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RAPID METHODS FOR MEASURING PRE-GERMINATION IN MALTING BARLEY AND ASSESSING ITS EFFECT ON MALT QUALITY

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RAPID METHODS FOR MEASURING PRE-GERMINATION IN MALTING BARLEY AND ASSESSING ITS EFFECT ON MALT QUALITY

by

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This report is dedicated to the memory of Dr Mike Proudlove (1953-1998), the leader of this project and many others funded by HGCA.

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OBJECTIVES

The objectives were threefold; firstly to examine the effectiveness of current methods used to estimate pregermination, secondly to design new rapid methods of identifying and quantifying pregermination and thirdly to assess the value of these methods for their ability to identify the actual problems created by pregermination rather than determining the degree of this phenomenon *per se*. These will allow an informed judgment to be made concerning the implications of the methods used as indicators of the value of barley for malting. The project has been divided into three stages:

a) A number of barley samples designated as pregerminated at grain intake sites were collected and analyzed for pregermination by a range of different tests.

b) The malting performance of these samples was assessed.

c) The two sets of data were analyzed for correlations.

DELIVERABLES

a) A new, rapid method of detecting pregermination in freshly harvested barley grains.

b) A comparison between new and existing methods.

c) A correlation relating the performance of barley grain in the chosen tests with the performance of the same six months later in recognized malting performance tests.

SUMMARY

Barley samples designated as "pregerminated" by a number of grain merchants and handlers have been tested for the extent of this phenomenon using a current IOB Recommended Method (fluoroscein dibutyrate staining), a variation of a previously Recommended Method (α-amylase activity) and two which are used routinely at various malting intake laboratories (tetrazolium and copper sulphate staining). All gave positive results when samples were visually pregerminated, with certain tests (in particular fluoroscein dibutyrate and tetrazolium) being more easy to interpret when intake/rejection were to be considered. Tetrazolium staining is still the cheapest, easiest and most rapid to perform and interpret method to determine signs of pregermination in barley grains. If time and equipment are available staining barley with fluoroscein dibutyrate gives reliable and repeatable results but estimates of pregermination are normally lower than those obtained by tetrazolium staining. In this study α-amylase did not prove a good test for pregermination as it only identified grains in which pregermination was fairly advanced. This was due to the time of development of the enzyme (it is synthesised only after one to two days of germination). In terms of cost and equipment, for early indications, speed and precision, the tetrazolium test was preferred.

New methods for determining pregermination in barley grains have included measuring levels of catalase, peroxidase, acid phosphatase and β-glucanase and examining seeds by NIR, conductivity, density and X-ray transflectance. Of the first two enzymes, catalase was undetectable in raw barley, whether it was pregerminated or not. Peroxidase was measurable but levels did not vary sufficiently for it to be reliably used as an estimate of pregermination. Acid phosphatase did show some correlation with results obtained by tetrazolium staining but more work needs to be carried out to corroborate these findings. Staining of barley endosperm, in particular the area adjacent to the scutellum, as a result of β-glucanase activity has been shown to be a reflect the extent of pregermination but again the repeatability of this method needs to be confirmed.

Prediction of barley pregermination by NIR spectroscopy showed good correlation with tetrazolium and copper sulphate staining and conductivity measurements of whole grains suggested that pregermination gave lower increases in current, particularly over the first 5 minutes of assessment. Grain density measured by flotation in a liquid with a density gradient suggested that the more pregerminated a grain the lower its density. This difference in density was not due to varying levels of total nitrogen between samples but did vary with variety. It was observed however, that the majority of densities from non-pregerminated grain were higher than those of the pregerminated samples again suggesting that pregermination reduced a grain's density. In order to establish whether the difference in density is due to pregermination rather than variety it will be necessary to compare the density of pregerminated grain to a sample of non-pregerminated grain from the same variety. X-ray transflectance clearly showed the development of roots and shoots in pregerminated grains but the cost of the equipment may preclude its routine use by grain handlers. Each of the new methods needs to be validated using a greater number of samples taken from different harvests.

Simulation of pregermination by steeping and drying of dried and undried barley grains cannot be used to generate samples mirroring those which occur naturally in the field. In particular, the degree of variability associated with wetting and heating of the growing ear on the plant, under different growing conditions, were not able to be reproduced in a laboratory-scale trial by steeping and then gently drying the grain.

Storage of small batches of pregerminated barleys at ambient temperatures for up to 6 months had very little effect on their malting performance or malt quality.

INTRODUCTION

Barley that is to be malted must be >96% viable (Germinative Capacity; GC) but preferably being nearer to 100%. Levels of GC less than these may result from problems in grain drying and/or storage, reducing its value, and can be exacerbated by the occurrence of pregermination. This phenomenon happens when barley corns begin to germinate before they have been harvested. That is, when the grain is still on the ear of the plant. This does not occur every year throughout the UK but certain barley growing regions are more susceptible than others are, particularly northern areas are. Pregermination may occur where harvest of the crop is delayed due to prolonged periods of wet weather and this can be exacerbated by concomitant elevated temperatures. This was particularly the case for the 1997 harvest with barleys from across the UK showing differing degrees of pregermination. Samples of these have been collected and used in the present study to estimate pregermination using established and new methods.

Pregermination may lead to several difficulties:

a) Grain that has germinated is usually killed when dried. Since it is normal to dry malting barley soon after harvest this could lead to a high proportion of dead grain in a sample[1].

b) During storage, which may be for as long as 18 months, the GC of pregerminated barley declines rapidly[2].

c) Pregerminated grain is more susceptible to fungal damage than that which is dormant[3]. This elevated fungal contamination, possibly due to leached endosperm nutrient reserves, can lead to difficulties in the brewery; notably with gushing. Serious contamination may also lead to higher levels of mycotoxins.

Several methods have been proposed and used to measure pregermination. These include detection of α-amylase[4-6] and staining with fluoroscein dibutyrate[7-10], methylene blue[9], tetrazolium[2], copper sulphate[11], acetic orcein[11] or an iodine/tetrazolium combination[12]. Each has advantages and disadvantages.

Synthesis of the hydrolytic enzyme α-amylase in the aleurone layer of barley grains occurs in response to plant growth regulators gibberellins. It is only after one or two days of germination that maximum activity becomes measurable and so detection normally indicates very pregerminated grain. Whilst once an IOB Recommended Method[6] relying on the production of a halo on a starch agar plate it was very time consuming. Even a more rapid method[13, 14], however, still relies on the time course of α-amylase production by the seed during germination.

Fluoroscein dibutyrate and methylene blue staining are Recommended Methods for IOB[9] and EBC[10] but are not rapid. Both involve embedding grains in either wax blocks or Araldite-type resin and sanding them down prior to incubation with the dye. Staining with flouroscein, normally seen as yellow colouration under UV light, relies on the activity of

lipases to cleave of the 'dibutyrate' part of the molecule and therefore relies on enzymatic activity. With methylene blue the endosperm only stains blue in pregerminated grains. Tetrazolium or copper sulphate staining are the easiest and most rapid methods to perform. The former involves cutting grains longitudinally, incubating them to the colourless dye under vacuum and scoring for the pattern of red colouration in the embryo, caused by dye reduction. The latter relies on placing corns in boiling copper sulphate, and examining the dorsal part of the embryo for blue-green colour, presumably the result of a reaction with reducing sugars, after peeling back the husk. Both take approximately 10-20 minutes to complete. Interpretation of staining patterns may be carried out by direct visual assessment by trained personnel although it is more usual to view under a low power, light microscope.

Staining with acetic orcein or iodine/tetrazolium are little, if at all, used by the UK grain industry.

Few of these methods have been correlated with each other and the same barley sample may be deemed acceptable by one method but unacceptable by another, in terms of perceived percentage pregermination. There is therefore a clear need for a rapid and easily interpreted procedure which may be used at barley intake at a maltings. Another important factor is how the initial estimation of pregermination may be related to the effect of storage on grain viability, malting performance and malt quality. Barley may be highly viable at intake but lose vigour and viability on storage making it unsuitable for malting after several months. This will have obvious cost implications for all members of the grain chain, from farmer to maltster. The effect of time on malting quality of pregerminated barley has therefore also formed part of the present study.

