

## Response to Selection for Increased Malt Extract

*Sophia Roumeliotis and Jason Eglinton*

School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond SA, 5064, South Australia

### Abstract

The level of malt extract remains a critical parameter for the success of new varieties in the malting and brewing industry. Physical and biochemical analyses have identified husk thickness as a key factor influencing malt extract. The potential negative impacts of thin husk, including propensity for skinning damage and pre-harvest sprouting, are outlined in the context of breeding strategies to minimise these risks. Genetic analysis of malt extract within the Galleon x Haruna Nijo mapping population confirmed the association of both traits with the short arm of chromosome 2H, and highlighted the ability of NIR to detect variation conferred by this locus. Subsequent research focused on the development of NIR calibrations for husk content and the validation of molecular markers linked to the 2HS locus. Crossing schemes to introgress the thin husk trait across a broad range of germplasm have been executed in conjunction with selection strategies using MAS for allele enrichment in segregating populations and whole grain NIR screening of early generation fixed lines. The response to genotypic and phenotypic selection for increased malt extract within the University of Adelaide Barley Program is examined over the last ten seasons (1997-2006). The results of the study are discussed in terms of the rate of genetic gain achieved within the breeding program and the impact of this approach in improving outcomes from wide crossing strategies.

### Keywords

Malt Extract, Marker Assisted Selection, Near Infrared Spectroscopy, Husk Content

### Introduction

Malt extract is the key economic parameter for maltsters and brewers in all markets. High levels of malt extract are therefore desired by the malting and brewing industries. Since the success of Schooner in the mid 1980's, mainstream Australian varieties have failed to match the malting quality standards set by European and Canadian varieties. Subsequently by the early 1990's, Australia's share of the important Japanese market for example, had fallen from 33% to 19% (Powell, 1997). This loss in market share can be largely attributed to the lower levels of malt extract in Australian varieties (Roumeliotis *et al*, 1999).

The Australian barley industry responded to this by conducting a major national research program on the genetics of quality, whilst simultaneously investing in infrastructure for quality evaluation to support an increased focus on breeding for malting quality.

This paper reports on the response to selection for increased malt extract by addressing the impact of improved genetic knowledge and specifically the implementation of Marker Assisted Selection (MAS) and Near Infrared Spectroscopy (NIR) selection strategies on breeding for quality, based on a long term analysis of malt extract. The response to genotypic and phenotypic selection for increased malt extract within the University of Adelaide (UA) Barley Program is examined over the last ten seasons (1997-2006). The genetic gain achieved for malt extract through the combination of MAS, NIR and pragmatic breeding is also analysed. In addition opportunities for future gains in malt extract are addressed including the effective deployment of very thin husk which may potentially offer a pathway to higher levels of malt extract. The potential of the next generation of elite malting quality is discussed through an introduction to the quality profile of the UA Barley Program line WI4262.

## Genetic analysis of malt extract

Within the UA Barley Program, molecular mapping efforts have focussed on identifying the genetic basis for malting quality within a range of international germplasm. The four loci that have been the most extensively characterised are located on chromosome 1H (Alexis derived), 2HS and 2HL (both Haruna niyo derived) and 5H (Harrington derived). These four now form the basis of routine MAS within the UA Barley Program. The relationship between malt extract and various factors contributing to malt extract was investigated in the Galleon x Haruna niyo mapping population. The interval map for barley chromosome 2H for Galleon x Haruna niyo shows that both malt extract and husk content are influenced by the same region, with Haruna niyo alleles conferring high malt extract and low husk content (Figure 1.) This work and subsequent validation studies confirm that the Haruna niyo 2HS locus increases malt extract by conferring decreased husk content.

