

Assessing Effects of Adverse Weather Conditions on Distilling Quality in Wheat

J.S. Swanston, P.L. Smith, A. Weir
Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA

Executive Summary

When alcohol yield measurements were compared with NIR predictions of alcohol yield for wheat samples from three trial sites, poor agreement was obtained for samples at a site where grain damage had resulted from heavy rain delaying harvest.

Elevated levels of α -amylase activity were observed in samples from the rain-damaged site, indicative of pre-harvest sprouting. Slightly elevated levels were also observed at one of the other sites, but distilling quality was not adversely affected.

As pre-harvest sprouting, followed by drying, resembles rudimentary malting, some malting tests were also applied to the samples. Samples from the rain-damaged site were characterised by higher original gravities, as breakdown of the grain structure had permitted solubilisation of some carbohydrate and nitrogenous material.

There appeared to be considerable variation between samples from two of the sites, for soluble nitrogen and reducing sugars but this may, in part, have reflected difficulties in accurately assaying small quantities. However, it was clear that samples from the site at which there was no elevation of α -amylase activity had lowest levels for both characters.

There was no significant, direct correlation between α -amylase activity and alcohol yield, either across all samples or among those from the rain-damaged site.

A regression equation, incorporating NIR predictions of alcohol yield and protein, plus soluble nitrogen and α -amylase activity was derived. This explained nearly 80% of the variation in alcohol yield at the rain-damaged site, compared to only 26% explained by the NIR prediction of alcohol yield. Another equation incorporating protein, grain size and α -amylase activity explained 82% of the variation in alcohol yield.

α -amylase made a negative contribution to both regression equations indicating that pre-harvest sprouting would have an adverse effect on distilling quality. Soluble nitrogen, in contrast, had a small positive effect, suggesting that ease of

solubilisation of protein may reflect aspects of composition or structure that are beneficial for distilling.

α -amylase testing was subsequently extended to all samples from the rain-damaged trial and considerable variation was observed, indicating that some lines were resistant to sprouting. This data will subsequently be used in seeking to locate genetic factors for resistance and to develop screening tests for breeders.

Introduction

Since 2004, SCRI has been one of the partners in the LINK project entitled 'Genetic Reduction of Energy and Emissions of Nitrogen in cereal growing' (acronym: GREEN Grain). This project has several strands but one, in which SCRI has taken a lead role, has been in seeking to determine the factors that control alcohol yield in wheat and their genetic control, with a view to developing screening techniques that can be exploited by plant breeders. Within this area, SCRI has collaborated with FOSS UK and the Scotch Whisky Research Institute (SWRI) in developing a Near Infra-Red Transmission (NIR) based method for predicting the alcohol yield obtainable from a given quantity of grain.

NIR predictions are developed through calibration of spectral data with results obtained by chemical analysis of the desired trait, in a range of samples. As the population on which the calibration is based is expanded, through inclusion of greater numbers, more environments and more seasons, the accuracy improves and NIR is now commonly used by processors to assess 'intake' samples of grain for compositional traits, particularly protein. However it is also possible to predict how samples will perform during processing and factors such as alcohol yield and residue viscosity (an indication of the ease of downstream processing) are of particular interest to grain distillers.

Within the GREEN Grain project, trials have been grown at SCRI and two sites managed by ADAS and a sub-set of samples, comprising a range of lines from each site, has been analysed by SWRI. The calibration derived from this sub-set is then used to enable prediction of the alcohol yield in all the other samples in the trials. An initial calibration was derived and utilised for material harvested in 2005 and it was intended that this would be tested and, if necessary, amended after data from 2006 was included. However, at one of the ADAS sites (High Mowthorpe in Yorkshire) heavy rain delayed harvest in 2006 and some evidence of damage to the grain, including precocious germination (pre-harvest sprouting), was observed in some samples.

