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QTL analysis, association mapping and marker-assisted selection for some quality traits in bread wheat - An overview of the work done at CCS University, Meerut

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Abstract

The present article summarises the results of our studies involving QTL mapping and marker-assisted selection (MAS) for three quality traits in bread wheat undertaken during the last more than a decade; however, most of these results were earlier published. The three quality traits include pre-harvest sprouting tolerance (PHST), grain protein content (GPC), and grain weight (GW). For PHST, a total of 13 QTLs were identified using two RIL populations and 30 QTLs were identified through association mapping, six QTLs being common in the two approaches (interval mapping and association mapping). Meta-QTL analysis for PHST was also conducted for 4 chromosomes (3A, 3B, 3D, 4A) and 8 meta-QTLs representing 36 earlier reported QTLs were identified. For GPC, a total of 13 QTLs were identified using interval mapping, but no association mapping was attempted. Similarly for GW, 10 QTLs were identified through interval mapping and 25 QTLs were identified through association mapping, four of these QTLs being common in both the approaches. MAS was also attempted for all the three traits. For PHST, seven BC_3F_4 progenies with high level of PHST were developed [these PHS tolerant lines also carried two *Lr* genes (*Lr*24 and *Lr*28) earlier introgressed through MAS]. For GPC, seven MAS-derived progenies (carrying the major gene *Gpc-B1*) with significantly higher GPC (14.83% to 17.85%) than their recipient parental genotypes were selected. Similarly for GW, 13 MAS-derived BC_3F_2 plants carrying different QTL combinations and having 1000 grain weight higher than that of the donor parent RS111 were selected. The results of these studies demonstrated successful tagging/mapping of QTL through QTL interval/association mapping and their subsequent use in marker-aided selection for wheat improvement.

Keywords: Bread wheat, QTL interval mapping, association mapping, marker-assisted selection, meta-QTL analysis

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops worldwide, occupying largest cultivated area, and supplying 40% of food globally and 25% of calories for the developing world. Although significant progress during the last 50 years has been made in increasing world wheat production, which reached ~690 mt in 2011, quality traits in this crop did not receive the desired attention of wheat breeders. Among quality traits, pre-harvest sprouting (PHS), grain protein content (GPC) and grain weight (GW) are three important quality traits. Pre-harvest sprouting (PHS) leads to reduced yield causing financial loss to growers (Kumar *et al.* 2010), GPC determines nutritional value, processing properties, quality of the end products (bread and pasta) and market value of wheat grain, while GW, an important component of grain yield (GY), has a favorable effect on flour yield (Sasaki *et al.* 1968; Ketata *et al.* 1976; Campbell *et al.* 1999). Therefore, determination of marker-trait associations for these three traits through QTL interval mapping and association mapping, and the subsequent use of associated markers for indirect marker-assisted selection (MAS) for these three traits are certainly desirable for wheat molecular breeding.

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Detailed genetic analyses of PHST, GPC and GW, conducted in the past, led to identification of a large number of QTL/genes, as evident from the following literature for each of these three traits: (i) PHST (Groos *et al.* 2002; Mares *et al.* 2002; Miura *et al.* 2002; Noda *et al.* 2002; Mori *et al.* 2005; Kulwal *et al.* 2004, 2005a, 2012; Mares *et al.* 2005; Mohan *et al.* 2009; Kumar *et al.* 2009; for a review Kulwal *et al.* 2010), (ii) GPC (Prasad *et al.* 1999; Harjit-Singh *et al.* 2001; Groos *et al.* 2003; Prasad *et al.* 2003; Sourdille *et al.* 2003; Kulwal *et al.* 2005b; Huang *et al.* 2006; Uauy *et al.* 2006a, b; Kunert *et al.* 2007) and (iii) GW (Huang *et al.* 2003, 2004, 2006; McCartney *et al.* 2005a; Kumar *et al.* 2006; Breseghello and Sorrells 2006, 2007; Sun *et al.* 2009). For PHST, QTLs on chromosomes 3A, 3B, 3D and 4A are considered to be important (Kato *et al.* 2001; Noda *et al.* 2002; Osa *et al.* 2003; Kulwal *et al.* 2005a; Mori *et al.* 2005; Mares *et al.* 2005; Torada *et al.* 2005; Chen *et al.* 2008; Liu *et al.* 2008; Fofana *et al.* 2009). Similarly for GW, important QTLs are known to be present on chromosomes 1A, 1B, 1D, 2B, 2D, 4B, 4D, 7A and 7D (Huang *et al.* 2003, 2004, 2006; McCartney *et al.* 2005a; Kumar *et al.* 2006; Wang *et al.* 2009). An important QTL on 7D (*QTgw.ipk-7D*) that explained 84.7% of the phenotypic variation for GW was earlier identified by Huang *et al.* (2003, 2004) and was also later fine-mapped (Roder *et al.* 2008). A number of leaf rust resistance genes (~60) are also known (Gupta *et al.* 2006), some of them (*Lr*9, *Lr*19, *Lr*24, *Lr*28 and *Lr*32) providing complete hypersensitivity against leaf-rust pathotypes in most of the wheat-growing regions of Asia and Europe (Tomar and Menon 1998; Huszar *et al.* 2001). However, for the same individual chromosome, non-overlapping or

partly overlapping set of QTLs/genes may be identified in different bi-parental populations, grown in different environments. Therefore, it is necessary to identify genomic regions that are common in different studies. Meta-QTL analysis helps in the identification of regions carrying a set of overlapping QTLs/genes from different studies. In the past meta-QTL analysis has been conducted for a number of traits in different crops (see Tyagi and Gupta 2012 for references).Meta-QTL analysis studies also have been conducted in wheat, but none has been conducted on the three traits used in our studies.

