Genetic Relationship between Kernel Discoloration and Grain Protein Concentration in Barley

Paulo C. Canci, Lexingtons M. Nduulu, Ruth Dill-Macky, Gary J. Muehlbauer, Donald C. Rasmusson, and Kevin P. Smith*

ABSTRACT

Kernel color and grain protein concentration (GPC) are two of the most important attributes of barley (*Hordeum vulgare* L.) grain intended for use in malting and brewing. Grain with protein levels that are too low or high or that have dark kernel color, which may result from the disease complex of kernel discoloration (KD), is unacceptable for the malting and brewing industries. The purpose of this study was to use molecular markers to map quantitative trait loci (QTLs) for KD and GPC in two recombinant inbred line (RIL) populations. One population was created by means of the high GPC and KD resistant cultivar Chevron. The other population was created from a KD resistant cultivar MNBrite that was derived from Chevron through eight generations of crossing and selection for bright kernels. The traits KD and GPC were evaluated in four and three environments, respectively, using the Chevron population and six environments using the MNBrite population. GPC and KD score were negatively correlated in both populations. A revised genetic map for the Chevron/M69 population, with an additional 45 simple sequence repeat (SSR) markers, was used to identify nine QTLs associated with KD on chromosomes 1(7H), 2(2H), 4(4H), 6(6H), and 7(5H), including two major QTLs on chromosome 6(6H). A single QTL for GPC identified in the Chevron mapping population, accounting for over 55% of the phenotypic variation, was located on chromosome 6(6H) and was coincident with one of the two major QTLs for KD. In this region, the Chevron allele increased GPC and decreased KD score. In the MNBrite mapping population, the same region of chromosome 6(6H) was mapped with SSR markers, and QTL analysis verified that this region was associated with both KD and GPC, indicating that MNBrite inherited this region of chromosome 6(6H) from Chevron. The results of this study suggest that if GPC and KD are controlled by tightly linked genes, then it should be possible to use SSR markers to identify recombinants in this region of chromosome 6(6H) and break the linkage to allow selection for KD resistance without high GPC. Alternatively, if GPC and KD are conditioned by the same gene, then it should be possible to select for KD resistance from Chevron by means of SSR markers and to use phenotypic selection to introgress other genes to reduce GPC to acceptable levels.

Kernel color and GPC are two of the most important selection criteria for determining whether barley grain is of sufficient quality for malting and brewing. Kernel color is negatively affected by KD, a disease complex caused by several fungal organisms including *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur [the perfect stage of *Bipolaris sorokiniana* (Sacc.) Shoemaker], *Fusarium graminearum* Schwab, and *Alternaria* spp. (Mathre, 1997). KD results in brown and black discoloration of the lemma and palea, which reduces the value of the grain for malting. In severe cases, grain may be rejected for malting and must be marketed for feed at a reduced price. The severity of KD varies depending on susceptibility of the cultivars and environmental conditions (Miles et al., 1987; Wilcoxson et al., 1980).

KD resistance is quantitatively inherited (Singh et al., 1995; Wilcoxson et al., 1980) and heritability estimates range from 0.27 to 0.76 (Miles et al., 1989). Previously, we studied the genetics of KD resistance in the cultivar Chevron (CIho 1111) and identified 11 QTLs associated with KD on the basis of evaluations in four environments (de la Peña et al., 1999). In this mapping population, Chevron × M69, these QTLs were distributed across the genome and were usually detected in only one of the environments. However, two QTLs on adjacent linkage groups of chromosome 6(6H) had large effects and were detected in three environments. It was not possible to determine whether these QTLs represented a single locus or two distinct loci because of low marker coverage in that area.

Grain protein concentration is also an important malting quality trait in barley and for six-rowed malting barley GPC that is too low (<115 g Kg^{-1}) or too high (>135 g Kg^{-1}) is generally not preferred by the malting and brewing industry. In particular, high GPC is associated with longer steep times, erratic germination, and haze formation in beer (Burger and LaBerge, 1985). Levels of GPC that are too low can result in low enzyme levels and poor yeast nutrition. GPC in barley is affected by genetic and nongenetic factors (Weston et al., 1993; Zubriski et al., 1970) and heritability estimates have been highly variable ranging from 0 to 0.80 in U.S. Midwest studies (Foster et al., 1967; Rasmusson and Glass, 1965). GPC is increased by applying nitrogen fertilizer (Zubriski et al., 1970) and growers producing malting barley must manage nitrogen application to achieve higher yields while maintaining acceptable levels of GPC for malting.

The genetics of GPC have been studied extensively because of its importance in grain quality. Genetic mapping studies have identified QTLs for GPC on all seven chromosomes of barley (Hayes et al., 1993, 1996; Larson et al., 1997; Marquez-Cedillo et al., 2000; Mather et al., 1997; Oziel et al., 1996). A recent study suggests that a major QTL for GPC exists on chromosome 6(6H) (See et al., 2002) and may be orthologous to a gene influencing protein concentration in *Triticum turgidum* subsp. *dicoccoides* (Korn. ex Asch & Graebn.) Thell (Joppa et al., 1997).

