With regards
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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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Yours sincerely,

Prof. Dr. G. [illegible]

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 · Mìcrosatellite· 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;

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

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*

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

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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References

Blanco A, De Giovanni C, Laddomoda B, Sciancalepore A, Simeone R, Devos KM, Gale MD (1996) Quantitative trait loci influencing grain protein content in tetraploid wheats. Plant Breeding 115: 310-316

Demeke T, Laroche A, Gaudet A (1996) A DNA marker for the *Bt-10* common bunt resistance gene in wheat. Genome 39: 51-55

Dhaliwal LS, Singh H, Nanda GS, Dhaliwal HS (1994) Inheritance of grain protein content in two high-protein lines of wheat. Rachis 13: 34-37

D' Ovidio R, Anderson OD (1994) PCR analysis to distinguish between alleles of a member of a multigene family correlated with wheat bread-making quality. Theor Appl Genet 88: 759-763

Edwards KJ, Barker JHA, Daly A, Jones C, Karp A (1996) Microsatellite libraries enriched for several microsatellite sequences in plants. BioTechniques 20: 758-760

Feuillet C, Messmer M, Schachermayr G, Keller B (1995) Genetic and physical characterization of the *Lr1* leaf rust locus in wheat (*Triticum aestivum* L.). Mol Gen Genet 248: 553-562

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. The Plant J 11: 45-52

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their application in wheat breeding. Plant Breeding (submitted)

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush GS (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using. RFLP and PCR. Theor Appl Genet 95: 313-320

Ichikawa N, Kishimoto N, Inagaki A, Nakamura A, Koshino Y, Yokozeki Y, Oka M, Samoto S, Akagi H, Higo K, Shunjyo C, Fujimura T, Shimada, H (1997) A rapid PCR-aided selection of rice line containing the *Rf-1* gene, which is involved in restoration of the cytoplasmic male sterility. Molecular Breeding 3: 195-202

Joppa LR, Changheng Du, Hart Gary E, Hareland Gary A (1997) Mapping gene(s) for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. Crop Sci 37: 1586-1589

Korzun V, Roder M, Worland AJ, Borner A (1997) Intrachromosomal mapping of genes for dwarfing (*Rht12*) and vernalization response (*Vrn1*) in wheat by using RFLP and microsatellite markers. Plant Breeding 116: 227-232

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185-199

Law CN, Young CF, Brown JWS, Snape JW, Worland AJ (1978) The study of grain protein control in wheat using whole chromosome substitution lines. In: Seed Protein Improvement by Nuclear Technique. IAEA, Vienna, pp. 483-502

Ma ZQ, Gill BS, Sorrells ME, Tanksley SD (1993) RFLP markers linked to two Hessian fly resistance genes in wheat (*Triticum aestivum* L.) from *Triticum tauschii* (wss) Schmalh. Theor Appl Genet 85: 750-754

Melchinger AE (1998) Advances in the analysis of data on quantitative trait loci. In: Chopra VL, Singh RB, Varma A (eds) Crop Productivity and Sustainability Shaping the Future. Oxford & IBH publishers, New Delhi, pp. 773-791

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci (USA) 88: 9828-9832

Morris R, Schmidt JW, Mattern PJ, Johnson VA (1973) Chromosomal locations of genes for high protein in the wheat cultivar Atlas 66. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Univ of Missouri, Columbia, pp 715-718

Naik S, Gill KS, Prakasa Rao VS, Gupta VS, Tamhankar SA, Pujar S, Gill BS, Ranjekar PK (1998) Identification of a STS marker linked to the *Aegilops speltoides*- derived leaf rust resistance gene *Lr28* in wheat. Theor Appl Genet 97: 535-540

Penner GA, Clarke J, Bezte LJ, Leisle D (1995) Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. Genome 38: 543-547

Qi L, Cao M, Chen P, Li W, Liu D (1996) Identification, mapping and application of polymorphic DNA associated with resistance gene *Pm21* of wheat. Genome 39: 191-197

Reddy JN, Baraoidan MR, Bernardo MA, George MLC, Sridhar, R (1997) Application of marker assisted selection in rice for bacterial blight resistance. Curr Sci 73: 873-875

Roy JK, Prasad M, Varshney RK, Balyan HS, Blake TK, Dhaliwal HS, Singh H, Edwards KJ, Gupta PK (1998) Identification of a microsatellite on chromosome 6B and a STS on 7D of bread wheat showing association with preharvest sprouting tolerance. Theor Appl Genet (accepted)

