

Mapping QTLs for pre-harvest sprouting traits in the spring wheat cross ‘RL4452/AC Domain’

G. Rasul · D. G. Humphreys · A. Brûlé-Babel ·
C. A. McCartney · R. E. Knox ·
R. M. DePauw · D. J. Somers

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Abstract Pre-harvest sprouting (PHS) in spring wheat (*Triticum aestivum* L.) is a major downgrading factors for grain producers and can significantly reduce end-use quality. PHS resistance is a complex trait influenced by genotype, environment and plant morphological factors. A population of 185 doubled haploid (DH) lines from the spring wheat cross ‘RL4452/AC Domain’ were used as the mapping population to detect quantitative trait loci (QTLs) associated with three PHS traits, germination index (GI), sprouting index (SI) and falling number (FN). Six major QTLs linked with PHS traits were mapped on bread wheat chromosomes 3A, 3D, 4A (2 loci), 4B and 7D. ‘AC Domain’ alleles contributed to PHS resistance

on 3A, 4A (locus-2) and 4B, and ‘RL4452’ alleles contributed resistance on 3D, 4A (locus-1) and 7D. QTLs detected on chromosome 4B controlling FN (*QFN.crc-4B*), GI (*QGI.crc-4B*) and SI (*QSI.crc-4B*) were coincident, and explained the largest amount of phenotypic variation in FN (22%), GI (67%) and SI (26%), respectively.

Keywords Falling number · Germination index · Microsatellite markers · Pre-harvest sprouting · Quantitative trait loci · Spring wheat · Sprouting index

Introduction

Pre-harvest sprouting (PHS) is a phenomenon of cereal crops when germination of grains occurs in the spikes before harvest. Downgrading of spring wheat due to PHS costs Canadian producers an average of \$100 million per year (Clarke et al. 2005). Pre-harvest sprouting negatively affects grain producers and the processing industry by reducing yield (Belderok 1968), test weight (Czarnecki and Evans 1986) and end-use quality (Buchanan and Nicholas 1980).

Because improved PHS resistance is often an important breeding objective dependable selection methods are essential (Paterson et al. 1989). Resistance to PHS is a complex trait which is controlled by genotype along with other factors, such as, spike and plant morphology (King and Richards 1984),

G. Rasul · D. G. Humphreys (✉) · D. J. Somers
Cereal Research Centre, Agriculture and Agri-Food
Canada, 195 Dafoe Road, Winnipeg, MB R3T 2M9,
Canada
e-mail: gavin.humphreys@agr.gc.ca

A. Brûlé-Babel
Department of Plant Science, University of Manitoba,
Winnipeg, MB R3T 2N2, Canada

C. A. McCartney
Department of Plant Sciences, College of Agriculture
and Bioresources, University of Saskatchewan,
Saskatoon, SK S7N 5A8, Canada

R. E. Knox · R. M. DePauw
Semiarid Prairie Agricultural Research Centre,
Agriculture and Agri-Food Canada, Box 1030,
Swift Current, SK S9H 3X2, Canada

germination inhibiting compounds in the bracts (Derera and Bhatt 1980), and stage of maturity (Gale et al. 1983). In addition, expression of PHS resistance is strongly influenced by the environment (Hagemann and Ciha 1987; Paterson et al. 1989) including temperature and humidity during grain ripening (Belderok and Habekotte 1980; Mares 1984). More recently, molecular techniques have been used to identify quantitative trait loci (QTLs) associated with PHS resistance in wheat (Roy et al. 1999; Kato et al. 2001; Groos et al. 2002; Osa et al. 2003; Mori et al. 2005; Mares et al. 2005; Torada et al. 2005).

Germination tests on threshed seeds have been used to screen germplasm for susceptibility to PHS (Hagemann and Ciha 1984). This is a straightforward and controlled method for evaluation of seed dormancy, and is insensitive to the spike characteristics. On the other hand, visual sprouting tests of intact spikes allow fast screening of many lines, and can detect not only dormancy but also other properties of the spikes which influence PHS under natural conditions (Hagemann and Ciha 1984; Paterson et al. 1989). Intact-spike sprouting tests and germination tests can produce results which are based on strong genetic control and the two types of testing can show a strong relationship, provided that spikes are sampled at a consistent maturity stage, usually physiological maturity, to minimize effects of differential dormancy (Paterson et al. 1989; Hucl 1994; Trethowan 1995). Artificial weathering of intact spikes using a rain simulator reduces environmental variation by controlling weathering conditions (Anderson et al. 1993; Humphreys and Noll 2002). A sprouting score based on spike wetting has been cited as a useful method to test segregating populations for PHS resistance (McMaster and Derera 1976; Paterson et al. 1989). Sprout damage can also be assessed and quantified by the Hagberg Falling Number test (AACC 2000), which is a measure of starch degradation presumably due to α -amylase activity before the appearance of any visible sprouting symptoms (Stoy 1982). While the various components of PHS resistance are not necessarily related to high falling number (FN) (Paterson et al. 1989), FN is an internationally accepted measure of sprout damage in wheat (Finney 1985).

From previously reported studies, QTLs for seed dormancy and PHS resistance have been identified using different evaluation techniques, and QTLs are reported separately for each screening method used.

Thus, breeders can select molecular markers for the desired component of PHS resistance.

Three QTLs for seed dormancy, scored as the percentage of germinated seeds, have been detected on group 4 chromosomes of wheat by Kato et al. (2001) using 119 doubled haploid (DH) lines derived from a cross between ‘AC Domain’ (a Canadian red spring wheat with high levels of dormancy) and ‘Haruyutaka’ (a Japanese red grained wheat with low levels of dormancy). More than 80% of the total phenotypic variance in seed dormancy was attributed to these three QTLs and was contributed by ‘AC Domain’. Mori et al. (2005) also reported QTLs on chromosome 4A and 4B associated with dormancy using germination tests in a Japanese red-grained wheat population, and fine mapped two putative QTLs: *QPhs.ocs-4A.1* on the long arm of chromosome 4A and *QPhs.ocs-4B.1* on the centromeric region of the long arm of chromosome 4B. Mares et al. (2005) reported that bread wheat with improved PHS resistance can be achieved through the introgression of putative grain dormancy gene/QTL located on chromosome 4A.

The three marker loci *Xfbb293*, *Xbcd131* and *Xgwm3* on the long arm of chromosomes 3A, 3B and 3D, respectively, have been reported by Groos et al. (2002) to be associated with PHS resistance evaluated by spike sprouting percentage. These loci co-localized with QTLs for grain seed coat colour in bread wheat where the “R-genes” were previously mapped. Two QTLs for seed dormancy designated as *QPhs.ocs-3A.1* and *QPhs.ocs-3A.2* evaluated by germination tests at 20°C were identified on the short and long arms of 3A, respectively (Osa et al. 2003).

Significant association with PHS tolerance rated by sprouting score was detected by Roy et al. (1999) on the long arm of chromosome 6B. Zanetti et al. (2000) reported QTLs for PHS resistance on chromosomes 5A which were associated with spelt ear morphology, and spelt-derived PHS QTLs on chromosomes 3B and 7B, and wheat derived PHS QTLs on chromosome 6A using a wheat x spelt cross as measured by FN and α -amylase activity. However, no QTLs have been reported to date using FN as a phenotyping method for PHS resistance in spring wheat. QTLs associated with high FN in bread wheat may be considered as a quality parameter for the processing industry.

