QTL analysis and marker assisted selection for improvement in grain protein content and pre-harvest sprouting tolerance in bread wheat

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ABSTRACT

During the past 10 years, QTL analyses and marker assisted selection (MAS) have been conducted by us for improvement of grain protein content (GPC) and pre-harvest spouting tolerance (PHST) in bread wheat. A number of QTL that were identified by us for GPC and PHST included both main-effect and epistatic QTL (E-QTL). For GPC, a major QTL (*GPC-B1*) on chromosome 6B identified at the University of California (Davis), and for PHST, a major QTL (*QPhs.ccsu-3A.1*) on chromosome 3A that was identified by us and explained up to 70% phenotypic variation were used for MAS. Introgression of these two QTL into 10 Indian elite wheat cultivars including those carrying either single or a combination of *Lr* genes for leaf rust resistance was also attempted. During backcrossing programme, foreground selection was performed using markers flanking the QTL/*Lr* genes and the whole-genome background selection was performed using SSR and AFLP markers. Selection was exercised for reconstituted BC_3F_1 plants, which contained the QTL allele for high GPC/PHST as well as the *Lr*-gene(s) and exhibited high genetic similarity (up to 100%) with the recipient parent (RP). Phenotypically, these selected plants exhibited increased GPC (up to 1.72% higher than the RP genotypes) or high level of PHS tolerance. The selected plants are being advanced to BC_3F_2 and progenies homozygous for GPC/PHST QTL showing leaf rust resistance in laboratory tests will be evaluated in replicated field trials over environments.

INTRODUCTION

In bread wheat (*Triticum aestivum* L.), grain protein content (GPC), preharvest sprouting (PHS) and resistance to leaf rust are important traits. During past 10 years, detailed genetic studies (QTL analysis) for these traits have been conducted word-wide, which led to tagging/mapping of genes/QTL that explained large proportion of phenotypic variation[1-7]. However, it has been recognized that the power of QTL discovery can be substantially improved by making provision for the detection and estimations of interactions among loci (epistatis) and between genes/QTL and environment. Therefore, statistical methods are being regularly developed and improved for the study of these interactions[8]. In wheat, these improved methods have been used for identification of interacting QTL for several important traits[2].

In recent years, QTL analysis in wheat led to identification of markers linked closely with desirable alleles of QTL for a number of agronomic traits, and their role in improvement of these traits through MAS has been suggested[3, 5-7, 9]. For instance, using MAS, two nematode resistance genes, *CreX* and *CreY* have been pyramided in one background that showed higher level of resistance compared to lines, which had only one of the two genes introgressed[10]. In the present communication, we report briefly the results of QTL analysis and marker assisted selection for GPC and PHS conducted in our laboratory during the last 10 years.

MATERIAL AND METHODS

Plant material

The three mapping populations [PI: PH132 (high GPC) × WL711 (low GPC), PII: W7984 (synthetic wheat) × Optata 85 (cultivar) and PIII: SPR8198 (PHS tolerant) × HD2329 (PHS susceptible)] used in the present study were each evaluated in 4~6 different environments comprising locations and years. The phenotypic data on RILs, and the whole genome framework genetic maps prepared by us for PI and PIII and ITMImap for PII were used for conducting QTL analysis,.

QTL analysis

The main effect QTL (M-QTL) were identified by single-locus QTL analysis using QTL Cartographer. A LOD (logarithm of odds) score of 2.5 was used for suggesting the presence of a putative QTL. Threshold LOD scores, calculated using 1,000 permutations, were used for declaring definitive QTL. Two-locus analysis was conducted using QTLMapper/QTLNetwork Version 2.0 (Table 2). The relative contribution of a genetic component was calculated as the proportion of the phenotypic variance explained (PVE) by that component.

