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QTL analysis for grain colour and pre-harvest sprouting in bread wheat

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ABSTRACT

A major objective in wheat breeding is the development of pre-harvest sprouting (PHS) tolerant wheat varieties with amber grain colour (GC), the latter being preferred both by the consumer in Asian markets, and by the processing industry globally. Quantitative trait loci (QTL) for these two traits were identified using a recombinant inbred line (RIL) population derived from an intervarietal cross, PH132 (red grain and PHS tolerant) × WL711 (amber grain and PHS susceptible). As many as 12 QTL for GC and 11 QTL for PHS were identified; 4 QTL for the two traits were co-localized. Most of the phenotypic variation (PV) for the two traits was explained by the main-effect QTL (M-QTL) having no interaction with environment, suggesting that selection may prove effective for improvement of both the traits. A major QTL for GC (PVE up to 40.42%), coincident with a minor QTL for PHS (PVE up to 8.10%), on the distal region of 3BL and a novel and major GC independent PHS QTL (PVE up to 29.47%) in proximal region of 6AL may prove useful for breeding PHS tolerant amber-grained wheat genotypes. Comparative genomic analysis revealed that the wheat genomic region carrying the major QTL for GC on 3BL is orthologous to a 1.63 Mb segment on rice chromosome 1, and the genomic region carrying the major QTL for PHS on 6AL is orthologous to a 5.47 Mb segment on rice chromosome 2. These rice genomic sequences may be exploited for fine mapping leading to map-based cloning of the above two major QTL, one each for GC and PHST.

1. Introduction

Pre-harvest sprouting (PHS) is a serious problem in wheat cultivation and leads to considerable damage to wheat grain in the event of rain just before the harvest. PHS is associated with increased α-amylase activity in the endosperm, which results in reduced yield due to harvest losses. In particular, it lowers the end-product quality of the grain. Therefore, incorporation of PHS tolerance (PHST) in high-yielding popular varieties has been a major objective for wheat breeders. However, PHST is often associated with red grain, which has relatively low acceptability among consumers, particularly in Asian markets, where products such as chapati, steamed bread and noodles are mostly consumed [1]. Amber wheat is also preferred for milling and for end-use quality in other parts of the world including USA [2]. Thus, while incorporating PHST in high-yielding cultivars, the breeder has to ensure that the associated red grain colour (GC) is not transferred; this has already been achieved in one reported case [3]. However, the genetic basis of the association between PHST and red GC is not fully understood and there is reason to believe that it is due to tight linkage, which can be broken [4–7].

In the past, both PHS and GC have been subjected to genetic studies in wheat. Several studies have shown that PHS is a complex trait, which is controlled by many genes/QTL identified on all the 21 wheat chromosomes, with group 3 and 4 chromosomes having major QTL [6–17]. QTL for PHS have also been identified on all chromosomes of rice and barley [18,19], suggesting a complex genetics of PHS in cereals. However, relative to PHS, only few studies have been conducted for GC. Three major loci for GC, named R_1, R_2 and R_3, were located cytogenetically on chromosome 3D, 3A and 3B, respectively [20–22]. Using diallel analysis, Reitan [23] suggested the presence of at least five different genes for GC. At least five loci for GC were also detected on five different chromosomes (3A, 3B, 3D, 5A and 7A) using QTL analysis [6,7,24]. The relationship between genetic systems controlling GC and PHS also suggested that group 3 QTL for GC and PHS are co-localized [6,7]. It is however apparent that association between PHST and red GC can be broken [3], if complete information on genetic systems controlling these two traits become available.

During the present study, a recombinant inbred line (RIL) population (designated as *PW*-population) derived from the cross PH132 (red-grained; PHS tolerant) × WL711 (amber-grained; PHS susceptible) was evaluated for GC and PHS in order (i) to identify

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unique QTL for each of these two traits, and (ii) to study digenic epistasis, and QTL × environment (QE) interactions involved in controlling these traits. In addition, attempts were made to identify rice genomic regions orthologous to major wheat QTL for GC and PHS that will help in high resolution mapping leading to map-based isolation of these QTL. A complete dissection of GC and PHS will eventually help in marker-aided development of amber wheats that are tolerant to PHS.

2. Materials and methods

2.1. Plant material and data recording

The *PW*-mapping population consisting of 100 recombinant inbred lines (RILs) derived from a cross PH132 × WL711 was available for this study. PH132 is a red-grained PHS tolerant line, and WL711 is an amber-grained, PHS susceptible cultivar. The mapping population along with the two parental genotypes was evaluated in replicated trials with simple lattice design at Meerut and Ludhiana (the two major wheat growing areas of Northern India) during 2003–2004 and 2004–2005, for both the traits. The population was evaluated for grain colour in four environments designated as I–IV (I = Meerut 2004, II = Meerut 2005, III = Ludhiana 2004 and IV = Ludhiana 2005) and for PHS in three of these (I–III) four environments.

