

Enhancing the identification of genetic loci and transgressive segregants for preharvest sprouting resistance in a durum wheat population

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Abstract Preharvest sprouting reduces grain quality and lowers grade. Characterization of preharvest sprouting resistance is important in selection in breeding for transgressive segregation and understanding the genetics of the trait for identifying QTL. Methods of measuring dormancy and other factors contributing to preharvest sprouting resistance are varied. The objective of this study was to demonstrate the requirement of multiple methods of measurement over multiple durations of germination to maximize understanding of transgressive segregation and QTL for preharvest sprouting resistance within a segregating durum wheat population grown in multiple environments. Ninety-eight durum wheat (*Triticum turgidum* L. var. *durum*) recombinant inbred lines

(RIL) from a cross of a minimally dormant line, Sentry, by a moderately dormant line, Kyle, and controls were grown in replicated field tests in 1996, 1997 and 1998 and in a growth chamber trial in 1998. Preharvest sprouting was measured from intact spikes as sprouting index or from hand threshed grain as germination index (GI), germination resistance (GR), and percent germination (PG). The threshed grain measures were evaluated using counts at 7, 14 and 21 days intervals from the start of germination. Correlations performed on the measure type and duration using lines within the RIL population showed some discontinuity across environments, type of measure and duration of measure, with counts at extended intervals for PG producing the lowest correlations. The number of transgressive segregant lines varied with environment, duration and type of measure. Different QTL were identified by different types of measures and duration of counts. GI calculated for 7, 14 and 21 days germination count intervals and GR calculated for 21 days identified a highly significant QTL on chromosome 1A (*QPhsd.spa.-1A.1*). GR calculated for 7 days identified a highly significant QTL on 2A (*QPhsd.spa.-2A.1*) in two different environments, and GI calculated for 21 days and PG at 7 days identified the same highly significant QTL on chromosome 7B (*QPhsd.spa.-7B.1*). The results indicated that multiple measures and durations of measure intervals must be applied to results collected across different environments to maximize the identification of QTL and transgressive segregants

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of the population segregating for preharvest sprouting resistance.

Keywords Dormancy · Sprouting index · Germination resistance · Germination index · Percent germination · QTL · Transgressive segregation

Introduction

In wheat, sprouting is a problem during wet or humid harvest conditions (McCaig and DePauw 1992), resulting in decreased grain yield and test weight and poor processing quality (Derera 1989). About 2 million ha of durum wheat are grown in western Canada each year (Statistics_Canada 2009) to supply the domestic and export pasta industry. Near and during harvest, wheat is exposed to a variety of abiotic factors that contribute to downgrading including sprouting damage. Periodically severe or widespread sprouting problems occur. Between 1978 and 1988, downgrading resulting from preharvest sprouting damage cost AUD\$100 million/year for four of the eleven years (Derera 1990). In recent years, substantial losses were experienced in Canada in 2000 and 2002 (Clarke et al. 2005a) and again in 2010. Incorporation of sprouting resistance maintains grade and reduces these losses. For example, if an additional 20% of the durum wheat crop were maintained at the top grade instead of dropping a grade, farm value would increase by \$12 million/year.

Preharvest sprouting resistance is not easy to characterize because of the complexity of the trait (Gerjets et al. 2010). Much of preharvest sprouting resistance is related to dormancy, which degrades through after-ripening from the time of seed maturity. Proper collection, handling and storage of wheat seed to be tested for sprouting response is imperative. Collection of material near physiological maturity to maximize dormancy (Gerjets et al. 2010), gentle handling to avoid seed damage (Clarke and DePauw 1989; Finkelstein et al. 2008), and storage of seed at low temperature to preserve dormancy (Stefani et al. 2000) until use are important to obtain reliable germination data. Seed damage from insects (for example wheat midge), disease and weathering reduce dormancy and complicate the evaluation of this trait (Singh et al. 2009).

Expression of preharvest sprouting resistance is affected by genotype, environment and interaction of the two (Flintham et al. 2002; Torada et al. 2005; Ogbonnaya et al. 2008). Multiple genetic loci are involved in controlling preharvest sprouting resistance (Flintham et al. 2002; Chao et al. 2010) and the expression of these loci varies across environments (Lunn et al. 2002; Chao et al. 2010). For breeders to make improvements for preharvest sprouting resistance from select germplasm, it is important for geneticists to fully characterize all facets of this complex trait within the germplasm being utilized for selection.

Defining measures of sprouting resistance or dormancy is also a challenge given that seed quality, time between harvest and testing, environmental conditions at the time of germination and variation in rate of germination over time affect the outcome. Dormancy can be characterized by counting the germination of seed each day for a series of days, from which germination resistance (GR) (Gordon 1971), germination index (GI) (Reddy et al. 1985), weighted GI (Walker-Simmons 1988; Walker-Simmons and Sesing 1990) or percentage germination (PG) are calculated (Belderok 1961, 1968; Lunn et al. 2002). Rain simulation treatments of intact spikes with bundles of spikes placed upright in rotating trays in an artificial rain chamber and counts of sprouted spikes are used to calculate a sprouting index (SI) (DePauw and McCaig 1991).

In genetic and breeding work, it is important to measure a trait in a way that maximizes selection for that trait. We need to determine if measurement methods differ for resolution of preharvest sprouting resistance. Ultimately we want to know how to best characterize the trait to maximize the identification of QTL and improved lines for breeding purposes. A simple hypothesis is that all measures of dormancy will identify all QTL; that is, different measures will identify the same QTL and the same lines for transgressive segregation.

The objective of this study was to demonstrate the need to apply multiple methods of measurement over multiple durations of germination to maximize understanding of transgressive segregation and QTL for pre-harvest sprouting. With the Sentry/Kyle random inbred durum population grown across multiple environments, identified QTL for preharvest sprouting resistance should maximize development of superior resistance in future durum breeding lines.