The objectives of this study were: (1) to detect QTLs for three PHS traits, falling number of whole milled flour, germination index of threshed seeds, and

sprouting index of intact spikes; (2) to investigate whether there are relationships between preharvest sprouting QTLs and QTLs of agronomic and quality traits previously identified (McCartney et al. 2005, 2006) on the microsatellite-based genetic map of the ‘RL4452/AC Domain’ DH population.

Materials and methods

Plant materials

The mapping population consisted of 185 DH lines from the cross ‘RL4452/AC Domain’, which was developed from F₁ plants using the maize pollen/embryo rescue technique (Fedak et al. 1997). ‘AC Domain’ (Townely-Smith and Czarnecki 2008) is a registered cultivar in the Canada Western Red Spring (CWRS) marketing class with a high level of sprouting resistance. ‘RL4452’ is a backcross derived unregistered line with a low to moderate level of sprouting resistance with the pedigree ‘Glenlea*6/’Kitt’. ‘AC Domain’, ‘RL4452’ and ‘Glenlea’ (Evans et al. 1972) were used as checks for phenotyping PHS response in all environments.

Experimental design

The DH lines and three checks were grown in two-row plots 1.5 m in length with 30 cm between rows. A randomized complete block (RCB) design with three replicates was used for the Glenlea (Manitoba) trial in 2003, and the Swift Current (Saskatchewan) trials in 2003 and 2004. For the Winnipeg (Manitoba) trial in 2004, and the Glenlea and Winnipeg trials in

2005, a 12 × 16 incomplete block design with three replicates was used, with eight incomplete blocks comprised of 16 plots and four incomplete blocks comprised of 15 plots. A separate randomization was used for each environment. About 60 spikes (15 at Swift Current) per plot were hand harvested by cutting the peduncle about 10 cm below the base of the spike, when about 80% of the spikes in a plot had reached at physiological maturity. Collapse of the last node of the peduncle when most green colour had been lost from the glumes was used as an estimator of physiological maturity. Spikes were air dried for two days prior to storage and then stored in a freezer at –20°C until phenotyping.

Phenotyping

The environments used and traits measured are summarised in Table 1. Three PHS traits, GI of threshed seeds, SI of intact spikes, and FN of whole milled flour, were evaluated on samples from Glenlea in 2003 (GL03), Winnipeg in 2004 (WP04) and two locations (Glenlea and Winnipeg) in 2005 (GL05 and WP05, Table 1). ‘Glenlea’ is the name of one of the locations as well as one of the check cultivars used in this study. Additionally, SI data of intact spikes was collected at Swift Current in 2003 and 2004 (SC03 and SC04).

Germination test

Ten spikes collected from each plot were carefully threshed in a bract remover to ensure that the seed coat was not damaged. Fifty seeds of each plot were placed into a disposable 100 × 15-mm Petri dish with Whatman® filter paper, 90 mm diameter, grade #3. To each

Table 1 Pre-harvest sprouting traits evaluated over multiple environments (site-years) in 185 doubled haploid lines of the ‘RL4452/AC Domain’ population

Pre-harvest sprouting trait	Abbreviation (trait)	Environments evaluated ^a	Abbreviation (environment)
Falling number	FN	Glenlea 2003, Winnipeg 2004	GL03, WP04
		Glenlea 2005, Winnipeg 2005	GL05, WP05
Germination index	GI	Glenlea 2003, Winnipeg 2004	GL03, WP04
		Glenlea 2005, Winnipeg 2005	GL05, WP05
Sprouting index	SI	Glenlea 2003, Winnipeg 2004	GL03, WP04
		Swift Current 2003, Swift Current 2004	SC03, SC04
		Glenlea 2005, Winnipeg 2005	GL05, WP05

^a Falling number and germination index were evaluated over four environments in Manitoba, Canada; sprouting index was evaluated over four environments in Manitoba, Canada and over two environments in Saskatchewan, Canada

Petri dish, 5 ml of tap water was added making the filter paper uniformly wet but not flooded. Tap water was used instead of distilled water to minimise mould growth during incubation. Seeds were positioned with the crease facing down to facilitate counting embryo germination. Petri dishes were arranged randomly into Rubbermaid® tubs, containing a thin layer of water in the bottom to maintain humidity (Strand 1980). Tub was placed in a growth cabinet set at 15°C in the dark with ambient relative humidity (Hagemann and Cihá 1984). Each dish was examined daily. Seeds were counted as ‘germinated’ when the white radicle or plumule was protruding and there was a visible tear in the seed coat. Counts were made over 21 days, everyday beginning on day 2 to day 7 and then every alternate day for the next 14 days. The day seed was plated is referred to as Day 0. The number of germinated seeds on each day was recorded and removed from the Petri dishes. The plastic tubs were checked everyday to ensure the presence of a shallow film of water on the bottom and then placed back in the growth cabinet. After 21 days, seeds that had not germinated were considered ‘non-viable’ and were excluded from the experimental total seed used.

A weighted GI as described by Reddy et al. (1985) with modification (after 7 days, day interval between two counts was adjusted in the formula) was calculated by giving maximum weight to the seeds that germinated first and progressively less weight to those seeds that germinated later (Eq. 1)

GI

$$= \frac{\{d \times n_1 + [(d-1) \times n_2] + [(d-2) \times n_3] + \dots + [1 \times n_d]\}}{(d \times N)} \quad (1)$$

where, d was the total number of days the seed was counted; $n_1, n_2, n_3, \dots, n_d$ were the number of seeds germinated on the 1st, 2nd, 3rd, ..., d th day, respectively; N was the total number of viable seeds plated for germination. Seed germination is inversely related to the degree of dormancy (Hagemann and Cihá 1984).

Rain simulation

Two sets of 15 intact spikes from each plot of the Manitoba trials, were placed separately in a rain simulator (Convicon, Model: S-10H) for FN and SI tests. The peduncles of the harvested spikes were cut

about 5–6 cm from the base of the spike and spikes were inserted upright into perforated aluminum trays with 15 spikes per row separated by 2.5–3 cm. Trays were placed in the rain simulator and spikes were subjected to a “standard wetting treatment” in a controlled environment chamber. The rain simulators were kept at 16°C temperature and 99% relative humidity. The “standard wetting treatment” consisted of fifty mm of rainfall applied in an initial 4-h wetting period followed by 6 mm in every 4 h period. To maximise uniformity of the wetting treatment, the trays were repositioned daily within the chamber. Samples to be used for the FN test were removed after 48–72 h while those to be used for the SI test were removed after 72–120 h once visible sprouting was observed. A “calibration” test in the rain simulator period was carried out with a subset of samples from each environment to maximize the phenotypic range (data not shown). Rain simulation duration for the FN and SI tests varied annually depending on level of field weathering before harvest.

Falling number test

For the FN test, artificially weathered spikes were dried for 48 h in a forced-air dryer at 45°C. Dried spikes from each plot were threshed in a single head thresher and the threshed grain was milled on a UDY Cyclone Sample Mill (UDY Corp., Fort Collins, CO). Sprouting damage caused by weathering was determined by Hagberg Falling Number according to method 56-81B (American Association of Cereal Chemists 2000) on a Perten Falling Number 1500 instrument (Perten Instruments Inc., Springfield, IL) using whole meal flour. A representative sample of 7 g flour was placed in a FN test tube and 25 ml of distilled water was added to form a slurry. The tube containing the slurry was shaken until no dry flour was observed at the bottom of the tube before being placed in the machine. During an initial time of 60 s, the plungers moved up and down in the tubes to ensure uniform mixing and heating of the slurry. The time required for the plungers to fall from the top to the bottom of the tube determined the FN in seconds.

