



Genetic and molecular basis of grain protein variation in malting barleys

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Introduction

Malting barley varieties inherently low in grain protein may have an advantage over those with high grain protein because in environments with adverse growing conditions they increase the likelihood that a grower will be able to produce barley with acceptable grain protein (Weston et al. 1993). Maltsters require barley in the protein range of 10.0 to 11.5 per cent for satisfactory malting and brewing, and sizeable discounts in price occur for barley marketed outside this narrow range. Apart from the use of barley for malting purposes, there is a market for low protein barley for the production of the distilled alcoholic beverage, 'shochu'.

Breeding for inherently low grain protein concentration is, however, difficult due to low trait heritability and marked genotype-environment interaction. Genetic linkage maps now available for major crop species permit the systematic mapping and localisation of genes that control such complex traits. These genes have been mapped to what has become known as quantitative trait loci (QTL). Once molecular markers within these regions are identified, breeders can select for desirable alleles without interference from environmental effects.

Grain protein and yield often exhibit a significant correlation, which is not genetic in origin. Because amino acid for protein synthesis and monosaccharides for starch synthesis are derived from largely separate biosynthetic pathways, on a per grain basis protein content and starch content should not be genetically related Jenner (1985). Available water and nutrients are more often the factors that affect deposition of starch and protein in the grain. Under poor growing conditions, water deficit during grain development reduces the availability of sucrose, which a grain may convert to starch, and prevents the dilution of protein by starch. While observed phenotypic variation for grain protein concentration in barley can vary from 6.5 to 20% (Moody, personal comm.), the actual range in genotypic variation may be much smaller, after necessary adjustment with respect to grain yield (Chery et al. 1981).

The objectives of this study were to determine the mode of inheritance of grain protein concentration in malting barleys, after adjusting observed phenotypes for yield

effects, and to identify chromosomal location of DNA markers linked to gene (s) that influence grain protein variability. Previous genetic studies on grain protein have been based on protein concentration uncorrected for yield. It is necessary to determine whether the mode of inheritance can be more clearly defined, and heritability increased for corrected protein concentration (Stoddard and Marshall 1990).

Materials and methods

Phenotypic variation

Grain protein and yield data were obtained from regional trials carried out between 1995 and 1999 in the States of Victoria and South Australia. Altogether, there were 3,636 complete data points for grain protein and yield, representing 80 genotypes and 129 environments (ie sites in each year).

Data analyses were performed using the method of generalised linear model (GLM) of Stoddard and Marshall (1992). The method adjusts the protein concentration of each genotype for the gross effects of the environment and any negative correlation with yield, according to the following model:

Phenotype (Observed protein) = Environment + yield (as a covariate) + Environment x yield + Genotype

Genotypic effects were calculated relative to 'Arapiles', which was used as the reference, low protein genotype.

F₂ Diallel

The genetics of grain protein concentration was studied using data from an 8-parent diallel cross. The genotypes were 'Arapiles', 'Schooner', 'VB9524*63', 'WI2875*17' and 'WI2808' all from Australian breeding programs, and 'Semal' and 'Vodka' from Europe and 'ND11231*12' from the North Dakota State University, Fargo, USA.

The genotypes were crossed in a half diallel to generate 28 F₁ hybrids. The hybrids were selfed to produce F₂ populations for each cross. The experimental materials, which comprised the eight parents and their 28 populations were grown under two nitrogen levels (0 and 60 Kg N/ha) in three replications during the 2000 season. At maturity, ten F₂ plants were sampled from each plot, and individually assessed for agronomic performance. Data on grain protein content were obtained on a bulked sample of F₂ plants from each plot by prediction using the Near Infrared Spectroscopy (NIRS) method.

Combining ability analyses were carried according to Griffing's method 4, fixed model (1956). Genetic analysis, based on an analysis of the array variances (V_{ri}) and covariances (W_{ri}), was also performed according to the procedure of Hayman (1954).

