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Mapping quantitative trait loci for preharvest sprouting resistance in white wheat

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Abstract The premature germination of seeds before harvest, known as preharvest sprouting (PHS), is a serious problem in all wheat growing regions of the world. In order to determine genetic control of PHS resistance in white wheat from the relatively uncharacterized North American germplasm, a doubled haploid population consisting of 209 lines from a cross between the PHS resistant variety Cayuga and the PHS susceptible variety Caledonia was used for QTL mapping. A total of 16 environments were used to detect 15 different PHS QTL including a major QTL, QPhs.cnl-2B.1, that was significant in all environments tested and explained from 5 to 31% of the trait variation in a given environment. Three other QTL QPhs.cnl-2D.1, QPhs.cnl-3D.1, and QPhs.cnl-6D.1 were detected in six, four, and ten environments, respectively. The potentially related traits of heading date (HD), plant height (HT), seed dormancy (DOR), and rate of germination (ROG) were also recorded in a limited number of environments. HD was found to be significantly negatively correlated with PHS score in most environments, likely due to a major HD QTL, QHd.cnl-2B.1, found to be tightly linked to the PHS QTL QPhs.cnl-2B.1. Using greenhouse grown material no overlap was found between seed dormancy and the four most consistent PHS QTL, suggesting that greenhouse environments are not representative of field environments. This study provides valuable information for marker-assisted breeding for PHS resistance, future haplotyping studies, and research into seed dormancy.

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Introduction

Premature germination of seeds while still attached to the mother plant is known as preharvest sprouting (PHS). In cultivated hexaploid wheat (*Triticum aestivum* L.) PHS is a common problem occurring when cool, wet weather persists around harvest maturity. The sprouting of grain prior to harvest lowers seed quality and test weight mainly through the breakdown of starch reserves in the endosperm by the enzyme α-amylase. Because of the reduction in quality for many end use products, the price paid for PHS affected wheat is severely downgraded at market and in more severe cases can be rejected outright.

Extensive seed dormancy is common in wild wheat relatives but has been lost during domestication. In general, PHS susceptibility is correlated with the low levels of seed dormancy commonly encountered in cultivated wheat. Red-grained wheat tends to be more resistant to PHS than white-grained genotypes due to an increased seed coat imposed dormancy (Flintham 2000). There is, however,

variation in embryo dormancy that can cause the most dormant white genotypes to be more dormant than the least dormant reds (Torada and Amano 2002; Wu et al. 1999). It has also been shown that the seed coat can have inhibitory effects separate from those related to coat color (Bewley and Black 1994). Because of the desire for separate classes of red and white wheat, breeders are forced to make use of genetic components other than red seed color in breeding for PHS resistance in white wheat. In addition to biological mechanisms that determine seed dormancy, preharvest sprouting is also influenced by seemingly unrelated plant physiology. It has been shown that accumulation of epicuticular wax, the absence of awns, and club-shaped spikes can reduce PHS by limiting water uptake (King and Richards 1984; King and von Wettstein-Knowles 2000).

Quantitative trait loci (QTL) for PHS have been found on all 21 wheat chromosomes. The most intensely studied locus, independent of the red kernel color loci on the long arm of group three chromosomes (R-A1, R-B1, R-D1), is a locus on chromosome 4A, often referred to as Phs and more recently Phs1 (Torada et al. 2008). The Phs1 locus on 4A was found to be a major locus controlling PHS in both red and white wheat from different areas of the world and was relatively stable over environments (Flintham et al. 2002; Kato et al. 2001; Mares et al. 2005, 2002; Mares and Mrva 2001; Mori et al. 2005; Torada et al. 2008). Other loci on the group 3 chromosomes, reportedly separate from the R and TaVp1 loci, have also been detected (Imtiaz et al. 2008; Kulwal et al. 2005; Osa et al. 2003). Studies have also reported QTL on the group 1 chromosomes (Anderson et al. 1993), both main and interaction effects on the group 2 chromosomes (Anderson et al. 1993; Imtiaz et al. 2008; Kulwal et al. 2004; Liu et al. 2008; Mares et al. 2002; Tan et al. 2006; Zanetti et al. 2000), and QTL on the group 6 chromosomes (Anderson et al. 1993; Imtiaz et al. 2008; Kulwal et al. 2004; Roy et al. 1999; Zanetti et al. 2000). Many of the QTL detected in these studies are significant in only certain environments and are not reported in other studies. The transient nature of the majority of PHS QTL may be due to interactions with the environment and/or genetic background, minor effects on the trait, germplasm specific alleles, or simply type I error.

Many of the previous genetic studies related to PHS resistance have focused on discrete loci (Kato et al. 2001; Kulwal et al. 2005; Mares et al. 2005; Mori et al. 2005; Ogbonnaya et al. 2008; Osa et al. 2003; Torada and Amano 2002), used minimal population sizes or environments (Anderson et al. 1993; Flintham et al. 2002; Roy et al. 1999; Tan et al. 2006) or involved crosses with red-seeded varieties (Flintham et al. 2002; Groos et al. 2002; Imtiaz et al. 2008; Kato et al. 2001; Kulwal et al. 2004, 2005; Mares et al. 2005; Miura et al. 2002; Mori et al. 2005; Osa et al. 2003; Roy et al. 1999). More comprehensive studies are needed to uncover novel sources of PHS resistance required to provide adequate protection for white wheat at harvest.

This study was undertaken to complement and extend the current knowledge about the genetics of PHS resistance in novel white wheat germplasm. A larger number of environments were used than in any previous PHS study to determine QTL loci consistent over locations and years. Extensive evaluation of other plant traits related to preharvest sprouting resistance but seldom reported, such as heading date (HD) and plant height (HT), were measured and compared to the PHS results. The comprehensive results presented herein provide valuable information for future PHS haplotyping studies, marker-assisted selection for PHS within and between wheat breeding programs, and research into the mechanisms governing seed dormancy in wheat.

Materials and methods

Plant material

In this study, a hexaploid wheat doubled haploid (DH) population consisting of 209 total lines was created from a cross between two elite soft white winter varieties that differ in PHS resistance. The female parent, Cayuga, is a PHS resistant variety derived from a cross between Geneva and Clark's Cream backcrossed to Geneva. The male parent, Caledonia, is a PHS susceptible variety that was selected as an off-type from Geneva. Both varieties are well adapted to the northeast United States. Caledonia carries the Rht-D1b dwarfing gene allele and is on average 85 cm tall, approximately 25 cm shorter than Cayuga, which carries the Rht-D1a allele. The HD of the two varieties are very similar with Caledonia typically heading one day earlier than Cayuga. Both varieties contain the photoperiod sensitive allele of Ppd-B1 on chromosome 2B and the insensitive allele Ppd-D1a on chromosome 2D based on the diagnostic markers reported in Beales et al. (2007). Seed for the parents and population is available from the National Small Grains Collection United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Aberdeen, Idaho.

