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ORIGINAL ARTICLE

Identification of markers linked to genes for sprouting tolerance (independent of grain color) in hard white winter wheat (HWWW)

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Abstract

Key message **Hard red wheats can donate genes to hard white wheats for tolerance to preharvest sprouting, the effects are quantitative in nature, and may be tracked with previously described DNA markers.**

Abstract Pre-harvest sprouting (PHS) of wheat (*Triticum aestivum* L.) can negatively impact end-use quality and seed viability at planting. Due to preferences for white over red wheat in international markets, white wheat with PHS tolerance has become increasingly desired for worldwide wheat production. In general, however, red wheat is more tolerant of sprouting than white wheat. The main objective of this study was the identification of PHS tolerance conditioned by genes donated from hard red winter wheat, using markers applicable to the Great Plains hard white wheat gene pool. Three red wheat by white wheat populations, Niobrara/NW99L7068, NE98466/NW99L7068 and Jagalene/NW99L7068 were developed, and white-seeded progenies were analyzed for PHS tolerance and used to identify markers for the trait. In the three populations, marker loci with significant allelic effects were most commonly located on chromosomes of group 2, 3, 4 and 5, though additional markers were detected across the wheat genome. Chromosome 3A was the only chromosome with significant markers in all three populations. Markers were inconsistent across the three populations, and markers linked to tolerance-inducing loci were identified in both tolerant and susceptible parents. Additive effects of marker loci were common. In the present investigation, a wide range of PHS tolerance was observed, even though all lines were fixed for the recently reported positive *TaPHS1* allele. PHS tolerance is controlled by additive major gene effects with minor gene effects where variations of minor gene effects were still unclear.

Introduction

The germination of wheat (*Triticum aestivum* L.) grain before harvest time is designated pre-harvest sprouting (PHS). The occurrence of PHS in wheat depends upon both the genetic background of the plant and the weather conditions during grain maturation. Warm temperatures and high humidity favor PHS, leading to economically important losses caused by reductions in both grain volume weight and end-use quality, due to the degradation of starch and protein (Flintham 2000). The annual financial loss of Canadian wheat alone from PHS is estimated to exceed $100 million (DePauw et al. 2012). Hence, PHS tolerant cultivars are needed in many production zones.

Hard red winter wheats have dominated production in the Great Plains of North America since the beginning of modern agriculture in the region. Hard white winter wheat development and production in the region, however, started in late 1980s. White wheats provide higher flour yield and

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lighter whole wheat flour color compared with red wheats. White wheats have two major markets, namely domestic production for whole wheat breads and as the wheat of choice in many export commercial markets. The Asian wet noodle market, for example, prefers white wheats (Graybosch et al. 2013). To expand wheat marketing potential, developing adapted white wheat cultivars in the United States is necessary, and such wheats require tolerance to PHS.

In cereal crops, seed dormancy and tolerance to PHS are correlated traits, or two manifestations of the same biochemical mechanism (Bewley and Black 1982). Lack of adequate seed dormancy is the major cause for PHS (Li et al. 2004). In wheat, PHS tolerance and/or longer seed dormancy are associated with red color pericarp and testa. Wheats with red grain color typically possess greater PHS tolerance than the white wheats (Morris and Paulsen 1992; Groos et al. 2002). The mechanism by which red grain color conditions PHS tolerance is still unclear (Flintham et al. 1999). The association between red grain color and PHS might be explained by a genetic linkage between PHS tolerance and *Red* genes or it may be due to a pleiotropic effect of the *Red* genes (Flintham et al. 1999). To insure tolerance to PHS, red wheats typically have been produced in high rainfall environments (Groos et al. 2002). However, genetic diversity for sprouting tolerance exists within both red and white wheats (Wu and Carver 1999) and both tolerant white wheats, and susceptible red wheats, are known.

Evaluation of tolerance to PHS in the field is not precise largely due to the inconsistency of the environmental conditions that favor it (Graybosch et al. 2013) in some geographic regions. Furthermore, environment and genotype × environment interactions influence tolerance to PHS (Kato et al. 2001). Therefore, more carefully controlled artificial or indirect methods to assess PHS tolerance have been developed. Examples include misting chamber assays, and measurement of starch quality and amylase activity (Clarke et al. 2005; Ross and Bettge 2009). These indirect means are time consuming, labor intensive and difficult to implement as error variances often are high. Germination tests at physiological maturity and germination with the inhibitor abscisic acid (ABA) are alternative approaches (Morris and Paulsen 1989; Wu and Carver 1999).

PHS is a complex trait controlled by quantitative trait loci (QTL) (Fofana et al. 2009; Knox et al. 2012). Given that phenotypic assays are laborious and often error-prone, marker-assisted selection is a desirable method to developing PHS tolerant lines in wheat breeding programs. Markers linked to PHS tolerance QTLs were identified in many prior studies (Kulwal et al. 2005; Mares et al. 2005; Sing et al. 2010, 2012). Kottearachchi et al. (2006) identified a QTL (QPhs-3As) on chromosome 3A using SSR makers. Some white progenies of recombinant inbred lines (RILs) derived from 'Zen'/'Spica' with the 'Zen' allele at QPhs-3As expressed higher dormancy than those with red grain. Liu et al. (2008), using greenhouse-grown Rio Blanco-derived populations, identified a QTL (*Qhs.pseru-3AS*) on chromosome 3A, which explained over 58 % of phenotypic variation for PHS tolerance. Genetic studies suggest that genes linked with PHS tolerance are mostly located on chromosome 2B, 3A and 4A (Graybosch et al. 2013). Recently, Singh et al. (2014), using simple sequence repeat (SSR) and Diversity Arrays Technology markers (DArT) on Double Haploid (DH) Canadian adapted durum wheats, detected QTL with small effects on chromosome 1A (*QPhs.spa-1A*), 1B (*QPhs.spa-1B*), 5B (*QPhs.spa-5B*), 7A (*QPhs.spa-7A*) and 7B (*QPhs.spa-7B*).

Nakamura et al. (2011) studied sequence variation in the promoter region of a wheat gene designated *TaMFT*, and suggested that the difference in seed dormancy between moderately and highly PHS tolerant genotypes is controlled by this dormancy gene. Liu et al. (2013) using comparative mapping and map-based cloning also isolated this locus (*Qphs.pseru.3As*) conditioning PHS tolerance. They renamed *TaMFT* (of Nakamura et al. 2011) to *TaPHS1* to better reflect the phenotype. Liu et al. found two mutations in *TaPHS1* that changed wheat from a PHS tolerant to a susceptible genotype. A GT-to-AT mutation in *TaPHS1* caused mis-splicing and further led to a premature stop codon that made the transcripts from the gene nonfunctional (Liu et al. 2013). Liu et al. (2013) suggested selection for positive alleles at this locus would be more predictive of PHS tolerance than seed color genes. Moreover, the levels of PHS tolerance of either PHS tolerant or susceptible genotype groups could be further modified by the seed color genes present. McKibbin et al. (2002) determined that mis-splicing of *Vp1* transcripts in wheat led to reduced *Vp1* protein production and malfunction of the gene, and diminished sprouting tolerance. Many major and minor genes, therefore, can impact tolerance to PHS in diverse genetic backgrounds.