Sprouting test

Sprouting induced through artificial weathering treatments was assessed using a visual sprouting test.

About 15 intact spikes were used for the visual sprouting test and sprouted kernels of the intact spike were counted after 72–120 h of rain simulation in all Manitoba environments. Any spikes exhibiting fungal growth were discarded because fungal growth could influence the sprouting data, and data for infected spikes was eliminated. The spikes of each plot were classified into 6 groups (0, 1, 2, 3–5, 6–10 and 10+) based on the number of visible sprouted kernels and the number of spikes in each group was recorded. A weighted sprouting index (SI) was calculated by using the formula (Townley-Smith 2008) with little modification (group 6 added later and formula adjusted; Eq. 2)

$$SI = \frac{(S_1 \times 1 + S_2 \times 2 + S_3 \times 3 + S_4 \times 5 + S_5 \times 7 + S_6 \times 9)}{N} \quad (2)$$

where, S_1 , S_2 , S_3 , S_4 , S_5 , and S_6 were the number of spikes with 0, 1, 2, 3–5, 6–10 and 10+ visible sprouted kernels, respectively; N was the total number of spikes observed for visible sprouted kernels. The SI is similar to the rating systems described by Paterson et al. (1989), and McMaster and Derera (1976), where a score of ‘0’ indicated no visible sprouting while ‘10’ reflected sprouting over the entire spike.

Statistical analysis

Analysis of variance (ANOVA) was carried out using PROC GLM, and PROC MIXED with REML and Type 3 as estimation methods, and the Satterthwaite method to determine degrees of freedom in the SAS[®] 9.1 for Windows statistical software (SAS Institute Inc., Cary, NC, USA). ANOVA for trials GL03, SC03 and SC04 were conducted with PROC GLM because a RCB design was used and trials WP04, GL05 and WP05 were analyzed with PROC MIXED because an incomplete block (lattice) design was used. Replicates and incomplete blocks within replicates were considered as random effects, and genotypes were considered as a fixed effect. The least square estimate of genotype means was used for further analysis for three sprouting traits in all environments, using the LSMEANS statement in PROC GLM and PROC MIXED. Pearson’s correlation coefficients between sprouting traits and between

environments for each trait were estimated by using PROC CORR of SAS[®] 9.1 to determine the relationships among traits and among environments for each trait using least square means. For correlation analysis between sprouting traits, overall estimates of least square means from the combined analysis of each trait was generated from least square means of all individual environments of each trait using the LSMEANS statement in PROC GLM.

Genotyping

The genetic linkage map was constructed by McCartney et al. (2005) as a part of Agriculture and Agri-Food Canada (AAFC) Crop Genomic Initiatives to investigate QTLs controlling agronomic traits (McCartney et al. 2005) and quality traits (McCartney et al. 2006) in spring wheat using 182 lines of the DH population ‘RL4452/AC Domain’. The map consisted of 369 loci on 27 linkage groups, covering 2,793 cM of length of 21 wheat chromosomes, including 356 microsatellite loci, five ESTs and eight genes.

QTL analysis

Composite interval mapping (CIM) (Zeng 1993, 1994) was used for QTL analysis using Windows QTL Cartographer 2.5 software (Statistical Genetics, NC State University, USA). The forward regression method was used by setting the threshold value at 0.05. Model 6 (Standard Model) was used as the CIM model with a 2 cM walk speed in a 10 cM window size and five control marker numbers. A QTL was declared when the LOD score was >3 at least in two environment datasets. MapChart 2.1 software (Voorrips 2002) was used to visualize the QTLs in the graphical chromosome maps.

Results

Phenotypic data

Highly significant ($P < 0.0001$) differences among lines in all four environments (GL03, WP04, GL05 and WP05; Table 2) were found for FN and clear phenotypic differences were detected between the parents ‘RL4452’ and ‘AC Domain’ (Fig. 1a; Table 3). The

Table 2 Analysis of variance of three pre-harvest sprouting traits evaluated over multiple environments in western Canada for 185 doubled haploid (DH) lines of the ‘RL4452/AC Domain’ population

Source	Environment ^a					
	GL03	WP04	GL05	WP05	SC03	SC04
Falling number						
Rep	*	NS	***	***	–	–
Intra block (Rep)	–	*	NS	NS	–	–
DH lines	***	***	***	***	–	–
Germination index						
Rep	NS	**	NS	**	–	–
Intra block (Rep)	–	*	***	***	–	–
DH lines	**	***	***	***	–	–
Sprouting index						
Rep	NS	***	*	**	*	*
Intra block (Rep)	–	NS	*	***	–	–
DH lines	***	***	***	***	***	***

Falling number and germination index were evaluated in Glenlea 2003 (GL03), Winnipeg 2004 (WP04), Glenlea 2005 (GL05) and Winnipeg 2005 (WP05); Sprouting index in Glenlea 2003 (GL03), Winnipeg 2004 (WP04), Glenlea 2005 (GL05), Winnipeg 2005 (WP05), Swift Current 2003 (SC03) and Swift Current 2004 (SC04)

*** $P < 0.0001$, ** $P < 0.001$, * $P < 0.05$; NS non-significant at $P > 0.05$

^a Randomized complete block design was used in GL03, SC03 and SC04; lattice design was used in WP04, GL05 and WP05

frequency distribution appeared normal in environments GL03 and WP04, and appeared slightly skewed towards low FNs in environments GL05 and WP05 (Fig. 1a). In all four environments, transgressive segregation was observed among DH lines for both lower and higher FNs compared to the parents except in the trial WP05, where only transgressive segregation for low FN was observed (Fig. 1a). Detection of transgressive segregants with higher FN than the resistant parent ‘AC Domain’ suggests that not only ‘AC Domain’ alleles but also ‘RL4452’ allele(s) contributed to the high FNs in population. ‘AC Domain’ had high FN in all environments ranging from 281 to 549 (Table 3). ‘RL4452’ had low to moderate FNs ranging from 113 and 210. Among DH

lines, FNs ranged from 61 to 620. Over all four environments, the minimum and the maximum mean FNs among DH lines were 101 and 371, respectively, and the parents ‘RL4452’ and ‘AC Domain’ had FNs 177 and 384, respectively (Table 4).

Highly significant variation for GI among DH lines was found for all four environments ($P < 0.001$; Table 2). The distributions for GI were generally skewed towards a preponderance of higher GI values. Transgressive segregates in DH lines were detected for both low and high GI in all environments (Table 3). The parents, ‘AC Domain’ and ‘RL4452’, showed marked differences in GI (Table 3; Fig. 1b). Mean GI among DH lines ranged between 0.78 and 0.96 across all environments (pooled data), and overall mean GIs were 0.77 and 0.94 for ‘AC Domain’ and ‘RL4452’, respectively (Table 4).

