Euphytica (2007) 156:185–194
DOI 10.1007/s10681-007-9365-y

Improvements in malting barley grain yield by manipulation of genes influencing grain protein content

Livinus C. Emebiri · David B. Moody · Cassandra Black · Maarten van Ginkel · Eduardo Hernandez

Received: 17 July 2006 / Accepted: 24 January 2007 / Published online: 18 February 2007

Abstract Malting barley (*Hordeum vulgare* L.) is traditionally grown under low soil fertility as a conservative approach to achieve the malting requirement of grain below a maximum prescribed limit for protein concentration, typically 12.0%. Such strategies severely limit grain yield. Traditionally, barley breeders have sought to improve yield in malting barley through selection for higher yield *per se*, without considering an alternative approach in which genetic manipulation of inherent grain protein concentration would allow higher yields through the adoption of an alternative farming system for malting barley production. The present study examined whether greater yield improvements could be achieved by manipulating genes influencing grain protein content, and allowing malting barley to be grown on crop rotations with higher fertility. Doubled haploid lines, selected from a mapping population based on allelic differences at two genes (quantitative trait loci; QTL) influencing grain protein content, were evaluated for productivity over a range of agronomic practices and environments (sowing date, nitrogen application, and a range of field histories). QTL effects were highly repeatable over the range of practices and environments, with individuals carrying two low-protein alleles ($q_1q_1q_2q_2$) producing grains with comparatively lower grain protein than individuals carrying the alternate allelic configuration ($Q_1Q_1Q_2Q_2$). Based on two years of data, the yield advantage of growing barley on legume stubble as opposed to wheat stubble was approximately 1.0 t ha^{-1}, or a yield increase of 53.3%. When grown after a pulse crop in 2005, average yields of those mapping population genotypes carrying low-protein alleles was 4.28 t ha^{-1}, with average grain protein of 12.0% whilst the average yield of their high protein counterparts was 3.76 t ha^{-1} with an average protein level of 12.7%. We conclude that, as an alternative means of malting barley yield improvement, varieties can be developed that allow barley to be grown in highly fertile conditions resulting in yields up to 53% over current farming practices, while maintaining protein levels within malting specifications.

Keywords Genetic manipulation · Malting barley (*Hordeum vulgare* L.) · Grain yield · Protein concentration · Quantitative Trait Loci (QTL)

L. C. Emebiri (✉) · D. B. Moody · C. Black · M. van Ginkel · E. Hernandez
Department of Primary Industries,
Grains Innovation Park, Natimuk Road, PB 260,
Horsham, Victoria, 3401, Australia
e-mail: Livinus.Emebiri@dpi.vic.gov.au

Introduction

Grain yield in rice and wheat has almost doubled over the last 50 years, largely through the incorpo-

ration of a small number of alleles that reduced height and thus reduced lodging, but most importantly changed assimilate partitioning resulting in an increased harvest index. The incorporation of dwarfing genes in modern rice and wheat cultivars changed harvest index from 30 to 45%, and increased yield potential (under fully irrigated conditions) from 4 to 10 t ha^{-1} (Khush 1999). Since 1966 when the first high-yielding variety of rice was released, the area devoted to rice production has increased only marginally by 17% while the average rice yield has increased by 71% (Khush 1999). In comparison, achieving yield increases in barley (*Hordeum vulgare* L) by genetic improvement have been somewhat slower, typically in the order of 0.8–1.0% per year (Thomas 2003). Semi-dwarf barley lines and cultivars have not fulfilled early expectations of increased yield, and in the case of dwarfing allele *sdw1*, the impact has ranged from variable (Eagles and Moody 2004) to negative for grain weight (Nedel et al. 1993; Hellewell et al. 2000).

The relative contributions of plant breeding and management to increased grain yield achieved by cereal growers are estimated to be about 35 and 65%, respectively (Abeledo et al. 2003). In the case of wheat and rice, yield improvements have resulted from the development of semi-dwarf cultivars, with improved harvest indices under conditions of high fertility (Khush 1999). Unlike rice and wheat, however, malting barley is traditionally grown under low soil fertility conditions as a conservative approach to achieve the malting industry requirement that grain protein is below a maximum prescribed limit, and such strategies obviously severely limit yields. In Australia, maltsters prefer GPC close to 10.5%, and barley delivered by growers outside a protein range of 9.5–12.0% is likely to be discounted or rejected from malting classification.