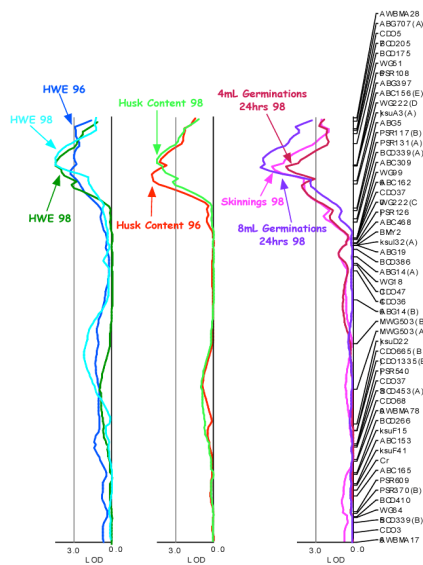


Figure 1. Interval map for barley chromosome 2H, for Galleon x Haruna niyo mapping population (Collins *et al*, 1999).

## Impact of Marker Assisted Selection on malt extract in Australian germplasm

Routine MAS within the UA Barley Program commenced in 1995 with disease resistance and abiotic stress tolerance the predominant target traits at that time. Since then the number of traits under selection and the scale of the Program has increased dramatically with a total of 45,000 assays across 18 traits completed in 2006. Routine marker screening for malt extract began in 2000. Table 1 shows the number of marker screens carried out between 2000 and 2006, demonstrating a rapid and significant increase.

Table 1. UA Barley Program marker screens for malt extract between 2000 and 2006.

Year	Number of marker screens for malt extract
2000	889
2001	1395
2002	1994
2003	2617
2004	2411
2005	3772
2006	9347

To determine the impact of MAS on malt extract in Australian germplasm, NIR whole grain analysis data from F<sub>3</sub> derived F<sub>4</sub> lines grown in Stage 0 double row trials was examined. This included all malting quality germplasm NIR scanned between 1997 and 2006 with the number of lines ranging in each year from 2,561 to 5,977, with a total of 38, 209 lines. The control variety Schooner was included in the control grid each year and this was used as the comparative basis.

Figure 2 shows the frequency of lines in Stage 0 with malt extract superior to Schooner. The plot illustrates that there has been a significant increase in the number of lines with high malt extract between 1997 and 2006. In 1997 only 21% of lines had higher malt extract, however the frequency has increased to 81% in 2006.

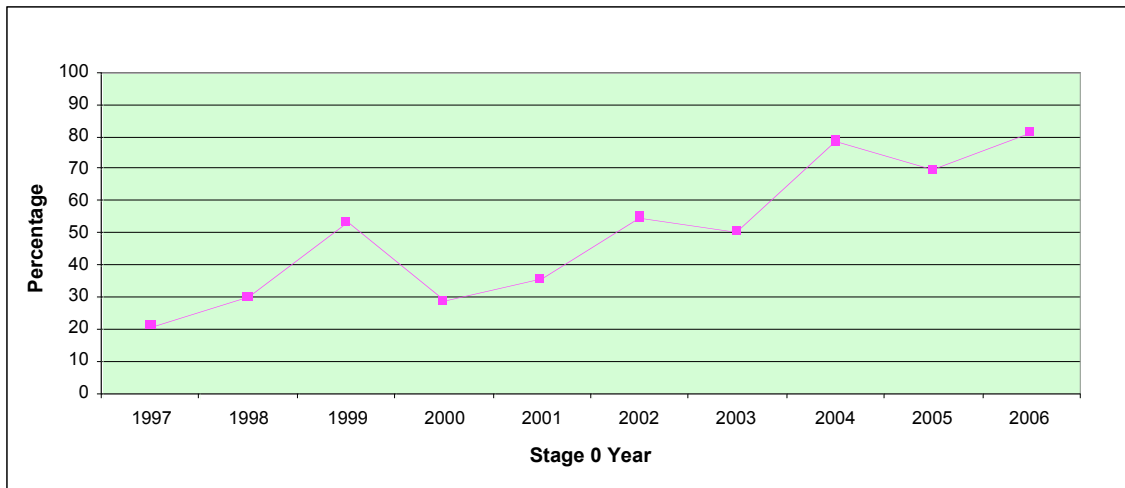


Figure 2. The frequency of lines in UA Barley Program Stage 0 trials between 1997 and 2006 with malt extract superior to Schooner.