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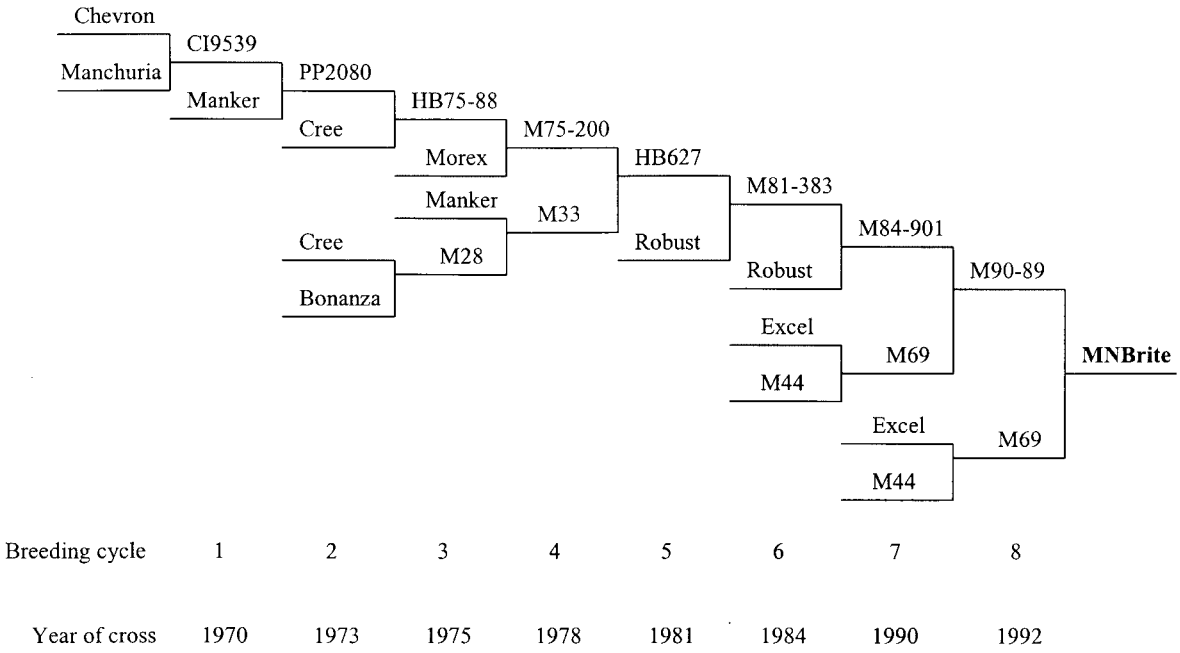

Fig. 1. MNBrite pedigree. A Chevron-derived progeny, CI9539, was the parent in the first cycle of breeding. M44 has the pedigree Nordic/Manker/3/Morex//Manker/63AB2987-32.

Evidence from breeding and genetic studies has shown that GPC and KD are genetically correlated. Breeding for KD resistance in six-rowed malting barley has relied mainly on the cultivar Chevron as a source of resistance. The cultivar MNBrite (PI 603050) was developed from a breeding program that utilized eight cycles of crossing and selection to incorporate Chevron-derived KD resistance into an improved malting quality background (Fig. 1; Rasmusson et al., 1999). MNBrite exhibits resistance to KD; however, it also has high GPC (Rasmusson et al., 1999) and therefore is not currently used by industry as a malting cultivar. This undesirable association has also been observed in breeding research to develop barley varieties with moderate GPC. In a study using three crosses to a low protein line derived from the cultivar Karl, kernel color was negatively correlated with GPC (Goblirsch et al., 1996).

New barley varieties with good KD resistance and moderate GPC are needed to allow producers to manage nitrogen to increase yield and still produce grain with good kernel color and acceptable protein levels. To explore further the genetics of these important traits, we have studied GPC and KD in two populations. The objectives of this study were to (i) increase molecular marker coverage in the Chevron × M69 population and refine the KD QTL positions on chromosome 6(6H); (ii) map QTLs for GPC in the Chevron × M69 population; (iii) map QTLs conditioning GPC and KD in a population created using the cultivar MNBrite; and (iv) determine the genetic relationship between KD and GPC.

MATERIALS AND METHODS

Parents and Populations

Two populations derived from crosses involving Chevron with M69 (CM) and MNBrite with M96 (MM) respectively, were used in this study. Chevron, introduced from Switzerland in 1914, exhibits resistance to KD and has high GPC. M69 and M96 are elite breeding lines developed at the University of Minnesota, are susceptible to KD, and have moderate GPC.

The CM mapping population consisted of 101 $F_{4:7}$ families obtained by single seed descent (de la Peña et al., 1999). The MM population consisted of 98 $F_{4:6}$ families obtained by single seed descent. After the F_4 generation, row increases in the field of individual lines were bulk harvested to generate seed used to evaluate KD and GPC in the $F_{4:6}$ and $F_{4:7}$ generations.

Disease Assessment

Kernel discoloration was previously evaluated in the CM population in four disease nurseries (de la Peña et al., 1999). KD was assessed on grain from two nurseries (Morris and Crookson) that were inoculated with *F. graminearum* and two nurseries (St. Paul) were inoculated with *B. sorokiniana* in single row plots as described below. For the MM population, KD was evaluated from grain harvested from six nurseries; two nurseries at St. Paul and one nursery at Crookston, MN in both years 1997 and 1998. Individual lines, MNBrite, M96 and Chevron were seeded in one-row plots, 1.8 to 2.4 m long, spaced 30 cm apart in a randomized complete block design with two replicates at each location. For the four trials conducted at St. Paul, in each year one nursery was inoculated with *F. graminearum* and one was inoculated with *B. sorokiniana*.