Numerous studies and grower experience have indicated that yields of barley following pulse crops or fallow are substantially higher than when following wheat or barley, and can be in the order of an additional yield of 20–40%. An alternative approach, therefore, is to provide growers with varieties, which are likely to produce high yields of grain under enhanced fertility conditions, but which do not exceed the maximum protein levels for malting specifications. Such varieties could successfully be grown as part of crop rotations previously thought to result in nitrogen levels too high for malting purposes. Previous research has shown that low-protein barley cultivars increase the likelihood that a grower will be able to maximise yield while maintaining grain protein within the acceptable range (Weston et al. 1993; Emebiri and Moody 2004a). An intriguing question, therefore, is whether greater yield improvements could be achieved in barley by manipulating a few genes influencing final grain protein content, compared to breeding for yield improvement *per se*, which involves the manipulation of hundreds of (undefined) genes (see reviews by Slafer 2003; Thomas 2003).

Research conducted over the past six years has elucidated a large part of the genetic basis for variation in grain protein concentration of two-rowed malting barley (Emebiri et al. 2003), and this was confirmed by independent studies conducted in Europe by Moralejo et al. (2004). We have focused on two independent regions associated with protein levels located on chromosomes 5H and 7H, and as part of this process have developed advanced barley lines which differ in the allelic constitution of the two QTL (quantitative trait loci) regions, influencing grain protein content. These lines have been evaluated intensively over a range of environments, field rotation histories and agronomic practices (e.g. different sowing dates, nitrogen application modes) in Australia, during 2004 and 2005 seasons. The fact that these lines share a common parental background make them unique experimental materials for investigating whether greater improvements in grain yield can be achieved by genetic manipulation of two grain protein genes, and allowing genetically low-protein barleys to be grown in high yield potential crop rotations.

Materials and methods

Germplasm

Doubled haploid (DH) lines, produced from the F_1 generation of a cross between VB9524 and ND11231–12, were used in this study. The parents

originated from breeding programs at the Department of Primary Industries, Victoria, Australia, and the North Dakota State University, Fargo, ND USA, respectively. This population has been well characterised for QTLs (Emebiri et al. 2003, 2004, 2005). The QTL associated with variation in grain protein at regions on chromosomes 5H and 7H were the subject of the present study.

Eight doubled haploid lines were selected from within the population, on the basis of different combinations of EST alleles at loci on 5H and 7H. One group of four lines had low-protein alleles from VB9524 and ND11231-12 ($q_1q_1q_2q_2$) and another, the alternate high-protein alleles ($Q_1Q_1Q_2Q_2$). Thus, each of the selected lines had known marker genotypes in the QTL regions, while carrying a random assortment of VB9524 and ND11231-12 alleles elsewhere in the genome (Igartua et al. 2000).

Field experiments

The selected lines were used to conduct a farming systems trial in 2004 and 2005. Other entries in the trials include parental lines, VB9524 (a close derivative of the Australian barley variety Arapiles) and ND11231-12 (a major derivative of the six-rowed barley, Karl), and the check varieties, Schooner, Gairdner, VB0229 and WI2875-17.

The experiments were conducted at the University of Melbourne's Longerenong campus (36°67′ S, 142°30′ E), near Horsham. DNA based soil tests indicated that the site was largely free of soil-borne disease pathogens, with risks of CCN (*Heterodera avenae* Woll.), Take-all (*Gaeumannomyces graminis* var. *tritici*) and *Rhizoctonia* classified as below detection limit, and risks of *Pratylenchus neglectus* and *P. thornei* damage as low to below detection limit. The trials were designed as a factorial experiment, consisting of a $4 \times 2 \times 14$ combinations of preceding (previous) crop type, time-of-sowing and lines (entries) in four replications.

The four preceding crop treatments included a cultivated 12 month fallow, a field pea crop, a *Medicago* pasture and a wheat crop. The field pea crop was seeded at the rate of 116 kg ha^{-1} (cv. Dundale), *Medicago truncatula* (cv. Mogul) at the rate of 10 kg ha^{-1}, and wheat (cv. Goldmark) at the rate of 70 kg ha^{-1}, all being the commercially recommended rates. Both the wheat and pea crops were harvested, and yielded an average of 3.1 and 1.7 t ha^{-1}, respectively, in the 2004 and 2005 seasons. In both seasons, the *Medicago* pasture was disc incorporated into the soil, while the wheat stubble was slashed. The *Medicago* dry matter production at soil incorporation averaged 6.9 t ha^{-1}.