Materials and methods

Genetic materials

Ninety-eight F₅ derived recombinant inbred lines (RIL) were developed from a cross of Sentry (Lebsock 1965) and Kyle (Townley-Smith et al. 1987) (Sentry/Kyle, SPARC cross identification number G9261). Preliminary observations indicated that Sentry was moderately susceptible to preharvest sprouting. Kyle is an adapted cultivar and intermediate for preharvest sprouting resistance (McLeod et al. 1991).

Phenotyping preharvest sprouting resistance

Field trials

The 98 RILs from Sentry/Kyle and varying numbers of controls were grown in field tests in a three replicate randomized complete block design near Swift Current in 1996, 1997 and 1998. In 1996, each replication contained two plots of Genesis (DePauw et al. 1989), Kyle, RL4137 (DePauw et al. 2009), SC8021-V2 (DePauw et al. 1992) and AC Karma (Knox et al. 1995), and one plot of Sentry and AUS1408 (Tan et al. 2006). In 1997 and 1998, each replication contained one plot of Genesis, RL4137, SC8021-V2 and AC Karma, and two plots of Sentry and Kyle.

Each plot comprised one or two rows 3 m long with approximately 200 seeds/row. Plots were 46 cm apart and separated by a row of spring-planted winter wheat, that remained vegetative throughout the growing season for erosion and weed control, and facilitated access for sampling.

Five to 10 spikes were sampled (depending on testing requirements) from a plot when 50% of the primary tillers in that plot had collapsed nodes on the stems (DePauw et al. 2009). Collapsed nodes of wheat stems is associated with about 16% grain moisture, which allows the grain to be stored and provides a level of after-ripening that allows a differential response in dormancy to be observed. These plots were identified followed by collection of spikes. Labels were made to tie spikes together (if the spikes were to be used in the rain simulator, waterproof labels were used). Care was taken to ensure that spikes were representative of the plot from which they were taken and that the nodes were collapsed on the stem. Spikes

showing prematurity blight or other disease problems were avoided, and an attempt was made to take spikes from primary culms. Spikes were placed in labeled boxes, and stored in a freezer at -23°C immediately after collection until threshing to minimize metabolic activity that would cause a loss of dormancy by after-ripening. In 1996, a second set of spikes was collected two weeks after the first set.

Growth chamber trial

An unreplicated test of 96 lines was grown in the growth chamber early in 1998 and harvested in June to July 1998. Spikes could not be collected for one line. The chamber was operated at 17°C with approximately $250\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ light intensity. One seed of each cultivar was grown in a 1 l milk carton ($10 \times 10 \times 25\ \text{cm}$) filled with soil. The cartons contained approximately 2 cm of peat moss in the bottom to cover drain holes, and were filled to approximately 7 cm from the top with a 2:1 ratio of field soil and soilless mix (Sungro Sunshine LB2 mix Basic). The substrate was packed tightly using a wooden paddle the same size as the opening to the carton. Cartons were filled to within 1.5 cm of the top with the soilless mix and packed again. Seeds were planted approximately 1.5 cm into the soil. Plants were provided with $200\ \text{mg l}^{-1}$ of 20–20–20 (N–P–K) at the time of watering. Four pots each of RL4137, Sentry, SC8021-V2 and Kyle, and three each of AC Karma and Genesis were grown. Two spikes/plant were harvested and stored in a freezer as previously described for field sampling.

Dormancy evaluation of threshed grain

In 1996 and 1997, seeds were germinated in an incubator at 15°C , and in 1998 in a growth chamber at 15°C , with lights off and relative humidity of about 50%. To ensure that the seed coat was not damaged, spikes were threshed by hand either by gently rolling the spikes on a rubber mat or by rolling the spikes within a piece of bicycle tyre tube and blowing off the chaff from a seed pan. Once the seeds were threshed they were packaged in envelopes and returned to the -23°C freezer. Spikes were processed in batches to minimize the time outside of the freezer. Seed of the five field spikes from a plot were bulked and sub-sampled for germination tests.

Two pieces of 12.5 cm Ahlstrom filter paper, grade 631, were inserted into each labeled 15 cm petri dish and double distilled water was added until the filter papers were uniformly wet and excess water was poured off. The petri dishes were left open and placed in a laminar flow hood under ultraviolet light for 20–30 min to help control microbial organisms. Seed samples were plated in a laminar flow hood. Thirty three seeds were germinated in 1996 and 30 seeds in 1997 and 1998 in the petri dishes with the crease of each seed facing down to make it easier to observe the germinated embryos. Daily, over the course of testing, the moisture content of the filter paper in each petri dish was visually checked, and water was added to moisten the filter paper if necessary but without flooding the dish. Seeds with embryos ranging in stage from the white radicle or plumule protruding with a visible tear in the seed coat to a plumule or radicle 2 mm long were counted as germinated and removed from the plate. Using a sterile chamber, counts were recorded daily up to 21 days and plates were re-randomized each day. The petri plates were stacked in plastic tubs in which a shallow film of water was maintained for humidity and a lid was placed on the tub.

After a final count on day 21, the ungerminated seeds in each petri dish were treated with 4.0–6.0 ml of 0.5 mM GA₃ solution for 1 h. The GA₃ solution was made by dissolving the salt in double distilled water. Excess solution was poured or blotted out, and petri dishes containing solution-treated seed samples were placed in a low temperature environment of approximately 2–5°C for 12 h or overnight. Daily counts of the germinated seeds were recorded for another 3 days. Any seed that was not germinated was considered to be nonviable, and was excluded from the experimental total seed used in calculations of dormancy.

