

With regards  
Manoj Prasad  
20.1.99

A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat

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**Abstract** This study was undertaken with a view to tag gene(s) controlling grain protein content (GPC) using molecular markers in bread wheat. For this purpose the genotype PH132 with high protein content (13.5%) was crossed with genotype WL711 with significantly lower protein content (9.7%) and 100 RILs were derived. These RILs showed normal distribution for protein content. The parental genotypes were analysed with 232 STMS primer pairs for detection of polymorphism. Of these 167 primer pairs gave scorable amplification products and 57 detected polymorphism between the parents. Using each of these 57 primer pairs, bulked segregant analysis on RILs representing the two extremes of the distribution was carried out. One primer pair for the locus *wmc41* showed association with protein content. This was further confirmed through selective genotyping. The co-segregation data on molecular marker (*wmc41*) and protein content on 100 RILs was analysed following single marker linear regression approach. Significant regression suggested linkage between *wmc41* and a QTL (designated as *QGpc1.ccsu-2D*) for protein content. The results showed that the above marker linked QTL accounted for 18.73% variation for protein content between the parents. The marker has been located on chromosome arm 2DL using nulli-tetrasomic lines and two ditelocentric stocks for chromosome 2D.

**Key words** Bread wheat · Grain protein content · Microsatellite · STMS · QTL analysis

## **Introduction**

In recent years the potential of molecular marker assisted selection in plant breeding has been demonstrated in several crops (D' Ovidio and Anderson 1994; Young et al. 1995;

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Huang et al. 1997; Ichikawa et al. 1997; Reddy et al. 1997). However, its utility in the hands of plant breeders has yet to be demonstrated in wheat, although molecular markers associated with dozens of genes controlling several traits of economic importance have been developed in this crop (Gupta et al. 1999). Majority of these traits, already tagged with molecular markers, are monogenic in their inheritance. These traits include dwarfing and vernalization response (Korzun et al. 1997), leaf rust resistance (Feuillet et al. 1995, 1997; Naik et al. 1998), kernel hardness (Sourdille et al. 1997), cadmium uptake (Penner et al. 1995), HMW glutenin (D' Ovidio and Anderson 1994), pre-harvest sprouting tolerance (Roy et al. 1998), Hessian fly resistance (Ma et al. 1993), resistance to common bunt (Demeke et al. 1996) and powdery mildew resistance (Qi et al. 1996).

The improvement in grain protein content (GPC) and its composition in bread wheat has been a major concern of plant breeders. This has been difficult for want of effective selection criteria and because selection was expensive and time consuming. In view of this, development of one or more molecular markers to be used for indirect selection for protein content/composition should be a convenient alternative. Keeping this in view, we selected two parents that significantly differed in GPC and developed a mapping population of recombinant inbred lines (RILs) to be used for identifying molecular markers that are closely associated to the quantitative trait loci (QTLs) for this trait. Through inheritance studies, we had earlier shown that the difference in GPC between the two parents was due to two major genes, even though the trait *per se* may be controlled by a number of QTLs. We screened the parents with a variety of available markers including MP-PCR, RAPDs, DAF, STS and SSRs (microsatellites). We found the SSRs to be the most promising of all the above classes of markers and therefore used them





Forward primer: 5'-TCCCTCTTCCAAGCGCGGATAG-3'

Reverse primer: 5'-GGAGGAAGATCTCCCGGAGCAG-3'

The two alleles of the molecular marker *wmc41* were designated as *hp* and *lp*, so that the genotypes of the RILs were classified as *hphp* or *lplp* on the basis of patterns observed in the parental genotypes (*hphp* = PH132; *lplp* = WL711).

#### QTL analysis

Single marker QTL analysis using linear regression was done following Tinker (1996).

The marker allele *hp* was coded 1 and the allele *lp* was coded 0 for conducting regression analysis.

#### Assignment of *wmc41* to a chromosome arm

Following the conditions described above, PCR amplification with *wmc41* primers was carried out initially with a set of all the 21 nulli-tetrasomic lines, and subsequently, with ditelocentrics 2DL and 2DS.