Highly significant variation ($P < 0.0001$) among DH lines was observed for SI in all environments (Table 2). Distributions were appeared normally distributed in GL05 and WP05 environments, whereas, distributions were skewed towards low SIs in SC03, SC04 and WP04, and towards high SIs in GL03 (Fig. 1c). ‘AC Domain’ and ‘RL4452’, were classified in the low and the high range groups of SI values, respectively, in all six environments. Numerous lines with higher SIs than ‘RL4452’ were observed in all environments except WP05. Lines with lower SIs than ‘AC Domain’ were detected in three environments (GL03, SC03 and WP04; Fig. 1c). ‘AC Domain’ had a low mean SI, ranging from 0.7 to 1.7 within five environments, but had an unexpectedly high mean SI (5.5) in GL03, although most lines had higher SI values in GL03. ‘RL4452’ had a mean SI range between 4.1 and 8.9. Correlations among six environments for the SI trait were significant (14 out of 15 correlations), except the correlation between GL03 and GL05 which was non-significant ($P = 0.05$). The overall mean SI in the DH population ranged from 1.6 to 7.4 (Table 4). The overall mean SI for ‘AC Domain’ was 1.9 and 6.8 for ‘RL4452’ across all six environments.

From the pooled datasets for each trait across all environments, FN was negatively correlated with GI ($r = -0.56$) and SI ($r = -0.80$), and GI was positively correlated with SI ($r = 0.59$). All correlation coefficients were highly significant ($P < 0.0001$; Table 4).

Fig. 1 Frequency distribution of pre-harvest sprouting responses (**a** Falling number; **b** Germination index; **c** Sprouting index) in 185 DH lines from the cross ‘RL4452/AC Domain’ along with their parents evaluated in multiple environments (GL03, Glenlea 2003; SC03, Swift Current 2003; WP04, Winnipeg 2004; SC04, Swift Current 2004; GL05, Glenlea 2005; WP05, Winnipeg 2005)

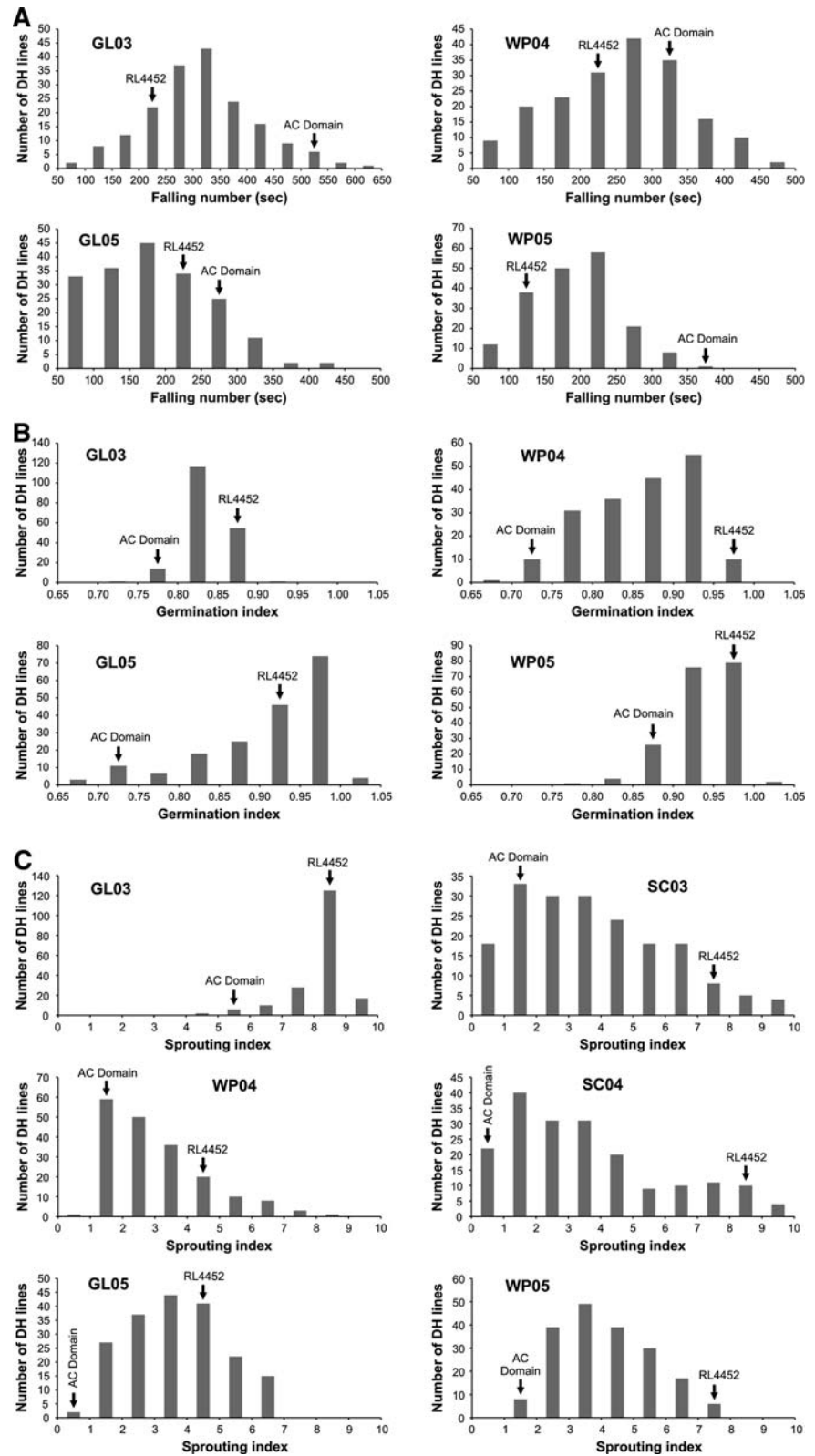


Table 3 Pearson's correlation coefficient between environments, overall mean and range in 185 doubled haploid lines of the 'RL4452/AC Domain' population along with check mean for three pre-harvest sprouting traits evaluated over multiple environments in Canada

Environment	Pearson's correlation coefficient					Doubled haploid population			Check means		
	WP04	GL05	WP05	SC03	SC04	Mean	Minimum	Maximum	AC Domain	Glenlea	RL4452
Falling number											
GL03	NS	-0.24**	0.17*	-	-	274	62	620	549	164	180
WP04	-	0.61***	0.43***	-	-	258	61	493	340	126	210
GL05	-	-	0.30***	-	-	184	62	442	281	142	206
WP05	-	-	-	-	-	192	65	332	364	76	113
Germination index											
GL03	0.19*	0.20*	0.28***	-	-	0.84	0.74	0.91	0.79	0.82	0.87
WP04	-	0.60***	0.62***	-	-	0.86	0.67	0.98	0.72	0.96	0.96
GL05	-	-	0.54***	-	-	0.91	0.67	1.01	0.70	0.94	0.94
WP05	-	-	-	-	-	0.94	0.79	1.00	0.87	1.00	0.99
Sprouting index											
GL03	0.19*	NS	0.27**	0.31***	0.25**	8.2	4.8	9.0	5.5	9.0	8.9
WP04	-	0.56***	0.51***	0.43***	0.40***	3.0	1.0	8.1	1.3	6.1	4.1
GL05	-	-	0.39***	0.27**	0.20*	3.7	0.9	6.9	0.7	5.1	4.1
WP05	-	-	-	0.52***	0.39***	4.1	1.0	7.6	1.6	6.7	7.9
SC03	-	-	-	-	0.56***	3.6	0.0	9.7	1.7	5.3	7.0
SC04	-	-	-	-	-	3.3	0.0	9.3	0.7	8.7	8.6

Falling number and germination index were evaluated in Glenlea 2003 (GL03), Winnipeg 2004 (WP04), Glenlea 2005 (GL05) and Winnipeg 2005 (WP05); Sprouting index in Glenlea 2003 (GL03), Winnipeg 2004 (WP04), Glenlea 2005 (GL05), Winnipeg 2005 (WP05), Swift Current 2003 (SC03) and Swift Current 2004 (SC04)