The weighted germination index (Reddy et al. 1985) was calculated as:

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

where ‘d’ is the total number of days for which the seed was counted; $n_1, n_2, n_3, \dots, n_d$ are the number of seeds germinated on 1st, 2nd, 3rd to d th day and ‘N’ is the total number of viable seeds plated for germination. This method of dormancy calculation weights

seeds that germinated early more heavily than seeds that germinated late. In 1997, a data factor required to calculate GI was not recorded, therefore no results were obtained for GI in 1997.

The GR (Gordon 1971) was calculated as:

$$GR = \{(d_1/2)(n_1) + [(d_2 + d_1)/2](n_2) + \dots + [(d_i + d_{i-1})/2](n_i)\} / N \text{days}$$

where d_1, d_2, \dots, d_i are the 1st, 2nd to i th day of the germination counts; n_1, n_2, n_3, \dots, n are the number of seeds germinated on 1st, 2nd, 3rd to d th day and ‘N’ is the total number of seeds germinated. This method calculates time when half the seed germinated.

Percent germination was calculated as the number of seed germinated on the day of interest divided by the total number of seed multiplied by 100. PG for 1997 was not calculated for the same reason as GI above.

Calculations for GI, GR and PG were performed for day 7, 14 and 21 to establish the effect of the duration of the test on resolution of preharvest sprouting response. The method-duration combinations are abbreviated as follows: GI7, GI14 and GI21 are germination indices for 7, 14 and 21 days, respectively; GR7, GR14 and GR21 are germination resistances for 7, 14 and 21 days, respectively; and PG7, PG14 and PG21 are percent germinations for 7, 14 and 21 days, respectively.

Sprouting of whole spikes

From the three replicate plots grown near Swift Current in 1997 and 1998, a second set of five spikes were sampled for rain simulator analysis. In 1997, one plot/replicate was sampled from 48 lines and the controls, Kyle, RL4137, SC8021-V2 and AC Karma. In 1998, all plots were sampled.

In preparation for rain simulator testing similar to that described by Fofana et al. (2008), the stems of the bundles of spikes were trimmed so they could be inserted in wire mesh in trays. The trays were placed in the sprouting chamber, a modified growth chamber equipped with spray nozzles and carousel to rotate the trays of spikes past the nozzles. The conditions within the rain simulator were a constant 17°C and maximum relative humidity. After an initial 5 h wetting period, water was sprayed at 8:30 a.m.–9:00 a.m. and then again at 8:30 p.m.–9:00 p.m. for 5 days at which time

the spikes were removed for scoring. The number of spikes/bundle with visible evidence of germination was recorded as the sprouting index for each line.

Genotyping

The DNA markers reported by Knox et al. (2005) along with other markers were applied to lines of the population as described by Houshmand et al. (2007) and Knox et al. (2002). DNA was extracted from single plants using a modified CTAB method (Saghai Maroof et al. 1984). Leaf tissue was sampled from up to two-week-old seedlings of the Sentry/Kyle population and controls that were grown at room temperature (approximately 21°C) in either petri dishes on filter paper or in 96 cavity (12 × 8) trays with cotton balls moistened with 0.2% KNO₃.

Wheat microsatellite markers

Parents were tested with approximately 1,000 microsatellite primer pairs with known sequences and chromosome locations. Those polymorphic between parents were evaluated on the whole population of lines. DNA was amplified and separated by either high resolution agarose gel electrophoresis or capillary electrophoresis. Amplification of DNA to be run on agarose gels was performed by creating a reaction mixture of 2.4 ng/μl DNA, 10%–10× PCR buffer without Mg [Invitrogen cat.# 18067-017: 200 mM Tris–HCl (pH 8.4), 500 mM KCl], 1.5 mM MgCl₂, 2 ng/μl forward and 2 ng/μl reverse primer, 0.2 mM of each dNTP, and 0.07 U/μl Taq (5 U of activity/μl) in Gibco nuclease-free water. The mixture was heated at 94°C for 3 min for initial denaturation, followed by 44 cycles of 94°C for 1 min, annealing temperature of specific primer for 1 min, and 72°C during extension for 1 min. This was followed by 72°C for 10 min for the final extension. The primers evaluated were wmc (Gupta et al. 2002), barc (Song et al. 2005), gwm (Röder et al. 1998), cfd (Sourdille et al. 2003), cfa (Guyomarc'h et al. 2002), and gdm (Pestsova et al. 2000) using reported conditions. PCR was done in a 96-well PTC-100 thermocycler (MJ Research Inc). PCR products were separated in 3% electrophoretic agarose gels (2:1 Metaphor/LE Agarose) in TBE buffer (0.045 M tris–borate, 0.045 M boric acid, and 0.001 M EDTA) at 200 v for 2–3 h depending on separation requirements. The size of DNA fragments

was determined by comparing against either a pGEM or 50 bp DNA size marker. The lines were scored for each of the polymorphic markers. For the capillary electrophoresis method, DNA amplification was performed by creating a mixture of 2.4 ng/μl DNA, 10%–10× PCR buffer without Mg [Invitrogen cat.# 18067-017: 200 mM Tris–HCl (pH 8.4), 500 mM KCl], 1.5 mM MgCl₂, 0.02 pmol/μl forward and 0.2 pmol/μl reverse primer, 0.18 pmol/ul M13 primer, 0.2 mM of each dNTP, and 0.05 U/μl Taq (5 U of activity/μl) in Gibco nuclease-free water. PCR conditions were the same as listed above. The M13 modified primers were utilized as described by Pozniak et al. (2007). The PCR products were separated using the Applied Biosystems 3130xl Genetic Analyser. The size of the bands was determined using either the ROX or LIZ size standards. The RILs and controls were scored for each of the polymorphic markers.

AFLP analysis

AFLP analysis was performed using the Invitrogen AFLP[®] Analysis System I Kit (Catalog no. 10544-013) following procedures defined for the system. Selective amplification of lines was performed using 20 *EcoR* I and *Mse* I selective amplification primer pair combinations. The amplified samples were denatured prior to loading into a 5% polyacrylamide sequencing gel. After electrophoresis for 2.5 h at 85 W, the gels were stained using the Promega SILVER SEQUENCE[™] DNA sequencing system (Catalogue no. Q4130) and visually scanned for polymorphic bands.

