J. Wheat Res. 3(1) : 14-23 (2011)

Mapping QTL associated with agronomic traits in bread wheat *(Triticum aestivum L.)*

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Abstract

The major focus of global wheat research efforts is to identify loci governing important agronomic and quality related traits and to understand their complex interactions responsible for the end phenotype. The objective of this study was to identify Quantitative trait loci (QTL) for agronomic traits such as Sedimentation volume (Sv), Grain protein content (Gpc), Thousand grain weight (Tgw) and Test weight (Tw) which play an important role in determining the end use and marketability of wheat. To accomplish this task, an RIL population from a cross between two Indian wheat varieties "HI977" and "HD2329"was used. The phenotypic data were collected from six environments including three different agro climatic zones for two consecutive years. Composite interval mapping revealed 68 QTL controlling Gpc, Sv, Tgw and Tw with individual phenotypic variation ranging from 7.0- 32.3% and total of 9 QTL clusters were detected on 7 chromosomes. AMMI model revealed significant contribution of G x E variance to all the traits. Furthermore, data analysis identified co-localization of few QTL affecting more than one trait.

Keywords: Protein content, Sedimentation volume, Grain weight, QTL, Wheat

Received: 18 July 2011/Accepted: 8 August 2011

Introduction

The quality traits such as Loaf volume (Lv), Sedimentation volume (Sv), Grain protein content (Gpc), Thousand grain weight (Tgw) and Test weight (Tw) play an important role in determining the end use and marketability of wheat which is a commonly grown cereal worldwide. Sv and Gpc have direct correlation with bread making quality (BMQ), while Tgw and Tw are yield components affecting its economic value. Reliable assessment of BMQ parameters with molecular markers has received considerable attention in recent years. As QTL governing these traits are influenced by the environment, identification of markers linked to these traits help in genetic dissection, as well as in Marker assisted selection (MAS) of these traits.

Sv, which is an indirect parameter of BMQ, is quantitatively inherited and influenced by environmental factors (Silvela *et al.* 1993). The Sodium dodecyl sulphate (SDS) sedimentation test is a simple, small-scale method that gives a quick estimate of wheat gluten strength. Bread making process requires Gpc to be above 12.5% and it is often environmentally influenced (Turner *et al.,* 2004). *Gpc-B1* gene mapped on chromosome arm 6BS (Joppa *et al.,* 1997) has shown promising increase in protein content in both tetraploid and hexaploid wheat (Mesfin *et al.,* 1999; Chee *et al.,* 2001). Though *Gpc-B1* accounts for 66% of protein content variation (Joppa *et al*., 1997), there are many loci on different chromosomes controlling Gpc which needs to be analysed. Tgw is positively correlated with flour yield and it is the most stable component of yield trait (Varshney *et al.,* 2000). It is influenced by many QTL located on different chromosomes (Giura and Saulescu, 1996; Varshney *et al.,* 2000). Tw measures relative plumpness of the grain and it is one of the important traits deciding the marketability of grain. It is a function of both kernel density and random kernel packing volume, often considered as an initial indicator of grain quality.

A breeder is, no doubt interested in enhancing the genotypic mean for any trait, but is also interested in its stability, which depends on Genotype x Environment interactions. These are considered to be important for quality traits in wheat; therefore, an assessment of stability of the trait of interest would permit better genotype characterization. Our group is engaged in mapping wheat quality traits such as thousand kernel weight, seed size and shape and loaf volume using molecular markers (Dholakia *et al.,* 2003; Elangovan *et al.,* 2008). In the present study, QTL for Sv, Gpc, Tgw, and Tw were identified through a framework map developed for a Recombinant inbred line (RIL) mapping population derived from a cross between HI977 (good BMQ) and HD2329 (poor BMQ) and attempted to understand the Genotype x Environment interactions (GEI) for these traits.

Materials and Methods

Plant material, field trial and phenotypic evaluation

A set of 105 RILs derived from a cross between the parents (HI977 x HD2329) was used in the present study. Details of field trial were as reported by Elangovan *et al*., (2008). Phenotypic data were collected in two successive years from all the RILs from 3 different agroclimatic zones (Karnal-North Western plain zone, Kota-Central zone and Pune-Peninsular zone). The data on Sv, Gpc, Tw and Tgw were recorded on the bulk yield of the lines. Gpc was determined using NIR with an Infrared analyzer 300 (Technicon, NY, USA) previously calibrated with Kjeldahl protein (N x 5.7) as described by "American Association of Cereal Chemists" (AACC 2000). The moisture content required for grain conditioning was determined simultaneously and protein content was calculated for standard 14% moisture content.

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Tw was measured by the hectoliter weight-measuring funnel of the SINARFP Auto 6080, while Tgw was determined by obtaining the weight of 1,000 grains in grams using an electronic counter (Misra and Gupta, 1995). Sv was measured using SDS sedimentation test to identify the gluten strength following the procedure of Dick and Donnelly (1980).

Data analyses

AMMI (Additive Main-effects and Multiplicative Interaction Model) analysis was performed with IRRISAT ver. 5.0 (IRRI, Philippines). Using crosstie module the analysis was restricted to a reduced AMMI model with four interaction principal component axes (IPCA) retained in the AMMI analysis of variance. Only four IPCAs were analysed because the limited information from the trials may restrict the interpretation of more than 4 axes. The IPCA values were near zero for stable genotypes which showed low environmental interaction and biplots were constructed by plotting the IPCA1 values with the mean value of the trait. ANOVA revealed significant difference among the genotypes of the population in each location for all the traits. HMW glutenin subunit analysis, the framework map and linkage groups construction were as reported by Elangovan *et al.,* (2008). The QTL analysis was performed according to the procedure described in Elangovan *et al.* (2008) and LOD value of 3.0 was considered as threshold to declare a QTL. Multitrait Composite Interval Mapping (MCIM) was conducted using the JZmap QTL module which is available in Windows QTL Cartographer v. 2.5.