Marker assisted selection (MAS)

Genotype Yecora Rojo containing a major QTL for GPC (kindly provided by Jorge Dubcovsky, University of California, Davis, USA) was used as donor for transferring high GPC into 10 elite Indian bread wheat cultivars [K9107, PBW343, HI977, Raj3765, HD2329 (*Lr24* + *Lr28*), HD2687, PBW373, PBW343 (620), PBW343 (702), PBW343 (721)]. Genotype SPR8198 containing a major QTL for PHST was used as a donor for transferring PHST into the PHS susceptible recipient elite bread wheat cv. HD 2329 carrying *Lr24* and *Lr28*. Foreground selection for QTL for GPC/PHST and for two major genes for leaf rust resistance was carried out using linked markers reported elsewhere[3, 6, 7, 9]. For background selection, a total of 35 SSR markers (representing 52 polymorphic loci) and 1035 AFLP markers (889 polymorphic between GPC parents + 146 polymorphic between PHST parents) involving 12 primer pairs were used for rapid reconstitution of the recipient genotype during marker assisted backcrossing.

Recording of phenotypic data

The grain protein content (%) in dry grains (10% moisture content) of each sample was directly obtained using Infratech Grain Analyser. Data on PHS were scored on the scale of 1 through 9 with score of 1 for genotypes with no visible sprouting and score of 9 for genotypes with complete sprouting (for details, see 1).

RESULTS AND DISCUSSION

QTL analysis for GPC and PHST

Results of single-locus and two-locus QTL analyses for GPC and PHST are summarized in Table 1; some of these results were published earlier[2, 11]. For GPC, following single locus analysis, only one definitive QTL (*QGpc.ccsu-2D.7*) detected in more than one environment was detected[2], but using two-locus analysis, 26 QTL were detected (14 QTL in PI and 12 QTL in PII). These QTL included M-QTL, E-QTL, along with QE and QQE interactions. However, none of the QTL was common between the two populations. M-QTL had little contribution to the phenotypic variation (7.22% to 7.24%), while QE and QQE interactions had substantial proportion (25.91% in PI and 47.99% in PII). It was inferred that, although, improvement in GPC is possible without the concurrent loss in grain yield, the available QTL in hexaploid wheat, at best, may lead to only marginal improvement of GPC through marker assisted selection (MAS), since no more than one eighth (PII) to a quarter (PI) of the total variation is fixable.

QTL analysis for PHST was carried out using two mapping populations (PII and PIII). Although as many as 5 QTL were detected in PII, but none of them could be detected in all the four environments. These five QTL individually explained a phenotypic variation ranging from 8.12% to 17.39%. In PIII, a major QTL for PHST on chromosome arm 3AL (Figure 1a), explaining 24.68% to 35.21% variation in individual environments and 78.03% variation in pooled environments, was detected[3]. Positive QTL effect suggested that an allele of the above QTL for PHST was derived from PHS tolerant genotype SPR8198. In this population, a QTL for PHST on chromosome 2A (Figure 1b), was also detected that explained 45.11% PV in pooled environments. This QTL contributed negative effect suggesting that an allele of this QTL for PHST is available in the PHS susceptible parental genotype HD2329. The marker allele associated with QTL for PHST in SPR8198 is currently being exploited by us in MAS for transfer of the linked QTL allele into elite Indian bread wheat cultivar.