In this communication, we describe the results of our studies, on QTL mapping (interval mapping/association mapping) for GPC, PHST and GW (Prasad *et al.* 1999, 2003; Kulwal *et al.* 2004, 2005a, b; Kumar *et al.* 2006; Kumar *et al.* 2009; Mohan *et al.* 2009; Mir *et al.* 2012, 2012; Jaiswal *et al.* 2012), identification of MQTLs for PHST on four chromosomes (Tyagi and Gupta 2012) and successful use of MAS for improvement of PHST (Kumar *et al.* 2010) GPC (Kumar *et al.* 2011) and GW in some elite Indian wheat cultivars.

Plant material for QTL interval mapping

For QTL interval mapping, three mapping populations were utilized. Two RIL mapping populations (designated as 'PHS pop-1' and 'PHS pop-2' in this communication) were used for PHST (for details see Kulwal *et al.* 2005a; Kumar *et al.* 2009; Mohan *et al.* 2009), one RIL population 'PHS pop-2' was also used for QTL mapping of GPC (Prasad *et al.* 1999; 2003) and one RIL mapping population (designated as GW pop) was used for genetic analysis of GW (Kumar *et al.* 2006), (see details in Table1).

Table 1. Details of plant materials and their evaluation for QTL analyses and association mapping

Descriptor	PHST	GPC	GW
1. QTL interval mapping			
Mapping population	(i) PHS pop-1 [SPR8198 (PHS tolerant) × HD2329 (PHS susceptible)] (ii) PHS pop-2 [PH132 (PHS tolerant) × WL711 (PHS susceptible)]	PHS pop-2 = PH132 (high GPC) × WL711 (low GPC)	GW pop = Rye Selection 111 (high GW) × Chinese Spring (low GW)
No. of RILs used	(i) 90(PHS pop-1), 100 (PHS pop-2)	100	92
No. of environments used for evaluation	(i) 6 (PHS pop-1), 3 (PHS pop-2)	5	6
Data scored	1-9 scale	GPC%	1000 grain weight
2. Association mapping			
No. of genotypes used	230 cultivars + 12 exotic lines	-	230 cultivars
Data available	2-4 years	-	3 years
Data scored	1-9 scale	-	1000 grain weight

Plant material for association mapping

A set of 230 wheat cultivars along with a set of 12 PHS tolerant exotic wheat genotypes (total 242 genotypes) was used for association mapping for PHST (see details in Table1). For association mapping of GW, the same set above 230 Indian bread wheat cvs. was used. The data on PHST were recorded by us at Meerut over 2-4 years on a scale of 1 to 9 with a score of 1 for genotypes with no visible sprouting and a score of 9 for the genotypes with complete sprouting and the data on GW (1000 kernel weight) from replicated trials for the above 230 cultivars were made available by DWR, Karnal (Kundu *et al.* 2006).

Plant material for marker-assisted selection

(i) ***MAS for pre-harvest sprouting tolerance and leaf rust resistance:*** For PHST, wheat genotype SPR8198 carrying a major QTL within a 17 cM region of chromosome arm 3AL (Kulwal *et al.* 2005a) was used as the donor parent. An elite cultivar, HD2329 carrying two leaf rust resistance genes (*Lr*24+*Lr*28) that were transferred earlier at IARI using marker-assisted backcrossing (MAB) was used as the recipient genotype for pyramiding of PHST QTL onto two *Lr* genes.

(ii) ***MAS for grain protein content:*** For GPC, 10 bread wheat genotypes [(RAJ3765, K9107, PBW373, PBW343, HD2687, HI977, PBW343 + *Lr*24 (three pre-bred lines), HD2329 (*Lr*24+*Lr*28)] were used as recipient genotypes. A bread wheat genotype Yecora Rojo containing *Gpc-B1* gene for high GPC (kindly provided by Jorge Dubcovsky, University of California, Davis, USA) was used as the donor parent.

(iii) ***MAS for grain weight:*** For introgression of GW QTLs, a cross was made between donor genotype RS111 (carrying high grain weight) and two recipient parents Raj3765 and K9107 (carrying low grain weight).