Results

Distribution of the traits in segregating RIL population

The parents, HI977 and HD2329 showed a statistically ignificant difference for all the traits across different environments (Table-1). Though both the parents have significant difference for Sv (51.82 for HI and 39.19 for HD) the difference was less for other traits like Gpc, Tw and Tgw. The presence of transgressive segregants for these traits denoted the contribution of alleles from both the parents. The parents HI977 (51.82 to 54.19) and HD2329 (38.76 to 39.63), showed a significant difference between them for Sv in each environment compared to other traits. Although the difference in Gpc between the two parents, HI977 (11.68 to 13.60) and HD2329 (11.52 to13.74) was rather small, significant difference was observed in the RIL population (9.35 to 14.91). Similarly, for Tgw and Tw the difference between the parents was less while range in population was found to be more (Table 1.)

Table 1 Agronomic traits distribution in the cross HI977 X HD2329

Trait	Description	HI977	HD2329	S. E.	Average	Range Min	Max
KarSv	Karnal Sedimentation volume cc.)	52.51	39.63	2.33	46.23	35.93	61.13
KotSv	Kota Sedimentation volume (cc.)	54.19	38.76	1.86	46.20	32.03	62.23
PunSv	Pune Sedimentation volume (cc.)	51.82	39.19	1.11	46.26	34.96	61.48
KarTw	Karnal Test weight (kg)	73.13	72.73	1.33	72.50	60.31	81.83
KotTw	Kota Test weight (kg)	79.29	79.79	0.54	79.77	75.52	82.78
PunTw	Pune Test weight (kg)	82.51	83.36	0.43	83.01	80.13	85.20
KarTgw	Karnal Thousand grain weight (g)	31.62	32.24	0.92	31.52	20.87	41.32
KotTgw	Kota Thousand grain weight (g)	39.86	40.08	0.76	39.57	31.53	46.92
PunTgw	Pune Thousand grain weight (g)	41.38	43.12	1.39	40.96	32.45	47.85
KarGpc	Karnal Grain protein content (%)	12.95	13.02	0.27	12.87	11.05	14.78
KotGpc	Kota Grain protein content (%)	11.68	11.52	0.36	11.55	9.35	14.02
PunGpc	Pune Grain protein content (%)	13.60	13.74	0.22	13.64	11.94	14.91

SE represents the standard error for the parents; average for each trait was calculated from overall population, and range represents minimum and maximum of the RILs

Correlation among the traits

Rank correlation among the traits recorded at different locations and Pearson's correlation between traits within same location were performed. Correlations were positive and significant between KarTgw and KotTgw (r=0.3**) for both the years. Correlation between PunTgw1 and KotTgw1 was significant (r=0.27**), while the correlation was negative and insignificant between PunTgw2 and KotTgw2. Similar observation was made for the trait Sv. The correlation for Gpc and Tw was insignificant between all the locations recorded in 2003-04, while it was positive and significant between KarGpc2 and KotGpc2 (r=0.26**) (in the year 2004-05) and also for PunGpc1 with KotGpc2 (r=0.36***). In case of the trait Tw, Karnal with Kota and Kota with Pune recorded positive and significant correlations. Sv recorded at Karnal and Kota location, showed significant positive correlation among them and also displayed a positive correlation with PunTgw2. The study on correlation among the traits revealed that Tw and Gpc had positive and significant correlation in Karnal location, while it was insignificant in the other two locations. Positive and significant correlation was identified between Gpc and Sv, only in Kota location. Some of these correlations were significant warranting multitrait QTL analyses for these correlated traits.

Table 2a QTL associated with agronomic traits

Composite interval mapping for Sv								
			Marker					
QTL	Chromosome	Trait	Left	Right	LOD	Position	Additive[a]	R^2 x 100
QSv.ncl-1B.1	1B	KarSv2	Xgwm131	XgluB1	3.59	180.21	-5.30	15.88
QSv.ncl-1B.2	1B	PunSv2	Xpsp3000	Xgwm1028	6.16	225.71	-1.21	14.88
QSv.ncl-1D.1	1D	KotSv1	Xgwm337	xgwm848	4.37	72.61	2.04	11.55
QSv.ncl-2A.1	2A	KarSv2	Xgwm830	Xgwm249A	3.35	24.01	-5.12	15.40
QSv.ncl-2B.1	2B	PunSv2	Xgwm120	Xgwm877	6.30	185.81	-1.12	16.24
QSv.ncl-3D.1	3D	KarSv1	Xgwm52	Xgwm664	4.15	7.71	2.35	14.14
QSv.ncl-5A.1	5A	PunSv2	Xbarc1	Xcfd20	4.12	86.91	-0.99	11.09
QSv.ncl-5D.1	5D	KarSv2	Xgwm736A	Xgwm1016	3.17	275.41	-5.01	14.50
QSv.ncl-6A.2	6A	KarSv2	Xgwm907	Xgwm1017	3.03	107.11	-4.90	15.86
QSv.ncl-6D.1	6D	KarSv2	Xcfd13	Xgwm1009C	3.90	12.01	-5.09	15.71
Composite interval mapping for Gpc								
			Marker					
QTL	Chromosome	Trait	Left	Right	LOD	Position	Additive[a]	R^2 x 100
QGpc.ncl-1B.1	1B	KarGpc1	Xpsp3000	Xgwm1028	3.12	63.11	0.45	20.73
QGpc.ncl-1B.2	1B	PunGpc1	Xbarc137	Xbarc187	3.64	88.41	0.23	11.04
QGpc.ncl-1B.3	1B	KarGpc1	Xcfd48A	XgluB1	5.07	141.21	0.24	12.49
QGpc.ncl-2B.1	2B	KotGpc2	Xgwm1128A	Xgwm429	6.68	48.61	-0.46	18.04
QGpc.ncl-3A.1	3A	KarGpc1	Xgwm369B	Xgwm369A	3.09	2.01	-0.39	25.44
QGpc.ncl-6A.1	6A	KotGpc2	Xgwm1296	Xgwm1150	8.08	84.51	0.47	18.00
QGpc.ncl-7D.1	7D	PunGpc2	Xgwm735	Xbarc184	3.70	26.01	-0.22	11.14