The year following the previous cropping, the 14 barley lines were sown on June 1 and July 5 in 2004, and July 8 and July 22 in 2005, for early and late planting dates, respectively. The experimental arrangement was a split-split plot design with Rotation System assigned to main plots, Time-of-sowing to sub-plots and Entries assigned to sub-subplots. Sub-subplots measured 8 m in length, with six rows of plants spaced 15 cm apart.

Data collection

In all trials, data were recorded on grain yield, protein content, kernel weight and grain plumpness. Grain protein content (grams protein per kilogram grain as a percentage) was determined on whole grain at harvest from each replication of the subplots using Near Infra-Red Spectroscopy that had been calibrated using the Dumas combustion method (RACI 2003). Average kernel weight was determined from the weight of 100 grains taken from unscreened samples, and expressed as the weight of 1,000 grains. Kernel plumpness was determined as the proportion of grains retained by passing a 100 g sample over a sieve with a 2.5 cm slotted opening on a Sortimat (Pfeuffer GmbH, Kitzingen, Germany) machine for two minutes.

Statistical analysis

Balanced analyses of variance (ANOVA) were carried using IRRISTAT v5 (IRRI, 1998–2005). We used a split-split plot model in a combined analysis for both years and a separate analysis for each year. In the ANOVA for grain protein content, grain yield was used as covariate to adjust for any correlations with yield (Stoddard and Marshall 1990). F-ratios used to test effects were determined according to LeClerg et al (1962).

Table 1 Mean squares and significance of main effects and interactions terms from the separate analysis for grain yield, grain protein content and grain physical attributes in cropping seasons 2004 and 2005

Source of variation	d.f.	Grain yield		Grain protein content		1000-kernel weight		Grain plumpness	
		2004	2005	2004	2005	2004	2005	2004	2005
Replications	3	1.04ns	1.31ns	19.58ns	0.88ns	144.97[a]	25.50ns	1177.78ns	1.83ns
Time-of-Sowing (ToS)	1	0.02ns	1.09ns	169.10[c]	5.19[a]	1.24ns	32.27ns	495.47ns	44.81ns
Error (a)	3	0.63	0.66	10.14	0.22	15.15	10.15	508.18	5.59
Rotation	3	27.83[c]	22.53[c]	728.74[c]	86.92[c]	1063.00[c]	54.11[c]	67603.20[c]	370.62[c]
Rotation × ToS	3	1.20[c]	0.37ns	56.09[c]	0.63[a]	49.11[c]	13.77ns	4570.71[c]	32.30ns
Error (b)	18	0.16	0.50	4.27	0.20	5.71	8.36	282.79	14.02
Entry	13	0.18[c]	3.64[c]	16.34[c]	5.27[c]	72.74[c]	65.91[c]	2184.29[c]	468.50[c]
Entry × ToS	13	0.50[c]	0.55[c]	9.25[c]	0.14ns	14.97[c]	13.63ns	520.79[c]	98.44[c]
Entry × Rotation	39	0.09[c]	0.23[c]	2.73[c]	0.23[c]	8.01[c]	16.29[a]	387.96[c]	18.38[c]
Entry × ToS × Rotation	39	0.09[c]	0.13ns	1.57[c]	0.16[a]	4.30ns	10.14ns	191.44[c]	5.36ns
Error (c)	312	0.04	0.12	0.77	0.10	3.80	10.88	95.55	4.33

[a], [b], [c] indicates significant effects at $P < 0.05$, 0.01 and 0.001 respectively

ns, not significant

Significant differences ($P < 0.05$) between means were determined by Fisher's protected least significant difference (LSD) test at 0.05%.

The GGE biplot software (Yan 2001) was used to carry out principal components analysis, as we dissected both the "environments" into years and rotation systems, plus the genotype responses into yield, GPC and, and grain physical attributes.

Results

In the separate analysis of variance (Table 1), Time-of-sowing main effects were not significant for grain yield, 1,000-kernel weight or grain plumpness, but were significant for grain protein content. Interactions with crop rotations had variable impacts, as the interaction components were highly significant in 2004 and not in 2005 (Table 1). Weather variables in 2004 and 2005 were very different. The absolute amount of rainfall was not markedly different for the two years, with a total of 376.8 mm observed in 2004 and 383.6 mm in 2005. However, total rainfall during the crucial period of grain filling (October–November) was very different, with a total of 52.4 mm for 2004 and 106.6 mm observed in 2005. Moreover, only 7 mm of rain was recorded in November 2004 during final grain filling, compared with 76.4 mm in 2005. Nevertheless error terms proved homogeneous and the two years were analyzed jointly.

