Identification of a Microsatellite on Chromosomes 6B and a STS on 7D of Bread Wheat Showing Association with Preharvest Sprouting Tolerance

With regards
Manoj Prasad
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Abstract In bread wheat, transfer of tolerance to preharvest sprouting (PHS) associated with genotypes having red kernel colour, to genotypes with amber kernels is difficult using conventional methods of plant breeding. Therefore, the present study was undertaken to identify DNA markers linked with tolerance to PHS. Such markers would allow indirect marker assisted selection of PHS tolerant genotypes with amber kernels. For this purpose, a set of 100 RILs were developed using a cross between a PHS tolerant genotype SPR8198, with red kernels and a PHS susceptible cv. HD2329 with white kernels. The two parents were analysed with 232 STMS (sequence-tagged-microsatellite site) and 138 STS (sequence-tagged site) primer pairs. A total of 300 (167 STMS and 133 STS) primer pairs proved functional giving scorable PCR products. Of these 57 (34%) STMS and 30 (23%) STS primer pairs detected reproducible polymorphism between the parent genotypes. Using these primer pairs, bulked segregant analysis was carried out on two bulked DNAs, one obtained by pooling DNA from five PHS tolerant RILs and other similarly derived by pooling DNA from five PHS susceptible RILs. Two molecular markers including one STMS primer pair for the locus *wmc104* and the other STS primer pair for the locus *MST101* showed apparent linkage with tolerance to PHS. This was confirmed following selective genotyping of individual RILs included in the bulks. Chi-square contingency tests for independence were conducted on the cosegregation data collected on 100 RILs involving each of the two molecular markers (*wmc104* and *MST101*) and PHS. The tests revealed strong association between each of the markers and tolerance to PHS. Using nullisomic-tetrasomic lines, *wmc104* and *MST101* were assigned to chromosomes 6B and 7D, respectively. The results also indicated that the

tolerance to PHS in SPR8198 is perhaps governed by two genes (linked with two molecular markers) exhibiting complementary interaction.

Key words Preharvest sprouting. Microsatellite. STMS. STS. Linkage. Bread wheat

Introduction

In wheat, preharvest sprouting (PHS) of grains in spike reduces grain yield, and leads to deterioration in the grain milling and baking quality (Varughese et al. 1987). The problem is wide-spread in major wheat growing areas of the world including those in India (Sharma et al. 1994; Iordanskaya and Pukhalskiy 1998). Tolerance to PHS is known to be associated with red kernels (Nilsson-Ehle 1914; Gfeller and Svejda 1960; Derera 1973), while susceptibility is associated with the white kernels (Derera et al. 1977), that are preferred by the consumers in the Indian sub-continent. This association between PHS and kernel colour may be either due to tight linkage between genes affecting the two traits or due to a pleiotropic effect of the genes for kernel colour (DePauw and McCaig 1983; Soper et al. 1989; McCaig and DePauw 1992). Attempts to break this association between red kernel colour and tolerance to PHS have only been partially successful (Noll et al. 1982; DePauw and McCaig 1983; McCaig and DePauw 1992; Sharma et al. 1994). Therefore, the transfer of tolerance to PHS from the germplasm with red kernels to that with amber kernels has been difficult.

At the biochemical level, higher level of tolerance to PHS in wheats with red kernels has been attributed variously to hypersensitivity of developing embryos to

abscisic acid (Walker-Simmons 1987), reduced levels of alpha-amylase in the grain (Bhatt et al. 1976), presence in the bracts of compounds inhibiting germination (Derera and Bhatt 1980) and slower water-uptake (King 1984). At the genetic level, inheritance of tolerance to PHS has been reported to be controlled either by minor polygenes (QTLs) or by major gene(s). For instance, Hagemann and Ciha (1987) and Anderson et al. (1993) showed that tolerance to PHS is expressed as a quantitative character, influenced by environment as well as by genotype × environment interaction. However, in another set of studies, tolerance to PHS has been reported to be controlled either by two (Bhatt et al. 1983) or by one (Mares and Ellison 1990; Iordanskaya and Pukhalskiy 1998) recessive gene(s). In still other studies, tolerance to PHS was shown to be controlled either by a single dominant gene, as shown in two wheat genotypes with red kernels (Sharma et al. 1994) or by partial dominance, as reported both in genotypes with red kernels (Noll et al. 1982) and in those with white kernels (Paterson and Sorrells 1990; Guo-Liang et al. 1997).