*** $P < 0.0001$; ** $P < 0.001$; * $P < 0.05$; NS non-significant at $P = 0.05$

Table 4 Pearson's correlation coefficient between pre-harvest sprouting traits, overall mean and range in 185 doubled haploid lines of the 'RL4452/AC Domain' population evaluated over four environments in Manitoba, Canada

Traits	Falling number	Germination index	Sprouting index	Doubled haploid population			Check means		
				Mean	Minimum	Maximum	AC Domain	Glenlea	RL4452
Falling number	-	-0.56***	-0.80***	227 ^a	101	371	384	127	177
Germination index	-	-	0.59***	0.89 ^b	0.78	0.96	0.77	0.93	0.94
Sprouting index	-	-	-	4.3 ^c	1.6	7.4	1.9	6.8	6.8

Pre-harvest sprouting traits were evaluated in Glenlea 2003, Winnipeg 2004, Glenlea 2005 and Winnipeg 2005

*** $P < 0.0001$

^a Standard error of mean falling number = 42

^b Standard error of mean germination index = 0.02

^c Standard error of mean sprouting index = 0.6

QTLs for falling number

Two QTLs controlling FN were detected by composite interval mapping in the DH population derived

from the cross 'RL4452/AC Domain' (Tables 5, 6; Fig. 2). A putative QTL for FN, *QFN.crc-4B*, was detected on chromosome 4B (Table 5). Based on R^2 values, the 4B QTL explained 14.9–22% of the FN

Table 5 Composite interval mapping analyses using QTL Cartographer for detecting QTLs controlling three pre-harvest sprouting traits in 185 doubled haploid lines of the ‘RL4452/AC Domain’ population evaluated over multiple environments in Canada

QTL ^a	Environment	Marker or interval	LOD	R ² (%)	Additive ^b
Falling number					
<i>QFN.crc-4A.1</i>	WP04	<i>Xwmc48</i>	3.0	6.1	−45
<i>QFN.crc-4B</i>		<i>Xwmc349</i>	9.5	22.0	86
<i>QFN.crc-4A.1</i>	GL05	<i>Xwmc48</i>	4.2	7.7	−43
<i>QFN.crc-4B</i>		<i>Xwmc349</i>	8.8	19.1	68
<i>QFN-4A.1</i>	Overall	<i>Xwmc48</i>	6.3	13.7	−40
<i>QFN-4B</i>		<i>Xwmc349</i>	7.7	14.9	42
Germination index					
<i>QGI.crc-4A.1</i>	GL03	<i>Xwmc48</i>	3.4	7.2	0.01
<i>QGI.crc-4B</i>	WP04	<i>Xwmc349</i>	41.6	66.6	−0.10
<i>QGI.crc-4B</i>	GL05	<i>Xwmc349</i>	18.1	33.5	−0.09
<i>QGI.crc-4B</i>	WP05	<i>Xwmc349</i>	14.8	28.2	−0.04
<i>QGI-4A.1</i>	Overall	<i>Xwmc48</i>	4.6	5.9	0.02
<i>QGI-4B</i>		<i>Xwmc349</i>	33.4	52.7	−0.06
Sprouting index					
<i>QSI.crc-3D</i>	GL03	<i>Xgwm383–Xgwm191</i>	3.0	8.6	0.5
<i>QSI.crc-3D</i>	WP04	<i>Xgwm191–Xbarc71</i>	4.1	8.5	0.9
<i>QSI.crc-4B</i>		<i>Xwmc349</i>	13.3	23.5	−1.5
<i>QSI.crc-4B</i>	GL05	<i>Xwmc349</i>	12.8	26.0	−1.5
<i>QSI.crc-4B</i>	WP05	<i>Xwmc349</i>	5.4	10.8	−0.9
<i>QSI.crc-7D</i>		<i>Xgwm482</i>	5.3	11.0	0.9
<i>QSI.crc-3A</i>	SC03	<i>Xwmc264</i>	5.4	8.7	−1.4
<i>QSI.crc-3D</i>		<i>Xgwm191–Xbarc71</i>	3.8	7.8	1.3
<i>QSI.crc-4A.2</i>		<i>Xgwm494</i>	3.2	6.3	−1.2
<i>QSI.crc-4B</i>		<i>Xwmc349</i>	3.9	6.2	−1.1
<i>QSI.crc-7D</i>		<i>Xgwm482</i>	11.3	–	2.2
<i>QSI.crc-4A.2</i>	SC04	<i>Xgwm494</i>	6.6	12.0	−1.6
<i>QSI.crc-7D</i>		<i>Xgwm482</i>	4.9	9.2	1.5
<i>QSI-3A</i>	Overall	<i>Xwmc264</i>	5.0	7.1	−0.6
<i>QSI-3D</i>		<i>Xgwm191–Xbarc71</i>	8.5	11.9	0.8
<i>QSI-4A.2</i>		<i>Xgwm494</i>	4.1	6.2	−0.6
<i>QSI-4B</i>		<i>Xwmc349</i>	14.9	26.9	−1.2
<i>QSI-7D</i>		<i>Xgwm482</i>	8.2	12.7	0.8

QTL was declared when LOD score was >3 at least in two environments

^a Falling number and germination index were evaluated in Glenlea 2003 (GL03), Winnipeg 2004 (WP04), Glenlea 2005 (GL05) and Winnipeg 2005 (WP05); Sprouting index in Glenlea 2003 (GL03), Winnipeg 2004 (WP04), Glenlea 2005 (GL05), Winnipeg 2005 (WP05), Swift Current 2003 (SC03) and Swift Current 2004 (SC04)

^b Additive effect of allelic contribution. A positive sign in falling number, and a negative sign in germination index and in sprouting index indicated that the ‘AC Domain’ allele at that locus contributed resistance in the respective trait relative to the RL4452 allele

phenotypic variation and the ‘AC Domain’ allele increased FN relative to the ‘RL4452’ allele. LOD score peaks fell between markers BF484674-297 and *Xbarc10* which map closely to the microsatellite

marker *Xwmc349* (Fig. 2). A second putative QTL, *QFN.crc-4A.1*, was detected in the terminal region of chromosome 4A (Table 5). *QFN.crc-4A.1* explained 6.1–13.7% of the phenotypic variation for FN.

Table 6 Chromosomes in which significant QTLs were detected for three pre-harvest sprouting traits in 185 doubled haploid lines of the ‘RL4452/AC Domain’ population evaluated over multiple environments in Canada

Environment	Falling number	Germination index	Sprouting index	Number of QTL
GL03	–	4A.1	3D	2
WP04	4A.1, 4B	4B	3D, 4B	3
GL05	4A.1, 4B	4B	4B	2
WP05	–	4B	4B, 7D	2
SC03	–	–	3A, 3D, 4A.2, 4B, 7D	5
SC04	–	–	4A.2, 7D	2
Overall	4A.1, 4B	4A.1, 4B	3A, 3D, 4A.2, 4B, 7D	6
Total	2	2	5	

Falling number and germination index were evaluated in Glenlea 2003 (GL03), Winnipeg 2004 (WP04), Glenlea 2005 (GL05) and Winnipeg 2005 (WP05); Sprouting index in Glenlea 2003 (GL03), Winnipeg 2004 (WP04), Glenlea 2005 (GL05), Winnipeg 2005 (WP05), Swift Current 2003 (SC03) and Swift Current 2004 (SC04)

Negative additive values indicate that ‘AC Domain’ allele reduced FN relative to the RL4452 allele. The LOD peak for *QFN.crc-4A.1* were located close to the microsatellite marker *Xwmc48* (Fig. 2).

