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Maximizing the identification of QTL for pre-harvest sprouting resistance using seed dormancy measures in a white-grained hexaploid wheat population

Sachin Kumar · Ron E. Knox · Fran R. Clarke · Curtis J. Pozniak · Ron M. DePauw · Richard D. Cuthbert · Stephen Fox

Abstract Pre-harvest sprouting in spring wheat causes significant financial loss to growers throughout the world and sprouting damage can be reduced by growing resistant genotypes. Several genetic factors, especially those related to seed dormancy, are involved in the control of pre-harvest sprouting resistance. The objective of this study was to identify quantitative trait loci (QTL) influencing pre-harvest sprouting resistance from multiple measures of dormancy at multiple germination intervals on seed harvested across multiple environments. A doubled haploid mapping population of 91 individuals derived from a cross of two Canadian white-seeded spring wheat genotypes, SC8021-V2 (pre-harvest sprouting resistant) and AC Karma (moderately susceptible to pre-harvest sprouting) was used for QTL mapping. Daily germination counts were analysed using germination index, germination resistance and percent germination at intervals of 3, 5, 7, 10, 14 and 21 days from spike samples collected from six field and one greenhouse environments in Saskatchewan, Canada. Continuous frequency distributions at certain measure-durations indicated genetic complexity of dormancy segregation in the SC8021-V2/AC Karma cross. Composite interval mapping detected significant ($p \leq 0.05$) QTL associated with resistance to pre-harvest sprouting on all 21 wheat chromosomes. Of the 26 total QTL, six were novel and the rest were detected either at the same marker or overlapping a marker interval reported in other studies. QTL expressed consistently for germination index, germination resistance and percent germination at different germination durations on chromosomes 2B, 4A, 5D and 6D. QTL identified on homoeologous chromosomes 4A, 4B and 4D with chromosome specific molecular variants of SSR marker *wmc617* suggest a conserved region for controlling dormancy on group four. The majority of QTL mapped in regions known to contain factors affecting different components of pre-harvest sprouting resistance like seed dormancy, seed coat colour, ABA responsiveness and alpha-amylase activity. This study demonstrated that using multiple measures of seed dormancy

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at multiple intervals of germination enhanced identification of QTL affecting dormancy in white-seeded hexaploid wheat.

Keywords Wheat · Dormancy · Pre-harvest sprouting · Germination index · Germination resistance · Percent germination · QTL

Introduction

Hexaploid wheat (*Triticum aestivum* L. em Thell.) is widely grown in a variety of climates the world over, and contributes up to 40 % of the world's food (Braun et al. 2010). The grain contains many essential nutrients including fiber, carbohydrates, proteins, vitamins, minerals, and other bioactive compounds that are important in human nutrition (Gillies et al. 2012; Balyan et al. 2013). Pre-harvest sprouting is the precocious germination of grains in the spike due to early breakage of seed dormancy usually the result of moist weather conditions that persist after physiological maturity. In Canada, the cost of downgrading because of pre-harvest sprouting damage was estimated at about 100 million dollars annually (Clarke et al. 2005). Seed dormancy in wheat plays a critical role in protection against pre-harvest sprouting (Mares 1987). Both too low or too high seed dormancy can be undesirable by either allowing pre-harvest sprouting after seed maturity if too low or delaying germination after seed sowing if too high. Therefore, development of wheat genotypes with a balanced degree of seed dormancy is needed. Temperature and moisture during grain development affect the expression of dormancy and pre-harvest sprouting resistance. Under Canadian conditions, DePauw and McCaig (1991) compared various assays to measure sprouting resistance, seed dormancy and alpha-amylase activity, and their relative components of variation. They reported significant correlation among traits, and the genotypic effects accounted for 44–90 % of the phenotypic variation. Heritability of the various traits varied from 0.59 to 0.93. Identification and incorporation of genetic factors underlying resistant genotypes is a key to improve resistance to pre-harvest sprouting (McCaig and DePauw 1992; Flintham 2000; Mares et al. 2005; DePauw et al. 2012).

Pre-harvest sprouting is a complex trait, but phenotypic evaluation of experimental genotypes is generally straightforward and is based on the sprouting rate. Evaluation of threshed seeds, instead of intact spikes where the embryo is concealed by the lemma and palea, is used to characterize dormancy level. Germination index (GI), germination resistance (GR) and percent germination (PG) are common measurements for characterizing resistance to pre-harvest sprouting based on seed dormancy (Rasul et al. 2009; Singh et al. 2010; Knox et al. 2005, 2012).

The complexity of control of pre-harvest sprouting is demonstrated by the wide range of factors involved in the trait. The majority of genetic factors, including morphologic, physiologic, and biochemical are extensively reviewed elsewhere (DePauw and McCaig 1991; Fang and Chu 2008; Kulwal et al. 2010; Graeber et al. 2012; DePauw et al. 2012; Gao et al. 2013). One facet of this complex trait deals with the balance of plant growth regulators abscisic acid (ABA) and gibberellic acid (GA) in their control of alpha-amylase enzyme activity. The level of viviparous-1 (*Vp1*) gene expression in mature embryos positively regulates ABA sensitivity and promotes seed dormancy. Huang and Brule-Babel (2012) transferred a *Vp1* homolog from maize (*Zea mays* L.) into an elite hexaploid wheat genotype, Zheng9023, through *Agrobacterium*-mediated transformation. After transformation, Zheng9023 had enhanced seed dormancy with significant reduction in alpha-amylase activity. Another factor reported by Nakamura et al. (2011) involved a candidate gene homolog of *MOTHER OF FT AND TFL1* (*MFT*) that acts in seed dormancy regulation during physiological maturity. In Arabidopsis, MFT was discovered as homolog of *FLOWERING LOCUS T* (*FT*), a floral promoter and *TERMINAL FLOWER 1* (*TFL1*), floral inhibitor (Hedman et al. 2009). Interestingly, in wheat, these MFT homologs (*TaMFT*) co-localized with the seed dormancy QTL *QPhs.ocs-3A.1* on chromosome 3A (Mori et al. 2005). In a subsequent study, Liu et al. (2013) cloned the gene *TaPHS1* underlying the QTL *Qphs.pseru-3AS* on the short arm of chromosome 3A in white-seeded wheat using comparative and map-based cloning approaches. They discovered two single nucleotide polymorphisms (SNPs) in the promoter region and three SNPs in the *TaPHS1* gene that altered pre-harvest sprouting resistance.

Another facet of pre-harvest sprouting in wheat relates to seed coat colour in which red coats are generally more resistant than seeds with white coats

(Nilsson-Ehle 1914; DePauw and McCaig 1983; Himi et al. 2002). It is thought that the relationship between pre-harvest sprouting resistance and grain colour is due to pleiotropic characteristics of three red alleles (Flintham 2000; Himi and Noda 2005). The presence of any one or more of the three red alleles (*R-A1b*, *R-B1b* and *R-D1b*) confers red grain colour, whereas non-red alleles are present in the homozygous condition at all loci in grains with white colour (McIntosh et al. 1998). DePauw and McCaig (1983) presented evidence for a mechanism for dormancy independent of seed coat color. However, understanding the biochemical mechanism behind red and non-red grain colour with respect to pre-harvest sprouting response remains a challenge.

The genetic control of pre-harvest sprouting resistance is complex, factors responsible for resistance are dispersed on almost every wheat chromosome and complex interactions occur between QTL ($Q \times Q$) and/or among environments ($Q \times Q \times E$) (Kulwal et al. 2004; Imtiaz et al. 2008; Kumar et al. 2009; Mohan et al. 2009). Also, the effects of QTL range from minor to major corresponding to a range in phenotypic expression from susceptible to resistant. As with DePauw and McCaig (1991) in the study of heritability, in order to deal with the complexity of pre-harvest sprouting resistance and identify associated QTL, different tests such as wetting spikes in an artificial rain simulator, threshed seed germination counts for dormancy, and alpha-amylase activity measured by falling number have been conducted (Roy et al. 1999; Mares et al. 2005; Imtiaz et al. 2008; Rasul et al. 2009). Knox et al. (2012) reported GI, GR and PG measures each at 7, 14 and 21 days intervals from the start of germination across multiple environments. The authors emphasized that measuring GR for longer and GI and PG for shorter duration of germination favor the selection of breeding lines with adequate (moderate to high) resistance to pre-harvest sprouting. They noted that multiple measures over environments allowed identification of several QTL that would not have been revealed using a single time interval or measure of pre-harvest sprouting resistance. Although a number of the QTL they identified were unique to durum, most were reported in research on hexaploid wheat demonstrating the validity of their approach. They identified three highly significant QTL on chromosomes 1A, 2B and 7B using a random inbred population of durum wheat. The complexity of pre-harvest sprouting resistance warrants a thorough examination of the trait from multiple perspectives for full elucidation of genetic factors.

QTL analysis is appropriate when multiple genes govern a trait i.e. complex genetic architecture. Identification and tagging of genetic loci is necessary for understanding the relationship among genes and the relationship with phenotype. Knox et al. (1998) performed genetic analysis of pre-harvest sprouting resistance on a white-seeded population of AC Karma and SC802 l-V2 in which AC Karma is moderately susceptible and SC8021-V2 is moderately resistant. They determined the segregation to be complex with at least seven effective factors involved. The present study with the same hexaploid wheat population is an extension of the previous study by Knox et al. (2012) in which GI, GR and PG measures were examined for three durations of germination counts in durum wheat. The objective of the present study was to maximize identification of QTL for pre-harvest sprouting resistance by analyzing three dormancy measures at multiple germination intervals across different environments.

Materials and methods

Mapping population

The SC8021-V2/AC Karma doubled haploid (DH) mapping population was derived from a cross between the Canada Prairie Spring (CPS) white wheat cultivar AC Karma (Knox et al. 1995), and the white-seeded germplasm line SC8021-V2 (DePauw et al. 1992). A population of 91 DH lines were developed from the F_1 plants using the maize pollination technique of Knox et al. (2000). The female parent, SC8021-V2 (PI-554142), developed as a pre-harvest sprouting resistance line, has the pedigree Kenya-321/Peck. The male parent, AC Karma, with the pedigree HY320*5/8W553//HY35, is moderately susceptible to pre-harvest sprouting.