QTL for agronomic traits

QTL were identified for agronomic performance in terms of yield and quality traits, using CIM analysis. In all, 68 QTL with LOD score ranging from 3.0- 9.05, were identified on 19 chromosomes for these traits, among which 11 to 26 ranged for each trait. The individual QTL effects (phenotypic contribution) ranged from 7.1-32.3 %. The B genome had the maximum number of QTL mapped (32) followed by D (19) and A (17) genome. The QTL distribution in homeologous

chromosomes for group 1 through group 7 was 21 (31 %), 11 (16 %), 6 (9 %), 5 (7 %), 11 (16 %), 11 (16 %) and 3 (4 %), respectively. The highest numbers of QTL were identified for the trait Tw (26), followed by Tgw (17), Sv (14) and Gpc (11). The parent HD2329 contributed for 42 QTL, while parent HI977 contributed for remaining QTL. The position of representative chromosomes with QTL co-located for respective traits above the threshold LOD 3.0 are as depicted in Fig. 1 and QTL with >10.0% phenotypic variation and LOD > 3.0 are listed in Table 2.

Table 2b QTL associated with agronomic traits

Composite interval mapping for Tgw

QTL	Chromosome	Trait	Marker		LOD	Position	Additive[a]	R^2 x 100
			Left	Right				
QTgw.ncl-1B.1	1B	KotTgw1	Xgwm1078	Xgwm1130	5.23	44.71	-1.25	12.34
QTgw.ncl-1B.2	1B	KotTgw1	Xpsp3000	Xgwm1028	3.58	57.11	-1.87	27.12
QTgw.ncl-1B.3	1B	PunTgw2	Xwmc419	Xgwm806	7.07	225.71	1.40	15.17
QTgw.ncl-1D.2	1D	PunTgw2	Xgwm1012	Xgwm957	3.76	192.61	1.31	11.40
QTgw.ncl-2B.2	2B	KarTgw2	Xgwm739	Xgwm1273	9.05	259.51	-2.42	26.52
QTgw.ncl-4B.1	4B	KotTgw2	Xgwm898	Xgwm113	5.75	2.01	1.16	14.64
QTgw.ncl-5A.2	5A	PunTgw2	Xbarc243	Xgwm4226	6.01	222.41	1.43	14.47
QTgw.ncl-5B.1	5B	PunTgw1	Xgwm443C	Xgwm234B	3.59	25.41	1.72	27.69
QTgw.ncl-5B.2	5B	KotTgw2	Xgwm843	Xgwm499A	3.67	79.01	-1.36	13.19
QTgw.ncl-6A.1	6A	KarTgw1	Xgwm1017	Xgwm427	3.71	126.31	-1.20	12.23
QTgw.ncl-6B.1	6B	PunTgw2	Xgwm1199B	Xbarc198	4.76	155.51	1.23	10.13

QTL for quality

A total of 14 QTL were identified for Sv on 11 chromosomes, with majority mapped on group 1, group 5 and group 6 chromosomes. Among these 14 QTL, 9 had negative additive effect, suggesting the contribution of alleles from inferior parent HD2329, especially for group 6 QTLs. Group 1 chromosome QTLs with positive and negative additive effect contributed 11.55-15.88% towards phenotypic variation, spanning through all the three locations. Similarly, total 11 QTL were detected on 7 chromosomes contributing 7.8- 25.44% for Gpc. Out of these, 7 were contributed by HI977 alleles and 4 by HD2329 alleles. The chromosome 1B had maximum QTL (4) followed by 6A (2). The highest contribution (25.44 %) was from *QGpc.ncl-3A.1* with allele from HD2329, followed by *QGpc.ncl-1B.1* (20.73 %) with allele from HI977.