MATERIALS AND METHODS

Materials

Samples of pregerminated barley were obtained from The Malting Barley Company, Melksham, Wiltshire, UK, Coastal Grain Ltd., Belford, Northumberland, UK, Robin Appel Ltd., and Pauls Malt Ltd., Bury St. Edmunds, Suffolk and Wallingford, Oxfordshire, UK, the last also supplying batches of undried grain. Reagents used were purchased from Rank Hovis MacDougall, Southampton, Hampshire, UK (β-limit dextrin), Polysciences, Incorporated, Warrington, PA 18976, USA (fluoroscein dibutyrate), Walters, Royston, Hertfordshire, UK (wax embedding blocks) and Megazyme, Bray, Co. Wicklow, Ireland (azo-barley glucan). All other chemicals were obtained from Merck Ltd., Lutterworth, Leicestershire, UK or Sigma, Poole, Dorset, UK and were of the highest purity available.

Methods

At intake all barley samples were tested for moisture, total nitrogen, germinative capacity (by the peroxide method) and germinative energy according to the IOB Recommended Methods[9]. They were subsequently micromalted at 16°C using a 7h wet, 17h air rest, 7h wet, 17h air rest and 1h wet steeping programme. Germination was for 4 days followed by drying in a forced draught oven for 8h at 45°C and 16h at 65°C. Selected samples were also remalted by the same regime after storage at ambient temperature for 6 months. All malts were analysed for chosen parameters, again using the IOB Recommended Methods[9].

To try and simulate pregermination barley was steeped in water for 2, 4, 6 or 8h at 16°C, germinated for 16, 24,40, 48 or 64h and finally dried in a forced draught oven at 35°C for 24h. Germination of dried grain was assessed by TTC staining[9].

Fluoroscein dibutyrate (FDB) staining was carried out according to IOB Recommended Methods[9] at Pauls Malt, Bury St. Edmunds, Suffolk, UK. Three lots of forty barley grains were individually embedded, ventral furrow side down, in a wax block, sanded down and flooded with 0.05% (w/v) FDB for 10 minutes. Samples were viewed under UV light and scored for yellow colouration by three people.

For **tetrazolium (TTC) staining** to detect pregermination[2] half corns were cut longitudinally through the ventral furrow and incubated under vacuum at 40°C for 10 minutes. Samples were drained, blotted on filter paper and individual corns examined under a light microscope (x40) or by direct visual interpretation. The scoring system used is outlined in Figure 1.

Copper sulphate staining of barley seed embryos[11] was followed by placing whole grains in a solution of boiling 20% (w/v) $CuSO_4$ for 10 minutes, draining and blotting dry on filter paper. The dorsal husk was peeled back to reveal the embryo for visual inspection and pregermination was scored as illustrated in Figure 2.

The levels of activity for a number of enzymes in barley samples were assessed, unless otherwise specified, by homogenizing whole seeds in a buffer of relevant pH using an

Ultraturrax T25 fitted with a S 25 N probe (Janke & Kunkel GmBH & Co., Staufen, Germany).

Catalase[15] was measured by adding 0.1ml of extract, equivalent to 10mg grain, to 2.9ml of 0.06% H_2O_2, in 50mM phosphate buffer, pH 5.0. The reaction was followed by monitoring the time taken for the A_{240} to decrease from 0.45 to 0.4.

Peroxidase[16] activity was estimated by the increase in absorbance at 405nm in response to the reaction of H_2O_2 with ABTS (2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulphonic acid)). Grain samples were frozen in liquid nitrogen, ground in a Moulinex coffee grinder and 5g of powder extracted in 40ml of 0.1M sodium acetate buffer, pH 5.0. This was centrifuged for 5 minutes at 3600g, the supernatant filtered through a Whatman no. 1 paper and 15µl added to the reaction mixture. This consisted of 2.79ml 70mM citrate/phosphate buffer, pH 4.0, 45µl ABTS solution (1 tablet dissolved in 375µl of the same buffer) and 150µl 100mM H_2O_2.

The levels of acid phosphatase[17] were determined by extracting 1g of grain in 10ml of 0.1M acetate buffer, pH 5.0, centrifuging at 3600g for 5 minutes and incubating 0.2ml of the supernatant with 1ml of 5.5mM *p*-nitrophenylphosphate, in 50mM citrate buffer, pH 4.8) for 30 minutes at 25°C. The reaction was stopped by the addition of 0.1M NaOH and the release of *p*-nitrophenol measured at 400nm.

Activity of α-amylase was monitored using a method adapted from Briggs (1961)[13], with β-limit dextrin as substrate[14], and following the decrease in A_{607} due to the breakdown and subsequent discolouration of an iodine-dextrin complex.

Staining of barley due to β-glucanase action was estimated by incubating 10 half grains in 1ml of azo-barley glucan at 30°C for a specific time[18]. After terminating the reaction with 3ml ethylene glycol monomethyl ester the solution was decanted off, centrifuged at 3600g for 10 minutes and the absorbance of the supernatant read at 590nm. The remaining grains were inspected for blue colouration at the scutellar-endosperm interface and in the embryo on the dorsal surface, seen by peeling back the husk.

Analysis of whole barley grains by NIR spectroscopy was carried out in a quartz cuvette, which holds approximately 80g of grain, over the wavelength range of 1100-2500nm. Scans were carried out using a Perstorp 6500 (Perstorp Analytical, Incorporated, Silver Spring, Maryland 20904, USA) and spectral data compared by Partial Least Squares Analysis (PLS)[19].

Conductivity values[20] were obtained using an Automatic Seed Analyser 610 (Agro Science, Incorporated, Ann Arbor, Michigan, USA), on loan from the National Institute of Agricultural Botany, Cambridge, UK. Samples of 100 individual barley grains were incubated in deionised water and current readings taken between 0-265µA, at 5µA intervals. The displayed current for each well was printed at 5, 15 and 25 minutes and the number of seeds which showed an increase in current at each time point also calculated.

Grain densities were measured by flotation in a liquid with a density gradient. Samples of 5 grains were incubated in 10ml of toluene at 20°C. 1ml aliquots of CCl_4 were then added until all the grains floated. The percentage of CCl_4 in the solution was calculated. Using this value, the density of the solution was determined using tables.

X-ray transflectance of individual grains[21], up to 96 at one time, was performed at Wakehurst Botanical Gardens, UK, using a Todd Research Ltd. radiation-proof cabinet with a high definition X-ray tube (Todd Research Ltd., Chelmsford, Essex, UK.). Photographs of each seed were taken after a 10s exposure at 13kV and 10mA, using Kodak Industrex MX film and processed in Kodak DX80 developer and Kodafix fixer. Data were assessed visually and selected examples scanned on a Hewlett Packard ScanJet IIC machine.

All data handling and correlations were processed using Microsoft Excel (version 7.0) run in Windows 95.

RESULTS AND DISCUSSION

Analysis of pregerminated and non-pregerminated barleys for moisture, total nitrogen (TN) and germinative capacity and energy[9] (Table 1) shows that the majority may have been accepted for intake on most of these parameters, the possible exceptions being 97/177 (Halcyon), 97/178 (Chariot) and 97/180 (Fanfare) all of which showed germinative capacities below that which would normally be acceptable. The germinative energy score for the sample of Alexis (97/173) would suggest that it was dormant yet it proved obviously pregerminated by all the tests applied, even by direct visual assessment. When barley samples were tested by TTC staining, which is also used at intake as a quick method to measure germinative capacity, there was good correlation with the latter but the majority of them proved positive for pregermination.