Sourdille P, Perretant MR, Charmet G, Leroy P, Gautier MF, Joudrier P, Nelson JC, Sorrells ME (1997) Linkage between RFLP markers and genes affecting kernel hardness in wheat (*Triticum aestivum* L em Thell). Theor Appl Genet 93: 580-586

Stein Ira S, Sears RG, Gill BS, Hoseney RC, Cox TS (1992) Heterogeneity of the 'Wichita' wheat monosomic set for grain quality and agronomic traits. Crop Sci 32: 581-584

Tegelstrom H (1992) Detection of mitochondrial DNA fragments. In: Hoelzel AR (eds) Molecular Genetic Analysis of Populations: A Practical Approach. IRL Press, Oxford, pp 89-114

Tinker NA (1996) New strategies for QTL mapping. In: Barley workshop (#15) at the Plant Genome IV conference held in San Diego on January 17, 1996

Warner MH, Jones Jr JB (1970) A rapid method for nitrogen determination in plant tissue. Communications in Soil Science and Plant Analysis 1: 106-114

Weising K, Nybom, H, Wolff K, Meyer W (1995) DNA Fingerprinting in Plants and Fungi. CRC press, Inc, Florida, 322 pp

Young ND, Denny RL, Concibido VC, Lange DA, Orf JH (1995) Marker assisted breeding in practice: RFLPs and soybean cyst nematode resistance. In: Induced Mutations and Molecular Techniques for Crop Improvement. IAEA, Vienna, pp. 245-251

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			

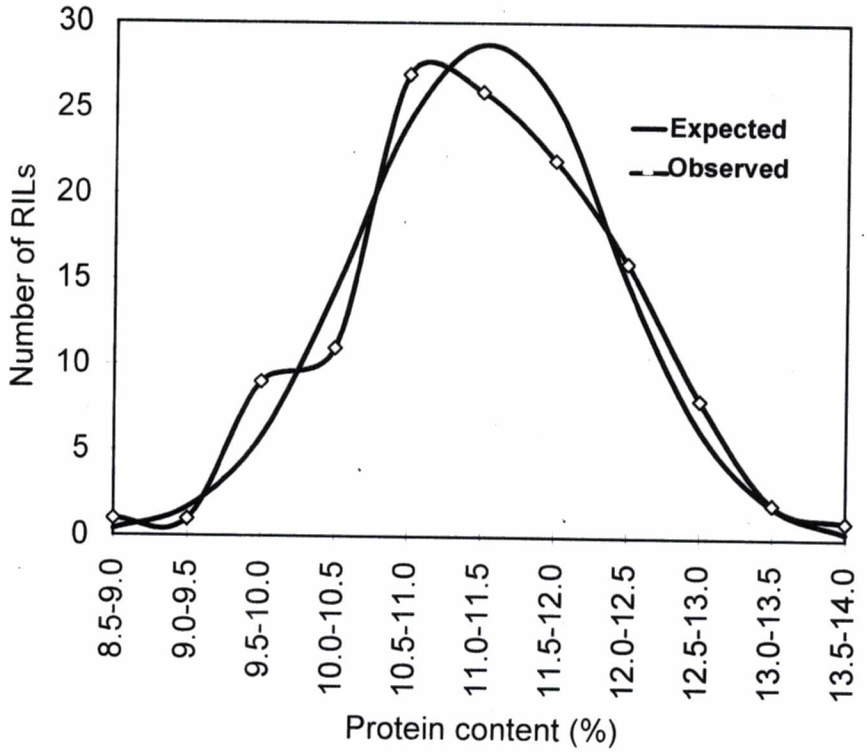
30
25
20
15
10
5
0
Number of RILs
Expected
Observed
8.5-9.0
9.0-9.5
9.5-10.0
10.0-10.5
10.5-11.0
11.0-11.5
11.5-12.0
12.0-12.5
12.5-13.0
13.0-13.5
13.5-14.0
Protein content (%)