Quantitative trait locus (QTL) analysis

QTL analyses were carried out using molecular marker data and phenotypic measurements obtained from 180 doubled haploid lines (DH). Parental genotypes used for the cross ('VB9524*63' and 'ND11231*12') were derived from pedigrees that are known to be inherently low in grain protein. The genotype, 'VB9524*63', is an advanced selection line from a cross of Arapiles with Franklin. Arapiles is an Australian variety, well characterised for its lower grain protein concentration (Moody 1994; Eagles et al. 1995). The genotype, 'ND11231*12', is a two-row barley lines derived from Karl, another source of the low-protein genes (Wesenberg et al. 1976).

Along with five check varieties ('Schooner', 'WI2808', 'WI2875*17', 'Semal', 'Gairdner'), the parents and the 180 DH lines were grown in a field trial carried out near Horsham (Lat. 36.5°S, alt. 135 m) in the Wimmera region of Victoria, in 1999. The experimental treatment comprised two levels of nitrogen application (0 and 60 Kg N/ha), and irrigated or normal dryland conditions, in a factorial combination. Trials were designed as a randomised complete block with split-plot arrangement and two replications. For logistical reasons, the irrigation and normal dryland treatments were grown in adjacent areas of the same irrigation bay, but were not randomised within the same design. Genotypes and nitrogen treatments were assigned to the mainplot and the subplot, respectively, for each of the irrigated and dryland experiments. Data on grain protein content were obtained by prediction using the Near Infrared Spectroscopy (NIRS) method.

Statistical methods used for QTL mapping

QTL analyses were carried out with a genetic linkage map constructed with molecular markers spaced at an average of 10 cM per chromosome, and adjusted grain protein data. The analyses were performed using the flanking marker regression technique described by Haley and Knott (1992), and implemented with the aid of the computer program, Qgene (Nelson 1997). The critical value for claiming QTL detection was a LOD of 3.0 determined by permutation (5000 reshuffles).

Results and discussions

Phenotypic variation in multi-location trials

Differences among sites were the most influential sources of variation, accounting for 88.5% of the total observed variation in grain protein. Although the yield effect was modest in comparison, it was nonetheless a significant source of variation, and accounted for a decrease of 0.7% in grain protein for every tonne increase in yield. Unadjusted grain protein varied by 4.5% amongst the 80 genotypes. When adjustments were made for yield effects, the range of variability decreased to 2.3%. This range in variation agreed with the observations of Chery et al. (1981) that the 'actual' genotypic variability for this character, after necessary adjustments with respect to grain yield, may be restricted to a low range of 1.5 to 2 points.

The range in adjusted grain protein for 8 of the genotypes considered is presented in Table 1. Of the standard varieties, only 'Gairdner' was significantly ($P < 0.01$) lower

than 'Arapiles' in grain protein concentration (Table 1). Two advanced selections from a cross between 'Arapiles' and the malting variety 'Franklin' ('VB9527' and 'VB9524') were also significantly lower than 'Arapiles'.

Table 1. Back-transformed means following adjustments for covariates, and estimates of regression parameters (differences compared with the reference low-protein genotype, 'Arapiles'), obtained using the generalised linear model: Protein = Site + Yield + Site*Yield + Name. Only the best 8 of the 80 varieties are represented.

Variety	Adjusted grain protein conc. (%)	Estimated regression parameter	SE	tpr
Arapiles	12.20	0.00	*	*
Barque	12.44	0.24	0.08	0.00
Sloop	12.40	0.20	0.08	0.01
Franklin	12.19	-0.01	0.08	0.88
Gairdner	11.81	-0.39	0.08	<0.001
Schooner	12.55	0.35	0.08	<0.001
Picola	12.18	-0.02	0.09	0.79
VB9524	11.69	-0.52	0.09	<0.001
VB9527	11.46	-0.74	0.10	<0.001

Quantitative genetics of grain protein from diallel analysis

In the analysis of variance, 1000-Kernel weight was used as a covariate to adjust grain protein because data for grain yield was unavailable. The results (not presented) showed a significant effect of the covariate ($P \leq 0.01$), indicating that the adjustments were justified. Plot means of the 28 F_2 full-sib families differed significantly ($P \leq 0.01$) for grain protein, but nitrogen application did not significantly influence grain protein concentration, and the genotype x nitrogen term was also not significant.

