

# Variation And Genetic Control Of Foam-Positive Proteins In Australian Barley Varieties.

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## Introduction

Beer foam quality is one of the critical characteristics that a consumer will use to determine the quality of dispensed beer. Brewers are particularly interested in optimising foam quality because it will impact upon customers purchase decisions. Beer foam quality is a combination of its stability, quantity, lacing (adhesion or cling), whiteness, 'creaminess', density, viscosity and strength (Bamforth, 1985).

Brewers current strategies for improving foam stability are to specify malt with relatively low Kolbach indices (KI, soluble/total protein) and supplement the beer produced with PGA (propylene glycol alginate), use foam generating "widgets" or use chemically hydrogenated hop extracts (i.e. "Tetra" hop). All these strategies are relatively costly and will impart visual or flavour effects on the beer that may not be desirable. Naturally occurring foam promotants in beer include proteins (>5kDa), isohumulone, metal cations, low levels of ethanol (<3%) and gums (presumably  $\beta$ -glucans and arabinoxylans) while lipids and high levels of ethanol are foam inhibiting (Bamforth, 1985). Foam-positive proteins can be divided into two fractions based on molecular weight, 1. high molecular weight (HMW, 35-50 kDa); 2. low molecular weight (LMW, 5-17 kDa). Both fractions originate primarily from malt (Asano & Hashimoto, 1980), with the HMW fraction containing mainly protein Z (Kaersgaard, P. & Hejgaard, 1979), and the LMW fraction containing LTP1 (lipid transfer protein 1) and a mixture of hordein and glutelin fragments (Sorensen *et al.*, 1993, Sheehan & Skerritt, 1997, Vaag *et al.*, 1999).

Protein Z may be further sub-divided into the protein Z4 and protein Z7 forms which are highly related and expressed from one or more genes on chromosomes 4H and 5H, respectively. In barley and malt, protein Z4 is the dominant isoform accounting for approximately 80 % of all protein Z. Evans *et al.*, (1998, 1999) applied quantitative enzyme-linked immunosorbent assays (ELISA) to measure the quantity of protein Z4, protein Z7 and LTP1 in malt. In small and pilot scale brewing trials, the level of protein Z4 was significantly and positively correlated to foam stability as measured by the Rudin head retention time test (Bishop *et al.*, 1975). It follows that if foam quality could be predicted by measurement of foam-positive proteins in barley and malt it would be possible to deliver malt to both domestic and international brewers that produce beer with high quality foam heads.

In this study, the quantity of protein Z4, protein Z7 and LTP1 were assessed in malt from a selection of Australian and international malting varieties. The genetic basis for the level of protein Z4 and Z7 were established by QTL analysis in two mapping populations.

## Materials and Methods

### *Malts*

Malt for the ELISA survey of foam-positive proteins was obtained from the Australian maltsters (Joe White Maltings, Barrett Burston Malting, Adelaide Malting Co. and Kirin Australia) and the Australian public barley improvement programs (NSW, QLD, SA, Victoria and WA). The barley for the malt was grown either in the 1996 or 1997 growing seasons.

### *ELISA for foam-positive protein*

Non-competitive double antibody sandwich-format ELISAs were used to quantify protein Z4, protein Z7 and LTP1 in malt flour extracted with phosphate buffered saline (PBS) and 1% mercapto ethanol by the methods described in Evans and Hejgaard, (1999).

### *Mapping populations and genetic mapping of protein Z level*

The level of malt protein Z4 and protein Z7 was measured by ELISA in 87 lines from the Chebec x Harrington (Kretchmer *et al.*, 1997) and 140 lines from the Morex x Harrington (National American Barley Genome Mapping Program) mapping populations. Trait marker associations were established using additive repression modelling with MapManager QTL (Manley & Elliot, 1993). Associations between molecular markers and the trait isoforms (protein Z) were also established using interval analysis (Lander & Botstein, 1989) with the graphical display of the associations being generated using QGENE (Nelson, 1997).

**Table 1.** Mean foam-positive protein contents of malt from barley varieties grown in 1997 from both commercial maltsters and the quality laboratories associated with the Australian breeding programs. Varieties ranked by protein Z4 content. Malt extracted with PBS/2-mercaptoethanol (combined extract).