### Results and Discussion

#### Grain protein content in recombinant inbred lines

One hundred RILs were developed from the cross PH132 × WL711 following single seed descent (SSD) method. The RILs were raised in a replicated trial at PAU, Ludhiana and the data on GPC was scored. The grain protein content (13.5%) of the parent PH132 differed significantly from that (9.7%) of the parent WL711. The GPC in the RILs ranged from 8.5% to 13.6%. Using this data on GPC of RILs, a frequency distribution curve was

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prepared (Fig. 1) and a chi-square test was used to test the goodness of fit for normal distribution. The data suggested a very high probability for good fit ( $P>0.60$ ) to the normal distribution. Earlier, based on the study of inheritance of GPC in the above cross it was concluded that the two parents differ by two partially dominant major genes with additive effect (Dhaliwal et al. 1994). However, a normal distribution for RILs suggested that the two parents may differ at several loci controlling this trait.

#### Marker identification

A total of 232 sequence tagged microsatellite site (STMS) primer pairs were used for detection of polymorphism between the two parental genotypes. Of these, 167 primers gave scorable amplification products. Fifty seven of these primers detected polymorphism between the parental genotypes. Using these 57 primers, bulked segregant analysis (Michelmore et al. 1991) was conducted on two pooled DNA samples, each consisting of 5-8 RILs, representing the two tails of the normal distribution. With 56 of the 57 STMS primer pairs, no apparent association between the markers and the protein content was observed. The solitary remaining *wmc41* primers exhibited amplification profiles (163 bp) characteristic of high and low protein parents in the corresponding bulks following bulked segregant analysis (Fig. 2). This suggested an association of this marker with GPC. To further confirm this association, selective genotyping (Lander and Botstein 1989) of individual RILs belonging to the two bulks was carried out (Fig. 2). The results revealed that out of the eight RILs belonging to high protein pool, seven showed profile similar to the high protein parent while out of six RILs belonging to low protein pool, four RILs gave profile similar to low protein parent. This confirmed an association between *wmc41*

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marker and GPC. Subsequently, all the 100 RILs were genotyped using the above STMS primer pair, and the data on segregation of the marker was recorded for conducting QTL analysis.

#### Assignment of *wmc41* to chromosome arm 2DL

The microsatellite locus *wmc41* (163 bp) was amplified in all nulli-tetrasomic lines, except the one nullisomic for 2D. Further, the amplification product was obtained using template DNA of ditelocentric 2DL and not that of 2DS, suggesting the presence of *wmc41* on chromosome arm 2DL.

#### QTL analysis and gene effects

Since the GPC data of RILs conformed with normal distribution (Fig. 1), the data on genotypes of these RILs, at the locus *wmc41*, were considered for QTL analysis using single marker linear regression approach (Tinker 1996). The regression of protein content on the *wmc41* marker was highly significant (Table 1) indicating a linkage between the molecular marker and a QTL for protein content (designated as *QGpc1.ccsu-2D*). The  $R^2$  value of 0.1873 suggested that *wmc41* linked QTL contributed to 18.73% of the total variation in protein content of RILs (Fig. 3). These results suggested that the marker *wmc41* may either be tightly linked to a QTL with small effect or loosely linked to a QTL with large effect (Melchinger 1998). The fact that the above QTL controls approximately one fifth of the total variation and the RILs showed a good fit to normal distribution, it may be concluded that there may be other QTLs controlling the difference in protein content between the parents. In bread wheat, genes for protein content have

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been located on chromosome 5D of 'Hope' (Law et al. 1978), chromosomes 5D and 5A of 'Atlas 66' (Morris et al. 1973), on chromosomes 1A, 1B and 7A of 'Plainsman V' and on chromosome 5B of 'Wichita' (Stein et al. 1992). Recently, in tetraploid wheat, six putative QTLs for grain protein content were located on chromosome arms 4BS, 5AL, 6AS, 6BS and 7BS (Blanco et al. 1996) and a major QTL accounting for 66% variation in grain protein content was located on chromosome 6B (Joppa et al. 1997). Our results together with the above reports suggest that several QTLs control grain protein content in wheat. We propose to identify more markers, particularly STMS markers, associated with the other QTLs. Using *wmc41* primers, PCR amplification with template DNA from all the 21 nulli-tetrasomics and the pair of ditelocentrics for chromosome 2D suggested that the *wmc41* linked QTL is located on chromosome arm 2DL.