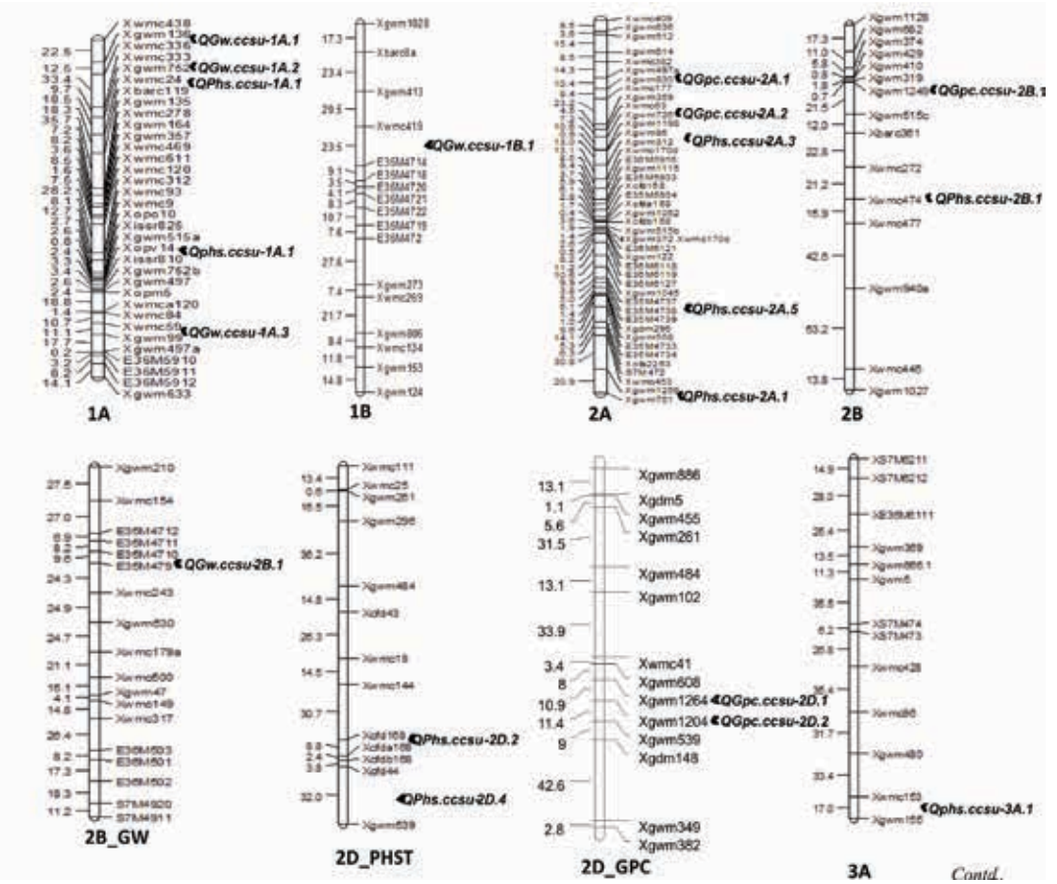

Fig. 1. Representative spikes showing PHS score at 1-9 scale, 1 showing complete tolerance and 9 showing complete susceptibility for PHS.

Phenotypic data

(i) ***Scoring for PHST:*** Sprouting index was estimated for calculating the tolerance level against PHS. SI based on the number of grains that germinate per spike, measured on a scale of 1-9, with a value of 9 meaning completely susceptible and a value of 1 meaning completely tolerant against sprouting (Fig. 1)

(ii) ***Test for leaf rust resistance:*** For the evaluation of leaf rust resistance, the material was grown in growth chambers, under controlled environmental conditions, at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi, India. Ten-day-old (single-leaf stage) seedlings were inoculated with pathotype 77-5 (the most virulent and predominant pathotype of leaf rust in South East Asia) following procedure reported earlier (Kumar *et al.* 2010). Disease reaction was recorded 12 days after inoculation following Stakman *et al.* (1962).

(iii) ***Data on grain protein content:*** The GPC (% grain weight) at 12% moisture content was estimated for each genotype from each replication using Infratech Grain Analyzer at Agharkar Research Institute, Pune.

(iv) ***1000 grain weight:*** A random sample of 1000-grains of a plant was weighed in g and recorded as 1000-grain weight.

Statistical analysis

(i) ***Construction of framework molecular linkage maps:*** The details of markers, and the construction of framework linkage maps for all the three mapping populations that were used for QTL mapping of PHST, GPC and GW are available elsewhere (Prasad *et al.* 2003; Kumar *et al.* 2009; Mohan *et al.* 2009; Mir *et al.* 2012). Consensus maps were also prepared for individual chromosomes from three mapping populations, wherever same chromosome in more than one populations was found to carry QTL for GPC, PHST and GW. These consensus maps were prepared using online software tool "MergMap" (http://138.23.178.42/mgmap/). For association mapping, markers were selected from the whole genome (Genome Wide Association Mapping).

(ii) ***QTL interval mapping :*** QTL interval mapping was conducted for PHST, GPC and GW for detection of main-effect QTL (M-QTL) with the help of softwares QTL Cartographer Ver. 1.21,Ver. 2.5 (Wang *et al.* 2007) and IciMapping Ver. 2.0 (Li *et al.* 2007, 2008) respectively. LOD score of 2.5 was used for suggesting the presence of a putative QTL. Threshold LOD scores, calculated using 1000 permutations, were used for declaring definitive QTL.