The combined analysis across years (Table 2) showed a strong influence of season and cropping history on all measured traits ($P < 0.001$). On the other hand, the effect of planting date and its interaction with years were rarely a significant source of variation, except for grain protein content (Table 2). Similarly, planting date did not strongly affect grain yield in the different crop rotations systems, but had a significant influence on protein content, kernel weight and plumpness. Entries were highly significantly different ($P < 0.001$), and showed significantly different responses to time-of-sowing and crop rotation.

The main effects of Time-of-Sowing and Rotation Systems on measured traits are presented in Table 3. GPC levels were higher in 2004, with a grand mean of 15.7%, compared with 12.4% in 2005. Conversely, yields were higher in 2005, with a grand mean of 3.8 t ha^{-1}, compared with 0.7 t ha^{-1} in 2004. Based on the 2 year average, the yield advantage of growing barley on legume stubble as compared to wheat stubble was approximately 1.0 t ha^{-1}, or a yield increase of 53.3% (Table 3). The yield advantage was much higher in 2004, which was a difficult year for achieving malting specifications, with higher protein levels and lower grain plumpness observed in barley following wheat (Table 3). In 2005, a more favourable year for malting barley production, a difference in grain yield of 0.9 t ha^{-1} or 28% was observed when barley was grown after a pulse crop, as opposed to growing barley on wheat

Table 2 Mean squares and significance of main effects, interactions and single-degree-of-freedom comparison terms from combined analysis for grain yield, protein content, and grain physical attributes measured in factorial experiments conducted in cropping seasons 2004 and 2005

Source of variation	d.f.	Grain yield	Grain protein content	Kernel weight	Grain plumpness
Main plots					
Replication	3	1.23ns	6.20ns	59.78ns	367.62ns
Year	1	2121.11^{c}	2461.75^{c}	30172.50^{c}	259455.00^{c}
Error (a)	3	1.12	14.26	61.20	416.63
Subplots					
ToS	1	0.41ns	116.79^{c}	52.42^{a}	329.32ns
Year × ToS	1	0.70ns	57.51^{a}	0.63ns	762.51^{a}
Error (b)	6	0.64	5.18	6.97	122.53
Sub-subplots					
Rotation	3	36.03^{c}	266.44^{c}	316.12^{c}	29194.50^{c}
Rotation × Year	3	14.33^{c}	549.22^{c}	620.32^{c}	34654.70^{c}
Rotation × ToS	3	0.19ns	22.52^{c}	98.75^{c}	3639.53^{c}
Rotation × Year × ToS	3	1.38^{a}	34.20^{c}	41.28^{a}	2737.99^{c}
Error (c)	36	0.33	2.23	12.53	230.11
Sub-sub-subplots					
Entry	13	2.11^{c}	18.29^{c}	127.11^{c}	1889.56^{c}
Entry × Year	13	1.72^{c}	3.32^{c}	21.57^{c}	830.35^{c}
$q_1q_1q_2q_2$ vs. $Q_1Q_1Q_2Q_2$ (2004)	1	0.06ns	49.93^{c}	532.56^{c}	15105.60^{c}
$q_1q_1q_2q_2$ vs. $Q_1Q_1Q_2Q_2$ (2005)	1	14.52^{c}	18.72^{c}	172.10^{c}	1128.25^{c}
Entry × ToS	13	0.65^{c}	4.82^{c}	21.13^{c}	168.23^{c}
$q_1q_1q_2q_2$ vs. $Q_1Q_1Q_2Q_2$ (Early)	1	7.88^{c}	49.46^{c}	141.11^{c}	4169.25^{c}
$q_1q_1q_2q_2Q$ vs. $Q_1Q_1Q_2Q_2$ (Late)	1	1.57^{c}	19.02^{c}	591.33^{c}	8450.15^{c}
Entry × Rotation	39	0.20^{c}	1.30^{c}	15.57^{c}	233.24^{c}
$q_1q_1q_2q_2$ vs. $Q_1Q_1Q_2Q_2$ (Fallow)	1	1.91^{c}	8.05^{c}	141.50^{c}	7435.89^{c}
$q_1q_1q_2q_2$ vs. $Q_1Q_1Q_2Q_2$ (Medic)	1	3.25^{c}	53.26^{c}	19.52ns	156.01ns
$q_1q_1q_2q_2$ vs. $Q_1Q_1Q_2Q_2$ (Pulse)	1	4.49^{c}	17.08^{c}	221.98^{c}	3088.56^{c}
$q_1q_1q_2q_2$ vs. $Q_1Q_1Q_2Q_2$ (Wheat)	1	0.19ns	3.41^{b}	399.08^{c}	4491.62^{c}
Entry × Year × ToS	13	0.40^{c}	4.57^{c}	15.50^{a}	517.12^{c}
Entry × Year × Rotation	39	0.12^{a}	1.66^{c}	13.25^{b}	240.78^{c}
Entry × ToS × Rotation	39	0.12^{a}	0.87^{c}	9.47ns	121.66^{c}
4-way interaction	39	0.10ns	0.85^{c}	7.45ns	131.62^{c}
Error (d)	624	0.08	0.43	8.24	56.77

