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Development, identification, and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat

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Abstract The wheat-*Stagonospora nodorum* pathosystem involves a number of pathogen-produced host-selective toxins that interact with host genes in an inverse gene-for-gene manner to cause disease. The wheat intervarietal recombinant inbred population derived from BR34 and Grandin (BG population) segregates for the toxin sensitivity genes *Tsn1*, *Snn2*, and *Snn3*, which confer sensitivity to the toxins ToxA, SnTox2, and SnTox3, respectively. Here, we report the addition of 141 molecular markers to the BG population linkage maps, the identification and/or development of markers tightly linked to *Tsn1* and *Snn2*, and the validation of the markers using a set of diverse wheat accessions. The BG population maps now contain 787 markers, and new simple sequence repeat (SSR) markers closely linked to *Snn2* on chromosome arm 2DS were identified. In an effort to target more markers to the *Snn2* locus, STS markers were developed from 2DS bin-mapped ESTs resulting in the development and mapping of 36 markers mostly to the short arms of group 2 chromosomes. Together, SSR and EST-STS markers delineated *Snn2* to a 4.0 cM interval. SSRs developed in related work for *Tsn1* were mapped in the BG population and delineated the gene to a 1.0 cM interval. Evaluation of the markers for *Tsn1* and *Snn2* in a diverse set of wheat genotypes validated their utility for marker-assisted selection, which is particularly efficient for removing toxin sensitivity alleles from elite germplasm and varieties.

Keywords *Triticum* · Marker-assisted selection · Molecular mapping · *Phaeosphaeria nodorum* · *Pyrenophora tritici-repentis*

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Introduction

Stagonospora nodorum blotch caused by the necrotrophic fungus *Stagonospora nodorum* (E. Mull.) Hedjar (anamorph; *Phaeosphaeria nodorum*) is a major disease of common wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD genomes) and durum wheat (*T. turgidum* L. ssp. *durum*, 2n = 4x = 28, AABB genomes), and it occurs in all major wheat growing areas of the world. The fungus has the ability to infect leaves causing Stagonospora nodorum leaf blotch (SNLB) and glumes resulting in Stagonospora nodorum

glume blotch (SNGB). Therefore, it has the ability to cause significant yield losses (Eyal 1981; King et al. 1983) and negatively impact grain quality (Eyal et al. 1987).

Recently, a number of host–toxin interactions have been characterized in the wheat-*S. nodorum* pathosystem (Liu et al. 2004a, b, 2006; Friesen et al. 2006, 2007, 2008). Liu et al. (2004a, b) identified and partially characterized the first *S. nodorum* host-selective toxin (HST), designated SnTox1, and mapped the corresponding host sensitivity gene (*Snn1*) on the short arm of chromosome 1B. In QTL analysis, the *Snn1* locus accounted for 58% of the variation in susceptibility to SNLB indicating that a compatible *Snn1*–SnTox1 interaction played an important role in causing disease.

The second *S. nodorum* HST was identified by Friesen et al. (2006) and designated SnToxA. This toxin was first identified in the tan spot fungus (*Pyrenophora tritici-repentis*) (Tomas and Bockus 1987; Lamari and Bernier 1989), another important necrotrophic wheat pathogen, and designated Ptr ToxA. Friesen et al. (2006) identified a gene (Sn*ToxA*) with 99.7% similarity to the Ptr *ToxA* gene in the *S. nodorum* genome and showed that the ToxA gene was horizontally transferred from *S. nodorum* to *P. tritici-repentis* prior to 1941. The *Tsn1* gene, which maps to the long arm of wheat chromosome 5B, was previously known to confer sensitivity to Ptr ToxA (Faris et al. 1996; Haen et al. 2004; Lu et al. 2006). Using *Tsn1*-disrupted mutants and an inter-varietal hard red spring wheat population of recombinant inbred (RI) lines derived from BR34 × Grandin (BG population), it was demonstrated that *Tsn1* confers sensitivity to both Ptr ToxA and SnToxA and that *Tsn1* accounts for as much as 68% of the phenotypic variation in susceptibility to SNLB (Friesen et al. 2006; Liu et al. 2006) indicating that a compatible *Tsn1*–SnToxA interaction plays a highly significant role in the development of SNLB.

The third *S. nodorum* HST to be identified was SnTox2, which causes necrosis on wheat genotypes harboring the *Snn2* gene on the short arm of wheat chromosome 2D (Friesen et al. 2007). Spore inoculation experiments of the BG population with a *S. nodorum* isolate that produced both SnToxA and SnTox2 indicated that the *Tsn1* and *Snn2* loci accounted for 20 and 47% of the variation in SNLB susceptibility, respectively. In a multiple regression model, the two loci together accounted for 66% of the phenotypic variation indicating that the effects of the *Tsn1*–SnToxA and *Snn2*–SnTox2 interactions are additive and that they are highly important in disease development.

More recently, a fourth *S. nodorum* HST, designated SnTox3, was identified and partially characterized (Friesen et al. 2008). Sensitivity to SnTox3 was governed by the *Snn3* gene, which was mapped to the short arm of chromosome 5B in the BG population. Evaluation of *S. nodorum* isolates that produced both SnTox2 and SnTox3 indicated that compatible *Snn2*–SnTox2 and *Snn3*–SnTox3 interactions were both important factors in the development of SNLB with the *Snn2* and *Snn3* loci explaining as much as 37 and 17% of the variation in disease, respectively.

Therefore, three toxin sensitivity loci—*Tsn1*, *Snn2*, and *Snn3*—have been mapped in the wheat BG population. We previously reported the construction of genetic linkage maps in the BG population consisting of 646 markers (Liu et al. 2005) with markers tightly linked to *Snn3* (Friesen et al. 2008), but rather loosely linked to *Snn2* (Friesen et al. 2007) and *Tsn1* (Liu et al. 2006). The identification of additional markers, particularly ones closely linked to toxin sensitivity loci would be especially beneficial for marker-assisted selection (MAS) of toxin insensitivity alleles. The objectives of this research were to further saturate the genetic maps in the BG population by incorporating additional simple sequence repeat (SSR; microsatellite) markers, identify or develop markers tightly linked to the toxin sensitivity genes *Tsn1* and *Snn2*, and to investigate the utility of the markers for MAS.

Materials and methods

Plant materials

The BG population of RI lines derived from the cross between the Brazilian hard red spring wheat variety BR34 and the North Dakota hard red spring wheat variety Grandin was developed and provided by Dr. James Anderson, University of Minnesota. A total of 118 $F_{7:9}$ RI lines were used for mapping as described in Liu et al. (2005). A set of 88 accessions representing *T. aestivum* and *T. turgidum* subspecies

(Table 1) was genotyped using markers developed for *Tsn1*, and a subset of 16 of the accessions from the same collection (Table 2) was used to evaluate markers closely linked to *Snn2* to validate the utility of the markers for MAS. Most of the 88 lines were obtained from the USDA-ARS National Small Grains Collection, Aberdeen, ID, except that seed of Scoop 1, Mexicali, and Altar 84 were obtained from the International Maize and Wheat Improvement Center (CIMMYT), Mexico; Arina and Forno were obtained from Dr. Beat Keller, University of Zurich, Switzerland; and the *T. turgidum* ssp. *dicoccoides* accessions 16-1, 16-29, 18-1, A-33, A-35, B-16, B-6, C-19, C-36, I-50, L-1, L-10, L-33, and L-40 were obtained from Dr. E. Nevo, University of Haifa, Israel. The *Tsn1* gene was originally mapped in an F_2 population derived from W-7976, which is a synthetic hexaploid wheat developed at CIMMYT derived from the durum wheat cultivar Mexicali, and the hard red spring wheat variety Kulm (Faris et al. 1996; Haen et al. 2004). Therefore, the marker alleles possessed by these two genotypes served as a reference for those observed in the 88 accessions.