Mean squares from combining ability analyses, based on adjusted and unadjusted grain protein, are presented in Table 2. For both data sets, only the general combining ability (GCA) mean squares were significant. The benefits from adjustment, however, were apparent in the estimates of the additive genetic variance and narrow sense heritability. Overall, the results suggested an additive-dominance model of inheritance for grain protein variation, with a predominantly additive genetic basis.

Table 2. GCA and SCA mean squares from the eight-parent diallel analysis of F₂ populations, based on genotypic values adjusted and unadjusted for starch-dilution effect, using 1000-Kernel weight as a covariate.

Source	DF	Adjusted grain protein conc.	Unadjusted grain protein conc.
GCA	7	0.799**	0.645**
SCA	20	0.130	0.162
Error	27	0.138	0.113
Variance components			
GCA		0.112 ± 0.07	0.080 ± 0.06
SCA		-0.009 ± 0.06	0.050 ± 0.06
Additive		0.223 ± 0.14	0.161 ± 0.12
Dominance		-0.009 ± 0.06	0.050 ± 0.06
Heritability (Narrow sense)		0.634	0.497

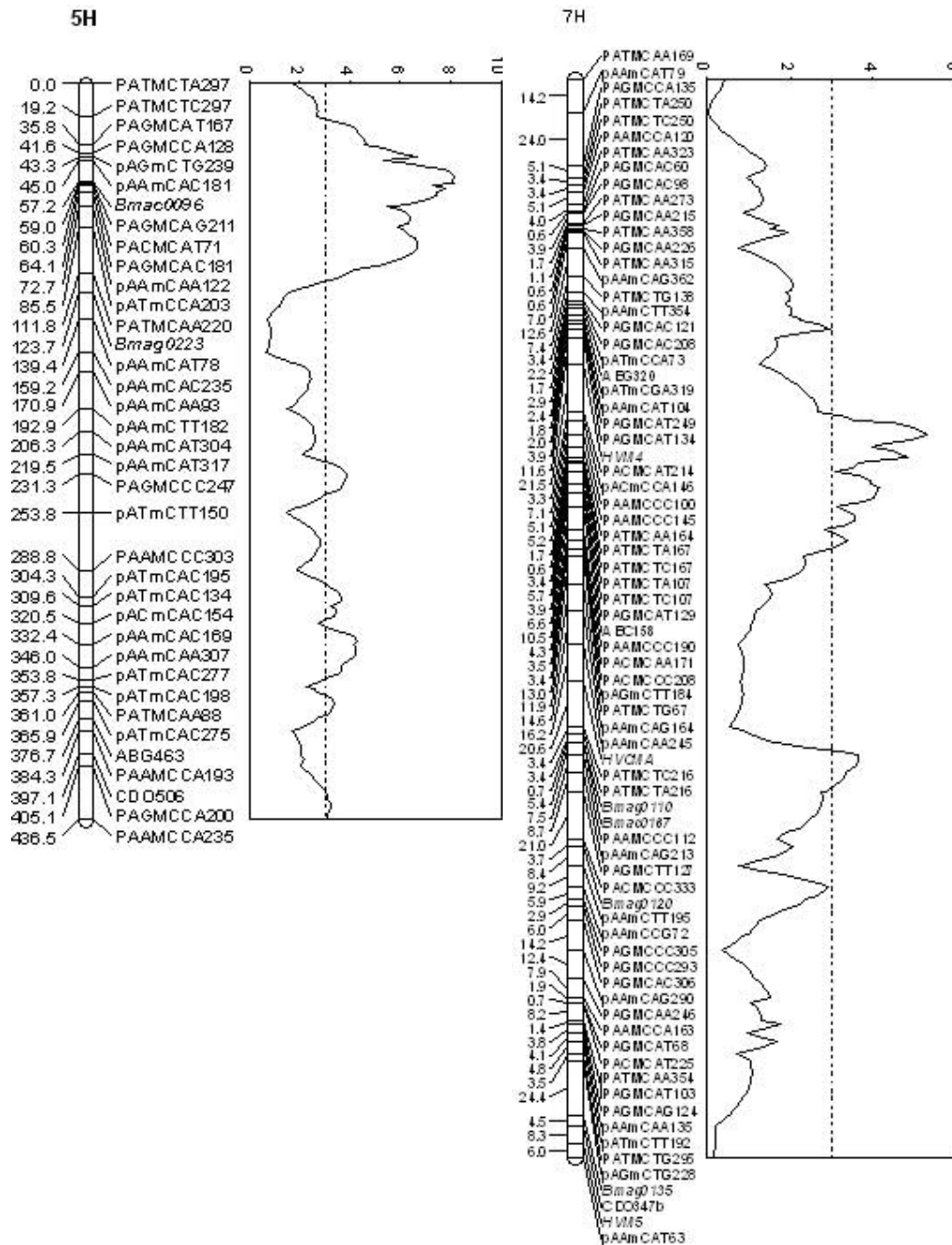
However, when data were analysed using the method of Hayman (1954), the WrVr graph had a slope of 0.57 ± 0.26 , which although significantly different from 0, was also significantly different from 1, suggesting the presence of non-allelic interaction. Removal of array 4 (WI2808), which had the highest variance, from the analysis gave a regression slope of 0.96 ± 0.26 which was significantly different from 0 and not from 1, indicating conformity to the additive-dominance model.