Fakthongphan (2015) used combining ability analysis for both PHS tolerance (measured in a misting chamber) and falling number from field-grown samples, to identify red wheat genotypes, capable of donating genes, independent of seed coat color, that could improve PHS tolerance of hard white wheats. 'Jagalene' consistently demonstrated high general combining ability (GCA) and 'Niobrara' showed moderately consistent GCA. Jagalene and Niobrara, therefore, might serve as sources of additional and previously undescribed genes contributing to PHS tolerance, independent of red seed color. With the need for molecular markers for efficient wheat breeding and the identified PHS tolerance in red wheat parents, additional research is needed to confirm and identify useful markers for improvement of the trait in white wheats. The goal of

Table 1 Pedigree and the origin of the parental lines

Name	Class	Pedigree	Origin
Niobrara	HRWW	TAM 105*4/Amigo//Brule	Nebraska Agricultural Experiment Station and USDA-ARS, Baenziger et al. 1996
Jagalene	HRWW	Jagger/Abilene	Syngenta, PVP 200200160
NE98466	HRWW	KS89H56-4/NE90518	University of Nebraska
NW99L7068	HWWW	KS84HW1968*RioBlanco/HBY762A//Halt	USDA-ARS, Lincoln, Nebraska

HRWW hard red winter wheat, *HWWW* hard white winter wheat

this study was to identify markers applicable to the Great Plains hard white wheat gene pool. DNA markers were tested to: (1) identify the marker(s) linked to genes from red wheats donating PHS tolerance (2) find the additional minor gene(s) for PHS tolerance and (3) determine the level of the variation in PHS tolerance in populations fixed for the favorable allele at *TaPHS1* identified by Liu et al. (2013).

Materials and methods

Plant materials and field experiment

Three breeding populations were developed: 'Niobrara/NW99L7068', 'NE98466/NW99L7068' and 'Jagalene/NW99L7068'. Niobrara, NE98466 and Jagalene, all red wheats, were mated to the hard white wheat, NW99L7068. Jagalene and Niobrara, as noted above, were selected as potential donors of genes for PHS tolerance. Furthermore, Jagalene previously was described as a PHS tolerant red wheat by additional researchers (Ibrahim et al. 2008). NW99L7068 was used as a susceptible white wheat parent. Origins of these parental lines are presented in Table 1.

Crosses were made in the University of Nebraska Agronomy greenhouse in the spring of 2003 and 2004. The F_1 generation was increased in the field at Yuma AZ, 2005. F_2–F_5 generations were advanced as bulks in the field at Mead, NE from 2006 to 2009. To obtain a pure hard white population, seeds of the F_2 and subsequent generations were sorted using a prototype version of an automated seed sorting device (Pearson et al. 2008). Subsequent to sorting, only white seeds were planted in each generation. From F_7 bulk populations, single-head selections were made and planted in the fall of 2011 as unreplicated F_8 head-rows with controls replicated and populations blocked in the field at Mead, NE. Advanced generations of 150–160 lines per population were planted as four row plots with controls replicated and populations blocked in the field at Mead and Lincoln, NE in 2013 for the F_9 generation, and again at Mead for the F_{10} generation in 2014. Jagalene, NE98466, Niobrara (hard red wheats), 'Antelope', NW99L7068 and 'Nuplains' (hard white wheats) were planted as controls.

Assessment of preharvest sprouting

At physiological maturity (PM), 40 heads from F_8 to F_{10} generations were snapped for assessment of PHS tolerance via a misting chamber assay. The assay was used to measure Delta Value, a variable estimating tolerance to PHS. Delta Value, the change in head area due to seedling growth after 7 days in misting chambers, was measured using a LiCor (Lincoln, NE, USA) Li-3100C leaf area meter (Fakthongphan 2015; Graybosch et al. 2013). High Delta Values are indicative of low tolerance to PHS.

DNA marker analysis

Thirty-six primers, previously associated with PHS tolerance, were selected from the literature (Guyomarc'h et al. 2002; Röder et al. 1998; Roy et al. 1999; Somers et al. 2004; Song et al. 2005) and evaluated for polymorphisms among the parents. DNA was extracted from experimental lines at the F_7 (2010 plant) generation. Young leaf tissue from a minimum of eight plants was used to isolate DNA with a CTAB (cetyltrimethyl ammonium bromide) method modified from Doyle and Doyle (1987). PCRs were conducted using a total volume of 20 µl and contained 10 pmol of each forward and reverse primer. The PCRs also contained 0.2 mM each dNTP, 2 mM $MgCl_2$, 1X Go Taq Buffer supplied by Promega, 0.5 U Go Taq Flexi Polymerase and 100 ng of template DNA in a Bio-Rad DNA Peltier Thermal Cycle. The PCR steps followed Guyomarc'h et al. (2002), Röder et al. (1998), Roy et al. (1999), Somers et al. (2004) and Song et al. (2005). Amplified PCR products were separated on 2 % agarose gels, and stained with ethidium bromide using UV light for visualization.

Twenty-seven primers demonstrating polymorphism in at least one of the three populations, as listed in Supplementary Materials, were selected for further analyses. All primers are simple sequence repeat (SSR) except those with the prefix "MST" were sequence-tagged sites (STS). DNA extraction, PCR preparation and amplification

Table 2 Analysis of variance of tolerance to preharvest sprouting (Delta Value) from control cultivars grown at four Nebraska environments 2012–2014

Source	DF	Type III SS	Mean square	*F* value	Pr > *F*
Control	5	91,541	18,308	16.58	<0.0001
Error	17	18,443	1104		
Error: 0.8165 × MS (env × control) + 0.1835 × MS (error)					
Environment	3	15,296	5098	2.14	0.133
Error	17	39,706	2377		
Error: 0.7704 × MS (rep(env)) + 0.9568 × MS (control × env) − 0.72728 × MS (error)					
Rep (environment)	8	14,330	1791	5.69	<0.0001
Environment × control	15	19,228	1281	4.07	<0.0001
Error: MS (error)	328	103,250	314		

Table 3 Mean and std error of control for tolerance to preharvest sprouting (Delta Value) from control cultivars grown at four Nebraska environments 2012–2014