Figure 3 shows the good repeatability of TTC staining to determine pregermination of the barley samples although 97/194 (Melanie) had to be retrialed because of significant differences between first and second assessment. It was tested a third time, confirming the decision for rejection in terms of pregermination. Barley 97/317 (Fanfare) was an undried sample known not to exhibit signs of pregermination, as confirmed by TTC staining This sample was subsequently used as a negative control and, unless shown, gave no response in the trials performed.

The same samples were then examined using other methods currently employed to detect signs of pregermination. Figure 4 outlines the repeatability of the IOB Method for FDB staining and it may be seen that, apart from 97/173 (Alexis) which stands out, most of the other samples may have been accepted for intake. Based on the number of times samples were analysed by this method those which may be deemed rejectable would include 97/173 (Alexis) and 97/179 (Optic). Barleys 97/175 (Chariot), 97/176 (Fanfare) and 97/177 (Halcyon) could be seen as borderline with 97/178 (Chariot) being a candidate for retesting and 97/180 (Fanfare) and 97/317 (Fanfare) being acceptable. An observation on this method was that samples must be examined rapidly. The fluorescence of the dye fades over several hours and restaining did not give the same estimate of pregermination.

A comparison between the results obtained from tetrazolium staining, both by the published[2] method or by staining grains which had been embedded in wax blocks as described for the FDB staining technique[9], copper sulphate staining and FDB staining are shown in Figure 5. Embedding grains and sanding them down presents a different plane of the embryo for assessment, probably losing the shoot initial and not fully exposing the root initial. This method is therefore not to be recommended for assessing pregermination. Scoring based on copper sulphate staining generally mirrored that seen for TTC but, apart from sample 97/180 (Fanfare), always gave lower results. When pregermination is high this is not really of concern but in marginal cases, *e.g.* 97/176 (Fanfare) and 97/177 (Halcyon), the barley would have been rejected by TTC but possibly accepted by copper sulphate. Apart from the most extreme case of pregermination (97/173 (Alexis)) the FDB staining method always gave lower estimates than either of the other two methods.

When levels of α-amylase were measured in the same barleys results were somewhat different, only two samples (97/173 (Alexis) and 97/179 (Optic)) showing any significant

appearance of pregermination (Figure 6), with all the others certainly being acceptable at intake. Together with the findings outlined in Figure 5 these results raise the question of whether various tests are under or over estimating the degree of pregermination. Certainly to be on the safe side, taken together with its rapidity, easiness to carry out and interpret (a microscope may not even be needed when an operator becomes acquainted with what to look for) and its overlap with the germinative energy test, staining with TTC is the current method that would be recommended for continued use, until an alternative is fully proven.

Alternative methods to estimate pregermination have been monitored in this study. These have been based on easy to perform and rapid enzyme assays[15-18] (Table 2).

Results from assaying either peroxidase or catalase showed very low levels in barley grains, pregerminated or not (Table 3). These enzymes do not seem good indicators of pregermination even though they are thought to be associated with early metabolic events in the seed.

Acid phosphatase was found to be measurable in unmalted barley grains, the change in absorbance in the assay correlating relatively well with the TTC staining of barley samples (Figure 7). An exception was probably 97/178 (Chariot), where much lower levels of acid phosphatase were determined than may have been expected, but this sample also showed the greatest variation when tested for TTC repeatability (Figure 3). It is envisaged that the development of this enzyme during the early stages of seed growth may well used to predict pregermination but further work is needed to confirm this.

The determination of β-glucanase[18] by the revised method described in Methods has the advantage that it may be used to provided two pieces of information. Firstly, the total level of pregermination in a sample may be estimated from the release of dye from the cross-linked azo-barley glucan substrate, from spectroscopic analysis of the supernatant. This is exemplified by results shown in Figure 8. From these it can be seen that there was a noticeable difference in A_{590} between control and pregerminated barleys which increases with time of incubation. The changes in relative absorbance values were, however, very small, questioning the merit of this part of the assay.

Secondly, however, an estimate of the number of individual seeds within a sample which showed activity of this enzyme was found easy to assess, even when relying on personnel who had never scored this method before (Figure 9). Scores were based primarily on the blue coloured band noticeable between the scutellum and the endosperm or colouration of the embryo when a section of the dorsal side husk was peeled back for the samples chosen and at range of incubation periods. The best results were obtained after a 15 minute incubation when scores from collaborators showed the least variation, percentage pregermination estimated closely mimicking that found using TTC staining.

The initial changes that occur during grain germination all involve mobilisation of seed reserves in the outermost parts of the grain, including those in the embryo and aleurone layer but also including the sub-aleurone area of the starchy endosperm. NIR specstroscopy[19] was therefore used to see if these could be picked up, based on the realignment of molecular bonds during seed reserve breakdown. Figure 10 shows that there was a good correlation between grains analysed by NIR compared to the extent of pregermination determined based on an average of TTC and copper sulphate staining.

The correlation appeared to be, indeed, somewhat better than with FDB staining (Figure 5). Scans of non-pregerminated samples (10 varieties from 5 geographically different sites) were used as the controls.

More pregerminated samples need to be examined, over a number of relevant harvests, before any firm conclusions can be drawn but there does seem to be some relationship between NIR spectra and the degree of pregermination.

A conductivity assay that has been used to measure the viability and damage caused by insects in various seeds[20] has also been investigated, based on the idea that germinating seeds may give higher values due to release/mobilisation of ions. In fact the opposite was found. It appeared that the more pregerminated the sample the lower was the increase in current over 15 minutes (Figure 11). From these data a cut-off point of between 50-70μA could possibly be used to determine pregermination. Those samples which show low or no current above these amperage readings are likely to be pregerminated. As with NIR, however, it would be advisable to examine more examples of both pregerminated and non-pregerminated seeds before a clear decision can be made.

Density analysis was carried out on four samples of varying levels of pregermination. It appeared that the more pregerminated the sample the lower the density (Table 4). These four samples all had differing levels of total nitrogen and this may have contributed to the variation in density. However, analysis of samples of the barley variety Rifle, each with different levels of total nitrogen indicated that the differences in density observed in first set of experiments was not due to total nitrogen (Table 5). It can be seen from Table 6 that variety influences density. These samples were all non-pregerminated and it was observed that the majority of the densities were higher than those of the pregerminated samples (Tables 4 and 6) suggesting that pregermination reduced a grain's density. In order to establish whether the difference in density is due to pregermination rather than variety it will be necessary to compare the density of pregerminated grain to a sample of non-pregerminated grain from the same variety.

In addition, an X-ray transflectance technique, which has been used for non-invasive examination of seeds for insect infestation[21] has also been investigated as a means of monitoring root and/or shoot growth as indicators of pregermination. Figure 12 highlights the noticeable differences between obviously pregerminated seed (with root initials being clearly visible; Figure 12a), a borderline case (where the root may just have started to grow; Figure 12b) and non-pregerminated seed (no evidence of embryo growth; Figure 12c). It proved easy to distinguish the different samples but the cost of the equipment[21] may preclude its routine use by grain handlers.

Analysis of malts made from barleys shortly after intake (Table 4a) showed that, apart from 97/174 (Melanie) and 97/180 (Fanfare), both of which appeared a little undermodified in terms of extract and viscosity, most gave acceptable products (based on the fact that they were micromalted). If these results are compared with those obtained from malting several of the same barleys 6 months (Table 4b) later it can be seen that the most pregerminated sample chosen, Chariot (97/175), did not seem to have deteriorated in its ability to malt adequately. Extract, TSN and viscosity were all virtually unchanged, within the error of the methods. Similarly, none of the other varieties showed any really significant decrease in malting performance although the extract value for Halcyon

(97/177) was lower after storage. The limited analyses are due to limiting amounts of barley available for micromalting.

CONCLUSIONS

Of the current methods available to predict pregermination in barley, staining with tetrazolium appears to be the most amenable. It is easy to carry out, rapid and, given sufficient experience, easy to interpret. Copper sulphate staining has many of the same qualities for estimating pregermination but was found to be more difficult to score by the personnel in this project. Staining by FDB was easy to interpret but was seen as time consuming and expensive to set up. It is, however, an IOB Recommended Method and should therefore be used in cases of dispute as the method of choice.