It was unknown what, if any, effect this might have on alcohol yield or on the accuracy with which alcohol yield might be predicted, when samples from High Mowthorpe were included in the 2006 sub-set sent to SWRI. The trial grown at

High Mowthorpe contained a population derived from a cross, between the wheat varieties Canterbury and Eclipse, on which a considerable amount of DNA sequence data had been obtained within the GREEN Grain project. It thus also presented an opportunity to identify and locate genetic factors associated with pre-harvest sprouting, which would provide useful additional data for future wheat breeding programmes. Quantifying pre-harvest sprouting within the 2006 sub-set and providing phenotypic data on trial samples from High Mowthorpe would thus be of considerable 'add-on' value to the GREEN Grain project but would require additional resources to achieve. For that reason, the Scottish Society for Crop Research was approached, and very kindly agreed, to support a small, additional project to meet these objectives.

Experimental

Experimental Material

i) 2007 Calibration Population

A total of 130 samples was chosen from trials at each of the three sites, SCRI near Dundee, ADAS Turrington, Norfolk and ADAS High Mowthorpe, Yorkshire. These were sent to SWRI for alcohol yield analysis and also scanned at SCRI using an Infratec 1241 Grain Analyser (FOSS, UK, Warrington, Cheshire). In addition, grain dimensions, that is length, width and length:width ratio (L:W) were determined, by scanning with a digital seed analyser, and thousand grain weight (TGW) calculated on each sample as described by Swanston et al. (2005).

ii) Population Trial at High Mowthorpe

192 entries comprising inbred lines from the cross Canterbury x Eclipse and a number of wheat varieties, as experimental controls, were included in a trial of three replicates, of which two were grown under an optimal nitrogen regime. The third replicate was grown without nitrogen application, for observation purposes, and was not included in this experiment.

Assessment of Pre-Harvest Sprouting

Quantification of pre-harvest sprouting was carried out by determining alpha-amylase activity. Grain was milled to pass through a 0.5mm screen and assayed for alpha-amylase activity using a 'kit' method, supplied by Megazyme International, Ireland Ltd., and based on a procedure described by McCleary and Sheehan (1987). This procedure was applied to all samples in the 2006 sub-set and to all entries in reps one and two of the High Mowthorpe population trial.

As sprouting followed by drying is similar to a very rudimentary form of malting, it was also decided to carry out some rapid small-scale malt tests, on the 2006 sub-set, to determine whether they could be useful and inexpensive predictors of pre-harvest sprouting. Approx. 5g of grain, from each sample, was milled in a coffee grinder, to produce a fairly coarse flour. From each flour sample, 4g were

extracted at 65°C, in 30mls of distilled water, with samples shaken every 10min to ensure thorough mixing. After 1hr, 10ml of distilled water, at 20°C was added to each sample, which was then filtered through Whatman's No4 filter paper. The specific gravity of the filtrate was determined using a density meter and the percentage extract was calculated by multiplying the excess degrees of gravity (taking the gravity of water at 20°C as 1000) by a factor of 2.63 (Dolan et al., 1981). The soluble nitrogen content of the filtrate was determined by a spectrophotometric method (Haselmore and Gill, 1995).

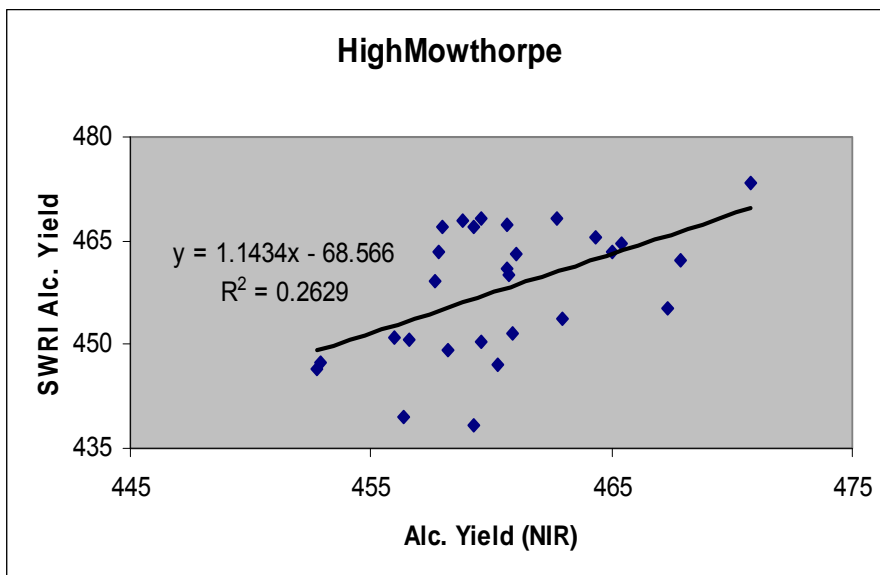
Reducing sugar content was determined by reduction of potassium ferricyanide under alkaline conditions. To 0.8ml of the filtrate, 0.2ml of 0.1N sodium hydroxide was added, followed by 5ml of 0.037% potassium ferricyanide. Sample tubes were placed in a boiling water bath for 4min, then cooled rapidly by placing in cold water, before the colour was determined, in a spectrophotometer, using the transmission mode at 420nm.