Cultivar	Mean ± std err				
	MD12	MD13	LNK13	MD14	Grand mean
White wheats					
Antelope	52.2 ± 6.7[a]	75.1 ± 7.2	79.1 ± 10.9	48.9 ± 4.2	57.2 ± 3.4
Nuplains	22.9 ± 6	40.9 ± 2	54.9 ± 6.8	53.0 ± 2.2	45.2 ± 2.5
NW99L7068	64.7 ± 6.8	98 ± 12.7	79.5 ± 8	59.9 ± 2.9	68.8 ± 3.2
Red wheats					
Jagalene	18.4 ± 1.7	16.0 ± 1.5	23.3 ± 5.6	19.4 ± 2.1	19.4 ± 1.6
NE98466	32.0 ± 5.4	44.8 ± 5.5	39.0 ± 3.8	22.4 ± 2.8	29.1 ± 2.3
Niobrara	18.4 ± 6.8	33.2 ± 3.7	42.9 ± 6.7	29.3 ± 2.4	31.4 ± 2

[a] Low Delta Values represent high tolerance to PHS

followed Graybosch et al. (2013). Fluorescent PCR fragment products were separated and detected using an ABI Prism 3730 DNA sequencer (http://www.appliedbiosystems.com) and peak data were extracted using GeneMarker version 1.95 (SoftGenetics.com). All lines of all three populations were also genotyped using the SNP markers for tolerant and susceptible alleles at *TaPHS1*, as described by Liu et al. (2013). No polymorphism at this locus was detected, and all lines were found to carry the "tolerant" allele. MapDisto Genetics Software version 1.7 was used to develop genetic maps in each population and establish chromosomal locations, when possible. LOD minimum 3, r-maximum or d-maximum 0.3 and minimum 10 % of missing data were employed (Lorieux 2012).

Statistical analysis

PC-SAS software version 9.2 (SAS Institute Inc., Car, NC, USA) was used for all statistical computations. In this study, genotypes (marker alleles) and entry (genotype) were considered fixed effects. Environments and the interaction between genotypes and environments were random effects. A separate analysis of wheat control genotypes was conducted. Analysis of variance (ANOVA), using SAS PROC GLM, was used to evaluate the source of variation for tolerance of PHS among controls. Mean and standard errors of Delta Values were calculated. The least significant difference (LSD) from the controls was calculated and used to compare Delta Values of lines in the three breeding populations.

Mean, standard error, minimum and maximum Delta Values for the three populations were calculated individually. PROC GLM was used to evaluate sources of variation for PHS tolerance, separately, within each population. Markers, environments and interaction between these variables were included in the model. Only confirmed homogeneous and homozygous lines were used for these analyses. Marker alleles (genotypes) were treated as fixed effects, the interaction between markers and environments were considered a random effect. For those loci displaying significant differences by ANOVA, mean and standard error of Delta Value were calculated separately for each locus within each population. Finally, when sample sizes were adequate, two-gene models were evaluated, again separately for each population, using all combinations of unlinked markers demonstrating significant genotypic effects at $P < 0.10$.

Table 4 Mean and std error of tolerance to preharvest sprouting (Delta Value) in three populations of red and white winter wheats

Population	Delta Value				
	Mean[a]	*n*	Std. err	Min	Max
Niobrara/NW99L7068	50.4	156	1.0	24.9	99.4
NE98466/NW99L7068	59.8	160	1.1	27.4	113.5
Jagalene/NW99L7068	64.4	160	1.1	29.5	127.5

Higher mean DV is indicative of lower tolerance to PHS

[a] Means from 4 environments; Mead 2012, Mead 2013, Lincoln 2013, Mead 2014, NE, USA

Results

Significant variation in PHS tolerance, as measured by Delta Value, due to environment, genotype and their interaction, was observed for the control genotypes (Table 2). Means by environments, grand mean and standard error of control cultivars are presented in Table 3. Among white wheats, Delta Values ranged from a low 45.2 (Nuplains) to a high of 68.8 (NW99L7068). Delta Values ranged from a low 19.4 (Jagalene) to a high of 29.1 (NE98466) among the red wheat controls. The hard red wheats had significantly higher tolerance to PHS (lower Delta Values) than the hard white wheat controls. Again, lower Delta Values are indicative of greater tolerance to PHS.

Table 4 presents mean and standard error of PHS tolerance in the three white wheat breeding populations. Mean Delta Values of individual lines from the same three populations are displayed in the histograms in Fig. 1. Observed Delta Value means of individual lines ranged from 24.9 to 99.4 (Niobrara/NW99L7068), from 27.4 to 113.5 (NE98466/NW99L7068) and from 29.5 to 127.5 (Jagalene/NW99L7068). Parental Delta Value means were 31.4 (Niobrara), 29.1 (NE98466), 19.4 (Jagalene) and 68.8 (NW99L7068). Progenies from all three populations showed wider ranges of Delta Value than their parents. Based on the LSD, 16.8, 20.1 and 23.1 % of lines were not significantly different in tolerance to PHS than the red parent in Niobrara/NW99L7068, NE98466/NW99L7068 and Jagalene/NW99L7068, respectively. Even though all entries of all three populations were fixed for the "favorable" allele at *TaPHS1* on chromosome 3AS, identified by Liu et al. (2013), a wide range of response in PHS tolerance still was observed.

The mapped chromosome locations based on both GrainGenes (http://wheat.pw.usda.gov/GG3) and confirmed in the present study are shown in Tables 5, 6 and 7, along with analyses of variance for each population. Significant environmental variation was observed for nearly all polymorphic markers in all three populations. In Niobrara/NW99L7068,