The activities of some enzymes which have been associated with seed growth were not found to be particularly good indicators of pregermination. Catalase was not detectable in pregerminated grains and peroxidase gave similar levels as found in non-pregerminated barley seeds. Levels of α-amylase were regarded to only identify grains which were obviously pregerminated, due to the time of development of this enzyme. β-glucanase activity, on the other hand, seems to offer a new way of estimating pregermination by staining the scutellar-endosperm interface and the dorsal side of the embryo in barley half seeds.

X-ray transflectance, conductivity, density measurements and NIR all show promise as methods for predicting barley pregermination but all need further study to fully evaluate their potential.

Storing the pregerminated barleys for 6 months at ambient temperatures did not, however, have any noticeable affect on malting quality.

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Table 1. Barley analysis for pregerminated samples at intake. All results were calculated using IOB Recommended Methods[9], as described in Methods.

Sample	Moisture (%m/m)	TN (%m/m)	GC (%)	GE (%)	TTC (%)
97/173 (Alexis)	15.8	1.41	98	69	70
97/174 (Melanie)	15.8	1.81	97	97	16
97/175 (Chariot)	13.2	1.54	98	89	19
97/176 (Fanfare)	15.7	1.8	96	94	7
97/177 (Halcyon)	14.8	1.87	92	95	9
97/178 (Chariot)	17.2	1.75	94	91	29
97/179 (Optic)	15.5	1.47	96	93	27
97/180 (Fanfare)	14.3	1.95	92	91	8
97/317 (Fanfare)	14	1.55	98	95	1
97/321 (Optic)	12.7	1.58	99	98	7
97/322 (Optic)	15.1	1.64	94	93	15

Table 2. Enzymes assayed as possible indicators of pregermination. The activity of each was measured as described in Methods.

- Catalase
- Peroxidase
- Acid Phosphatase
- β-glucanase

Table 3. Levels of oxidative enzymes in barley samples.

Sample	Catalase[a] (A_{240})	Peroxidase (units[15])
97/173 (Alexis)	0.807	39
97/174 (Melanie)	0.704	68
97/175 (Chariot)	0.617	70
97/176 (Fanfare)	0.695	64
97/177 (Halcyon)	0.714	84
97/178 (Chariot)	0.744	87
97/179 (Optic)	0.692	68
97/180 (Fanfare)	0.752	64
97/317 (Fanfare)	0.721	57
96/92 (Chariot)[b]	0.743	64

[a] Initial absorbance, no change after 5 minutes
[b] Stock barley

Table 4. Comparison of the densities of four barley samples of differing degrees of pregermination.

Sample	**Level of Pregermination**	**CCl_4 (% vol)**	**Density[a] (g/ml)**
97/317 (Fanfare)	None	71	1.383
97/175 (Chariot)	Low	68	1.361
97/179 (Optic)	Moderate	64	1.332
97/173 (Alexis)	High	50	1.230

[a]obtained from density tables (CCl_4 and toluene at 20°C)

Table 5. Effect of total nitrogen content of Rifle on its density.

Total Nitrogen (m/m)	**CCl_4 (% vol)**	**Density[a] (g/ml)**
1.65	66	1.346
1.75	69	1.368
1.78	66	1.346
1.89	68	1.361
2.11	66	1.356

[a]obtained from density tables (CCl_4 and toluene at 20°C)

Table 6. Effect of variety on density.

Sample	Total Nitrogen (%m/m)	$\%CCl_4$	Density
Fanfare	1.80	64	1.332
Intro	1.80	74	1.405
Pastoral	1.80	66	1.346
Sunrise	1.79	62	1.317
Regina	1.81	72	1.390
Spice	1.81	66	1.346

[a]obtained from density tables (CCl_4 and toluene at 20°C)

Table 7. Malt analyses from samples processed at intake and after 6 months storage.

a) Barley malted at intake

Sample	Moisture (%m/m)	H.W.E_2 (L°/kg)	H.W.E_7 (L°/kg)	F/C diff (L°/kg)	Colour (°EBC)	T.S.N. (%m/m)	FAN (%m/m)	Viscosity (mPa.s)	DP (°IOB)
97/173 (Alexis)	5.1	311	309	2	3.00	0.82	0.15	1.47	84
97/174 (Melanie)	5.3	302	299	3	2.60	0.76	0.11	1.61	140
97/175 (Chariot)	4.8	310	309	1	2.60	0.62	0.10	1.44	89
97/176 (Fanfare)	5.1	307	303	4	2.30	0.70	0.10	1.55	72
97/177 (Halcyon)	5.4	306	302	4	2.50	0.72	0.10	1.60	88
97/178 (Chariot)	5.8	312	310	2	2.30	0.76	0.11	1.46	121
97/179 (Optic)	4.3	305	303	2	3.00	1.10	0.16	1.46	140
97/180 (Fanfare)	4.9	303	295	8	2.30	0.66	0.09	1.66	72
97/317 (Fanfare)	4.8	310	307	3	3.20	0.52	0.09	1.61	67

b) Barley malted after 6months storage at ambient temperatures

Sample	Moisture (%m/m)	H.W.E_7 (L°/kg)	T.S.N. (%m/m)	Viscosity (mPa.s)
97/175 (Chariot)	5.1	312	0.55	1.42
97/176 (Fanfare)	5.6	304	0.59	1.54
97/177 (Halcyon)	5.5	296	0.54	1.65
97/180 (Fanfare)	5.4	294	0.46	1.64

FIGURE LEGENDS

Figure 1. Diagrammatic representation of pregermination as detected by TTC staining. 1. Pass – root and shoot initials not developed. 2. Pass – Root initial has started to develop but has not broached the micropyle. 3. & 4. Fails – Root and shoot have started to develop and root has broached the micropyle. 5. Fail – Root has broached the micropyle and shoot has penetrated through the scutellum.

Figure 2. Copper sulphate staining test for barley pregermination – patterns of recognition. Passes – either no or minimal staining, the root has not broached the micropyle. Fails – stains blue-green and root shows signs of penetrating the micropyle.

Figure 3. Repeatability of TTC staining of pregerminated and non-pregerminated barley. Each result is the score from an individual evaluation done in duplicate (except 97/174, Melanie done in triplicate. Samples were – 97/173, Alexis; 97/174, Melanie, 97/175, Chariot; 97/176, Fanfare; 97/177, Halcyon; 97/178, Chariot; 97/179, Optic; 97/180, Fanfare; 97/317, undried Fanfare.

Figure 4. Fluoroscein dibutyrate (FDB) staining of pregerminated and non-pregerminated barleys. All samples were assessed for fluorescence by embedding 40 seeds in each of three wax blocks, sanding down and flooding with FDB. Three individuals assessed all three blocks and results are the means of their scores. A zero score equates to no fluorescence being detectable for any seed in the block.

Figure 5. Comparison of methods used to detect pregermination in barley. The methods used to predict and score for each of the methods are detailed in the Methods section.

Figure 6. Levels of α-amylase in samples of pregerminated barleys. Enzymic activity was determined using β-limit dextrin as substrate, as outlined in the Methods section.

Figure 7. Comparison of ranking of barley sample pregermination by TTC staining and acid phosphatase activity. Numbers above each point and bar relate to the relative ranking of that sample by that method. 97/322, Optic.

Figure 8. Levels of β-glucanase in the supernatant of barley half corns incubated with azo-barley glucan. Samples were of Alexis (97/173) and Fanfare (97/317).

Figure 9. β-glucanase activity, measured as staining of the scutellar endosperm, using azo-barley glucan as substrate. Independent assessments were made of twenty seeds by three individuals on Alexis (97/173), Optic (97/179) and Fanfare (97/317).

Figure 10. Correlation between NIR spectra of pregerminated and non-pregerminated barley grains with reference data obtained from TTC and copper sulphate staining. NIR spectra for non-pregerminated was obtained from fifty samples from the 1997 harvest.