The relationship between the intensity of MAS and the malt extract levels across the germplasm base is shown in Figure 3. This plot illustrates the frequency of lines in Stage 0 with malt extract superior to Schooner, overlaid with the corresponding marker screens for malt extract, which were conducted on the preceding complex cross F<sub>1</sub> populations. As marker screen numbers have increased so has the frequency of lines with higher malt extract. The close relationship between the level of marker screening and the percentage of lines with malt extract greater than Schooner, illustrates the major impact of MAS in selecting for improved levels of malt extract.

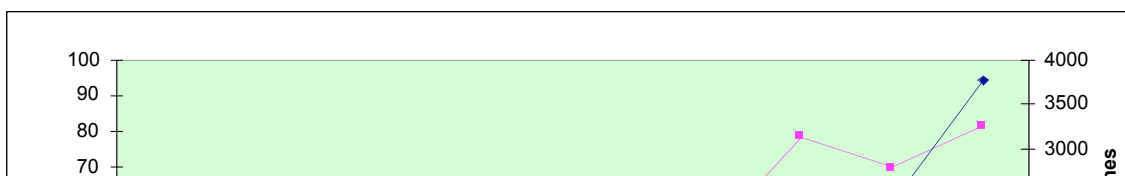


Figure 3. The frequency of lines in UA Barley Program Stage 0 trials between 1997 and 2006 with malt extract superior to Schooner overlaid with the corresponding marker screens for malt extract, which were conducted on the preceding complex cross F1 populations.

### **Impact of Near Infrared Spectroscopy on malt extract in Australian germplasm**

The second key strategy used to select for increased malt quality is NIR. This technology is used to predict malting quality in early generation material. Samples are scanned and the results are used as a basis for selecting lines for promotion to Stage 1 yield trials.

NIR uses both visible and near infrared light which is reflected in the range 400nm-2500nm at 2nm intervals. The reflected light at known wavelengths indicates the relative composition of the barley. It is a high throughput, non destructive technology. 'In-house' calibrations have been developed and used in the UA Barley Program since 1995, with the database now containing over 3,000 samples. Sixteen calibrations have been developed since 1995, with both whole grain and whole malt applications.

In order to maintain robust calibrations, a representative subset of the season samples is added to the database on an annual basis to account for new germplasm and for differing seasonal conditions, In addition, this group of samples acts a validation set. For malt extract, the correlations between the laboratory results and the NIR are very high;  $R^2=0.925$  for whole grain and  $R^2=0.953$  for whole malt, providing high confidence in the predictive power of the technology.

To assess the impact of NIR on malt extract in Australian germplasm, malt quality analysis of  $F_3$  derived  $F_5$  lines from Stage 1 yield trials was examined. This included all malting quality germplasm, micromalted and analysed between 1997 and 2005 with the number of lines ranging in each year from 196 to 391, with a total of 2,583 lines. The control variety Schooner was included in the control grid each year and this was used as the comparative basis.

Figure 4 illustrates the frequency of lines in Stage 1 with malt extract superior to Schooner. The plot shows that there has been a significant increase in the number of lines with higher malt extract between 1997 and 2005. In 1997 only 42% of lines had higher malt extract however this has increased to 91% in 2005. Importantly, this data validates the selection of lines for promotion using whole grain NIR.