Phenotypic evaluations for reaction to ToxA and SnTox2

Cultures of ToxA were purified as described in Zhang et al. (1997) and provided by Dr. Steven W. Meinhardt, Department of Plant Pathology, North Dakota State University. Cultures of SnTox2 were partially purified as described in Friesen et al. (2007). The BG population was previously screened with both ToxA and SnTox2 and the results were presented in Liu et al. (2006) and Friesen et al. (2007), respectively. Here, we used ToxA to screen the 88 accessions listed in Table 1, and SnTox2-containing cultures for screening the 16 accessions listed in Table 2. More lines were not evaluated with the SnTox2-containing cultures because protocols for purifying SnTox2 have not yet been established, and it is possible that the partially purified cultures could contain additional, yet unidentified, toxins. Plants were infiltrated at the second leaf stage as described in Liu et al. (2006) and were scored as sensitive or insensitive 3 days later based on the presence or absence of necrosis, respectively. These experiments were repeated twice.

Molecular mapping

SSR markers not previously screened by Liu et al. (2005) were chosen from the SSR primer sets CFA, CFD (Sourdille et al. 2004), KSUM (Yu et al. 2004), GDM (Pestsova et al. 2000), GWM (Röder et al. 1998), and WMC (Somers et al. 2004). All PCR primer sequences are available in the Graingenes database (http://wheat.pw.usda.gov/GG2/index.shtml).

We are in the process of using a map-based cloning approach to isolate the *Tsn1* gene and we have assembled a physical BAC contig spanning the locus (Lu and Faris 2006; Faris et al., unpublished). In the initial stages of BAC contig assembly, Lu et al. (2006) mined flanking BAC sequences for SSRs and developed two markers, *Xfcp1* and *Xfcp2* that delineated *Tsn1* to a 0.8 cM interval in a tetraploid mapping population. Here, we mined new, more closely associated, BAC sequences flanking the *Tsn1* locus for SSRs using the SSRIT software (Temnykh et al. 2001). Two SSRs flanking the *Tsn1* candidate gene region were identified for which PCR primers were developed (Table 2). These two SSR markers designated *Xfcp394* and *Xfcp620* delineate the *Tsn1* locus to a 0.07 cM genetic interval, which corresponds to a 344 kb segment (Faris et al., unpublished). Here, *Xfcp394* and *Xfcp1* were mapped in the BG population (*Xfcp620* was monomorphic) and *Xfcp1*, *Xfcp394*, and *Xfcp620* were all used to genotype the set of 88 wheat lines. The physical order of these markers relative to *Tsn1* is known to be *Xfcp1–Xfcp620–Tsn1–Xfcp394*.

We also identified an SSR within the 3′ untranslated region of the 5B homoeoallele (*q-B1*) of the domestication gene *Q* on chromosome 5A (Zhang et al., unpublished) and developed the marker *Xfcp621*. All FCP SSR primer sets were designed using the MacVector v8.0 software (Accelrys, Inc. San Diego, CA).

To target additional markers to the *Snn2* locus on chromosome arm 2DS, we downloaded the sequences for 147 ESTs mapped to the 2DS5-0.47-1.00 deletion bin by the NSF-funded EST project (http://wheat.pw.usda.gov/NSF/). All EST sequences were subjected to BLASTn searches of the Dana Farber Cancer Institute (DFCI) gene indices database (http://compbio.dfci.harvard.edu/tgi/) to identify corresponding tentative consensus (TC) sequences. PCR primers were designed from TC sequences (or EST sequences when

Table 1 Accessions of *Triticum aestivum* and *T. turgidum* subspecies tested for reaction to ToxA and genotyped with markers *Xfcp1*, *Xfcp620* and *Xfcp394*

Cultivar or accession ID	PI or CI	*Triticum* species	Subspecies[a]	Genotype			
				Xfcp1[b]	*Xfcp620*[c]	ToxA[d]	*Xfcp394*[e]
Atlas 66	CItr 12561	*aestivum*	*aestivum* (w)	S	I	I	I
BR34	N/A	*aestivum*	*aestivum* (s)	Null	S	I	I
Cheyenne	PI 192268	*aestivum*	*aestivum* (w)	S	S	S	I
Chinese Spring	CItr 14108	*aestivum*	*aestivum* (s)	I	I	I	I
Jagger	PI 593688	*aestivum*	*aestivum* (w)	S	S	S	I
Salamouni	PI 182673	*aestivum*	*aestivum* (s)	I	I	I	I
TAM 105	CItr 17826	*aestivum*	*aestivum* (w)	S	S	S	I
Dapps	PI 633862	*aestivum*	*aestivum* (s)	I	I	I	I
Grandin	PI 531005	*aestivum*	*aestivum* (s)	S	S	S	S
Hope	CItr 8178	*aestivum*	*aestivum* (s)	S	S	S	S
Katepwa	N/A	*aestivum*	*aestivum* (s)	S	S	S	S
Opata 85	PI 591776	*aestivum*	*aestivum* (s)	I	I	I	I
Selkirk	CItr 13100	*aestivum*	*aestivum* (s)	S	S	I	I
Steele ND	PI 634981	*aestivum*	*aestivum* (s)	S	S	S	S
Sumai 3	PI 481542	*aestivum*	*aestivum* (s)	S	S	S	S
Thatcher	CItr 10003	*aestivum*	*aestivum* (s)	I	I	I	I
Kulm	PI 590576	*aestivum*	*aestivum* (s)	S	S	S	S
Glenn	PI 639273	*aestivum*	*aestivum* (s)	S	S	S	S
Bobwhite	PI 520554	*aestivum*	*aestivum* (s)	S	S	S	S
Fielder	CItr 17268	*aestivum*	*aestivum* (s)	S	S	S	S
Glenlea	Cltr 17272	*aestivum*	*aestivum* (s)	S	S	S	S
Timstein	PI 168688	*aestivum*	*aestivum* (s)	S	S	S	S
Renan	PI 564569	*aestivum*	*aestivum* (w)	I	I	I	I
Arina	N/A	*aestivum*	*aestivum* (w)	I	I	I	I
Forno	N/A	*aestivum*	*aestivum* (w)	S	S	S	I
Norstar	CItr 17735	*aestivum*	*aestivum* (w)	S	S	S	I
Langdon	CItr 13165	*turgidum*	*durum*	S	S	S	I
Ben	PI 596557	*turgidum*	*durum*	S	S	S	I
Altar84	N/A	*turgidum*	*durum*	I	I	I	I
Mountrail	PI 607530	*turgidum*	*durum*	I	I	I	I
Scoop1	N/A	*turgidum*	*durum*	I	I	I	I
Mexicali	PI 433760	*turgidum*	*durum*	I	I	I	I
Termok	PI 41023	*aestivum*	*compactum*	S	S	S	S
Moco de Espiga Quadrada	PI 56213	*aestivum*	*compactum*	I	I	I	I
Sinai No. 3	PI 60740	*aestivum*	*compactum*	S	S	S	S
Gluclub	PI 114638	*aestivum*	*compactum*	S	S	S	S
Premier	PI 278581	*aestivum*	*compactum*	S	I	I	I
Tincurrin	PI 434642	*aestivum*	*compactum*	S	S	S	S
132	PI 42014	*aestivium*	*sphaerococcum*	I	I	I	I
52	CItr 8610	*aestivium*	*sphaerococcum*	S	S	S	S
219	PI 70711	*aestivium*	*sphaerococcum*	I	I	I	I
I12	PI 83402	*aestivium*	*sphaerococcum*	S	S	S	S