In the *F. graminearum* inoculated nurseries, macroconidia (1.8×10^6 macroconidia mL^{-1}), or *F. graminearum* colonized wheat (*Triticum aestivum L.*) and/or maize (*Zea mays* L.) kernels were used as sources of inoculum (de la Peña et al., 1999). In St. Paul 1997, each plot received approximately 30 mL of macroconidia suspension (12 isolates) applied three times between anthesis and physiological maturity with a tractor-mounted sprayer. In St. Paul 1998, individual plots were inoculated with a suspension of *F. graminearum* macroconidia (18 isolates) at a concentration of 2×10^5 spores mL^{-1} with a compressed CO_2 powered backpack sprayer that dispersed 9.5 mL s^{-1} at 0.28 Pa (40 psi). Plots were sprayed for 6 s, which delivered approximately 6×10^6 macroconidia m^{-1} of row. Three applications were made starting at heading with

the second and third application at 3-d intervals. Genotypes with similar heading date were grouped and inoculum was applied according to the maturity class. The Crookston nurseries were artificially inoculated by scattering wheat or maize seeds colonized with between 12 and 15 different *F. graminearum* isolates collected in the Red River Valley, MN, in previous years. Inoculum was dispersed at or within a few days of jointing at a rate of 10 to 20 g/plot.

The nurseries inoculated with *B. sorokiniana* were conducted at St. Paul in 1997 and 1998. A conidial suspension of *B. sorokiniana* (5×10^4 conidia mL^{-1}) was applied 2 or 3 times at a rate of 33 mL $plot^{-1}$ with a tractor-mounted sprayer. The first inoculation was applied at anthesis, with later inoculations between anthesis and physiological maturity at 7- to 10-d intervals. Plots were sprinkler irrigated, except when it rained, after the first inoculation for 15 to 30 min twice a day until approximately 5 d before maturity.

Kernel discoloration was scored on harvested grain by a 1-to-5 scale, where 1 was the brightest and 5 the most discolored grain as described by Miles et al. (1987). To enhance assessments, samples were examined on 15-cm diam white paper plates under fluorescent lights. A set of control samples representing the range of KD reactions was used as a reference for assigning scores.

Grain Protein Concentration Assessment

In the CM population, GPC was determined on whole grain samples harvested at Crookston in 1995 and St. Paul in 1995 and 1997 from the trials described previously (de la Peña et al., 1999). Grain protein concentration was determined for the Chevron/M69 population with a Grainspec grain analyzer (Foss North America, Eden Prairie, MN). Barley protein calibration for near infrared reflectance (NIR) was based on GPC values of samples provided by T&W Agriculture Services (Fargo, ND) from multiple years and locations in the USA, determined by the Kjeldahl procedure (William, 1984). For the MM population, GPC was determined from the six St. Paul and Crookston nurseries grown in 1997 and 1998 with a Model 6500 NIR Spectrometer (Foss North America). In this case, barley protein calibration for NIR was based on the Kjeldahl GPC values of 200 samples from different trials grown in 1997 and 1998 at Crookston and St. Paul, MN.

Phenotypic Data Analysis

Analysis of variance (ANOVA) was performed under a randomized complete block design with genotype and replicate as sources of variation for the individual environments by Proc GLM (SAS Institute, 1988). Analysis of variance was conducted across environments for traits in which the error mean squares were homogeneous and used environments, replicates within environments, genotypes and genotype × environment (G×E) as sources of variation. All factors were considered random effects. Correlation coefficients between GPC and KD were computed from the genotype means in each environment in which both traits were evaluated by Proc CORR (SAS Institute, 1988).

DNA Markers

To enhance marker coverage of the CM map, 208 SSR markers obtained for barley (Liu et al., 1996; Ramsay et al., 2000) were screened for polymorphism between Chevron and M69 following the protocol described by Liu et al. (1996). DNA was isolated from a bulk of at least 10 $F_{4:5}$ plants per line as described by de la Peña et al. (1996). Products from PCR amplification with SSR primers were resolved on either silver stained gels (Bassam et al., 1991) or on an infrared detection instrument (Global IR^2 System, LI-COR Biosciences, Lincloln, NE). Markers identifying polymorphisms were screened on the entire population.

To test for markers associated with GPC and KD in the MM population, 87 genomic or cDNA probes previously mapped in the CM population were screened for restriction fragment length polymorphisms (RFLPs) between MNBrite and M96 by means of the restriction endonucleases *Eco*RI, *Eco*RV, *Hin*dIII, and *Dra*I. Barley genomic DNA was isolated and DNA gel blot analyses were performed as previously described (de la Peña et al., 1999). Twenty-four of the 45 SSR markers placed on the CM map were screened for polymorphism between MNBrite and M96. Three additional SSR markers thought to map to chromosome 6(6H), but that were not polymorphic between Chevron and M69, also were screened for polymorphism between MNBrite and M96. Markers that were polymorphic between parents were subsequently scored in the MM population and used for linkage analysis.