PHS experimental design

Within each experimental field the Cayuga × Caledonia population was planted in 1 m rows in a randomized complete block design with two replications and parental checks. The population was planted in 2 or 3 fields near Ithaca, NY, USA in each year from 2001 to 2006 for a total

of 16 environments. The field location designations, number of lines with phenotypic data, the location means, parental means, and standard deviations can be found in Table 1. The locations from 2001 had fewer lines due to insufficient seed for a portion of the lines. Five spikes from each line were harvested at physiological maturity, as evidenced by the loss of green color from the glumes, and dried indoors for 5 days at ambient humidity and temperature. Due to slight differences in maturity, spikes were not always harvested on a single day. When the harvest was split among different days the harvest date for the line was recorded and taken into account as described in the QTL analysis section. After drying for 5 days PHS phenotyping was conducted as described by Anderson et al. (1993). For the McGowan 2004 location an additional three spikes were collected, dried for 5 days, and then placed at −20°C to preserve dormancy. The spikes were stored for approximately 4 months and then used for subsequent germination experiments. The seeds used for the afterripened germination tests were from a single replication in the Snyder location near Cornell University, Ithaca, NY in 2003. The greenhouse germination experiments were conducted in the spring of 2004. A randomized complete block design was used with two replicates. Three seeds of each line were sown in 15 cm plastic pots containing Cornell mix soil. The pots were rotated each week to avoid any bench edge effects. Plants were grown under 16 h days with natural and supplemented light from 1,000 W mercury lamps. A total of five spikes from each line and replication were harvested at physiological maturity and dried at room temperature and humidity for 10 days before being used for germination testing. For all the germination tests a subset of lines taken from the first 175 lines of the population were used. This was done to limit the number of lines tested to a manageable number and still retain statistical power. The lines selected represent a random sampling of both genotypes and phenotypes.

Germination tests

The germination tests on afterripened seeds used bulk belt-threshed seeds for each line that had been stored at room temperature and humidity for 8 months. All other germination tests were done on the primary and secondary seeds selected from the middle two-thirds of the spike. All seeds that had blackpoint, were shriveled, diseased, or showed evidence of sprouting were discarded. A total of 20 seeds from each spike were surface sterilized by soaking in 50 mL of 10% Clorox bleach for 10 min and were then rinsed with five 50 mL washes of sterile ddH_2O. The seeds were then placed crease down on 9 cm diameter germination paper soaked with 3 mL of sterile ddH_2O in plastic Petri plates. The plates were sealed in plastic bags with a wet paper towel to maintain high humidity and prevent drying. All germination tests were done at 22°C in the dark. Germination was assessed every 24 h, as evidenced by radicle protrusion, and all germinated seeds were discarded. Seeds contaminated by fungal growth were promptly removed and not included in the germination calculations. Following the germination test time period all remaining seeds were treated with 1 mL of 10 mM gibberellic acid and were placed at 4°C for 3 days to break dormancy. The seeds were then placed back at 22°C in the dark and assessed for germination 5 days later. All seeds that had not germinated were considered not viable and were excluded from calculations. The McGowan 2004 germination tests used seeds from three replicate spikes and were carried out for 10 days. The germination index (GI) was calculated by: $GI = (10 \times g_1 + 9 \times g_2 + \ldots + 1 \times g_{10})/(\#\text{ of viable seeds})$, where g is the number of seeds that germinated on the designated day (Walker-Simmons 1987). In the greenhouse 2004 germination experiment seeds were assessed for germination over 35 days and the GI was calculated as above. The germination tests on the McGowan 2004 and greenhouse 2004 environments were considered to be a measure of seed dormancy (DOR). The afterripened germination experiment was carried out for 4 days. The afterripened seeds germinated very rapidly with most lines exhibiting 100% germination of viable seeds after 48 h. Due to the rapid germination the percent germination after 1 day was used. Because the afterripened seeds lacked seed dormancy any difference in germination was considered to be due to the rate of germination (ROG).

Heading date and plant height

All HD and HT traits were measured on the same plants used for PHS testing. HD was measured by recording the date on which spike emergence was complete for 50% of the row. Plant height was measured to the top of the spike for the mean of the row. For all traits the measurements for the two replications within a location were averaged for further analyses.

DNA isolation and molecular marker genotyping

Isolation of DNA was conducted as previously described (Heun et al. 1991). More than 2,000 primers or clones were screened and 484 loci were originally mapped including 215 simple sequence repeats (SSRs), 147 Diversity Array Technology markers (DArT) (Triticarte Pty. Ltd., Yaralumla, Australia), 72 amplified fragment length polymorphisms (AFLPs), 31 target region amplification polymorphisms (TRAPs), 16 restriction fragment length polymorphisms (RFLPs), three expressed sequence tag-SSRs (EST-SSRs), and one sequence tagged site (STS).

Table 1 The table shows trait data and locations used for preharvest sprouting (PHS), seed dormancy (DOR), rate of germination (ROG), heading date (HD), and plant height (HT)