Statistical analysis

Germination resistance, GI, PG and SI (rain simulator) results were analyzed with SAS (version 8.2, Littell et al. 1996). Diagnostics which included the *rstudent* (Rawlings 1988, p. 250) identified possible outliers. The mixed model analysis of variance was performed on each environment with PROC MIXED. Genotypes were considered fixed and replications were random.

Within each test, differences among parents and checks were tested and genotypes were compared with parents to identify transgressive segregants using the *t* test in the PDIFF option with the appropriate error term. Calculation of means was based on all entries. The highest standard error of the difference was used

for presentation in the figures for a conservative comparison of parents and parents with controls when unbalanced. Transgressive segregants were identified as genotypes significantly greater than the high parent or less than the low parent.

Pearson's correlation was performed among measurement methods and within measurement methods for each test duration. Control cultivars were used to evaluate performance of a measurement method. The highest standard error of the difference for pairs of comparisons within environments is reported in the figures.

QTL analysis was performed on genotypic data for 156 SSR and 74 AFLP markers on 96 lines by first generating a linkage map using JoinMap 3.0 (Van Ooijen and Voorrips 2001). Simple interval mapping (SIM) and composite interval mapping (CIM) were then performed using Multiple Quantitative Trait Loci (MQTL) software (Tinker and Mather 1995) on least square means to determine the significance of the association of markers to preharvest sprouting resistance measures. MQTL was run using 1,500 permutations, 5 cM walk speed and a 5% type I error level. Null amplicon results were treated as missing values in the MQTL analysis. Each of the methods of measuring preharvest sprouting resistance was used in the QTL analysis to assess differences in precision of detection of the QTL.

Results

The greatest differential between check cultivars was 21 days for GR, 7 days for GI and 7 days for PG (Fig. 1), GR for 21 days (GR21) of Sentry was consistently less resistant than Kyle and the two sprouting resistant controls, white-seeded SC8021-V2 and red-seeded RL4137, ($P < 0.05$) except in 1997 when all four were similar (Fig. 1a). Sentry was significantly more sprouting resistant than low sprouting resistance controls Genesis and AC Karma ($P < 0.05$) except for 1998 when Sentry and AC Karma showed no difference (Fig. 1a). The GR of Kyle at 21 days was not significantly different ($P < 0.05$) from SC8021-V2, but was significantly ($P < 0.05$) less sprouting resistant than RL4137 in two out of four environments. In 1996 for sampling time T2, Kyle was significantly ($P < 0.05$) more sprouting resistant than AUS1408. The trends of GR for 14 and

7 days were similar to GR for 21 days. RL4137 tended to be more resistant than Kyle and SC8021-V2 except in the 1998 growth chamber study where the three lines were similar with the GR7, GR14 and GR21 measures ($P < 0.05$). Differences in GR were much more compressed in the 1997 environment. The same general pattern among the check cultivars was observed for GI (Fig. 1b), but rank changed between Kyle and SC8021-V2 in the growth chamber 1998 in both GR14 and GI14. Like GR, the GI for RL4137 was similar to the GI of Kyle and SC8021-V2 in the 1998 growth chamber trial. The ranks of the more resistant checks for PG7 were similar to GI7; however, the susceptible checks, AC Karma and Genesis, were little differentiated (Fig. 1). Except for the growth chamber in 1998 and to a lesser extent the 1998 field environment, there was little discrimination between check cultivars in PG for 14 and 21 days measures. Seed collected two weeks after the first sampling date tended to take fewer days for 50% germination (GR) whereas the difference in sampling date was less obvious with GI and PG. The SI from rain simulator trials ranked the parents and control cultivars for 1998 similarly to the majority of other measures (Fig. 1d). In 1997, RL4137 and SC8021-V2 were significantly ($P < 0.05$) more sprouting resistant than Kyle, whereas Kyle was not different from Sentry, AC Karma or Genesis (Fig. 1d).

Correlations were significant at $P < 0.05$ unless otherwise reported. Correlations between GR for the 21 days germination count of the population and GR for 14 days within the same test were $r = 0.99$ for all field environments. The correlation between the 14 and 21 days GR for the growth chamber, $r = 0.94$, was somewhat lower than field values. Correlations between 21 and 7 days GR values across environments (range $r = 0.82$ – 0.91) were lower than between 21 and 14 days, but higher than those for the growth chamber ($r = 0.78$). Correlations between the 14 and 7 days GR ($r = 0.86$ – 0.94) were slightly higher than between 21 and 7 days. Correlations of GI measures among the germination periods of 7, 14 and 21 days trended similar to GR ($r = 0.94$ – 1.00 across environments) except the growth chamber correlations appeared somewhat higher ($r = 0.83$ – 0.99 among combinations of durations). Correlations of PG among the germination periods of 7, 14 and 21 days ($r = 0.19$ – 0.98) were generally lower than respective correlations for GR and GI. The correlations between