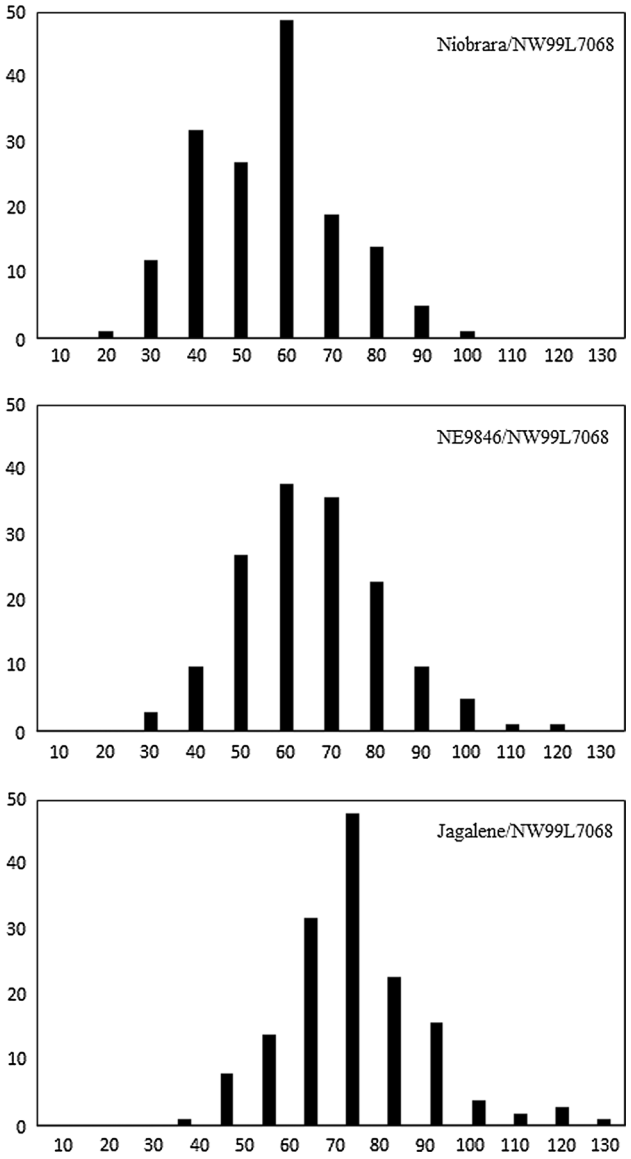

Fig. 1 Histograms of Delta Value of lines in three breeding populations of hard white winter wheat; LSD = 13.8; Parental lines: Nio = Niobrara, NW = NW99L7068, NE = NE98466, Jag = Jagalene

significant effects due to markers (allele) at $P < 0.10$ were observed for Xbarc10, Xbarc77 and Xwmc594; in NE98466/NW99L7068 for Xbar59, Xbarc105, Xcfd10 and Xgwm132; in Jagalene/NW99L7068 for Xbarc59, Xgwm155, Xgwm261, Xgwm371and Xgwmc428. Markers with significant effects due to genotype (allele) were found on several chromosomes, though significant markers on chromosome 3A were most frequent. Allelic effects of the observed significant markers are given in Table 8. The interaction between environment and marker was rarely significant in any of the three populations (Tables 5, 6, 7).

Table 5 Mean square from analysis of variance of putative markers for preharvest sprouting tolerance (Delta Value) in population 1 (Niobrara/NW99L7068)

Marker	Chromosome[b]	Mean square[a]				
		Environment	Genotype	Entry (genotype)	Genotype × environment	Error
Xbarc10	2B[c], 4B[c], 5A[c], 7B[c]	4079***	4450***	670***	47.5	345
Xbarc57	3A[c]	7481***	345*	883***	155.1	403
Xbarc77	3B[c]	12,298*	3392**	815***	389*	422
Xbarc59	2D[c], 5B[c]	20,409***	1080*	845***	600**	417
Xbarc321	3A[d], 3D[c]	17,566***	2983*	810***	914.1**	407
Xgwm132	6B[c], 6D[c]	9538***	756	762***	543	403
Xgwm261	2D[c]	17,932***	2674*	786***	1343***	417
Xgwm429	2B[c]	12,614***	2239*	792***	982**	407
Xwmc48	4A[c], 4B[c]	16,058***	320	826***	334	394
Xwms319	2B[c,c], 7A[c]	5389*	471	704***	673*	384
Xwmc428	3A[c]	15,431***	1722*	864***	680*	407
Xwmc594	3A[c,d]	11,718**	8552**	772***	1211***	421
Xwms429	2B[c,d]	11,220**	1495*	871***	791*	413

***, **, * Significant effects or difference observed at $P < 0.05$, $P < 0.10$, $P < 0.25$ level

[a] *F* value was calculated using different error term in the Type 3 analysis of PROC MIXED Delta Value from 4 environments; Mead 2012, Mead 2013, Lincoln 2013, Mead 2014, NE, USA

[b] Chromosomal location

[c] From "GrainGene (http://wheat.pw.usda.gov/GG3)

[d] Confirmed from this study

Table 6 Mean square from analysis of variance of putative markers for preharvest sprouting tolerance (Delta Value) in population 2 (NE98466/NW99L7068)

Marker	Chromosome[b]	Mean square[a]				
		Environment	Genotype	Entry (genotype)	Genotype × environment	Error
Xbarc10	2B[c], 4B[c], 5A[c], 7B[c]	16,175***	223	1073***	336	368
Xbarc12	3A[d]	24,906***	5	1015***	830*	633
Xbarc54	3A[d], 6D[c]	23,996***	800*	986***	331	610
Xbarc55	1B[c], 2B[c], 5B[c]	21,934***	324	1015***	357	616
Xbarc59	2D[c], 2B[c]	26,455***	7865***	979***	982*	624
Xbarc77	3A[d], 3B[c]	12,272*	198	969***	830	650
Xbarc105	3A[d], 7A[c], 7D[c]	19,292***	2252**	952***	504	603
Xbarc321	3A[c], 3D[c]	24,462***	117	989***	884	621
Xcfd10	3A[d], 5A[c], 5B[c]	23,150***	4948***	957***	241	658
Xgwm132	3A[b], 6B[c], 6D[c]	13,743***	3120***	800***	317	649
Xgwm261	2D[c]	72,497***	1146	996***	1211*	583
Xwmc48	4A[c], 4B[c]	10,233***	381	849	426	614
Xwmc428	3A[d]	30,710***	669.9	849***	426	614
Xwmc552	3A[c], 3D[c]	22,864***	1443*	1014***	72	649
Xwmc319	2B[c], 7A[c]	15,021**	1926*	981***	504	582

***, **, * Significant effects or difference observed at $P < 0.05$, $P < 0.10$, $P < 0.25$ level

[a] *F* value was calculated using different error term in the Type 3 analysis of PROC MIXED Delta Value from 4 environments; Mead 2012, Mead 2013, Lincoln 2013, Mead 2014, NE, USA

[b] Chromosomal location

[c] From "GrainGene (http://wheat.pw.usda.gov/GG3)

[d] Confirmed from this study

Table 7 Mean square from analysis of variance of putative markers for preharvest sprouting tolerance (Delta Value) in population 3 (Jagalene/NW99L7068)

Marker	Chromosome[b]	Mean square[a]				
		Environment	Genotype	Entry (genotype)	Genotype × environment	Error
Xbarc59	2D[c], 5B[c]	38,908***	2767***	1089***	377	480
Xbarc105	3A[c], 7A[c], 7D[c]	27,598***	524	1041***	437	486
Xcfd10	5A[c], 5B[c]	37,876***	24	1162***	129	490
Xgwm155	1D[c], 3A[c]	40,534***	4729***	1035***	342	507
Xgwm261	2D[c]	41,694***	2142**	1149***	790	492
Xgwm371	5B[c], 5D[c]	40,700***	4268**	1087***	454	492
Xgwm494	3A[c], 4A[c]	37,856***	2551*	1129***	564	504
Xgwmc48	4A[c], 4B[c]	39,930***	998	1111***	530	475
Xgwmc428	3A[c]	39,079***	3593**	1111***	529	495
Xwmc594	3A[c]	40,151***	1862*	1099***	513	482
Xwmc59	1A[c]	33,873***	1902	1121***	2424***	478
Xwms429	2B[c]	33,510***	7	931***	1317***	482

***, **, * Significant effects or difference observed at $P < 0.05$, $P < 0.10$, $P < 0.25$ level

[a] *F* value was calculated using different error term in the Type 3 analysis of PROC MIXED Delta Value from 4 environments; Mead 2012, Mead 2013, Lincoln 2013, Mead 2014, NE, USA

[b] Chromosomal location

[c] From "GrainGene (http://wheat.pw.usda.gov/GG3)

To further define the utility of marker-assisted selection for PHS tolerance, all potential possible combinations of unlinked loci, showing significant differences ($P < 0.10$) in single gene models (Table 8), were examined using two gene models (Tables 9 and 10). Putative alleles contributing to PHS tolerance, or lower Delta Value, were designated as "T", while "S", designated alleles contributed to susceptibility to PHS, or higher Delta Value.