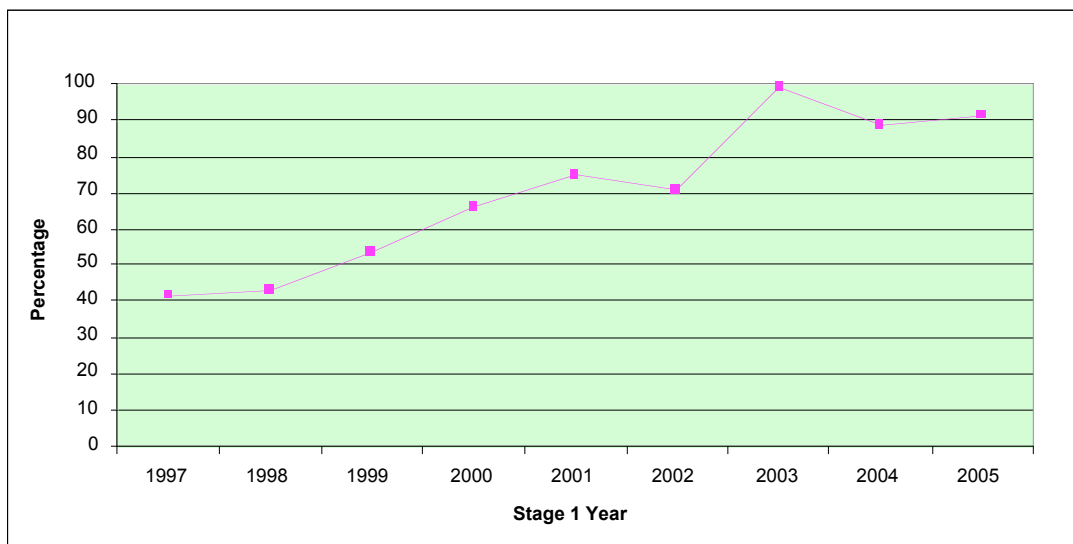


Figure 4. The frequency of lines in UA Barley Program Stage 1 trials between 1997 and 2005 with malt extract superior to Schooner.

The relative effectiveness of NIR selection across each season is shown in Figure 5. The frequency of lines in Stage 1 trials with higher malt extract than Schooner is overlaid with the corresponding frequency of lines in Stage 0 with malt extract superior to Schooner. These samples had been NIR scanned the previous year as F<sub>3</sub> derived F<sub>4</sub> lines. NIR selection has increased the frequency of lines with high malt extract in all years, with the increase ranging between 16 and 161%, depending on the season.

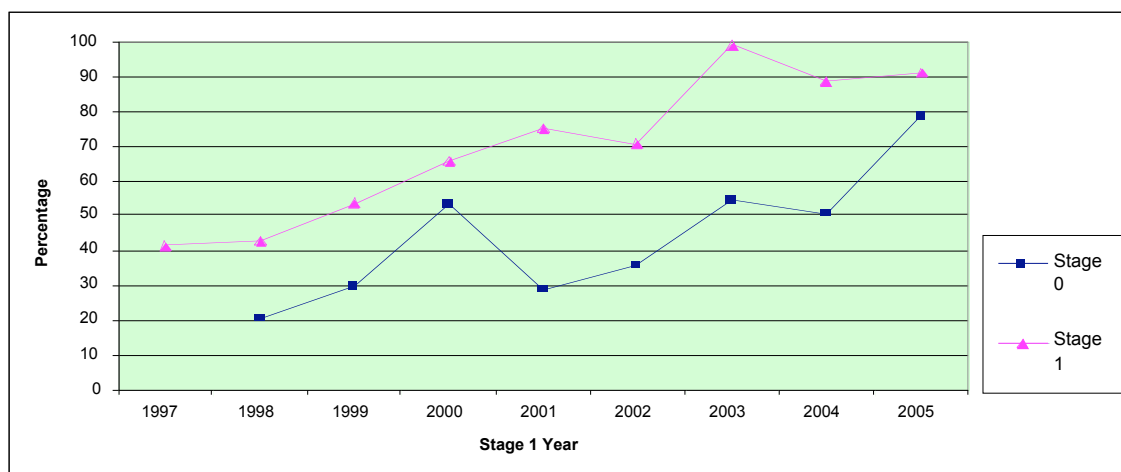


Figure 5. The frequency of lines in the UA Barley Program Stage 1 trials with higher malt extract than Schooner, overlaid with the corresponding frequency of lines in Stage 0 with malt extract superior to Schooner.

There are however some limitations in using NIR selection, particularly in seasons resulting in poor physical grain quality. In drought affected seasons as was the case in 1999 and 2004, NIR selection was shown to be less effective, with a lower frequency of lines with higher malt extract than Schooner evident in 2000 and 2005 when samples were micromalted and analysed in Stage 1 trials.

## Validation of combined MAS, NIR and traditional selection for malt extract in Australian germplasm

To validate the combined effects of MAS, NIR and traditional selection for malt extract, the malt quality analysis of advanced lines from Stage 3 replicated yield plots at multiple sites was examined. All malting quality germplasm, micromalted and analysed between 1997 and 2005, comprising a total of 326 lines was assessed. This analysis was based on 22 site by season combinations and all analyses were carried out using small scale EBC standard methods (Roumeliotis and Tansing, 2003). The control variety Schooner was included each year and this was used as the comparative basis.