Field experiments

Doubled haploid lines, SC8021-V2, AC Karma and three check lines AC Vista (DePauw et al. 1998), Genesis (HY355, DePauw et al. 1989) and RL4137 (DePauw et al. 2009) were grown in field trials near Swift Current and Saskatoon, Saskatchewan in 1997,

1998 and 1999 in a randomized complete block design with three replications. Of the 91 DH lines, 84 were grown in 1997 and 1998 near Swift Current and Saskatoon. AC Vista was not grown in 1997 and 1998 Swift Current, and Genesis was not grown in 1999 Saskatoon that contained 111 lines. Plots were one or two rows, 3 m long with 200 seeds per row. Rows were 23 cm apart and plots were separated by a row of spring-planted winter wheat that remained vegetative throughout the growing season to help control weeds and soil erosion, and to facilitate access for sampling. Five to ten spikes were harvested from each plot when half of the primary tillers had collapsed nodes on the stem that indicates about 16 % grain moisture. Harvested spikes were air dried and then stored at -20 °C in a freezer to preserve dormancy.

Greenhouse experiment

An alpha-lattice randomized complete block trial involving three replications that included the 91 DH lines was conducted in a greenhouse (GH) near Swift Current in 2004. The DH lines, parents and check lines RL4137, Genesis and AUS1408 (Mares 1987) were seeded in early spring 2004 and grown at temperature 20 ± 5 °C with a long day photoperiod of 16 h. Due to early sowing, plants reached physiological maturity in long natural day length with minimum use of supplemental light. The check cultivar AC Vista was not planted in the GH trial. To evaluate dormancy, two spikes per plant were harvested from each replication at physiological maturity. Harvested spikes were then ripened at 30 °C for 3 days and stored at −20 °C.

Phenotypic evaluation

The 5–10 spikes were threshed as a bulk for each experimental unit and seed germinations for evaluation with GI, GR and PG measures were performed as described by Knox et al. (2012) for each of the seven environments. The trait measures, environments (locations by year) and days on which germination data were assessed are presented in Fig. 1. The number of seeds plated was 41–51 in 1997, 13–72 in 1998 and 26–128 in 1999 for the trials near Saskatoon. Similarly for Swift Current, 31–60 seeds were plated in 1999. Due to an oversight, the number of seeds plated for
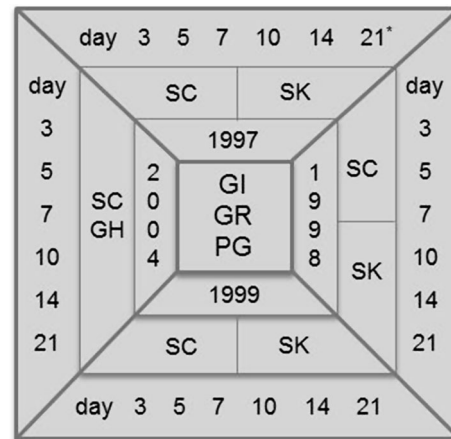
Fig. 1 Diagram of dormancy trait measures examined at different count durations in days from tests performed over multiple environments on 91 lines of the SC8021-V2/AC Karma doubled haploid mapping population where GI is germination index; GR: germination resistance, PG: percent germination, SC: Swift Current, SK: Saskatoon and GH: greenhouse. The *asterisk* indicates that data for PG at day 21 were not recorded for the SC and SK locations in 1997

Swift Current was not recorded during 1997 and 1998. In other tests there was little difference in number of viable seed and number of seed plated; therefore we used number of viable seed as totals for Swift Current 1997 and 1998. Seeds from the Swift Current trials were counted until day 28 in 1997 and day 21 in 1998 and 1999. For the Saskatoon trials, seeds were counted until day 28 in 1997 and 1998 and day 40 in 1999. The number of ungerminated seeds was recorded in 1997, 1998 and moldy seeds was recorded in all the 3 years for Saskatoon. The number of ungerminated and moldy seeds was not recorded for Swift Current.

After the final counts for Swift Current 1999, the ungerminated seeds were treated with 4.0–6.0 ml of 0.5 mM gibberellic acid (GA_3) solution for 1 h to test the seeds viability as described by Knox et al. (2012). Daily counts of germinated seeds were recorded for another 3 d. Any seed that failed to germinate was considered to be non-viable, and was excluded from the experimental total seeds. The measurements of seed germination for each DH line, parents and check lines were calculated as follows:

(i) Germination index (GI): GI (Reddy et al. 1985; Walker-Simmons 1988), places greater weight on seeds that germinate earlier and was calculated as:

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

where 'd' indicates the total number of days for which the seed was counted; n_1, n_2, n_3, …, n_d are the number of seeds germinated on the 1st, 2nd, 3rd to dth day and 'N' is the total number of viable seeds plated for germination. This equation of dormancy calculation provides a maximum index of 1.0 when all seeds germinate by day 1, while an index of less than 1.0 indicates increased dormancy.

(ii) Germination resistance (GR): The equation of GR reported by Gordon (1971) calculates time when 50 % of the seeds germinate:

PG. The PG21d (PG on day 21) was not determined for the 1997 Swift Current and Saskatoon environments (Fig. 1). The year-location combinations were abbreviated as follows: 1997SC, 1998SC, 1999SC and 2004SC-GH were field trials during 1997, 1998, 1999 and GH experiment during 2004 near Swift Current, respectively. Similarly 1997SK, 1998SK and 1999SK were field trials during 1997, 1998 and 1999 near Saskatoon, respectively.

$$GR = \frac{\left\{\left(\frac{d_1}{2}\right)(n_1) + \left[\left(\frac{d_2 + d_1}{2}\right)(n_2)\right] + \left[\left(\frac{d_3 + d_2}{2}\right)(n_3)\right] + \cdots + \left[\left(\frac{d_i + d_{i-1}}{2}\right)(n_i)\right]\right\}}{N} days$$

where d_1, d_2, d_3, …, d_i are the 1st, 2nd, 3rd to ith day of the germination counts; n_1, n_2, n_3, …, n_i are the number of seeds that germinated on the 1st, 2nd, 3rd to d_ith day, respectively and 'N' is the total number of seed germinated.

(iii) Percent germination (PG): PG was calculated as the number of seeds germinated on the day of interest divided by the total number of seed multiplied by 100.

$$PG = \left(\frac{n}{N}\right) \times 100$$

where 'n' is the cumulative number of seed germinated on the day the calculation was made and 'N' is the total number of viable seed.

To establish the effect of duration of the test on resolution of pre-harvest sprouting response, calculations for GI, GR and PG were performed at day 3, 5, 7, 10, 14 and 21, and respective measure-duration combinations were abbreviated as GI3d, GI5d, GI7d, GI10d, GI14d and GI21d for GI; GR3d, GR5d, GR7d, GR10d, GR14d and GR21d for GR; and similarly PG3d, PG5d, PG7d, PG10d, PG14d and PG21d for

Genotyping

Fresh young leaves (2–5 cm) of the parents and the 91 DH lines were sampled. DNA was extracted and simple sequence repeat (SSR) markers were applied as described by Knox et al. (2012). The markers used were those described by Somers et al. (2004).

Statistical analysis

GI, GR and PG results were analyzed using Statistical Analysis Software (SAS) version 8.2 (Littell et al. 1996). The diagnostic test rstudent (Rawlings 1988) was performed to identify outliers. The mixed model analysis of variance was performed on each environment with PROC MIXED. Genotypes were considered fixed and replications were random. Pearson's correlation (r) was performed among sprouting measurement methods and within measurement methods for seed counts and durations of each test. The checks provided a reference with which to evaluate the performance of the measurement methods over DH lines of the SC8021-V2/AC Karma population.

Linkage analysis, using JoinMap v4.1, was performed with 534 polymorphic SSR markers (Van Ooijen 2011). Those showing co-segregation were removed from further analysis. The Chi square goodness-of-fit test ($P \leq 0.01$) implemented in Join-Map software was performed to test conformity to the expected Mendelian segregation ratio of 1:1. Markers showing segregation distortion from the expected ratio were excluded before grouping markers. The SSR markers were ordered with the regression mapping algorithm and were classified into linkage groups using a minimum LOD score threshold of 3.0 at an increment of 0.5. Linked markers were only considered as constituting a linkage group if more than five markers collapsed into a single group. The optimal marker order was estimated using the 'Ripple' command with a value of 1. Recombination frequencies were converted to map distances in centimorgans (cM) using the Kosambi mapping function (Kosambi 1944).

QTL analysis on phenotypic measures was conducted using the linkage map and genotypic data of SSR markers from the 91 DH lines. Composite interval mapping analysis was performed on the least square means data to determine the significant association of markers to traits using WinQTL Cartographer v2.5 (Wang et al. 2012). The ZmapQTL function with a window size of 10 cM, 1 cM walk speed and forward and backward regression of Standard Model 6 was used for QTL discovery. A significant QTL was declared when the LOD score was greater than the threshold value that was obtained based on a permutation test ($P \leq 0.05$) using 1000 iterations independently for each trait. For this study, two QTL positions in the same linkage group were considered as a single QTL if the distance of the nearest marker of each QTL was ≤ 50 cM. We applied this definition of discrete QTL because QTL are marker-trait associations that vary among different datasets simply from random sampling variation. Therefore, it can be difficult to know which QTL in the same region among different data sets are a result of a common gene. QTL were accepted as novel if markers associated with QTL identified in this study did not coincide with markers reported in previous studies.

Results

Phenotypic evaluation

The mean values of the parents and check lines (from here on simply referred to as checks) for the three dormancy measures varied across multiple environments. Within each germination count duration interval, the checks showed significant variation in dormancy level (Supplementary Fig. 1a–c). RL4137 and SC8021-V2 were sprouting resistant; AC Vista was moderately resistant, while AC Karma and Genesis were the most susceptible throughout the study. In each year of field trials, lower dormancy was observed at Saskatoon compared to the Swift Current trials (Supplementary Fig. 1a–c). The ranking from sprouting resistant to sprouting susceptible was consistent with previously published results (Knox et al. 2012).

GI discriminated among the less dormant genotypes in a short germination test, such as 3 days (GI3d), but discriminated better among more dormant genotypes if the test was run longer, such as 14 days (GI14d). For instance, AC Karma had a GI of 0.72 after 3 days, but SC8021-V2 needed more than 14 d to achieve an equivalent germination in the Swift Current trial of 1997 (Supplementary Fig. 1a). Moreover, dormant RL4137 required more than 3 weeks to reach a comparable GI. A similar trend for GI was observed in all field environments, but not with the GH environment where relatively low discrimination between genotypes was observed. Both AC Karma and SC8021-V2 showed no germination within the GH trial assessed on the 3rd day (GI3d = 0.00); however, AC Karma germinated more quickly than SC8021-V2 with increasing number of days.

GR tended to discriminate between genotypes the longer the seeds were germinated, which was more apparent for the highly dormant genotypes. For example, lines were more differentiated at GR21d compared to GR3d (Supplementary Fig. 1b). AC Karma, in all the field environments, showed a consistently low resistance to germination (GR21d = 2.07), which means it germinated quickly, while SC8021-V2 germinated less quickly and exhibited a higher GR (GR21d = 5.85) (Supplementary Fig. 1b).