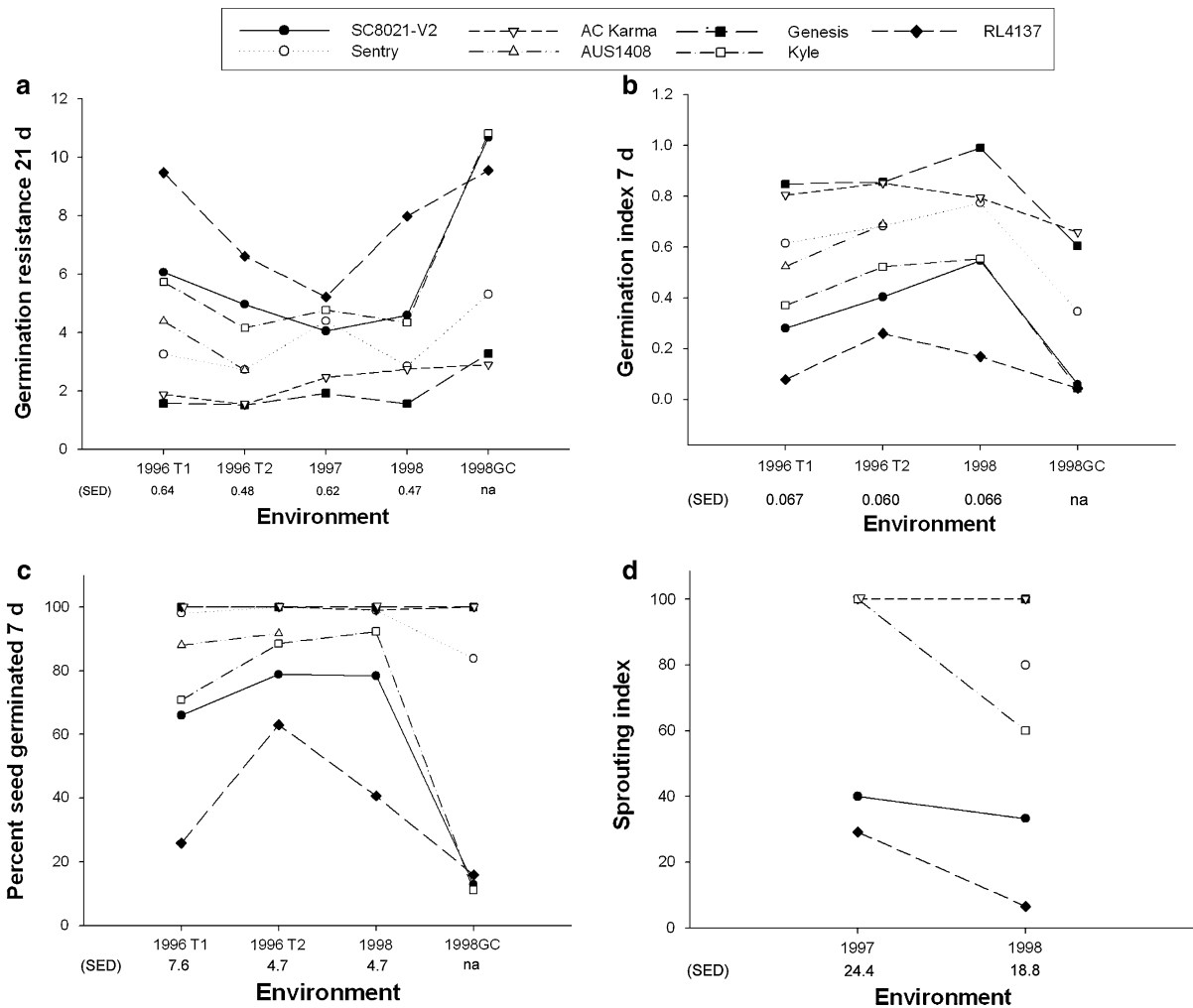


Fig. 1 Distribution of parental and control cultivars across environments for measures of preharvest sprouting resistance: **a** germination resistance at 21 days; **b** germination index at 7 days; **c** percent seed germinated at 7 days; **d** rain simulator

sprouting index, with their standard errors of difference (SED) except for the unreplicated growth chamber test (na = not available)

PG at 21 and 7 days were very low for 1996-T1 ($r = 0.21$) and 1996-T2 ($r = 0.19$).

Correlations between GR and GI were high for each field environment measured at 21, 14 and 7 days ($r = -0.82$ to -1.00). The correlations between GR with GI was lowest for GI at 21 days and GR at 7 days for the field environments ($r = -0.80$ to -0.89) and with the growth chamber results ($r = -0.61$). The correlation declined for the growth chamber test when fewer days were used to calculate GR.

Germination resistance and PG were poorly to moderately correlated across environments within 21, 14 and 7 days (significant correlations ranged from

$r = -0.19$ to -0.92) with some correlations involving PG21 being not significant, the highest being those with PG7 ($r = -0.56$ to -0.93). Percent germinated seed showed the lowest correlations with GR at the longest duration (21 days) ($r = -0.19$ to -0.76). For example, between PG at 21 days and GR at 21 days, the range across environments was from essentially no correlation of $r = -0.04$ (not significant) to a moderate correlation of $r = -0.76$, explaining just 58% of the variation, whereas PG at 7 days with GR at 21 days ranged over the various environments from $r = -0.86$ to -0.93 . The correlation of GI with PG followed a similar pattern to GR with PG, except for

the growth chamber results, which showed moderately high to high correlations ($r = 0.61\text{--}0.96$).

The SI of rain simulator sprouted spikes was moderately correlated with GR21, GR14 and GR7 in both 1997 and 1998 field environments ($r = -0.58$ to -0.70). GI among duration of germination counts in 1998 provided similar moderate correlations with SI ($r = 0.68\text{--}0.70$). For the 1998 field environment, rain simulator SI results were moderately correlated with PG at 7 days ($r = 0.59$), but correlations dropped off for PG at 14 days ($r = 0.46$) and 21 days ($r = 0.38$). Correlations tended to be lower with the 1998 growth chamber (range of GR by SI $r = -0.31$ to -0.37). Among all measures and durations of counts, correlations between Time 1 and Time 2 assessments in 1996 were similar.

Test means varied from environment to environment (Table 1). Within a type of measure, the variation depended on the duration seeds were allowed to germinate. The magnitude of the test means increased from the 7–21 days count duration of germination for GR, GI and PG. As expected, seeds germinated quicker at sample Time 2 compared to sample Time 1 in 1996 for all three measurement methods.