QTL for yield

For Tgw, 17 QTL were identified on 11 chromosomes with majority mapped on group 1 (6) followed by group 5 (5). The chromosomes 2B, 5A and 5B each had 2 QTL controlling Tgw. *QTgw.ncl-5B.1* explained 27.69% of variation with HI977 allele, followed by *QTgw.ncl-1B.2* (27.12%) and *QTgw.ncl-2B.2* (26.52%) contributed by poor parent, suggesting the importance of alleles from HD2329. Two QTL (*QTgw.ncl-1B.3* and *QTgw.ncl-4B.1*) with positive additive effect contributed 15.1 % and 14.6 %, respectively, towards phenotypic variation of Tgw. In case of Tw, together 26 QTL were identified from 16 chromosomes. Three common QTL (*QTw.ncl-5D.2, QTw.ncl-6B.2,* and *QTw.ncl-6D.1*) were detected in two locations, Pune and Karnal. The contribution of the phenotypic variation ranged from 7.1- 32.3 %. HD2329 contributed for Tw through 16 QTL and HI977 for the remaining 10 QTL. Majority of QTL were on group 1 chromosomes (8) followed by group 2 (5) and group 6 (4).

QTL clusters for yield and quality traits

A total of 68 QTL were detected for Gpc, Sv, Tgw and Tw in the present analysis. A total of 9 QTL clusters were detected on 7 chromosomes of which two clusters were identified on chromosomes 1B and 5D, while single clusters were on 2A, 3D, 4B, 6D and 7D chromosomes. Also, Tw QTLs cluster more often co-located with Sv than with others, while the QTL cluster of Tgw and Sv, was identified only on chromosome 1B.

Table 2c QTL associated with agronomic traits

Composite interval mapping for Gpc								
			Marker					
QTL	Chromosome	Trait	Left	Right	LOD	Position	Additive	R2 x 100
QTw.ncl-1A.1	1A	KotTw2	Xgwm99	Xgwm3036B	3.95	248.11	-0.51	10.00
QTw.ncl-1B.1	1B	PunTw2	Xgwm131	Xglu1B	3.59	180.21	-5.30	15.88
QTw.ncl-1B.2	1B	PunTw1	Xgwm153A	Xpsp3100	5.19	251.51	-0.44	9.51
	1B	KotTw1	Xgwm153A	Xpsp3100	4.39	251.51	-0.50	11.19
QTw.ncl-1B.4	1B	KarTw2	Xwmc406	Xbarc181	3.52	82.91	1.46	12.23
QTw.ncl-1D.2	1D	PunTw1	Xgwm4810	Xglu1D	5.50	114.21	-0.37	11.12
QTw.ncl-2A.1	2A	PunTw2	Xgwm830	Xgwm249A	3.35	24.01	-5.12	15.40
QTw.ncl-2B.1	2B	KarTw2	Xbarc24	Xgwm429	3.87	29.01	-4.07	32.30
QTw.ncl-2B.2	2B	KotTw2	Xgwm1128A	Xgwm148	3.25	60.61	-0.55	12.48
QTw.ncl-2D.1	2D	KotTw1	Xgwm988	Xgwm484	3.95	73.61	0.46	10.02
QTw.ncl-3A.1	3A	KotTw1	Xgwm1038	Xgwm1071A	4.91	126.11	-0.96	15.52
QTw.ncl-4D.1	4D	KotTw2	Xgwm194	Xgwm609	4.80	40.71	-0.54	13.27
QTw.ncl-5B.1	5B	KotTw1	Xgwm540	Xbarc88	5.90	49.61	0.75	18.68
QTw.ncl-5D.1	5D	KarTw1	Xgwm4265B	Xgwm1462	4.33	86.71	-1.59	15.47
QTw.ncl-5D.2	5D	PunTw2	Xgwm736A	Xgwm1016	3.17	275.41	-5.01	14.50
	5D	KarTw2	Xgwm736A	Xgwm1016	4.27	277.41	-3.65	27.38
QTw.ncl-6B.1	6B	KarTw2	Xbarc178	Xgwm921	3.29	40.01	-4.09	21.36
QTw.ncl-6B.2	6B	KarTw2	Xbarc14	Xgwm1199C	3.70	107.11	-3.92	23.13
	6B	PunTw2	Xbarc14	Xgwm1199C	3.03	107.11	-4.90	15.86
QTw.ncl-6D.1	6D	KarTw2	Xcfd13	Xgwm1009C	3.13	10.01	-3.51	25.54
	6D	PunTw2	Xcfd13	Xgwm1009C	3.90	12.01	-5.09	15.71
QTw.ncl-6D.2	6D	KarTw2	Xgwm459	Xgwm1103B	3.03	70.71	-4.07	21.21

Left and Right represent flanking markers to the corresponding QTL
Kar- Karnal, Kot- Kota, Pun- Pune; 1- Season 2003-04, 2- Season 2004-05

AMMI and G x E analysis

The AMMI analysis of variance of Tw tested in six environments showed that 79.70% of the total sum of squares (TSS) was attributable to environmental effect, only 4.5% to genotypic effect and 15.8% to GEI effect (Table 3), while for Sv the environmental effect was 23.23% of the TSS, 28.73% to genotypic effects and 48.03% to GEI effect. Similarly, the Gpc showed that 62.38% of the TSS was due to the environmental effects, only 9.11% due to the genotypic effect and 28.51% to the GEI effect. The highest contribution of TSS was realized for Tgw through environment (68.25%), the genotypic effects and GEI were 7.91and 23.83 %, respectively.

