The development and implementation of near infrared calibrations for predicting malting quality in barley

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Introduction

The South Australian Barley Improvement Programmeme (SABIP) encompasses barley breeding, agronomy, pathology, variety testing, quality evaluation and research, with the aim of producing new malting barley varieties which are adapted to the climatic and agronomic conditions of South Australia. Early generation material (Stage 0–1) includes trials up to F5, introduced lines, cross evaluation experiments and lines from the doubled haploid programme. Intermediate material (Stage 2–3) includes lines up to the F6–F7 generations. Late generation (advanced) trials (Stage 3–4) include the most advanced stages of the breeding programme. Detailed malt quality data is required from advanced trials to help discriminate between lines over a range of sites and seasons. Advanced testing is followed by commercial evaluation through pilot brewing and full-scale commercial trials.[1]

In the development of improved barley cultivars for malting end uses, selection for quality characteristics has traditionally involved small-scale (micro) malting and subsequent wet chemistry analysis. Micro-malting and analysis are destructive on grain samples, time-consuming and resource-demanding. These time and resource limitations largely determine the number of malting quality assays possible in a given season of a barley breeding programme—currently 3,500 per annum. Furthermore, malting is not possible until post-harvest dormancy is broken, so quality data is not available in time for selection of lines prior to seeding. Micro-malting based quality selection is, therefore, largely restricted to late generations where the number of individuals is substantially reduced following selection for other important traits such as maturity, plant type, disease resistance and grain yield. Selection, based on micro-malting, has been shown to be effective in the development of improved malting quality cultivars but, typically, genetic progress has been slow and improvements have generally been small. The confinement of micro-malting based quality evaluation to the already highly selected, genetically narrow, late generations of breeding programmes is likely to have substantially reduced the overall potential genetic gain. To improve the rate of genetic improvements in malting quality, it is necessary to select early generations, which are characterised by large population sizes and high genetic variance.

Two quality parameters of major importance to the malting and brewing industries are hot water extract (HWE) and grain protein (GP). HWE provides the brewer with an indication of the amount of extractable material in the malt (carbohydrate and proteins) that can be utilised during the brewing process. Low levels of GP (9.0–11.8%) are desirable as the lower the proportion of protein present in the grain the greater the proportion of carbohydrate and therefore fermentable sugars.[2]

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High throughput, efficient malting quality screening methods are required for selection among the large number of individuals typical of early generation stages of a breeding programme. Near infrared (NIR) spectroscopy-based malting quality prediction and molecular marker assisted (not discussed in this paper) selection appear to be the most promising tools to facilitate early generation malting quality selection. NIR transmission spectroscopy has been used to measure malt quality parameters in wort.[3,4] Furthermore, NIR has been applied to ground malt flour and ground barley flour for the analysis of HWE, milling energy, beta-glucan and protein.[5] The most efficient screening option would be to use whole barley grain, yet few studies[6] have reported attempts to develop calibrations of this type.

In this study, whole grain NIR calibrations were developed to predict HWE and GP. The implementation of NIR calibrations for HWE and GP in a practical breeding programme is discussed.

Experimental

Calibration development

Grain samples

172 barley samples were obtained from the 1994 Stage 0 early generation trials, grown at Charlick in South Australia, along with appropriate control samples. These lines were specifically chosen to represent a range of genetic backgrounds and crosses available in the SABIP.

Micro-malting and malting quality analysis

Barley samples were screened over a 2.2 mm screen, and 30 g of each was micro-malted in a Phoenix Automatic Micro-malting System without the use of additives. The micro-malting schedule has three main stages: (a) Steep and Air Rest, 7 : 8 : 9 : 6 : 0.5 hours (wet : dry : wet : dry : wet) at 15°C, (b) Germination, 88.5 hours at 15°C and (c) Kilning, 30–40°C for nine hours, 40–60°C for four hours, 60–70°C for two hours and 70–80°C for four and a half hours.[1]

Malt quality parameters were assessed using standard analytical methods. HWE was analysed by a small-scale version of the recommended EBC fine grind extract method.[7] GP was measured using a small-scale variation of the standard Kjeldahl assay and a Kjeltec auto distillation unit.[8]

Table 1. Whole grain calibration and validation statistics for HWE and GP, developed from 1994 early generation lines and validated with 1995 season samples.