The 1997 environment revealed many more transgressive segregants, both high and low, than other environments (Table 2). Unfortunately, only GR could be calculated for 1997. Except for 1997, transgressive segregation of susceptible lines was minimal to not

significant depending on environment and measure, whereas in resistant lines, the occurrence of significant transgressive segregation consistently occurred.

For each environment and type of measure, the same data was used to calculate the different durations of germination. Therefore differences in results for comparisons between durations show that different numbers of lines were identified as transgressive segregants depending on the duration. Comparing durations for each measure method, GR7 consistently identified the greatest number of lines more resistant than the more sprouting resistant parent Kyle and less resistant than the more susceptible parent Sentry except for 1996-T2 (Table 2). Percent germination identified resistant transgressive segregants even at 14 and 21 days. With limited results across environments, the rain simulator identified a high number of sprouting resistant transgressive segregants. Considering the identity of the transgressive lines, only a few appeared transgressive to the mean of the resistant parent across more than one measure or more than one duration or more than one environment. More often different lines segregated as significantly more sprouting resistant than Kyle depending on measurement type, duration or environment.

QTL analysis of the Sentry/Kyle population (Knox et al. 2005) for each type of measure indicated a strong QTL for preharvest sprouting resistance on chromosome 1A for GR and GI (Table 3). The QTL was

Table 1 Test mean, standard error (SE) of the test mean and number of observations (N) for each measurement type and duration across environments

Environment	Measure Duration (days)	GR			GI			PG		
		N	Mean	SE	N	Mean	SE	N	Mean	SE
98	7	104	3.43	0.049	104	0.64	0.010	104	94.00	0.359
98	14	104	3.69	0.056	104	0.82	0.006	104	98.76	0.359
98	21	104	3.75	0.064	104	0.88	0.004	104	99.20	0.175
97	7	104	3.82	0.022	–	–	–	–	–	–
97	14	104	4.42	0.039	–	–	–	–	–	–
97	21	104	4.52	0.043	–	–	–	–	–	–
96T1	7	105	3.79	0.037	105	0.46	0.009	105	85.22	0.949
96T1	14	105	4.44	0.079	105	0.71	0.006	105	98.34	0.143
96T1	21	105	4.53	0.090	105	0.80	0.004	105	99.26	0.094
96T2	7	105	2.93	0.052	105	0.62	0.008	105	93.98	0.322
96T2	14	105	3.22	0.071	105	0.80	0.005	105	99.02	0.109
96T2	21	105	3.25	0.061	105	0.86	0.003	105	99.36	0.092

Table 2 Number of lines (excluding checks) significantly more susceptible than the susceptible parent (Sentry) or significantly more resistant than the resistant parent (Kyle) for preharvest sprouting resistance for each measurement method across environments

Measure ^b	Parent	Number of lines ^a			
		1996-T1	1996-T2 ^c	1997	1998
GR21	Sentry	0	0	13	0
GR21	Kyle	1	5	14	4
GR14	Sentry	0	0	14	0
GR14	Kyle	3	4	17	4
GR7	Sentry	0	5	6	0
GR7	Kyle	6	3	22	1
GI21	Sentry	0	0	–	0
GI21	Kyle	3	2	–	5
GI14	Sentry	0	0	–	0
GI14	Kyle	3	3	–	5
GI7	Sentry	0	0	–	0
GI7	Kyle	3	6	–	3
P21	Sentry	0	0	–	0
P21	Kyle	5	6	–	3
P14	Sentry	0	0	–	0
P14	Kyle	1	5	–	4
P7	Sentry	0	0	–	0
P7	Kyle	3	5	–	6
RS-SI	Sentry	–	–	–	0
RS-SI	Kyle	–	–	15	7

^a Counts exclude checks; differences were declared at the $P = 0.05$

^b GR germination resistance; GI germination index; decayed seed were excluded from calculation (could not be calculated for 1997)

^c 1996 Time 2 = second spike collection (10 days after first collection)

identified in 1996 Time 1 ($P < 0.001$) using GI at three durations of counts and in 1996 Time 2 with GR21 ($P < 0.001$). A highly significant QTL was identified ($P < 0.001$) in more than one environment on chromosome 2A (interval *Xgwm515-Xgwm425*) for GR7. Percent germination at 7 days identified a QTL on chromosome 7B ($P < 0.001$), that was also identified by GI at 21 days in 1996 Time 2 ($P < 0.001$) using composite interval mapping (CIM) in interval *Xgwm297-Xwmc532*. No QTL at a significance level of 1% or better was identified in the 1998 field environment or from the rain simulator. Only one QTL on chromosome 7A using PG14 was identified at the 1% level of significance with the growth chamber environment. QTL were observed across measures and measurement durations at 1, 5 or 10% significance levels on chromosomes 6B, 4A, 2A, 7B, 7A and an unidentified linkage group for the AFLP markers

TGAC80-AAACC190. When not limited by significance level, the QTL on chromosome 2A and 1A were observed in most environments.