PG did not discriminate effectively among genotypes with low dormancy, but differentiated among genotypes with greater dormancy and those with short to moderate durations of 3–7d. Clear differences were observed between dormant and non-dormant genotypes using the PG measure; Genesis and AC Karma both exhibited rapid germination by day 3 (PG3d = 96 %) whereas RL4137 (PG3d = 0.00) and SC8021-V2 (PG3d = 14 %) showed greatest resistance to germination (Supplementary Fig. 1c). SC8021-V2 germinated earlier than RL4137 and this difference was best observed at days 5 and 7. In contrast, PG was a poor measure for genotypes possessing greater dormancy if germination continued for a longer duration. For example, the dormant parent SC8021-V2 had the same PG (96 %) by day 14 as AC Karma had on day 3 in 1997 near Swift Current. Unlike field trials, germination among genotypes when tested in the GH trial continued to show some variation for more dormant lines at longer germination durations. Maximum difference between less dormant and dormant genotypes was observed with measures GI5d, GR21d and PG5d for field environments and GI10d, GR21d and PG7d for the GH environment.

Frequency distributions of GI, GR and PG by germination time intervals (Supplementary Fig. 2a–l) revealed that the lines were commonly skewed with the peak positioned over the less dormant portion of the distribution; although normal-shaped and bimodal distributions were also observed, mostly for intermediate durations of germination. Bi-directional transgressive segregation was evident across environments and types of measure-durations; however, transgressive genotypes varied. Depending on the environment, a differential in germination rate was higher at an earlier or later duration. For example, in 1999SC the greatest differential in PG occurred at days 3 and 5, whereas the differential was greatest for PG7d in the 2004 GH.

Correlations (Supplementary Table 1) within environments among trait germination measure-durations tended to show a reduced relationship among and within measures for more disparate germination durations. The range in correlation coefficient between GI and PG among germination durations varied from 0.38 to 0.99 for field, whereas a slightly greater range of 0.17–0.99 was observed among measures associated with the GH environments. The GI and GR were negatively correlated with a maximum range of −0.43
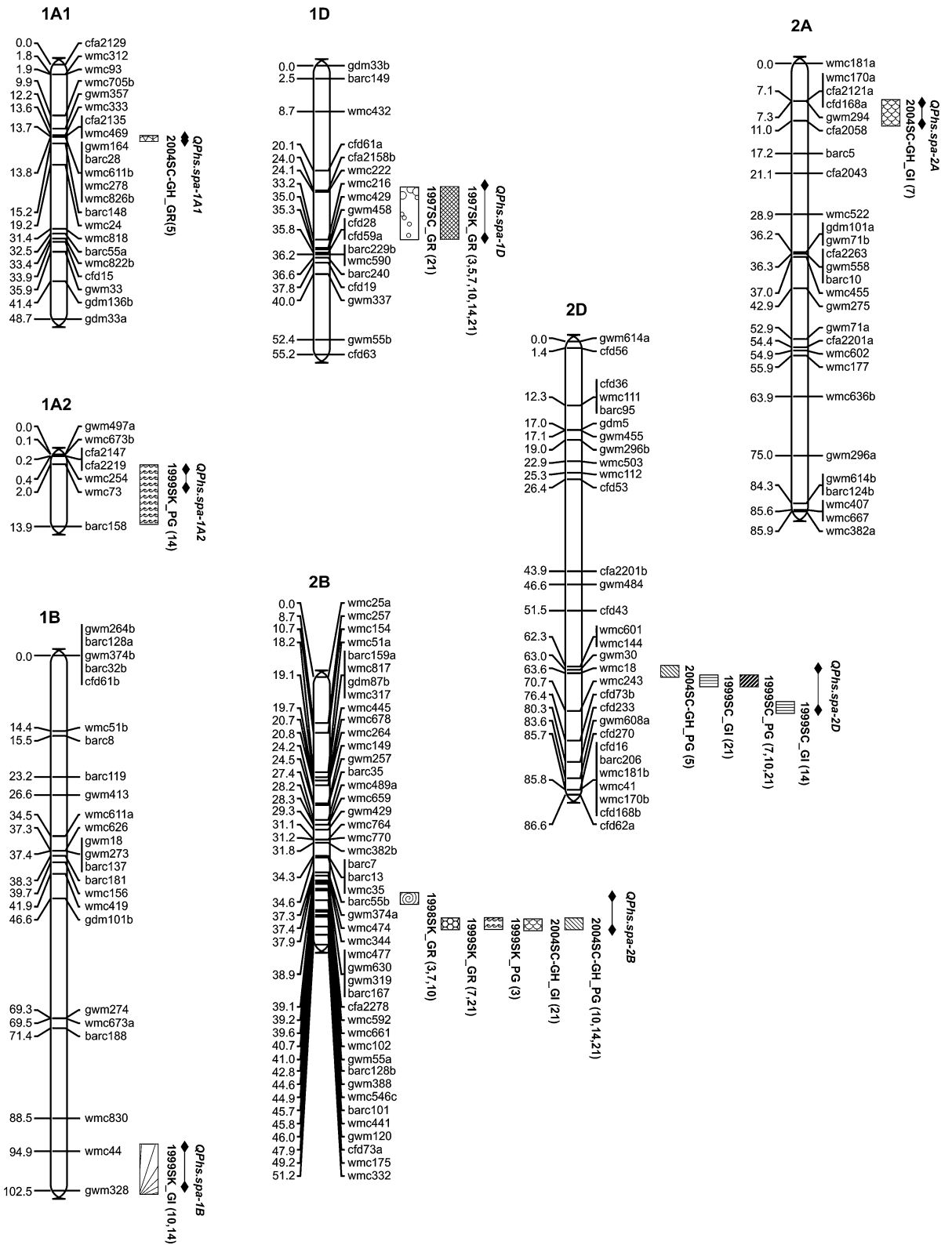
to −1.00 for field and again a greater range of −0.18 to −0.98 for the GH trials. The negative correlations between PG and GR measures ranged from −0.37 to −0.97 with field environments and −0.22 to −0.98 with the GH environments.

Genetic map and QTL for pre-harvest sprouting resistance

The SC8021-V2/AC Karma genetic map of SSR markers consisted of 485 loci distributed over 24 linkage groups covering 1411.5 cM (Supplementary Table 2). The remaining 49 SSRs either showed segregation distortion or remained ungrouped and hence were excluded from the map. The order of the markers resembled the wheat microsatellite consensus genetic map published by Somers et al. (2004). Two linkage groups were associated with each of chromosomes 1A, 7A and 7D and were labelled as 1A1 and 1A2, 7A1 and 7A2, and 7D1 and 7D2 (Fig. 2).

The genome-wide significant LOD thresholds for QTL declaration obtained by the 1000 iterations for each data set ranged from 2.08 to 3.97 at $p \leq 0.05$. At these thresholds, 33 chromosomal regions were associated with pre-harvest sprouting resistance, of which the six chromosomes 2B, 2D, 3A, 4A, 4D and 6D exhibited more than one QTL peak that were within 50 cM of each other. Each QTL is listed with the associated chromosome arm, environment-measure-duration, associated marker-interval, LOD values of QTL peak, percent phenotypic variation explained (%PVE), and favorable parental allele in Table 1. The QTL on the same chromosome within 50 cM of each other were considered as one and designated by a common name giving a total of 26 QTL and representing all 21 wheat chromosomes (Fig. 2). However, three chromosomes namely 3D, 7B and 7D1 each contained two QTL peaking more than 50 cM from one another and were considered separate QTL. In this case, the QTL were designated *QPhs.spa-3D.1*, *QPhs.spa-3D.2*; *QPhs.spa-7B.1*, *QPhs.spa-7B.2* and *QPhs.spa-7D1.1*, *QPhs.spa-7D1.2* (Fig. 2; Table 1). All seven environments (six field and one GH) for all three measures by six germination durations, except for the GI measure from the 1998 Saskatoon environment, were involved in revealing the 26 QTL.

The explained portion of phenotypic variation for the QTL ranged from minor, with a low of 6 %, to

1A1

Position	Marker
0.0	cfa2129
1.8	wmc312
1.9	wmc93
9.9	wmc705b
12.2	gwm357
13.6	wmc333
13.6	cfa2135
13.7	wmc469
	gwm164
	barc28
13.8	wmc611b
	wmc278
	wmc826b
15.2	barc148
19.2	wmc24
31.4	wmc818
32.5	barc55a
33.4	wmc822b
33.9	cfd15
35.9	gwm33
41.4	gdm136b
48.7	gdm33a

QPhs.spa-1A1
2004SC-GH_GR(5)

1A2

Position	Marker
0.0	gwm497a
0.1	wmc673b
0.2	cfa2147
	cfa2219
0.4	wmc254
2.0	wmc73
13.9	barc158

QPhs.spa-1A2
1999SK_PG (14)

1B

Position	Marker
0.0	gwm264b
	barc128a
	gwm374b
	barc32b
	cfd61b
14.4	wmc51b
15.5	barc8
23.2	barc119
26.6	gwm413
34.5	wmc611a
37.3	wmc626
	gwm18
37.4	gwm273
	barc137
38.3	barc181
39.7	wmc156
41.9	wmc419
46.6	gdm101b
69.3	gwm274
69.5	wmc673a
71.4	barc188
88.5	wmc830
94.9	wmc44
102.5	gwm328

QPhs.spa-1B
1999SK_GI (10,14)

1D

Position	Marker
0.0	gdm33b
2.5	barc149
8.7	wmc432
20.1	cfd61a
24.0	cfa2158b
24.1	wmc222
33.2	wmc216
35.0	wmc429
35.3	gwm458
35.8	cfd28
	cfd59a
36.2	barc229b
	wmc590
36.6	barc240
37.8	cfd19
40.0	gwm337
52.4	gwm55b
55.2	cfd63

QPhs.spa-1D
1997SK_GR (3,5,7,10,14,21)
1997SC_GR (21)

2B

Position	Marker
0.0	wmc25a
8.7	wmc257
10.7	wmc154
18.2	wmc51a
	barc159a
19.1	wmc817
	gdm87b
	wmc317
19.7	wmc445
20.7	wmc678
20.8	wmc264
24.2	wmc149
24.5	gwm257
27.4	barc35
28.2	wmc489a
28.3	wmc659
29.3	gwm429
31.1	wmc764
31.2	wmc770
31.8	wmc382b
	barc7
34.3	barc13
	wmc35
34.6	barc55b
37.3	gwm374a
37.4	wmc474
37.9	wmc344
	wmc477
38.9	gwm630
	gwm319
	barc167
39.1	cfa2278
39.2	wmc592
39.6	wmc661
40.7	wmc102
41.0	gwm55a
42.8	barc128b
44.6	gwm388
44.9	wmc546c
45.7	barc101
45.8	wmc441
46.0	gwm120
47.9	cfd73a
49.2	wmc175
51.2	wmc332