Results

2007 Calibration Population

Comparison of alcohol yield measurements made at SWRI and NIR predictions of alcohol yield showed relatively poor agreement in the samples from High Mowthorpe (Fig. 1) although the prediction was accurate for samples from the other two sites (data not shown). There is a strong negative correlation between alcohol yield and grain protein

Fig 1. Alcohol yield plotted against NIR prediction of alcohol yield for 28 winter wheat samples grown at High Mowthorpe in 2005-06.



content (Swanston et al., 2005) and data from the first year of the GREEN Grain project suggested that protein content correlated even more closely with NIR predictions of alcohol yield than with measured alcohol yield. This is probably due to slight under-estimation of the extent to which some lines deviate from the expected relationship between the two parameters. However, when NIR predictions of alcohol yield were plotted against grain protein for the samples from High Mowthorpe, no significant correlation was observed (Fig. 2). It was clear, therefore that there were problems associated with these samples that precluded accurate prediction of compositional and processing character through scanning by NIR

Although there was variation in α -amylase activity between samples from all three sites, it was clear (Fig. 3) that there were also large differences between the sites. The samples from the SCRI site, at which there had been no sprouting, showed very low levels of α -amylase activity while, at Terrington, where there had been some rain prior to harvest, but samples had not appeared problematic in alcohol yield analysis, α -amylase levels were slightly elevated. The highest levels of α -amylase were observed, however amongst samples from High Mowthorpe site and, although there was some variation between the samples, it was clear that significant levels of pre-harvest sprouting had occurred at this site. A similar pattern of results was observed for hot water extract

Fig 2. NIR predictions of alcohol yield plotted against grain protein content for 28 winter wheat samples grown at High Mowthorpe in 2005-06.