Multitrait composite interval mapping

Single locus multitrait composite interval mapping (MCIM) was performed to identify stable QTL across environments and also to detect QTL affecting more than one trait. The MCIM analysis was performed in two different ways. In the first case, each trait was analyzed separately with all the six environments together to check stability of QTL across all the environments. This analysis did not reveal any such QTL in all the environments. However, 3 QTL were detected on chromosomes 1B and 6B affecting Sv based on data from 3 locations (Karnal, Kota and Pune) in season 2004-05 (Table 4a). This revealed presence of stable loci governing Sv in 3 different agroclimatic zones. No such QTL was detected in more than 2 environments for the remaining traits (Gpc, Tgw and Tw), which might be due to strong environment influence on these traits. In order to identify QTL affecting more than one trait, data was analyzed from each environment separately, with all the 4 traits together. Total 6 such QTL were detected on chromosomes 5A, 5B, 6A, 6B and 7D (Table-4b). The region between Xwms1296-Xwms1150 on chromosome 6A showed effect on Gpc, Sv

and Tw at Karnal location in the second season. Similarly, 6B had one QTL which showed effect on Tw and Sv, while Gpc and Sv were affected by 7D QTL. Additionally, 2 QTL were detected on 5A and 5B chromosomes, which revealed effect on Tgw and Sv at Pune location. Further, chromosome 6B had 2 different QTL affecting Tw and Sv based on different environment. No such QTL was detected at Kota location for both the seasons.

Discussion

Growing genotypes under well adapted conditions with strong phenotypic expression can lead to over estimation of the genetic component and it could be avoided by including contrasting environments and seasons in which observations are made. In accordance the experimental materials consisting of an RIL population developed with the cross HI977 x HD2329 were grown in three different agro climatic conditions in India for two consecutive years. Using these data we identified 68 QTL through CIM for four traits. Continuous phenotypic variation and transgressive segregation for all the four traits observed in the RIL population revealed the quantitative inheritance of these traits, presence of alleles with good and poor BMQ related traits in both the parents, and usefulness of this population for QTL analysis.

Considerable difference in Sv was observed between the parental lines compared to the other traits (Table-1). We detected 14 QTL for Sv on chromosomes 1B, 1D, 2A, 2B, 3D, 4B, 5A, 5B, 5D, 6A and 6D. Blanco *et al.* (1998) reported a positive and significant relationship between *Glu-B1* locus and Sv. In our study two QTL influencing Sv were identified on 1B chromosome. The *QSv.ncl-1B.1* co-locating with *Glu-B1* locus and the *QSv.ncl-1B.2* below the *Glu-B1* locus, stressed the importance of *Glu-B1* loci on Sv and also both these QTL were contributed through HD2329 alleles. Zanetti *et al.* (2001) and Huang *et al.* (2006) detected a QTL at *Glu-B1* locus with considerable contribution to Sv. The *QSv.ncl-1B.1* near the Glu-B1 might be a comparable locus to the above reported other QTL. At Karnal location, 7 QTLs (*QSv.ncl-1B.1*, *QSv.ncl-2A.1*, *QSv.ncl-3D.1, QSv.ncl-4B.2, QSv.ncl-5D.2, QSv.ncl-6A.2* and *QSv.ncl-6D.1*) were detected for SV on chromosomes 1B, 2A, 3D, 4B, 5D and 6D, respectively. Kota location had only 1 QTL (*QSv.ncl-1D.1*), but for Pune