Figure 11. Conductivity measurements on pregerminated and non-pregerminated barley grains. The increase in current in 100 individual cells, each containing one barley grain, was measured after 15 minutes incubation in deionised water.

Figure 12. Pictures of individual barley grains showing different levels of pregermination taken using an X-ray transflectance method.

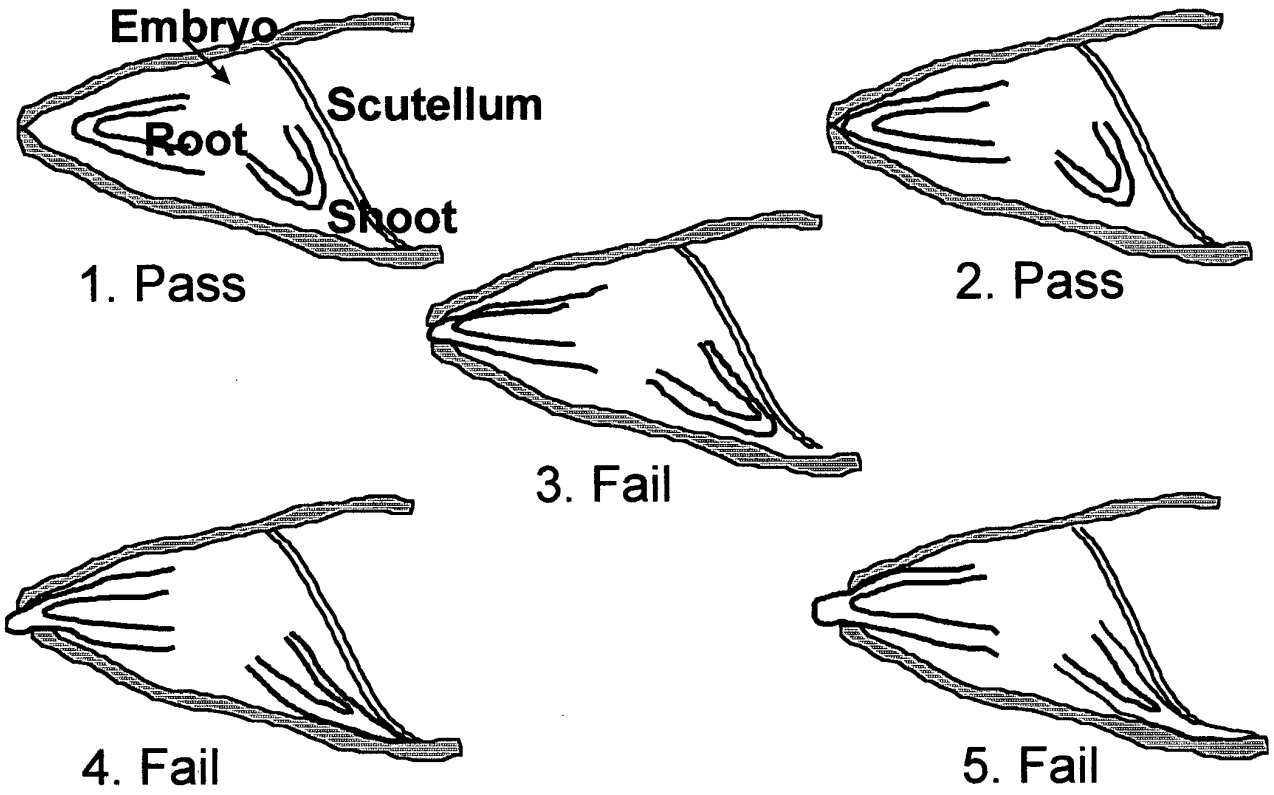


Figure 1. Diagramatic representation of pregermination as detected by TTC staining.

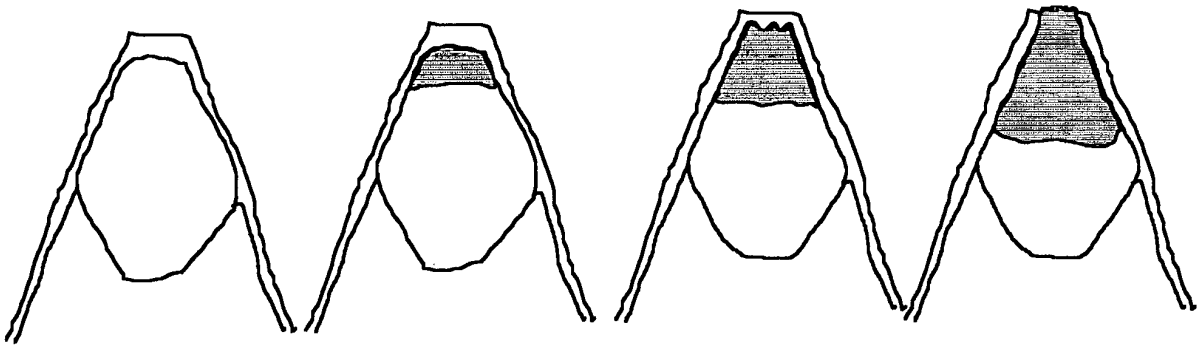


Figure 2. Copper sulphate staining test for barley pregermination - patterns of recognition.

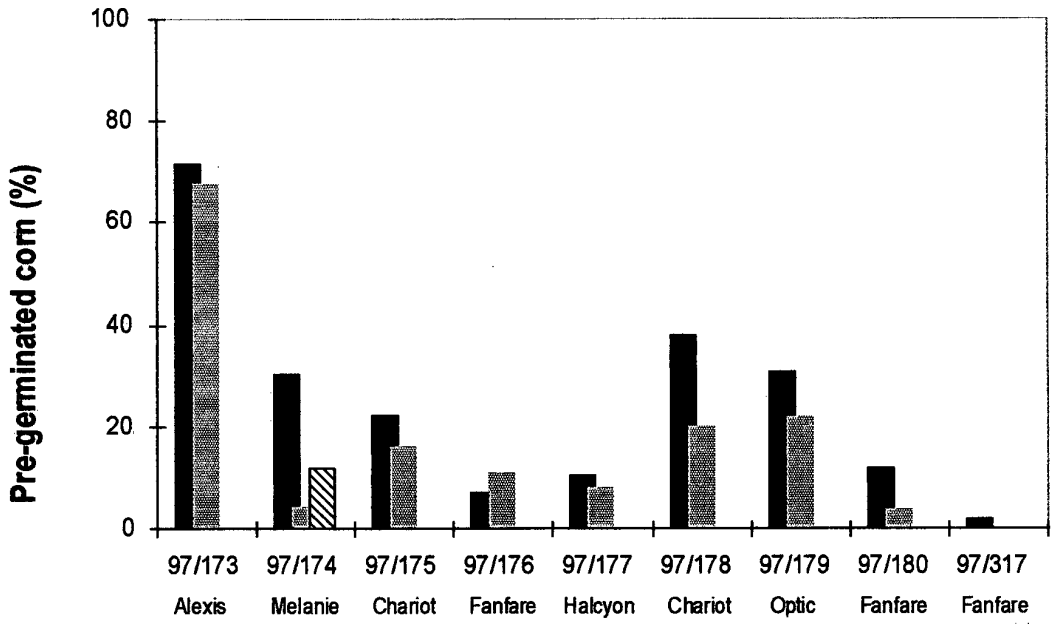


Figure 3. Repeatability of TTC staining of pregerminated and non-pregermiated barley. Each bar represents a separate determination and all samples were tested in duplicate except 97/174 (Melanie). See Figure Legends for details about sample varieties and text for the method.

