

The heritability of malt extract predicted using NIR spectroscopy

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Introduction

In southern Australia, genetic progress for malting quality characteristics, particularly malt extract, has been slow. Part of the problem lies with the nature of conventional micro-malting and subsequent quality analysis which are time consuming, expensive and destructive. These assays have often restricted selection for malt quality to highly selected, genetically narrow, mid-late generations of breeding programs. To hasten improvements in malting quality, high throughput assay systems are required. These must be suitable for early generations of the breeding program, which are characterised by large population sizes and high genetic and environmental variance. Whole grain NIR spectroscopy offers the opportunity for efficient early generation selection. NIR calibrations for hot water (malt) extract (HWE) and grain protein (GP) have been developed and reported at the last BTS (Roumeliotis *et al.*, 1997). NIR calibrations for HWE and GP correlated closely with EBC extracts ($R=0.851$, $P<0.005$) and Kjeldahl proteins ($R=0.982$, $P<0.005$) respectively. These calibrations have been validated and successfully implemented into a practical breeding program with over 6000 samples scanned per annum.

In this study, aspects of the implementation of NIR calibrations for HWE and GP in a practical breeding program are discussed. Special reference is made to heritability of NIR predicted HWE, response to selection, the power of discrimination between cultivars of known quality and the potential application in predicting the breeding outcomes of specific cultivar crossing combinations.

Materials and methods

Micromalting and Malting Quality Analysis

Barley samples were screened over a 2.2mm screen, and thirty grams of each was micromalted in a Phoenix Automatic Micromalting System without the use of additives. The micromalting 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 9 hours, 40-60°C for 4 hours, 60-70°C for 2 hours and 70-80°C for 4.5 hours (Anon, 1999). HWE was analysed by a small scale version of the recommended EBC fine grind extract method (MacLeod *et al.*, 1991). GP was measured using a small scale variation of the standard Kjeldahl assay and a Kjeltex auto distillation unit (Anon., 1998).

NIR calibrations and scanning

Calibrations were previously developed on a NIRSystems 6500 scanning spectrophotometer in conjunction with NSAS v3.30 software (Roumeliotis *et al.*, 1997). Samples were scanned as whole grain and absorbance data was measured in reflectance mode using the whole 400-2500nm range.

Early generation rows 1996-98

In each year, experiments were sown at Charlick Experiment Station, near Strathalbyn in South Australia. Each experiment consisted of 900 plots, 4m long by two rows wide. Lines were grouped by family and F4 lines were derived from F3 single plants. A grid of six control varieties was placed every sixth plot.

Skiff / Gimpel population : Parent – Offspring study

103 F₄ derived individuals from the cross Skiff (low-medium HWE) x Gimpel (medium-high HWE) were grown at Charlick Experiment Station in 1996, 1997 and 1998. The experiments were single replicate with a control grid of Australian varieties every sixth plot.

Results

Correlation of EBC HWE (from micromalting) and NIR predicted HWE

EBC HWE (from micromalting) and NIR predicted HWE were highly correlated ((Roumeliotis *et al.*, 1997 and Table 1). More importantly, varieties ranked similarly as shown by highly significant Spearman rank correlation coefficients.

Table 1. Correlation coefficients for HWE and GP prediction by NIR for the 1996 and 1997 seasons. (***) P < 0.001)

Malt Quality Trait	Simple Correlation		Spearman Correlation	
	1996 season	1997 season	1996 season	1997 season
HWE	0.82***	0.66***	0.84***	0.64***
GP	0.85***	0.90***	0.87***	0.88***

Ranking of 'control' varieties

Commercial varieties, which were well characterised for malt extract, were scanned to ensure they rank as expected. High extract varieties such as Harrington, Chariot and Franklin, ranked either first or second, whilst feed varieties such as Galleon, Chebec and Barque consistently have the lowest ranking (Table 2).

Table 2. Mean % HWE and mean GP adjusted HWE values for six controls from the 1996, 1997 1998 season Stage 0 early generation trials.

Variety/Line	Mean % HWE			Ranking		
	1996	1997	1998	1996	1997	1998
Harrington	83.31			1		
Franklin	82.45	80.45	81.61	2	2	3
Schooner	81.62	79.23	81.43	3	3	4
Skiff	80.90	78.52		4	4	
Chebec	79.96	77.61	80.10	5	5	5
Galleon	80.12			6		
Chariot		82.04	81.76		1	2
Barque		76.81	79.45		6	6
Alexis			82.14			1

Broad sense heritability of NIR HWE

HWE predicted by NIR is highly heritable. Heritability estimates based on four replicates of each of the control varieties listed in Table 2, grown in early generation experiments range from 0.51 to 0.92 over the period 1996-1998 (Table 3). Lower estimates were observed

where either missing values occurred (Exp87/1996), or feed varieties (with low malt extract) were not included (exp80/1996).

Table 3. Broad sense heritability estimates for predicted HWE, based on nine ‘control’ varieties grown in early generations trials.

1996 season		1997 season		1998 season	
Exp 80	Exp 87	Exp 37	Exp 40	Exp 43	Exp 45
0.65	0.51	0.92	0.92	0.66	0.84

Skiff / Gimpel population: Parent – Offspring study

The heritability of NIR predicted HWE was calculated as the parent-offspring correlation in 103 F₄ derived individuals from the cross Skiff (low-medium HWE) x Gimpel (medium-high HWE), grown at Charlick Experiment Station in 1996, 1997 and 1998. Correlation coefficients between the NIR prediction and laboratory method were significant in all years (Table 4). HWE predicted by NIR was highly correlated with EBC HWE in this population for the 1996 and 1997 seasons (Table 5, 1998 data unavailable at time of publication).