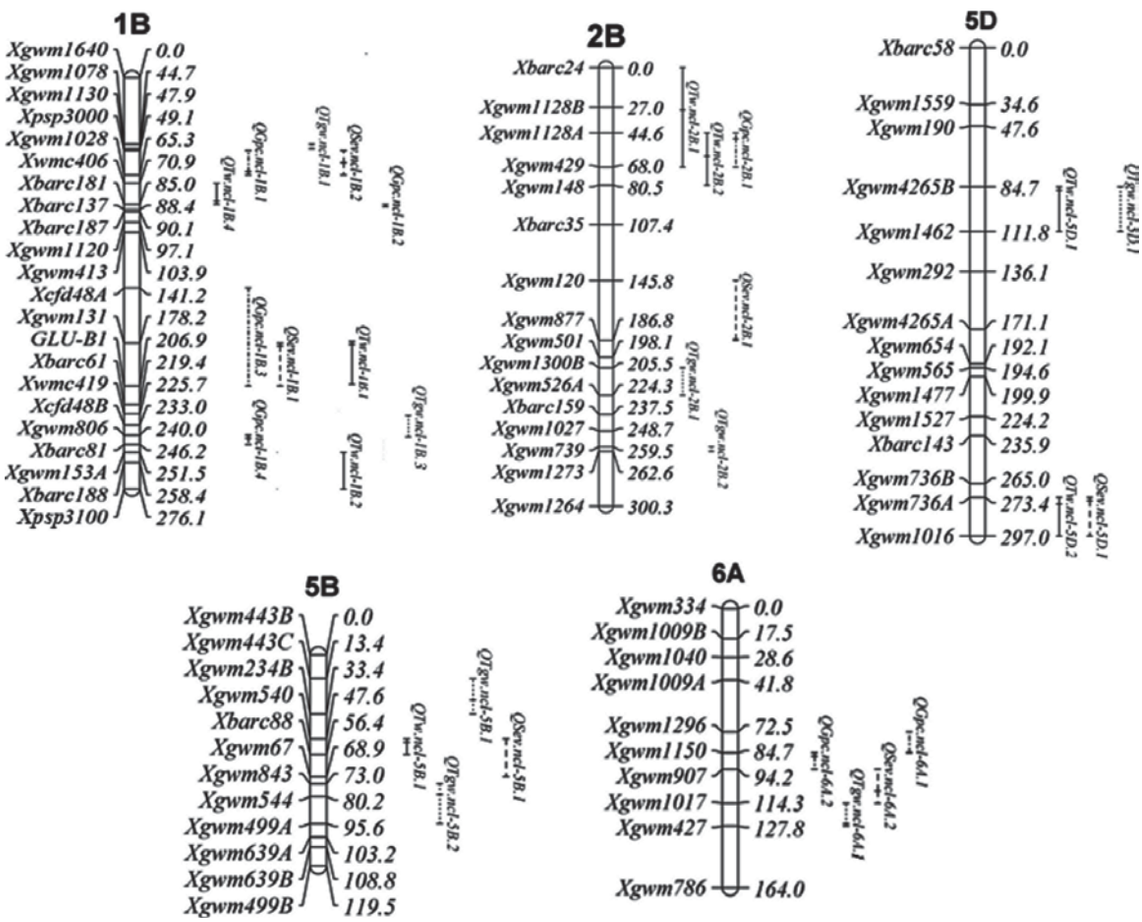

Fig. 1 Important chromosomes with locations of overlapping QTLs for yield and quality parameters on the genetic map of HI977 x HD2329 RIL population. Mapped markers are indicated on the left and their corresponding genetic distances (cM) are indicated on the right, QTL confidence interval with a LOD score ‡ 3 is indicated by a vertical bar.

Table 3 Analysis of variance for Sv, Gpc, Tgw and Tw

	Sv					Tgw				
Source	***df***	***S.S.***	***M.S.***	***F***	***% explained***	***df***	***S.S.***	***M.S.***	***F***	***% explained***
Genotype (G)	104	5554.49	53.41		28.73	104	1489.51	14.32		7.91
Environment (E)	5	4491.09	898.22		23.23	5	12850.20	2570.04		68.25
GxE	520	9285.46	17.86		48.03	520	4487.56	8.63		23.84
IPCA 1	108	3034.29	28.10	1.852***	15.70	108	1483.38	13.74	1.884***	7.88
IPCA 2	106	2896.88	27.33	2.493***	14.99	106	1196.56	11.29	1.911***	6.36
IPCA 3	104	1723.32	16.57	2.052***	8.91	104	754.31	7.25	1.391*	4.01
IPCA 4	102	1263.76	12.39	3.374***	6.54	102	613.85	6.02	1.37	3.26
GXE residual	100	367.20				100	439.46			
Total	629	19331				629	18827.30			
	Gpc					Tw				
G	104	72.52	0.70		9.11	104	736.67	7.08		4.50
E	5	496.65	99.33		62.38	5	13044.90	2608.99		79.70
GxE	520	226.98	0.44		28.51	520	2586.36	4.97		15.80
IPCA 1	108	67.87	0.63	1.627***	8.53	108	1373.86	12.72	4.322***	8.39
IPCA 2	106	59.39	0.56	1.719***	7.46	106	933.70	8.81	9.668***	5.70
IPCA 3	104	38.87	0.37	1.24	4.88	104	132.59	1.27	1.761***	0.81
IPCA 4	102	30.56	0.30	0.99	3.84	102	87.52	0.86	1.462*	0.53
GXE residual	100	30.29				100	58.69			
Total	629	796.15				629	16368			

The AMMI components are denoted as IPCA1, IPCA2, IPCA3 and IPCA4 ANOVA is calculated from the values of RILs across all the six environments, significance of AMMI components are indicated with asterisk symbol (*)

***P<0001 **P<001 *P<005

Table 4a Multitrait composite interval mapping analysis for stable QTL

Traits	Chromosome	Marker interval	Position (cM)
KarSv2+ KotSv2+ PunSv2	1B	cfd48B-wms806	238.1-239.9
	1B	barc61- wmc419	221.0-226.3
	6B	wms1199C-wms1199A	115.1-118.8

Table 4b QTL based on multitrait composite interval mapping analysis

Traits	Chromosome	Marker interval	Position (cM)
KarGpc2+KarTw2+KarSv2	6A	wms1296-wms1150	78.5-84.5
KarTw2+KarSv2	6B	wms921-wms132B	56.1-64.7
KarGpc2+ KarSv2	7D	wms735-wms974	9.5-10.6
PunTgw2+PunSv2	5A	barc1-wms156	79.5-82.6
PunTgw2+PunSv2	5B	wms234B-wms540	37.7-39.0
PunTw2+PunSv2	6B	wms1199A-wms1199B	124.9-127.0

location 6 QTL (*QSv.ncl-1B.2, QSv.ncl-2B.1, QSv.ncl-4B.1, QSv.ncl-5A1, QSv.ncl-5B.1* and *QSv.ncl-6A.1*) were observed. Rousset *et al.* (2001) reported a Sv QTL on *Glu-D1* locus, while Martin *et al.* (2001) identified a positive and significant correlation of Glu-D1d (5+10) with Sv compared to Glu-D1a (2+12), in 1B/1R translocated lines. However, Zanetti *et al.* (2001) and Huang *et al.* (2006) could not identify any QTL on Glu-D1 locus. Similarly in our study, though the parental lines carried Glu-D1d (HI977) and Glu-D1a (HD2329), we could not identify any QTL on Glu-D1 locus. This result supported that group 7 and group 6 chromosomes along with other chromosomes, regulated the expression of the HMW glutenin genes (Wanous *et al.,* 2003). Two QTL were detected on chromosomes 2A *(QSv.ncl-2A.1)* and 2B *(QSv. ncl-2B.1)*, with 15.4 and 16.24 % PVE which are in similar to those reported by Zanetti *et al.* (2001) on chromosome 2A. Three Sv QTLs each were detected on group 5 and group 6 chromosomes, with negative additive effects except the *QSv. ncl-5D.1,* which was contributed by HI977. The *QSv.ncl-5A.1* explained 11.09 % of PVE due to Sv and contributed by an allele from HD2329, which was comparable to Sv QTL earlier reported on 5A (Blanco *et al.,* 1998; Zanetti *et al.,* 2001). The highest contributing QTL (15.86 %) for Sv was detected on 6A *(QSv.ncl-6A.2)* with negative additive effect, but its position was different from the QTL stated by Blanco *et al.* (1998). Among the 14 QTLs identified for Sv, 9 QTLs

were contributed by the poor parent (HD2329), suggesting the importance of allele from the inferior parent.