Trait	Environment number	Field name	Year	Lines with data	Mean trait score ± SD	Trait score range	Cayuga score ± SD	Caledonia score ± SD
PHS	1	Caldwell	2001	159	4.03 ± 1.57	0.80–7.80	1.60 ± 0.53	6.40 ± 0.74
PHS	2	Ketola	2001	152	4.17 ± 1.42	0.20–7.60	2.30 ± 0.88	5.70 ± 0.62
PHS	3	Snyder	2001	154	3.50 ± 1.44	0.20–6.80	2.10 ± 0.64	5.40 ± 1.09
PHS	4	Caldwell	2002	206	2.76 ± 1.35	0.00–6.40	1.90 ± 0.58	4.10 ± 1.43
PHS	5	New Ketola	2002	204	3.88 ± 1.22	0.20–7.00	2.80 ± 0.95	5.80 ± 0.37
PHS	6	Helfer	2003	209	3.81 ± 1.34	0.20–7.00	1.10 ± 0.87	6.40 ± 1.04
PHS	7	McGowan	2003	209	4.02 ± 1.40	0.40–8.20	2.50 ± 1.21	6.10 ± 1.01
PHS	8	Helfer	2004	209	3.68 ± 1.59	0.20–8.00	1.70 ± 0.34	6.40 ± 0.52
PHS	9	Ketola	2004	209	3.90 ± 1.15	0.60–8.20	2.20 ± 1.18	5.60 ± 0.77
PHS	10	McGowan	2004	209	3.48 ± 1.32	0.20–7.80	1.60 ± 0.95	6.00 ± 0.52
PHS	11	Helfer	2005	209	5.68 ± 1.22	1.80–8.60	3.20 ± 0.85	7.30 ± 0.82
PHS	12	Ketola	2005	209	4.23 ± 1.61	0.00–7.80	2.40 ± 0.43	5.60 ± 1.06
PHS	13	McGowan	2005	209	5.26 ± 1.31	0.60–8.80	3.50 ± 0.57	6.90 ± 0.34
PHS	14	Caldwell	2006	209	2.93 ± 1.37	0.00–7.20	1.60 ± 0.75	5.00 ± 1.45
PHS	15	Helfer	2006	209	3.66 ± 1.75	0.00–8.00	2.50 ± 1.89	5.90 ± 0.57
PHS	16	Snyder	2006	209	1.29 ± 1.20	0.00–6.20	0.30 ± 0.20	2.60 ± 1.64
DOR	10	McGowan	2004	160	7.63 ± 0.56	5.49–8.92	7.26 ± 0.31	7.76 ± 0.66
DOR	18	Greenhouse	2004	170	9.26 ± 4.82	0.00–31.25	12.15 ± 2.01	15.43 ± 8.90
ROG	17	Snyder	2003	161	0.63 ± 0.16	0.26–0.86	0.64 ± 0.10	0.46 ± 0.12
HD	11	Helfer	2005	209	0.00 ± 0.86	−2.53–2.47	−0.28 ± 0.50	0.47 ± 0.00
HD	12	Ketola	2005	209	0.00 ± 0.95	−2.42–2.58	0.33 ± 0.50	0.33 ± 0.50
HD	13	McGowan	2005	209	0.00 ± 0.95	−2.22–2.78	0.28 ± 0.58	−0.03 ± 0.50
HD	15	Helfer	2006	209	0.00 ± 1.48	−2.20–3.80	0.05 ± 0.96	−0.45 ± 1.5
HD	16	Snyder	2006	209	0.00 ± 1.09	−1.67–4.33	0.08 ± 0.50	−0.17 ± 1.00
HT	11	Helfer	2005	208	82.41 ± 9.66	60.00–103.00	92.00 ± 0.00	72.00 ± 0.00
HT	12	Ketola	2005	209	80.54 ± 9.15	62.50–100.00	85.75 ± 2.99	65.50 ± 1.00
HT	15	Helfer	2006	209	89.99 ± 10.56	67.50–115.00	98.75 ± 3.50	74.25 ± 2.99
HT	16	Snyder	2006	209	106.83 ± 11.82	85.00–130.00	117.75 ± 3.30	91.25 ± 2.50

The HD trait is plus or minus days based on the location mean set equal to zero. The HT trait is in cm and SD is the standard deviation

Southern hybridization was conducted as previously described (Anderson et al. 1992). TRAP markers were amplified according to the protocol of Liu et al. (2005), separated on 4% denaturing polyacrylamide gels, and stained with silver nitrate. The random and telomere specific primers were used as previously described (Liu et al. 2005). Other specific primers for the TRAP technique were designed to candidate genes using the program Primer3 (Rozen and Skaletsky 2000) or were from publicly available SSR primer sets found to be monomorphic between the parents. Development of AFLP markers followed the protocol of Vos et al. (1995) using *Eco*RI and *Mse*I restriction enzymes and primers with three selective bases. The amplified products were run on 4% denaturing polyacrylamide gels and stained with silver nitrate. The marker nomenclature follows the standardized naming code (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html). The SSR markers used were BARCs (Song et al. 2005; http://wheat.pw.usda.gov), CFDs (Guyomarc'h et al. 2002), GDMs (Pestsova et al. 2000), GPWs (Sourdille et al. 2004; http://wheat.pw.usda.gov), GWMs (Röder et al. 1998), and WMCs (Gupta et al. 2002). All DArT markers were genotyped by Diversity Array Technology Pty Ltd, Yarralumla, Australia and designated with the prefix wPt (http://www.triticarte.com.au). EST-SSR markers included KSUMs and CNLs as described by Yu et al. (2004). Primers for STS markers were designed using the Primer3 software (Rozen and Skaletsky 2000) and included primers for the CDO64 Oat cDNA clone (BE439283). The STS primers for the Rht-D1b genes were described previously (Ellis et al. 2002). The TaVp1 primers were based on Yang et al. (2007b).

Linkage map construction

The linkage map was constructed with the Map Manager QTXb20 computer program (Manly et al. 2001) using the Kosambi mapping function with a linkage threshold significance of $p < 0.0001$. Following the initial map construction the "ripple" command was used to find the optimal marker order within linkage groups. Any remaining linkages with LOD <3.0 were split into separate groups. For all unlinked markers the allele phase was flipped and another attempt to integrate the markers into the linkage groups was done using the "distribute" command. Chromosome designations were assigned based on previous wheat map locations (http://wheat.pw.usda.gov) and information available on DArT marker locations (http://www.triticarte.com.au). Any remaining groups were designated as unknown. After assigning chromosome designations redundant markers were removed from the map. For cosegregating markers the marker with less missing data was retained. Markers were then removed from the map to achieve marker spacing of ~5–10 cM and preference was given to markers with less missing data. The "ripple" command was used following the removal of each marker to obtain the new optimal marker order. The final map was used for all subsequent analyses.

QTL analysis

Prior to QTL analysis the PHS scores were regressed on the harvest date and the mean of the residuals from the two replications were used for subsequent QTL analyses. This procedure was done to correct for the effects of different harvest dates and to improve the normality of the data. In cases where all lines were harvested on the same day, the mean of the PHS scores from each replicate was used.

The QTL analyses were done using the software package QTL Cartographer for Unix compiled for Mac OS10.4 (Basten et al. 1994). The initial QTL discovery used stepwise regression (SR) implemented by the SRmapqtl command using model 2 with both forward and backward regression at a significance threshold of $p < 0.01$ (Basten et al. 2002). These data were then used to conduct composite interval mapping (CIM) using the Zmapqtl command with model 6, 2 cM steps, and a 10 cM window. Significance thresholds were calculated at $p < 0.05$ by 1,000 permutations (Churchill and Doerge 1994). QTL confidence intervals were determined by one-LOD intervals surrounding the QTL peak. This interval roughly approximates a 95% confidence level (Mangin et al. 1994). The QTL were named following the recommended rules for gene symbolization in wheat (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm).