Within Niobrara/NW99L7068 (Table 9), mean Delta Value scores of all TT classes (two putative PHS tolerant loci) were lower, and, hence, PHS tolerance was higher, than the SS classes (two putative susceptible loci). The same observations were evident in NE98466/NW99L7068 (Table 10) and Jagalene/NW99L7068 (Table 11). The TT class was always significantly lower in mean Delta Value than the SS class. In the two gene models, tolerance of PHS of the ST and TS classes (one putative tolerant locus and one putative susceptible locus) commonly showed intermediate results, indicating the action of additive genes.

Discussion

Previous studies have demonstrated that PHS tolerance is a quantitative trait (Fofana et al. 2009; Groos et al. 2002; Lawson et al. 1997; Liu et al. 2008). Herrmann (2007) and Jiang and Xiao (2005) explained that the genetic architecture associated with PHS in wheat is controlled by many genes with additive effects. Liu et al. (2008), Liu and Bai (2010) found that *Qphs.pseru-3AS*, a QTL showed a major effect on tolerance to PHS. Liu et al. (2013) cloned a major locus (*TaPHS1*) and identified both positive (increasing tolerance) and negative (decreasing tolerance) alleles, and speculated that selection for or against these would improve PHS tolerance. In the present investigation, a wide range of PHS tolerance was observed, even though all lines were fixed for the positive *TaPHS1* allele. This study did demonstrate that PHS QTLs from previously studied wheat populations will be useful in developing Great Plains white wheats with enhanced PHS tolerance, and that the red wheat gene pool contains alleles, independent of seed coat color, that can be exploited in white wheat breeding. In the three breeding populations, loci contributing to PHS tolerance were located on chromosomes 2B, 3A, 3B, 4B, 5A and 7B in Niobrara/NW99L7068, chromosomes 2B, 2D, 3A,5A, 5B, 6B, 6D, 7A and 7D in NE98466/NW99L7068 and chromosomes 1D, 2D, 3A, 5B and 5D in Jagalene/NW99L7068. In the Niobrara/NW99L7068 population, two linkage groups were established: Xgwm429 and Xwmc319 on chromosome 2B; Xbar321, Xgwm132 and Xwmc594 on chromosome 3A. One linkage group was identified in the NE98466/NW99L7068 population with Xbar77, Xbar54, Xbar12, Xbar105, Xgwm132, Xbar321, Xcfd10, Xwmc428, Xwmc552 and Xwmc96 located on chromosome 3A. A linkage group including Xcfd10 and Xwms429 was found in Jagalene/NW99L7068, however, the genomic location of these loci was unclear. To

Table 8 Mean Delta Value for lines with respective parental alleles at loci with significant ($P < 0.10$) effects in three populations of hard white winter wheat

Population[a]	Loci	Chromosome[b]	Parental allele			Parent	Putative effect
			No. lines[e]	Mean[f]	Std. err		
I = Niobrara/NW99L7068	Xbarc10	2B[c], 4B[c], 5A[c], 7B[c]	39	55.3	1.9	Niobrara	S
			7	37.2	3.6	NW99L7068	T
	Xbarc77	3B[c]	29	46.8	2.2	Niobrara	T
			109	52.5	1.2	NW99L7068	S
	Xwmc594	3A[c,d]	36	45.5	1.7	Niobrara	T
			99	54.1	1.3	NW99L7068	S
II = NE98466/NW99L7068	Xbarc59	2D[c], 2B[c]	78	57.4	1.5	NE98466	T
			72	64.4	1.8	NW99L7068	S
	Xcfd10	3A[c], 5A[c], 5B[c]	79	64.1	1.6	NE98466	S
			60	60.0	1.9	NW99L7068	T
	Xgwm132	3A[d], 6B[d], 6D[d]	30	52.5	2.4	NE98466	T
			81	63.0	1.6	NW99L7068	S
	Xbarc105	3A[d], 7A[c], 7D[c]	86	63.4	1.6	NE98466	S
			37	54.9	2.1	NW99L7068	T
III = Jagalene/NW99L7068	Xbarc59	2D[c], 5B[c]	67	62.7	1.6	Jagalene	T
			73	66.9	1.8	NW99L7068	S
	Xgwm155	1D[e], 3A[d]	65	68.5	2.0	Jagalene	S
			75	62.8	1.5	NW99L7068	T
	Xgwm261	2D[c]	83	67.1	1.7	Jagalene	S
			60	63.4	1.8	NW99L7068	T
	Xgwm371	5B[c], 5D[c]	61	62.6	1.9	Jagalene	T
			78	67.8	1.7	NW99L7068	S
	Xwmc428	4A[a], 4B[a]	70	67.7	1.8	Jagalene	S
			71	62.8	1.7	NW99L7068	T

T allele associated with greater tolerance to pre-harvest sprouting, *S* allele associated with greater susceptibility

[a] Delta Value from 4 environments; Mead 2012, Mead 2013, Lincoln 2013, Mead 2014, NE, USA

[b] Chromosomal location

[c] From "GrainGene (http://wheat.pw.usda.gov/GG3)

[d] Confirmed from this study

[e] Only homogeneous, homozygous lines included

[f] Means are presented only for those markers with significant ($P < 0.10$) differences indicated by ANOVA

Table 9 Mean sprouting tolerance of genotypes identified by two independent loci (Niobrara/NW99L7068)

Locus 1				Locus 2				Delta Value		
Loci	Chromosome	Genotype[b]	Effect	Loci	Chromosome	Genotype	Effect	No. lines	(Tolerance) mean	Std. err
Xbarc77[a]	3B	Niobrara	T	Xwmc594	3A	Niobrara	T	14	47.5ab	2.9
		Niobrara	T			NW99L7068	S	11	49.2ab	4.0
		NW99L7068	S			NW99L7068	S	83	55.2b	1.4
		NW99L7068	S			Niobrara	T	22	44.3a	2.2

Means followed by the same letter were not significantly different, $P < 0.10$

[a] Putative phenotyic effect from single-locus analysis (see Table 6)

[b] Genotye = parental origin of respective alleles

Table 10 Mean sprouting tolerance of genotypes identified by two independent loci (NE98466/NW99L7068)