Figure 6 illustrates the frequency of lines in the UA Barley Program Stage 3 trials with malt extract superior to Schooner between 1997 and 2005. The period 1997 to 1999 is the product of traditional selection alone, with the frequency of lines exhibiting acceptable quality oscillating between 11 and 39% only. In addition, these lines were equally variable in their agronomic performance.

The combined impact of MAS and NIR selection strategies can be measured from 2000 onwards, with the frequency of lines with higher malt extract rising from 15% in 2000 to 96% in 2005. It is also significant to acknowledge that this improvement in malt quality has been achieved with simultaneous improvements in grain yield and disease resistance.

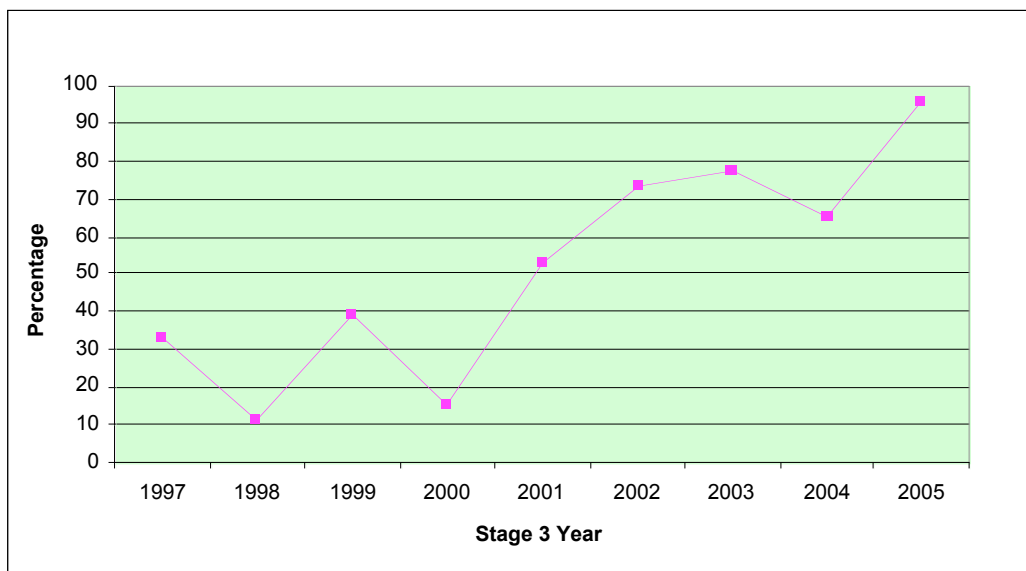


Figure 6. The frequency of lines in the UA Barley Program Stage 3 trials with malt extract superior to Schooner between 1997 and 2005.

## Genetic gain achieved for malt extract in elite Australian germplasm through the combination of MAS, NIR and pragmatic breeding

The actual mean malt extract increase in the UA Barley Program, Stage 3 trials is shown in Figure 7. In 2000, from when the impact of combined MAS and NIR selection strategies can be measured, the mean extract genetic gain was only 0.16%. Since then, there has been a significant increase with results showing that the mean malt extract levels have increased by 2.5% in 2005.

MAS, NIR and sustained pragmatic selection pressure has increased the frequency of lines with high malt extract across the entire UA Barley Program and the absolute extract levels in advanced lines have also increased significantly.