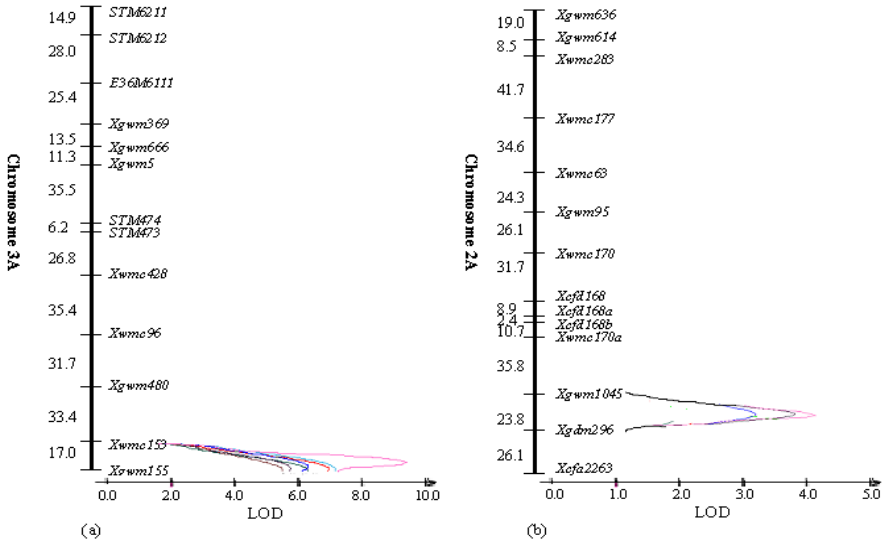

Figure 1. A QTL Cartographer plot for chromosome (a) 3A and (b) 2A obtained following composite interval mapping (CIM) for pre- harvest sprouting tolerance (PHST) in population PIII.

Table 1. A summary of the results of single- and two-locus QTL analysis in three mapping populations of bread wheat

Mapping population	Trait	Single-locus analysis		Two-locus analysis				
		No. of QTL identified (chromosome)	PVE (%)	M-QTL	PVE (%)	No. of digenic QQ epistasis interactions	PVE (%)	PVE (%) by QE & QQE interactions
PI	GPC	10 (2A, 2B, 2D, 3D, 4A, 6B, 7A)	2.95-32.44	5	7.24	2	2.68	25.91
PII	GPC	7 (1D, 2D, 2A, 5A, 3A, 7D)	8.38-16.58	5	7.22	3	6.04	47.99
PII	PHST	5 (2B, 2D, 3B, 3D, 3B)	8.12-17.39	8	47.95	4	28.73	-
PIII	PHST	7 (1A, 2A, 3A, 2D, 3B)	9.00-78.03	4	37.28	7	27.03	-

Marker assisted introgression of QTL for GPC

Most Indian bread wheat varieties have low to medium GPC (10.9% to 12.14%), and thus have poor nutritional value. Therefore, a major QTL (*GPC-B1*) for GPC was introgressed into 10 elite Indian bread wheat cultivars using marker assisted selection (MAS). A high GPC bread wheat genotype Yecora Rojo carrying *GPC-B1* was used as the donor parent. MAS was exercised using foreground and background selections. Foreground selection for *GPC-B1* QTL was carried out using an allele specific marker *Xuhw89*, which is tightly linked (0.1 cM) to the *GPC-B1* QTL. Background selection was carried out using SSR/AFLP markers. Selection led to the identification of BC_3F_1 plants carrying *GPC-B1* QTL, showing higher GPC (up to 1.72% higher than the recipient parent genotypes), and high genomic similarity (up to 100%) with the recipient parent genotype.

Marker assisted introgression of QTL for PHST

The desirable allele of the PHST QTL *QPhs.ccsu-3A.1* identified by us earlier was introgressed through MAS into elite but PHS susceptible Indian bread wheat cultivar HD2329, which also carried two alien leaf rust resistance genes (*Lr24* + *Lr28*) that were introgressed into this cultivar earlier using MAS. Foreground selection was performed using markers (*gwm155* and *wmc153)* flanking the QTL, and background selection for the whole-genome was performed using SSR/AFLP markers. The desirable alleles of the above two leaf rust resistance genes were also tracked in each backcross generation using linked SCAR markers earlier developed by us. In BC_3F_1 generation, the reconstituted plants were selected, which exhibited 94.3%-97.3% genetic similarity with the recipient bread wheat genotype and contained the QTL allele for PHST. Phenotypically, these selected plants exhibited high level of PHS tolerance (PHS scores ranged from 1 to 3). For both traits, plants selected through MAS are being advanced to BC_3F_2 and progenies homozygous for GPC/PHST QTL will be evaluated in replicated field trials over environments.

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