Tests for epistatic and interaction QTL were conducted using the software program QTLNetwork 2 (Yang et al. 2007a, 2008). The 1D search for main effect QTL was done with a 10 cM testing window, 1 cM walking speed, and a 5 cM filtration window. Both the 1D and 2D genome scans were done with $p < 0.05$ significance threshold based on 1,000 permutations.

Table 2 The table shows the correlation coefficient (r) for the mean PHS score over all locations and the other traits measured in the study

Trait	Comparison	r
PHS	Between environments	0.49[a]
PHS mean	DOR McGowan 2004	0.35[a]
PHS mean	DOR Greenhouse 2004	−0.13
PHS mean	ROG Snyder 2003	−0.55[a]
PHS mean	HD mean	−0.39[a]
PHS mean	HT mean	−0.33[a]

[a] $P < 0.01$

Statistical analyses

All correlations used the Pearson product-moment correlation in the JMP statistical software package (http://www.jmp.com). Significance thresholds were based on a Bonferroni corrected p-value for 95 and 99% confidence levels to take into account multiple testing. All references to correlation significance levels are based on the corrected confidence levels.

Results

Evaluation of PHS

The mean correlation coefficient (r) for the PHS scores between environments was 0.49 (Table 2). Correlation coefficients between any two PHS environments ranged from 0.27 to 0.7 and all but two comparisons were significantly correlated (corrected $p < 0.05$). No clear trend was observed in the PHS correlations between locations within a single year or between the same field from different years (Supplementary S1).

Analysis of variance revealed significant ($p < 0.0001$) sources of variation for genotype, environment, genotype by environment, and replication within environment terms. The average score for the PHS resistant parent, Cayuga, ranged from 0.30 in the Snyder 2006 location to 3.50 in the McGowan 2005 location. The average score for the PHS susceptible parent Caledonia was as low as 2.60 in the Snyder 2006 location to as high as 7.30 in the Helfer 2005 location. The three locations from 2005 had the three highest PHS means while the Caldwell and Snyder locations in 2006 had the first and third lowest means, respectively. In

all locations the highest and lowest PHS scores in the population were outside the mean of the parents (Table 1). The heritability of the PHS trait was found to be 0.44 as calculated using the variance components from the ANOVA.

PHS and germination tests

To determine the relationship between the PHS trait, as measured by whole spike visual index, and DOR, the McGowan 2004 location was sampled for both PHS and DOR as measured using the GI (Table 1). The mean PHS score over all locations was significantly correlated with DOR McGowan 2004 ($r = 0.35$), representing a significant correlation between PHS resistance and seed dormancy (Table 2). Comparisons between the PHS score from individual locations and DOR McGowan 2004 were variable ranging from $r = 0.02$ to $r = 0.5$ (Supplementary S1).

To assess whether the ROG, and not DOR, was contributing to PHS susceptibility, afterripened seed from the Snyder 2003 location was used in germination tests (Table 1). Contrary to expectation, an increased ROG was significantly ($p < 0.01$) negatively correlated ($r = -0.55$) with the mean PHS score over all locations (Table 2). Thus, PHS resistance was associated with a faster ROG. The r values in the individual location comparisons ranged from -0.28 to -0.55 (Supplementary S1).

In order to determine if the greenhouse environment could be used in place of field-testing to assess PHS, the population was grown in the greenhouse in 2004 (Table 1). Germination tests were used instead of the whole spike PHS tests, because the relatively high level of seed dormancy in greenhouse grown material does not allow for whole spike assessments without the interference from considerable fungal growth on the wetted spike. The greenhouse 2004 GI was not significantly correlated with the mean PHS score over all locations (Table 2).

PHS and heading date

Heading date was recorded for the three locations in 2005 and the Helfer and Snyder locations in 2006 (Table 1). Table 2 shows the correlations between the mean HD and PHS scores over the tested locations. The HD trait was highly correlated ($p < 0.01$) between locations with r values from 0.73 to 0.84 (Supplementary S1). The mean HD and PHS scores were significantly ($p < 0.01$) negatively correlated ($r = -0.39$). Therefore, PHS resistance was associated with later HD in this population.

PHS and plant height

Plant height (HT) was recorded for the Helfer and Ketola locations in 2005 and the Helfer and Snyder locations in 2006 (Table 1). The HT trait was highly correlated ($p < 0.01$) between locations with correlation coefficients between 0.88 and 0.90. The mean HT and PHS scores were significantly ($p < 0.01$) negatively correlated ($r = 0.33$). Thus, increased plant height was associated with PHS resistance in this population.

Linkage map

A total of 484 loci were originally mapped on 209 lines of the Cayuga × Caledonia DH population. The map was then trimmed to 221 non-redundant loci composed of 134 SSR, 50 DArT, 25 AFLP, 7 TRAP, 4 RFLP, and 1 STS locus. The total map length was 1552.6 cM spread over 42 linkage groups with an average spacing of 9.3 cM between markers. All 21 wheat chromosomes were represented by at least one linkage group. Chromosomes 1D, 4D, 5A, and 7B had the least marker coverage (Supplementary S2). Only two markers showed a skewed segregation ratio (χ^2, $p < 0.01$) and were located on different linkage groups. The minimal number of skewed markers indicated that the mapped portion of the genome segregated normally and that the mapping and scoring were of high quality.

PHS QTL

Composite interval analysis (CIM) was used to detect QTL for PHS in the 16 different environments. A total of 15 different QTL across the genome were detected at $p < 0.05$ in at least one environment (Table 3a; Supplementary S3). Of those 15 QTL, four QTL, QPhs.cnl-2B.1, QPhs.cnl-2D.1, QPhs.cnl-3D.1, and QPhs.cnl-6D.1, were found to be significant at $p < 0.01$ in four or more environments and all had PHS resistance coming from the Cayuga allele (Table 3a; Fig. 1). The QPhs.cnl-2B.1 QTL was significant ($p < 0.01$) in all 16 environments and explained from 5% of the PHS variation in the Helfer 2004 location to 31% of the variation in the Ketola 2001 location (Supplementary S3). Using the mean PHS score over all locations the QPhs.cnl-2B.1 QTL was found to contribute 24% of the trait variation (Table 3a). The peak of the QPhs.cnl-2B.1 QTL varied between environments from 2 cM in the Caldwell 2001 and Caldwell 2002 locations to 31 cM in the Snyder 2006 location. The QTL peak for the PHS mean over all environments was 14 cM between the SSR markers BARC55 and WMC474. Often the QPhs.cnl-2B.1 peak was quite broad and appeared to have more than one peak in the region. However, separate QTL were not resolved using CIM analysis so the QTL was treated as a single QTL.