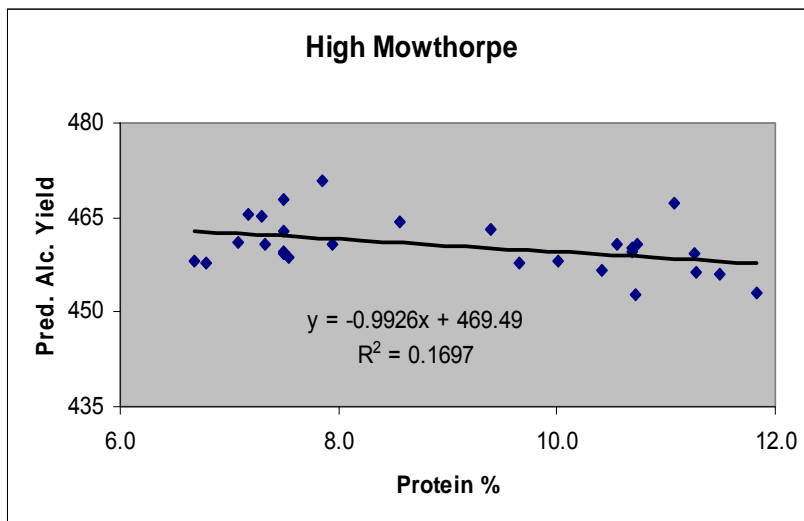
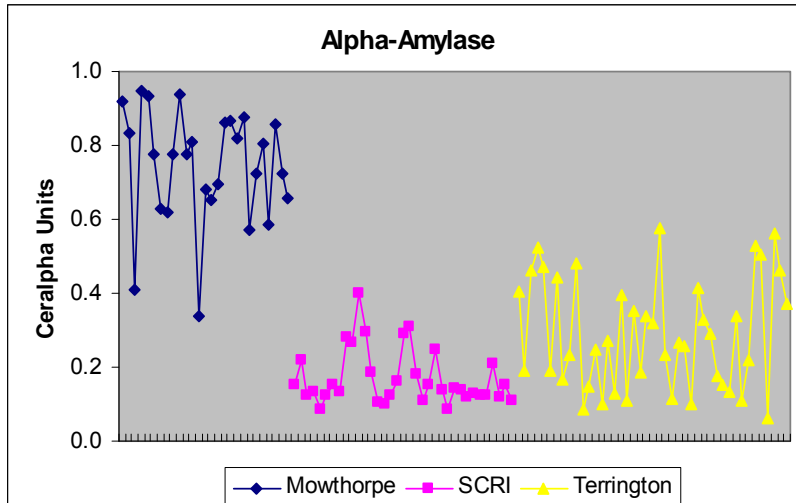
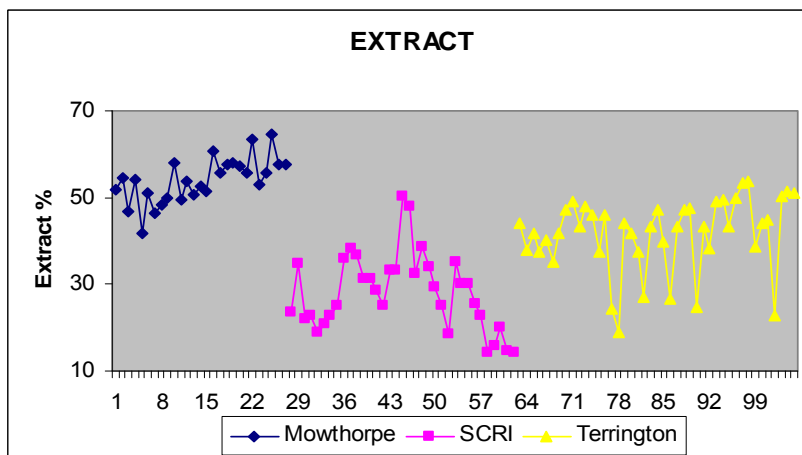


Fig 3. Variations in α -amylase activity amongst winter wheat samples grown at three sites.



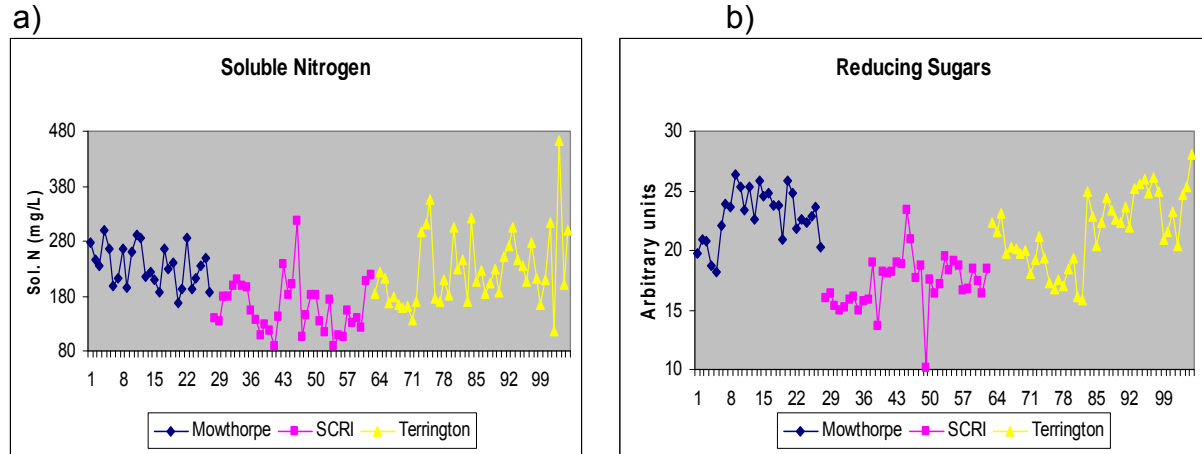
(Fig 4), with lowest extracts observed amongst the samples from SCRI and highest levels amongst those from High Mowthorpe. Again, the Terrington samples were intermediate. These data indicated differences between the sites in the degree of modification; that is breakdown of the endosperm structure, as a result of sprouting, allowing differing amounts of material to become soluble during hot water extraction.