(iii) ***Association mapping:*** For association mapping (PHST and GW), population structure was worked out using STRUCTURE 2.2, and kinship matrix was calculated from

the marker data using TASSEL 2.1. Association between markers and traits was worked out through application of General Linear Model (GLM) and Mixed Linear Model (MLM) (Yu *et al.* 2006) approaches using software TASSEL 2.1 Significance of marker-trait associations were described in terms of p-values ($p \leq 0.05$ for significant markers).

(iv) ***Meta-QTL analysis for PHST:*** A bibliographic review was conducted and 30 studies reporting QTLs for PHST or related traits were used for meta-QTL analysis with the help of BioMercator software (Goffinet and Gerber 2000). For conducting meta-QTL analysis, consensus maps for four individual chromosomes, namely 3A, 3B, 3D and 4A were developed and all the QTLs of a particular map were positioned onto the respective consensus map by means of homothetic function (monotonic transformation of homogeneous function), using common markers between framework maps and the consensus map. The details of the procedure followed for development of consensus maps and projection of QTLs are described elsewhere (Tyagi and Gupta, 2012).

Marker-assisted selection

For marker-assisted selection involving PHST and GPC with leaf rust resistance, both foreground and background selections were exercised. Molecular markers either linked with the desired gene/QTL or flanking the gene/QTL were used for the foreground selection. The SSRs used for background selection were distributed throughout the wheat genome in a reference map (Somers *et al.* 2004; details of SSRs available on request). For both the traits, in each backcross generation, the proportion of the genome from the recipient parent was estimated following Sundaram *et al.* (2008).

(i) ***MAS for PHST and leaf rust resistance (LRR):*** Foreground selection for PHST was exercised using two SSR markers (Xgwm155 and Xwmc153) flanking the QTL for PHST (Kulwal et al. 2005a). For leaf rust resistance, SCAR markers, SCS73719 linked to Lr24 (Prabhu et al. 2004) and SCS421570 linked to Lr28 (Cherukuri et al. 2005), were used to confirm the retention of Lr24 and Lr28 in the backcross progenies. For background selection involving PHST, 61 SSR markers polymorphic between the donor and the recipient genotypes were used in three successive backcross progenies (BC1 to BC3) for rapid reconstitution of the genome of the recipient genotype.

(ii) ***MAS for GPC:*** For GPC, either the marker (NorB2) flanking the gene or the perfect marker (Xuhw89) present within the gene "Gpc-B1" were used for foreground selection. For GPC, background selection in each of the three backcross progenies (BC1 to BC3) was carried out using a total of 92 SSRs polymorphic between each pair of the donor and the recipient genotypes.

(iii) ***MAS for GW:*** For GW, foreground selection was carried out with two markers viz. *Xwmc24* and *Xwmc59* (associated with two separate QTL for grain weight on chromosome 1A) in three backcross progenies (BC1F1, BC2F1, and BC3F1) involving two genetic backgrounds, namely Raj3765 and K9107.

Marker-trait associations for PHST, GPC, and GW

For each of the three traits, marker-trait associations (MTA) were determined using one or more (upto four) mapping populations and making use of one or more of the following approaches: bulk segregant analysis (BSA), interval mapping (IM) and association mapping (AM). BSA and IM were used for GPC, while IM and AM both were used for PHST and GW.

Table 2. Summary of QTL/markers identified through QTL interval mapping and association mapping for PHST, GPC and GW

Descriptor	PHST*	GPC	GW
1. QTL Mapping			
No. of QTLs identified	7 (6)	16	10
LOD range	2.80-6.41 (2.84-9.51)	2.6-6.5	3.25-20.50
Chromosomes involved	1A, 2A, 2D, 3A, 3B (1A,2A, 2B, 3B, 6A, 6B)	2A, 2B, 2D, 3D, 4A, 6B, 6D, 7A, 7D	1A, 1B, 2B, 5A, 6A, 6B, 7A, 7D
Range of PVE (%)	15.2-45.11 (8.41-29.47)	0.63-35.80	4.37-23.27
2. Association mapping			
No. of sub-populations identified by structure analysis	15	-	13
No. of QTLs identified	30	-	9
Chromosomes involved	1A, 1B, 2A, 2B, 2D, 3B, 3D, 4A, 4B, 4D, 5B, 5D, 6B, 6D, 7A, 7B, 7D	-	1A, 1B, 2A, 3B, 4A, 4B, 4D, 5A, 6D, 7A, 7B, 7D
Level of p-values	<0.05	-	<0.05

* values given outside and inside the parentheses are the results obtained using PHS pop-1 and PHS pop-2 respectively.