Table 1 continued

Cultivar or accession ID	PI or CI	*Triticum* species	Subspecies[a]	Genotype			
				Xfcp1[b]	*Xfcp620*[c]	ToxA[d]	*Xfcp394*[e]
971	PI 278650	*aestivium*	*sphaerococcum*	S	I	I	I
128	PI 367199	*aestivum*	*spelta*	Null	S	S	S
Arrancada	PI 191826	*turgidum*	*polonicum*	I	I	I	I
148	PI 225334	*turgidum*	*polonicum*	S	S	S	S
22	PI 70738	*turgidum*	*carthlicum*	S	S	S	S
7282	PI 182471	*turgidum*	*carthlicum*	S	I	I	I
6332	PI 140191	*aestivum*	*macha*	I	I	I	S
I-1-3544	PI 272555	*aestivum*	*macha*	I	I	I	I
Letshchumicum	PI 352466	*aestivum*	*macha*	I	I	I	I
69Z5.193	PI 355514	*aestivum*	*macha*	I	I	I	I
DN-2378	PI 361862	*aestivum*	*macha*	I	I	I	I
G532	PI 428146	*aestivum*	*macha*	I	I	I	I
G1260	PI 428148	*aestivum*	*macha*	Null	I	I	I
G866	PI 428178	*aestivum*	*macha*	Null	I	I	I
1744	PI 378469	*aestivum*	*spelta*	Null	I	I	I
I-1-599	PI 272573	*aestivum*	*spelta*	I	I	I	I
ts060	PI 286060	*aestivum*	*spelta*	I	I	I	I
Steiners Roter Tiroler Dinkel	PI 355651	*aestivum*	*spelta*	S	I	I	I
Tv342	PI 428342	*aestivum*	*vavilovii*	I	I	I	I
ELS 6304-72	CItr 14621	*turgidum*	*dicoccum*	Null	I	I	I
425b	CItr 14454	*turgidum*	*dicoccum*	S	I	I	I
35900	PI 74108	*turgidum*	*dicoccum*	S	I	I	I
372	PI 94680	*turgidum*	*dicoccum*	S	I	I	S
Kathiawar	PI 40919	*turgidum*	*dicoccum*	I	I	I	I
Early Spelt	PI 94664	*turgidum*	*dicoccum*	I	I	I	I
265	PI 94648	*turgidum*	*dicoccum*	I	I	I	I
258	PI 94641	*turgidum*	*dicoccum*	I	I	I	I
ELS6404-129-2	CItr 14824	*turgidum*	*dicoccum*	I	I	I	I
301	PI 94747	*turgidum*	*dicoccum*	I	I	I	I
Khapli	PI 101971	*turgidum*	*dicoccum*	I	I	I	I
I-1-2708	PI 272582	*turgidum*	*dicoccoides*	I	I	I	I
Schweinfurthii	PI 352328	*turgidum*	*dicoccoides*	I	I	I	I
16-1	N/A	*turgidum*	*dicoccoides*	I	I	I	S
16-29	N/A	*turgidum*	*dicoccoides*	Null	I	I	S
18-1	N/A	*turgidum*	*dicoccoides*	Null	I	S	S
A-33	N/A	*turgidum*	*dicoccoides*	Null	I	I	I
A-35	N/A	*turgidum*	*dicoccoides*	I	I	I	I
B-16	N/A	*turgidum*	*dicoccoides*	I	I	I	I
B-6	N/A	*turgidum*	*dicoccoides*	S	S	I	I
C-19	N/A	*turgidum*	*dicoccoides*	I	I	I	I
C-36	N/A	*turgidum*	*dicoccoides*	I	I	I	I
I-50	N/A	*turgidum*	*dicoccoides*	Null	I	I	I

Table 1 continued

Cultivar or accession ID	PI or CI	*Triticum* species	Subspecies[a]	Genotype			
				Xfcp1[b]	*Xfcp620*[c]	ToxA[d]	*Xfcp394*[e]
L-1	N/A	*turgidum*	*dicoccoides*	Null	I	I	S
L-10	N/A	*turgidum*	*dicoccoides*	Null	I	I	S
L-33	N/A	*turgidum*	*dicoccoides*	Null	I	I	S
L-40	N/A	*turgidum*	*dicoccoides*	Null	I	I	S

[a] Subspecies followed by (w) or (s) indicate winter or spring type, respectively

[b] For marker *Xfcp1*, 'I' indicates the 374 bp allele and 'S' indicates the 402 bp allele

[c] For marker *Xfcp620*, 'I' indicates the 226 bp allele and 'S' indicates the 252 bp allele

[d] 'I' indicates insensitive to ToxA (contains *tsn1* allele) and 'S' indicates sensitive to ToxA (contains *Tsn1* allele)

[e] For marker *Xfcp394*, 'I' indicates the 383 bp allele and 'S' indicates the 328 bp allele

Table 2 Hexaploid wheat cultivars infiltrated with SnTox2-containing cultures and genotyped with markers *XTC253803* and *Xcfd51*, which flank the *Snn2* locus on chromosome arm 2DS

Cultivar[a]	Genotype		
	XTC253803[b]	SnTox2[c]	*Xcfd51*[b]
Atlas 66	194	I	Null
BR34	194	I	165 + 194
Cheyenne	194	I	163 + 192
Chinese Spring	194	I	159 + 188
Jagger	197 + 202	I	163 + 192
Salamouni	194	I	165 + 194
TAM 105	194	I	165 + 194
Dapps	197	S	153 + 182
Grandin	197	S	153 + 182
Hope	197	S	153 + 182
Katepwa	197	S	153 + 182
Opata 85	197	I	157 + 186
Selkirk	197	S	153 + 182
Steele ND	197	S	153 + 182
Sumai 3	196	S	157 + 186
Thatcher	197	S	153 + 182

[a] Information regarding the accession numbers, genus, species, and subspecies for these cultivars is given in Table 1

[b] Fragment sizes detected by the markers given in base pairs

[c] 'I' indicates insensitive to the SnTox2-containing culture (has the *snn2* allele) and 'S' indicates sensitive to the SnTox2-containing culture (has the *Snn2* allele)

no corresponding TC was identified) using the program PRIMER3 (Rozen and Skaletsky 2000) (Table 3).

Protocols for DNA isolation, PCR, and genotyping of SSR and EST-STS markers were as described in Liu et al. (2005) and Lu et al. (2006), respectively. PCR products for all markers were either separated on 6% polyacylamide gels, stained with SYBR green II, and digitally scanned using a Typhoon 9410 variable mode imager (GE Healthcare, Waukesha, WI), or separated on 2% agarose gels, stained with ethidium bromide and photographed.