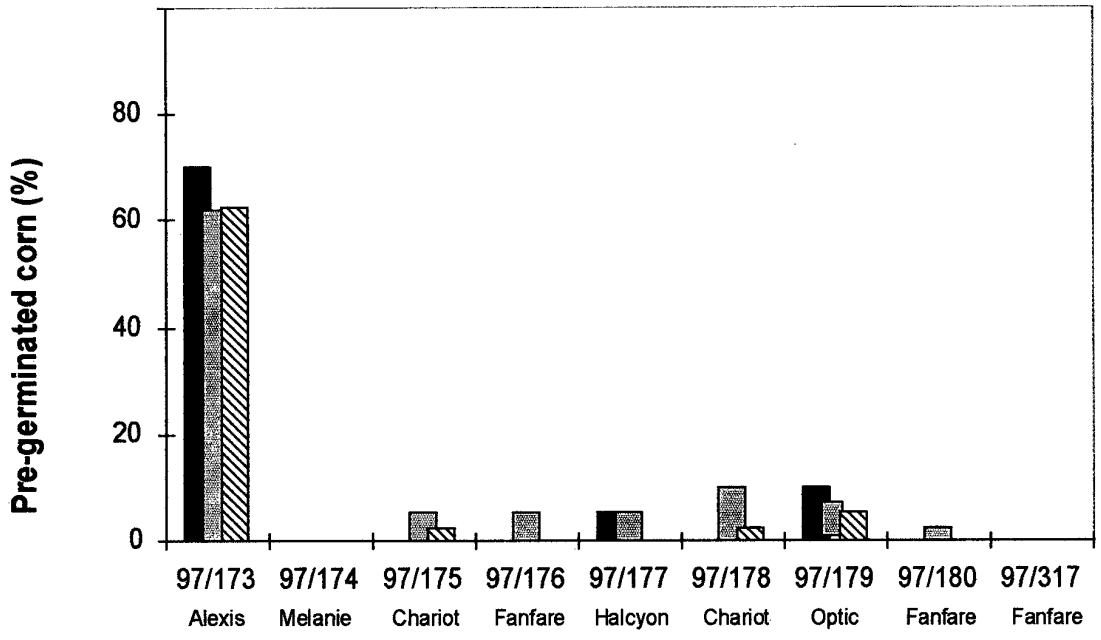


Figure 4. FDB Staining of pregerminated and non-pregerminated barleys. Each bar represents individual determinations in triplicate. A score of zero means no fluorescence was detected in that sample. Varieties are as described for Figure3. See text for method details.

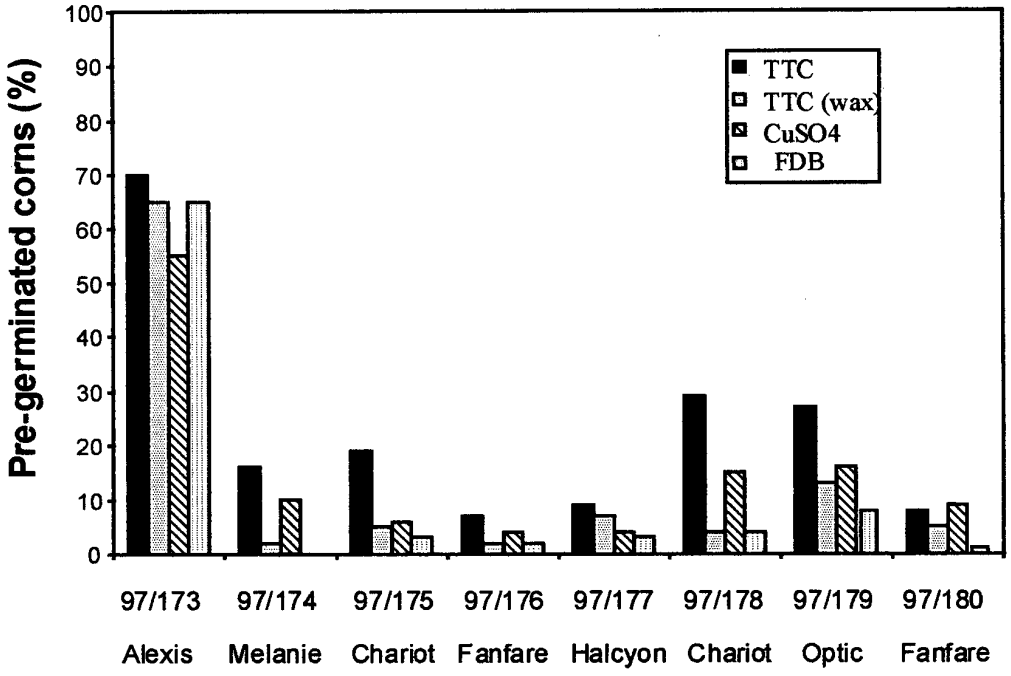


Figure 5. Comparison of methods used to detect pregermination in barley. Varieties are as described for Figure 3 and each method was carried out as outlined in the text.

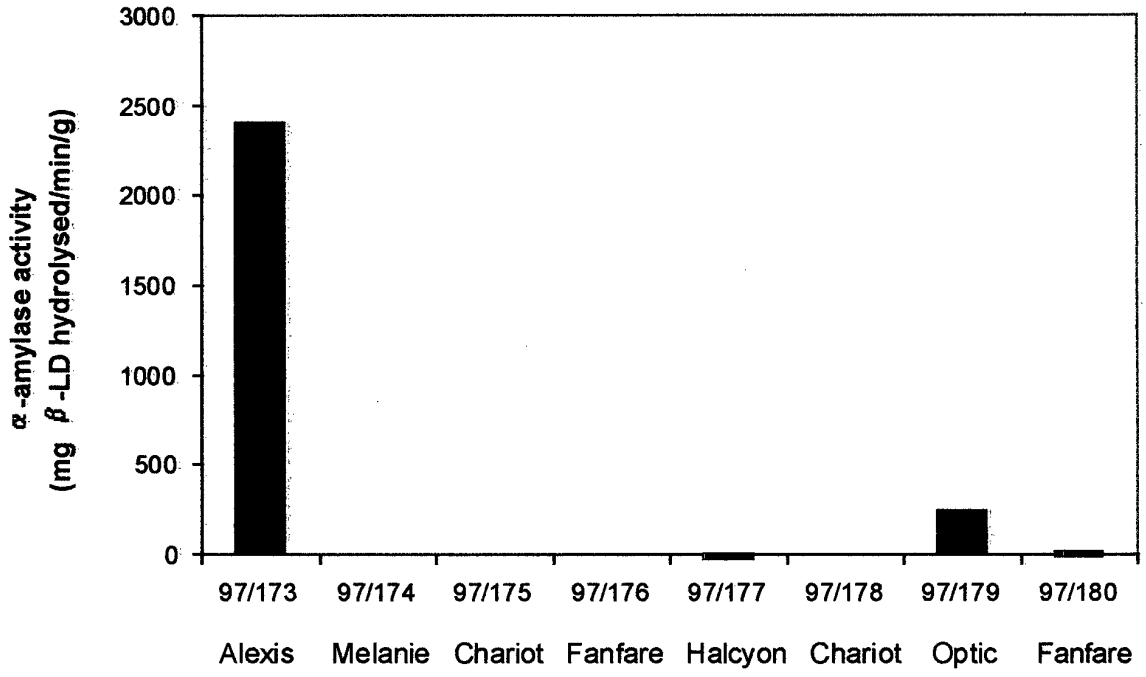


Figure 6. Levels of α-amylase in samples of pre-germinated barleys. Varieties are those described for Figure 3 and the method was as detailed in the text.