Graphical analysis of the relationship between the standardised parental measurements, Y_r, and the standardised array values for Wr+Vr was then carried out on the remaining seven parental lines. From the distribution of array points on the graph, it was concluded that low grain protein concentration was controlled by dominant alleles, with 'ND11231-12' and 'VB9524' possessing an excess of dominant alleles over recessive alleles, while 'Semal' possessed an excess of recessive alleles, over dominant alleles.

Chromosomal regions influencing grain protein concentration in mapping population study

Two regions of the barley genome, located on chromosome 5H and 7H, were detected with peak LOD scores ranging from 5 to 8 (Fig. 1). The region on chromosome 5H spanned about 71 cM on either side of the centromere, and the alleles that reduced grain protein were derived from 'ND11231-12'. Regression estimates of allelic effects, calculated as the difference in mean phenotype when compared with alleles from 'VB9524', showed that 'ND11231-12' alleles in this region decreased grain protein by approximately 1%.

Fig. 1. Linkage map of chromosome 5H and 7H, showing the location of QTL with significant effects on grain protein concentration.



The region on chromosome 7H showed two peak LOD scores that exceeded the genome wide error threshold. The first QTL region spanned a length of about 75-cM on the long arm of the chromosome, flanked by the SSR marker, HVM4, and an RFLP marker, ABC158. The second region was located in an adjacent interval flanked by an SSR marker, Bmag0110, and an AFLP marker, PAGMCA C306. The

alleles for low-protein in both QTL regions were inherited from 'VB9524', contributing to a combined decrease of 0.9% in grain protein concentration.

Comparison with results from other mapping studies

Genes or gene complexes affecting grain protein concentration in barley have been mapped on all seven barley chromosomes (Zale et al. 2000), giving the impression of a complex genetic system. However, such reports have been based on protein concentration uncorrected for yield, making comparisons difficult. For instance, Thomas et al. (1996) reported a QTL associated with the *denso* gene on chromosome 3H that produced a marked reduction in grain nitrogen due to dilution effect. We found no evidence of a QTL for adjusted protein on chromosome 3H, or on chromosome 1H.

It is worthy to note, however, that some aspects of our results are very consistent with those of other researchers. For instance, the centromeric location of QTL on chromosome 5H has been reported in crosses involving Harrington and Morex (Marquez-Cedillo et al. 2000), and confirmed in studies by Igartua et al. (2000). Also QTL for fermentability has been localised within the same region (Swanston et al. 1999). The existence of a low protein gene in this location seems logical, since fermentability and grain protein exhibit negative relationships (Swanston et al. 2000).

The QTL detected on chromosome 7H is also supported by evidence from such similar mapping studies. Further studies have been initiated to validate the QTL on chromosome 5H and 7H, and to determine their sensitivity to environmental stress and the physiological basis of their action.

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