Locus 1				Locus 2				Delta Value		
Loci	Chromosome	Genotype[a]	Effect	Loci	Chromosome	Genotype	Effect	No. lines	(Tolerance) Mean	Std. err
Xbarc59	2B, 2D	NE98466	T	Xbarc105	3A, 7A, 7D	NE98466	S	22	60.1ab	2.8
		NE98466	T			NW99L7068	T	50	56.3a	1.9
		NW99L7068	S			NW99L7068	T	51	63.8ab	2.0
		NW99L7068	S			NE98466	S	12	66.4b	4.9
Xbarc59	2B, 2D	NE98466	T	Xcfd10	5A, 5B	NE98466	S	35	63.6b	2.4
		NE98466	T			NW99L7068	T	33	51.1a	2.3
		NW99L7068	S			NW99L7068	T	24	65.6b	3.2
		NW99L7068	S			NE98466	S	40	65.1b	2.4
Xbarc59	2B, 2D	NE98466	T	Xgwm132	6B, 6D	NE98466	T	21	51.4a	3.1
		NE98466	T			NW99L7068	S	33	58.4a	2.3
		NW99L7068	S			NW99L7068	S	45	66.2b	2.3
		NW99L7068	S			NE98466	T	10	56.8ab	4.1
Xbarc105	3A, 7A, 7D	NE98466	S	Xcfd10	5A, 5B	NE98466	S	12	70.0b	4.4
		NE98466	S			NW99L7068	T	60	62.4ab	1.8
		NW99L7068	T			NW99L7068	T	36	56.6a	2.5
		NW99L7068	T			NE98466	S	17	58.8ab	3.2
Xbarc105	3A, 7A, 7D	NE98466	S	Xgwm132	6B, 6D	NE98466	T	9	56.2ab	4.5
		NE98466	S			NW99L7068	S	9	61.9ab	4.2
		NW99L7068	T			NW99L7068	S	64	62.7b	1.8
		NW99L7068	T			NE98466	T	20	52.9a	3.1
Xcfd10	5A, 5B	NE98466	S	Xgwm132	6B, 6D	NE98466	T	7	55.1ab	5.3
		NE98466	S			NW99L7068	S	50	65.3b	2.1
		NW99L7068	T			NW99L7068	S	24	59.2ab	2.9
		NW99L7068	T			NE98466	T	18	50.0a	3.1

Means followed by the same letter were not significantly different, $P < 0.10$

[a] Genotype = parental origin of respective alleles

develop lines with greater tolerance to PHS, these genes can be pyramided with each other, as per Graybosch et al. (2013).

The contribution of parental alleles to PHS tolerance of progeny genotypes was not always predicted by the parental phenotypes. For example, NW98466 contributed both T and S alleles (Tables 9, 10). Similar observations were made for parental genotypes Niobrara, Jagalene and NW99L7068. NW99L7068, the susceptible white wheat parent, still contributed some positive alleles. The pedigree of NW99L7068 includes RioBlanco, a PHS tolerant cultivar (Morris and Paulsen 1992), and perhaps it is able to contribute positive alleles once they are free of other negative alleles within the genome. Jagalene, a PHS tolerant wheat, still produced both susceptible and tolerant progenies. While significant effects were observed via pyramiding of T alleles, it was clear the effects were slight. All observations still confirm the quantitative and additive nature of the inheritance of PHS tolerance, and suggest pyramiding of multiple T alleles should be conducted to improve the chances of identifying PHS tolerant cultivars.

The polymorphic markers did not consistently identify loci across populations due to the difference of genetic background and different genes involved in each population. Tolerance to PHS is also strongly affected by environmental factors (Zanetti et al. 2000) and genetic, gene interactions and G × E interaction (Flintham 2000; Mares et al. 2005), though significant interactions between marker loci and environment rarely were observed in the present study. Additional loci in wheat cultivars must contribute to the interactions observed with environment. (Gu et al. 2004; 2006) noted that tolerance to PHS is controlled by many QTLs with various effects. Hence, these results can be explained by additive gene effects. No doubt, additional markers need to be identified to explain the observed variation both within and across these and other breeding populations. In all populations, transgressive segregation

Table 11 Mean sprouting tolerance of genotypes identified by two independent loci (Jagalene/NW99L7068)

Locus 1				Locus 2				Delta Value		
Loci	Chromosome	Genotype[a]	Effect	Loci	Chromosome	Genotype	Effect	No. lines	Mean	Std. err
Xbarc59	2B, 2D	Jagalene	T	Xgwm155	1D, 3A	Jagalene	S	26	64.1ab	2.2
		Jagalene	T			NW99L7068	T	37	62.0b	2.2
		NW99L7068	S			NW99L7068	T	36	62.5b	2.2
		NW99L7068	S			Jagalene	S	33	70.6a	2.9
Xbarc59	2B, 2D	Jagalene	T	Xgwm261	2D	Jagalene	S	38	63.4a	2.2
		Jagalene	T			NW99L7068	T	25	61.8ab	2.6
		NW99L7068	S			NW99L7068	T	29	62.5b	2.6
		NW99L7068	S			Jagalene	S	42	69.8a	2.6
Xbarc59	2B, 2D	Jagalene	T	Xgwm371	5B	Jagalene	T	24	60.0b	2.9
		Jagalene	T			NW99L7068	S	39	65.5a	2.7
		NW99L7068	S			NW99L7068	S	34	69.8a	2.7
		NW99L7068	S			Jagalene	T	36	64.1ab	2.6
Xbarc59	2B, 2D	Jagalene	T	Xwmc594	3A	Jagalene	S	30	63.3a	2.3
		Jagalene	T			NW99L7068	T	32	61.5b	2.6
		NW99L7068	S			NW99L7068	T	33	62.6a	2.5
		NW99L7068	S			Jagalene	S	39	70.2a	2.6
Xgwm155	1D, 3A	Jagalene	S	Xgwm261	2D	Jagalene	S	45	68.5a	2.4
		Jagalene	S			NW99L7068	T	16	68.1a	4.1
		NW99L7068	T			NW99L7068	T	41	61.3b	2.0
		NW99L7068	T			Jagalene	S	34	64.4ab	2.3
Xgwm155	1D, 3A	Jagalene	S	Xgwm371	5B	Jagalene	T	27	62.9b	3.1
		Jagalene	S			NW99L7068	S	32	73.3a	2.8
		NW99L7068	T			NW99L7068	S	44	62.8b	2.0
		NW99L7068	T			Jagalene	T	28	62.3b	2.6
Xgwm155	1D, 3A	Jagalene	S	Xwmc594	3A	Jagalene	S	32	71.9a	2.9
		Jagalene	S			NW99L7068	T	27	63.4b	3.0
		NW99L7068	T			NW99L7068	T	41	62.3b	2.1
		NW99L7068	T			Jagalene	S	43	62.6b	2.3
Xgwm261	2D	Jagalene	S	Xgwm371	5B	Jagalene	T	36	63.1b	2.6
		Jagalene	S			NW99L7068	S	43	71.1a	2.3
		NW99L7068	T			NW99L7068	S	32	63.2b	2.3
		NW99L7068	T			Jagalene	T	36	61.4b	2.3
Xgwm261	2D	Jagalene	S	Xwmc428	3A	Jagalene	S	46	71.0a	2.4
		Jagalene	S			NW99L7068	T	32	61.2b	2.6
		NW99L7068	T			NW99L7068	T	36	64ab	2.3
		NW99L7068	T			Jagalene	S	21	60.0b	2.8
Xgwm371	5B	Jagalene	T	Xwmc428	3A	Jagalene	S	29	66.0ab	2.9
		Jagalene	T			NW99L7068	T	30	58.5bc	2.6
		NW99L7068	S			NW99L7068	T	37	65.9ab	2.4
		NW99L7068	S			Jagalene	S	36	70.0a	2.5

