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# Identification of a microsatellite on chromosomes 6B and a STS on 7D of bread wheat showing an association with preharvest sprouting tolerance

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**Abstract** In bread wheat, the transfer of tolerance to preharvest sprouting (PHS) that is associated with genotypes having red kernel colour to genotypes with amber kernels is difficult using conventional methods of plant breeding. The study here was undertaken to identify DNA markers linked with tolerance to PHS as these would allow indirect marker-assisted selection of PHS-tolerant genotypes with amber kernels. For this purpose, a set of 100 recombinant inbred lines (RILs) was developed using a cross between a PHS-tolerant genotype, SPR8198, with red kernels and a PHS-susceptible cultivar, '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 STMSs and 133 STSs) primer pairs proved functional by 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, we carried out bulked segregant analysis on two bulked DNAs, one obtained by pooling DNA from 5 PHS-tolerant RILs and the other similarly derived by pooling DNA from 5 PHS-susceptible RILs. Two molecular markers, 1 STMS primer pair for the locus *wmc104* and a 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 a strong association between each of the markers and tolerance to PHS. Using nullisomic-tetrasomic lines, we were able to assign *wmc104* and *MST101* 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

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## Introduction

In wheat, preharvest sprouting (PHS) of grains in the 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 white kernels (Derera et al. 1977), which are preferred by consumers in the Indian sub-continent. This association between PHS and kernel colour may be due either 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 germplasm with red kernels to that with amber kernels has been difficult.

At the biochemical level, the higher level of tolerance to PHS in wheats with red kernels has been attributed variously to the hypersensitivity of developing embryos to abscisic acid (Walker-Simmons 1987), reduced levels of alpha-amylase in the grain (Bhatt et al. 1976), the presence of compounds in the bracts that inhibit 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 (quantitative trait loci, 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 that is influenced by the environment as well as by genotype × environment interaction. However, in another set of studies, tolerance to PHS was reported to be controlled by either two (Bhatt et al. 1983) or 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, the 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 study presented here was undertaken for tagging gene(s) for tolerance to PHS using sequence-tagged microsatellite site (STMS) and sequence-tagged site (STS) markers. One microsatellite marker on chromosome 6B and another STS marker on chromosome 7D were identified as showing strong association with tolerance to PHS.

## Materials and methods

### Plant material

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

### Evaluation for PHS

Both the parent genotypes and 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 then 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 2–3 h to prevent them from drying out. Observations were recorded after 10 days following complete sprouting in the susceptible parent 'HD2329'. Lines with no visible signs of sprouting of grains in the spikes were scored as tolerant.

### DNA isolation

DNA was isolated from 10- to 15-day-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 the 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 the 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.

### Polymerase chain reaction (PCR) amplification

DNA amplification was carried out in a Perkin-Elmer Thermal Cycler 4800 using 25-µl (STMS)/50-µl (STS) reaction mixtures, each containing 100 ng template DNA, 2 µ*M* (STMS)/0.6 µ*M* (STS) primers, 200 µ*M* each of the dNTPs, 2.5 m*M* (STMS)/1.5 m*M* (STS) $MgCl_2$, 1 × PCR buffer and 2 U of Stoffel fragment (Perkin Elmer). For STMS-PCR, the following profile was followed: initial denaturation at 95°C for 5 min; followed by 40 cycles of 95°C for 60 s, 51°C/61°C for 60 s, 72°C for 60 s with a ramp at the rate of 0.5°C/s, and a final extension at 72°C for 5 min. The PCR profile for STS was similar except that the following thermal cycle was used: 94°C for 78 s, 50°C for 78 s, 72°C 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 parental 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 $\chi^2$-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 accomplished through PCR amplification using template DNA from each of the 21 nullisomic-tetrasomic lines.

## Results and discussion

### Polymorphism between parental genotypes

A total of 232 STMS and 138 STS primers were used on two parental 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 parental genotypes. This was considered encouraging in view of the reports of the detection of low levels of polymorphism in bread wheat using other molecular markers including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (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; Röder 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 Röder 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 parental genotypes were used for conducting bulked segregant analysis (Michelmore et al. 1991) using two bulked DNAs, one obtained by pooling DNA from 5 PHS-tolerant RILs and the other similarly obtained from 5 PHS-susceptible RILs. Of all the primers used with the two bulk DNAs, only 1 STMS primer pair (*wmc104*) and 1 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 approximately 140 bp and 160 bp in length, and those obtained in the susceptible parent ('HD2329') were approximately 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 425 bp in length in the 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 the PHS-susceptible bulk DNA, STMS primer pair *wmc104* gave a characteristic amplification profile of the susceptible parent, but such a consistent pattern was not observed with STS primer pair *MST101*, which gave PCR products characteristic of both 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 the *wmc104* primer pair, we observed that 4 out of 5 RILs from each of the two bulks showed amplification profiles characteristic of the corresponding parents, indicating an association between *wmc104* and tolerance to PHS. With the *MST101* STS primer pair, on the other hand, 4 out of 5 RILs belonging to the tolerant bulk showed the characteristic amplification profile of the tolerant parent and 2 RILs out of 5 showed the characteristic amplification profile of the susceptible parent.