Discussion

Genotypes with published information on their level of preharvest sprouting resistance were included as controls to place the study of measures of resistance in the Sentry/Kyle durum RIL population in context. The ranking of the controls by the different methods of measure relative to their published performance provides some indication of the reliability of the measure. Lines RL4137, SC8021-V2 and AUS1408 were described as sprouting resistant (DePauw et al. 2009; DePauw et al. 1992; Tan et al. 2006), whereas Genesis and AC Karma are sprouting susceptible

Table 3 QTL at different levels of significance, in percent, for preharvest sprouting resistance measurement methods and durations across environments using simple and composite interval mapping

	GI						GR						PG						SI	
	7		14		21		7		14		21		7		14		21		S	C
	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C				
6B Xgwm508-Xgwm156																				
1998GC	N	5	N	5	N	10	N	10	10	5	10	5	N	5	N	N	N	N		
4A Xgwm397																				
1998GC	10	N	5	10	5	5	N	N	10	N	10	10	5	N	N	5	N	10		
1A Xwmc611-Xwmc333																				
1996T1	0.1	N	0.1	N	0.1	N	N	N	1	N	1	N	1	N	N	N	N	N		
1996T2	1	N	1	N	1	N	5	N	1	N	0.1	N	5	N	N	N	N	N		
1997							N	N	N	N	10	N							N	N
2A Xgwm47-Xgwm630																				
1996T1	N	N	N	N	N	N	5	10	N	N	N	N	N	N	N	N	N	N		
1997							1	5	N	N	N	N							N	N
1998	10	N	N	N	N	N	N	5	N	10	N	N	N	N	N	N	N	N	N	N
2A Xgwm515-Xgwm425																				
1996T1	N	N	N	N	N	N	0.1	1	N	N	N	N	N	N	N	N	N	N		
1997							0.1	5	N	N	N	N							N	N
1998	N	10	N	N	N	N	N	5	N	N	N	N	N	N	N	N	N	N	N	N
XTGACC80^a-AAACC19^a																				
1996T1	N	N	N	N	10	10	N	N	N	N	N	N	5	10	1	5	N	N		
1996T2	10	N	10		10	N	N	N	N	N	10	N	N	N	N	N	N	N		
7B Xbarc72-Xgwm333																				
1996T1	N	10	N	10	N	10	N	N	N	1	N	5	N	5	N	N	N	N		
1996T2	N	5	N	1	N	5	N	N	N	1	N	1	N	1	N	N	N	N		
7B Xgwm297-Xwmc532																				
1996T1	N	5	N	5	N	5	N	N	N	1	N	5	N	1	N	N	N	N		
1996T2	N	5	N	1	N	0.1	N	N	N	1	N	1	N	0.1	N	1	N	N		
7A Xgwm276																				
1998	N	N	N	N	N	N	N	N	N	N	N	N	10	N	N	N	N	N	N	N
1998GC	N	N	10	N	5	N	N	N	N	N	N	N	N	N	1	10	5	N		
2B XTGAGC165^a-Xwmc592																				
1997							N	N	N	N	N	N							N	5
1A, 1B, Xgdm33																				
1998	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	5	N

S simple interval mapping, C composite interval mapping, N no association

^a AFLP

(Clarke et al. 2005b; DePauw et al. 2009). GR and GI, for the three durations of counts, and PG7 consistently ranked the control cultivars in the order RL4137, 8021-V2, Kyle, AUS1408, Sentry, AC Karma and Genesis from most resistant to most susceptible. This

ranking of controls is consistent with the literature (Nyachiro et al. 2002). These measures seem similarly reliable based on their performance in ranking the controls, with most detecting subtle differences in ranking between field studies and the growth chamber

study. Kyle was moderately sprouting resistant similar to the white-seeded hexaploid line SC8021-V2. Sentry has minimal preharvest sprouting resistance, superior to the susceptible white-seeded hexaploid control Genesis. The low discrimination of lines using PG14 and PG21, as expected, suggests PG with increased length of time of germination is a poor measure of moderate preharvest sprouting resistance, which can be attributed to many more seeds having germinated in dormant lines over the longer time frame. Therefore, a dormant line could have the same PG as a non-dormant line after a long germination period. The results for the controls indicated little was gained by evaluating seed beyond 7 days, and PG discrimination dropped off after 7 days. However, a longer duration for PG may be useful in identifying transgressive segregants.

The overall experimental means typically showed differences in measures for the duration of germination counts (Table 1). The close correlations among environments between GR14 and GR21 indicated that there is little differentiation of preharvest sprouting resistance between these two measures. However, lower correlations of GR21 or GR14 with GR7 indicated a difference. The generally good correlation between GI and GR measures of preharvest sprouting resistance and the similar correlation between GI 7, 14 and 21 days counts indicated that GI and GR would provide similar understanding of the material being measured. With the requirement of a total seed count for GI, the issue of whether seed are dormant or dead is a factor that could complicate the interpretation of this method of measuring dormancy. Reasonably consistent correlations of duration of counts for GR and for GI among field environments suggest that conclusions would be independent of environment, although conclusions drawn from the growth chamber are somewhat different.

Some poor and moderate correlations among the 7, 14 and 21 days duration of counts with PG indicated timing of counts can have a substantial effect on the conclusions using the PG measure. Although GR and GI showed similar correlations for the different durations of counts for the various environments, correlations of PG with GR or GI were often quite low. One would expect the higher correlations between GR or GI and PG7, and particularly GR21 with PG7, to generate similar interpretation of lines for preharvest sprouting resistance; whereas the other two durations

could be expected to place more emphasis on the detection of lines with greater dormancy. Except for PG14 and PG21, the measures detected the drop off in dormancy between Time 1 and Time 2 within the controls in 1996.

The moderate correlations of SI among GR and GI values for the same environment indicated that the rain simulator method is only somewhat interchangeable with GR and GI for evaluating preharvest sprouting resistance in the Kyle/Sentry population. We used only five spikes for our evaluation of SI, although an increased number may improve precision of the SI. The correlation of SI with PG at any duration of count is less than the correlations of SI with GR and GI. These lower correlations indicate rain simulator results are not interchangeable with PG. This could be due to the low precision of the SI test protocol but also that the rain simulator potentially measures additional factors involved in preharvest sprouting resistance compared to GR, GI and PG. Because the rain simulator test uses intact spikes, there is the potential for chaff and morphological characteristics to influence preharvest sprouting in addition to the seed dormancy that is measured with GR, GI and PG (Derera and Bhatt 1980; King and Richards 1984).