^{a} , ^{b} , ^{c} indicates significant effects at $P < 0.05$, 0.01 and 0.001 respectively

ns, not significant

stubble. However, grain protein levels were also higher after a pulse crop (Table 3).

Genotype × trait profiling

The GGE biplot methodology is a useful tool to analyse and visualise the multi-trait profile of entries, when different combinations of traits are desired. In the present study, the principal component analysis was based on trait-standardised data to account for the different units of trait measurement. The overall pattern of trait interrelationships captured by the biplot agreed with expectations. For instance, grain yield and protein content were negatively correlated, as shown by the obtuse angle between their respective vectors. Similarly, the acute angle between grain yield, kernel weight and plumpness suggested a positive interrelationship, but the 90° angle between vectors for grain protein and heading date suggested that, in these materials, the two traits were largely independent.

The biplot display was used to compare entries on the basis of their multi-trait profile and to identify clusters sharing good characters in certain aspects (Yan and Rajcan 2002). The first two principal components explained 74% of the multivariate complex, and all measured traits were equally important, as indicated by the relative length of their vectors (Fig. 1). The first axis

Table 3 Means of grain yield, protein content, 1000-kernel weight and plumpness, as affected by planting date and crop rotation system for cropping seasons 2004 and 2005

	Grain yield (t ha^{-1})		Grain protein Content (%)		1000-kernel weight (g)		Grain plumpness (%)	
Treatment	2004	2005	2004	2005	2004	2005	2004	2005
Time of sowing								
Early	0.74	3.88	15.06	12.25	33.73	45.35	55.04	90.84
Late	0.75	3.78	16.34	12.52	33.57	45.89	55.15	90.20
5%LSD	0.24	0.24	0.70	0.12	1.71	0.96	9.98	0.71
Crop rotation								
Fallow	0.68	4.13	16.17	12.96	33.49	45.71	53.43	88.41
Medic pasture	0.40	3.96	11.39	12.80	35.49	45.99	76.66	90.41
Pulse crop	1.47	4.05	17.94	12.57	36.57	44.62	71.77	90.40
Wheat	0.44	3.16	17.29	11.19	29.05	46.17	18.52	92.85
5%LSD	0.11	0.20	1.84	0.23	0.49	0.81	3.32	1.05

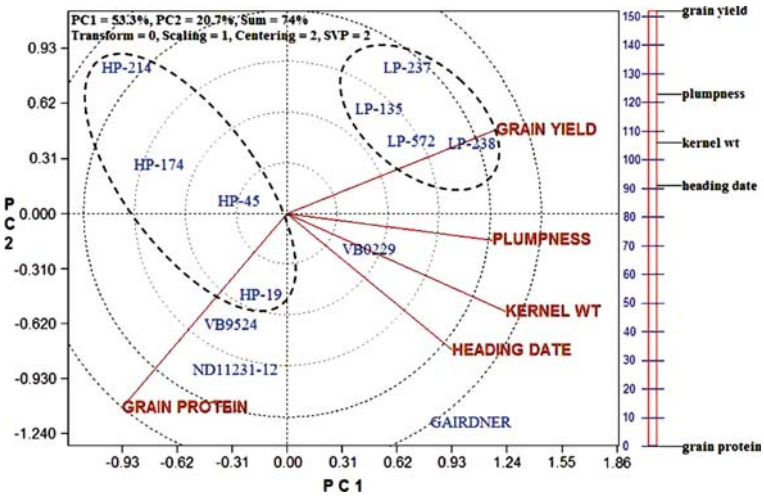

Fig. 1 Biplot analysis of genotype × trait data. Plot shows the degree of genetic correlation among traits (angle in degrees along vertical bar) and clustering of entries on the basis of their multi-trait profile. Lines LP-237, LP-135, LP-238 and LP-572 carry two alleles ($q_1q_1q_2q_2$) for low-protein, derived from parents, VB9524 and ND11231–12