Framework linkage maps consisting of 214 marker loci for PHS pop-1, 217 marker loci for PHS pop-2, 173 markers for GPC pop and 294 marker loci for GW pop were prepared and used for QTL interval mapping (Prasad *et al.* 2003; Kumar *et al.* 2009; Mohan *et al.* 2009; Mir 2012). The results of genome-wide single-locus QTL interval mapping for PHS, GPC and GW are summarised in Table 2 and **Fig. 2**. For PHST, 7 QTLs in PHS pop-1 and 6 QTLs in PHS pop-2 were identified following CIM; for GPC, 16 QTLs including 7 QTLs following SMA, 7 QTLs following SIM and 13 QTLs following CIM were identified, for GW: 10 QTL were identified following ICIM . In addition to the above M-QTL identified by CIM/ICIM, a large number of epistatic QTLs (E-QTLs) were also identified for two of the three traits (namely, PHST and GW) by using mixed-model-based composite interval mapping implemented in QTL Network (Yang *et al.* 2007). QTL × environment interactions were absent for both the traits. The important feature of the above QTL mapping studies was the identification of two major and stable QTL for PHST on chromosome arms 2AL (PVE up to 31.52%) and 3AL (PVE up to 32.81%) in PHS pop-1 and one major and stable QTL on 6AL (PVE up to 29.47 %) in PHS pop-2. Similarly, four major QTL for GW were identified on chromosomes 1A, 5A, 6A and 6B (each explained >20% PV; **Fig. 2**).

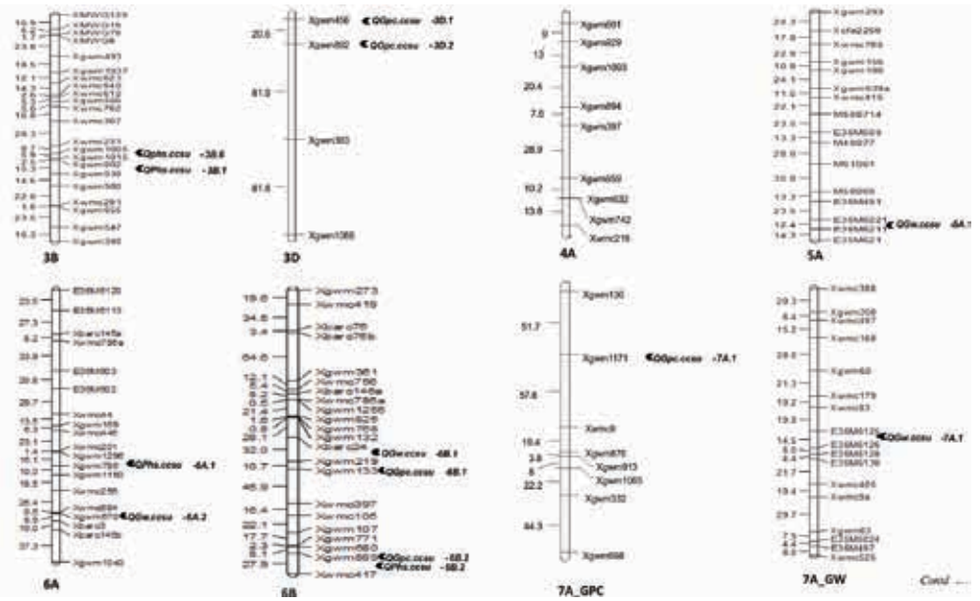

Fig. 2 Framework linkage maps of individual chromosomes showing main-effect QTL (M-QTL), identified in all the three RIL mapping populations for PHST, GPC and GW. Each QTL is represented by arrow head followed by the name of the QTL. Chromosome 1A is mergemap from all the three mapping populations; 2A, 2B and 3B are the mergemaps from two PHST populations, and chromosomes 6A and 6B are mergemaps from PHS pop-2 and GW pop.

Following association mapping, 30 markers were found to be associated with PHST. Only eight SSR markers associated with QTL for PHST were such, which were located within the marker intervals that were earlier reported to carry QTLs for PHST. The remaining 22 markers that were found to be associated with PHST could not be associated with any of the genomic regions known to carry QTLs for PHST (QTL for PHST are known to occur on all the 42 chromosome arms of wheat genome). Association mapping for GW allowed validation of 8 known markers linked with QTL for GW, identification of 6 new markers (with relatively more tight linkage) in the genomic regions/marker intervals previously reported to harbour QTL for GW and 11 markers in genomic regions that were not known to carry any QTL for GW.

Eight Meta-QTLs for PHST

Using 50 original QTLs from 15 different individual studies, 8 meta-QTLs (MQTLs) were identified: 7 MQTLs were located on chromosomes of homoeologous group 3 including 3A (2 MQTL), 3B (3 MQTL) and 3D (2 MQTL) and 1 MQTL was located on chromosome 4A. Confidence interval (C.I.) for each of these 8 MQTLs was particularly narrow. Co-localizations between candidate genes for dormancy/ PHST (*taVP1* and *TaGA20-ox1*) and MQTL positions were also reported (Tyagi and Gupta; 2012).

Marker-assisted selection (MAS)

(i) ***MAS for PHST:*** The PHST score of the donor (SPR8198) and recipient (HD2329 *Lr24+Lr28*) genotypes used during the present study was 1 and 9, respectively. In BC_3F_3 seven lines were selected exhibiting high tolerance (PHS score = 2-3). Selfed progenies (BC_3F_4) of the above seven PHS tolerant lines exhibited hypersensitive reaction to leaf rust when tested under controlled conditions.