QPhs.spa-2B
1998SK_GR (3,7,10)
1999SK_GR (7,21)
1999SK_PG (3)
2004SC-GH_GI (21)
2004SC-GH_PG (10,14,21)

2D

Position	Marker
0.0	gwm614a
1.4	cfd56
12.3	cfd36
	wmc111
	barc95
17.0	gdm5
17.1	gwm455
19.0	gwm296b
22.9	wmc503
25.3	wmc112
26.4	cfd53
43.9	cfa2201b
46.6	gwm484
51.5	cfd43
62.3	wmc601
63.0	wmc144
63.6	gwm30
	wmc18
70.7	wmc243
76.4	cfd73b
80.3	cfd233
83.6	gwm608a
85.7	cfd270
	cfd16
	barc206
85.8	wmc181b
	wmc41
	wmc170b
	cfd168b
86.6	cfd62a

QPhs.spa-2D
2004SC-GH_PG (5)
1999SC_GI (21)
1999SC_PG (7,10,21)
1999SC_GI (14)

2A

Position	Marker
0.0	wmc181a
	wmc170a
7.1	cfa2121a
	cfd168a
7.3	gwm294
11.0	cfa2058
17.2	barc5
21.1	cfa2043
28.9	wmc522
	gdm101a
36.2	gwm71b
	cfa2263
36.3	gwm558
	barc10
37.0	wmc455
42.9	gwm275
52.9	gwm71a
54.4	cfa2201a
54.9	wmc602
55.9	wmc177
63.9	wmc636b
75.0	gwm296a
84.3	gwm614b
	barc124b
85.6	wmc407
	wmc667
85.9	wmc382a

QPhs.spa-2A
2004SC-GH_GI (7)

major, with a high of 60 %. Nineteen QTL were specific to field environments, three were detected in the GH environment only, and four were common in both GH and field environments. Five QTL were identified with GI and PG, seven QTL with GR and PG, and four QTL with GI, GR and PG measures (Fig. 3). The same QTL were not detected for both GI and GR measures in this study. Based on individual trait measures, three QTL each were identified with GI and with GR and four QTL were identified with PG (Fig. 3). Across experiments, the number of QTL associated with each germination measure-duration and associated chromosome broken down by year, location, trait measure, and the day of germination is summarized in Table 2. A total of 125 measure-duration combinations contributed to detection of the 26 QTL. For example, a maximum of 31 measure-durations involving GI, GR and PG identified a QTL on chromosome 5D, while 12 identified a QTL on 4B, 11 on 7D, and 10 on 2B (Table 2).

QPhs.spa-5D, a consistent and major QTL observed through dormancy measures GI, GR and PG, was identified on chromosome 5D at SSR locus *gdm133b*. Except for 1999SC, this QTL was detected in each environment and explained 11–29 % of the phenotypic variation of the traits measured (Tables 1, 2; Fig. 2). The *QPhs.spa-5D* allele for greater seed dormancy was contributed by SC8021-V2, the parent with greater seed dormancy. Another consistent QTL, *QPhs.spa-2B* on chromosome 2B observed with GI, GR, and PG was detected in 1998SK, 1999SK and 2004SC-GH trials. Although two LOD peaks were observed for this QTL, they were considered as a single QTL because they differed by merely 4 cM.

QPhs.spa-2B had a moderate effect, compared to *QPhs.spa-5D*, explaining 8–16 % of the variation. SC8021-V2 contributed the *QPhs.spa-2B* greater seed dormancy allele. Two other QTL, one on chromosome 2D (*QPhs.spa-2D*) and the other on 6D (*QPhs.spa-6D*), produced moderate effects and were detected with the GH and at least one field environment. The resistant alleles for both of these QTL came from dormant parent, SC8021-V2.

Other QTL detected in response of reduced GI and PG measures were mapped on chromosomes 2D, 4B, 7A2 (resistant allele contributed by SC8021-V2), 4D and 7D2 (resistant allele contributed by AC Karma) (Figs. 2, 3). *QPhs.spa-2D* was observed as three peaks associated with different environments within the 8.2 cM interval of *wmc144-wmc243*.

In the field environments, GI at three durations of germination and PG at all six durations identified a major QTL, *QPhs.spa-4B*, which was mapped on chromosome 4B in marker interval *wmc617b-wmc48a* that spanned 11.6 cM. This QTL, with resistance from SC8021-V2, explained 35-60 % of the phenotypic variation. Another major QTL, *QPhs.spa-7D2*, observed with GI and PG in field environments was revealed on chromosome 7D2 in a 4 cM region flanked by *barc76* and *cfa2257a*. *QPhs.spa-7D2* explained 14–47 % of the phenotypic variation, and the resistance came from AC Karma. A QTL, *QPhs.spa-4A*, was specific to the 1999SC trial and was observed on chromosome 4A with GI5d, and GR and PG at multiple durations. *QPhs.spa-4A* was identified as two peaks that differed by 12 cM in marker intervals *barc170-wmc617a*. AC Karma contributed the resistance of *QPhs.spa-4A*, and this source explained 17–33 % of the phenotypic variation. Quantitative trait locus, *QPhs.spa-1D*, on chromosome 1D was observed with GR21d in 1997SC and GR3-21d in 1997SK. This QTL explained 30–44 % of the phenotypic variation and resistance was contributed by SC8021-V2.

The marker *wmc617* amplified DNA in all three homoeologous group four chromosomes. *QPhs.spa-4A* was detected in interval *barc170-gwm397-wmc617a* on chromosome 4A, *QPhs.spa-4B* was detected in the interval *wmc617b-wmc48* on chromosome 4B and *QPhs.spa-4D* was detected in interval *wmc617c-wmc89b* on chromosome 4D. *QPhs.spa-4B* had largest effect explaining up to 60 % of the phenotypic
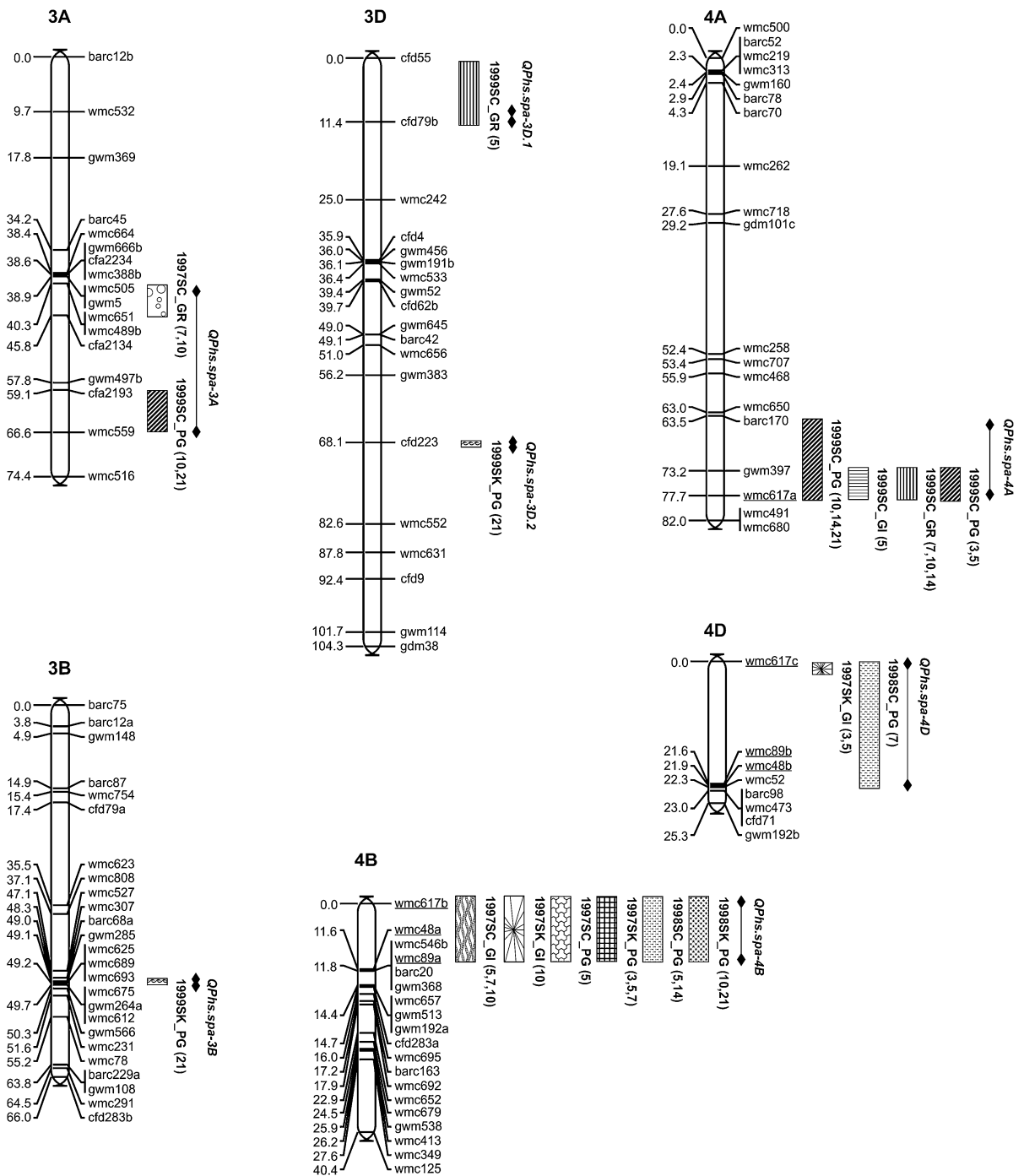
Fig. 2 continued

variation. Other QTL observed in the SC8021-V2/AC Karma DH population had minor to moderate effects for GI, GR and PG measures at various durations (Table 1; Fig. 2).