Means followed by the same letter were not significantly different, $P < 0.10$

[a] Genotype = parental origin of respective alleles

for PHS tolerance was observed. Similar to the results reported here for hexaploid wheat, Knox et al. (2012) noted that transgressive segregation occurred for greater PHS tolerance in a durum wheat population from a cross between moderately susceptible and intermediate PHS tolerant parents.

Conclusions

In three breeding populations in which all lines were fixed for the major PHS tolerance allele of *TaPHS1*, a wide range of variation was observed, indicating that there are many minor genes involved with PHS tolerance in all three populations. Chromosome 3A, upon which *TaPHS1* resides, still was demonstrated to house additional loci influencing the trait. This chromosome should be subjected to intensive investigation to further define its critical role in PHS tolerance.

QTLs from previously studied wheat populations were found to influence PHS in Great Plains white wheats. These alleles were derived from hard red wheats, and are independent of seed coat color. Due to the different genes and differences of genetic backgrounds, markers indicating positive alleles will not consistently identify positive phenotypes across populations. In addition, crossing PHS tolerant parental genotypes may not guarantee good tolerance to PHS in their progenies, due to negative transgressive segregation. Crossing susceptible PHS parental genotypes could contribute some PHS tolerance, a result of positive transgressive segregation. In addition, environmental effects modulate the expression of PHS tolerance, and might mask genotypic effects. However, genotype × environment effects were minimal, confirming the potential use of marker-assisted selection for PHS tolerance improvement.

Author contributions statement Juthamas Fakthongphan conducted field and laboratory experiments, analyzed data and drafted manuscript. R.A. Graybosch and P.S. Baenziger developed genetic materials, designed the experiments, analyzed data and drafted manuscript. Paul St. Amand and Guihua Bai conducted DNA marker analyses and assisted with manuscript preparation.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Supplementary material

Supplementary Table S1 Primers used in DNA marker analysis

Primer	Sequence	DNA SequenceTailed	Locus/ Putative Chromosomes as per Grain genes	Putative chromosome (this study)
BARC10F18	GCGTGCCACTGTAACCTTTAGAAGA	ACGACGTTGTAAAACGACGCGTGCCACTGTAACCTTTAGAAGA	Xbarc10, 2B, 4B, 5A, 7B	
BARC10R	GCGAGTTGGAATTATTTGAATTAAACAAG	GCGAGTTGGAATTATTTGAATTAAACAAG		
BARC12F18	CGACAGAGTGATCACCCAAATATAA	ACGACGTTGTAAAACGACCGACAGAGTGATCACCCAAATATAA	Xbarc12, 3A	Pop2: 3A
BARC12R	CATCGGTCTAATTGTCAATGTA	CATCGGTCTAATTGTCAATGTA		
BARC54F18	GCGAACAGGAGGACAGAGGGCACGAGAG	ACGACGTTGTAAAACGACGCGAACAGGAGGACAGAGGGCACGAGAG	Xbarc54, 3A, 6D	Pop2: 3A
BARC54R	GCGCTTTCCCACGTTCCATGTTTCT	GCGCTTTCCCACGTTCCATGTTTCT		
BARC55F18	GCGGTCAACACACTCCACTCCTCTCTC	ACGACGTTGTAAAACGACGCGGTCAACACACTCCACTCCTCTCTC	Xbarc55, 1B, 2B, 5B	
BARC55R	CGCTGCTCCCATTGCTCGCCGTTA	CGCTGCTCCCATTGCTCGCCGTTA		
BARC57F18	GCGACCACCTCAGCCAACTTATTATGT	ACGACGTTGTAAAACGACGCGACCACCTCAGCCAACTTATTATGT	Xbarc57, 3A	
BAR0057R	GCGGGGAGGCACATTCATAGGAGT	GCGGGGAGGCACATTCATAGGAGT		
BARC59F18	GCGTTGGCTAATCATCGTTCCTTC	ACGACGTTGTAAAACGACGCGTTGGCTAATCATCGTTCCTTC	Xbarc59, 2D, 5B	
BARC59R	AGCACCCTACCCAGCGTCAGTCAAT	AGCACCCTACCCAGCGTCAGTCAAT		

Primer	Sequence	DNA Sequence Tailed	Locus/ Putative Chromosomes as per Grain genes	Putative chromosome (this study)
BARC77F18	GCGTATTCTCCCTCGTTTCCAAGTCTG	ACGACGTTGTAAAACGACGCGTATTCTCCCTCGTTTCCAAGTCTG	Xbarc77, 3B	Pop2: 3A
BARC77R	GTGGGAATTTCTTGGGAGTCTGTA	GTGGGAATTTCTTGGGAGTCTGTA		
BARC105F18	CAGGAAGAAAAGGAAAGCATGCGACAA	ACGACGTTGTAAAACGACCAGGAAGAAAAGGAAAGCATGCGACAA	Xbarc105, 3A, 7A, 7D	Pop2: 3A
BARC105R	GCGGTGTGGCAATAATTACTTTTT	GCGGTGTGGCAATAATTACTTTTT		
BARC31CF18	GGGCGGCGCATGTGCACCTA	ACGACGTTGTAAAACGACGGGCGGCGCATGTGCACCTA	Xbarc310, 3A	
BARC31CR	GCGTGGAAGCGACTAAATCAACT	GCGTGGAAGCGACTAAATCAACT		
BARC321F18	TGCACTTCCCACAACACATC	ACGACGTTGTAAAACGACTGCACTTCCCACAACACATC	Xbarc321, 3A, 3D	
BARC321R	TTGCCACGTAGGTGATTTATGA	TTGCCACGTAGGTGATTTATGA		Pop1: 3A; Pop2: 3A
CFA2193F18	ACATGTGATGTGCGGTCATT	ACGACGTTGTAAAACGACACATGTGATGTGCGGTCATT	Xcfa2193, 3A	
CFA2193R	TCCTCAGAACCCCATTCTTG	TCCTCAGAACCCCATTCTTG		
DUP398F18	CTGAGCCCTCTTTGCTATGC	ACGACGTTGTAAAACGACCTGAGCCCTCTTTGCTATGC	Xdup398, 2B	
DUP398R	TCGGTGAGATTGAAAGGTCC	TCGGTGAGATTGAAAGGTCC		
CFD10F18	CGTTCTATGACGTGTCATGCT	ACGACGTTGTAAAACGACCGTTCTATGACGTGTCATGCT	Xcfd10, 5A, 5B	
CFD10R	TCCATTTTCAAAAACACCCTG	TCCATTTTCAAAAACACCCTG		Pop2: 3A; pop3:3A