Map Construction and QTL Analysis

Linkage analyses of the CM and the MM populations were performed by GMendel 3.0 (Holloway and Knapp, 1994) by evaluating the populations as an F_4 generation using a LOD score (base-10 log likelihood ratio test statistic) of 4.0. The linkage groups were assigned according to published maps (Kleinhofs et al., 1993; Graner et al., 1991; Qi et al., 1996). Composite interval mapping (CIM) was employed on the CM population by the software PLABQTL (Utz and Melchinger, 1996) to identify genomic regions associated with KD and GPC. Markers to be used as cofactors were selected by a stepwise regression procedure with the default selection parameters. A LOD score of 3.35 was used for detection of QTLs, corresponding to experiment-wise and comparison-wise error of $P = 0.05$ and $P = 0.0004$, respectively, as calculated by the Bonferroni Chi-square approximation suggested by Zeng (1994). The proportion of phenotypic variance explained by each QTL was estimated by the coefficient of determination (r^2), which is based on the partial correlation of putative QTL with the trait adjusted for cofactors in the multilocus model. In addition, an estimate of the additive effect (α) of the "Chevron" allele was obtained by the regression coefficient from the multilocus model. Alpha values are relative to the mean of phenotypic trait values used in the analysis. An α value of 0.5 for a GPC QTL would indicate that a "Chevron" allele increases GPC by half a percentage point in that environment. Since only a single linkage group spanning part of chromosome 6(6H) was constructed for the MM population, simple interval mapping (SIM) was employed to identify genomic regions associated with KD and GPC using PLABQTL. The LOD score threshold for detection was set at 2.5, corresponding to P values of 0.05 and 0.007, for experiment-wise and comparison-wise error, respectively.

RESULTS AND DISCUSSION

Refined Mapping of KD Resistance in the CM Population

In our previous study of KD resistance in Chevron, we identified two major QTLs at the adjacent ends of two adjacent linkage groups corresponding to chromosome 6(6H) (de la Peña et al., 1999). Low marker coverage in this area left us unable to determine if these were two distinct QTLs or a single locus. To increase the resolution of the map for this population, we added 45 SSR markers and regenerated the linkage map (Fig. 2). In general, the marker positions are consistent with the