The QPhs.cnl-2D.1 QTL was detected in six environments and contributed from 7% to 11% of the variation in the trait (Supplementary S3). The QPhs.cnl-2D.1 QTL peak was found between 33 and 51 cM with the peak for the

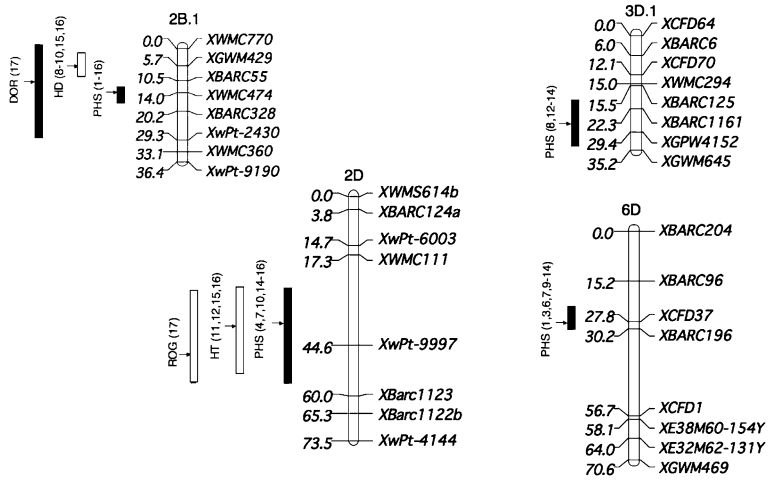

Fig. 1 The locations of the most replicable preharvest sprouting (*PHS*) QTL and the overlapping QTL for seed dormancy (*DOR*), rate of germination (*ROG*), plant height (*HT*), and heading date (*HD*). The numbers following the trait name indicate the locations in which the QTL was significant using thresholds based on 1,000 permutations ($p < 0.05$). The location numbers and corresponding locations can be found in Table 1. The *bar* to the left of the linkage group indicates the 1-LOD confidence interval for the QTL of the trait mean and the *arrow* indicates the peak position. *Black bars* indicate increasing trait value coming from Caledonia and *white bars* indicate increasing trait value coming from Cayuga

PHS mean, falling at 37 cM between the SSR marker WMC11 and the DArT marker wPt-9997 (Fig. 1). No direct comparisons between the 2B.1 and 2D linkage maps from this study could be made and attempts to use data from the wheat consensus map (Somers et al. 2004) to bridge between the two homoeologs were inconclusive in determining whether QPhs.cnl-2B.1 and QPhs.cnl-2D.1 were in homoeologous positions.

The QPhs.cnl-6D.1 QTL was significant in 10 environments. This QTL explained from 4% of the trait variation in the Snyder 2001 location to 25% of the variation in the Ketola 2005 location (Supplementary S3). For the mean PHS score over all locations the QPhs.cnl-6D.1 QTL contributed 14% of the trait variation (Table 3a). The peak of the QPhs.cnl-6D.1 QTL was relatively stable ranging from 27 cM in the Helfer 2005 location to 38 cM in the McGowan 2004 location (Supplementary S3). The peak for the PHS mean over all locations was found to be 28 cM between the SSR markers CFD37 and BARC196 (Table 3a; Fig. 1).

The QPhs.cnl-3D.1 QTL was significant in four environments and contributed between 5 and 10% of the trait variation in any one environment. The QPhs.cnl-3D.1 QTL peak fell between 22 and 36 cM and the peak for the mean was located at 26 cM between the SSR markers BARC1161 and GPW4152 (Fig. 1). The other PHS QTL were detected in three or less environments (Table 3a).

Using the mean PHS score for all environments eight of the 15 QTL were detected, including the four most significant QTL QPhs.cnl-2B.1, QPhs.cnl-2D.1, QPhs.cnl-3D.1, and QPhs.cnl-6D.1. Three additional QTL, QPhs.cnl-5B.1, QPhs.cnl-5B.2, and QPhs.cnl-5D.1, were detected when using the overall mean but were not detected in any one location (Table 3a). For all three unique QTL the Caledonia allele contributed to PHS resistance.

Seed dormancy and rate of germination QTL

To assess whether the same QTL were responsible for seed dormancy (DOR) in the field and greenhouse, CIM analysis was used to detect two DOR QTL from material grown in the greenhouse in 2004 (Table 3b). The QDor.cnl-4A.1 QTL was the most significant and contributed 10% of the variation in the trait. Surprisingly increased dormancy came from the Caledonia allele for both QPhs.cnl-4A.1 locus and the other significant QTL, QDor.cnl-7D.1 (Table 3b). The QDor.cnl-4A.1 QTL appeared to be shifted in relation to the QPhs.cnl-4A.1 locus, which had increased PHS resistance from the Cayuga allele in the Helfer 2003 location (Supplementary S3). The peak of the QDor.cnl-7D.1 QTL was shifted slightly in relation to the QPhs.cnl-7D.1 QTL peak from the McGowan 2004 location but had an overlapping confidence interval, however, the allele effects were in opposite directions.

To directly relate QTL for PHS and DOR in field grown material, CIM was used with the GI on the same samples from the McGowan 2004 location. By using the seed germination test instead of whole spike PHS test, it should be possible to detect seed dormancy QTL in the absence of complicating factors from spike related traits. Only two QTL, QDor.cnl-2B.1 and QDor.cnl-4D.1, were detected for the DOR trait from the McGowan 2004 location contributing

Table 3 The table shows the QTL detected for the traits preharvest sprouting (PHS), seed dormancy (DOR), rate of germination (ROG), heading date (HD), and plant height (HT)

