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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, we carried out bulked segregant analysis on RILs representing the two extremes of the distribution. One primer pair for the locus *wmc41* showed association with protein content. This was further confirmed through selective genotyping. The co-segregation data on the molecular marker (*wmc41*) and protein content on 100 RILs was analysed by means of a single-marker linear regression approach. Significant regression suggested linkage between *wmc41* and a QTL (designated as *QGpc.ccsu-2D.*) for protein content. The results showed that this marker-linked QTL accounted for 18.73% of the 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

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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; 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, even though molecular markers associated with dozens of genes controlling several traits of economic importance have been developed in this crop. The majority of these traits already tagged with molecular markers are monogenic in their inheritance and 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. 1999), 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. It has been difficult to achieve for want of effective selection criteria and because selection is expensive and time-consuming. In view of this, the development of one or more molecular markers to be used for indirect selection for protein content/composition should be a convenient alternative. With this in mind, 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 carried out earlier, we had 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 simple-sequence repeats (SSRs, microsatellites). We found the SSRs to be the most promising of all the above classes of markers and therefore used them extensively for the study of polymorphism between the parents and subsequently between two bulks of RILs representing high and low grain protein content. Following bulked segregant analysis, a marker was identified that showed an association with GPC. Subsequent analysis identified a QTL for GPC which was assigned to chromosome arm 2DL using all 21 nulli-tetrasomic lines and the two ditelocentrics for chromosome 2D.

Materials and methods

Plant material

Two bread wheat genotypes differing for GPC, namely PH132 (high GPC) and WL711 (low GPC), and a set of 100 RILs derived from these parents were used in the present study. The RIL population was developed following single-seed descent (SSD) method at Punjab Agricultural University (PAU), Ludhiana.

Evaluation of parents and RILs for grain protein content

The parents along with RILs were raised in two replications at PAU, Ludhiana. Protein content in seeds harvested on individual plant basis was determined on a dry weight basis by estimating nitrogen content in each grain sample using a Technicon autoanalyser (Warner and Jones 1970) and the following formula:

Protein content (%) = Nitrogen content (%) × 5.7.

DNA isolation and sequence-tagged microsatellite site (STMS) primers

DNA was extracted from 10- to 15-days-old seedlings raised in a growth chamber using a modified CTAB method (Weising et al. 1995). A set of 232 STMS primers were made available to us as a member of the Wheat Microsatellite Consortium (WMC) under an international collaborative project. These STMS primers were designed using sequence data of clones containing microsatellites. The genomic clones were isolated from a microsatellite-rich library (Edwards et al. 1996) and were sequenced by members of the WMC.

Polymerase chain reaction (PCR)

DNA amplifications were carried out in 25-µl reaction mixtures, each containing 100 ng template DNA, 2 µ*M* STMS primer, 200 µ*M* each of the dNTPs, 2.5 m*M* $MgCl_2$, 1 × PCR buffer and 2 U Stoffel fragment (Perkin Elmer) using the following PCR profile in a Perkin Elmer DNA Thermal Cycler: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 1 min, 51°C/61°C for 1 min, 72°C for 1 min with a ramp at the rate of 0.5°C and a final extension at 72°C for 5 min. The amplification products were resolved on 10% polyacrylamide denaturing gels following silver staining (Tegelstrom 1992).

Evaluation of fragment patterns

Amplification products were obtained using *wmc41* primers, developed by J. Dubcovsky, University of California, Davis Campus, USA (Dubcovsky, unpublished results; personal communication), having the following sequences:

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

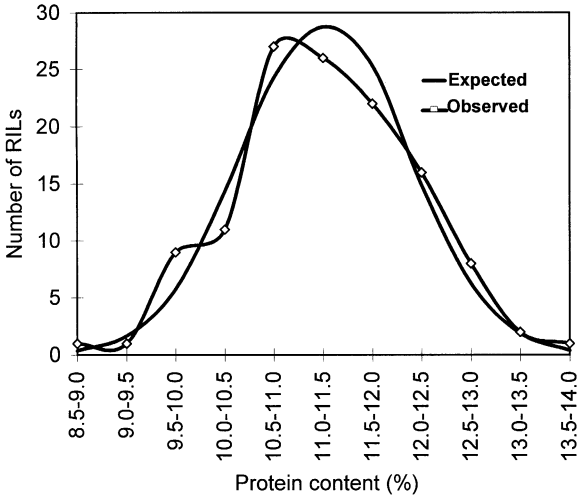

Fig. 1 Frequency distribution of grain protein content in RILs showing a good fit to the normal distribution