Fig 4. Variations in hot water extract amongst winter wheat samples grown at three sites.



There was considerable variation within sites for soluble nitrogen (Fig 5a) and reducing sugar (Fig 5b) contents, although this may have reflected the difficulty in accurately measuring relatively low values, compared to those observed in malt, on such a small scale. Differences between the Mowthorpe and Terrington sites were not clear, but the lowest values were observed among the SCRI samples.

Fig 5. Variations in a) soluble nitrogen (in mg per L) and b) reducing sugar (arbitrary units) contents amongst winter wheat samples grown at three sites.



To determine whether there was a clear effect of α -amylase activity on alcohol yield, the two parameters were plotted against each other over all sites (Fig 6a) and for the Mowthorpe samples only (Fig 6b). However, for both data sets, there was a wide scatter, with no significant correlation. This was not surprising as alcohol yield was likely to be affected by a number of factors, including protein content. Several combinations of factors were therefore tested to obtain a regression equation which improved on the original NIR prediction (Fig 1). The most effective combined predicted alcohol yield (PAY) with grain protein content (GP) α -amylase activity (AA) and soluble nitrogen, measured in mg/litre (SN). The regression equation was as follows:

$$y = 288.01 + 0.46\text{PAY} - 4.61\text{GP} - 11.46\text{AA} + 0.044\text{SN}$$

When this was used to calculate an amended PAY (predicted alcohol yield) and plotted against alcohol yield determined by SWRI (Fig. 7), there was a highly significant correlation, with $R^2 = 0.79$. This prediction therefore explained nearly 80% of the variation in alcohol yield, compared to only 26% explained by the original NIR prediction (Fig. 1).

Fig 6. Association between alcohol yield and α -amylase activity for a) all samples and b) samples from the Mowthorpe site only.

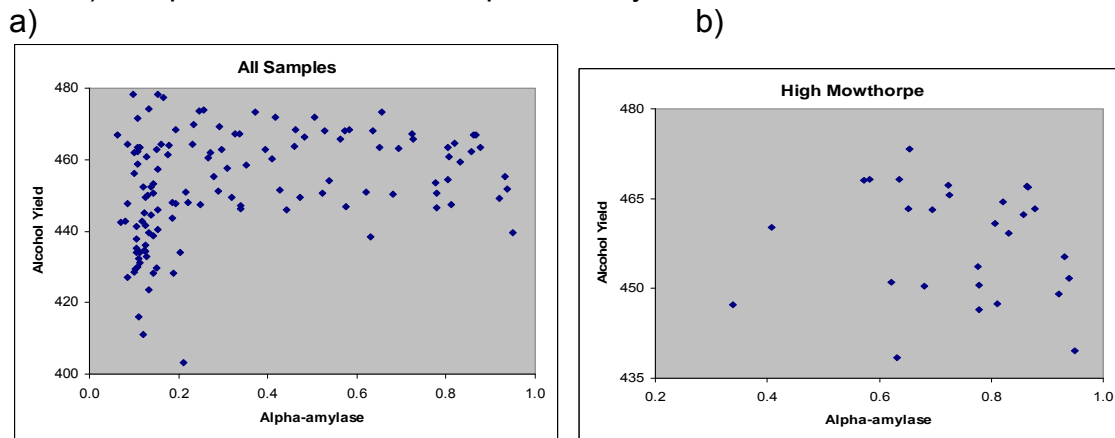
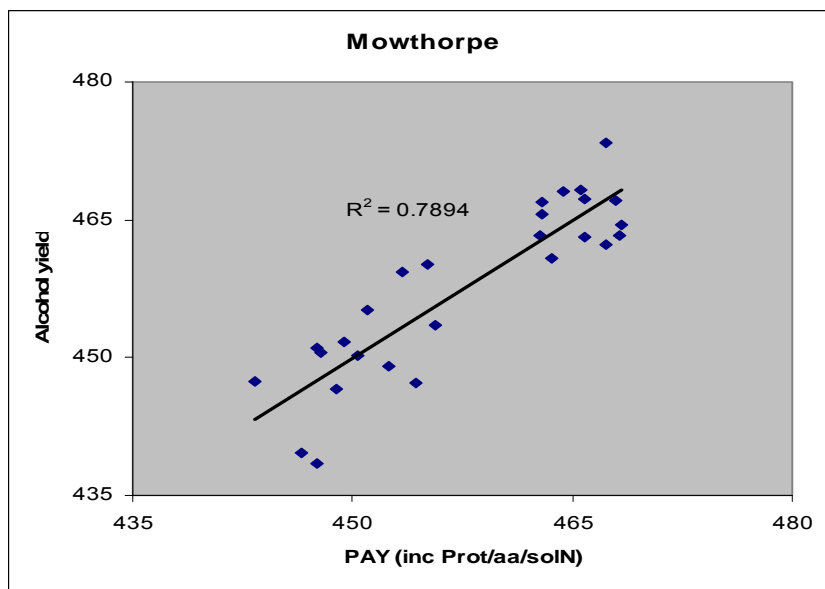