Screening for tolerance to PHS in segregating populations during breeding exercises has been difficult. Therefore, identification of DNA markers linked to PHS tolerance should facilitate indirect marker aided selection of genotypes which are amber grained and tolerant to PHS. In view of this, the present study was undertaken for tagging gene(s) for tolerance to PHS using STMS and STS markers. One microsatellite marker on chromosome 6B and another STS marker on chromosome 7D were identified showing strong association with tolerance to PHS. The results of this study are presented in this communication.

Materials and methods

Plant material

A mapping population in the form of RILs (F_6 lines), developed from the cross SPR8198 × HD2329, following single seed descent (SSD), was utilized. The genotype SPR8198 was tolerant to PHS and had red kernels (Sharma et al. 1994), while HD2329, a widely grown cultivar in India, was susceptible to PHS and had amber kernels.

Evaluation for PHS

The parent genotypes as well as RILs were raised at Punjab Agricultural University, Ludhiana. At the time of maturity, five spikes of each genotype were harvested. The harvested spikes were scored for tolerance using the laboratory test of Baier (1987). The spikes were immersed in water for 4-6 h and were then kept in laboratory at room temperature on a 7.5 cm thick layer of moist sand covered with a double layer of moist jute bags. To prevent drying, the spikes were sprinkled with water every 2-3 h. Observations were recorded after 10 days following complete sprouting in the susceptible parent HD2329. Lines with no visible signs of sprouting of grains in spikes were scored as tolerant.

DNA isolation

DNA was isolated from 10-15 days old seedlings raised in a growth chamber using a modified CTAB method (Weising et al. 1995).

STMS Primers

A set of 232 STMS primer pairs were made available to us as a member of Wheat Microsatellite Consortium (WMC) under an international collaborative project. These STMS primers were designed using DNA sequences of clones containing microsatellites. The genomic clones were isolated from a microsatellite rich library (Edwards et al. 1996) and were sequenced by members of WMC.

STS Primers

A set of 138 STS primer pairs derived from genomic and cDNA clones of barley, wheat, oat and *Triticum tauschii* were developed and synthesized at Montana State University, Bozeman, USA.

PCR - amplification

DNA amplification was carried out in Perkin-Elmer Thermal Cycler 4800 using 25µl (STMS)/50µl (STS) reaction mixtures, each containing 100ng template DNA, 2µM (STMS)/0.6µM (STS) primers, 200µM each of the dNTPs, 2.5mM (STMS)/1.5mM (STS) $MgCl_2$, 1×PCR buffer and 2U of Stoffel fragment (Perkin Elmer). For STMS-PCR, the following profile was followed: initial denaturation at 95^0C for 5 min, followed by 40 cycles of 95^0C for 60 sec, 51^0C/61^0C for 60 sec, 72^0C for 60 sec with a ramp at the rate of 0.5^0C/sec and final extension at 72^0C for 5 min. The PCR profile for STS was similar except that the following thermal cycle was used: 94^0C for 78 sec, 50^0C for 78 sec, 72^0C for 2 min. The STMS amplification products were resolved on 10% polyacrylamide

denaturing gels following silver staining (Tegelstrom 1992), while the STS amplified products were visualized on 2% agarose gels following ethidium bromide staining.

Linkage analysis

All the STMS and STS primer pairs showing polymorphism between parent genotypes were used for genotyping the RILs. Data recorded from amplification profiles using each primer pair and data on PHS on the RILs was analysed using χ^2 - contingency test for independence to identify the primer(s) showing association with tolerance to PHS.

Chromosome assignment of STMS and STS markers

Assignment of identified markers to specific chromosomes was done through PCR amplification using template DNA from each of the 21 nullisomic-tetrasomic lines.

Results and Discussion

Polymorphism between parent genotypes

A total of 232 STMS and 138 STS primers were used on two parent genotypes, namely SPR8198 (tolerant to PHS) and HD2329 (susceptible to PHS) to detect polymorphism. Of the above primers, 167 STMS and 133 STS primers (a total of 300 primers) proved to be functional primers giving scorable amplification products. Of these 300 functional primers, 57 (34%) STMS and 30 (23%) STS primers detected reproducible polymorphism between the two parent genotypes. This was considered encouraging in view of the reports of the detection of low level of polymorphism in bread wheat using

other molecular markers including RFLPs, RAPDs and oligonucleotide in-gel hybridization (Chao et al. 1989; Kam-Morgan et al. 1989; Liu et al. 1990; Devos and Gale 1992; Cadalen et al. 1997; Varshney et al. 1998). The results of the present study and those of other studies thus clearly indicate that in bread wheat, STMS (Plaschke et al. 1995; Roder et al. 1995; Ma et al. 1996; Bryan et al. 1997; our unpublished results) and STS markers are more informative than RFLPs (detecting polymorphism at <10% loci; see Roder et al. 1998) and RAPDs (detecting polymorphism at 3.3% loci; Penner et al. 1995).