Markers were placed on the existing framework maps using the computer program Mapmaker v2.0 for Macintosh (Lander et al. 1987). The 'GROUP' command was used to identify the correct linkage group and the 'TRY' command was used to determine the best marker interval for new markers. The 'RIPPLE' command was then used to validate the marker order. Those markers mapping at an LOD < 3.0 were placed in the most likely intervals.

Results

Mapping additional SSR markers in the BG population

Of the 182 SSR primer sets screened for polymorphism between BR34 and Grandin, 75 (41%) detected polymorphisms and led to the identification of 106 SSR marker loci for an average of 1.4 loci per primer set (Table 4, Fig. 1). New SSR marker loci were detected on all chromosomes with the exception of chromosome 3D, which is non-recombinant in this population (Liu et al. 2005). The number of new SSR markers per chromosome ranged from one on chromosomes 3A and 4D to 21 on chromosome 5B for an average of 5.4 per chromosome (Fig. 1). The number

Table 3 Newly developed PCR-based markers, their primer sequences and annealing temperatures, and their chromosomal locations in the population of recombinant inbred lines derived from the hard red spring wheat varieties BR34 and Grandin

Marker type	Marker designation	F primer	R primer	Annealing temp	Chrom[b]
SSR	*Xfcp1*[a]	ATAACTCCGTCACGACCACCTCCTCTCAAG	CAGTCTGAAAACGCCATACCCG	65	5B
SSR	*Xfcp394*	GTAGCCTGCAGGTACAAACTGGA	CAGTGTTAAGAAGTGTGTTCTGGTC	60	5B
SSR	*Xfcp620*	CATAACCTTCATACGGACTTGCTCAC	TATTCTTGCCAGTGTTGGGAGGG	65	5B
SSR	*Xfcp621*	GTTTGGAAAACGTGAGCAATGG	GCATCATCAGGATTCTCTACCGC	58	5B
EST-STS	*XBE483620*	CTCCAGCCGATACGTGTGAT	TGGAATTCCCACAAGCAGAC	55	2A
EST-STS	*XBE489611*	ACCATGAGAGCAACCCTGAC	CGACCGAAGATTTGAAGGAG	55	2D
EST-STS	*XBE495012*	ACCAAACGCGAGATTCACAG	GCTCACCGATGACTTGAAGG	55	2A, 2B
EST-STS	*XBF201415*	GGCTGCAGAAAGAAGACACC	AGGTCTGCGCTCGTTATCAT	55	2B
EST-STS	*XBG236447*	TTGCTCCATCATCACCCTCCTC	GCGTCAATGTTGCTTTCGTCC	55	2B, 2D
EST-STS	*XBG604604*	AACGTGCCAGGTTGGTAGAC	ACCACGCTCGATTTCACTCT	55	2A
EST-STS	*XBQ162243*	CGTCGTCTGCCAAAACTGAC	CAGCAGCAGCATCGTGAGTA	55	2B
EST-STS	*XBQ162449*	TATCACTTCGACCGCATAGC	CGGGATTCAGTTTAGCTCGT	55	2D
EST-STS	*XBQ167022*	GCGGAGGGAGTATTCTCCAA	CATCTTTGTGCAAGGAGCAA	55	2B
EST-STS	*XCD373606*	GGTGATCCGATTCCGACAAT	AAGGGTGCCATTACCACCAC	55	2B
EST-STS	*XTC232230*	TGTGCTCCTGCCTGAGTATG	CGACAGAAAGTCTGGCAACA	55	2A, 2B
EST-STS	*XTC236178*	CTCGCAAACCCAAACAACAT	GCAGCCAGCTTCTATGAACG	55	2B
EST-STS	*XTC236248*	GCCATTTTGTTCCAATGGTT	GAGGGAGAGGGATTCTTCGT	55	1B, 2D
EST-STS	*XTC236991*	GTCAACCGGCAAAGCATCTA	CGTTTAGCACATCCTGCACA	55	2B
EST-STS	*XTC239909*	ATCAGGTCGTGGTTTCGATG	AAAGGCGAGCAGATTCACAA	55	2A (2)[c]
EST-STS	*XTC240114*	GGAATTAACCGAGCTGCGTA	GCGGCCTCTGTATCTTGATT	60	2D
EST-STS	*XTC242566*	CTTGCTGGATCAGGTCACAA	TGGGAGGAGGCTGTTCTCTA	55	2B, 2D
EST-STS	*XTC247418*	GGAACATCCTTGGTTCAGGA	TTGGTGGATGTGAACTTGGA	55	2A
EST-STS	*XTC253803*	TGCTTTTGTGCCAGATGATG	CCACCGGGACAAGTCAGATA	58	2D
EST-STS	*XTC255653*	TGCAGAATCATCACAGCACA	AGACGGATCTTTCGTGCATC	55	2B
EST-STS	*XTC255898*	CGACGGTGCTTACATCGAAT	TGCATGAATGAGGCACTCTG	55	5B
EST-STS	*XTC257526*	AAGGACGTCTTCGTGCACTT	ATCCACATTCCTTGCAAACC	55	2B
EST-STS	*XTC262321*	GCCTAACCAGTCGGACACAT	TGCTCTCTCCTTCCGTCTTC	55	2A
EST-STS	*XTC262844*	AAACCTTTGCTTCAGCCACT	TGATCAAGGCAGCAAGACTC	55	2A, 6B

Table 3 continued

Marker type	Marker designation	F primer	R primer	Annealing temp	Chrom[b]
EST-STS	*XTC263791*	AGACTACCTGATCCGCTCCA	CATATGCATGCTCCACAACC	55	2D
EST-STS	*XTC265669*	ACTCCTTCACTTGCCGAGAA	TGCCACACCAGCTTCTAATG	55	2B
EST-STS	*XTC269810*	GATGGTCCTGGAAGTGGACA	CAGCTCACTCGGAATCTTGC	55	2A (2)[c]
EST-STS	*XTC270911*	GGACGTCTTCGTCCACTTCA	TATTACGCATCGACGCAGGT	55	2B

[a] *Xfcp1* was reported in Lu et al. (2006)

[b] Chrom. = chromosome

[c] Markers detected two loci on chromosome 2A

of SSR loci detected per primer pair ranged from 1 to 3 (Table 4). Among the three subgenomes, 29, 52, and 25 SSR loci were assigned to the A, B, and D genomes, respectively. The 106 new SSR loci were added to the existing framework linkage maps of the BG population. A total of 75 of the new markers mapped at an LOD > 3.0, whereas 33 markers did not map at an LOD > 3.0 and were placed in the most likely marker intervals (Fig. 1). However, 23 previously mapped markers that did not map at an LOD > 3.0 now meet this criterion with the addition of the 75 new markers.

Markers tightly linked to *Tsn1*

As stated above, *Xfcp620* was monomorphic between BR34 and Grandin and thus was not mapped in the BG population. The marker *Xfcp1* mapped 0.5 cM proximal to *Tsn1* in the BG population and *Xfcp394* mapped 0.5 cM distal to *Tsn1* (Fig. 1). Therefore, these two SSR markers delineated *Tsn1* to a 1.0 cM interval in the BG population.