Variety	Sample number	Grain Protein (%)	KI (%)	Protein Z4 ( $\mu\text{g/g Dwt}$ )	Protein Z7 ( $\mu\text{g/g Dwt}$ )	LTP1 ( $\mu\text{g/g Dwt}$ )
<b>High protein Z4 level</b>						
Gairdner	9	10.6	39.6	1357	108	465
Picola	3	10.8	31.0	1303	121	460
Alexis	2	9.3	50.7	1250	93	313
Lindwall	5	9.2	45.8	1235	85	286
Grimmett	13	10.4	43.4	1214	229	376
Unicorn	2	9.8	46.0	1203	198	541
Harrington	15	10.0	48.8	1190	246	468
Arapiles	18	9.7	47.2	1188	100	421
Tallon	10	10.0	41.8	1161	148	383
Chariot	2	9.2	46.6	1157	136	518
Franklin	27	10.3	42.5	1101	237	369
Sloop	9	10.0	50.7	1058	78	621
<b>Intermediate protein Z4 level</b>						
Manley	2	8.9	55.8	884	81	415
Fitzgerald	7	10.7	42.7	855	112	457
Schooner	30	10.9	48.3	829	116	477
Stirling	14	10.4	43.6	745	187	513
Parwan	5	10.1	43.8	659	234	297
Chebec	7	10.4	44.7	570	91	437

## Results and Discussion

### *Survey of level of foam-positive proteins in Australian malts*

The level of the foam-positive proteins, protein Z4, Z7 and LTP1 from the 1997 growing season of Australian and some international malting varieties malted by both Australian maltsters and the public breeding programs are presented in Table 1. Overall the results show that Alexis, Arapiles, Chariot, Franklin, Gairdner, Grimmatt, Harrington, Lindwall, Picola, Sloop, Tallon and Unicorn have high levels of protein Z4 (means 1058-1357 µg/g) while Chebec, Fitzgerald, Manley, Parwan, Schooner and Stirling have intermediate levels of protein Z4 (means 532-870 µg/g). These results were consistent with results obtained from the 1996 growing season (Table 2). Table 2 also identifies that Pirkka (and Morex, data not shown) form a third category that have low levels of protein Z4 (means 55-112 µg/g). Figure 1 shows that there is a trend for the level of protein Z4 and protein Z7 to increase with grain protein. This is in agreement with Giese and Hejgaard (1984) who investigated protein Z accumulation in grain using detached spikes in liquid-culture. Occasionally some samples give results that were substantially higher than predicted by this relationship (Fig. 1). At present time an explanation for these observations is not available. Kolbach index also appears to be negatively correlated with protein Z4 and Z7 level (data not shown). This may in part be due to a further relationship with grain protein where grain with lower protein contents tends to be more modified. Tables 1 and 2 also show that the level of protein Z7 was seen to be high in Grimmatt, Franklin, Harrington, Parwan, Pirkka, Stirling, and Unicorn (means 175-301 µg/g) and low in Gairdner, Picola, Alexis, Arapiles, Tallon, Chariot, Sloop, Manley, Fitzgerald, Schooner, Chebec (means 81-169 µg/g). The level of LTP1 was somewhat lower in all varieties grown in the 1997 season (approx. 300-500 µg/g) compared to the 1996 season (approx. 500-800 µg/g) except for those grown in WA (data not shown) and Sloop which had contents more typical of those from the 1996 growing season. The explanation for the generally lower levels of LTP1 is not understood at this stage.

**Table 2.** Mean foam-positive proteins contents of micro-malted barley from seven varieties grown at five sites in South Australia in 1996. Varieties ranked by protein Z4 content. Malt extracted with PBS/2-mercaptoethanol (combined extract).