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

The details of the sequences of the *wmc104* primer pair (designed by WMC on the basis of the sequence provided by G. Penner, Agriculture and Agri-Food, Winnipeg, Canada) and *MST101* primer pair (Blake et al. 1996) are as follows: (1) *wmc104*: forward primer: 5′- TCTCCCTCATTAGAGAGTTGTCCA-3′; reverse primer: 5′-ATGCAAGTTTAGAGCAACACCA-3′, (2) *MST101*: forward primer: 5′-CCACCATGAAGA CCTTCCTC-3′; reverse primer: 5′-ACCTTGCAT-GGGTTTAGCTG-3′.

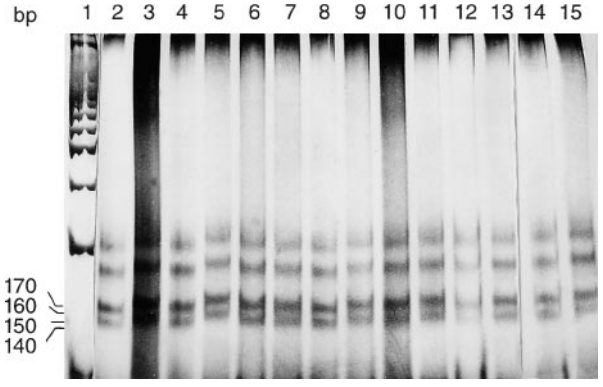


**Fig. 1** Selective genotyping of the RILs used in bulked segregant analysis with STMS *wmc104. Lane 1* 100-bp ladder DNA marker, *lanes 2, 3* parents SPR8198 (tolerant) and HD2329 (susceptible), *lanes 14, 15* bulked segregants tolerant and susceptible to PHS, *lanes 4–8* RILs tolerant to PHS, *lanes*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

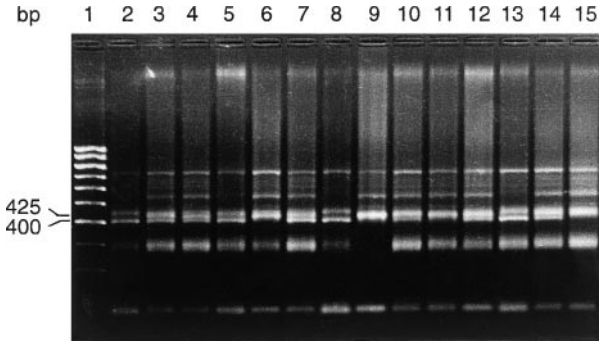


**Fig. 2** Selective genotyping of RILs used in bulked segregant analysis with STS *MST101. Lane 1* 100-bp ladder DNA marker, *lanes 2, 9* parents SPR8198 (tolerant) and HD2329 (susceptible), *lanes 3, 10* bulked segregants tolerant and susceptible to PHS, *lanes 4–8* RILs tolerant to PHS, *lanes 11–15*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

## Chromosome assignment of STMS and STS markers

Chromosomal assignment of each of the 2 markers linked with PHS was accomplished 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), tolerance to PHS in SPR8198 was shown to be controlled by one dominant gene. It is possible that the two genes for tolerance to PHS detected during the present study are complementary genes in SPR8198 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 previous studies that one (Mares and Ellison 1990; Iordanskaya and Pukhalaskiy 1998) or two (Bhatt et al. 1983) major genes control 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 that is influenced by the environment and genotype × environment interaction. Through RFLP analysis, Anderson et al. (1993) identified eight regions of the wheat genome showing significant association with resistance to PHS. One of these regions, identified by RFLP marker *Xcnl.BCD1426*, was located on the long arm of 6B, similar to the findings of the present study. 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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**Note added in proof** Consistent with our results, a gene *Phs* (Preharvest sprouting), conferring high level of dormancy in red-grained winter wheat showing association with 2 AFLP markers and 1 microsatellite *Xpsp3003* locus was also recently identified on the long arm of chromosome 7D by Flintham et al. (1999).

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