J. Wheat Res. 3(1) : 33-36 (2011)

Molecular marker analysis reveals Yellow berry tolerance loci on chromosomes 2D and 5D in bread wheat (*Triticum aestivum L.*)

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Abstract

Yellow berry (YB) is a physiological kernel disorder in wheat, which manifests as yellow, mottled, non-vitreous and starchy kernels. It occurs due to deficient soil nitrogen levels and affects milling and end-product quality of wheat. Genetic control of this disorder and association of some of the chromosomes with YB tolerance has been shown previously. However, the location of genes associated with YB tolerance on such chromosomes was not known. In view of this, we developed a simple sequence repeat marker based linkage map of hexaploid wheat using a recombinant inbred line population that segregated for YB tolerance and mapped the YB-linked genes on two wheat chromosomes. A major gene associated with YB was mapped on the short arm of chromosome 5D. In addition, a minor locus on chromosome 2D was detected that showed 6.9% contribution to the phenotypic variance in YB. The markers associated with the major gene for YB could be used for developing better varieties of wheat by molecular breeding.

Key words: Starchy wheat, piebald wheat, mottled kernels, SSR, linkage mapping, QTL analysis

Received: 5 August 2011 / Accepted : 18 August 2011

Introduction

Yellow berry (YB) is a kernel disorder in wheat often associated with deficiency in nitrogen fertilization (Robinson *et al*., 1979; Anderson 1985; Gianibelli *et al*., 1990). Its symptoms are the presence of light yellow, opaque, soft and starchy kernels whose normal appearance should be hard, vitreous and translucent (Roberts and Freeman, 1908). Yellow berry is also manifested as piebald kernels due to starchy zones in them. These starchy zones are opaque, and due to the presence of air pockets in them, appear chalky when the kernels are sectioned (Dexter *et al*., 1989). Yellow berry has been reported in diploid (D'Edigio *et al*., 1993), tetraploid (Bnejdi and El Gazzah, 2008) and hexaploid wheats (Dhaliwal *et al*., 1986; Ammiraju *et al*., 2002) and is negatively correlated with grain protein content (Dhaliwal *et al*., 1981; Sharma *et al*., 1983; Ammiraju *et al*., 2002). The reduced protein content in YB wheat adversely affects its end use potential. According to Dexter and Matsuo (1981), the increase in relative starch content causes finer semolina granulation and increase in flour production during milling in YB durum wheat. Furthermore, the reduced protein content results in deterioration of spaghetti cooking quality. In case of Canadian western red winter wheat, the higher incidence of yellow berry kernels may reduce its market value to levels that are comparable to that of feed wheat (http://www.grainscanada.gc.ca). Though varietal differences in vitreousness in durum and bread wheat (Hadjichristodoulou 1979; Dhaliwal *et al*., 1986, Gupta *et al.* 2008) are reported, the knowledge about the genetic basis of YB tolerance is minimal.

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Previously, Dhaliwal *et al.* (1986) evaluated the inheritance of YB in six intervarietal hexaploid wheat crosses and found the trait to be controlled by either two or three dominant genes. By monosomic studies, the presence of two major dominant genes on chromosomes 1A and 7A and four modifiers on 4A, 4B, 6A and 6D was indicated. Using PCR-based markers, Ammiraju *et al.* (2002) reported two simple sequence repeat (SSR) markers on chromosome 5D; an ISSR and a RAPD marker, both mapped on 6B, to be associated with YB tolerance. In durum wheat, resistance to YB was investigated using generation mean analysis in four resistant or intermediate-resistant × susceptible crosses and a complex gene action controlling this trait with additive, dominance and epistatic effects was suggested (Bnejdi and El Gazzah, 2008). In addition to this, cytoplasmic effects on resistance to YB in durum wheat have also been reported (Bnejdi *et al*., 2010). In view of the paucity of information on the genetic basis of YB tolerance in wheat, the information on the precise chromosomal locations of loci contributing to this trait was needed. Hence, we performed genetic analysis of YB tolerance in hexaploid wheat with the help of an SSR marker based linkage map of a recombinant inbred line (RIL) population that showed variation for YB tolerance and identified a major locus on chromosome 5D and a minor locus on chromosome 2D for tolerance to YB.

Materials and Methods

Plant material and phenotypic evaluation

A population of 185 RILs derived from the cross of the hexaploid wheat genotypes, Rye Selection 111 (YB tolerant) and Chinese Spring (YB susceptible), was used for the phenotypic analysis and construction of the SSR linkage map. The parental genotypes and the RIL population were grown in a randomized block design with two replications under a low nitrogen input at the vegetative stage (60 kg

N/ha) as suggested by Dhaliwal *et al.* (1981) to allow the YB trait to express in such nitrogen stress conditions. The kernels from parental genotypes and RILs were inspected visually and the number of YB kernels (yellow or mottled) was recorded. YB phenotypic data were recorded as percentage of YB kernels for each of the parental genotypes and the RILs.