Variety	Barley protein (%)	Protein Z4 (µg/g dwt)	Protein Z7 (µg/g dwt)	LTP1 (µg/g dwt)
<b>High protein Z4 level</b>				
Alexis	10.4	1353	124	532
Sloop	10.4	1206	90	779
Franklin	10.1	1182	273	519
Arapiles	10.0	1024	169	709
Harrington	9.8	919	175	548
<b>Intermediate protein Z4 level</b>				
Schooner	10.0	532	96	747
<b>Low protein Z4 level</b>				
Pirkka	10.5	55	301	540

### *Genetic analysis of protein Z4 and protein Z7 levels in malts*

Polymorphisms for the level of protein Z4 and Z7 were measured by ELISA in the Chebec (intermediate protein Z4, low protein Z7) x Harrington (high Z4 and Z7) and Harrington x Morex (low Z4, high Z7) mapping populations. Interval mapping analysis at confidence level  $P = 0.001$  showed that protein Z4 mapped to a single located on chromosome 4H (4B) with highly significant likelihood of difference (LOD) scores of 19.2 and 32.3 in the Chebec x

Harrington and Harrington x Morex populations, respectively (Fig 2A,B). The map location was consistent with that reported by Evans *et al.*, (1995) where the protein Z4 gene was located on the short arm of chromosome 4H (4B). Interval mapping analysis at confidence level  $P = 0.001$  showed that protein Z7 content in the Harrington x Morex population mapped to a single location on the long arm of chromosome 5H (7B) with a highly significant LOD score of 13.4 (Fig 2C). This result is consistent with that found by Hejgaard (1984). These mapping analyses demonstrate that the level of protein Z4 and protein Z7 are both determined by the expression of a small number of major gene/s with at least two alleles.

## Conclusions

The quantities of the malt protein Z and LTP1 have been found to influence foam quality. ELISAs developed for protein Z4, protein Z7 and LTP1 were used to survey the levels of these proteins in a range of Australian and international malting barley varieties. Substantial variation was observed in the protein Z4 and protein Z7 levels which was shown by genetic analysis to be the result of the expression of a small number of major gene/s and a positive association with barley protein content. Since the level of these proteins is under simple genetic control and positive alleles are available in adapted germplasm, it should be possible for barley breeders to readily increase their levels. Increasing the level of the foam-positive proteins in malt is expected to reduce the necessity of brewers to use foam supplementation measures. This should improve the desirability, shelf-life, cost and wholesomeness of beer produced from malt.

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Figure 1: Comparison of the relationship between malt protein content and the level of protein Z4 ( □ ) and protein Z7 ( ◆ ) for A. Schooner malts and B. Harrington malts.

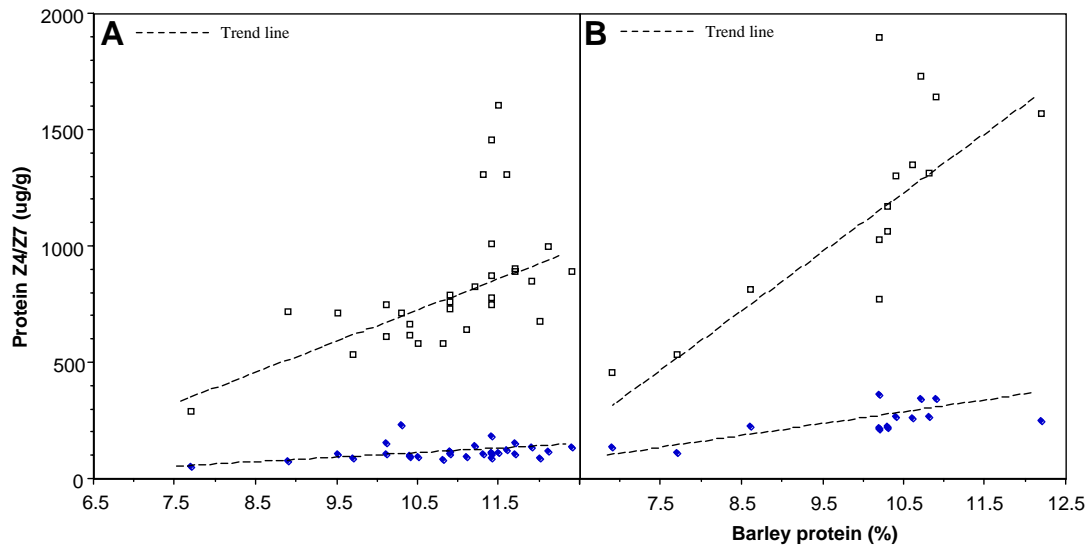


Figure 2: Interval mapping analysis of A. Morex (low protein Z4) x Harrington (high protein Z4) population, B. Chebec (intermediate protein Z4) x Harrington (high protein Z4) population (87 lines), C. Chebec (low protein Z7) x Harrington (high protein Z7) population.