Table 4. Correlation (= heritability) of HWE by laboratory EBC and NIR between 103 individuals in Skiff x Gimpel population grown in 1996, 1997 and 1998 (all correlations significant at P<0.001).

	1996 EBC HWE	1997 EBC HWE
1996 NIR	0.71	0.32
1997 NIR	0.50	0.51
1998 NIR	0.38	0.57

Table 5. Comparison of laboratory analysis and NIR prediction for HWE and GP in 103 F₄ derived lines from the cross Skiff x Gimpel. (***) P< 0.001)

Malt Quality Trait	Simple Correlation		Spearman Correlation	
	1996 season	1997 season	1996 season	1997 season
HWE	0.71***	0.57***	0.72***	0.53***
GP	0.77***	0.84***	0.72***	0.80***

Discussion

A barley breeder must be convinced that implementation of NIR screening will result in a consistent response to selection. Specifically the breeder must know the heritability of the trait, its power to discriminate between varieties, any correlated traits and environmental influences. All of these issues were addressed over the period 1996-1998. We are now confident that we can separate genotypes that have similar or greater HWE than Schooner from low extract, feed types. Since many crosses still involve feed types as a source of disease resistance and /or grain yield, it is important to be able to discard poor quality segregants as early as possible. Furthermore, large population sizes are required to recover desirable recombinants and currently NIR prediction is the only suitable option for screening of very large populations.

HWE values are currently adjusted for grain protein, so that selection for HWE is independent of protein. In breeding malting barley for the low rainfall areas of South Australia, simultaneous selection for low protein would also be useful. Current calibrations are suitable for this task.

The NIR calibration used to predict HWE is complex and reads across a wide range of wavelengths. We have confidence that it is however incorporating key traits determining malt extract. Firstly, we know that wavelengths that detect starch and sugars feature heavily in the calibration equation. Secondly, mapping studies in the population Galleon x Haruna nijo show a major QTL in common for laboratory and NIR HWE, which also is associated with husk content.

The current breeding system used by the S.A. Barley Improvement Program can also use NIR screening as a cross prediction tool. Many programs have separate cross prediction experiments where a small (approximately 25) number of individuals are field tested and then malted in the next season before a decision to proceed with the cross is made. This is costly in time and resources since the program subsequently has two cohorts of material from the same cross. NIR screening applied to early generation rows provides simultaneous cross prediction and real selection data, prior to the deadline for seeding the following crop. Crosses of known 'high' and 'low' extract potential have been detected using NIR (Table 6). The low predicted extract potential is conditioned by alleles from the feed varieties Barque, Mundah, and/or Forrest. Conversely, Alexis, Fitzgerald and Harrington contribute to the high predicted extract potential of the superior crosses.

Table 6. The use of NIR as a cross prediction tool – Mean predicted HWE adjusted for protein effects and expressed as deviation from the site mean HWE for three high extract potential and three low extract potential crosses from early generation plots.

High predicted extract crosses			Low predicted extract Crosses		
Cross	N	Mean HWE	Cross	n	Mean HWE
WI2875-1/Harrington//VB9624	37	+0.42	Forrest/Chariot//Barque	7	-0.51
Waveney/9104//Alexis/Chebec	9	+1.21	Waveney/9104//Barque	10	-1.20
Waveney/Chebec//Fitzgerald	13	+0.63	WI2875-2/Mundah//Barque	20	-0.70

The calibrations developed so far have been specifically for the Charlick Experimental Station. We are not confident of extrapolating to samples from other sites but observe that control varieties among samples grown at Pinery in 1997 in Stage 1 trials gave consistent results. The most obvious environmental effect is protein. The early generation trials are grown as a single replication in 2 row x 4 metre in large blocks of 900 rows. Variation in protein within an experiment is often in the range 2-5% . While it is possible to adjust HWE for protein, the environmental variation in protein is still one of the main factors reducing selection efficiency.

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 successfully used to predict the malting quality of 3000 lines from the 1996 season, 7100 lines from the 1997 season and 6100 from the 1998 season early generation trials. 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 early generation trials of 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 micromalting, 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. Based on agronomic data, percentage screenings and NIR testing, 70% of early generation lines have been culled from the breeding program for both the 1996 and 1997 seasons. Selection at Stage 0 can now be made on maturity, lodging, screenings and predicted HWE and GP. This more efficient culling procedure results in the same number of lines entering Stage 1, however they should be of significantly better malting quality.

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References

- Anon. (1998). Analysis Committee of the EBC, *Analytica EBC*, Verlag Hans Carl Getranke-Fachverlag, Section 3 Barley Method 3.3.1.
- Anon., (1999). S.A Barley Improvement Program, *Barley Quality Report 1997 Season*, University of Adelaide, Waite Campus.
- Roumeliotis, S., Logue, S.J., Barr, A.R. and Jefferies. S.P. (1997). *Proc. 8th Aust. Barley Tech. Symp.*, Gold Coast, Queensland, p. 2.4.27 – 2.4.33.
- Macleod, L.C., Lance, R.C.M., Dowling, M.A., Logue, S.J. and Sparrow, D.H.B. (1991). *Proceedings of the 5th Aust. Barley Tech. Symp.*, Horsham, Victoria, p. 80.