(ii) ***MAS for GPC:*** Means for GPC (%) and yield in the 124 MAS-derived progenies carrying *Gpc-B1*, were compared

with those of the recipient parents. There were 71 progenies, which exhibited high GPC (%) at all the three locations with no yield penalty, although improvement in GPC (%) was not statistically significant. Only three progenies one at each location showed significantly higher GPC (%) without any yield penalty relative to their respective recipient parental genotypes. However, similar significant change in protein yield (calculated as t/ha) was not observed in these three selected progenies.

When pooled data from three locations was examined five progenies involving three of the 10 recipient parents [two each belonging to genotypes HD2329 (*Lr*24 + *Lr*28) and Raj3765 and one belonging to HI977] had significantly higher GPC (%) with no significant reduction in yield; only one of the above five MAS derived progenies, had significantly higher GPC at one of the three locations, so that altogether there were seven progenies (two showing higher GPC at one location only, one showing higher GPC at one location and also in pooled data and four showing higher GPC only in pooled data but not at any individual location), which either had higher GPC at one of the three locations or exhibited higher GPC in pooled data. The selected lines were again subjected to multiplications trail in 2010-11 and 2011-12 to confirm earlier results; the results of these trails are being analysed.

(iii) ***MAS for GW:*** As many as 13 BC_3F_2 plants each from 13 corresponding progenies were selected following foreground (carrying either one or both the above QTLs) and phenotypic selection (TGW for each progeny exceeded that of the donor parent).

QTL mapping for PHST, GPC, and GW

The results of our study on PHST are in agreement with the concept of quantitative nature of PHST, since several QTL with both major and minor effects were identified by us on a number of different chromosomes. The identification of several QTL for PHS in this study also underlined the importance of genome-wide QTL analysis, since only a solitary major QTL (*Qphs.ccsu-3A.1*) for PHS was reported by us earlier in PHS pop-1 (Kulwal *et al.* 2005a). However, the polygenic control of PHST in bread wheat, involving only a few major QTL and a large number of minor QTL, limits the chances of success for improvement of PHST in bread wheat through classical methods of plant breeding. Hence, molecular markers linked with PHST may be used for marker-assisted selection (MAS) to accelerate development of cultivars with high level of PHST.

A comparison of the genomic locations of QTL for PHST identified by us with those identified in earlier studies in wheat suggested several novel QTL, reported for the first time (for details, see Kumar *et al.* 2009; Mohan *et al.* 2009). For example, the major QTL (PVE up to 29.47%) in the centromeric bin of 6AL (*QPhs.ccsu-6A.1*) in PHS pop-2 was reported for the first time (Kumar *et al.* 2009). QTL identified on 2AL and 2DL in PHS pop-1 which are not homoeo-QTL were also reported by us for the first time.

The results of association mapping of PHST were also compared with earlier studies. Out of 30 SSR loci that were detected by us to be associated with PHST on the basis of association mapping, only 8 were associated with six known PHST QTLs. The remaining 22 SSRs could not be associated with any of the QTLs reported earlier, although QTLs for PHST perhaps occur everywhere in the genome (>165 QTLs spread over all 42 chromosome arms have been reported). Also, among the markers that were found to be associated with known QTLs, 7 of the 8 SSRs were new, since only one (gwm526) of these 8 SSRs was known earlier to be associated with PHST. Thus our association mapping studies validated six earlier reported QTLs for PHST and also helped in the identification of 7 more closely linked markers for five of these six QTLs. Of the above validated QTLs for PHST, the QTL *QGi-crc.3B* reported by Fofana *et al.* (2009) is a major QTL controlling several traits such as germination index, sprouting index and falling number that are related to PHST/dormancy. Two of the 7 new markers (namely, wmc418 and gwm131) were associated with this major QTL (*QGi-crc.3B*). These two markers were associated with the above PHST QTL more closely than the earlier known markers and may prove useful for MAS during improvement of PHST in wheat.

During the last two decades, a number of QTL studies on the genetics of GPC in bread wheat have been conducted (Snape *et al.* 1995; Blanco *et al.* 1996; Prasad *et al.* 1999, Harjitsingh *et al.* 2001; Khan *et al.* 2000). Of the 16 QTLs reported, 7 QTLs located on chromosome 2A, 2B, 2D, 3D and 7A were also reported in earlier studies (Kuspira and Unrau 1957; Levy and Feldman 1989). A QTL (*QGpc.ccsu-2D.*1) located on chromosome 2D with PVE 11.0% to 20.0% in different environments was found to be a major QTL and could be important for MAS. A major gene *Gpc-B1* derived from tetraploid wheat was also mapped on short arm of chromosome 6B (Distelfeld *et al.* 2006). For GPC, since no major QTL comparable to *Gpc-B1* was detected through our own studies, we had to use *Gpc-B1* gene for introgression into 10 Indian wheat cultivars.