QTL	Linkage group	Peak (cM)	Closest marker	LOD	R^2 (%)	Source of PHS resistance	Significant environments
(a) QTL for PHS							
QPhs.cnl-1A.1	1A.1	12	CFA2129	3.79	6	Caledonia	2, 3
QPhs.cnl-1B.1	1B.1	15	BARC240	5.36	4	Cayuga	9, Mean
QPhs.cnl-1B.2	1B.2	12	wPT-2094	4.82	7	Cayuga	1, 3, 12
QPhs.cnl-2B.1	2B.1	14	WMC474	27.10	24	Cayuga	1–16, Mean
QPhs.cnl-2B.2	2B.2	4	wPT-2397	4.31	4	Cayuga	8, Mean
QPhs.cnl-2D.1	2D	37	wPT-9997	7.59	7	Cayuga	4, 7, 10, 14–16, Mean
QPhs.cnl-3A.1	3A.1	0	wPT-1596	3.62	6	Cayuga	5, 8
QPhs.cnl-3D.1	3D.1	26	GPW4152	6.38	4	Cayuga	8, 12–14, Mean
QPhs.cnl-4A.1	4A.1	68	WMC617	3.56	3	Cayuga	6, Mean
QPhs.cnl-4D.1	4D	12	RHT-DF-MR2	6.00	9	Cayuga	3, 4
QPhs.cnl-5B.1	5B.1	2	wPT-3661	5.23	4	Caledonia	Mean
QPhs.cnl-5B.2	5B.1	48	wPT-1457	9.02	6	Caledonia	Mean
QPhs.cnl-5D.1	5D.1	21	BARC347	3.96	3	Caledonia	Mean
QPhs.cnl-6D.1	6D.1	28	CFD37	17.87	14	Cayuga	1, 3, 6, 7, 9, 10–14, Mean
QPhs.cnl-7D.1	7D.1	24	BARC278	3.76	4	Cayuga	10
QPhs.cnl-7D.2	7D.2	44	BARC172	3.19	4	Cayuga	6
QPhs.cnl-7D.3	7D.2	88	BARC154	3.45	6	Caledonia	4
QPhs.cnl-7D.4	7D.2	134	wPT-0934	5.29	5	Caledonia	2, 8, 9, Mean

QTL	Linkage group	Peak (cM)	Closest marker	LOD	R^2 (%)	Source of increased DOR or ROG	Significant environments
(b) QTL for DOR and ROG traits							
QDor.cnl-2B.1	2B.1	13	WMC474	3.63	8	Cayuga	10
QDor.cnl-4A.1	4A	44	BARC170	4.38	10	Caledonia	18
QDor.cnl-4D.1	4D	12	RHT-DF-MR2	5.1	13	Cayuga	10
QDor.cnl-7D.1	7D.1	12	WMC396	3.75	8	Caldeonia	18
QRog.cnl-2D.1	2D	47	wPT-9997	3.97	7	Cayuga	17
QRog.cnl-4D.1	4D	8	RHT-DF-MR2	4.17	7	Cayuga	17

QTL	Linkage group	Peak (cM)	Closest marker	LOD	R^2 (%)	Source of later HD or increased HT	Significant environments
(c) QTL for HD and HT traits							
QHd.cnl-1B.1	1B.1	11	BARC240	6.95	4	Cayuga	16, Mean
QHd.cnl-2B.1	2B.1	6	GWM429	47.95	46	Cayuga	11–13, 15, 16, Mean
QHd.cnl-3A.1	3A.2	16	BARC346	16.37	12	Cayuga	11, 13, 15, 16, Mean
QHd.cnl-3B.1	3B.2	13	GWM493	4.72	3	Caledonia	16, Mean
QHd.cnl-4D.1	4D	10	RHT-DF-MR2	13.57	9	Caledonia	11–13, Mean
QHd.cnl-7D.1	7D.2	5	WMC671	6.68	5	Caledonia	16, Mean
–	Unknown.1	0	TRAP_GWM165Lto5	6.12	17	Caledonia	11–13
QHt.cnl-2D.1	2D	40	wPt-9997	27.96	17	Cayuga	11, 12, 15, 16, Mean
QHt.cnl-4D.1	4D	12	RHT-DF-MR2	74.96	60	Cayuga	11, 12, 15, 16, Mean

The peak, closest marker, LOD, and R^2 are based on the QTL results of the mean over all environments. For the QTL that were only detected in individual locations and not by the mean, the mean was calculated from the results of the QTL analysis for the individual locations. Significance was based on 1,000 permutations with $p < 0.05$. The environment codes can be found in Table 1

8 and 13% of the trait variation, respectively (Table 3b). The peak for QDor.cnl-2B.1 was within 1 cM of the QPhs.cnl-2B.1 peak from the mean over all environments and had increasing dormancy contributed by the Cayuga allele (Fig. 1). The QDor.cnl-4D.1 peak was found to be 2 cM from the Rht-D1 gene, which is known to be segregating in the Cayuga × Caledonia population. A QTL for PHS, QPhs.cnl-4D.1, was also detected in two environments but not in the McGowan 2004 environment. Both QTL had increasing dormancy coming from the Cayuga allele (Supplementary S3).

To determine whether the rate of seed germination (ROG) influenced the measure of seed dormancy and PHS resistance, CIM analysis was used to detect QTL for seed germination in afterripened seeds from the Snyder 2003 location. Extensive afterripening is known to alleviate seed dormancy in wheat and differences in seed dormancy could be attributed to different rates of germination in lines with higher PHS scores. Using CIM analysis two QTL, QRog.cnl-2D.1 and QRog.cnl-4D.1, each contributed 7% of the trait variation (Table 3b). The peak for the QRog.cnl-4D.1 QTL was located 2 cM from the Rht-D1 gene. A QTL for PHS, QPhs.cnl-4D.1, was detected in two environments near Rht-D1 as was the QDor.cnl-4D.1 QTL from the McGowan 2004 location. For both ROG QTL the Cayuga allele contributed to increased ROG.

Heading date QTL

Heading date was recorded in five locations and was used in CIM analysis to detect five different QTL (Supplementary S4). The QHd.cnl-2B.1 QTL was detected at in all five environments tested and had a consistent peak at 6 cM from the top of the linkage group near the SSR marker GWM429 (Table 3c). This QTL contributed from 27 to 41% of the variation in the HD trait. The QHd.cnl-3A.1 QTL was detected in four environments contributing from 8 to 12% of the trait variation. The Cayuga allele at both the QHd.cnl-2B.1 and QHd.cnl-3A.1 QTL contributed to later HD. The Caledonia allele contributed to later HD at the QHd.cnl-4D.1, QHd.cnl-7D.1, and the unknown.1 group QTL.

Plant height QTL

Using CIM analysis two consistent QTL were detected for plant height on chromosome 2D and 4D (Table 3c). The peak of QHt.cnl-4D.1 QTL was located at the major plant height related gene Rht-D1 and contributed from 59 to 69% of the variation in the trait. The QHt.cnl-2D.1 QTL contributed from 15 to 25% of the trait variation and was located in the interval between the SSR marker WMC111 and the DArT marker wPt-9997. Though the diagnostic marker for the Rht8 dwarfing gene, GWM261, was monomorphic in the Cayuga × Caledonia population, comparisons with the wheat consensus map (Somers et al. 2004) show that GWM261 would likely be contained in the WMC111 to wPt-9997 interval. For both HT QTL the Cayuga allele contributed to increased plant height.