Markers linked to the major QTL for GC and PHS were assigned to specific chromosome arms using nullisomic–tetrasomic (NT) and ditelosomic (DT) lines for 3B and 6A. Later, physical mapping of these linked markers to specific regions of the arms was achieved using deletion stocks for 3BL and 6AL [25]. The seed material of NT and DT lines was kindly provided by B.S. Gill, Kansas State University, Kansas, USA and the seed material of deletion stocks was kindly provided by T.R. Endo, Kyoto University, Japan.

For scoring GC, five spikes (per replication) from each of the two parents and from each of the 100 RILs were harvested as and when they reached physiological maturity. For scoring the data on grain colour, few seeds of each line were soaked in 5% (w/v) sodium hydroxide (NaOH) solution for 30–45 min in small Petri dishes and placed against a white background. Data on red/amber grain colour were scored visually, on a scale of 1 through 5, with score of 1 for genotypes with amber grain and a score of 5 for the genotypes with dark red grain colour.

For scoring PHS, at maturity, five random spikes from each genotype in each replication were harvested and immediately immersed in water for 4–6 h (see Ref. [11]). After immersion, the spikes were kept in the laboratory at room temperature on a 7.5 cm thick layer of moist sand covered with a double layer of moist jute bags. The spikes were sprinkled with water every 3–4 h to prevent drying. After 10 days, data on PHS were scored on the scale of 1–9 with a score of 1 for genotypes with no visible sprouting and a score of 9 for the genotypes with complete sprouting (modified after McMaster and Derera [26]).

2.2. Framework genetic map

A framework genetic map consisting of 173 loci was earlier prepared by Prasad et al. [27] using the same mapping population. In the present study, 47 new markers (genotype data provided by NCL, Pune, India), which included SSR, ISSR and RAPD markers, were added to this framework map while three previous markers were removed making the total number of mapped loci to 217.

DNA isolation and SSR analysis were performed following Prasad et al. [27]. The details of ISSR and RAPD markers used in the present study are reported in an earlier study by Dholakia et al. [28]. The new markers were integrated into the previous map [27] using MAPMAKER v. 2.0 computer program [29] based on Kosambi's mapping function [30] and using a minimum LOD score of 3.0.

2.3. Statistical analysis

Frequency distributions and mean values for GC and PHS of RILs in each case were obtained using the demo version of software package SPSS. Simple correlations and rank correlations were also obtained using the same software. Analyses of variances involving estimations of genotype × environment interactions were conducted using a statistical software package SPAR1 (IASRI, New Delhi) using genotypes as fixed effects and the other sources of variation as the random effects. Graphical genotypes were prepared using the software GGT (available at http://en.bio-soft.net/other/GGT.html). Single-locus QTL analysis for GC and PHS was carried out by composite interval mapping (CIM) using QTL Cartographer V2.5 [31]. A LOD score of 2.5 was used for suggesting the presence of putative QTL. The threshold LOD scores for detection of definitive QTL were also calculated based on 1000 permutations [32]. Confidence intervals (CI) were obtained using positions ± 1 LOD away from the peak. More than one QTL with overlapping CI were treated as one QTL. QTLNetwork 2.0 [33], which is based on mixed-model-based composite interval mapping (MCIM) [34,35], was used to conduct two-locus QTL analysis. This allows identification of QTL involved in main-effects (M-QTL), epistatic interactions (QQ or E-QTL) and environmental interactions (QE or QQE). A significance level of $P < 0.05$ was used to select associated markers and to declare putative M-QTL or E-QTL.

2.4. Comparative genomic analysis of major GC and PHS QTL with rice

The availability of large number of RFLP markers on *ITMI*-linkage map [24,36,37] provides a useful resource for comparative mapping with other members of grass family. The map used in the present study and the *ITMI*-linkage map were used for identification of rice genomic regions that are orthologous to wheat genomic regions containing major QTL detected in the present study. The nucleotide sequences for RFLP markers, ESTs and *TaDFR* gene [38] were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/). The nucleotide sequences for rice markers and BAC/PAC clones flanking the QTL for grain colour were obtained from Gramene (http://www.gramene.org/). Rice–wheat comparative genomic analysis was carried out using BLASTN. A significant match was declared when there was at least 80% nucleotide identity for not less than 100 bases and with an *e* value of less than e^{-20}.

3. Results and discussion

Although a number of studies have been conducted in wheat to understand the genetic architecture of PHS [6–12,15–17], attempts were rarely made to identify genomic regions/molecular markers associated with GC [6,7,24]. Also, the relationship between genetic systems controlling GC and PHS has been examined only sparingly [6,7]. In view of the above, the available *PW*-population was utilized for a study of the genetics of these two traits with the hope that new QTL and interactions may be discovered.

3.1. Phenotypic analyses

The parental genotypes of the *PW*-population differed significantly for GC and PHS (PH132: GC score = 5, PHS score = 1; WL711: GC score = 1, PHS score = 8). The mean scores for these two traits in RILs also differed significantly (Table 1) and showed continuous variation, although the mean scores for PHS were skewed towards PHS tolerance suggesting non-uniform distribution of loci controlling this trait. For each of the two traits, a few