Like PHST, for GW also, both QTL mapping and association mapping approaches suggested that GW is controlled by a large number of small effect QTLs, and only few major QTLs are known (Kumar *et al.* 2006; Mir *et al.* 2012). Three major and stable QTL (*QGw.ccsu-1A.3, QGw.ccsu-5A.1 and QGw.ccsu-6A.2*) identified through QTL mapping may prove useful for MAS for the improvement of GW in bread wheat. The identification of significant marker-GW associations identified through association mapping (AM) by us largely confirmed the results of QTL analysis carried out in our own laboratory and elsewhere, thus validating earlier results. On chromosome 1A, AM identified four SSR markers (*Xwmc336*, *Xwmc24*, *Xgwm99* and *Xgwm135*). Among these four markers, *Xgwm99* is a flanking marker of the major and stable QTL (*QGw.ccsu-1A.3*) identified by us through interval mapping. A closely linked marker (*Xwmc89*) on chromosome 4D was also identified in a genomic region harbouring QTLs for six important traits (grain weight, test weight, grain yield,

plant height, days to maturity and lodging), earlier reported through interval mapping (McCartney *et al.* 2005). Similarly, closely linked markers were also identified for some other QTLs including one important QTL on chromosome 7D identified by us. The results of association mapping during our study confirmed that association mapping has a higher power of resolution than bi-parental linkage mapping. The closely linked markers as well as new markers identified through association mapping may prove efficient for the improvement of GW also through MAS. Thus complementary strengths of both linkage–based interval mapping and LD-based association mapping approaches will allow efficient development of markers for molecular breeding. Joint linkage-association mapping may further improve the power and precision of these genetic studies.

Meta-QTL analysis for PHST

It may be recalled that in our study involving meta-QTL analysis, 36 reported QTLs were condensed into 8 MQTLs, each with a relatively narrow confidence interval (CI), thus providing more closely associated markers for these reported QTLs. Since these MQTLs are based on QTLs detected in different environments, these MQTLs may also be more stable across environments. Three of the 8 MQTLs were located on long arms of chromosome 3A, 3B and 3D (MQTL 2, MQTL 5 and MQTL 7) and are believed to be homeo-triplicate MQTLs. Interestingly, these three MQTLs are located in the region, where the triplicate loci of the gene *taVp1* are located. Therefore, *taVp1* may be a candidate gene for PHST/dormancy. Similarly position of MQTL-8 on 4AL suggested the possibility of GA20-oxidase gene (*TaGA20-ox1*) to be a candidate for PHST/seed dormancy. Another intresting feature of this study was that out of 8 MQTLs, four MQTLs resulted due to clustering of dormancy and PHST QTLs, suggesting that these may represent pleiotropic or closely linked genes, which influence both these traits (PHST and dormancy). Two MQTLs also resulted due to co-localisation of QTLs for PHST and grain colour (GC), suggesting that QTLs for PHST and GC may be closely linked or pleiotropic in nature. A solitary MQTL (MQTL8) on chromosome 4A was based on 11 individual QTLs (each contributing to 8-45% of PVE) and therefore can be exploited for marker-assisted selection (MAS). However, further studies need to be conducted to fully understand the genetic architecture of PHST and dormancy, since only 4 of 21 chromosomes of wheat were used in the present study, and QTLs for PHST are located on all the 21 chromosomes.

Marker-assisted Selection (MAS)

Among cereals, successful stories of marker-assisted pyramiding of genes for resistance to leaf rust and powdery mildew in wheat (Kloppers and Pretorius 1997; Liu *et al.* 2000), and those for other disease resistance genes in crops like rice and barley are already available (Huang *et al.* 1997; Hittalmani *et al.* 2000; Sanchez *et al.* 2000; Singh *et al.* 2001; Werner *et al.* 2005). In wheat >60 QTL/genes are being currently tracked with molecular markers by one or more breeding program worldwide (Gupta *et al.* 2010a). As a result, a number of cultivars have been released and a large number of improved pre-bred wheat genotypes have been developed through MAS. However, MAS for improvement of PHST and GPC has only been used sparingly, although two cultivars/varieties (Lassik and Farnum) with improved for GPC through MAS recently became available in USA (for reviews see Gupta *et al.* 2010a, b).

(i) *MAS for PHST:* During the present study, we attempted to bring together a major QTL for PHST and two important *Lr* genes in the background of cultivar HD2329 using MAS (Kumar *et al.* 2010). For this purpose, we successfully introgressed a major PHST QTL (*QPhs.ccsu-3A.1*) mapped on chromosome arm 3AL (Kulwal *et al.* 2005a) into the cultivar HD2329, to which *Lr24* and *Lr28* genes for leaf rust resistance were already introgressed using MAS. Both foreground and background selections were exercised. The background selection helped us to reconstitute the recipient parent genome (HD2329) to as high as 83.3% in BC_1F_1 and to as high as 93.4% in BC_3F_1. Similar studies were also reported earlier in rice (Sundaram *et al.* 2008). As many as 60% of the reconstituted plants were found to be PHS tolerant, when phenotyping for PHST was done on these plants following MAS. The remaining ~40% positive plants which had a higher PHS score suggest that there are other QTL, which control PHST and that MAS should involve several QTL for PHST and need to be used in combination with phenotyping evaluation.