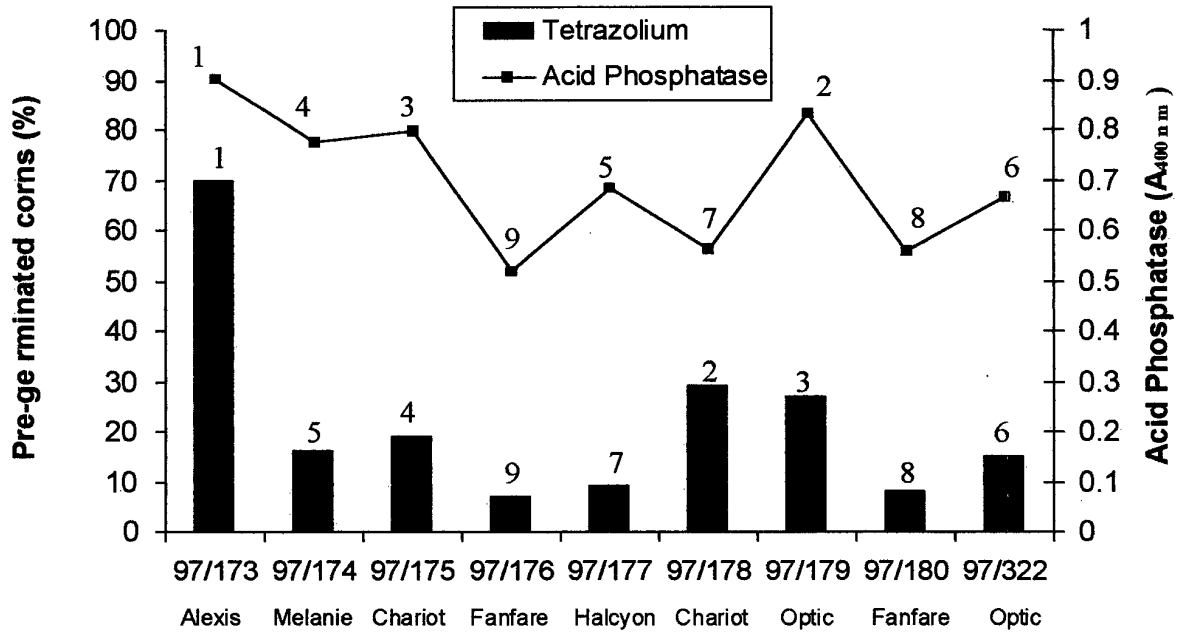


Figure 7. Comparison of ranking of barley sample pregermination by TTC staining and acid phosphatase activity. Methods for each are described in text.

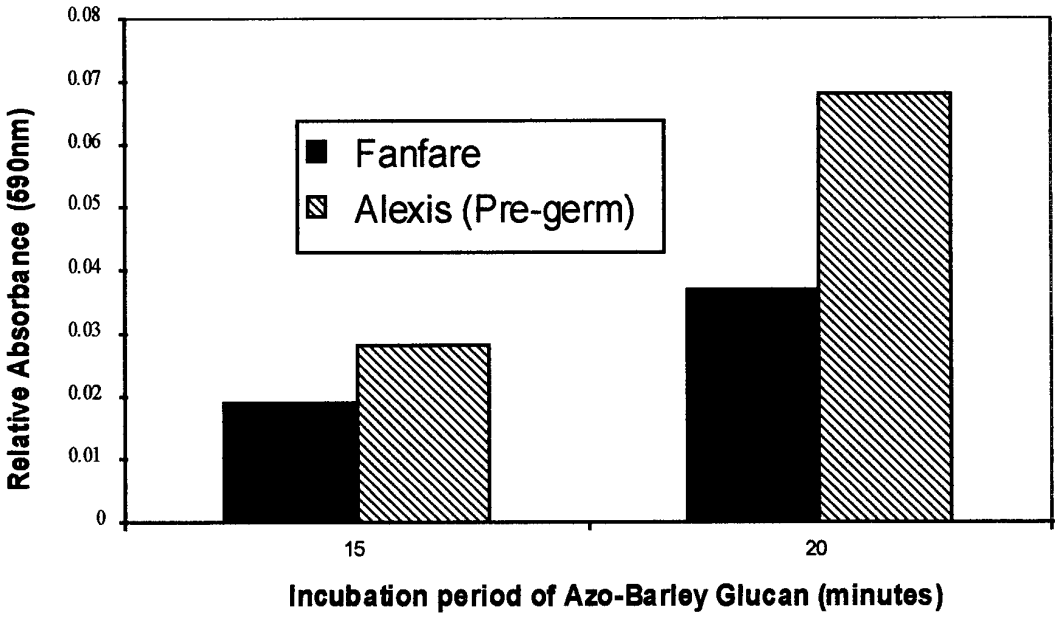


Figure 8. Levels of β-glucanase in the supernatant of barley half corns incubated with azo-barley glucan. Samples were 97/317 (Fanfare) and 97/173 (Alexis). See text for method details.

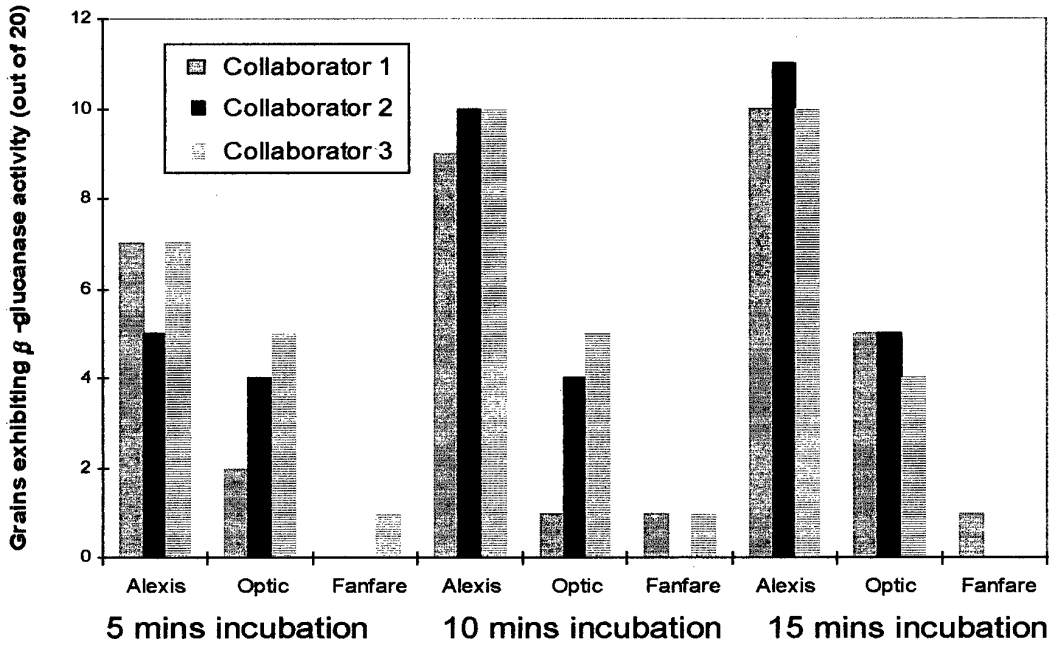


Figure 9. Comparison of β-glucanase activity staining of pregerminated and non-pregerminated barley corns. Three collaborators each scored samples independently.

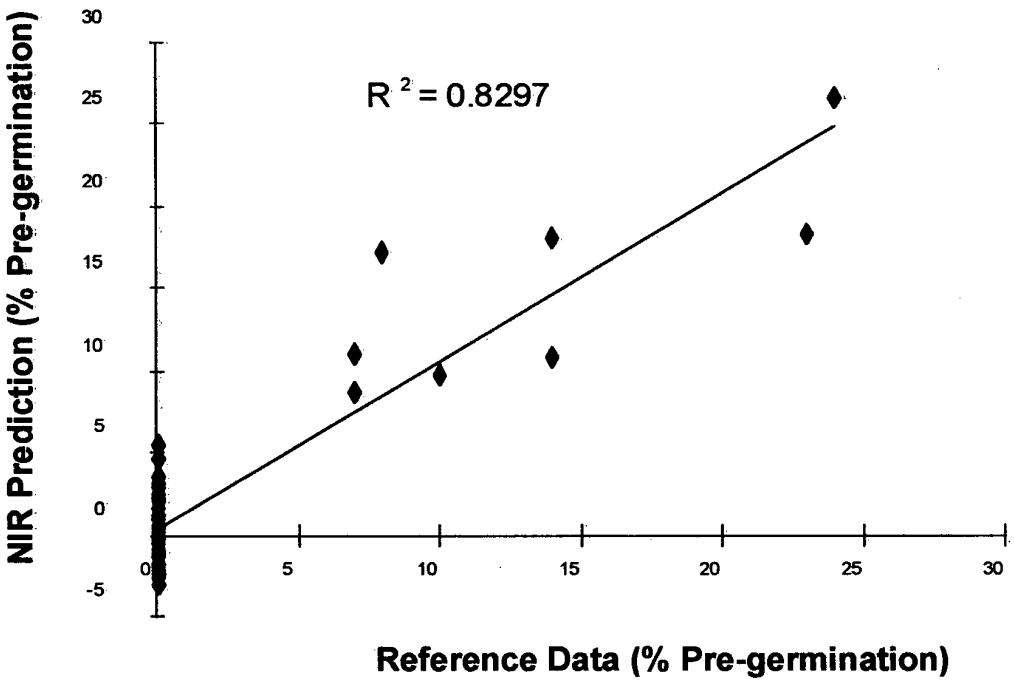


Figure 10. Correlation between NIR spectra of non-pregerminated and pregerminated whole barley seeds. Reference data was taken from the mean calculated by TTC and copper sulphate staining.