QTLs for germination index

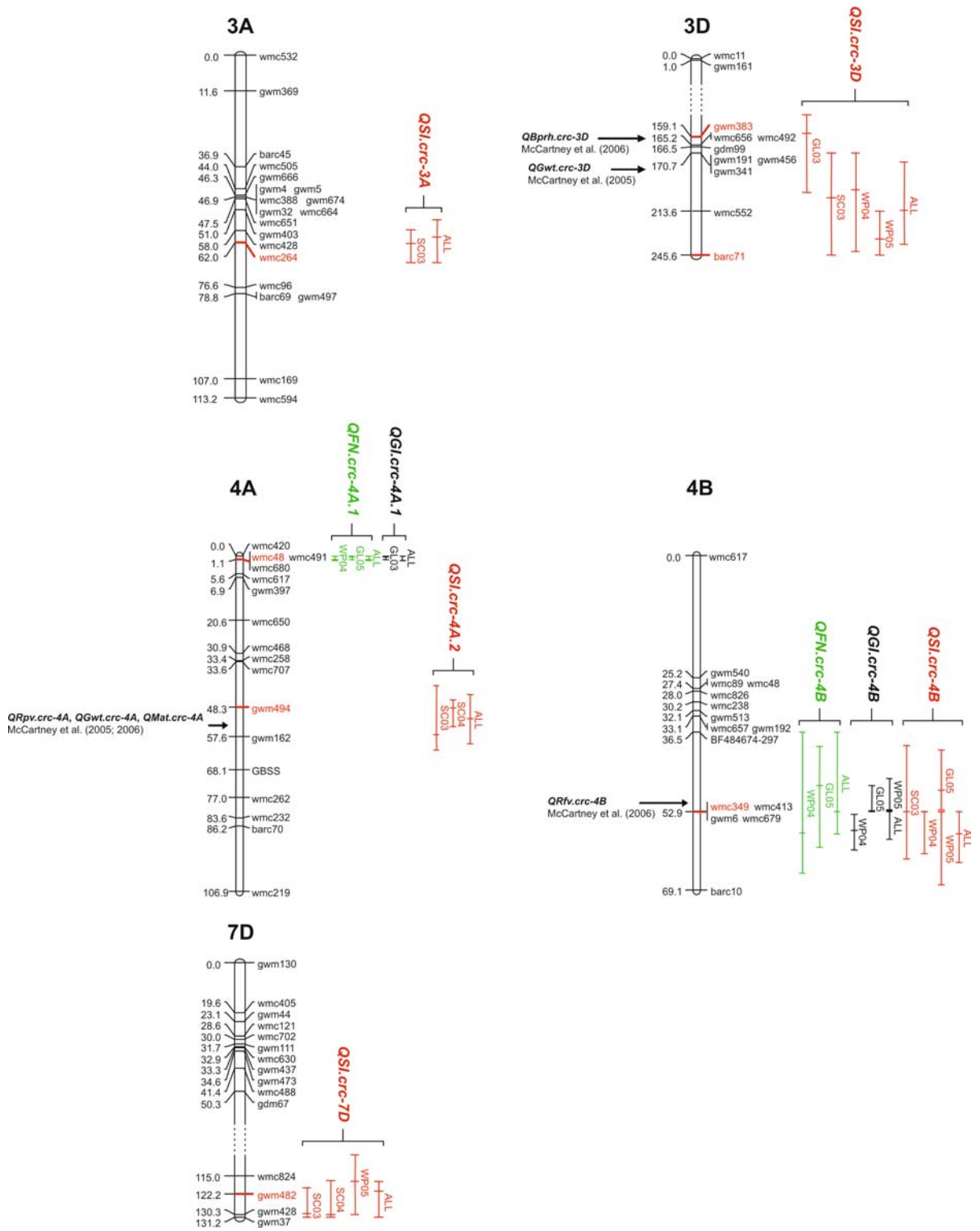
Two GI QTLs were identified in the DH population ‘RL4452/AC Domain’ (Tables 5, 6; Fig. 2). *QGI.crc-4B*, on chromosome 4B, was detected in three of four individual environments and in the combined dataset (Table 5). This QTL had the largest effect on GI and explained 28.2–66.6% of the phenotypic variation. Negative additive values indicate that the ‘AC Domain’ allele at the 4B locus tended to reduced GI compared to the ‘RL4452’ allele. *QGI.crc-4B* was coincident with the FN QTL *QFN.crc-4B* on chromosome 4B (Fig. 2). A second putative QTL for GI, *QGI.crc-4A.1*, was detected on chromosome 4A and this QTL was coincident with the FN QTL *QFN.crc-4A.1*. The ‘AC Domain’ allele increased GI values at this locus compared to the ‘RL4452’ allele (Table 5).

QTLs for sprouting index

Five significant QTLs for SI were found. *QSI.crc-4B* was observed on chromosome 4B (Table 5; Fig. 2) and it explained between 10.8 and 26.9 percent of the phenotypic variation for SI. Negative additive values for this QTL indicated that the ‘AC Domain’ allele contributed to lower SI compared to the ‘RL4452’. A putative QTL on chromosome 3D, *QSI.crc-3D*, explained between 7.8 and 11.9 percent of the

phenotypic variation for SI (Table 5; Fig. 2). *QSI.crc-7D* was detected on chromosome 7D, and explained between 11.0 and 12.7 percent of the phenotypic variation. Positive additive values for loci *QSI.crc-3D* and *QSI.crc-7D* suggested that ‘AC Domain’ alleles at both loci resulted in increased SI values. An additional QTL for SI, *QSI.crc-3A*, was mapped to chromosome 3A at marker locus *Xwmc264* and explained 7.1 to 8.7 percent of the phenotypic variation (Table 5; Fig. 2). The ‘AC Domain’ allele at this locus was found to decrease SI relative to the ‘RL4452’ allele. On chromosome 4A, *QSI.crc-4A.2*, was detected solely in the Saskatchewan environments (SC03, SC04). *Xgwm494* was the closest marker to *QSI.crc-4A.2*, and the ‘AC Domain’ allele at the *QSI.crc-4A.2* locus reduced SI values relative to the ‘RL4452’ alleles (Table 5).

