			
All samples	0.56	0.55	0.50
Samples with >94% germination	0.57 (47)[b]	0.61 (12)	0.66 (2)
Samples with <95% germination	0.45 (6)	0.50 (12)	0.48 (21)

[a] Pregermination and viability data were generated four months after harvest.
[b] Values in parentheses = number of samples.

TABLE VIII
Germination and Pregermination Data on Two- and Six-Rowed Barleys Generated About Two Months After Harvest (1989 Crop)

	Pregermination		
Barley Type	Negative	Slightly Positive	Highly Positive
Two-rowed (n = 238)[a]			
n	136	24	78
Mean germination, %	99	97	91 (74)[b]
Six-rowed (n = 135)[c]			
n	56	35	44
Mean germination, %	99	99	94 (81)

[a] 220 Harrington, 10 Ellice, 8 Klages.
[b] Values in parentheses = mean of the lowest 20% of the samples in this group.
[c] 62 Bonanza, 73 Argyle.

In addition to the barley series shown in Table I, many other barleys of the 1987–89 crop years were tested. Results for all barleys were consistent with those in Tables VII and VIII and further showed that the germination potential of a barley in storage at any given time is affected by pregermination, viability, and storage time. Depending on the age of a barley when tested, it could fall into one of five possible groups of test results with regard to the presence of α-amylase, viability, and germination potential, as follows,: 1) negative for α-amylase, high in viability, and high in germination potential; 2) negative for α-amylase, medium to low in viability, and high in germination potential; 3) positive for α-amylase (Fig. 5, lp to c), high in viability, and high in germination potential; 4) positive for α-amylase, low in viability, and low in germination potential; 5) negative for α-amylase, very low in viability, and very low in germination potential.

The barleys in group 1 would be the most desirable for malting. They did not pregerminate in the field, are high in viability, and should retain a high germination potential indefinitely. Barleys in group 2 also appear to retain their germination capabilities for long periods despite only mediocre viability readings. Unlike barleys in group 5 (discussed below), barleys in this group would never have experienced any pregermination. Barleys in group 3 are typical of those of a wet harvest tested soon after harvest. Many of the later selections of the 1989 crop in western Canada were this type. The moist conditions at harvest caused many barleys to pregerminate. However, because they were tested soon after harvest, they tested high in viability and germination. If retested after several months in storage, many of these barleys would fall into group 4, and if tested after about two or more years in storage, some would fall into group 5. The α-amylase that was present earlier in storage would denature and no longer be detected by the test. Although this phenomenon was observed in the present work, few of the barleys deteriorated to this extent. The rate at which a barley progresses from group 3 to group 4 or 5 probably depends on degree of pregermination and initial viability of the barley.

No attempt was made in the present work to explain the phenomena observed. Whether dormancy and viability act independently or are related in some way and share a common mechanism is unknown to the author. Presumably, the reason the barleys pregerminated under adverse conditions at harvest was that they lacked dormancy. However, in discussing the work of Gordon, Brooks (5) points out that pregermination could be affected by physical or external factors. Pregermination occurred in dormant grains showing rupture of the pericarp-testa, whereas intact grains showed no pregermination. Canadian malting barleys, both two- and six-rowed, are susceptible to kernel peeling and damage, and their propensity to pregerminate in the field under moist conditions at harvest is consistent with the observations of Gordon.

In the present work, moist conditions at harvest not only gave rise to pregermination but also were conducive to microbial development. The α-amylase detected in some cases may have been of microbial rather than incipient origin, although this is unlikely. Studies on possible interrelationships among dormancy, viability, kernel damage, and microbes on pregermination were beyond the scope of the present study but remain a challenge for future work.

Commercial Application of TZ Viability and Pregermination Tests

The TZ viability and starch-iodine α-amylase pregermination tests detailed in the present work seem to provide a reliable estimate of a barley's germination potential at the time of testing; they also predict the potential after several months in storage. Therefore, the objective of the study was largely met, and it would behoove selectors to apply these techniques in their selection process. Including both tests in the process would represent maximum use and benefit. If testing were done at or shortly after harvest, consistent with the objective of the test, selectors would encounter only the scenarios of groups 1–4 (discussed in the previous section). Based on present knowledge, including data in the present work, the barleys of choice would be those in group 1 and possibly group 2. If supply shortages require selectors to consider barleys that test positive for α-amylase, those barleys should be further subjected to the TZ test; barleys testing low in TZ should be rejected. Those that test high in viability could be selected but should be malted as soon as possible after harvest.