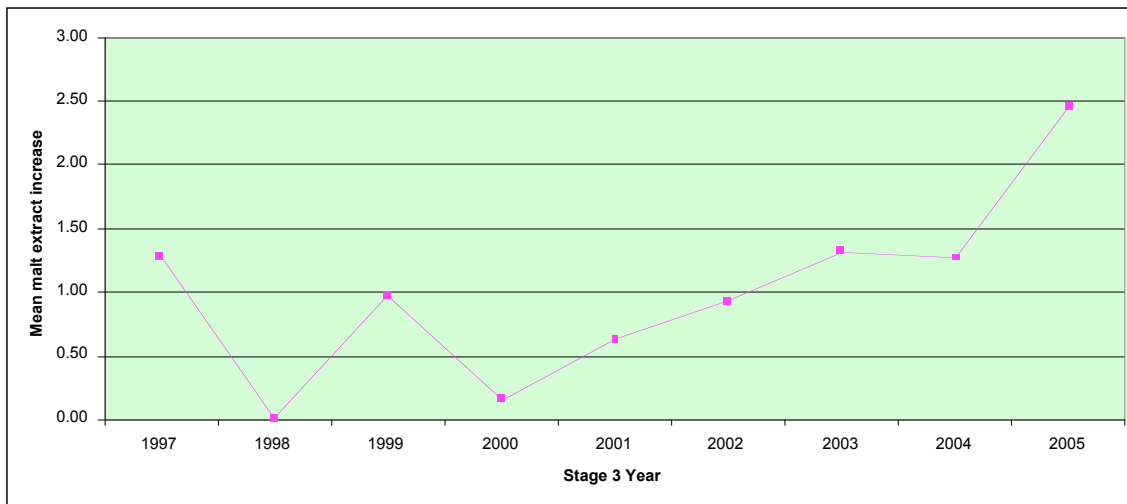


Figure 7. The mean malt extract increase in the UA Barley Program Stage 3 advanced trials, between 1997 and 2005.

### Opportunities for future gains in malt extract

There are a number of factors responding to the selection for increased malt extract levels. The data from this study suggests that grain protein levels are trending lower but this is not a consistent trend and the results are for the most part inconclusive. In addition, although the data is limited, this study suggests that husk content is trending lower, however the magnitude of the decrease in husk is quite modest.

Of these factors, lower husk content potentially offers a pathway to higher levels of malt extract. The understanding of the genetic control of husk content has been relatively well characterised with research showing a strong relationship between high malt extract and thin husk. However there are a number of potential problems associated with thin husk. As previously reported in Roumeliotis *et al*, 1999, these include an increased tendency for weather damage and pre-harvest sprouting, an increased likelihood of embryo damage and skinning during harvest and subsequent grain handling, and over modification during the malting process. Husk also plays a part in forming the filter bed during lautering and low levels may impact on the brewing process. Most of these issues relate to skinning damage and are magnified by poor husk adherence.

In a previous study by Roumeliotis *et al*, 2001, 103 lines derived from Dhow within 2 crosses were classified by their husk adherence (excellent, good or poor). In addition, these lines were analysed for percent skinning, husk content and malt extract. The results in Table 2 show that although the selections characterised by poor husk adherence had on average 15% higher skinning and 0.8% lower husk content, they only added 0.6% to malt extract levels. Most importantly, the highest malt extract levels were actually achieved by those lines characterised by good and excellent husk adherence. The key finding is that husk adherence segregates independently with the results demonstrating that it is possible to breed and select for lines with low husk content and good hull adherence to achieve very high malt extract levels.

Table 2. The effect of husk adherence on skinning, husk content and malt extract in a study of 103 selections from Dhow crosses.

Husk adherence	Mean % Skinning	Range Skinning	Mean % Husk Content	Range Husk Content	Mean % Malt Extract	Range Malt Extract	N
Excellent	8	2-17	9.76	7.95-11.70	78.2	75.5-81.0	24
Good	13	5-32	9.41	7.24-11.75	78.3	74.8-80.8	55
Poor	23	4-53	8.94	6.35-10.27	78.8	77.1-80.5	24
<b>LSD 5%</b>	<b>3.5</b>		<b>0.47</b>		<b>NS</b>		

Deployment of the thin husk trait within the UA Barley Program has involved a deliberate and systematic crossing and selection approach to further improve malt extract levels. Marker Assisted Selection is used to combine the chromosome 2H QTL along with other loci. In 2002, an NIR calibration was developed to measure husk content. The correlation between the laboratory reference EBC method (Analysis Committee of the EBC, 1998) and the NIR is  $R^2=0.829$ . All lines NIR scanned in Stage 0 double row trials are assessed for husk content. NIR operators also visually inspect samples for skinning damage. In addition, plot harvester set up is constantly monitored and adjusted using Schooner as the control sample to keep skinning risk constant. Utilising this coordinated approach, the aim is to increase malt extract levels through exploiting thin husk, without increasing the risk of skinning damage.