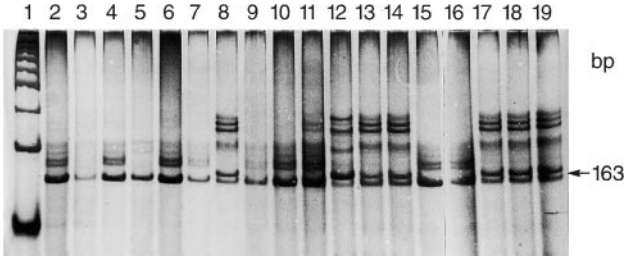

Fig. 2 Selective genotyping of RILs (representing extreme groups) with *wmc41* primers. *Lane 1* 100-bp ladder marker; *2, 12* parents, PH132 and WL711; *3, 13* bulked segregants for high and low grain protein content; *4-11* RILs with high GPC; *14-19* RILs with low GPC

Marker identification

From a total of 232 STMS primer pairs used for the detection of polymorphism between the two parental genotypes 167 gave scorable amplification products. Of these primers, 57 detected polymorphism between the parental genotypes. Using these 57 primers, we conducted bulked segregant analysis (Michelmore et al. 1991) on two pooled DNA samples, each consisting of 5–8 RILs and 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* primer 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, we carried out selective genotyping (Lander and Botstein 1989) of individual RILs belonging to the two bulks (Fig. 2). The results revealed that out of the 8 RILs belonging to the high protein pool, 7 showed a profile similar to that of the high protein parent while out of 6 RILs belonging to the low protein pool, 4 RILs gave a profile similar to that of the low protein parent. This confirmed an association between the *wmc41* marker and GPC. Subsequently, all 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 of the nulli-tetrasomic lines, except the 1 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 a normal distribution (Fig. 1), the data on the genotypes of these RILs, at the locus *wmc41*, were considered for QTL analysis using the 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 *QGpc.ccsu-2D.1*). The R^2 value of 0.1873 suggested that the *wmc41*-linked QTL contributed to 18.73% of the total variation in protein content among the RILs (Fig. 3). These results suggested that the marker *wmc41* may either be tightly linked to a QTL with a small effect or loosely linked to a QTL with a large effect (Melchinger 1998). The fact that the above QTL controlled approximately one-fifth of the total variation and that the RILs showed a good fit to a normal distribution indicates that there may be other QTLs controlling the difference in protein content between the parents. In bread wheat, genes for protein content have 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. When the *wmc41* primers, were used for PCR amplification with template DNA from all the 21

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			

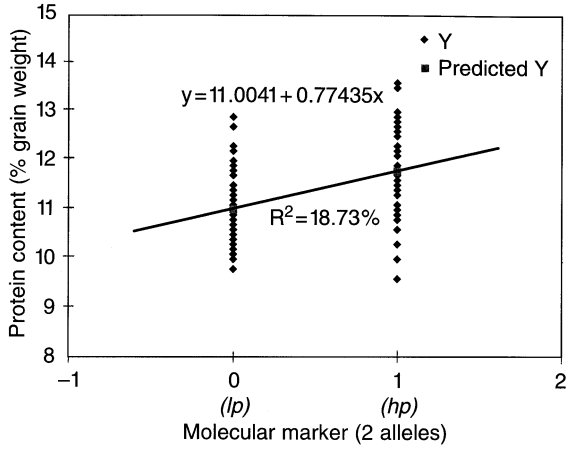

Fig. 3 Regression slope drawn using single-marker linear regression QTL analysis

nulli-tetrasomics and the pair of ditelocentrics for chromosome 2D, it suggested that the *wmc41*-linked QTL is located on chromosome arm 2DL.

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