If resources or barley supplies do not allow selectors to be as critical as they would like to be, at least cursory estimates of pregermination should be made. A rapid, less sensitive version of the test should be used to identify and reject at least the most severely pregerminated barleys. Test sensitivity could be lessened by increasing the concentration of starch or reducing the volume of enzyme extract used in the infusion. As previously discussed, larger numbers of samples could be tested each day if flow-injection analysis were used or if various short-cuts were implemented.

Effect of Poor Germination on Malt Quality

The minimum germination capacity generally considered acceptable for malting barley is 95% (5). The present work and that of others (1,17) show that some barleys test well below this level, especially after several months in storage. Although blending of barleys in shipping and in the malthouse tends to mask barleys with poor germination and maintain average germination counts well over 90%, batches of barley at the malthouse may have germinations well below 90%. This is particularly true for barleys of crop years with extended periods of rainfall at harvest.

The 1985 crop year in western Canada was the most notorious in recent years for producing barleys with poor germination. Modification was erratic, and many malts produced worts with viscosities in excess of 1.60 cp. However, the adverse effects of poor germination on malt quality often are not readily apparent, and it is difficult to obtain reliable estimates of the extent to which poor germination affects quality. To gain more of an appreciation of the effects of poor germination on malt quality, and hence an appreciation of the importance of exercising more critical selection, several laboratory maltings were conducted, including the following trial.

About 90 kg of barley with 83% germination was sampled from a commercial lot and malted in a pilot malting unit. At the end of germination, green malt was sampled, and dead berries were manually separated from live berries. Both fractions, along with another sampling of the parent green malt, were then kilned simultaneously in a laboratory-sized micromalting kiln. The kilned malts were then subjected to standard and special analyses.

The data in Table IX show that the 100% live fraction produced the best overall analysis. However, the parent fraction, with a germination of only 83%, also produced a fairly typical analysis (hazy wort was the only atypical item). Even the analysis of the dead-berry fraction far exceeded expectation. The fine-coarse difference was high, the wort was very hazy, and the amylase levels were lower than ever observed commercially. However, fine-grind extract, protein solubilization, wort viscosity, and malt friability were comparable to levels sometimes obtained on commercial malts, especially those made from barleys typical of the 1985 crop year.

Regarding the friabilimeter data, much lower friable and much higher whole-kernel values were expected. In fact, the latter fraction should theoretically have been 100% instead of 7.8%. Also, since the kernels were supposedly dead, they should have shown 100% fluorescence the full length of the kernel when analyzed by the Carlsberg Calcofluor homogeneity test (22). Instead, only 32% of the kernels showed fluorescence from one fourth to full length of the kernel, and 44% showed no fluorescence, indicating that these kernels were in fact fully

modified.

There are two possible explanations as to why analyses of the parent and dead berry fractions exceeded expectations. First, berries were considered dead if no evidence of germination was apparent—specifically, if no rootlets or acrospires were visible after four days of germination. In fact, closer analysis using a hot chlorine solution to remove the husk revealed that 12% of the dead berries had developing acrospires in the 0–1/4 category. Furthermore, although no outward manifestations of germination were evident (visible rootlet and acrospire development), some modification apparently was occurring. This would explain the significant amount of modification revealed by the Calcofluor and friabilimeter homogeneity tests. The second possible explanation is that the live berries modified well and produced an excess of enzymes that tended to carry the dead berries. This would apply particularly to indexes of modification such as wort viscosity, where individual kernels can affect others during mashing and raise the average modification value of the malt sample.

Therefore, it is difficult to assess the extent to which poor germination adversely affects malt quality in commercial malt production. The data in Table IX probably present a realistic estimate, but chances are they underestimate the extent. The effects may be accentuated when a barley is malted en masse in a malthouse versus a small-scale laboratory, as in the present study. As previously mentioned, commercial malts with viscosities around 1.60 cp are sometimes produced, and germination often is the only barley quality or processing parameter that is suspect.

In the absence of accurate estimates, however, perhaps it should be accepted that poor germination adversely affects quality, and if measures are available to control the germination capacity of barleys delivered to the malthouse, they should be used. The measures not only should include use of more critical selection using procedures such as those proposed in the present work, but also should attempt to incorporate dormancy into varieties. The latter is not an easy task; there appears to be an inverse relationship between dormancy and maltability. Present-day Canadian malting barley varieties are very susceptible to pregermination and, consequently, loss of germination capacity during storage. Nevertheless, the overall quality of malts produced from today's barleys far exceeds that of older varieties with dormancy. Given the choice, maltsters and brewers are much better off with today's barleys. However, this realization has not precluded breeders from trying to develop good malting barley varieties with dormancy; efforts have been underway for some time.