Identification and development of markers for the *Snn2* region

The initial mapping of *Snn2* by Friesen et al. (2007) indicated that it resided on chromosome arm 2DS in a 13.5 cM interval flanked by markers *Xgwm614* and *Xbarc95*. The mapping of the additional 106 markers in this work led to the identification of markers *Xcfd56* and *Xcfd51*, which delineated *Snn2* to an 8.1 cM interval with *Xcfd51* being linked at only 0.4 cM (Fig. 1).

In an effort to target more markers to the *Snn2* region, the wheat ESTs mapped to the 2DS5-0.47-1.00 deletion bin were used. Corresponding TC sequences were identified for 107 of the 147 ESTs, and primers were designed for these and the 40 ESTs for which no TCs were identified. Of the 147 primer pairs surveyed, 28 (19%) amplified fragments that were polymorphic between BR34 and Grandin (Table 3) and were mapped in the BG population (Fig. 1). The 28 primer pairs detected 36 loci of which 11, 14, and 8 resided on chromosomes 2A, 2B, and 2D, respectively. Three loci were detected on chromosomes 1B, 5B, and 6B. The EST-STS markers *XTC253803*, *XTC240114*, and *XBE489611* were all linked to *Snn2* on 2DS. Among these, *XTC253803*

Table 4 Chromosomal locations of markers generated by 72 SSR primers derived from the CFA, CFD, GDM, GWM, KSUM, and WMC sets and mapped in the population of recombinant inbred lines derived from the hard red spring wheat varieties BR34 and Grandin[a]

Primer set	Chrom.[b]	Primer set	Chrom.	Primer set	Chrom.	Primer set	Chrom.
CFA2019	7A	CFD53	2D, 7B	GWM66	3B, 7B	KSUM69	5B, 5D
CFA2026	4A, 5B	CFD56	1A, 2D	GWM114	2A	KSUM253	1B
CFA2040	7B	CFD58	1A, 1D, 5B	GWM131	3A, 3B, 6B	WMC28	2B
CFA2104	5D	CFD59	1B, 6B	GWM165	4B	WMC47	2B, 4B
CFA2106	7B	CFD65	1D	GWM191	2B, 5B	WMC149	5B
CFA2147	1A, 3B	CFD67	5D	GWM205	4A, 5A, 5D	WMC160	5B
CFA2173	4A	CFD71	2A, 4D	GWM234	1A, 5B	WMC235	5B
CFA2187	5B	CFD76	6B, 6D	GWM285	3B	WMC258	4A
CFA2240	1A, 7A	CFD77	2D	GWM332	2B, 7A	WMC318	5A, 5D
CFD4	3B	CFD81	5D	GWM369	6A	WMC405	5B, 7A
CFD14	7D	CFD86	5B	GWM389	2A, 2B, 3B	WMC597	2B
CFD18	5D	CFD102	5D	GWM427	6A	WMC605	7B
CFD20	2A, 5B	CFD165	5D	GWM448	1A, 2A	WMC612	3B
CFD21	1B	CFD175	3B, 5B(2)[c]	GWM533	1B, 5B	WMC630	5D, 7D
CFD25	1B	GDM116	5B, 5D	GWM547	3B	WMC640	5B
CFD37	6D	GDM136	5B	GWM570	6A	WMC728	1B
CFD40	5D	GDM153	5D	GWM617	6A	WMC773	6A
CFD51	2D	GWM63	7A	KSUM44	2A	WMC799	5D

[a] A total of 75 SSR primer sets were used. The three FCP SSR primer sets are presented in Table 3

[b] Chrom. = chromosome

[c] Primer set CFD175 detected two markers on chromosome 5B

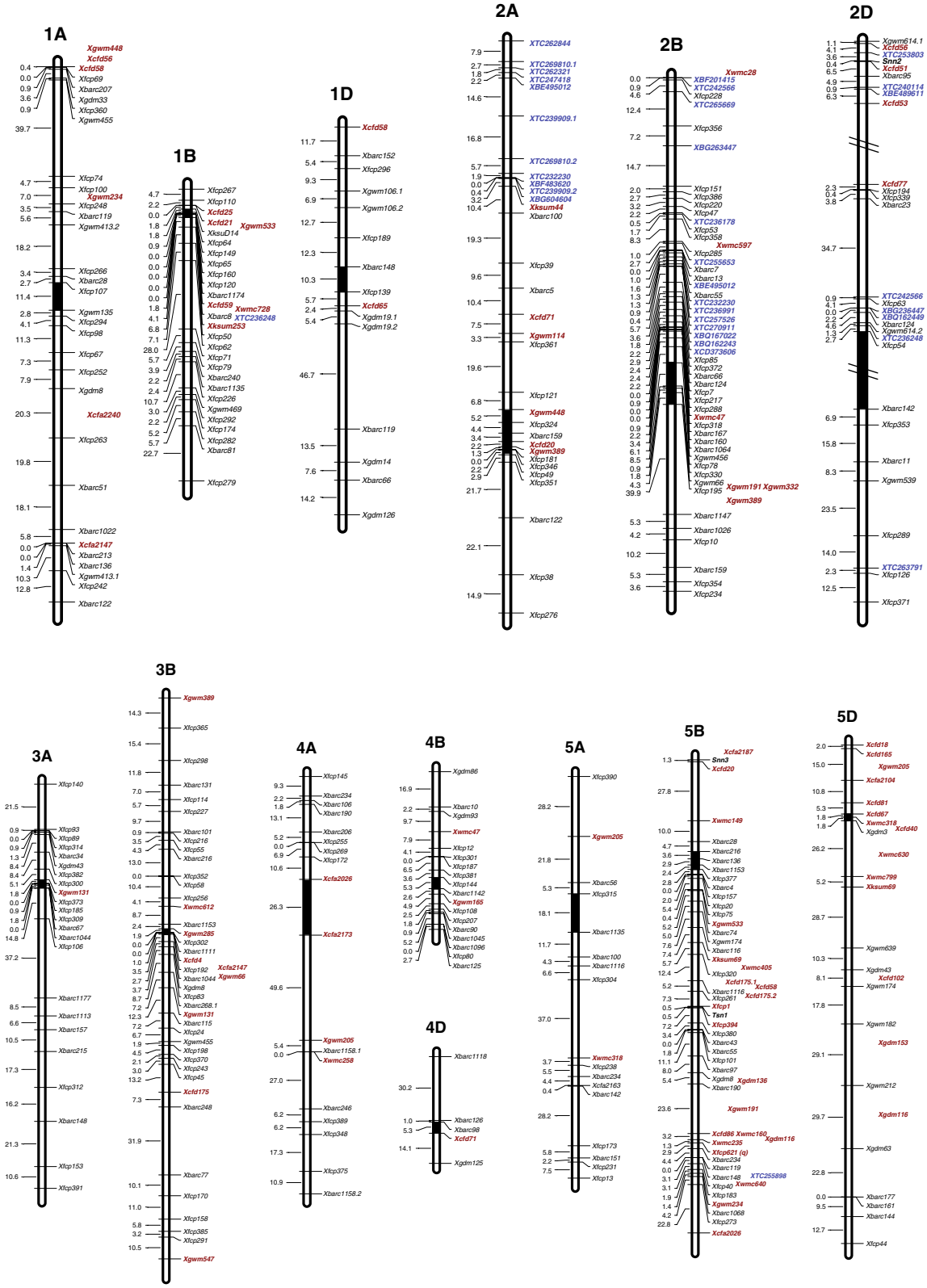
was the closest at 3.6 cM on the distal side of *Snn2* (Fig. 1). Therefore, *Snn2* is delineated to a 4.0 cM interval by *XTC253803* and *Xcfd51*.