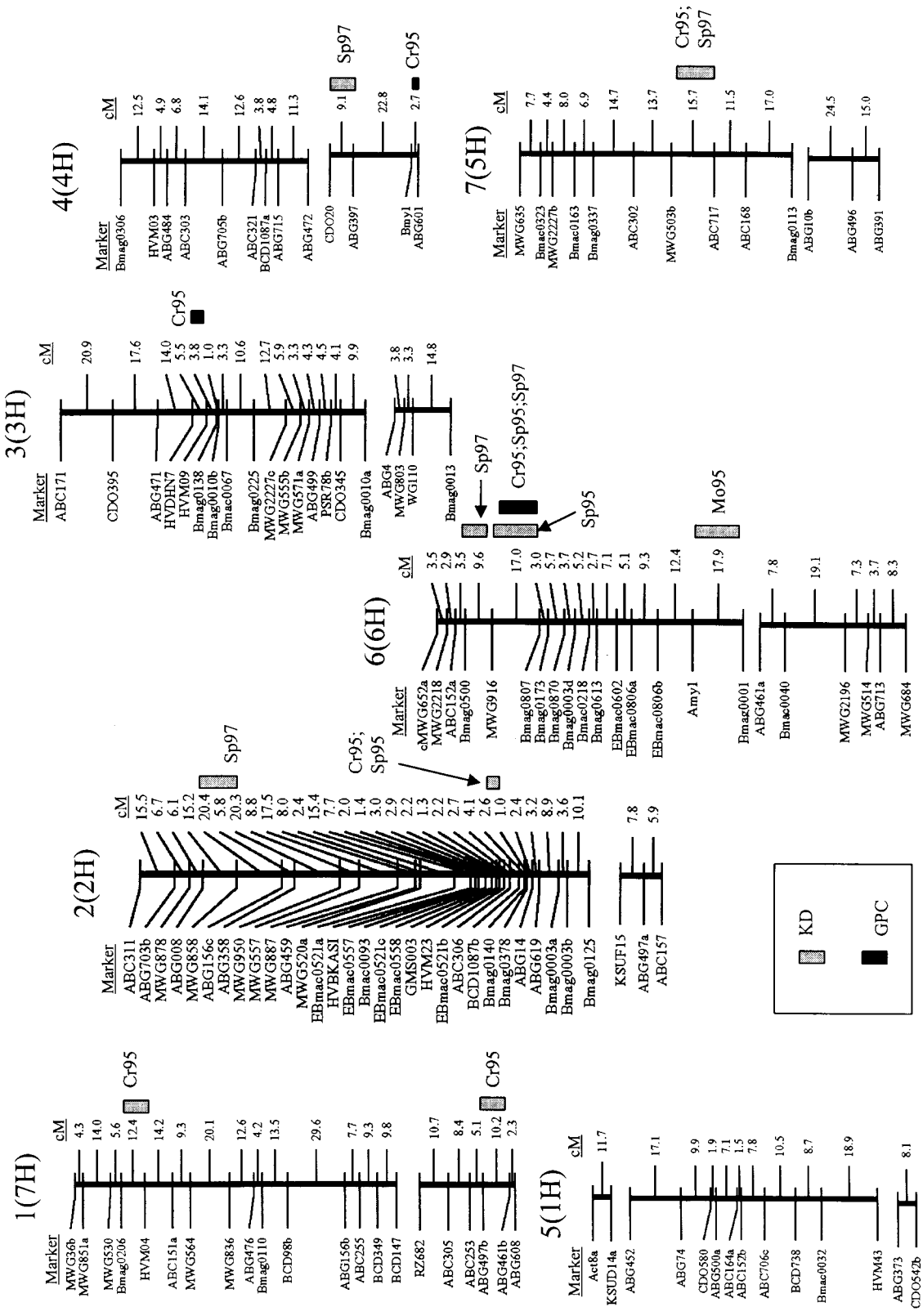

Fig. 2. Revised genetic linkage map of the Chevron × M69 (CM) mapping population originally published in de la Peña et al. (1999). The bars are adjacent to the marker interval on the map in which the QTL was detected. QTL for kernel discoloration (KD) and grain protein concentration (GPC) are shown for individual environments and are coded to identify the environments in which they were detected (Cr95 = Crookston 1995; Mo95 = Morris 1995; Sp95 = St. Paul 1995; Sp97 = St. Paul 1997). See Tables 2 and 4 for parameter estimates for the QTLs identified for KD and GPC, respectively.

Table 1. Mean squares from analysis of variance for kernel discoloration (KD) severity and grain protein concentration (GPC) for Chevron/M69 and MNBrite/M96 populations.

	Chevron/M69		MNBrite/M96	
Source	**KD**	**GPC**	**KD**	**GPC**
Environments	**26.8****	**169.7****	**2.32****	**69.1****
Reps (in environments)	**0.6**	**11.6**	**1.10**	**109.0**
Genotypes†	**1.5****	**39.9****	**0.64****	**12.2****
Genotypes × Environment‡	**0.6***	**10.8**	**0.37**	**03.3**
Error	**0.4**	**08.8**	**0.36**	**03.2**

*** Significant at the 0.05 probability level.**
**** Significant at the 0.01 probability level.**
† Significance of mean square for genotype was tested using the MS from Genotypes × Environments as the error term.
‡ Genotypes × Environment was tested using residual error as the denominator in the F test.

previously published map of the CM population (de la Peña et al., 1999) and other published barley genetic maps (Kleinhofs et al., 1993; Qi et al., 1996; Ramsay et al., 2000; Costa et al., 2001). Nine of the 45 SSR markers mapped between MWG916 and *Amy1*, thus joining the two adjacent linkage groups of chromosome 6(6H) described in the original paper.

We observed significant G×E interaction for KD in the CM population (Table 1), so we conducted the QTL analysis by location. Using this revised map, we identified nine QTLs for KD resistance (Table 2; Fig. 2). Six of these QTLs were among the 11 QTLs that had been described in the original analysis of the CM population (de la Peña et al., 1999). Three additional QTLs, on chromosomes 1(7H), 2(2H), and 6(6H), were identified in this study, while five QTLs from the original study were not detected at the higher LOD threshold imposed to account for the additional markers. All of the QTLs identified, except for one QTL identified on chromosome 7(5H) and one QTL on chromosome 2(2H), were detected in only a single environment. The lack of QTLs that are reproducible across environments was observed by de la Pẽna et al. (1999) in the original analysis of KD and for QTLs associated with Fusarium head blight (FHB). In this study, the screening environments for KD assessment differed considerably, particularly with respect to inoculum (*B. sorokiniana* or *F. graminearum).* Apparently, this factor does not explain differences among environments, since in the two instances where KD QTLs were observed in multiple environments, the environments were not similar with respect to year or method of inoculation. At this time, there are no other published QTL mapping studies of KD resistance in barley. However, several studies have observed inconsistent detection of QTLs across environments for FHB resistance (Ma et al., 2000; Zhu et al., 1999). An important purpose of this study is to validate QTLs for KD resistance that were identified in the CM population by means of the MM population. This validation is essential before utilizing markers to augment selection in breeding.

We identified three QTLs for KD resistance on chromosome 6(6H) in the CM population (Fig. 3). The increased marker coverage in this region places the two KD QTLs, which were previously located on adjacent linkage groups, on a single linkage group with a distance of over 50 centimorgans (cM) between them. These two QTLs on chromosome 6(6H), flanked by the markers MWG916-Bmag0807 and *Amy1*-Bmag0001, explain the largest amount of the variation for KD, 21.5 and 30.2%, respectively (Table 2).

Mapping GPC in the CM Population

To investigate the relationship between KD and GPC, we mapped GPC in the CM population. Chevron had 35 g kg^{-1} higher GPC than M69 (Table 3). No significant G×E interaction was detected for GPC (Table 1). There were significant differences among lines in the population for GPC and the mean of the lines was very near the mean of the parents (Table 3). The correlation coefficient (r) values between KD score and GPC for the three environments tested ranged from −0.21 to −0.41. Three QTLs for GPC were detected with the major QTL (MWG916-Bmag0807) detected in all three environments (Table 4). This major GPC QTL was detected in the same region (MWG916-Bmag0807) as one of the QTLs for KD resistance on chromosome 6(6H) (Table 2, Fig. 3). This QTL for GPC explains over 55% of the phenotypic variance in the population with the Chevron allele conditioning high GPC (Table 4).

Table 2. Quantitative trait loci associated with kernel discoloration (KD) severity at four environments in the Chevron/M69 (CM) population.