Primer	Sequence	DNA Sequence Tailed	Locus/ Putative Chromosomes as per Grain genes	Putative chromosome (this study)
FBB0335F18	AACAGCTATGACCATG	ACGACGTTGTAAAACGACAACAGC TATGACCATG	FBB0335	Pop1: 3A; Pop2: 3A
FBB0335R	GTAAAACGACGGCCAGT	GTAAAACGACGGCCAGT		
GWM132F18	TACCAAATCGAAACACATCAGG	ACGACGTTGTAAAACGACTACCAA ATCGAAACACATCAGG	Xgwm132, 6B, 6D	
GWM132R	CATATCAAGGTCTCCTTCCCC	CATATCAAGGTCTCCTTCCCC		
GWM155F18	CAATCATTTCCCCCTCCC	ACGACGTTGTAAAACGACCAATCA TTTCCCCCTCCC	Xgwm155, 1D, 3A	
GWM155R	AATCATTGGAAATCCATATGCC	AATCATTGGAAATCCATATGCC		
GWM261F18	CTCCCTGTACGCCTAAGGC	ACGACGTTGTAAAACGACCTCCCT GTACGCCTAAGGC	Xgwm261, 2D	
GWM261R	CTCGCGCTACTAGCCATTG	CTCGCGCTACTAGCCATTG		
GWM371F18	GACCAAGATATTCAAACTGGCC	ACGACGTTGTAAAACGACGACCAA GATATTCAAACTGGCC	Xgwm371, 5B, 5D	
GWM371R	AGCTCAGCTTGCTTGGTACC	AGCTCAGCTTGCTTGGTACC		
GWM429F18	TTGTACATTAAGTTCCCATTA	ACGACGTTGTAAAACGACTTGTAC ATTAAGTTCCCATTA	Xgwm429, 2B	
GWM429R	TTTAAGGACCTACATGACAC	TTTAAGGACCTACATGACAC		
GWM494F18	ATTGAACAGGAAGACATCAGGG	ACGACGTTGTAAAACGACATTGAA CAGGAAGACATCAGGG	Xgwm494, 3A, 4A	
GWM494R	TTCCTGGAGCTGTCTGGC	TTCCTGGAGCTGTCTGGC		
MST0101F18	CCACCATGAAGACCTTCCTC	ACGACGTTGTAAAACGACCCACCA TGAAGACCTTCCTC	MST0101, 7D	Pop1: 3A; Pop2: 3A
MST0101R	ACCTTGCATGGGTTTAGCTG	ACCTTGCATGGGTTTAGCTG		
WMC48F18	GAGGGTTCTGAAATGTTTTGCC	ACGACGTTGTAAAACGACGAGGGT TCTGAAATGTTTTGCC	Xwmc48, 4A, 4B	
WMC48R	ACGTGCTAGGGAGGTATCTTGC	ACGTGCTAGGGAGGTATCTTGC		

Primer	Sequence	DNA Sequence Tailed	Locus/ Putative Chromosomes as per Grain genes	Putative chromosome (this study)
WMC59F	ACGACGTTGTAAAACGACTCATTCGTTGCAGATACACCAC	ACGACGTTGTAAAACGACTCATTCGTTGCAGATACACCAC	Xwmc59, 1A	
WMC59R	TCAATGCCCTTGTTTCTGACCT	TCATTCGTTGCAGATACACCAC		
WMC96F18	TAGCAGCCATGCTTAGCATCAA	ACGACGTTGTAAAACGACTAGCAGCCATGCTTAGCATCAA	Xwmc96, 3A, 4A, 5A, 5D, 6A, 7A	Pop2:3A
WMC96R	GTTTCAGTCTTTCACGAACACG	GTTTCAGTCTTTCACGAACACG		
WMC307F18	GTTTGAAGACCAAGCTCCTCCT	ACGACGTTGTAAAACGACGTTTGAAGACCAAGCTCCTCCT	Xwmc307, 3B	
WMC307R	ACCATAACCTCTCAAGAACCCA	ACCATAACCTCTCAAGAACCCA		
WMC349F18	ACACACACTCGATCGCAC	ACGACGTTGTAAAACGACACACACACTCGATCGCAC	Xwmc349, 4B	
WMC349R	GCAGTTGATCATCAAAACACA	GCAGTTGATCATCAAAACACA		Pop2:3A
WMC428F18	TTAATCCTAGCCGTCCCTTTTT	ACGACGTTGTAAAACGACTTAATCCTAGCCGTCCCTTTTT	Xwmc428, 3A	
WMC428R	CGACCTTCGTTGGTTATTTGTG	CGACCTTCGTTGGTTATTTGTG		Pop1: 3A; Pop2:3A
WMC552F18	ACTAAGGAGTGTGAGGGCTGTG	ACGACGTTGTAAAACGACACTAAGGAGTGTGAGGGCTGTG	Xwmc552, 3D	
WMC552R	CTCTCGCGCTATAAAAGAAGGA	CTCTCGCGCTATAAAAGAAGGA		
WMC594F18	CCCCTCACTGCCG	ACGACGTTGTAAAACGACCCCCTCACTGCCG	Xwmc594, 3A	
WMC594R	ATATCCATATAGTACTCGCAC	ATATCCATATAGTACTCGCAC		Pop1: 2B
WMS319F18	GGTTGCTGTACAAGTGTTCACG	ACGACGTTGTAAAACGACGGTTGCTGTACAAGTGTTCACG	Xwmc319, 2B, 7A	
WMS319R	CGGGTGCTGTGTGTAATGAC	CGGGTGCTGTGTGTAATGAC		Pop1: 2B; Pop3: 2B or 5A, 5B

Primer	Sequence	DNA Sequence Tailed	Locus/ Putative Chromosomes as per Grain genes	Putative chromosome (this study)
WMS429F18	TTGTACATTAAGTTCCCATTA	ACGACGTTGTAAAACGACTTGTAC ATTAAGTTCCCATTA	Xwms429, 2B	
WMS429R	TTTAAGGACCTACATGACAC	TTTAAGGACCTACATGACAC		