Discussion

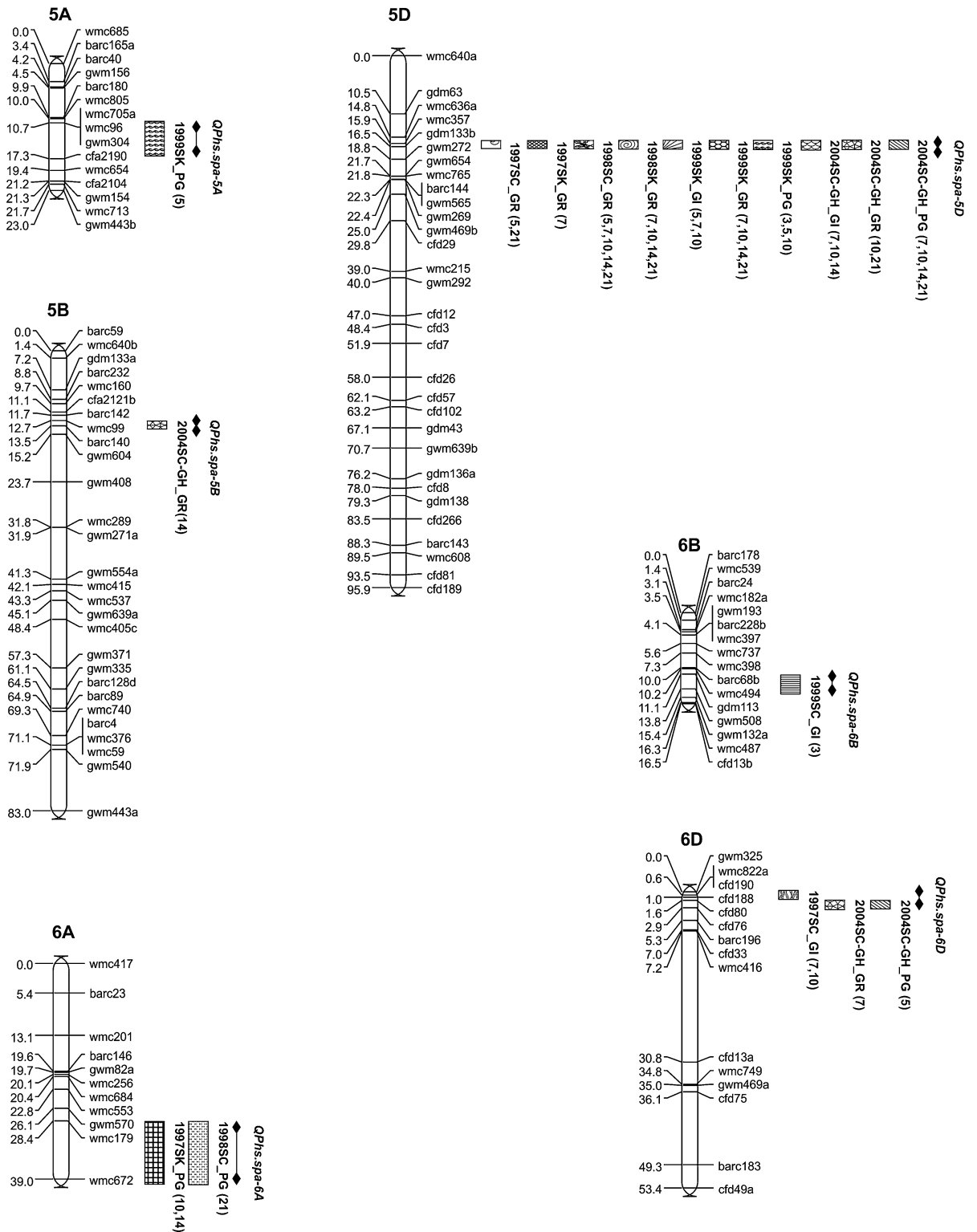No previous reports were found for the six QTL mapped on chromosomes 1B, 2D, 5D, 6A, 7D1.2 and
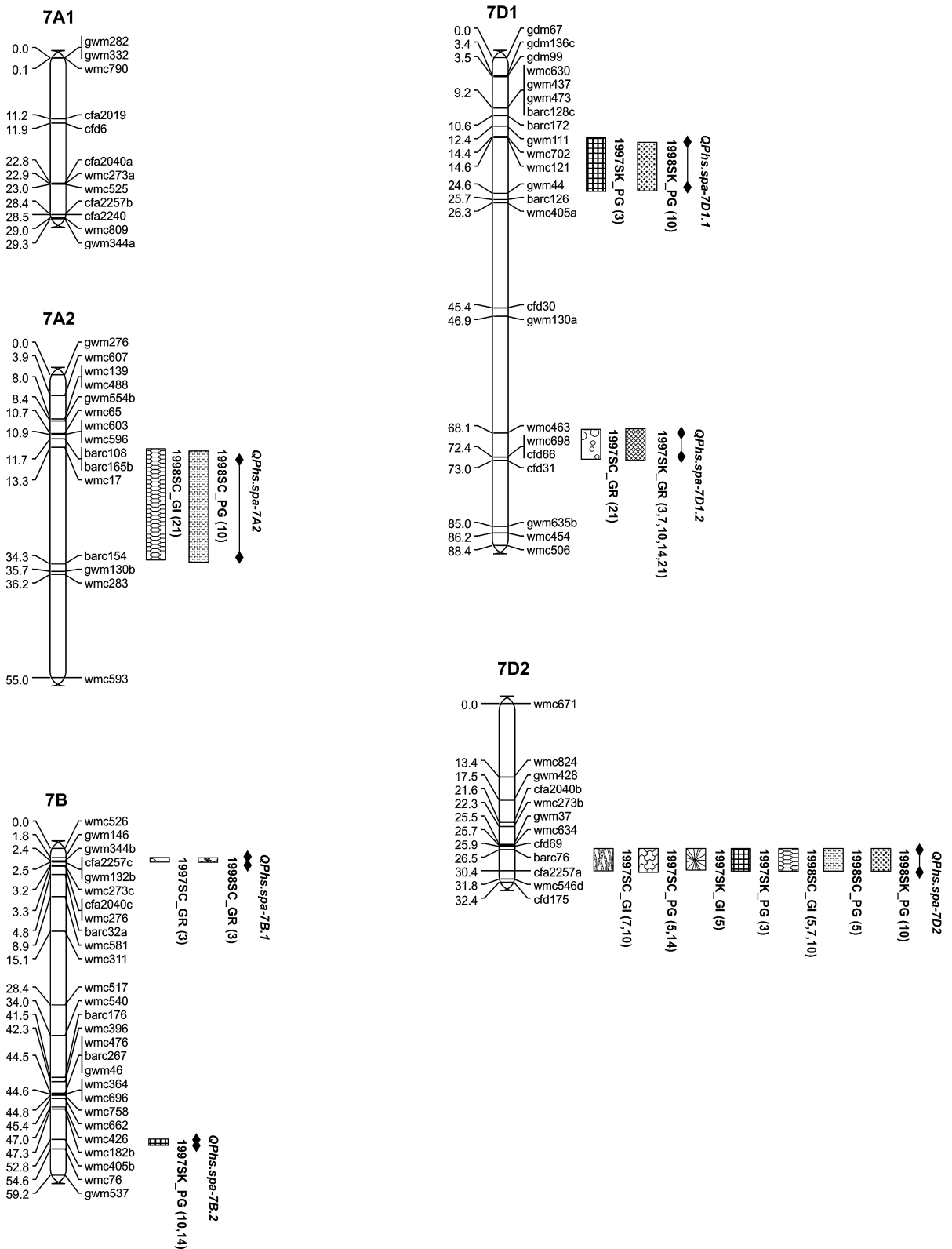
Fig. 2 continued

7A1

Position	Marker
0.0	gwm282
	gwm332
0.1	wmc790
11.2	cfa2019
11.9	cfd6
22.8	cfa2040a
22.9	wmc273a
23.0	wmc525
28.4	cfa2257b
28.5	cfa2240
29.0	wmc809
29.3	gwm344a

7A2

Position	Marker
0.0	gwm276
3.9	wmc607
8.0	wmc139
	wmc488
8.4	gwm554b
10.7	wmc65
	wmc603
10.9	wmc596
11.7	barc108
	barc165b
13.3	wmc17
34.3	barc154
35.7	gwm130b
36.2	wmc283
55.0	wmc593

1998SC_GI (21)
1998SC_PG (10)
QPhs.spa-7A2

7B

Position	Marker
0.0	wmc526
1.8	gwm146
2.4	gwm344b
	cfa2257c
2.5	gwm132b
3.2	wmc273c
3.3	cfa2040c
	wmc276
4.8	barc32a
8.9	wmc581
15.1	wmc311
28.4	wmc517
34.0	wmc540
41.5	barc176
42.3	wmc396
	wmc476
44.5	barc267
	gwm46
44.6	wmc364
	wmc696
44.8	wmc758
45.4	wmc662
47.0	wmc426
47.3	wmc182b
52.8	wmc405b
54.6	wmc76
59.2	gwm537

1997SC_GR (3)
1998SC_GR (3)
QPhs.spa-7B.1

1997SK_PG (10,14)
QPhs.spa-7B.2

7D1

Position	Marker
0.0	gdm67
3.4	gdm136c
3.5	gdm99
	wmc630
	gwm437
9.2	gwm473
	barc128c
10.6	barc172
12.4	gwm111
14.4	wmc702
14.6	wmc121
24.6	gwm44
25.7	barc126
26.3	wmc405a
45.4	cfd30
46.9	gwm130a
68.1	wmc463
72.4	wmc698
	cfd66
73.0	cfd31
85.0	gwm635b
86.2	wmc454
88.4	wmc506

1997SK_PG (3)
1998SK_PG (10)
QPhs.spa-7D1.1

1997SC_GR (21)
1997SK_GR (3,7,10,14,21)
QPhs.spa-7D1.2

7D2

Position	Marker
0.0	wmc671
13.4	wmc824
17.5	gwm428
21.6	cfa2040b
22.3	wmc273b
25.5	gwm37
25.7	wmc634
25.9	cfd69
26.5	barc76
30.4	cfa2257a
31.8	wmc546d
32.4	cfd175

1997SC_GI (7,10)
1997SC_PG (5,14)
1997SK_GI (5)
1997SK_PG (3)
1998SC_GI (5,7,10)
1998SC_PG (5)
1998SK_PG (10)
QPhs.spa-7D2

Fig. 2 continued

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Table 1 Significant QTL for dormancy (i.e. pre-harvest sprouting resistance) measurement methods and durations across environments using composite interval mapping