	Calibration set						Validation set			
Malt character	*N*	Range	No. of factors	Regression method & math treatment	*R*	*SEC*	*N*	Range	*R*	*SEP*
Hot water extract	172	76.0–85.9	6	PLS 2nd deriv segment 10 gap 0	0.893	0.8	12	73.0–82.8	0.851	0.69
Grain protein	105	8.9–13.9	11	PLS 2nd deriv segment 10 gap 0	0.982	0.22	12	8.6–17.0	0.959	0.19

N = Sample number
R = Correlation coefficient
SEC = Standard error of calibration
SEP = Standard error of prediction

NIR calibrations

An NIRSystems 6500 scanning spectrophotometer, in conjunction with NSAS v3.30 software, was used to develop the calibrations. Samples were scanned as whole grain and absorbance data was measured in reflectance mode using the whole 400–2500 nm range. All samples were scanned in duplicate with the second scan being a repack of the first. A second derivative mathematical treatment was subsequently applied with segment and gap sizes of 20 and 0 and 10 and 0, respectively. The spectra were averaged and Partial Least Squares (PLS) Regression applied to find the model best fitting the data.

Calibration development and validation

Whole grain calibration and validation statistics are presented in Table 1. A calibration was produced for HWE, using six PLS derived factors, which gave a correlation of 0.893 and a standard error of 0.68. The laboratory standard error of this method is 0.69, which shows that the results are comparable. When this calibration was validated, a correlation of 0.851 between laboratory and NIR values was calculated.

For GP, a calibration was generated using 11 PLS factors, giving a correlation of 0.982 and a standard error of 0.22. Laboratory kjeldahl analysis gave a standard error of 0.19. Validation gave a correlation of 0.959 between laboratory and NIR values.

Implementation

NIR based screening of early generation breeders lines, using the calibrations for whole grain HWE and GP prediction was first implemented into the SABIP for the 1996 season and subsequently expanded for the 1997 and 1998 season early generation trials. These calibrations have been used successfully to predict the malting quality of 3,000 lines from the 1996 season, 7,100 lines from the 1997 season and 6,100 from the 1998 season early generation trials.

The growing season in South Australia takes place from May through to December, with harvest from November to January. Grain samples are then cleaned and screened and subsequently NIR tested between January and April. Entry decisions for new season trials, using malt quality information provided through NIR testing and agronomic data can, therefore, be made just prior to sowing.

There are a number of factors that need to be taken into consideration when using NIR for routine testing. Several environmental factors have been identified which can affect results and introduce variation. Sample temperature has been found to affect NIR prediction.[9] A significant positive correlation ($R = 0.78$***, $P < 0.001$) was found between the mean Schooner control standard deviation and maximum daily temperature. Humidity, pigmentation, skinning (either due to harvest or threshing damage) and sample cleanliness have also been found to influence NIR prediction. For skinned samples in particular, NIR predictions are always 1–3% higher than normal. As a result, within the SABIP, several processes have been put in place to provide efficient throughput of samples, careful handling, cleaning and storage of grain and ensure accuracy and reliability of results.

Routine NIR testing involves several stages that are described below.

Sample preparation

In readiness for NIR testing, samples are cleaned and sieved over a 2.2 mm screen using a Perten Sample Cleaner model SLN3. This instrument has the advantage of cleaning and grading by size simultaneously. The gentler deawning action of this machinery also helps prevent skinning. During busy periods, or times of instrument breakdown, the SABIP also utilises a Labofix Indent Cylinder screening machine. However, this method of sample preparation is more labour-intensive, as it requires the sample to be previously cleaned using a thresher which increases the chances of skinning the grain.

Table 2. Validation correlation coefficients for HWE and GP prediction by NIR for the 1996 and 1997 seasons.

Malt quality trait	Simple correlation		Spearman correlation	
	1996 season	1997 season	1996 season	1997 season
Hot water extract	0.82***	0.66***	0.84***	0.64***
Grain protein	0.85***	0.90***	0.87***	0.88***

*** $P < 0.001$

NIR testing

Before NIR testing, samples are placed overnight, in the same room as the NIR instrument to allow them to equilibrate to room temperature (21–24°C). Using the NSAS software, both HWE and GP are analysed with a single scan.

Validation of NIR testing

To validate NIR calibrations, samples are randomly selected from all experiments within the early generation trials. These samples are micro-malted and analysed for GP and HWE. Simple correlation analysis is carried out to compare laboratory results to NIR predicted results. The Spearman Rank Correlation Coefficient is also calculated to determine whether the laboratory method and the NIR testing are ranking barley varieties/lines in similar order. To validate the 1996 season Stage 0 experiments, laboratory testing was carried out on 140 samples randomly selected from 3000 NIR tested. Similarly, for the 1997 season validation, 300 samples were chosen and laboratory analysed. Table 2 presents the validations for the 1996 and 1997 seasons.