Validation of markers tightly linked to *Tsn1* and *Snn2*

The 88 accessions listed in Table 1 were evaluated for reaction to purified ToxA and genotyped with markers *Xfcp1*, *Xfcp394* and *Xfcp620* to evaluate the utility of the markers in predicting reaction to the ToxA. Among the 88 accessions, *Xfcp1* detected two different alleles in addition to a null allele, and *Xfcp394* and *Xfcp620* detected two alleles each. *Xfcp1* detected a 402 bp fragment most often associated with the ToxA sensitive (dominant *Tsn1* allele) genotype and a 374 bp fragment most commonly associated with the ToxA insensitive (recessive *tsn1* allele) genotype. One allele of *Xfcp394* consisted of a 328 bp fragment, which was most commonly associated with the *Tsn1* allele (ToxA sensitivity) (Fig. 2). The other allele detected by *Xfcp394* consisted of a 383 bp fragment most commonly associated with the recessive *tsn1* allele (ToxA insensitivity) (Fig. 2). *Xfcp620* detected alleles of 226 and 252 bp, which were most commonly associated with the *tsn1* and *Tsn1* alleles, respectively.

Among the 88 accessions, 56 (64%) harbored "sensitive" or "insensitive" alleles at all three marker loci in ToxA sensitive and insensitive accessions, respectively (Table 1). In other words, no recombination was observed within the segment harboring the three SSR markers and *Tsn1* among

Fig. 1 Updated genetic linkage maps generated in the BG▶ population with 142 new marker loci. New SSR markers are shown in red and EST-STS markers are shown in blue. Anchor markers, mapped at an LOD > 3.0, are shown with lines drawn across the chromosome. Of the markers mapping at an LOD < 3.0, only newly mapped markers are shown and are placed in the most likely positions. Double diagonal lines through chromosomes indicate grouping at an LOD < 3.0. Approximate positions of centromeres are indicated by black regions on the chromosomes


1A
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Xcfd56
Xcfd58
Xfcp69
Xbarc207
Xgdm33
Xfcp360
Xgwm455
Xfcp74
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Xgwm234
Xfcp248
Xbarc119
Xgwm413.2
Xfcp266
Xbarc28
Xfcp107
Xgwm135
Xfcp294
Xfcp98
Xfcp67
Xfcp252
Xgdm8
Xcfa2240
Xfcp263
Xbarc51
Xbarc1022
Xcfa2147
Xbarc213
Xbarc136
Xgwm413.1
Xfcp242
Xbarc122
1B
Xfcp267
Xfcp110
Xcfd25
Xcfd21
Xgwm533
XksuD14
Xfcp64
Xfcp149
Xfcp65
Xfcp160
Xfcp120
Xbarc1174
Xcfd59
Xwmc728
Xbarc8
XTC236248
Xksum253
Xfcp50
Xfcp62
Xfcp71
Xfcp79
Xbarc240
Xbarc1135
Xfcp226
Xgwm469
Xfcp292
Xfcp174
Xfcp282
Xbarc81
Xfcp279
1D
Xcfd58
Xbarc152
Xfcp296
Xgwm106.1
Xgwm106.2
Xfcp189
Xbarc148
Xfcp139
Xcfd65
Xgdm19.1
Xgdm19.2
Xbarc119
Xgdm14
Xbarc66
Xgdm126
2A
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XTC247418
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XTC239909.1
XTC269810.2
XTC232230
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XTC239909.2
XBG604604
Xksum44
Xbarc100
Xfcp39
Xbarc5
Xcfd71
Xgwm114
Xfcp361
Xfcp121
Xgwm448
Xfcp324
Xbarc159
Xcfd20
Xgwm389
Xfcp181
Xfcp346
Xfcp49
Xfcp351
Xbarc122
Xfcp38
Xfcp276
2B
Xwmc28
XBF201415
XTC242566
Xfcp228
XTC265669
Xfcp356
XBG263447
Xfcp151
Xfcp386
Xfcp220
Xfcp47
XTC236178
Xfcp53
Xfcp358
Xwmc597
Xfcp285
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Xbarc13
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Xfcp372
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Xbarc124
Xfcp7
Xfcp217
Xfcp288
Xwmc47
Xfcp318
Xbarc167
Xbarc160
Xbarc1064
Xgwm456
Xfcp78
Xfcp330
Xgwm66
Xfcp195
Xgwm191 Xgwm332
Xgwm389
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Xfcp10
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Xfcp354
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2D
Xgwm614.1
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XTC253803
Snn2
Xcfd51
Xbarc95
XTC240114
XBE489611
Xcfd53
Xcfd77
Xfcp194
Xfcp339
Xbarc23
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Xfcp63
XBG236447
XBQ162449
Xbarc124
Xgwm614.2
XTC236248
Xfcp54
Xbarc142
Xfcp353
Xbarc11
Xgwm539
Xfcp289
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Xfcp93
Xfcp89
Xfcp314
Xbarc34
Xgdm43
Xfcp382
Xfcp300
Xgwm131
Xfcp373
Xfcp185
Xfcp309
Xbarc67
Xbarc1044
Xfcp106
Xbarc1177
Xbarc1113
Xbarc157
Xbarc215
Xfcp312
Xbarc148
Xfcp153
Xfcp391
3B
Xgwm389
Xfcp365
Xfcp298
Xbarc131
Xfcp114
Xfcp227
Xbarc101
Xfcp216
Xfcp55
Xbarc216
Xfcp352
Xfcp58
Xfcp256
Xwmc612
Xbarc1153
Xgwm285
Xfcp302
Xbarc1111
Xcfd4
Xfcp192
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Xfcp304
Xwmc318
Xfcp238
Xbarc234
Xcfa2163
Xbarc142
Xfcp173
Xbarc151
Xfcp231
Xfcp13
5B
Xcfa2187
Snn3
Xcfd20
Xwmc149
Xbarc28
Xbarc216
Xbarc136
Xbarc1153
Xfcp377
Xbarc4
Xfcp157
Xfcp20
Xfcp75
Xgwm533
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Xwmc405
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Xfcp394
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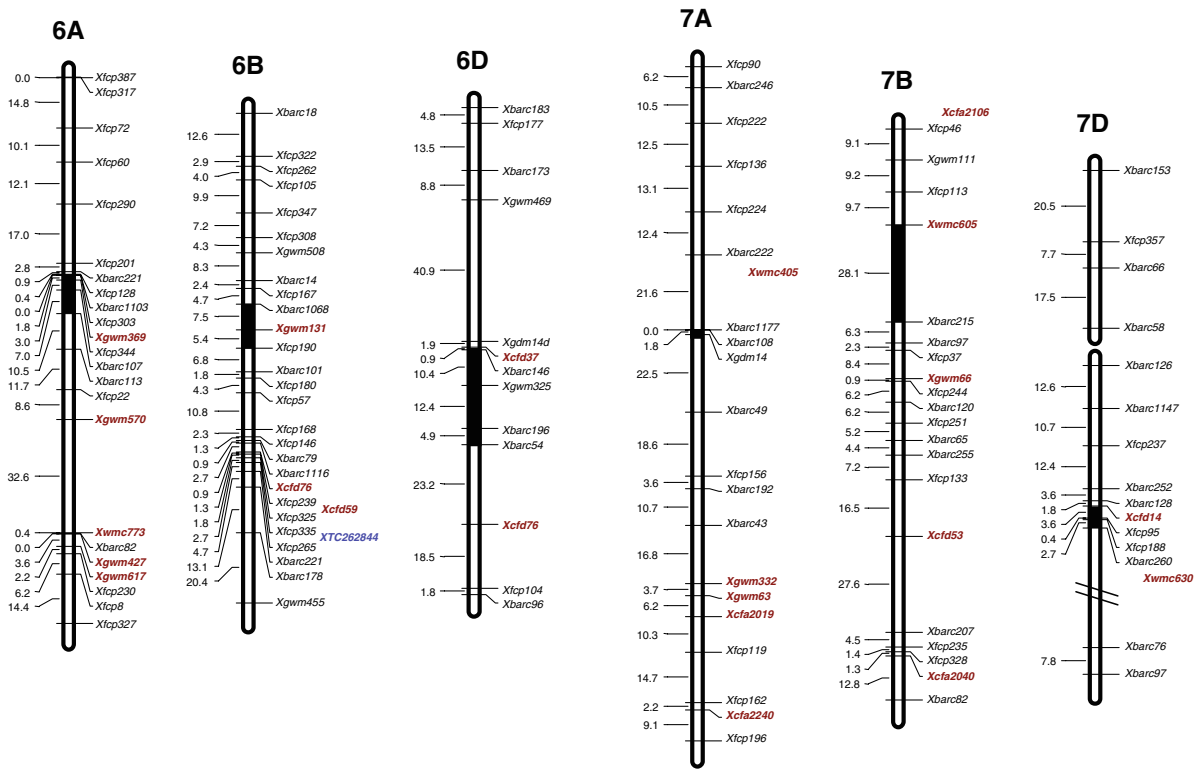