Molecular marker analyses

The RILs were genotyped with 193 polymorphic SSR markers and a linkage map consisting of 169 markers representing twelve wheat chromosomes, 1A, 1B, 1D, 2B, 2D, 4B, 5A, 5B, 5D, 6B, 7A and 7B, was constructed (Ramya *et al*., 2010). This linkage map spanned 1020.75 cM with the average marker interval of 6.04 cM. Linkage analysis of YB was performed using the SSR marker and YB segregation data of the RIL population with the software JoinMap v. 4.0 (Van Ooijen 2006). The SSR markers and YB were initially grouped with LOD thresholds from 3.0 to 5.0, and after inspecting the grouping results, the final grouping was done using a minimum conservative LOD threshold of 4.0. During grouping, marker linkages with recombination frequency <0.45 were used. The "goodness-of-fit" threshold for locus removal was set to 5.0; the "ripple" command was used each time after adding a locus to a linkage group and three "mapping rounds" were performed for each linkage group. The Kosambi mapping function (Kosambi, 1944) was used to calculate the map distances. QTL analysis was conducted using QTLNetwork v. 2.0 in the framework of mixed linear model (Wang *et al*., 1999; Yang *et al*., 2007 & 2008). During one dimensional (1D) genome scans, a walk speed of 1 cM, testing window size of 10 cM and filtration window of 10 cM were set. During the analyses, *F*-statistic was computed and the critical *F* value was calculated by 1000-permutation tests (Doerge and Churchill, 1996) with $P \leq 0.05$ as experiment-wise significance level for candidate interval selection, putative QTL detection and determination of QTL effects. MapChart v. 2.0 (Voorrips, 2002) was used for graphical representation of the linkage map.

Results and Discussion

RIL frequency distribution suggested YB determination by major gene(s)

Evaluation of the parental genotypes showed higher tolerance to YB by Rye Selection 111 (RS) with only 10% of the kernels showing the phenotype. In contrast, Chinese Spring (CS) kernels showed 100% incidence of YB. Among the RILs, 39.2% displayed greater tolerance with only 0-10% of the kernels indicating YB (Figure 1). On the contrary, a high degree of YB incidence with 90-100% YB kernels was shown by 31.4% of the RILs. The remaining RILs (29.4%) displayed continuous variation and showed 10-90% YB incidence. The frequency distribution, displaying two major classes of tolerant and susceptible RILs, along with many minor classes with for intermediate phenotype, suggested the involvement of major genes with few modifiers. Similar observation of the involvement of a small number of genes in determining YB was also made by Dhaliwal *et al.* (1986) and Bnedji and El Gazzah (2008). Since the RILs with 0-10% YB showed increased tolerance similar to the parent RS, they were designated by the parental genotype 'a' and the highly susceptible RILs with 90-100% YB were scored as 'b', similar to CS. The RILs showing intermediate YB phenotype of 10-90% were scored as missing data '-'. The ^{2} test of goodness of fit suggested a good fit to the 1:1 (a:b) segregation ratio ($^2 = 1.5$).

Chromosome 5D harbors a major gene for YB

Linkage analysis using the SSR markers and YB segregation data in the RILs mapped the major YB locus on distal end of 5DS (Figure 2). The closest marker was Xcfd18 at a distance of 5.26 cM. To test the reliability of the linkage of YB at this position, linkage analysis was performed at progressively higher LOD values and the linkage was intact up to the LOD value of 19.5. Previously, the association of YB with Xgwm190 was reported using single marker analysis with 113 RILs of the RS×CS population (Ammiraju *et al.* 2002). In the present study, we have clearly demonstrated the position of the YB locus on chromosome 5DS using a linkage map of SSR markers using a larger RIL population and at very high LOD value. The short arm of the chromosome 5D harbors the well-studied Ha locus associated with the determination of kernel texture (Bhave and Morris 2008). However, the deletion bin locations of Xcfd18 and Xgwm190 on 5DS are 5DS1-0.63-0.67 and C-5DS1-0.63, respectively, while the Ha locus is in the most distal 5DS deletion bin, 5DS2-0.78-1.00 http://wheat.pw.usda.gov/ggpages/ SRclub/ GeneticPhysical/). Hence, we expect that the Ha locus is present at the distal end of 5DS, after the YB locus, although we could not conclusively demonstrate this as we have not evaluated kernel texture in this study.

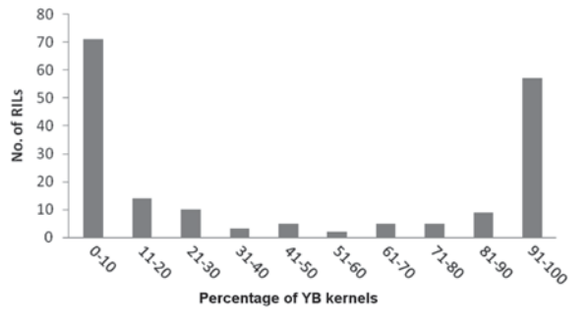

Fig. 1 Frequency distribution of Yellow Berry in the RILs of the Rye Selection 111 × Chinese Spring population.