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**Table 1** Regression analysis of protein content on *wmc41* STMS marker

Source	Degrees of freedom	Mean squares	F- value	P- value
Regression	1	14.98444	22.59003	< 0.01
Residual	98	0.663321		
Total	99			



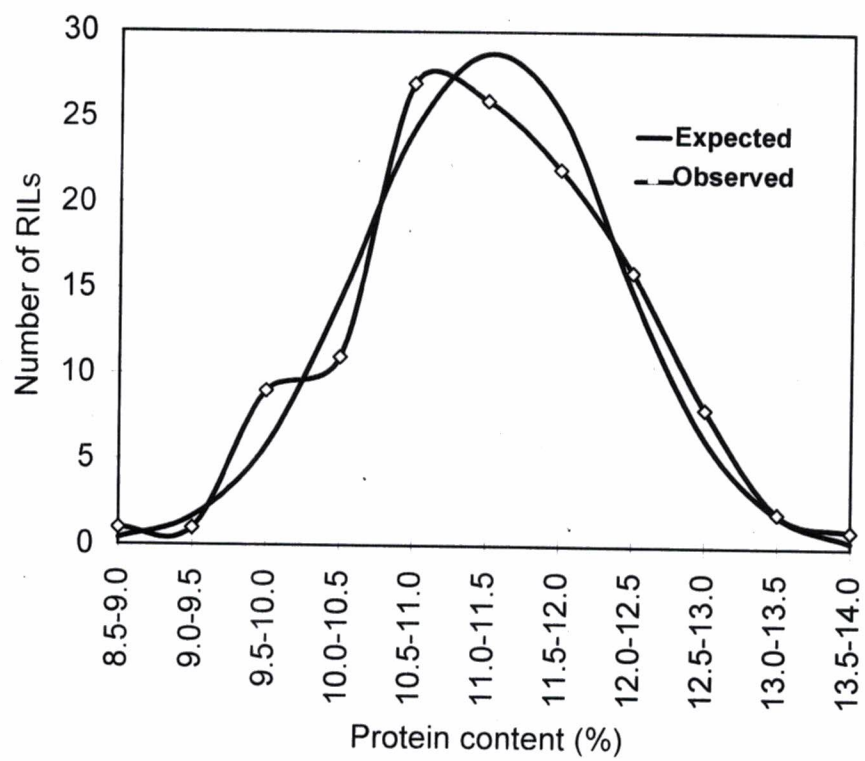
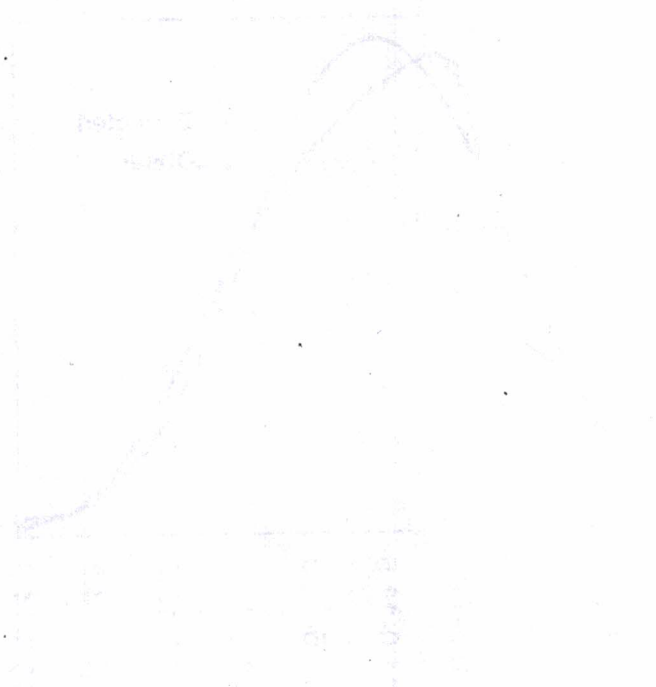