Monitoring of calibrations

Validation of NIR testing is not often possible until after breeders' selection decisions have been made. As a result, calibrations need to be constantly monitored whilst samples are being NIR tested. To assist in monitoring calibrations, control varieties are checked to ensure they rank correctly. Table 3 shows the mean percentage HWE and mean GP-adjusted HWE values for six controls from the 1996, 1997 and 1998 seasons. HWE results are adjusted for within-site variation in GP, therefore the protein-adjusted values represent the deviation in % HWE from the regression of HWE on GP. The results in Table 3 are very reassuring as they show that varieties are being ranked correctly. High extract varieties such as Harrington, Chariot and Franklin, rank either first or second, whilst feed varieties such as Galleon, Chebec and Barque consistently have the lowest ranking.

Conclusion

NIR prediction has shown substantial promise as an early generation screening technique. Calibrations to predict HWE and GP have been developed and validated and have subsequently been successfully implemented into the SABIP. Provided care is taken with grain handling, sample storage and monitoring of controls, validations suggest that these calibrations are adequate for application within the SABIP. Whole grain NIR has numerous benefits. Up to 350 whole grain samples can be analysed by NIR per day. In contrast, only 20 EBC HWE samples can be laboratory-analysed per day when micro-malting, sample preparation, grinding and analysis are taken into account. The ability to predict HWE and GP from whole grain by NIR has triggered fundamental changes to the breeding strategy employed by the SABIP. Selection in early generations (Stage 0–1) can now be made on agronomic pa-

Table 3. Mean % HWE and mean GP adjusted HWE values for six controls from the 1996, 1997 and 1998 season Stage 0 early generation trials.

Growing season	Variety/line	Mean % HWE	Mean GP adjusted HWE value	Ranking
1996	Harrington	83.31	1.49	1
	Franklin	82.45	1.45	2
	Schooner	81.62	0.28	3
	Skiff	80.90	–0.22	4
	Chebec	79.96	–0.19	5
	Galleon	80.12	–0.78	6
1997	Chariot	82.04	2.25	1
	Franklin	80.45	1.41	2
	Schooner	79.23	0.68	3
	Skiff	78.52	–0.55	4
	Chebec	77.61	–0.89	5
	Barque	76.81	–1.60	6
1998	Alexis	82.14	0.93	1
	Chariot	81.76	0.91	2
	Franklin	81.61	0.82	3
	Schooner	81.43	0.54	4
	Chebec	80.10	–0.70	5
	Barque	79.45	–1.46	6

rameters such as maturity, lodging and screenings percentage, as well as predicted HWE and GP (Table 4). Based on a combination of agronomic data and NIR testing, 70% of early generation lines have been culled from the breeding programme for both the 1996 and 1997 seasons. This increase in selection efficiency has allowed the breeders to increase population sizes in Stage 0 from 8,000 to 15,000 (Table 4). Similar numbers of lines are entering Stage 1. However, they should be of significantly better malting quality.

Although whole grain NIR has obvious advantages over whole malt or barley and malt flour NIR prediction, the predictive capabilities of whole grain NIR for quality parameters other than HWE and GP has yet to be assessed. The capability to predict other malt quality parameters using NIR would be advantageous. As a result, with the purchase of ISI software in 1997, as part of a national standardisation initiative, an experiment has been designed to develop whole grain calibrations for a broader range of malt quality parameters. Whole malt calibrations will also be investigated as they may prove more successful for quality parameters only expressed in malt for example, alpha-amylase. It is anticipated that calibrations will be developed to predict HWE (EBC and IOB), viscosity, diastatic power, beta-amylase, alpha-amylase, beta glucan, free amino nitrogen, grain colour, grain protein, malt protein and friability.

Table 4. Where NIR technology fits into the SABIP quality evaluation strategy.

Stage	Entry number before culling			Traditional malt quality tests	NIR testing
	1997	1998	1999		
4	25	25	25	Micro-malt, HWE, viscosity, diastatic power, alpha-amylase, beta-amylase, malt protein, GP, free amino nitrogen, apparent attenuation limit, grain colour, friability	None
3	150	180	150	Micro-malt, HWE, viscosity, diastatic power, alpha-amylase, beta-amylase, malt protein, GP, free amino nitrogen,	None
2	500	500	500	Micro-malt, HWE, viscosity, diastatic power, malt protein	None
1	2,500	2,600	3,000	Micro-malt, HWE, viscosity, diastatic power, malt protein	Whole grain NIR
0	8,000	12,000	15,000	None	Whole grain NIR

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