Fig 7. Alcohol yield plotted against a predicted alcohol yield (PAY) based on NIR prediction of alcohol yield + grain protein, α -amylase and soluble nitrogen.

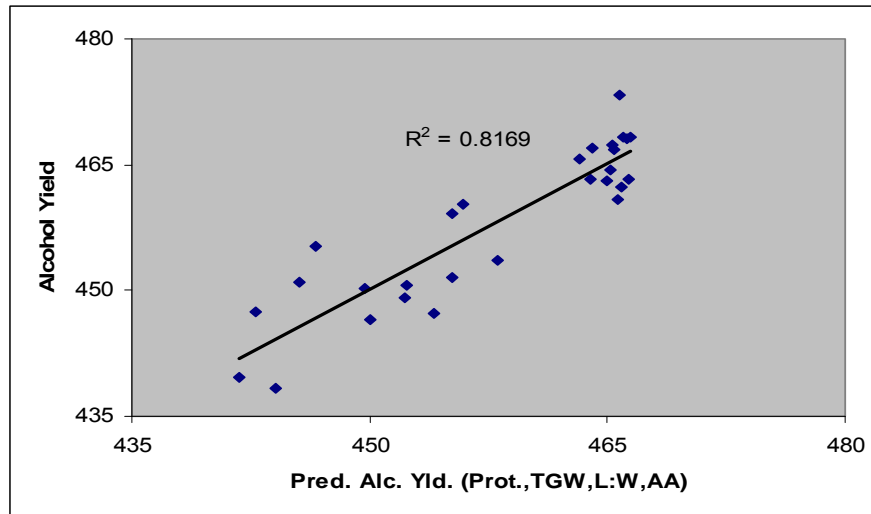


An alternative approach was based on the suggestion of Swanston et al (2007) that the inclusion of grain size parameters such as thousand grain weight and grain length to width ratio could improve the utility of grain protein as a predictor of alcohol yield. Direct application of the equation derived by Swanston et al. (2007) was, however, ineffective in predicting alcohol yield in the samples from High Mowthorpe (data not shown). A new equation was therefore derived, by multivariate regression, with α -amylase (AA) as an additional parameter, as follows:

$$y = 581.89 + 2.30TGW - 14.31GP - 18.36L:W - 3.55AA$$

This equation was used to derive predicted alcohol yields, which were plotted against the values obtained at SWRI (Fig 8) and gave a highly significant correlation with $R^2 = 0.82$.

Fig 8. Alcohol yield plotted against a predicted alcohol yield (PAY) based on grain protein, thousand grain weight, grain length:width ratio and α -amylase.