Fig. 4



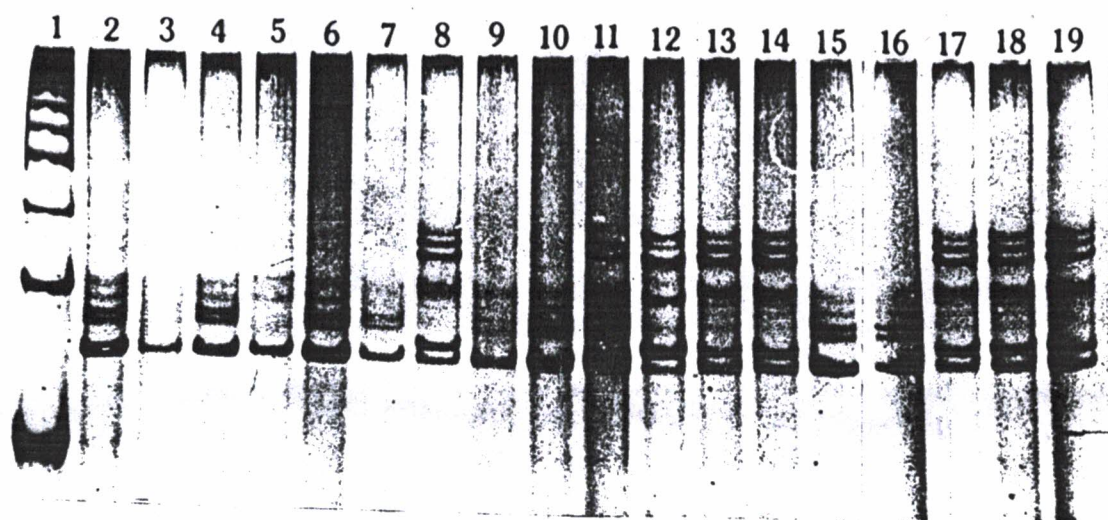


Fig. 2



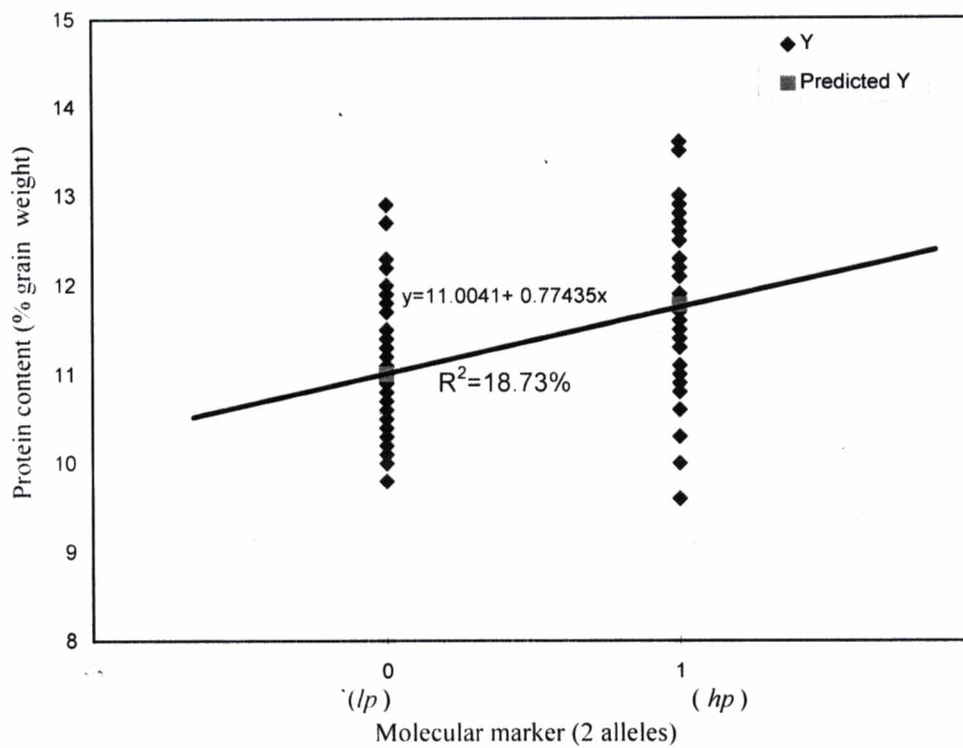


Fig. 3





## Figure Legends

**Fig. 1** Frequency distribution of grain protein content (GPC) in RILs showing a good fit to the normal distribution

**Fig. 2** Selective genotyping of RILs (representing extreme groups) with *wmc41* primers (lane 1=100bp ladder marker; 2, 12= parents, PH132 & WL711; 3, 13= bulked segregants for high and low proteins; 4 -11=RILs with high GPC; 14 -19=RILs with low GPC)

**Fig. 3** Regression slope drawn using single marker linear regression QTL analysis