QTL year/ location	Trait measure and duration (in days)	Chromosome arm[a]	QTL associated marker-interval[b]	LOD threshold[c]	CIM LOD[d]	%PVE[e]	Additive value[f]
QPhs.spa-1A1 (SC8021-V2)[g]							
2004/SC-GH	GR5	1AL	cfa2135	3.54	3.60	6	0.10
QPhs.spa-1A2 (SC8021-V2)							
1999/SK	PG14	1AL	wmc73-barc158	2.78	4.97	25	−0.52
QPhs.spa-1B (SC8021-V2)							
1999/SK	GI10,14	1BL	wmc44-gwm328	3.08–3.23	3.59–3.63	18	−0.03
QPhs.spa-1D (SC8021-V2)							
1997/SC	GR21	1DS	wmc222-wmc216	2.93	6.58	37	1.14
1997/SK	GR3-21	1DS	wmc222-wmc216	2.90–3.08	3.76–6.76	30–44	0.62–0.74
QPhs.spa-2A (AC Karma)							
2004/SC-GH	GI7	2AL	gwm294-cfa2058	3.19	3.92	13	0.04
QPhs.spa-2B (SC8021-V2)							
2004/SC-GH	GI21	2BL	wmc546c	2.82	3.72	9	−0.05
1998/SK	GR3,7,10	2BL	gwm55a	3.23–3.31	3.89–4.97	13–16	0.28–0.41
1999/SK	GR7,21	2BL	wmc546c	3.25–3.50	3.37–4.04	11–13	0.21–0.25
1999/SK	PG3	2BL	wmc546c	3.44	3.67	12	−6.53
2004/SC-GH	PG10-21	2BL	wmc546c	2.58–2.99	3.37–3.52	8–10	−5.83 to −6.85
QPhs.spa-2D (SC8021-V2)							
1999/SC	GI14,21	2DL	wmc18-wmc243	3.09–3.40	3.89–4.95	13–14	−0.07
1999/SC	PG7,10,21		wmc18	3.00–3.39	2.97–4.91	9–14	−3.49 to −7.45
2004/SC-GH	PG5	2DL	wmc144	2.21	8.41	43	−19.27
QPhs.spa-3A (SC8021-V2)							
1997/SC	GR7,10	3AL	wmc489b-cfa2134	2.89–2.98	4.05–4.72	25–30	1.28–1.81
QPhs.spa-3A (AC Karma)							
1999/SC	PG10,21	3AL	cfa2193-wmc559	2.43–3.00	3.65–5.42	24–25	8.91–13.71
QPhs.spa-3B (AC Karma)							
1999/SK	PG21	3BL	wmc307	2.08	2.22	8	0.09
QPhs.spa-3D.1 (AC Karma)							
1999/SC	GR5	3DS	cfd55-cfd79b	3.77	5.78	23	−0.36
QPhs.spa-3D.2 (SC8021-V2)							
1999/SK	PG21	3DL	cfd223	2.08	2.82	10	−0.09
QPhs.spa-4A (AC Karma)							
1999/SC	GI5	4AL	gwm397-wmc617a	3.91	4.84	17	0.13
1999/SC	GR7-14	4AL	gwm397-wmc617a	3.72–3.83	4.62–5.31	17–25	−0.56 to −0.83
1999/SC	PG3,5,10-21	4AL	barc170-wmc617a	2.43–3.97	3.22–5.22	19–33	8.96–14.69
QPhs.spa-4B (SC8021-V2)							
1997/SC	GI5-10	4BL	wmc617b-wmc48a	3.07	4.50	35	−0.26
1997/SK	GI10	4BL	wmc617b-wmc48a	2.86	15.57	56	−0.45

Table 1 continued

QTL year/ location	Trait measure and duration (in days)	Chromosome arm[a]	QTL associated marker-interval[b]	LOD threshold[c]	CIM LOD[d]	%PVE[e]	Additive value[f]
1997/SC	PG5	4BL	wmc617b-wmc48a	2.96	10.11	48	−43.80
1997/SK	PG3-7	4BL	wmc617b-wmc48a	2.75–3.03	7.80–17.57	45–59	−40.29 to −48.63
1998/SC	PG5,14	4BL	wmc617b-wmc48a	2.85	9.81–17.89	50–60	−45.20 to −49.10
1998/SK	PG10,21	4BL	wmc617b-wmc48a	2.99	17.06–18.69	54–56	−46.51 to −47.82
QPhs.spa-4D (AC Karma)							
1997/SK	GI3,5	4DS	wmc617c	2.82–3.04	3.22–3.28	10–12	0.09–0.10
1998/SC	PG7	4DS	wmc617c-wmc89b	2.91	8.55	42	43.31
QPhs.spa-5A (SC8021-V2)							
1999/SK	PG5	5AS	gwm304-cfa2190	3.12	4.33	18	−3.63
QPhs.spa-5B (SC8021-V2)							
2004/SC-GH	GR14	5BL	wmc99	3.15	4.99	11	0.44
QPhs.spa-5D (SC8021-V2)							
1999/SK	GI5-10	5DL	gdm133b	3.08–3.51	3.83–4.05	13	−0.05
2004/SC-GH	GI7-14	5DL	gdm133b	3.09–3.27	6.27–10.81	16–29	−0.04 to −0.08
1997/SC	GR5,21	5DL	gdm133b	2.93–2.98	3.63–4.35	12–18	0.41–0.89
1997/SK	GR7	5DL	gdm133b	3.08	3.27	11	0.57
1998/SC	GR5-21	5DL	gdm133b	3.05–3.27	3.89–5.29	14–18	0.42–0.81
1998/SK	GR7-21	5DL	gdm133b	3.14–3.38	4.23–6.08	14–19	0.33–059
1999/SK	GR7-21	5DL	gdm133b	3.25–3.50	3.93–4.51	13–15	0.21–0.27
2004/SC-GH	GR10,21	5DL	gdm133b	3.19–3.21	4.51–6.91	11–17	0.41–0.53
1999/SK	PG3,5,10	5DL	gdm133b	2.85–3.44	3.37–5.23	12–18	−0.41 to −6.88
2004/SC-GH	PG7-21	5DL	gdm133b	2.58–3.51	7.23–8.31	20–25	−9.20 to −14.34
QPhs.spa-6A (AC Karma)							
1997/SK	PG10,14	6AL	wmc179-wmc672	2.83	7.34	50	47.43
1998/SC	PG21	6AL	wmc179-wmc672	2.86	8.23	52	46.73
QPhs.spa-6B (SC8021-V2)							
1999/SC	GI3	6BS	gdm113-gwm508	3.90	5.00	25	−0.16
QPhs.spa-6D (SC8021-V2)							
1997/SC	GI7,10	6D	gwm325	2.83–2.98	3.04	15	−0.28
2004/SC-GH	GR7	6D	cfd80	3.03	4.04	10	0.14
2004/SC-GH	PG5	6D	cfd80	2.21	2.60	8	−3.27
QPhs.spa-7A2 (SC8021-V2)							
1998/SC	GI21	7AS	wmc17-barc154	2.81	4.60	48	−0.39
1998/SC	PG10	7AS	wmc17-barc154	2.90	5.95	49	−40.91
QPhs.spa-7B.1 (SC8021-V2)							
1997/SC	GR3	7BL	gwm146-gwm344b	2.80	2.92	13	0.60

Table 1 continued

QTL year/ location	Trait measure and duration (in days)	Chromosome arm[a]	QTL associated marker-interval[b]	LOD threshold[c]	CIM LOD[d]	%PVE[e]	Additive value[f]
1998/SC	GR3	7BL	gwm146-gwm344b	3.02	3.10	12	0.27
QPhs.spa-7B.2 (SC8021-V2)							
1997/SK	PG10,14	7B	wmc405b	2.83	3.32	13	−12.59
QPhs.spa-7D1.1 (SC8021-V2)							
1997/SK	PG3	7DS	wmc121-gwm44	3.03	8.99	48	−39.18
1998/SK	PG10	7DS	wmc121-gwm44	2.99	14.54	55	−44.85
QPhs.spa-7D1.2 (AC Karma)							
1997/SC	GR21	7DS	wmc463-wmc698	2.93	4.61	21	−0.87
1997/SK	GR3,7-21	7DS	wmc463-wmc698	2.90–3.08	5.23–5.75	22–27	−0.55 to −0.63
QPhs.spa-7D2 (AC Karma)							
1997/SC	GI7,10	7DL	barc76-cfa2257a	2.83–2.98	5.71–8.21	40–46	0.36–0.40
1997/SK	GI5	7DL	barc76-cfa2257a	2.82	10.92	41	0.38
1998/SC	GI5-10	7DL	barc76-cfa2257a	2.90–3.23	3.51–8.00	14–46	0.33–0.41
1997/SC	PG5,14	7DL	barc76-cfa2257a	2.87–2.96	7.26–9.72	44–47	43.68–47.94
1997/SK	PG3	7DL	barc76-cfa2257a	3.03	6.69	39	38.48
1998/SC	PG5	7DL	barc76-cfa2257a	2.85	7.48	45	44.78
1998/SK	PG10	7DL	barc76-cfa2257a	2.99	3.23	30	38.27

SC: Swift Current; SK: Saskatoon; GH: greenhouse; CIM: composite interval mapping

[a] Based on Somers et al. (2004); Kumar et al. (2013), S: short arm; L: long arm

[b] Nearest SSR markers to the QTL peak is underlined

[c] Based on 1000 permutations (values given in range for respective trait measure with multiple durations)

[d] Maximum likelihood LOD score for the QTL (values given in range for respective trait measure with multiple durations)

[e] Phenotypic variation explained by the QTL (values given in range for respective trait measure with multiple durations)

[f] Positive sign in germination resistance (GR) and negative sign in germination index (GI) and in percent germination (PG) indicated that SC8021-V2 allele contributed resistance for respective trait measure-duration relative to the AC Karma allele (values given in range for respective trait measure with multiple durations)

[g] QTL designation (favourable parental allele)

7D2; therefore these QTL are considered novel. They had medium to large effects in enhancing seed dormancy for protection against pre-harvest sprouting. The remaining 20 QTL were detected either at the same marker or overlapping marker intervals reported previously.

Several QTL were anticipated based on previous research into the population by Knox et al. (1998) who indicated a conservative minimum of seven effective factors. The two parents, SC8021-V2 and AC Karma, are genetically diverse with a low coefficient of parentage (Knox et al. 2014) suggesting they possess a diversity of factors for dormancy. As expected from previous research (DePauw and McCaig 1991; McCaig and DePauw 1992; Knox et al. 2012),

genotype SC8021-V2 was sprouting resistant but less so than the red-seeded cultivar RL4137, and AC Karma was more sprouting susceptible. The considerable variation for dormancy across the various durations of germination and transgressive segregation beyond SC8021-V2, suggested AC Karma possessed genetic factors contributing to dormancy. AC Karma contributed QTL for increased dormancy on chromosomes 2A, 3A, 3B, 3D, 4A, 4D, 6A and 7D. The continuous normal and skewed distributions of the population indicated that multiple minor alleles are involved in controlling seed dormancy. This complex genetic control of dormancy is consistent with earlier studies (Knox et al. 1998; Kulwal et al. 2004; Imtiaz et al. 2008; Mohan et al. 2009; Fofana et al. 2009). The
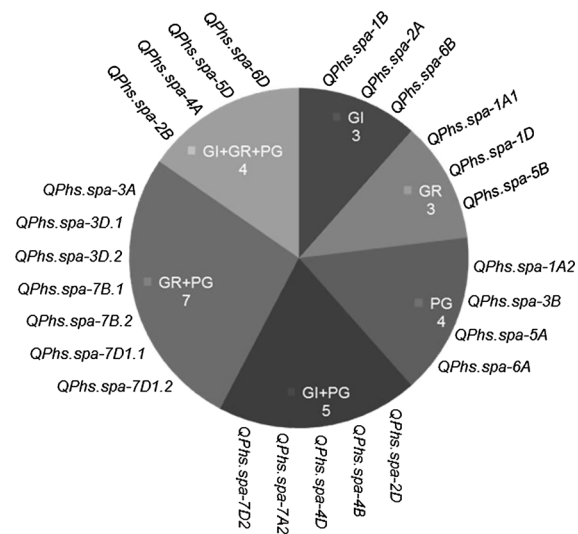
Fig. 3 Pie chart illustrating the number of pre-harvest sprouting resistance associated QTL detected in SC8021-V2/AC Karma doubled haploid mapping population for combinations of measurement methods of dormancy where GI: germination index, GR: germination resistance, PG: percent germination

different durations of germination maximized differential under some conditions, revealing a bimodal distribution that indicated, in addition to minor genes, the presence of a major gene.