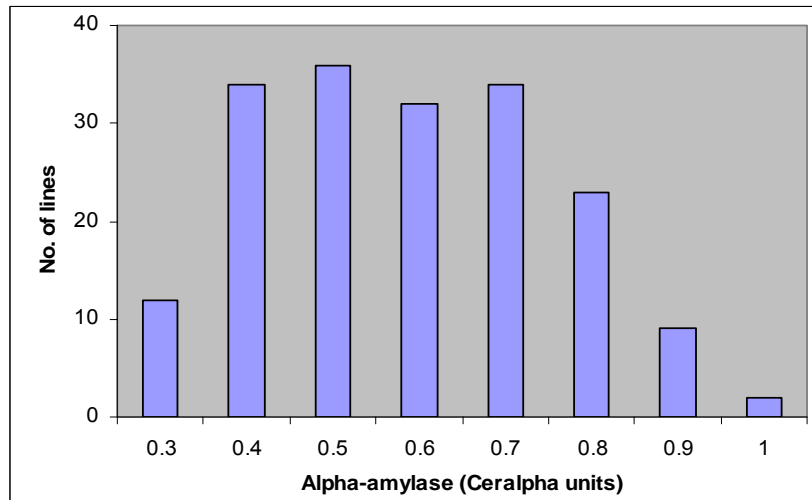
From both equations, it is clear that grain protein and α -amylase had negative effects on alcohol yield, so pre-harvest sprouting would therefore have a deleterious effect on distilling quality. This would be expected to result from a loss of fermentable material to the embryo during germination. However, the effect of soluble nitrogen was a small positive one. This suggests that protein solubilisation during sprouting is not only associated with increases in enzyme activity and that ease of solubilisation may reflect aspects of protein structure or composition that are favourable for distilling quality. This will require further investigation.

High Mowthorpe Population Trial

Measurements of α -amylase activities were made on a total of 384 samples representing two replicates of 182 inbred lines and 10 control varieties. The inbred lines showed a normal distribution (Fig 9), covering a fairly wide range of values, indicating that some of the inbred lines were demonstrating resistance to pre-harvest sprouting, whereas others were particularly susceptible. Due to problems with seed production, it had not been possible to include the parental variety, Canterbury within the trial, but the other parent, Eclipse, gave an α -amylase activity of 0.52 Ceralpha units. Irrespective, therefore, of whether Canterbury had high or low activity, there would be transgressive segregation in at least one direction. This is indicative that the α -amylase activities observed

were due to more than one genetic factor, so this characteristic would be suited to future studies aimed at locating the genetic factors involved.

Fig 9. Distribution of α -amylase activity within 182 inbred lines from the cross Canterbury x Eclipse.



Discussion and Conclusions

These preliminary data indicate that weather damage to wheat crops that leads to fairly high levels of pre-harvest sprouting is likely to cause two major problems for distillers. Firstly, it leads to a reduction in alcohol yield and, secondly, it adversely affects the capacity of NIR calibrations to accurately predict alcohol yield. The latter problem may, in effect, be the greater, since it will cause problems at intake and may lead to rejection of grain consignments. Results here indicated that grain protein was a more accurate predictor of alcohol yield than the existing NIR alcohol yield calibration and could be enhanced when factors relating to grain size were added. However, the regression equation derived by Swanston et al (2007) proved inaccurate, so it would appear that different equations may need to be calculated for each data set. This would clearly not be practicable for the distilling industry, so the most appropriate means of assessment may be to rely on protein determination, whilst ensuring that there have been no adverse effects on grain filling.

While high α -amylase levels were associated with a reduction in spirit yield, the effect was comparatively small. There was no significant, direct calibration between α -amylase and alcohol yield (Fig 6 a and b) and removing α -amylase from the calculation of PAY, as shown in Fig 8, had a very small effect on the R^2 value (data not shown). Values obtained for alcohol yield at Mowthorpe are high, especially among the samples with low grain protein levels, so it would appear that the effect of pre-harvest sprouting on alcohol yield is less than that associated with different environments (Swanston et al., 2007). Wet weather

immediately prior to harvest is clearly problematic, but drought conditions earlier in the season, which may restrict grain filling and lead to low starch and high protein levels are likely to have a greater adverse effect on distilling quality.

The variations in α -amylase observed in the inbred lines (Fig 9) indicates that there is genetic variation for the character. Locating the genetic factors involved could offer two major benefits to wheat breeders. Firstly, there would be the opportunity to identify DNA-based markers linked to, or associated with, these factors which could be used to screen breeding populations and assist selection for a reduced risk of pre-harvest sprouting. Secondly, it would be possible to determine whether genetic factors for α -amylase were independent of those influencing alcohol yield. Any evidence of association would have implications for the choice of parents and selection strategies for the progenies. The data collected in this project will therefore be of great value to future work on the genetic control of distilling quality that will constitute a major part of the GREEN Grain project.

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