Fig. 2 Genetic linkage map of the cross ‘RL4452/AC Domain’ showing 29 QTLs in six environments for three sprouting traits over five wheat chromosomes. *Green, black and red vertical lines* indicate the QTLs for falling number, germination index and sprouting index, respectively. *Vertical text* on the right of each QTL indicates the environment where QTL is detected (GL03, Glenlea 2003; SC03, Swift Current 2003; WP04, Winnipeg 2004; SC04, Swift Current 2004; GL05, Glenlea 2005; WP05, Winnipeg 2005; ALL, Overall environments). Two *horizontal lines* intersecting two ends of each *vertical line* indicate the QTL confidence interval defined by 1 LOD drop-off from the peak. *Horizontal line* in between two *horizontal lines* or *bold horizontal line* if with confidence interval indicates the LOD peak. Molecular markers and their genetic map positions in Kosambi centimorgans are indicated on the *right* and on the *left* side of each chromosome, respectively. Markers in *red color* indicate the closest markers or marker interval for the detected QTLs



Combined effect of the QTLs

Doubled haploid lines of the cross ‘RL4452/AC Domain’ could be categorized into resistant and susceptible groups based on SI values (Fig. 1). The five most resistant and five most susceptible lines were chosen, and their allelic combinations at six marker loci linked with PHS QTLs on chromosomes 3A, 3D, 4A (2 loci), 4B and 7D were determined (Table 7). The mean FN, GI and SI of those lines from the pooled datasets over all environments are also presented in Table 7. The lowest SI and the highest FN were observed in the line 93E54*C53 with the allelic combination of the ‘AC Domain’ alleles at *gwm264*, *gwm494*, *wmc349* and *gwm482*, and ‘RL4452’ alleles at *gwm191* and *wmc48* (Table 7). Five out of six alleles in 93E54*C53 contributed to PHS resistance. Four out of five most resistant lines had the combination of ‘AC Domain’ alleles at *gwm494* and *wmc349* on chromosomes 4A (locus-2) and 4B, respectively. The three most

resistant lines with the lowest SI also had the highest FN, although the five lines with the lowest SI did not have the lowest GI (Table 7).

The line with the highest SI, 93E54*F13, had ‘AC Domain’ alleles at *gwm494* and *gwm482*, and ‘RL4452’ alleles at *gwm264*, *gwm191*, *wmc48* and *wmc349*, where three out of six alleles were undesirable for PHS resistance (Table 7). This line (93E54*F13) had the highest SI but only the third lowest FN. The line with the lowest FN (93E54*B74) had the allelic combination of the ‘AC Domain’ alleles at *wmc48*, *wmc349* and *gwm482*, and ‘RL4452’ alleles at *gwm264*, *gwm191* and *gwm494*, in which four alleles contributed to reduce PHS resistance.

The four most resistant lines all possessed the ‘AC Domain’ alleles at the 4A.2 and 4B loci and none of the most susceptible lines possessed this combination. Further, the ‘RL4452’ allele at *wmc48* on chromosome 4A.1 was absent in four out of the five most susceptible lines (Table 7).

Table 7 The five most resistant and the five most susceptible doubled haploid lines of the cross ‘RL4452/AC Domain’ with their allelic combinations at the six marker loci and their mean

sprouting index, germination index and falling number evaluated overall environments

Doubled haploid line ^a	Alleles at the marker loci ^b						Falling number	Germination index	Sprouting index
	<i>gwm264</i> (3A)	<i>gwm191</i> (3D)	<i>wmc48</i> (4A.1)	<i>gwm494</i> (4A.2)	<i>wmc349</i> (4B)	<i>gwm482</i> (7D)			
Most resistant									
93E54*C53	A+	R+	R+	A+	A+	A–	371	0.82	1.6
93E54*B43	R–	A–	R+	A+	A+	R+	350	0.89	2.0
93E54*C41	A+	A–	A–	A+	A+	A–	353	0.84	2.3
93E54*D58	R–	R+	R+	A+	A+	R+	315	0.85	2.3
93E54*F29	R–	A–	A–	A+	R–	A–	335	0.84	2.3
Most susceptible									
93E54*B74	R–	R+	A–	R–	A+	A–	101	0.94	6.9
93E54*J21	R–	A–	A–	A+	R–	–	179	0.93	7.1
93E54*D55	R–	A–	A–	R–	A+	R+	145	0.95	7.2
93E54*A99	A+	A–	A–	A+	R–	R+	132	0.92	7.2
93E54*F13	R–	R+	R+	A+	R–	A–	112	0.93	7.4

Falling number and germination index were evaluated over four environments (Glenlea 2003, Winnipeg 2004, Glenlea 2005 and Winnipeg 2005); Sprouting index was evaluated over six environments (Glenlea 2003, Winnipeg 2004, Glenlea 2005, Winnipeg 2005, Swift Current 2003 and Swift Current 2004)

^a The most resistant and the most susceptible lines were selected based on their sprouting index values

^b A, ‘AC Domain’ allele; R, ‘RL4452’ allele. +, desirable allele increased pre-harvest sprouting resistance; –, undesirable allele reduced pre-harvest sprouting resistance. The designated chromosome for each marker locus is in parenthesis

Discussion

Measurements of weathering traits varied greatly across environments. This variability among environments is likely associated with differential field weathering and genetic responses to environmental cues during the development of dormancy, which can make it difficult to determine the level of artificial weathering required to clearly show phenotypic differences. The amount of dormancy and field weathering before harvest must be considered prior to artificial weathering in a rain simulator to assure maximum differentiation in phenotypic expression of sprouting traits. Time periods in the rain simulator for FN and visual sprouting tests should be adjusted to account for level of dormancy and field weathering of samples. In this study, effective differentiation among lines for FN was usually obtained 24–48 h earlier than that of SI by artificial weathering. An additional 1–2 days are required for radicle growth beyond the spikelets in the visible sprouting tests. Low falling numbers can result earlier even though there are no visible signs of sprouting. This has been previously reported by Mares and Mrva (2001).

Among the six PHS QTLs detected, the QTLs on chromosome 4B had the highest LOD scores; explained the largest proportion of phenotypic variation; and were the most consistently detected QTLs for all three traits in both individual and overall environments. The ‘AC Domain’ allele(s) at the 4B locus increased FN, and decreased GI and SI values, explaining up to 67% of the phenotypic variation. It is possible that the QTL on chromosome 4B is a single gene controlling all three measures of PHS resistance (FN, GI, SI), which could be mapped as a Mendelian trait in future studies. Moreover, the SSR marker *wmc349* should be evaluated for its potential in marker-assisted breeding for improved PHS resistance. Kato et al. (2001) reported a seed dormancy QTL, *QPhs.ocs-4B.2*, in the same genomic region of chromosome 4B using DH lines from a cross ‘AC Domain/Haruyutaka’, and the ‘AC Domain’ allele at that QTL contributed to increased seed dormancy. McCartney et al. (2006) mapped a QTL for rapid visco-analyzer (RVA) final viscosity (*QRfv.crc-4B*) approximately 10 cM from *QFN.crc-4B* and *QSI.crc-4B*, on the short arm of chromosome 4B in the same ‘RL4452/AC Domain’ population. The stirring number performed with RVA has been used to monitor

the viscosity and starch degradation in a flour sample as a result of sprouting, and is highly correlated with FN (Ross et al. 1987).

The second most important PHS QTL (*QFN.crc-4A.1* and *QGI.crc-4A.1* associated with FN and GI) mapped to chromosome 4A (locus-1) and has not been previously reported. ‘RL4452’ alleles improved sprouting resistance at the 4A.1 locus. Thus, desirable PHS QTL alleles were detected in both parents (AC Domain on 4B and RL4452 on 4A.1). Transgressive segregants for PHS resistance likely resulted from the combined effects of the desirable alleles from both ‘AC Domain’ and ‘RL4452’ (Table 7).