Linkage of PHS tolerance with markers

Fifty seven (57) STMS and 30 STS primers detecting polymorphism between the parent genotypes were used for conducting bulked segregant analysis (Michelmore et al. 1991) using two bulked DNAs, one obtained by pooling DNA from five PHS tolerant RILs and the other similarly obtained from five PHS susceptible RILs. Among all the primers used with the two bulk DNAs, only one STMS primer pair (*wmc104*) and one STS primer pair (*MST101*) exhibited apparent association with tolerance to PHS. Two PCR amplified products that were obtained using *wmc104* in the tolerant parent (SPR8198) were ~140 and ~160 bp in length and those obtained in the susceptible parent (HD2329) were ~150 and 170 bp in length. The PCR products obtained using *MST101* STS primers gave several bands on agarose gel, but only a solitary band representing a product of 400 bp in length in the tolerant parent (SPR8198) and 425bp in length in susceptible parent (HD2329) was consistently polymorphic. These primers when used with the PHS tolerant bulk DNA, gave characteristic amplification profiles of the tolerant parent (Figs.

1 and 2). With PHS susceptible bulk DNA, although STMS primer pair *wmc104* gave characteristic amplification profile of the susceptible parent, such a consistent pattern was not observed with STS primer pair *MST101*, which gave PCR products characteristic of both the parents. The expected association of these two markers with tolerance to PHS was confirmed using selective genotyping of individual RILs belonging to the two bulks. Using *wmc104* primer pair, the results revealed that four out of five RILs each belonging to each of the two bulks showed amplification profiles characteristic of the corresponding parents indicating an association between *wmc104* and tolerance to PHS. With *MST101* STS primer pair, on the other hand, four out of five RILs belonging to the tolerant bulk showed characteristic amplification profile of the tolerant parent and two RILs out of five showed characteristic amplification profile of the susceptible parent.

A set of 100 RILs were also genotyped using the above two primer pairs. The segregation data on PHS and the data on PCR products obtained with each of the two primer pairs were separately analysed using chi-square contingency test for independence to find out the association between tolerance to PHS and each of the two markers. The chi-square value for independence involving PHS with *wmc104* (χ^2=19.07; p<0.0001) and that involving PHS with *MST101* (χ^2=17.36; p<0.0001) were both highly significant. This suggested that *wmc104* and *MST101* are strongly associated with PHS.

The details of the sequences of *wmc104* primer pair (designed by WMC on the basis of sequence provided by G. Penner, Agriculture and Agri-Food, Winnipeg, Canada) and *MST101* primer pair (Blake et al. 1996) are as follows: (i) *wmc104*: Forward primer: 5'-TCTCCCTCATTAGAGAGTTGTCCA-3'; Reverse primer: 5'-ATGCAAGT

TTAGAGCAACACCA-3', (ii) *MST101*: Forward primer: 5'-CCACCATGAAGACCTT CCTC-3'; Reverse primer: 5'-ACC TTGCATGGGTTTAGCT G-3'.

Chromosome assignment of STMS and STS markers

Chromosomal assignment of each of the two markers linked with PHS was done using template DNA from 21 nullisomic-tetrasomic lines. *wmc104* and *MST101* markers were thus assigned to chromosomes 6B and 7D, respectively indicating the presence of at least two major genes for tolerance to PHS, one each on 6B and 7D. However, in an inheritance study involving the cross SPR8198 × WL711 (Sharma et al. 1994), the tolerance to PHS in SPR8198, was shown to be controlled by one dominant gene. It is possible that the above two genes in SPR8198 for tolerance to PHS detected during the present study are complementary genes and that WL711 differs only at one locus, carrying at the other locus the same allele which is present in SPR8198. Evidence is available from the previous studies that one (Mares and Ellison 1990; Iordanskaya and Pukhalaskiy 1998) or two (Bhatt et al. 1983) major genes control the tolerance to PHS in wheat. However, Hagemann and Ciha (1987) and Anderson et al. (1993) showed that tolerance to PHS is expressed as a quantitative character, influenced by environment and genotype × environment interaction. Through RFLP analysis, Anderson et al. (1993) identified eight regions of wheat genome showing significant association with resistance to PHS. One of these regions similar to the findings of the present study was located on the long arm of 6B identified by RFLP marker Xcnl.BCD1426. This region on 6BL was shown to account for 10.7% of the phenotypic variance due to PHS. At present, it is

difficult to say if the RFLP marker Xcnl.BCD1426 and STMS marker *wmc104* fall in the same region of chromosome 6B.