Epistatic interactions

All of the traits used in CIM analysis for main effect QTL were also tested for epistasis using the software program QTLNetwork2. No significant epistatic interactions were detected for any trait except the plant height trait from the Snyder 2006 location where one additive × additive interaction was observed between linkage group 2B.2 and 3D.1. The lack of significant epistatic interactions for the PHS trait indicates that PHS variation in the Cayuga × Caledonia population was primarily controlled by simple additive effects.

Discussion

QTL for PHS

A total of 15 QTL for PHS resistance were detected in the Cayuga × Caledonia population. Four of those QTL were found across multiple environments including the most significant QTL QPhs.cnl-2B.1, which was detected in all environments. Using the mean score over all environments, the QPhs.cnl-2B.1 QTL contributed 24% of the trait variation and had a peak at 14 cM between the markers BARC55 and WMC474. The stability and significance of the QPhs.cnl-2B.1 QTL suggests that it is a major QTL affecting PHS. Across map comparisons were inconclusive in determining if the QPhs.cnl-2B.1 QTL and the QPhs.cnl-2D.1 QTL are in homoeologous locations. Fine mapping of both loci would help to determine the relationship.

The QPhs.cnl-3D.1 QTL was detected in four of the 16 environments and contributed from 5 to 10% of the variation in the trait. There was no QTL for any of the other traits located on the 3D.1 linkage group. The viviparous-1 homolog in wheat (TaVp1) is known to be located on the long arm of the group 3 chromosomes (Bailey et al. 1999). Based on comparisons between the Bailey et al. (1999) and other previously published maps it is possible that the QPhs.cnl-3D.1 and QPhs.cnl-3A.1 QTLs are in a similar location as the TaVp1 gene. Repeated attempts to map the TaVp1-D gene in the Cayuga × Caledonia population were unsuccessful, however, TaVp1-B was mapped on 3BL.

The QPhs.cnl-6D.1 QTL was significant in 10 of the 16 environments. Of the 10 locations where the QPhs.cnl-6D.1 QTL was detected, the QTL from 2005 had the largest

effects. In the Ketola 2005 location, the effect of QPhs.cnl-6D.1 was greater than QPhs.cnl-2B.1. The three locations from 2005 also had the highest PHS means for the Cayuga × Caledonia population. In 2005, the seed development period, June 1 to July 1, was characterized by average temperatures 2.4°C higher than any other year included in this study. The average daily temperature departure from normal was 3.1°C, which was 2.3°C higher than any other year (http://met-www.cit.cornell.edu/climate/ithaca/). The specifics regarding the interaction of PHS and seed dormancy with temperature are not known, but it has been shown that high temperatures increase embryo sensitivity to the dormancy inducing hormone abscisic acid (ABA) (Walker-Simmons 1988). Preharvest sprouting susceptible wheat genotypes appear to be lacking the secondary dormancy response to high temperatures due to their decreased ABA sensitivity relative to PHS resistant varieties (Walker-Simmons 1988). Specific genetic studies focused on dormancy acquisition under elevated temperatures and ABA sensitivity could help to confirm the relationship.

Seed dormancy and rate of germination QTL

The germination experiment from the McGowan 2004 location was intended to identify those PHS QTL that contribute directly to seed dormancy, but should not be considered a comprehensive search for seed dormancy QTL. The major PHS QTL QPhs.cnl-2B.1 was also shared with a QTL for seed dormancy, QDor.cnl-2B.1, indicating that the QTL influences seed dormancy and is not due to factors related to the spike. The QPhs.cnl-2B.1 QTL could not be detected in the DOR Greenhouse 2004 environment. The lack of shared DOR QTL between the field and greenhouse environments, that were both assayed using germination tests, suggests that the greenhouse environment is not suitable for predicting seed dormancy in field grown material. The only other PHS QTL that was also detected as a DOR QTL was the minor QPhs.cnl-4D.1 locus. There are two main reasons why the other PHS QTL were not detected as seed dormancy QTL. The first reason may be that the other loci control factors such as spike morphology or the physiology of water uptake, which can influence PHS resistance separate from seed dormancy. The other possibility is that the germination test was not sensitive enough to detect the other smaller seed dormancy QTL, because dormancy was lost in the cold storage prior to germination testing. Though the dormant seeds were stored at −20°C to preserve dormancy, some dormancy was lost in the 4 months before the germination tests were conducted. The means of the GI for Cayuga and Caledonia were 7.26 and 7.76, respectively, which is a much smaller difference than what has been observed in Cayuga and Caledonia seeds tested immediately without cold storage (data not shown). This partial loss of dormancy could have reduced the significance of the seed dormancy QTL such that only those QTL that contributed the most to the trait variation were detected. The inability to detect a DOR QTL near the QPhs.cnl-6D.1 locus suggests that perhaps the QTL is due to factors other than seed dormancy. However, further testing that directly compares PHS and seed dormancy would help to confirm the role of the QPhs.cnl-6D.1 QTL.

Interestingly the 4D locus near the dwarfing gene Rht-D1 played a role in dormancy and the ROG, with the Cayuga allele promoting DOR but conversely increasing ROG after dormancy was lost through afterripening. It has been previously shown that the GA-insensitive Rht-D1b allele promotes the down-regulation of α-amylase but has no effect on seed dormancy (Mrva and Mares 1996). The finding that a locus near the Cayuga Rht-D1a gene contributes to seed dormancy is new and appears contradictory to its function of increasing α-amylase expression. It is possible that the Rht-D1 gene plays a role in seed dormancy separate from α-amylase expression, which is important for the ROG when there is no dormancy. It is also possible that the DOR and ROG traits are being controlled not by Rht-D1 but by closely linked genes.

The seed dormancy and ROG results should be viewed only as a starting point for further investigation due to the limited number of environments used. However, the identification of QDor.cnl-2B.1 at the same locations as QPhs.cnl-2B.1 provides good preliminary data that increased PHS resistance from the Cayuga allele at this locus is due to increases in seed dormancy.

QTL for heading date and plant height

Heading date is often a confounding factor in QTL studies and variety testing and is especially important when analyzing a trait such as PHS, where the trait can differ greatly in seeds of different maturities. Harvesting spikes at physiological maturity is intended to offset some of these differences. The two parents used in this study, Cayuga and Caledonia, have very similar HD with an average difference of only 1 day. The QTL analysis for HD in this population uncovered QTL from both parental varieties including a major QTL, QHd.cnl-2B.1, which had a later HD effect from the Cayuga allele (Table 3c). This QTL is located very close to the putative location of the Ppd-B1 gene based on map comparisons with previous studies (Hanocq et al. 2004). The diagnostic marker for the Ppd-B1 gene was found to be monomorphic between Cayuga and Caledonia, but because the marker is not based on the functional polymorphism determining photoperiod sensitivity it is unknown whether the two parents have different functional alleles (Beales et al. 2007). Consequently, the QTL could

be either the Ppd-B1 gene or a different one that is closely linked.