The impact of this approach and the initial response to selection for thinner husk can be seen in Figure 8. The plot illustrates absolute mean malt extract in UA Barley Program Stage 0 double row trials overlaid with absolute husk content, compared to Schooner between 1997 and 2006. Absolute malt extract levels have risen from 0.92% lower than Schooner in 1997 to 1.18% higher in 2006. Simultaneously, husk content levels have decreased by 0.3% since 2003 and have remained constant for the last 3 seasons. This initial assessment of the response to selection for thinner husk shows that it has made some impact with a positive change seen from 2003 onwards.

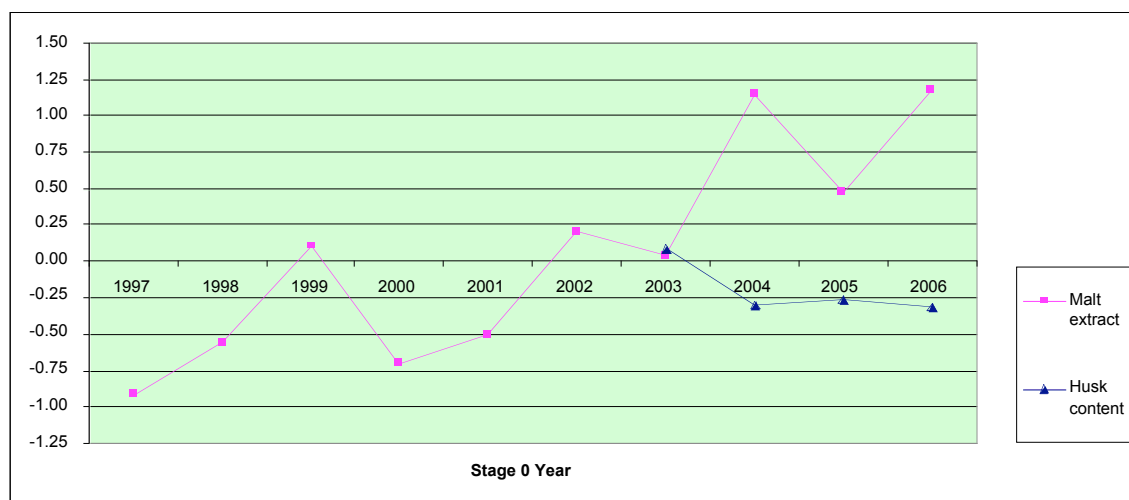


Figure 8. Absolute malt extract and husk content levels compared to Schooner in the UA Barley Program Stage 0 double row trials, between 1997 and 2006.

## WI4262-The next generation of malt extract

Recent varieties released by the UA Barley Program reflect the advances in malt extract achieved across the germplasm base. Listed in Table 3 are the major varieties released by the UA Barley Program since 1968, their year of release and relative malt extract levels. When Clipper was released in 1968, this variety was producing malt extract levels of 79%. The successful variety Schooner was released in 1983 producing malt extract levels of 80%. However, despite the releases of Sloop and the CCN resistant version SloopSA in 1997 and 2002 respectively, malt extract levels had only increased by 0.5%. The release of Dhow in 2002 was a breakthrough in terms of malt extract levels. This is a variety which produced very high levels of malt extract (84%) however it was also very thin husked and highly prone to skinning damage. As a result it never became a mainstream variety. Consequently it is with Flagship released in 2006 and the new elite line WI4262 which is currently undergoing commercial scale testing that we can see a new generation of malt quality varieties that reflect this shift in quality and increase in absolute malt extract levels.