Fig. 1

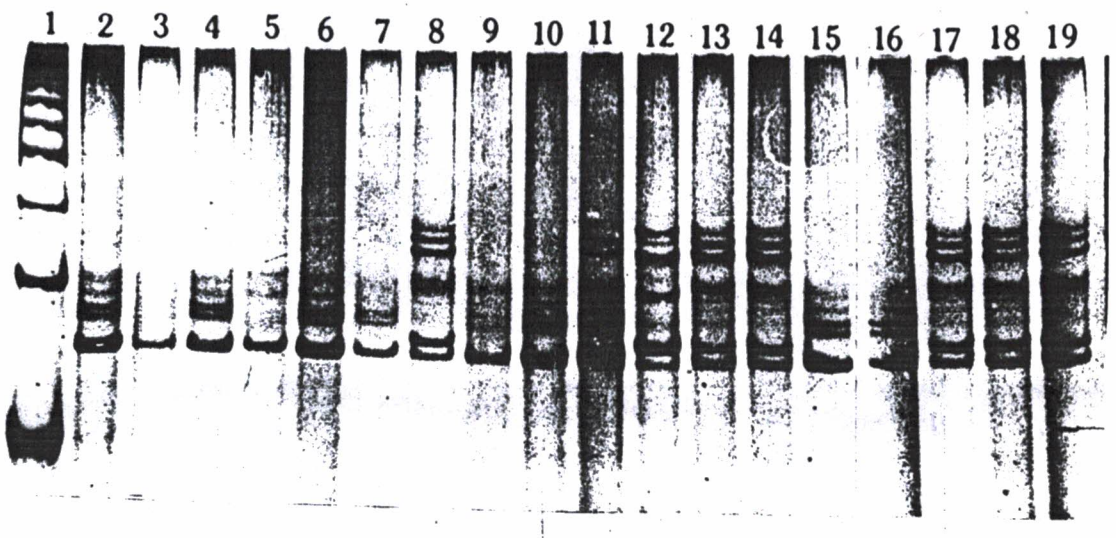

Fig. 2

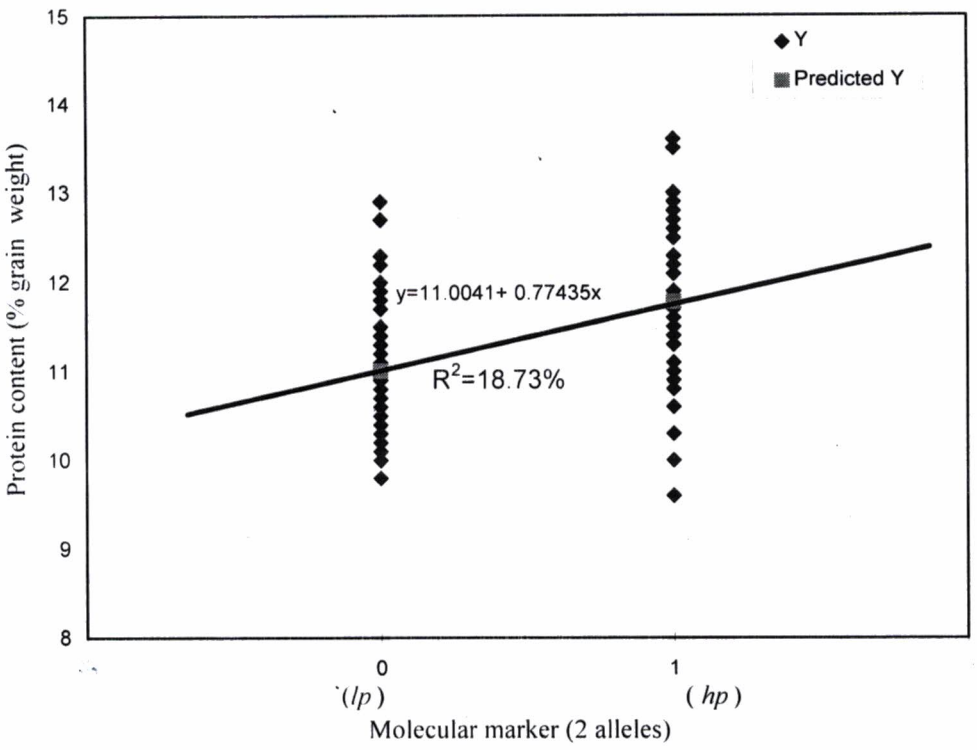
15
14
13
12
11
10
9
8
Protein content (% grain weight)
Y
Predicted Y
y=11.0041+ 0.77435x
R2=18.73%
0
1
(lp)
(hp)
Molecular marker (2 alleles)

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