QTL analysis for Gpc revealed 11 QTL with phenotypic variation explained (PVE) ranging from 7.88- 25.44 % located on 7 chromosomes (1B, 2A, 2B, 3A, 3D, 6A and 7D), which was in well accordance with the previous reports that Gpc is influenced by many chromosomes of wheat (Joppa *et al.,* 1997; Mesfin *et al.,* 1999; Blanco *et al.,* 2002; Groos *et al.,* 2003; Prasad *et al.* 2003; Huang *et al.,* 2006). Though the difference in protein content between the parents was less, transgressive segregants were observed for Gpc. These transgressive segregants for high Gpc might be due to minor genes segregating in the population (Chee *et al.* 2001) and the different Gpc controlling alleles in the parents, confirming the suitability of this population for QTL analysis of Gpc. At Karnal location, 2 major QTL (*QGpc.ncl-1B.1* and *QGpc.ncl-3A.1*) were identified on chromosome 1B and 3A, respectively. Similarly, 2 major QTL (*QGpc.ncl-2B.1* and *QGpc.ncl-6A.1*) were found on chromosome 2B and 6A at Kota location. The chromosome 1B had maximum number of QTL (4), with one QTL (*QGpc.ncl-1B.3*) near *Glu-B1* locus. Perretant *et al.* (2000) and Turner *et al.* (2004) had also reported a QTL on chromosome 1B, at a comparable location to the above QTL. Similarly, *QGpc.ncl-2A.1* controlling PunGpc2 had similar location reported by Groos *et al.* (2003). Also the *QGpc.ncl-2B.1* detected in Kota location was similar to the QTL reported by Turner *et al.* (2004) on chromosome 2B. The *QGpc.ncl-3A.1* accounted for the highest PVE of 25.44 % and was close to the Gpc loci identified by Groos *et al.* (2003) and homeologous to QTL reported by Zanetti *et al.* (2001). The *QGpc.ncl-3D.1* identified in our study was on 3DL, while Prasad *et al.* (2003) reported *QGpc.ccsu-3D.1* on 3DS in the population of WL711 x PH132 cross. The *QGpc.ncl-6A.1* on chromosomes 6A was in comparable location to the reported Gpc QTL (Perrentent *et al.,* 2000 and Groos *et al.,* 2003). The QTL (*QGpc.ncl-7D.1*) was identified in Pune location and the position of this QTL was different from the 7D Gpc QTL reported by Prasad *et al.* (2003) however, was near to the loci reported by Groos *et al.* (2004). In our study the maximum PVE explained by the QTL was 25.44 %, while Joppa *et al.* (1997) identified a QTL on 6B (Gpc-B1) accounting for 66 % variation in diploid wheat. Such single major QTL was not identified in our study, as well as in the report by Prasad *et al.* (2003) and Groos *et al.* (2004).

Tgw is one of the important yield components and selection for this trait directly increases the yield (Quarrie *et al.*, 2005). Though its correlation with quality parameters is reported (Zanetti *et al.*, 2001), selection for quality trait alone will not help in improving this trait. A pronounced and significant variation for Tgw suggested several genes with major and minor effects that were involved in the phenotypic expression of this trait. Tgw was controlled by few chromosomes, with 17 QTLs identified in our study, covering all groups of chromosomes except group 3. Tgw QTL each identified on 1B, 2B and 5B (Table-2), viz; QTgw.ncl-1B.2, QTgw.ncl-2B.2 and QTgw.ncl-5B.1 explained maximum phenotypic variation (27.12 %, 26.52 % and 27.69 %, respectively). Tgw QTL on 2B chromosome were identified as important (Groos *et al.*, 2003; Huang *et al.*, 2006), since granule bound starch synthase genes were identified on this chromosome (Vrinten and Nakamura, 2000). Interestingly these two QTL had negative additive variance, implying the positive role of BMQ inferior parent (HD2329) to Tgw. Further, it supported the absence of correlation between Tgw and dough quality parameters (Zanetti *et al.*, 2001), though Tgw and Gpc are often negatively correlated.