Significant correlations were detected between PHS resistance and later HD, and the PHS QTL QPhs.cnl-2B.1 was found to be in close proximity with the HD QTL QHd.cnl-2B.1. The peak and confidence interval for the QPhs.cnl-2B.1 QTL overlapped with the confidence interval for QHd.cnl-2B.1 in some environments. The QTL analysis using the mean PHS score over all environments located the peak for the QPhs.cnl-2B.1 QTL 8 cM away from the consistent peak for the QHd.cnl-2B.l QTL. Using this comparison there was no overlap in the 1-LOD confidence intervals between the two QTL. Using the mean over all environments can be expected to give a more accurate estimation of QTL position and effect. The close linkage of QTL for the PHS and HD traits could explain the significant correlations.

Comparisons with other PHS QTL studies

The most closely related PHS QTL study was that of Anderson et al. (1993). In that study two white winter wheat populations were used to detect PHS QTL across the genome. One of the parents from that study was Clarks Cream (CC), a PHS resistant variety that was used in the original cross during the development of Cayuga. The Anderson et al. (1993) study found QTL on chromosome 1A and one of the group 2 chromosomes for the CC × NY6432-18 recombinant inbred population. It was later learned that the CDO64 locus found to be significant in that study was located on chromosome 2B (data not shown). A PCR-based marker designed from the CDO64 clone sequence was found to cosegregate with GWM429, which is located near the QPhs.cnl-2B.1 locus detected in this study. Based on comparisons between marker alleles for CC and Cayuga it was determined that the Cayuga allele was not inherited from Geneva. Because CC was apparently heterogeneous for the 2B region, the original genotype used to create the population was not recovered. Other populations are being genotyped to identify the original allele, however, based on the results from this study and the Anderson et al. (1993) study, the 2B PHS QTL appear to be functionally similar.

In a PHS QTL mapping study of a cross between the hard white winter wheat varieties Rio Blanco and NW97S186, two separate QTL, QPhs.pseru-2B.1 and QPhs.pseru-2B.2, were detected on chromosome 2B from greenhouse grown materials (Liu et al. 2008). The QPhs.pseru-2B.2 locus was found to be 18 cM from the SSR marker WMC474, which is near the peak for QPhs.cnl-2B.1 QTL from the mean over all environments. Without additional markers in common between the two studies it is difficult to determine whether the QPhs.pseru-2B.2 and the QPhs.cnl-2B.1 QTL are due to the same locus. An examination of the pedigrees of Rio Blanco and CC does not reveal common ancestry going back at least three generations (http://genbank.vurv.cz/wheat/pedigree).

Using the International Triticeae Mapping Initiative (ITMI) population (synthetic W7984 × Opata85), Kulwal et al. (2004) detected a QTL on chromosome 2B, QPhs.ccsu-2B.1, in one environment. The QPhs.ccsu-2B.1 peak was located between the SSR marker GWM55 and the RFLP marker BCD1119. Neither marker was mapped in this study, however, BARC18 is contained in the GWM55–BCD1119 interval in the ITMI population. This marker was found to be located between WMC474 and BARC328 in the Cayuga × Caledonia population full map. This places the QPhs.ccsu-2B.1 and QPhs.cnl-2B.1 QTL in the same vicinity on chromosome 2B but a more direct relationship cannot be discerned due to the lack of shared markers between the maps.

Several studies have identified PHS QTL on chromosome 2D (Imtiaz et al. 2008; Mares et al. 2002; Tan et al. 2006). The Mares et al. (2002) study used a chromosome substitution set between Oxley and AUS1408 (dormant parent) and found lower dormancy in the chromosome substitution line containing the AUS1408 chromosome 2D. Tan et al. (2006) also used AUS1408 in a DH population from a cross with the variety Cascades. A significant QTL was found on chromosome 2D in the marker interval WMC112–GWM102 with increased dormancy from the Cascades allele, confirming the less dormant 2D allele found previously in the AUS1408. The marker interval on 2D found in the Tan et al. (2006) study appears to be centromeric as compared to the QPhs.cnl-2D.1 interval between WMC111 and BARC1123 found in this study, however, a direct comparison cannot be made due to a lack of shared markers. Imtiaz et al. (2008) detected an epistatic interaction QTL between chromosome 2D and 3D and between 2D and 4A. There was no main effect QTL detected at the 2D locus and the nearest marker GWM30 is centromeric to the QPhs.cnl-2D.1 locus in this study.

A number of studies have detected QTL on the group 3 chromosomes including chromosome 3D, however, several of the reported QTL can be attributed to the red kernel color gene R-D1 (Groos et al. 2002; Imtiaz et al. 2008; Kulwal et al. 2004; Kulwal et al. 2005; Osa et al. 2003).

The major PHS resistance locus identified on chromosome 4A appears to play a very limited role in PHS resistance in the Cayuga × Caledonia population. In this study a PHS QTL was detected on 4A in only one field environment contributing only 5% of the trait variation. A similar locus was also detected for the seed dormancy trait in the greenhouse environment. The inability to detect the 4A Phs1 locus could be due to a number of reasons including small or no difference between alleles, weak Phs1 alleles,

reduced QTL expression in the study environments, or a combination of these factors. The slightly larger effect of the Phs1 locus on seed dormancy in the greenhouse indicates that there is allelic variation at the locus but the QTL may not be expressed in the study field environments. It is also likely that neither Cayuga nor Caledonia contain the strong PHS resistance alleles on 4A as seen in other materials. A haplotyping study by Ogbonnaya et al. (2007) included the US winter wheat variety Clarks Cream, which is in the pedigree of Cayuga. Though PHS resistant, Clarks Cream had a different Phs1 allele than the other varieties known to carry the Phs1 resistance. Unfortunately, the study used proprietary SSR markers limiting the ability to extend the haplotyping to Cayuga and Caledonia (Ogbonnaya et al. 2007).

The detection of the consistent large-effect QTL QPhs.cnl-2B.1 adds important information to the growing knowledge concerning PHS resistance and seed dormancy in wheat. The fact that QTL have been found in a similar location in the other two PHS QTL studies involving white winter wheat from North American germplasm suggests novel PHS resistance alleles still exist. As more information about germplasm specific major loci is gathered it may be possible to pyramid PHS resistance alleles through marker-assisted breeding. The QTL identified in this study are well-suited to marker-assisted breeding due to the lack of significant epistatic interactions that can reduce progress.

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