			Crookston 1995			St. Paul 1995			Morris 1995			St. Paul 1997		
LG†	**QTL Position‡**	**Marker Interval§**	**LOD**	**$R^2 \times 100$¶**	**Alpha#**	**LOD**	**$R^2 \times 100$**	**Alpha**	**LOD**	**$R^2 \times 100$**	**Alpha**	**LOD**	**$R^2 \times 100$**	**Alpha**
1(7H).1	**24**	**Bmag0206-HVM04**	**3.4**	**6.4**	**−0.20**									
1(7H).2	**34**	**ABG497-ABG461b**	**3.8**	**10.1**	**−0.26**									
2(2H).1	**38**	**ABG008-MWG858**										**4.4**	**15.3**	**−0.28**
2(2H).1	**160, 162**	**Ebmac0558-HVM23**	**5.1**	**7.5**	**−0.24**	**4.2**	**7.0**	**−0.30**						
4(4H).2	**0**	**CDO20-ABG397**										**3.6**	**10.6**	**−0.20**
6(6H).1	**14**	**Bmag0500-MWG916**										**4.3**	**19.7**	**−0.30**
6(6H).1	**36**	**MWG916-Bmag0807**				**4.3**	**21.5**	**−0.50**						
6(6H).1	**102**	**Amy1-Bmag0001**							**8.7**	**30.2**	**−0.48**			
7(5H).1	**70,64**	**MWG503b-ABC717**	**3.4**	**10.8**	**0.28**							**4.4**	**8.4**	**0.20**
		Multi-locus	**7.3**	**28.5**		**6.6**	**26.4**		**nd**	**nd**	**nd**	**12.0**	**42.1**	

† The number before the decimal is the chromosome and the number following the decimal is the linkage group for that chromosome.
‡ Distance in centimorgans from the top of the linkage group as displayed in Fig. 2. Multiple positions correspond to different environments in the same order as they appear in the table from left to right.
§ Markers flanking the peak of the LOD scan.
¶ Percentage phenotypic variance explained by QTL.
 # Effect of Chevron allele on KD expressed as regression coefficient.

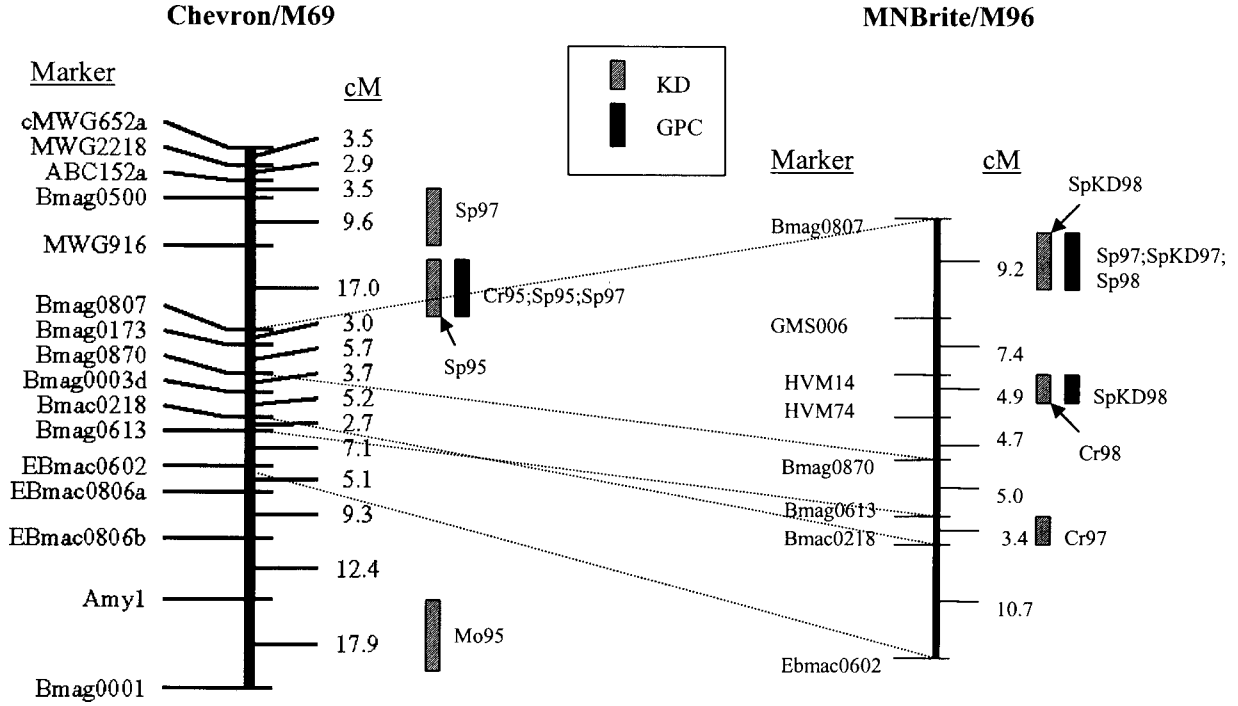

Fig. 3. Quantitative trait loci associated with kernel discoloration (KD) and grain protein concentration (GPC) on chromosome 6(6H) for Chevron/M69 (CM) and MNBrite/M96 (MM) mapping populations. QTL for KD and GPC are shown for individual environments and are coded to identify the environments in which they were detected. Cr95 = Crookston 1995; Sp95 = St. Paul 1995; Mo95 = Morris 1995; Sp97 = St. Paul 1997; Cr97 = Crookston 1997; SpKD97 = St. Paul *B. sorokiniana* nursery 1997; Cr98 = Crookston 1998; Sp98 = St. Paul 1998; SpKD98 = St. Paul *B. sorokiniana* nursery 1998). See Tables 5 and 6 for parameter estimates for the QTLs identified for KD and GPC.