Altogether, we were successful in developing seven lines that were PHS tolerant and leaf rust resistant. These lines are presently being evaluated for yield in multi-location trials. However, all these lines have red grain colour due to possible association of the red grain colour with the QTL for PHST introgressed by us. Since, amber grain colour is preferred in the Asian markets, we are currently introgressing through MAS, the QTL for PHS tolerance that are independent of grain colour with the aim of producing PHS tolerant amber grained wheat genotypes.

(ii) *MAS for GPC:* High GPC gene *Gpc-B1* was also introgressed through MAS into 10 Indian bread wheat genotypes for the first time. Variation in the magnitude of GPC (%) was noted between the progenies derived from a common recipient parent genotype as well as between the progenies involving different recipient parents. Variation was also observed when the same progeny was evaluated at three different locations. This was also evident from ANOVA (data not sown), which suggested significant interaction of the *Gpc-B1* gene with the recipient parent genotype and also with the environments.

As described in the results earlier, there were 71 progenies, which exhibited high GPC (%) at all the three locations with no yield penalty, although improvement in GPC (%) was marginal (increment 0.14% to 9.81%) and not statistically significant. Together the results of the present study indicated the role of genotype-by-environment interaction in determining GPC (%) and grain yield in wheat and the role of phenotypic selection in identification of progenies

combining high GPC with high grain yield. The GPC (%) of the seven improved progenies varied from 14.83% to 17.85% representing an increment of 12.93% to 29.62% over the GPC of their respective recipient genotypes. The scatter plots of the values of grain yield vs. GPC (%) and grain yield vs. protein yield also suggested that it is possible to combine high GPC due to the *Gpc-B1* with high yield. This is consistent with earlier reports that *Gpc-B1* has limited negative impact, if any, on wheat yield (Kade *et al*., 2005; Brevis and Dubcovsky, 2010). It may be speculated that these progenies with high GPC and no yield penalty may have an efficient nitrogen uptake and/or nitrogen re-mobilization from leaf and stem tissues contributing to grain development leading to breakage of the known negative correlation between grain yield and GPC.

The grain yield of the MAS-derived high GPC progenies was in the range of 5.32 to 6.10 t/ha, which is within the range of the wheat yields recovered in the experimental fields in India. The mean values of the plant height and yield contributing traits of the above seven MAS-derived progenies also did not differ from their respective recipient genotypes and together contributed to observed comparable grain yield in the MAS-derived progenies (For details see Kumar *et al*. 2011).

It may be recalled that the recovery of the genome of recipient parent in the seven selected lines was not as high as one would expect after three backcross generations. This is not surprising in view of the limited population size that was used as a trade-off due to limited resources, and the 10 backcross populations that we handled simultaneously. Further, each of the seven progenies having high GPC without any yield penalty had 72.00% to 95.71% of the genome of recipient parent suggesting that full restoration of the recipient genotype may not always be necessary, and that a restricted backcross breeding programme may be followed for the selection of the superior genotypes.

(iii) ***MAS for GW:*** In order to obtain wheat progenies with improved grain weight, we carried out MAS involving foreground selection in two backcross populations (involving Raj3765 and K9107 as the recurrent parents and Rye Selection 111 as the donor parent) over three generations (i.e. BC_1 to BC_3) using SSRs *wmc59* and *wmc24* associated with two separate QTLs for grain weight located on chromosome 1A (Mir *et al*. 2012). Following three cycles of foreground selection, 13 BC_3F_2 plants (seven in the background of Raj3765 and six in the background of K9107) with significantly higher grain weight (range 53.68g to 61.44g) than the respective recurrent parent genotypes and also the donor genotype (Rye Selection 111) were recovered suggesting successful application of MAS in improvement of grain weight in wheat. The higher grain weight (range 2.24% to 17.00%) of the selected progenies over the donor parent further suggested that the two introgressed QTLs (*QGw.ccsu-1A.1* and *QGw.ccsu-1A.3*) also had complimentary interaction with the background genotypes of the recurrent parents suggesting the importance of the two QTLs for grain weight in marker-assisted breeding for higher grain weight in wheat. The BC_3F_3 progenies derived from the above selected 13 BC_3F_2 plants will be tested for yield and yield related traits in replicated trials with a view to identify high yielding wheat progenies with bold grains.

Conclusions and Perspective

In this communication, we present summary of our studies on successful study of marker-trait association through both QTL interval and association mapping for quality traits. Major QTLs/genes are now available for all these quality traits on all the 21 chromosomes. Since several QTL studies are available for the same trait using different populations, an important exercise of meta-QTL analysis was also undertaken for PHST. In future we plan fine mapping of QTLs, which should give more reliable QTLs and associated markers for wheat molecular breeding and map-based cloning . We also plan to undertake joint linkage and association studies to obtain more robust markers associated with genuine QTL. Another desirable area of research at the international level would be genome-wide selection (GWS) or genomic selection (GS), which is yet to be tried at a large scale to become effective for wheat breeding.

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