Fig. 1 continued

these 56 accessions. Only four genotypes including two *T. aestivum* ssp. *aestivum* spring wheat varieties (BR34 and Selkirk), and two *T. turgidum* ssp. *dicoccoides* accessions (18-1 and B-6) had recombination events between *Xfcp620* and *Tsn1*. Null alleles were present for *Xfcp1* in 14 accessions. Disregarding these, eight accessions including one *T. aestivum* ssp. *aestivum* winter wheat cultivar (Atlas 66), one *T. aestivum* ssp. *compactum* cultivar (Premier), one *T. aestivum* ssp. *sphaerococcum* accession (971), one *T. turgidum* ssp. *carthlicum* accession (7282), one *T. aestivum* ssp. *spelta* accession (Steiners Roter Tiroler Dinkel), and three accessions of *T. turgidum* ssp. *dicoccum* (425b, 35900, and 372) showed recombination between *Xfcp1* and *Xfcp620*. There were 15 genotypes with recombination events between *Xfcp394* and *Tsn1*. These included five of the eight *T. aestivum* ssp. *aestivum* winter wheats evaluated (Cheyenne, Jagger, TAM105, Forno, and Norstar), two durum varieties (Ben and Langdon), one accession each of *T. aestivum* ssp. *macha* (6332) and *T. turgidum* ssp. *dicoccum* (372), and six accessions of *T. turgidum* ssp. *dicoccoides* (16-1,16-29, L-1, L-10, L-33, and L-40). No accession harbored recombination events immediately flanking *Tsn1*, but the *T. turgidum* ssp. *dicoccum* accession 372 harbored recombination events between *Xfcp1* and *Xfcp620* and between *Tsn1* and *Xfcp394*.

Sixteen of the 88 lines were evaluated for reaction to partially purified cultures of SnTox2 and genotyped with markers *Xcfd51* and *XTC253803*, which flank the *Snn2* locus on chromosome arm 2DS (Table 2, Fig. 2). As mentioned above, it is possible that the partially purified SnTox2 cultures may contain additional, yet unidentified toxins. Therefore, sensitivity to the partially purified cultures is not proof that a line is sensitive to SnTox2. However, an insensitive reaction is indicative of SnTox2 insensitivity. The hexaploid wheat cultivars Atlas 66, BR34, Cheyenne, Chinese Spring, Jagger, Salamouni, TAM105, and Opata 85 were insensitive to the SnTox2-containing culture indicating they contain the recessive *snn2* allele, whereas the remaining eight lines were sensitive and may carry the dominant *Snn2* allele (Table 2).

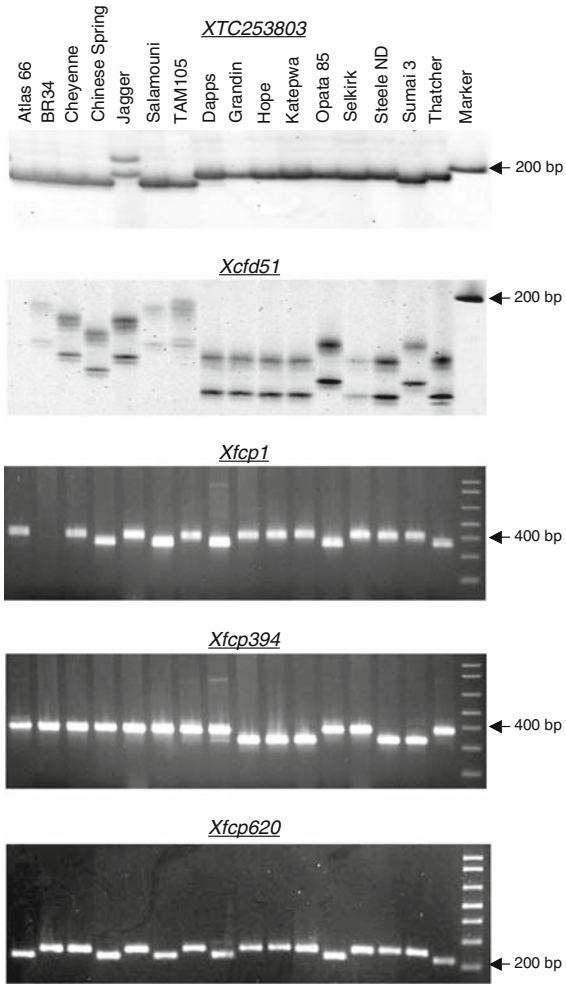

Fig. 2 Molecular profiles of 16 hexaploid wheat varieties revealed using markers *XTC253803*, *Xcfd51*, *Xfcp1*, *Xfcp394*, and *Xfcp620*. Fragments amplified by *XTC253803* and *Xcfd51* are separated on 8% polyacrylamide gels and those by *Xfcp1*, *Xfcp394*, and *Xfcp620* are separated on 2.0% agarose gels. Cultivar names are shown across the top. Marker fragments of known size are indicated by arrows along the right of each image

XTC253803 detected four different alleles among the 16 cultivars (Table 2, Fig. 2). Among the eight cultivars that were sensitive to the SnTox2-containing culture, all had the same 197 bp allele with the exception of Sumai 3, which had a 196 bp allele. Six of the eight SnTox2 insensitive cultivars (Atlas 66, BR34, Cheyenne, Chinese Spring, Salamouni, and TAM 105) all had a 194 bp allele. Of the remaining two SnTox2 insensitive cultivars, Jagger had a 197 + 202 bp fragment pair and Opata 85 had a 197 bp fragment, which was the same as that observed in the SnTox2 sensitive cultivars.