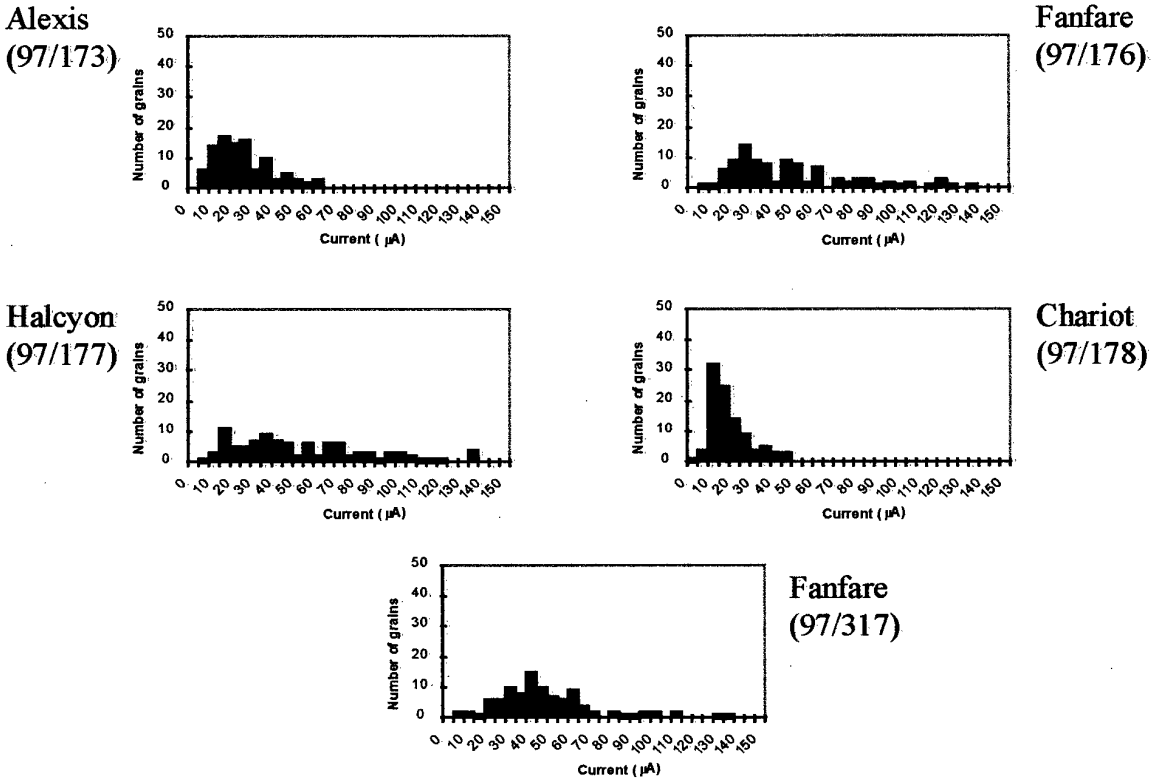
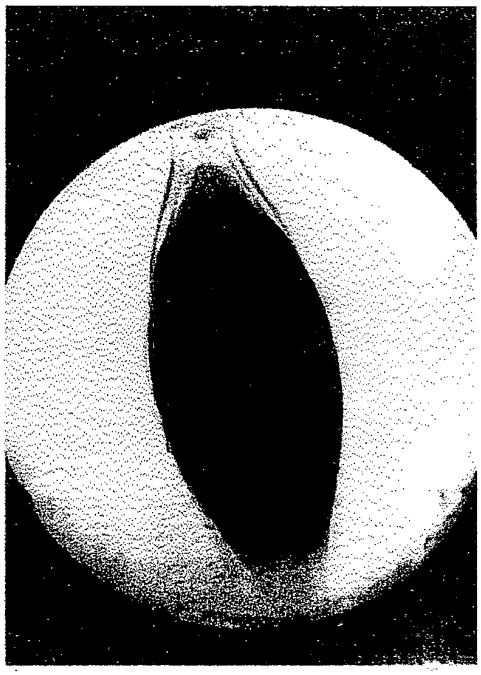


Figure 11. Conductivity measurements on 100 seeds of pregerminated and non-pregerminated barley. Increase in current was measured after 15 minutes incubation.
a) 97/173; b) 97/176; c) 97/177; d) 97/178; e) 97/317.

a)



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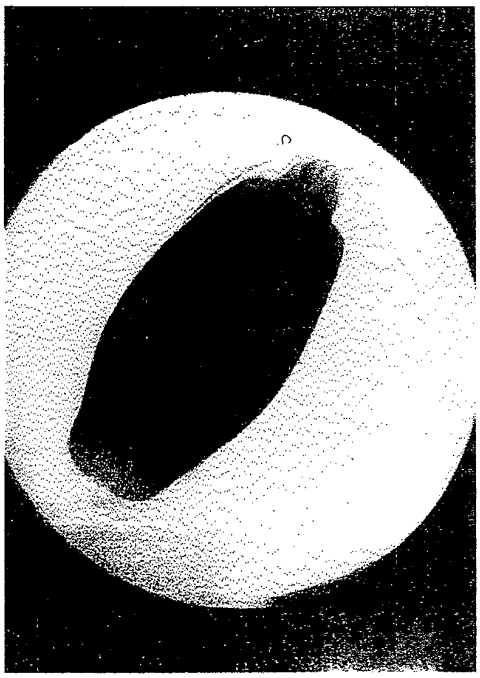

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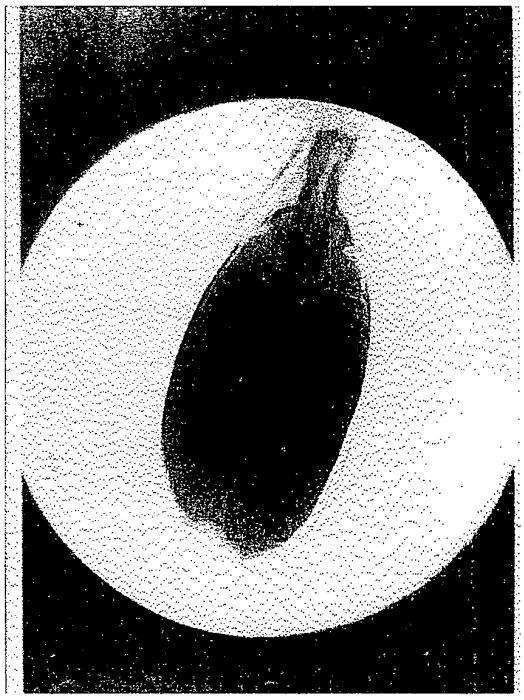

Figure 12. X-ray tranflectance photographs of pregerminated and non-pregerminated barley grains. a) non-pregerminated (97/317); b) slight pregermination (97/179); c) pregerminated (97/173).