(PC1) split the protein entries into two groups, which showed good agreement with their genotypic classification as high (HP) and low (LP) protein lines. The analyses clustered all four LP genotypes together in the upper right, grain yield quadrant and opposite to the grain protein vector (Fig. 1). This indicates a tendency towards higher grain yield and lower protein content for these genotypes, and because grain protein and heading date were largely independent in these materials, this behaviour might be unaffected by maturity. In contrast, the low-protein capacity of the check variety, Gairdner, might be compromised by maturity. In the biplot display, Gairdner was placed close to the vector for heading date, which is consistent with its known characteristic as a late-maturity line. Gairdner was included in the trials as one of the reference low-protein checks, because it is notably a low-protein achiever in this environment (Bedggood and Bedggood 2003).

Comparison across rotations

In both the separate and combined analyses of variance (Table 1 and 2), the Entries and Entries × Rotation interaction terms were highly significant ($P < 0.001$), indicating an inherent genetic difference in the way barley lines responded to the previous cropping rotations. Although Time-of-Sowing (ToS) was rarely a significant factor, the Entries × ToS terms were largely significant (Table 1 and 2), and single-degree-of-freedom contrast tests were therefore conducted to check whether the differences persisted when genotypes when sown early or late, and in the different crop rotation systems. With a

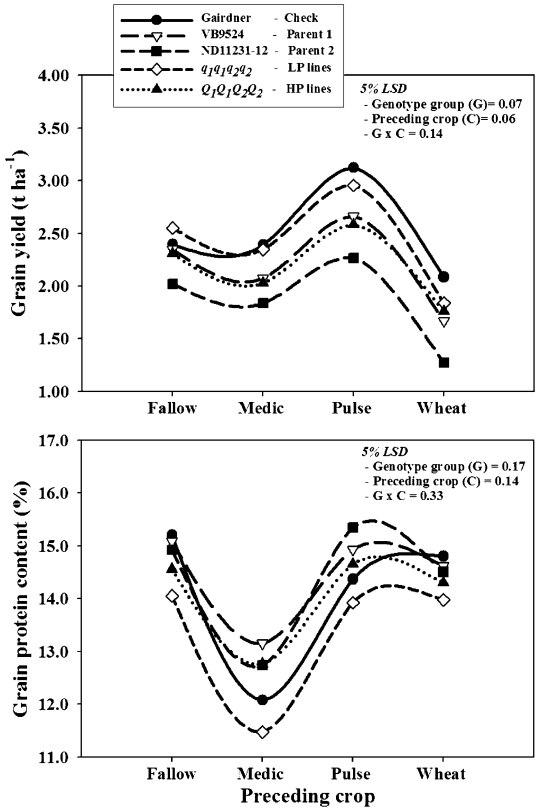

Fig. 2 Mean grain yield and protein content under different crop rotation systems. Plots represent genotypes possessing low protein alleles, parents, and check variety, Gairdner, averaged over years and planting dates

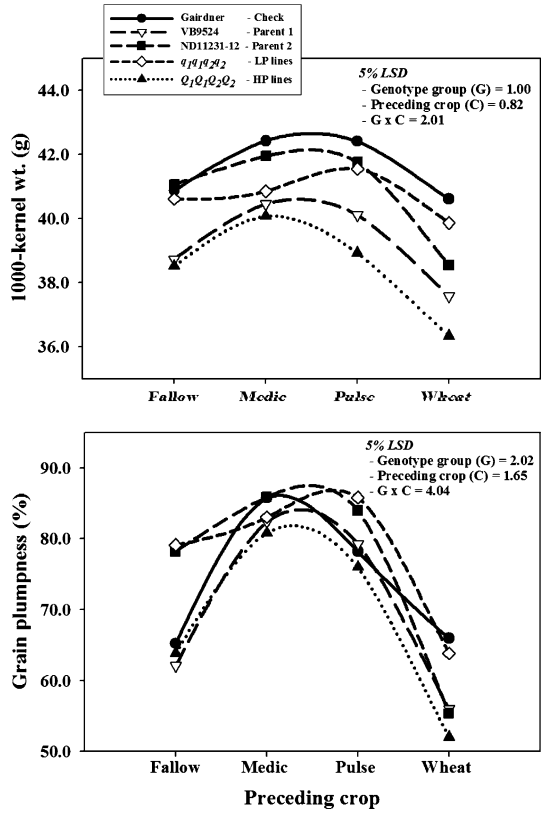

Fig. 3 Mean kernel weight and plumpness under different crop rotation systems. Plots represent genotypes possessing low protein alleles, parents, and check variety, Gairdner, averaged over years and planting dates