TABLE IX
Malt Analysis Applicable to Three Malts Produced from Three Subsamples of a Green Malt Differing in Percent Germination[a]

	Subsample or Fraction of Kernels		
Sample	Parent	Live	Dead
Barley			
Germination, %	83	100	0
Pregermination	Positive	NA	NA
Malt analysis			
Extract (fine) %, db	80.7	81.1	79.4
Extract (coarse) %, db	79.5	80.2	74.8
Fine-coarse difference, %	1.2	0.9	4.6
Wort color (°SRM)	1.51	1.54	2.89[b]
Wort clarity	Hazy	Hazy	Very hazy
Diastatic power (°ASBC)	117	121	61
α-Amylase (DU)	58	63	19
Malt protein, %	11.64	11.64	11.64
Wort protein, %	5.33	5.63	4.24
Soluble-total protein, %	45.8	48.3	36.4
Wort viscosity, cP	1.41	1.38	1.56
Malt friability			
Percent friable	80	83	58
Percent whole kernels	2.4	0.2	7.8
Calcofluor fluorescence, %[c]			
0	71	66	44
0–1/4	22	18	20
1/4–3/4	6	16	32
3/4–1	1	0	0

[a] All analyses were performed according to ASBC methods (3).
[b] Erroneous high color reading due to haze.
[c] A fully modified kernel would show zero fluorescence when tested with Calcofluor. At the other extreme, a dead or totally nonmodified kernel would fluoresce the full length of the kernel. For example, 71% of the kernels in the parent fraction showed no fluorescence and were therefore fully modified. The remainder of the kernels in the fraction showed varying degrees of fluorescence and hence varying degrees of nonmodification.

SUMMARY

Efforts to develop tests to estimate barley viability based on conductivity and principles of resazurin dye reduction were unsuccessful. The conductivity approach investigated consistently showed wide differences among extracts of different barleys, but the data did not adequately relate to the germination potential of barleys. Conductivity readings generated on 100 barleys four months after harvest produced weak positive correlation coefficients, with germination counts generated 2 and 12 months after harvest.

Approaches investigating the resazurin reduction principle were unsuccessful because of subjectivity and repeatability concerns. Even triplicate testing did not adequately compensate for erratic readings. In general, however, barleys that tended to render resazurin solutions colorless were those with the greatest decrease in germination potential during storage. This observation was contrary to expectation but consistent with the barleys that pregerminated.

A rapid, objective test for viability was developed using the TZ dye. Barley grist was slurried with 1% TZ for 30 min; the red formazan that developed between TZ and viable embryos was extracted with propanol on standing overnight at 40°C and the absorbance determined at 480 nm. More immediate estimates were obtained by replacing the overnight extraction procedure with extraction by homogenizer or wrist shaker.

Two approaches to estimating pregermination were investigated, both based on α-amylase. The ICC falling-number approach yielded data that would predict the germination potential of a barley after several months in storage but was considered too time-consuming to meet the volume requirements of barley selectors. More suitable was a starch-iodine assay that consists of incubating an aqueous extract of barley with dilute starch and, after 30 min, testing for residual starch, using iodine.

Many barleys of the 1987–89 crop years were tested for viability and pregermination. Barleys that pregerminated were able to regerminate after harvest, but their ability to do so decreased as storage time increased. Barleys that did not pregerminate retained high germination counts even after two years in storage. In one barley series consisting of 100 Harrington barleys from the 1987 crop, 53 tested negative for pregermination, 24 tested slightly positive, and 23 tested highly positive. The average germination of these barleys after 22 months in storage was 97, 85, and 66%, respectively. The rate at which pregerminated barleys lose their germination potential during storage seems related to degree of pregermination and to viability. In general, the higher a barley's viability, the greater is its longevity.

Adverse effects of poor germination on malt quality were investigated in a pilot-scale malting trial. Malt analyses were generated on three kilned-dried subsamples of a barley sampled at the end of germination on the basis of percent germination. The fraction with all live berries produced the best overall malt analysis; the dead-berry fraction showed the least modification

and the poorest overall analysis. However, analysis of the latter fraction far exceeded expectations based on commercial malting experience.

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