Testing the population under different environments provided the opportunity to sample the effect of differentially expressed genes, while analysis of germination measures and durations provided opportunity to reveal the QTL. The complexity of dormancy was demonstrated with the revelation of many genetic loci differentially responding to varied environmental cues. First, the variation in dormancy of genotypes was revealed among environments for each of the three trait measures. For example, Swift Current and Saskatoon field trials showed a clear difference in level of dormancy. Second, the variation was revealed by differences in correlations among the germination measure-duration combinations over environments. Even when correlations were high among measures, variation existed for gene expression. For example, PG21d revealed more significant QTL than PG14d, indicating greater differences in information content between these durations than the high correlation suggested. Based on the frequency distributions, the greatest differential between less dormant and more dormant genotypes was with GI5d, GR21d and PG5d for field environments and GI10d, GR21d and PG7d for

the GH environment. Even though these germination measures-durations tended to reveal the most QTL, many exceptions occurred. As indicated by Knox et al. (2012), a variety of germination measures and counting intervals should be used to maximize identification of QTL. The continuous distributions, transgressive segregation, low correlations between certain combinations of germination measure-durations, and variation of response among the different environments demonstrate the complexity and support the resolution of 26 QTL involving all 21 chromosomes.

Four pre-harvest sprouting QTL expressed consistently for GI, GR and PG measures at different germination durations: *QPhs.spa-2B*, *QPhs.spa-5D* and *QPhs.spa-6D* across multiple environments and *QPhs.spa-4A* in a single environment. *QPhs.spa-4A* was considered real because it explained up to 33 % of the phenotypic variation for that environment. The environment insensitive *QPhs.spa-5D* is near the region Fofana et al. (2009) identified a QTL, *QSi.crc-5D*, on chromosome 5D associated with lower sprouting index. *QSi.crc-5D* was independent of seed coat colour but sensitive to environments. QTL on chromosome 5D were also reported by Anderson et al. (1993), Kulwal et al. (2004) and Jaiswal et al. (2012).

QTL *QPhs.spa-2B* identified in interval *gwm55a-wmc546c* on chromosome 2B is merely 2.1 cM from the *TaSdr-B1* gene (Zhang et al. 2014) between *wmc477-barc55* which regulates seed dormancy. Given that mapping of markers and QTL analysis uses best fit algorithms and noise in the data affects precision, it is possible that *QPhs.spa-2B* is identifying the gene *TaSdr-B1*. Knox et al. (2012) also discovered a SSR marker (*wmc592*) for pre-harvest sprouting resistance on chromosome 2B of durum wheat near *QPhs.spa-2B* QTL. The *QPhs.spa-2B* is in close proximity to *gwm55a*, where Hanocq et al. (2004) reported a photoperiod sensitive QTL for the *Ppd-B1* gene. Munkvold et al. (2009) found a pre-harvest sprouting resistance QTL (*QHd.cnl-2B.1*) overlapped with dormancy and heading date at *gwm429* on chromosome 2B. It is possible that *QPhs.spa-2B* corresponds to *Ppd-B1*. Pre-harvest sprouting resistance QTL on chromosome 2B were also reported by Kumar et al. (2009) and Miao et al. (2013).

The *QPhs.spa-2D* QTL is 16 cM from *Qphs.sau-2D* discovered by Ren et al. (2008) in a synthetic hexaploid × bread wheat cross. Several other studies reported pre-harvest sprouting QTL on chromosome

Table 2 The number of QTL and associated chromosome across the 125 measure-duration combinations which contributed to the identification of 26 QTL in SC8021-V2/AC Karma doubled haploid mapping population assessed across seven environments

Year	1997		1998		1999		2004 GH	Total
Location	Swift Current	Saskatoon	Swift Current	Saskatoon	Swift Current	Saskatoon	Swift Current	
Total	17	25	16	11	18	19	19	125
Germination index								
3	–	1 (4D)	–	–	1 (6B)	–	–	2
5	1 (4B)	2 (4D,7D2)	1 (7D2)	–	1 (4A)	1 (5D)	–	6
7	3 (4B,6D,7D2)	–	1 (7D2)	–	–	1 (5D)	2 (2A,5D)	7
10	3 (4B,6D,7D2)	1 (4B)	1 (7D2)	–	–	2 (1B,5D)	1 (5D)	8
14	–	–	1 (7A2)	–	1 (2D)	–	1 (5D)	3
21	–	–	–	–	1 (2D)	1 (1B)	1 (2B)	3
Germination resistance								
3	1 (7B.1)	2 (1D,7D1.2)	1 (7B.1)	1 (2B)	–	–	1 (1A1)	5
5	1 (5D)	1 (1D)	1 (5D)	–	1 (3D.1)	–	–	5
7	1 (3A)	3 (1D,5D,7D1.2)	1 (5D)	2 (2B,5D)	1 (4A)	2 (2B,5D)	1 (6D)	11
10	1 (3A)	2 (1D,7D1.2)	1 (5D)	2 (2B,5D)	1 (4A)	1 (5D)	1 (5D)	9
14	–	2 (1D,7D1.2)	1 (5D)	1 (5D)	1 (4A)	1 (5D)	1 (5B)	7
21	3 (1D,5D,7D1.2)	3 (4B,7D1.1,7D2)	1 (5D)	1 (5D)	–	2 (2B,5D)	1 (5D)	10
Percent germination								
3	–	1 (4B)	–	–	1 (4A)	2 (2B,5D)	–	6
5	2 (4B,7D2)	1 (4B)	2 (4B,7D2)	–	1 (4A)	2 (5A,5D)	2 (2D,6D)	10
7	–	2 (6A,7B.2)	1 (4D)	–	1 (2D)	–	1 (5D)	4
10	–	2 (6A,7B.2)	1 (7A2)	3 (4B,7D1.1,7D2)	3 (2D,3A,4A)	1 (5D)	2 (2B,5D)	12
14	1 (7D2)	2 (6A,7B.2)	1 (4B)	–	1 (4A)	1 (1A2)	2 (2B,5D)	8
21	–	–	1 (6A)	1 (4B)	3 (2D,3A,4A)	2 (3B,3D.2)	2 (2B,5D)	9

Name of chromosome given in parenthesis for each measure-duration on which QTL were identified; GH: greenhouse

2D (Anderson et al. 1993; Mares et al. 2002; Mrva and Mares 2002; Hanocq et al. 2004; Tan et al. 2006; Munkvold et al. 2009). *QPhs.spa-2D* may be related to previously reported QTL. The *QPhs.spa-6D* QTL, detected in field and GH environments, mapped in the interval *gwm325-cfd80* which is near the location of the germination inhibitor phytohormone ABA responsiveness gene *TaABA8′OH1* (ABA 8′-hydroxylase activity) on chromosome 6D. The gene plays a key role in seed dormancy regulation (Kobayashi et al. 2010; Chono et al. 2013; Iehisa et al. 2014).

Chromosomes of homoeologous group 4 are considered to have an effective role in pre-harvest sprouting resistance in wheat through dormancy control [Kulwal et al. 2010 (review); Lohwasser et al. 2013]. Noda et al. (2002) investigated another ABA responsive gene located on the long arm of chromosome 4A. *QPhs.spa-4A,* located in interval *barc170-gwm397-wmc617a* on 4A, is in a region associated with dormancy and pre-harvest sprouting resistance in numerous other QTL studies of hexaploid wheat (Flintham et al. 2002; Torada et al. 2005; Mares et al. 2005; Tan et al. 2006; Ogbonnaya et al. 2008; Imtiaz et al. 2008; Munkvold et al. 2009; Rasul et al. 2009; Kulwal et al. 2010; Singh et al. 2010, 2012; Lohwasser et al. 2013). The locus was also identified in tetraploid (Knox et al. 2012) and diploid (Nakamura et al. 2007) wheat populations, and in an association mapping study (Kulwal et al. 2012). It is possible that *QPhs.spa-4A* harbors the gene related to ABA responsiveness. Because a common SSR marker *wmc617* amplifies DNA in all three homoeologous chromosomes (4A, 4B and 4D), it is reasonable to expect gene homoeologues for pre-harvest sprouting resistance among the group four chromosomes (Fig. 2). It is noteworthy that this region of chromosome 4A and its probable homoeologous location on 4B (*QPhs.spa-4B*; *wmc617b-wmc48*) and on 4D (*QPhs.spa-4D*; *wmc617c-wmc89b*) detected three major QTL for pre-harvest sprouting resistance in multiple environments. Our study appears to be the first report of QTL for pre-harvest sprouting resistance in the same homoeologous region of all three group four chromosomes. Given that *QPhs.spa-4B* had the largest effect, explaining on average 51 % of the phenotypic variation, SC8021-V2, the source of this resistance, is a good candidate for further ABA responsiveness gene analysis. Studies also show that the dwarfing genes (i.e. *Rht-1, Rht-2, Rht-3*) located on the group four chromosomes have pleiotropic effects on many plant characters including pre-

harvest sprouting resistance (King et al. 1983; McIntosh 1988; Wu et al. 2011). The *Rht-B1c* variant exhibited insensitivity to GA_3 which caused reduction in alpha-amylase activity in seeds, increased ABA responsiveness and increased Hagberg falling number, resulting in improved resistance to sprouting damage (Flintham et al. 1997; Gooding et al. 2012). However, the location of *Rht-B1c* allele at *wmc125* on chromosome 4B (Navarro et al. 2014) is distant from the *QPhs.spa-4B* QTL.