Although the correlations indicate measures differ in the characterization of lines, there is little to indicate a single superior method. Precision among measures and durations may affect the level of correlation, but correlations may actually be different because different measurement methods are quantifying different sprouting resistance parameters.

The slightly greater resistance of Sentry durum than the white seeded hexaploid controls Genesis and AC Karma demonstrates that Sentry has some preharvest sprouting resistance to contribute to transgressive segregation. Sentry may have some alleles in common with Kyle which would explain why Sentry is not fully sprouting susceptible and that there were generally few susceptible transgressive segregants. Kyle was intermediate to the white seeded hexaploid sprouting resistant lines AUS1408 and the more resistant SC8021-V2, which suggests Kyle is a reasonable source of preharvest sprouting resistance.

Contrary to the several high correlations, the many differences in the number of lines significantly different from the parents observed with the various measures and durations of the measures across environments (Table 2) indicates no one measure or

duration of measure fully characterizes the lines. The difference in number of transgressive segregants for the three durations of a measure type demonstrated that different aspects of preharvest sprouting resistance are revealed depending on the duration of counts. That transgressive segregation for greater preharvest sprouting resistance did occur for all measures in the population indicated that progress can be made through the cross of Sentry and Kyle.

The simple hypothesis is that all measures of dormancy identify the same QTL is not supported by the results of our study. As expected for a trait as complex as preharvest sprouting resistance, there is interaction with environment such that QTL may express more strongly in one environment but not another (Tan et al. 2006). This complexity appears to be further demonstrated by different measure types and durations identifying different QTL. Given that the QTL identified appear to relate to those previously reported, albeit several identified only in *Triticum aestivum*, strengthens the argument that to fully understand the genetic control of preharvest sprouting resistance, multiple types of measures and durations of germination for each of those measures must be implemented. We noted potential QTL on chromosomes 2A and 7B as did Zanetti et al. (2000) in a hexaploid wheat cross. In a study of substitution lines in durum, Chao et al. (2010) noted chromosome 7B as having a role in preharvest sprouting resistance. We observed two adjacent QTL intervals on chromosome 7B near a QTL reported by Gelin et al. (2006) in durum. Mohan et al. (2009) reported two epistatic QTL on chromosome 7B of hexaploid wheat. We observed two intervals on chromosome 2A. The *Xgwm515-Xgwm425* interval is very close to the QTL near *Xgwm95* reported by Gelin et al. (2006) in the durum cross IACT12/Ben. The putative QTL on 4A is in the same region as *QPhs.dpi.vic.4A.1* reported by Ogbonnaya et al. (2008) and near the QTL on 4A reported by Rasul et al. (2009) in hexaploid wheat. Roy et al. (1999) discovered a marker for preharvest sprouting resistance on chromosome 6B of bread wheat near the QTL that we report. A QTL in the same region was reported by Gelin et al. (2006) in durum wheat. A QTL for preharvest sprouting resistance was reported by several researchers in hexaploid wheat in the same region of chromosome 1A (Anderson et al. 1993; Mohan et al. 2009; Zanetti et al. 2000) as we found in this durum cross. Gelin et al. (2006) also reported a QTL for preharvest sprouting on 1A in durum, but distal to the location we observed. The *QPhs.ccsu-2B.1* QTL

reported by Kumar et al. (2009) and *QPhs.cnl-2B.1* reported by Munkvold et al. (2009) on chromosome 2B are in the same region as the weak durum QTL that we identified. The *QPhs.ccsu-7A.1* QTL reported by Mohan et al. (2009) in bread wheat is in the same region as the 7A QTL we identified.

By using multiple measures of preharvest we were able to more fully characterize preharvest sprouting resistance loci segregating in Kyle/Sentry. This information will be valuable in selecting for transgressive segregants in crosses involving these two parents or superior offspring. Although all QTL regions were reported previously in hexaploid wheat, some were not previously reported in durum.

Despite the different methods, consistently ranking controls and in many cases correlating well, the different measurement methods and duration of counts within the methods appear to reflect different aspects of preharvest sprouting resistance based on QTL and transgressive segregation results. These findings are consistent with results reported by Gerjets et al. (2010) suggesting a single point measure of dormancy at harvest may not adequately capture all facets of preharvest sprouting resistance. Those genotypes performing as most resistant across measures for different environments probably have the most robust and strongest levels of preharvest sprouting resistance.

The moderate correlations of preharvest sprouting resistance measured through the rain simulator with that of seed dormancy could be because of intact spike factors involved in preharvest sprouting resistance that are not detected in standard germination tests. Consistent with SI measuring other attributes is that it produced QTL that did not overlap with those identified by the dormancy measures, although the SI did not generate highly significant QTL. This suggests that SI may have low preharvest sprouting resistance resolving power, but with a count of only five spikes precision may be lower than if more spikes had been used.

This study looked only at three durations: 7, 14 and 21 days. The results from each method of characterizing sprouting resistance could be further analyzed to see if there are other durations that could be incorporated into the germination process for identifying transgressive segregation and QTL. Also, other methods of characterizing sprouting resistance have been used (Singh et al. 2008) and these could also be assessed for providing additional information on QTL and transgressive segregants.

The results demonstrate that preharvest sprouting resistance in durum wheat is under complex genetic control, which allows for transgressive segregation. Furthermore, multiple QTL and the varied appearance of those QTL from one environment to another further indicate the complexity of the trait. GI identified a highly significant QTL on 1A (*QPhsd.spa.-1A.1*) in the interval *Xwmc611-Xwmc333* as did GR at 21 days in 1996. GR identified a highly significant QTL on 2A (*QPhsd.spa.-2A.1*) in the interval *Xgwm515-Xgwm425* in 1996 and 1997. Germination index calculated at 21 days and PG calculated at 7 days identified the same highly significant QTL on chromosome 7B (*QPhsd.spa.-7B.1*) in the interval *Xgwm297-Xwmc532*.

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