A total of 26 QTL were identified for Tw of which 4 QTL were consistent in at least two locations. The large number of QTL detected for this trait, may be due to the fact that, Tw also affects Tgw, grain length and grain width. At Kota, 2 major QTL (*QTw.ncl-3A.1* and *QTw.ncl-5B.1*) on chromosomes 3A and 4D and at Pune 2 major QTL (*QTw.ncl-1B.1* and *QTw.ncl-2A.1*) on 1B and 2A were detected. At Karnal, 7 major QTLs were detected from which 3 (*QTw.ncl-5D.2*, *QTw.ncl-6B.2* and *QTw.ncl-6D.1*) were also detected at Pune location. Although Tw often has positive correlation with yield and Tgw (Huang *et al.,* 2006), in our study, correlation between Tgw and Tw were insignificant for most of the locations due to the Q x E interaction. High environmental interactions for traits such as Tgw and Tw were also reported by Zhang *et al.* (2005). Hence, independent and simultaneous improvement in Tgw and Tw both could well be achieved by selection. The highest contribution was from *QTw.ncl-2B.1*, which accounted for 32.3 % variation for Tw, followed by 5B, 6D and 6B chromosomes (Table-2). The QTL reported by Campbell *et al.* (1999) on 2BS chromosome explained 31 % variation due to Tw and was located in comparable location with *QTw.ncl-2B.2*. The *QTw.ncl-6B.2*, *QTw.ncl-6D.1* and *QTw.ncl-6D.2* were new loci and not reported earlier. Interestingly, it was observed that all the QTL with contribution > 20 % were from the parent HD2329, suggesting that it has some good alleles.

In wheat, associations of qualitatively inherited genes together represent gene-rich regions and they form the hot spots of recombination. QTL are usually spread over all the chromosomes, but clusters of QTL in certain chromosomal regions have been observed as well (Huang *et al.,* 2006). QTL affecting several traits are common and may be due to pleiotropy or close linkage. Since most of the QTL clusters in this study were located in the centromere region of the chromosomes, clustering may be explained by the suppression of recombination in these regions (Tanksley *et al.,* 1992). Like single genes, these QTL for different traits were mapped in the same genomic regions forming clusters (Huang *et al.,* 2006). Several clusters of yield QTL have also been identified previously in wheat, either controlling yield itself or a yield component (Groos *et al.,* 2003; Quarrie *et al.,* 2005). Though chromosome 1B had 2 clusters, total 13 QTLs, controlling Gpc, Sv, Tgw and Tw were detected on it. Similarly, 6 QTLs were mapped on 2B, 5 QTLs on 5D and 6A; and 4 QTLs each on 1D, 2A, 4B, 5B and 6B chromosomes. QTL cluster of Tgw and Sv was identified only on chromosome 1B, detected on Pune location, with

significant negative correlation. The correlation between Sv and Tw was insignificant in all the locations, but in the clusters, they often co-located, compared to other traits. This may be due to absence of epistatis among these traits or low effects of these QTLs to traits, being a frame work map and small population, the sampling effects affect the magnitude of confidence interval (Darvasi *et al.,* 1993). On chromosome 5D, within the interval of *Xgwm4265B* and *Xgwm1462*, two significant QTL one each for Tgw (*QTgw.ncl-5D.1*) and Tw (*QTw.ncl-5D.1*) were detected. Similarly, the cluster comprising *QTw.ncl-5D.2* and *QSv.ncl-5D.2* within the interval of *Xgwm736A* and *Xgwm1016* was observed. It was also observed on chromosome 5D within the interval of *Xcfd13* and *Xgwm1009C*, comprising *QSv.ncl-6D.1*, and *QTw.ncl-6D.1*. Among these clusters, both Tw and Sv have negative additive effect, while Tgw had positive effect. Saturating these regions with more markers will help in identifying close flanking markers for these QTL and pyramiding of these QTL clusters will result in overall improvement.

In an attempt to identify the environmental variation, we analyzed the population with AMMI. Transgressive segregants were realized across all the environments, but their performance was not stable for all the traits, across different environments. A large sum of environmental effect was diverse, with large differences among environmental means causing most of the variation in all the traits. The magnitude of the GEI to TSS for Tw, Sv, Gpc and Tgw were 3.51, 2.26, 3.12 and 2.27, respectively. This indicated the substantial differences in genotypic response across environments.

MCIM analysis was performed to find QTL stability as well as to find QTL affecting more than one trait. Although many QTL were detected in CIM, majority of them were not identified in MCIM analysis. This may indicate more stringent or higher level of confidence in MCIM compared to CIM and similar results were observed for yield traits in wheat (Kumar *et al.* 2007). Although, no QTL was detected in all the environments for any trait, we could identify 3 QTL for Sv, which showed presence in 3 different locations. Further, 6 QTL were detected on 5 different chromosomes affecting minimum 2 traits. The QTL identified by MCIM suggest pleiotropy rather than linkage as the possible cause of correlations among these traits. This may be taken into consideration while planning the experiments with molecular breeding which are aimed to simultaneous improvement in more than one trait. Also to get significant improvement in the preferred trait using MAS, it's necessary to use QTL with moderate or small effect as well compared to only large effect QTL usage. Some of the identified QTL showed low phenotypic contribution which might indicate the possible involvement of additional unidentified loci either because of incomplete genome coverage or the smaller size of the population and they could only be detected by using more markers spanning the entire genome. This study revealed the importance of combination of stable QTL with region specific QTL for better phenotype and QTLs presented in our study will be useful in MAS efforts for improvement of wheat grain quality.

Acknowledgement

ME acknowledges the Council of Scientific and Industrial Research (CSIR) for financial support through Junior and Senior Research Fellowship and DAAD-IAESTE (International Association for Exchange of Students for Technical Experience) for support to carry out part of research work with Dr. M. S. Roder at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany. Some of the SSR primers were kindly provided by the company Trait Genetics GmbH, IPK, Germany. The Director, Agharkar Research Institute, Pune is profusely thanked for field facility. The financial assistance for this project from the Department of Biotechnology (DBT), New Delhi is gratefully acknowledged.

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