A SI QTL was detected on the long arm of chromosome 4A (*QSI.crc-4A.2*). Kato et al. (2001) also reported a QTL for preharvest sprouting resistance on 4AL associated with dormancy in red-grained wheat using a DH population from the cross ‘AC Domain/Haruyutaka’. Kato et al. (2001) reported that *QPhs.ocs-4A.1* was homoeologous to the barley gene SD4 on chromosome 4H. In this study and in the study by Kato et al. (2001), the ‘AC Domain’ allele contributed the resistance to sprouting. Others have reported QTLs on chromosome 4AL associated with seed dormancy measured by germination test (Mares and Mrva 2001; Flintham et al. 2002; Torada et al. 2005; Mares et al. 2005; Mori et al. 2005). Lohwasser et al. (2005) identified a QTL in a similar location on 4AL linked to the PHS by measuring sprouting of intact spikes. RVA peak viscosity (*QRpv.crc-4A*) was mapped on the same marker interval as *QSI.crc-4A.2* and *QFN.crc-4A.2* (McCartney et al. 2006). Since RVA peak viscosity measures starch breakdown, it is indirectly related to FN because of the effects of sprouting on starch. QTL analysis from this study demonstrates the close relationship between RVA peak viscosity and sprouting resistance since these two QTLs (*QRpv.crc-4A* and *QSI.crc-4A.2*) mapped to the same interval. McCartney et al. (2005) also reported that a QTL associated with 1000-grain weight (*QGwt.crc-4A*) overlapped a time to maturity QTL (*QMat.crc-4A*) at the same locus as *QSI.crc-4A.2* and *QFN.crc-4A.2* (Fig. 2). The ‘AC Domain’ allele increased 1000-grain weight and reduced time to maturity at this locus (McCartney et al. 2005). It is not evident whether the association between seed mass, time to maturity and high sprouting resistance is direct or pleiotropic. Later maturing genotypes in western Canada may be more likely to experience wet and cool

harvest conditions. Earlier maturing genotypes may escape the autumn weather conditions which favour PHS. Nevertheless, the QTL interval *Xgwm494/Xgwm162* is very important for PHS influencing sprouting index, falling number, RVA peak viscosity, 1000-grain weight and time to maturity.

A SI QTL was detected on chromosome 3A (*QSI.crc-3A*). Kulwal et al. (2005) reported a major QTL (*QPhs.ccsu-3A.1*) for PHS tolerance on 3AL; however, it was approximately 24 cM distal (Somers et al. 2004) from the 3A QTL reported here. It has been reported that a grain colour gene, *R-A1*, and wheat *taVp1* gene, orthologous to the maize *Vp1* gene which encodes a dormancy related transcription factor, are also located on the long arm of 3A chromosome (Nelson et al. 1995; Bailey et al. 1999).

A SI QTL was detected on chromosome 3D. *QSI.crc-3D* was identified in several environments spanning a large interval between markers *Xgwm383* and *Xbarc71*. The ‘RL4452’ allele reduced SI at this locus. Groos et al. (2002) mapped a QTL for PHS on the long arm of chromosome 3D close to the SSR marker *Xgwm3* which was co-localized with grain colour genes *R-D1*. SSR marker *Xgwm3* mapped in the marker interval between *Xgwm191* and *Xwmc552* on the consensus map (Somers et al. 2004). The *QPhs.crc-3D* QTL was also detected in this interval. Therefore, pleiotropic effects of the *R*-genes and PHS resistance genes or linkage between them cannot be ignored. McCartney et al. (2005) mapped a 1000-grain weight QTL (*QGwt.crc-3D*) and a QTL controlling plant lodging (*QLd.crc-3D*) in the same region as the QTL controlling SI (*QSI.crc-3D*). The ‘RL4452’ alleles increased both 1000-grain weight and plant lodging. Pleiotropic effects between larger seed size, plant lodging and sprouting performance may explain the coincidence between these traits since the 3D QTL interval is quite large (Fig. 2).

A baking quality QTL for proof height (*QBprh.crc-3D*) was also reported on 3D at marker locus *Xwmc492* (McCartney et al. 2006) which coincided with SI QTL (*QSI.crc-3D*). The ‘AC Domain’ allele lowered proof height, and increased SI values at this locus, suggesting that high baking proof height might be dependent on sprouting resistance, assuming that samples in the quality study (McCartney et al. 2006) were affected by sprouting. Baking quality is affected by the proteolytic enzymes released during sprouting which causes a change in

the gluten protein functionality. Excessive hydrolytic enzymes in sprouted grains make dough stickier and harder to handle. Thus, flour from sprouted grains may reduce the baking proof height. Mansour (1993) reported a smaller volume and a compact interior of breads baked from sprouted wheat.

Only QTLs associated with SI were detected on chromosome 7D and sprouting resistance was contributed by ‘RL4452’ alleles. It is possible that the 7D QTLs are associated with later events in the sprouting process, because the SI test permits a greater development of sprouting compared to GI and FN tests. Roy et al. (1999) identified a PHS QTL on 7D chromosome linked to a sequence tagged site (STS) marker *MST101*; however, the SSR-based map location was not available.

Results from the combined effect of the six QTLs linked with PHS suggest that lines with a high level of PHS resistance can be achieved when the desirable QTL alleles are combined from both parents. QTL alleles on group 4 chromosomes appear to be the most important in maintaining high levels of PHS resistance. Others have reported similar findings (Kato et al. 2001; Torada et al. 2005; Mares et al. 2005; Mori et al. 2005). Candidate markers *gwm494* on chromosome 4A (locus-2), *wmc349* on 4B and *wmc48* on 4A (locus-1) may be useful for marker-assisted selection for PHS resistance. Phenotypic data in the most resistant and in the most susceptible lines also suggested that SI is closely related to FN. Both SI and FN are measured from samples following artificial weathering; whereas, GI was measured in the samples without artificial weathering. This may partly explain the close association of the SI and FN QTLs.

GI and FN QTLs were only detected on group 4 chromosomes; whereas, SI QTLs were detected on group 4, group 3, and group 7 chromosomes in multiple environments. It may be that the number of QTLs detected is associated with the degree of damage due to sprouting. GI was measured when the pericarp/testa was ruptured by the swollen embryo and the embryo was visible. This stage can be reached by embryo cell elongation after imbibition of grains and may not require a thorough breakdown of endosperm reserves or mobilization of the reserves (Bewley and Black 1994). SI was evaluated after 72–120 h when radical growth was visible beyond the spikelets. At this stage, the mobilization of endosperm solutes is required to nourish the growing

embryo. Hence, the degree of sprouting damage at the time of SI evaluation would likely be higher than that of the GI and FN tests. More QTLs were detected for SI which may also indicate that more genes are involved in SI than FN and GI, possibly because SI is measured at a more developed phase of the sprouting process. Analysis of gene expression profiling of a time series during the germination and sprouting processes using microarray technology would permit the association of gene expression changes to sprouting damage.

Summary

A DH mapping population derived from the spring wheat cross ‘RL4452/AC Domain’ was used to detect QTLs associated with three PHS traits: germination index (GI), sprouting index (SI) and falling number (FN). QTLs linked with PHS traits were mapped on bread wheat chromosomes 3A, 3D, 4A (2 loci), 4B and 7D. ‘AC Domain’ alleles on 3A, 4A (locus-2) and 4B, contributed to PHS resistance while ‘RL4452’ alleles contributed resistance on 3D, 4A (locus-1) and 7D. QTLs detected on chromosome 4B controlling FN (*QFN.crc-4B*), GI (*QGI.crc-4B*) and SI (*QSI.crc-4B*) were coincident, and explained the largest amounts of phenotypic variation in FN (22%), GI (67%) and SI (26%), respectively. The DH lines with the highest levels of PHS resistance generally had desirable PHS resistance alleles from both ‘AC Domain’ and ‘RL4452’. Thus, improved PHS resistance in spring wheat can be obtained through pyramiding PHS resistance QTLs from different sources.

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