The *QPhs.spa-7A2* QTL strongly identified in the field environment and mapped to the short arm of chromosome 7A at interval *wmc17-barc154* which is in a similar region as *QPhsd.spa-7A* reported by Knox et al. (2012) and *QPhs.spa-7A* reported by Singh et al. (2014) in durum wheat, and *QPhs.ccsu-7A.1* reported by Mohan et al. (2009) in hexaploid wheat. This consistency suggests the 7A QTL is important for imparting pre-harvest sprouting resistance in different genetic backgrounds. The LOD peak of the chromosome 7B *QPhs.spa-7B.1* QTL in interval *gwm146-gwm344b* was very close to the marker *wmc526* reported by Jaiswal et al. (2012). Two QTL, *QPhs.spa-7B.1* (1.9 cM) and *QPhs.spa-7B.2* (52.8 cM) are the first and second occurrences of QTL on chromosome 7B. Based on SSR markers in a consensus map of 7B from the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml), *QPhs.spa-7B.2* at *wmc405b* is near to the QTL *QPhs.spa-7B* reported by Singh et al. (2014) and *QPhsd.spa-7B.1* reported by Knox et al. (2012) in durum wheat. Kobayashi et al. (2010) reported a QTL for ABA responsiveness on 7B which is 9 cM away from *QPhs.spa-7B.2*. We considered the two QTL LOD peaks on chromosome segment 7D1 that spaced more than 50 cM as two different QTL [*QPhs.spa-7D1.1* (21.6 cM) and *QPhs.spa-7D1.2* (72.1 cM)]. The *QPhs.cnl-7D2* QTL reported by Munkvold et al. (2009) on 7D is in the same region as *QPhs.spa-7D1.1*. The second QTL on chromosome 7D, *QPhs.spa-7D1.2*, in interval *wmc463-wmc698* for the GR measure at day 3, 7, 10, 14 and 21 was novel. A major QTL *QPhs.spa-7D2* on chromosome 7D2 (another linkage group of 7D) at interval *barc76-cfa2257a* was also novel. Using bulk segregant analysis in a DH population, Flintham (2000) also reported a gene, *Phs*, on chromosome 7D. These reports along with our findings indicate that factors on chromosome 7D have the capacity to control sprouting.

Dormancy and thus pre-harvest sprouting resistance are associated with red pericarp (Groos et al. 2002). However, DePauw and McCaig (1983) and DePauw et al. (1992) recovered sprouting resistant white-seeded progeny from crosses of dormant red-seeded by white-seeded sprouting susceptible parents which demonstrated that at least two mechanisms controlled pre-harvest sprouting resistance. The *R* genes responsible for controlling red seed coat are present on the homoeologous group three chromosomes. The three QTL *QPhs.spa-3A*, *QPhs.spa-3B* and *QPhs.spa-3D.2* detected for GR and PG measures were co-localized with the three major QTL namely, *QCL.crc-3A*, *QCL.crc-3B* and *QCL.crc-3D* associated with red seed coat color identified by Fofana et al. (2009). These findings demonstrate substantial complexity with the same QTL being responsible for pre-harvest sprouting resistance in white and red-seeded wheat. No QTL were detected on group three chromosomes with GI, and those detected with GR and PG were weak and appeared in few environments. Perhaps different alleles of the seed coat color genes occur in white seed coat genotypes that still weakly affect dormancy but do not impart red color. Alternatively, the dormancy genes are only linked to the seed coat colour genes. The 3A chromosome was previously associated with a pre-harvest sprouting resistance QTL (Rasul et al. 2009), *Qphs.pseru-3AS*, reported by Liu et al. (2008) and Liu and Bai (2010), although maps could not be cross-referenced. Liu et al. (2013) cloned a gene from white-seeded wheat which they designated *TaPHS1*. Another gene on chromosome 3A reported to be involved in controlling seed dormancy and germination is *TaMFT-A1*, a homoeologue of *MOTHER OF FT* (Nakamura et al. 2011; Lei et al. 2013). The structure of the *TaMFT* and *TaPHS1* genes are the same except for some SNPs within the promoter and exon–intron sequences, and their relevance with the QTL we identified needs further work.

The QTL *QPhs.spa-5A,* with moderate effect in interval *gwm304-cfa2190,* is consistent with the interval of chromosome 5A detected for a pre-harvest sprouting QTL by Groos et al. (2002) and Glein et al. (2006). A QTL for ABA responsiveness (Iehisa et al. 2014) maps 7 cM away from *QPhs.spa-5A* and was associated with dehydration tolerance and seed dormancy in a F_2 population of the cultivar Chinese Spring crossed with Chinese Spring (Hope5A).

Chinese Spring (Hope5A) is a chromosome substitution line of Chinese Spring with chromosome 5A of Hope. The putative QTL *QPhs.spa-5B* detected only for GR14d from the GH environment appears in the vicinity (11–13 cM) of the QTL reported in hexaploid wheat by Fofana et al. (2008) and in durum wheat by Singh et al. (2014).

The *QPhs.spa-6A* QTL mapped on chromosome 6A was revealed by PG from field environments and appears to map at least 30 cM from the *QPhs.ccsu-6A.1* reported by Kumar et al. (2009) based on the markers position on the consensus map of wheat by Maccaferri et al. (2014). Thus, *QPhs.spa-6A* may be a novel QTL. However, another pre-harvest sprouting QTL on 6A was reported by Zanetii (2000) using restriction fragment length polymorphism (RFLP) probes but it is difficult to relate this locus to *QPhs.spa-6A*. The minor *QPhs.spa-6B* QTL in interval *gdm113-gwm508* is in the same region as a QTL reported by Glein et al. (2006) and Knox et al. (2012) in durum wheat populations.

The QTL, *QPhs.spa-1D*, that expressed strongly for the GR3-21d in interval *wmc222-wmc216* is in the same region which Kumar et al. (2009) reported for grain colour (*wmc222*). As with the red seed coat genes of the group three chromosomes, one may hypothesize the overlap as being related in function. Lohwasser et al. (2013) also reported dormancy QTL *QD20.ipk-1D* on chromosome 1D just 2.2 cM from *QPhs.spa-1D*. The minor effect *QPhs.spa-1A1* QTL at *cfa2135* coincides with a QTL reported by Knox et al. (2012) and Jaiswal et al. (2012), and is near to QTL reported in many other studies (Glein et al. 2006; Munkvold et al. 2009; Mohan et al. 2009; Singh et al. 2010; Rehman et al. 2012; Singh et al. 2014). Singh et al. (2010) and Zanetti et al. (2000) detected a QTL on chromosome 1A that was possibly involved with alpha-amylase activity, an important predictor of pre-harvest sprouting. *QPhs.spa-1B* in interval *wmc44-gwm328* that explained 18 % of the phenotypic variation for GI10d and GI14d is in a different position from QTL reported by Munkvold et al. (2009), Jaiswal et al. (2012) and Singh et al. (2014). Therefore *QPhs.spa-1B* may be independent of their 1B QTL.

QTL, *QPhs.spa-2A,* for GI7d from the GH environment was in interval *gwm294-cfa2058* on chromosome 2A, a different position from the previous reports (Mohan et al. 2009; Kumar et al. 2009; Kobayashi

et al. 2010; Jaiswal et al. 2012; Knox et al. 2012). The marker coverage was low on chromosome 2A which may affect the QTL position and this conclusion.

Our study differed from other studies (Biddulph et al. 2008; Singh et al. 2008; Knox et al. 2012) with not only the application of multiple measures of seed dormancy but also with multiple time intervals during germination up to 21 days across multiple environments. This is the first report in which QTL associated with the seed dormancy component of pre-harvest sprouting resistance were detected and mapped on all the chromosomes. This intense set of measurements was intended to capture maximum expression of genes in chromosomal regions (QTL) influencing pre-harvest sprouting resistance. Knox et al. (1998) first used the phenotypic data collected on the SC8021-V2/AC Karma population to estimate that more than seven effective factors segregated with traditional quantitative analysis. The addition of marker data for QTL analysis further elucidated the complexity of sprouting resistance and demonstrated that far more QTL were involved. The majority of the QTL align with previously reported QTL. This alignment not only validates the QTL, but that the intensive measuring protocol and analysis is important to revealing dormancy QTL segregating in a population. The QTL identified in this study, through comparisons in the literature of commonly mapped markers, were associated with important components of pre-harvest sprouting resistance such as seed coat colour, ABA responsiveness, and alpha-amylase activity. The QTL ranged from minor to major, some were previously reported, some were identified in different environments and genetic backgrounds, some appear to be validated based on common locations with previously reported QTL, and others appear to be novel. Six QTL we describe as novel in the sense that they were previously unidentified yet influence control of pre-harvest sprouting. Further validation is required for several of the QTL.

As demonstrated, the genetic control of dormancy and thus pre-harvest sprouting resistance is complex, and the genes at several loci across the whole genome are greatly influenced by the environment. Even though many more QTL were identified in the SC8021-V2/AC Karma population than previously reported in a single mapping population, several QTL reported by others were not detected here. The possible reasons for not detecting QTL relate to (i) lack of presence of alleles in the parental genotypes,

(ii) effective alleles were common between the two parents so no segregation occurred, (iii) sampled environments were not conducive to expression, and (iv) unsaturated coverage of the genome due to lack of polymorphic markers and therefore low-resolution of the genetic map. Variation in pre-harvest sprouting resistance may also exist beyond main effect QTL, such as epistatic interactions. Such analysis remains to be done. Challenges remain, such as determining why apparent homoeologous QTL located on the group three chromosomes, which are associated with red grain colour, also appear to control pre-harvest sprouting in red- and white-seeded wheat. Similarly, the relationship of homoeologous QTL variants detected on 4A, 4B and 4D need to be confirmed using other populations or by sequencing of the regions of interest.

Understanding the major QTL for pre-harvest sprouting resistance detected on chromosomes 1A, 1D, 2B, 4A, 5D, 6D, 7D observed through measuring different durations of germination with GI, GR and PG may aid in marker-assisted breeding for improved pre-harvest sprouting resistance in wheat. The study also revealed that favourable alleles for improving sprouting resistance were present in both SC8021-V2 and AC Karma parents. The markers associated with the transgressive segregant genotypes for sprouting resistance from the SC8021-V2/AC Karma population can be used to further develop more resistant white-seeded wheat cultivars.

This study with hexaploid wheat confirmed the approach reported by Knox et al. (2012) with durum wheat to maximize identification of QTL contributing to dormancy. Our results support conclusions of other researchers that QTL for pre-harvest sprouting resistance are located on all the wheat chromosomes (Flintham et al. 2002). Pyramiding of these QTL will boost the level of resistance to pre-harvest sprouting in white-seeded wheat cultivars. In this era of genomics, the availability of chromosome-based sequences and high-throughput genotyping platforms for construction of high-resolution genetic maps will allow further fine mapping and better understanding of pre-harvest sprouting resistance QTL.

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