Relationship between High GPC and Low KD in the Cultivar MNBrite

Since the KD resistance in the cultivar MNBrite is presumably derived from Chevron, we used molecular markers to try to validate the KD and GPC QTLs identified in the CM population. MNBrite was more KD resistant than M96, but less resistant than Chevron (Table 3). Similarly, MNBrite was intermediate to Chevron and M96 for GPC. The MM population mean for both traits was intermediate to the values for the two parents. For both traits, there was no significant G×E interaction in the population. Mean square errors were homogenous for KD so analyses using both individual environments and means across environments were conducted. In four of the six environments, there were significant negative correlations between KD score and GPC ranging from $r = -0.22$ to -0.28 in the MM population.

Since MNBrite was developed from crosses between related elite breeding lines (Fig. 1), we anticipated low marker polymorphism between MNBrite and M96. Both parents include the cultivars Robust and Excel in their pedigrees. Of the 97 molecular markers tested on MNBrite and M96, only 17 exhibited polymorphism. Ten of these markers mapped to chromosome 6(6H) of which eight formed a single linkage group of 45 cM in the region between markers MWG916 and *Amy1* (Fig. 3). Two of these markers, Bmag0807 and Bmac0218, showed slight segregation distortion toward the MNBrite allele. The other two polymorphic markers mapped to the short arm of chromosome 6(6H) distinct from the regions associated with GPC and KD in the CM population. The seven polymorphic markers that did not map to chromosome 6(6H) are located on chromosomes 1(7H), 2(2H), and 5(1H).

Table 3. Mean kernel discoloration score for the MNBrite/M96 population, and mean grain protein concentration for the Chevron/M69 and MNBrite/M96 populations and parents across multiple environments.

	Kernel discoloration		Grain protein concentration (g kg^{-1})			
	MNBrite/M96†		Chevron/M69‡		MNBrite/M96†	
Genotype	Mean	Range	Mean	Range	Mean	Range
Chevron	1.0a§	1.0–1.0	178a†	149–184	137a	132–158
M69	–	–	143b	128–157	–	–
MNBrite	2.6b	2.0–3.0	–	–	121a	108–131
M96	3.7c	3.0–4.0	–	–	109c	102–118
$F_{4:5}$ Families	3.1	2.0–5.0**	160	124–205**	115	92–139**

** Significant variation among F_4 derived$_{:5}$ families at the 0.01 probability level.
† Mean of six environments.
‡ Mean of three environments.
§ Means within the same column followed by the same letter are not significantly different as determined by Fisher's protected LSD ($P = 0.05$).

Table 4. Quantitative trait loci associated with grain protein content (GPC) at three environments in the Chevron/M69 (CM) population.

LG†	QTL Position‡	Marker Interval§	Crookston 1995			St. Paul 1995			St. Paul 1997		
			LOD	$R^2 \times 100$¶	Alpha#	LOD	$R^2 \times 100$	Alpha	LOD	$R^2 \times 100$	Alpha
3(3H).1	56	HVDHN7-HVM09	6.8	26.9	0.50						
4(4H).2	34	Bmy1-ABG601	3.5	18.7	−0.42						
6(6H).1	30,34,36	MWG916-Bmag0807	18.1	56.6	1.30	16.2	59.2	1.06	19.7	59.3	0.63
		Multi-locus	19.6	59.5		nd	nd		nd	nd	

† The number before the decimal is the chromosome and the number following the decimal is the linkage group for that chromosome.
‡ Distance in centimorgans from the top of the linkage group as displayed in Fig. 2.
§ Markers flanking the peak of the LOD scan.
¶ Percentage phenotypic variance explained by QTL.
# Effect of Chevron allele on GPC expressed as regression coefficient.

A LOD scan for KD QTL in the MM population by individual environments detected significant QTLs in the region between Bmag0807-Bmac0218 in three environments as three separate peaks (Fig. 3, Table 5). When the analysis is conducted using the mean across environments, a single QTL for KD was detected in the marker interval Bmag0807-GMS006, 7 cM from Bmag0807, explaining 14% of the phenotypic variation (data not shown). This is very near the KD QTL detected in the CM population which was less than 1 cM from Bmag0807 in the adjacent marker interval MWG916-Bmag0807 (Fig. 3, Table 2). The data suggest that this represents a single KD QTL that is linked to Bmag0807 identified in both the CM and MM population. As in the CM population, this KD QTL was also coincident with a major GPC QTL that explains between 22 to 35% of the variation for GPC (Table 6). In this region, the MNBrite allele conditioned higher GPC and lower KD. These data indicate that this region of chromosome 6(6H) donated from Chevron is at least partially responsible for the higher GPC and lower KD observed in the cultivar MNBrite.

Implications for Breeding KD Resistance and Acceptable GPC

Breeding for resistance to KD in the development of the cultivar MNBrite resulted in the introgression of a region of chromosome 6(6H) from the cultivar Chevron. This region of chromosome 6(6H) is also associated with GPC; however we cannot determine at this point whether this association is due to tight linkage of KD and GPC genes or the pleiotropic effect of a single gene. In either case, selection for KD resistance appears to have also resulted in higher GPC. Interestingly, the other region on chromosome 6(6H) associated with KD (*Amy1*-Bmag0001) was not introgressed from Chevron into MNBrite. This region had a similar effect on KD but no effect on GPC. This region was probably lost during the breeding process because very few lines were selected for crossing to generate the next cycle and little or no selection for GPC was imposed during the development of MNBrite.