Table 3. UA Barley Program malt variety releases 1968-2006

Variety	Year of release	% Malt Extract
Clipper	1968	79
Schooner	1983	80
Sloop	1997	80.5
SloopSA	2002	80.5
Dhow	2002	84
Flagship	2006	83
WI4262	Currently in commercial scale testing	83.8

WI4262 (Chieftain/VB9624/4/Keel/3/Sahara/WI2723//Chebec/5/BX98A;080-375) is a CCN resistant semi dwarf line with Gairdner maturity. It has good grainsize and good resistance to spot form net blotch and scald with excellent boron tolerance. Table 4 shows the performance of WI4262 from the 2005 season, expressed as a percentage of Schooner, with analysis carried out by both industry (Joe White Maltings (JWM), Barrett Burston Maltings (BBM), International Malting Company (IMC) and the UA Quality laboratories showing comparable results. WI4262 is suited to the domestic market, exhibiting very high extract (83.8%), modest diastatic power, and low fermentability, consistent with the Sd2L beta-amylase allele (Table 5).

Table 4. WI4262 Malt quality data, expressed as a percentage of Schooner

	Malt Extract	Diastatic Power	Apparent Attenuation Limit	Kolbach Index	Malt Protein
JWM*	104	119	95	95	93
BBM*	104	129	99	88	95
IMC*	107	78	96	90	96
UA#	107	99	100	91	89

\*Industry lab data from 2005 season Stage 3 samples from Brentwood, mean of 2 reps

#UA data from 2005 season Stage 3 samples from Yeelanna, Swan Hill, Clinton, Weetulta, mean of 2 reps

Table 5. WI4262 UA Quality Lab data, 2003-2005 seasons

	<b>% Malt Extract</b>	<b>Diastatic Power</b> ( $\mu$ moles maltose equiv/min/gm)	<b>% Apparent Attenuation Limit</b>	<b>% Kolbach Index</b>	<b>% Malt Protein</b>
WI4262	83.8	367	82.7	41.0	9.84
Schooner	78.3	361	82.5	45.1	10.74
% of Schooner	107	102	100	91	92

All malt quality parameters were assessed using standard analytical methods (Roumeliotis and Tansing, 2003)

## Conclusion

Improvement in malt extract is an important goal of the UA Barley Program since high levels are required by the malting and brewing industries. Both MAS and NIR based selection have increased the frequency of lines with high malt extract. In addition, the combined impact of MAS, NIR and pragmatic breeding has increased the absolute levels of malt extract in advanced lines. The initial response and impact of effective deployment of very thin husk suggests that it may offer the possibility of developing varieties with further improvements in malt extract levels. The recently released variety Flagship and WI4262, currently undergoing commercial scale testing signal a new generation of malting lines reflecting the shift in quality and increase in absolute malt extract levels.

## References

- Analysis Committee of the EBC (1998) *Analytica EBC*, Verlag Hans Carl Geranke-Fachverlag
- Collins H.M., Logue S.J., Jefferies S.P., Stuart I.M., and Barr A.R (1999) A Study of the Physical, Biochemical and Genetic Factors Influencing Malt Extract. *Proceedings of the 9<sup>th</sup> Australian Barley Technical Symposium*, Melbourne, Victoria, pp.2.44.1-2.44.6
- Powell G.E (1997) Quality Requirements for Australian Malting Barley. *Proceedings of the 8<sup>th</sup> Australian Barley Technical Symposium*, Gold Coast, Queensland, p.2.2.2 (a)-2.2.2(h)
- Roumeliotis S., Collins H.M., Logue S.J., Willsmore K.L., Jefferies S.P. and Barr A.R (1999) Implications of Thin Husk. *Proceedings of the 9<sup>th</sup> Australian Barley Technical Symposium*, Melbourne, Victoria, pp.3.5.1-3.5.6
- Roumeliotis S., Logue S.J., Hunt C. and Barr A.R. (2001) Pre-release characterisation of the malting profile of WI-3102. *Proceedings of the 10<sup>th</sup> Australian Barley Technical Symposium*, Canberra, ACT
- Roumeliotis S. and Tansing P. 2003 Season Barley Quality Report. The University of Adelaide, Waite Campus

## Acknowledgements

The authors would like to acknowledge all former and current staff of the UA Barley Program, ABB Grain, Joe White Maltings, Barrett Burston Maltings, International Malting Company, Australian Associated Brewers, and Australian grain growers and the Commonwealth Government through the Grains Research and Development Corporation.