few exceptions, differences between the $q_1q_1q_2q_2$ and $Q_1Q_1Q_2Q_2$ genotypes were highly significant for all measured traits, irrespective of years, time of sowing or rotation system (Table 2). Graphical plots of the combined average performances of the genotypes, relative to parents and the most relevant check variety are presented in Fig. 2 and 3.

In general, all lines showed similar responses to the preceding crop (Fig. 2 and 3), with higher yields and higher protein content observed when barley followed a pulse crop. Genotypes with the low-protein genes ($q_1q_1q_2q_2$), however, consistently maintained comparatively lower grain protein contents, and a yield advantage of 11–24% over their high-protein counterparts, when barley followed a legume crop, depending on the season. The results for kernel weight and plumpness (Fig. 3) also showed a similar tendency of low-protein genotypes to have higher attributes, and to maintain superiority over their high-protein counterparts, irrespective of the preceding crop.

Mean values obtained for the individual entries are presented in Table 4. For brevity, only data from pulse and wheat crops are presented, and only from the 2005 trial. Gairdner was the highest yielding of the check varieties (Table 4), producing over 4 t ha^{-1} but it only made malting specifications when grown after a wheat crop. In comparison, two of the low-protein genotypes (97-020D-237 and 97-020D-238) produced grain yields of >4 t ha^{-1}, following a pulse crop, and maintained grain protein content within malting specifications (Table 4). The principal component analyses confirmed the consistent (clustered) pattern of the four low-protein lines and their ability to express consistent behaviour across years and rotation systems (Fig. 1).

Table 4 Mean grain protein, grain yield, kernel weight and plumpness of 14 barley genotypes grown after pulse and wheat crops in 2005.

Entry	Genotype	Grain yield (t ha^{-1})	Grain protein content (%)	1000-kernel wt. (g)	Grain plumpness (%)	Grain yield (t ha^{-1})	Grain protein content (%)	1000-kernel wt. (g)	Grain plumpness (%)
		Pulse crop				Wheat crop			
97-020D-135	$q_1q_1q_2q_2$	3.91	12.26	45.36	93.75	3.25	10.74	48.07	95.33
97-020D-237	$q_1q_1q_2q_2$	4.55	11.87	43.90	90.94	3.44	10.45	46.21	93.65
97-020D-238	$q_1q_1q_2q_2$	4.53	11.98	45.64	94.12	3.42	10.93	49.56	95.54
97-020D-572	$q_1q_1q_2q_2$	4.14	12.10	45.32	90.22	3.11	10.45	46.23	91.81
97-020D-19	$Q_1Q_1Q_2Q_2$	3.78	12.89	45.45	90.47	2.83	11.05	46.58	92.72
97-020D-45	$Q_1Q_1Q_2Q_2$	3.93	12.90	45.67	92.96	3.35	11.37	48.21	95.70
97-020D-174	$Q_1Q_1Q_2Q_2$	3.74	12.52	41.66	82.06	2.99	11.01	41.57	88.78
97-020D-214	$Q_1Q_1Q_2Q_2$	3.60	12.59	37.93	83.89	2.87	10.95	43.93	86.47
VB9524	Parent 1	3.92	13.11	44.88	88.25	2.97	11.87	44.68	89.30
ND11231-12	Parent 2	3.32	12.72	46.22	91.29	2.14	11.07	47.07	94.31
Gairdner	Check	4.47	12.96	46.15	87.64	3.63	10.83	47.15	92.65
VB0229	Check	4.39	12.58	45.19	94.15	3.48	10.88	47.28	94.45
Schooner	Check	4.09	13.05	45.61	93.15	3.29	11.68	45.94	95.24
WI2875-17	Check	4.28	13.00	45.71	92.79	3.49	11.90	43.88	94.00
Mean	–	4.04	12.61	44.62	90.41	3.16	11.08	46.17	92.85
5% LSD	–	0.43	0.34	3.11	2.36	0.43	0.34	3.11	2.36