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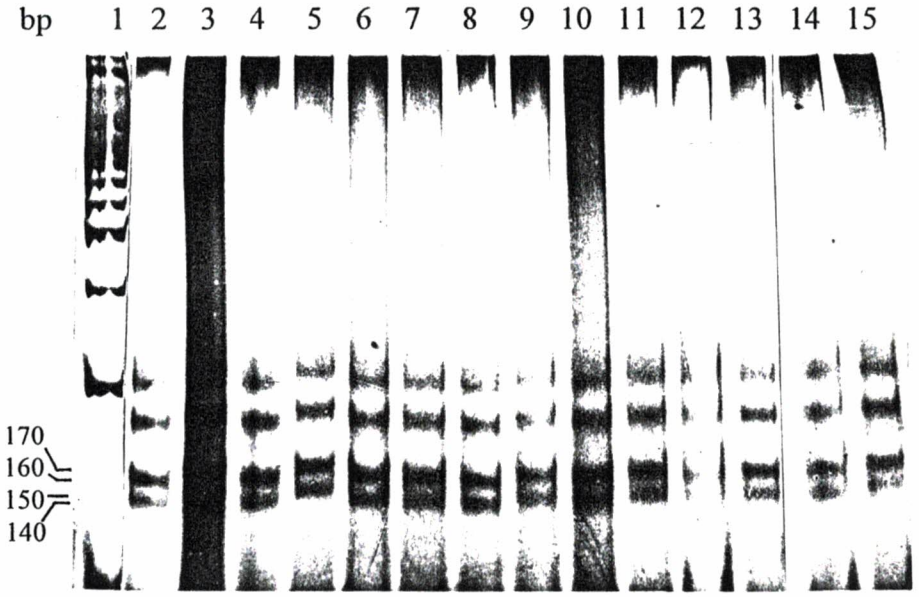

Fig. 1

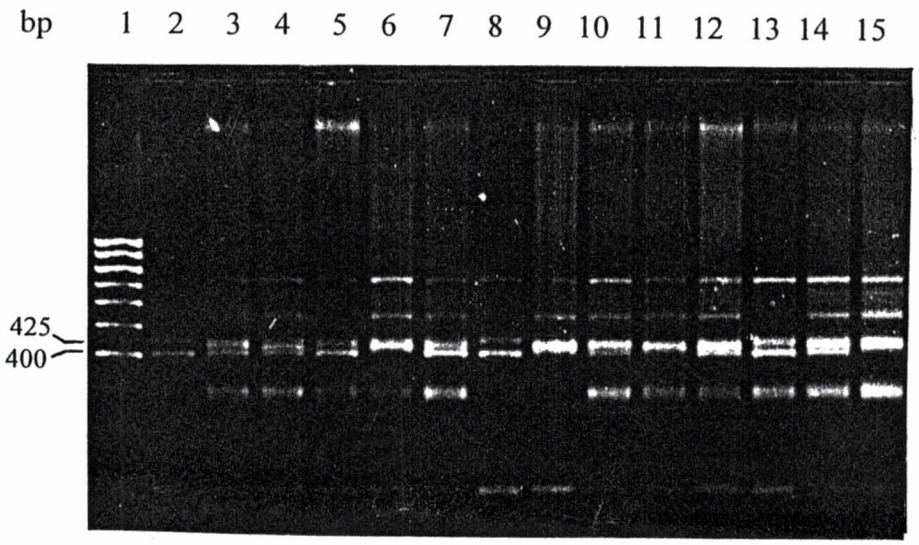

Fig. 2

Legend to figures

Fig. 1 Selective genotyping of RILs, used in bulked segregant analysis, with STMS *wmc104* (lane 1 = 100 bp ladder DNA marker; 2, 3 = parents SPR8198 (tolerant) and HD2329 (susceptible); 14, 15 = bulked segregants tolerant and susceptible to PHS; 4 - 8 = RILs tolerant to PHS; 9 - 13 = RILs susceptible to PHS)

Fig. 2 Selective genotyping of RILs, used in bulked segregant analysis, with STS *MST101* (lane 1 = 100 bp ladder DNA marker; 2, 9 = parents SPR8198 (tolerant) and HD2329 (susceptible); 3, 10 = bulked segregants tolerant and susceptible to PHS; 4 - 8 RILs tolerant to PHS; 11 - 15 = RILs susceptible to PHS)