Xcfd51 detected six different alleles, including a null-allele, among the 16 cultivars (Table 2, Fig. 2). The null and four other alleles were present among the SnTox2 insensitive lines Atlas 66, BR34, Cheyenne, Chinese Spring, Jagger, Salamouni, TAM105, and Opata 85. With the exception of Sumai 3, which had the same allele as Opata 85, *Xcfd51* detected a common allele in all the cultivars that were sensitive to the SnTox2-containing culture, which differed from those observed among the insensitive lines.

Discussion

High-density genetic linkage maps developed in intervarietal or interspecific wheat populations are particularly useful because MAS is typically performed in such populations. The development of wheat intervarietal linkage maps in the past has been ever challenging due to the inherent low levels of polymorphism. However, with the development and employment of SSR markers, maps generated in several interspecific populations have recently been published (Sourdille et al. 2003; Suenaga et al. 2005; Liu et al. 2005; Torada et al. 2006). The BG population is particularly useful because it segregates for a number of important agronomic traits including disease resistance and end-use quality.

In the original mapping of the BG population, Liu et al. (2005) screened 600 SSR primer sets for polymorphism between BR34 and Grandin, of which 175 (30%) revealed polymorphisms and yielded 328 marker loci. Including SSR and target region amplified polymorphic (TRAP) markers, Liu et al. (2005) reported a total of 646 markers, of which 352 mapped at an LOD > 3.0 and were used to construct the initial linkage maps. The maps spanned a genetic distance of 3,045.8 cM and had an average density of one marker per 8.7 cM. In this work, we added 141 new marker loci to the maps. The framework maps in the BG population now consist of 480 marker loci that span 3595.8 cM and have an average marker density of one marker per 7.5 cM. This is an increase of 18% in map length and a 14% increase in marker density compared to the original BG maps published by Liu et al. (2005). The increase in map length was primarily due to the extension of linkage groups to include the short arms of chromosomes 1A, 2A, and 5D, which were previously uncovered by markers (Liu et al. 2005).

The wheat-*S. nodorum* system appears to rely largely on compatible interactions between host-selective toxins and corresponding host genes that confer sensitivity, and these components interact in an "inverse" gene-for-gene manner. In this scenario, a compatible host–toxin interaction relies on the direct or indirect recognition of the toxin by a dominant host gene product, which leads to toxin sensitivity and enhanced disease susceptibility. Absence of either the toxin or the dominant host gene precludes recognition and results in an incompatible, or resistant, response. The chromosomal locations of the genes *Tsn1*, *Snn2*, and *Snn3*, which confer sensitivity to the *S. nodorum* host-selective necrosis toxins ToxA, SnTox2, and SnTox3 were previously reported in the BG population and found to reside on chromosome arms 5BL, 2DS, and 5BS, respectively (Liu et al. 2006; Friesen et al. 2007, 2008). Friesen et al. (2008) tagged the *Snn3* locus with the marker *Xcfd20*, demonstrated that this marker was linked to *Snn3* at a distance of 1.4 cM, and indicated its usefulness for MAS.

Friesen et al. (2007) defined *Snn2* to a 13.5 cM interval flanked by markers *Xgwm614* and *Xbarc95* on chromosome arm 2DS. Although these flanking markers are both user-friendly SSRs, the interval of 13.5 cM is rather large for efficient MAS. In the current work, the mapping of additional SSRs and 2DS bin-mapped ESTs led to the identification and development of PCR-based markers that reduced the *Snn2* interval to a more desirable 4.0 cM.

The *Tsn1* gene was previously delineated to a 15.3 cM interval in the BG population by markers *Xfcp261* and *Xfcp380* (Liu et al. 2006). Initial work toward the positional cloning of *Tsn1* led to the development of SSR markers *Xfcp1* and *Xfcp2*, which delineate *Tsn1* to a 0.8 cM interval in a tetraploid wheat mapping population (Lu et al. 2006). Subsequent positional cloning work led to the development of two additional SSR markers, *Xfcp620* and *Xfcp394*, which we validated in the current work. These markers delineate *Tsn1* to a 0.07 cM interval, which corresponds to 344 kb, in a tetraploid mapping population (Faris et al., unpublished). We also validated the utility of *Xfcp1* described by Lu et al. (2006) who also suggested that *Xfcp2* would be suitable for MAS of *Tsn1*. Therefore, the availability of four user-friendly and effective SSR markers (*Xfcp1*, *Xfcp2*, *Xfcp394*, and *Xfcp620*) tightly linked to *Tsn1* provide wheat researchers multiple options in the event one or more of the markers are monomorphic in given breeding materials. The importance of developing multiple user-friendly markers for a given gene was realized in this work due to the fact that *Xfcp620* was not polymorphic in the BG population. Once the *Tsn1* gene has been isolated, we will work to develop "perfect" allele specific markers for the gene itself.

Marker-assisted selection against toxin sensitivity loci in backcrossing schemes is particularly beneficial because sensitivity is dominant, and backcrosses to sensitive recurrent parents yield only sensitive plants. Heterozygous BC_1 individuals can easily be selected using the codominant markers developed in this work, and used in subsequent backcrosses to the recurrent parent without progeny testing or test crossing. Given the importance of the *Snn2*–SnTox2 interaction in susceptibility to SNLB and of the *Tsn1*–ToxA interaction in susceptibility to both tan spot and SNLB, it would be desirable to remove the toxin sensitivity alleles from elite germplasm and cultivars, and the markers identified/developed in this work will be useful for removing the dominant *Tsn1* and *Snn2* alleles. However, among the 88 accessions tested, linkage disequilibrium was not as high as expected at the *Tsn1* locus with 19 (22%) of the lines having undergone apparent recombination events between *Tsn1* and either *Xfcp620* or *Xfcp394*. It is interesting to note that, among the eight *T. aestivum* winter wheat cultivars evaluated, five (63%) showed recombination between *Tsn1* and *Xfcp394* (Table 1). Therefore, caution is needed when selecting based on these marker profiles, and it is important that the reaction to ToxA is known for breeding materials before the markers are employed.

For *Snn2*, numerous alleles were detected by both *XTC253803* and *Xcfd51* among the eight lines insensitive to SnTox2-containing cultures, but all except one of the eight sensitive lines had the same allele (Table 2, Fig. 2). The cultivar Sumai 3 was the one exception, and it had the same allele as the SnTox2 insensitive line Opata 85 at the *Xcfd51* locus and a unique allele at the *XTC253803* locus. Therefore, either Sumai 3 has undergone recombination events flanking *Snn2*, or it is actually insensitive to SnTox2 and the sensitive reaction was due to the presence of a yet unidentified toxin in the partially purified SnTox2 cultures. Among the cultivars with sensitivity to the SnTox2-containing culture, Grandin

is the only cultivar proven to be sensitive to SnTox2, and it is possible that other "sensitive" cultivars with the same marker alleles as Grandin are not actually sensitive to SnTox2, but rather a yet unidentified toxin. However, the likelihood of that scenario is low. Once the SnTox2 protein has been purified, a more robust effort to characterize germplasm for presence of *Snn2* can be undertaken. In the mean time, *Xcfd51* and *XTC253803* should be useful for selection of genotypes lacking the dominant *Snn2* alleles and eliminating SnTox2 sensitivity.

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