Discussion

There is substantial evidence in the literature showing that genetic manipulation of grain protein genes in cereals changes the components of grain yield and ultimately grain yield itself. At the University of Illinois at Urbana-Champaign, USA, a divergent recurrent selection program based solely on grain protein concentration has been going on for over 100 years, and results were recently reviewed by Below et al. (2004). One of the many highlights of the review is that, on average, the low-protein plants (ILP) produce double the grain yields compared to the high-protein (IHP) counterparts (96 vs. 45 g plant^{-1}). While both selections have similar amounts of whole-shoot (all above-ground plant parts) dry matter at silking, they differ greatly in their dry matter accumulation after silking, with the ILP accumulating twice as much dry matter as the IHP. The wide variation in protein and dry matter production of these selections intuitively implies that it must have been accompanied by corresponding changes in nitrogen and carbon metabolisms in the plant (Below et al. 2004).

In other cereal crops, previous studies have shown that genotypes with genetically high and low grain protein content also show relative differences in grain yield. In a backcross oat (*Avena sativa* L.) population subjected to intense selection for protein percentage, Takeda and Frey (1985) showed that the selection resulted in lines with high protein percentage but low grain yields. In wheat, Mesfin et al. (2000) observed that, in two different genetic backgrounds, the difference in mean grain yield between low- and high-GPC lines was in the range of 12.2 and 68.4 kg ha^{-1}.

The present study is based on the concept of breeding varieties with other attributes (ie inherently lower grain protein) that allow those varieties to be grown in crop rotations with higher inherent fertility and hence increasing the "on-farm" yields of malting barley. The research suffers from the fact that genotypes used were not near-isogenic but rather selected for presence/absence of marker alleles at two grain protein QTLs. Other QTLs that might be present, therefore, would behave like additional environmental effects and reduce the significance of any association. This was clearly demonstrated by the aberrant behaviour of the HP lines in the biplot analysis (Fig. 1), but the LP lines formed a uniquely different cluster from the parents, and were negatively correlated with grain protein

content. It is noteworthy to observed that VB0229 was placed close to the LP lines. VB0229 was derived from a second set of doubled haploids not used in the original mapping efforts. In multi-location field trials conducted in southern Australia, VB0229 was identified as consistently low in GPC (Moody 2005).

Despite a marked contrast in GPC levels observed in 2004 and 2005, and the negative impact of delayed planting (Table 3), none of these environmental factors changed genotypic ranking. Based on two years of experimentation, the yield advantage of growing barley on legume stubble compared to wheat stubble was approximately 0.96 t ha^{-1}, or a mean yield increase of 53.3% (Table 3). Grain proteins were also high on legume stubble (Table 3; Fig. 2), but genetically low-protein lines, such as 97-020D-237 and 97-020D-238, achieved higher grain yields of over 4 t ha^{-1}, while maintaining protein content within malting specifications (Table 4).

These positive results can be explained as due to physiological determinants of low grain protein content that favour higher grain yield. Lorenzoni et al. (1978) showed that low-GPC maize strains resulting from the divergent selection program at University of Illinois, Urbana, tended to have a longer duration of the linear phase of grain fill, and generally exhibit greener leaves during the later stages of grain fill. In our study no significant differences in maturity were found. Reggiani et al. (1985) reported that the low protein strains (ILP) showed a higher capacity to transport sugars, whereas the high-protein strains (IHP) had a higher capacity to transport amino acids. Lohaus et al. (1998) confirmed the superior capacity of IHP to transport amino acids, with higher concentrations of amino acids in the leaves, xylem, phloem and grains of IHP lines compared to those with ILP. This enhancement was primarily due to higher levels of asparagine, which the authors attributed to greater assimilation of nitrate by IHP in the roots.

The physiological bases for the observed yield increases in the present study have been examined in a previous report (Emebiri and Moody 2004b). In summary, there were no differences among genotype classes for tillering, number of fertile tillers or dry matter accumulation in the straw. However, there were highly significant differences in spike morphology (weight, length and kernels per spike), and on average, the low-GPC barley genotypes produced heavier and longer spikes, and had more kernels per spike than the respective high-GPC counterparts. Similar changes from genetic manipulation of protein concentration in maize were reported by Uribelarrea et al. (2004, 2007), who assessed the yield potential and nitrogen use of Illinois protein selection strains in hybrid combinations with an elite tester. In general, hybrids containing parents selected for low protein concentrations had the heaviest individual kernels, whereas kernels of hybrids that had a parent selected for high protein concentration were smaller.

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