A minor QTL on Chromosome 2D for YB tolerance

Though the number of RILs displaying intermediate YB phenotype of 10-90% was much smaller (29.4%) than the tolerant and susceptible RILs (70.6%), their distribution showed continuous variation. This suggested the presence of modifier genes with minor effects in addition to the major locus on 5DS in the expression of YB. In earlier reports,

Dhaliwal *et al.* (1986) too suggested the presence of minor genes or modifiers in the control of YB. Ammiraju *et al.*

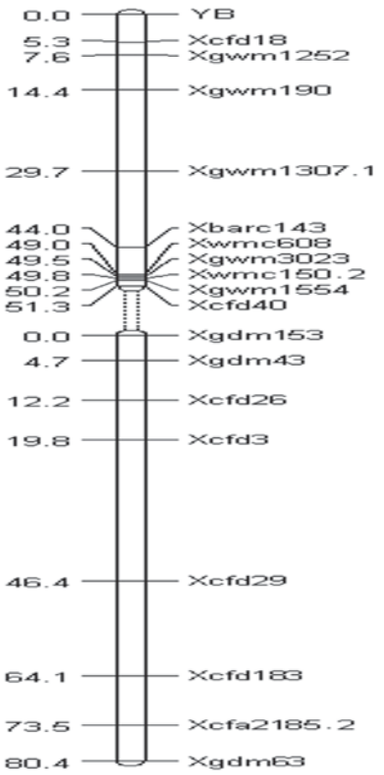

Fig. 2 Linkage map of chromosome 5D in Rye Selection 111 × Chinese Spring population showing the YB locus. Short arm is towards the top.

(2002) identified marker-trait associations on chromosome 6B with approximately 4.5% contribution to YB. In durum wheat, epistasis was suggested in addition to additive and dominance effects (Bnedji and El Gazzah, 2008). Hence, QTL analysis with the software QTLNetwork was employed utilizing the percentage of YB kernels recorded for the RILs, in order to identify the QTLs and possible epistatic effects for YB. Similar to linkage analysis, QTLNetwork showed the association of YB with 5DS (Figure 3a). The closest marker was *Xgwm1252* with 36.8%contribution to YB variance. In addition, a QTL on chromosome 2D was identified with *Xgwm539* as the closest marker, which explained 6.9% contribution to YB variance (Figure 3b). Both these regions on chromosomes 2D and 5D showed main-effect QTLs with significant individual effects and epistatic interactions for YB were not detected. It is interesting to note that both the QTLs for YB identified in the present study are located on the D genome and hence, the genes conferring tolerance to YB in durum and diploid wheats could be entirely different from the presently identified loci.

In this study, we present for the first time, the molecular genetic mapping of a major locus for YB in hexaploid wheat. The information revealed by the present study will be useful for the better understanding of this physiological disorder and for breeding superior wheat varieties with increased tolerance to YB by marker assisted selection.

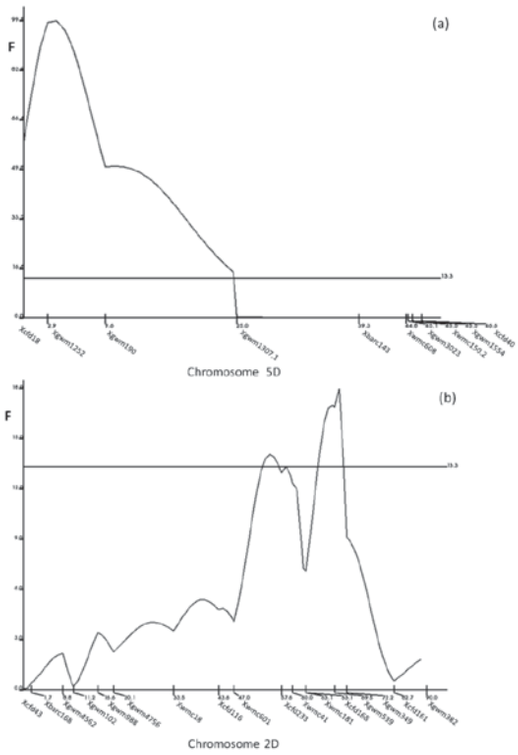

Fig. 3 *F*-statistic plots of the linkage groups representing chromosome 5DS (a) and 2D (b) displaying QTLs for YB tolerance.

Acknowledgements

We thank the Department of Biotechnology, India, for financial assistance for the project. We also thank the Leibniz Institute of Plant Genetics and Crop Plant Research and TraitGenetics, Germany, for 21 *gwm* primers and Prof. MD Gale for *psp* primers. RP acknowledges the Council of Scientific and Industrial Research, India, for Research Fellowship.

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