The region of chromosome 6(6H) near marker Bmag0807 has also been associated with GPC in genetic studies using parents with low GPC. In a study using a population from the low protein cultivar Karl and a higher protein cultivar Lewis, the marker HVM74 was near a QTL for GPC that accounted for 40% of the phenotypic variation (See et al., 2002). Substituting the Karl alleles at this locus for the Lewis alleles reduced GPC 13 g kg^{-1}. In another mapping study using a parent derived from Karl and the Midwest six-rowed cultivar Azure, the marker HVM31 was associated with GPC and also kernel color (Garcia et al., 2000). In reference to our genetic maps, the SSR marker HVM31 appears to be in the middle of the interval MWG916-Bmag173 (Ramsay et al., 2000), which we found to be associated with GPC and KD in both the CM and MM population. The Karl allele conditioned low GPC and dark kernel color. This would suggest that this region on chromosome 6(6H) harbors a major locus for GPC and that there exists a low GPC allele (Karl), moderate GPC allele (Midwest six-rowed), and a high GPC allele (Chevron).

KD resistance is also important because of its link to FHB resistance. These two diseases share the causal organism *F. graminearum.* Interestingly, a QTL associated with FHB resistance has recently been mapped on chromosome 6(6H) between markers Xwg719d-Xcdo785d in a Chevron/Stander population (Ma et al., 2000). These markers are located within the marker interval MWG916-*Amy1*, which we found to be associated with KD resistance in the CM and MM population. In a related study, utilizing two populations derived

Table 5. Quantitative trait loci associated with kernel discoloration (KD) severity on chromosome 6(6H) at three environments in the MNBrite/M96 (MM) population.

QTL Position†	Marker interval‡	Crookston 1997			Crookston 1998			SPKD# SpKD 1998		
		LOD	$R^2 \times 100$§	Alpha¶	LOD	$R^2 \times 100$	Alpha	LOD	$R^2 \times 100$	Alpha
2	Bmag0807-GMS006							2.6	12.0	−0.20
14	HVM14-HVM74				5.1	21.5	−0.31			
40	Bmag0613-Bmac0218	3.0	17.0	−0.32						

† Distance in centimorgans from the top of the linkage group as displayed in Fig. 3.
‡ Markers flanking the peak of the LOD scan.
§ Percentage phenotypic variance explained by QTL.
¶ Effect of MNBrite allele on KD expressed as regression coefficient.
# SPKD = St. Paul *B. sorokiniana* KD nursery.

Table 6. Quantitative trait loci associated with grain protein content (GPC) on chromosome 6(6H) at four environments in the MNBrite/M96 (MM) population.

QTL		SPKD SpKD 1997			SPFHB sp. 1997			SPKD# SpKD 1998			SPFHB# sp. 1998		
Position†	Marker interval‡	LOD	$R^2 \times 100$§	Alpha¶	LOD	$R^2 \times 100$	Alpha	LOD	$R^2 \times 100$	Alpha	LOD	$R^2 \times 100$	Alpha
2,4,2	Bmag0807-GMS006	4.8	22.2	0.35	8.2	34.8	0.43				6.8	28.7	0.24
14	HVM14-HVM74							7.2	29.3	0.38			

† Distance in centimorgans from the top of the linkage group as displayed in Fig. 3.
‡ Markers flanking the peak of the LOD scan.
§ Percentage phenotypic variance explained by QTL.
¶ Effect of MNBrite allele on GPC expressed as regression coefficient.
# SPKD = St. Paul *B. sorokiniana* KD nursery; SPFHB = St. Paul Scab nursery.

from progeny of the Chevron/M69 population, we validated KD and FHB resistant QTLs that are coincident on chromosome 6(6H) (Canci, 2001). The detection of KD and FHB QTLs in the same chromosomal region indicates that the Chevron-derived chromosome 6(6H) region between markers MWG916-*Amy1* provides resistance to KD as well as FHB. The presence of FHB and KD resistance QTLs on chromosome 6(6H) suggests that resistance to these diseases may be controlled by alleles at the same locus.

Bright kernels and GPC between 115 g kg^{-1} and 135 g kg^{-1} are requirements of the malting and brewing industries. Therefore, the ability to manipulate the genes controlling these traits is economically important. Finding resistance to KD and moderate GPC in populations derived from Chevron may require a new approach. If the coincident QTLs for KD and GPC are tightly linked genes, then one approach would be to use large populations to identify recombinants that are both KD resistant and low or moderate in GPC. Markers mapped to this region could be used to identify individuals in which recombination events in this chromosome region have occurred. The resulting selected set of individuals could then be screened for GPC and KD to identify the desired recombinants. If KD and GPC are controlled by a single gene, then it will be necessary to find other genes that lower GPC. Markers could be used to select for the Chevron allele at the GPC/KD region while using phenotypic selection to introgress other genes for lower GPC. Another possibility would be to use the markers *Amy1* and Bmag0001 to select for the KD region on chromosome 6(6H) that was not associated with GPC. However, this region should be validated before proceeding with marker assisted selection. Finally, additional effort is necessary to clarify the location of the KD and GPC QTLs. Fine mapping of these regions is underway to refine further the locations of QTLs and potentially distinguish tightly linked genes. The identification of markers that differentiate KD resistance and GPC will be useful for malting barley breeding.

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