Euphytica (2005) 143: 261–264
DOI: 10.1007/s10681-005-7874-0

Genetic analysis of pre-harvest sprouting in a durum wheat cross

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Key words: dormancy, durum, genotype × environment interaction, QTL

Summary

Pre-harvest sprouting of durum wheat (*Triticum turgidum* L. var *durum*) reduces commercial grade, although the actual effects on processing quality are controversial. Little is known about the genetics of the dormancy component of pre-harvest sprouting resistance in durum. We studied the segregation of dormancy in 98 recombinant inbred lines from a cross of a relatively non-dormant line, CI13102, with a moderately dormant line, Kyle. The lines and parents were grown in field tests over three years, 1996, 1997 and 1998. Spikes were collected at approximately 20% moisture and stored at −23 °C. Hand-threshed grain of the lines was germinated, and number of seeds germinated was counted each day. A germination resistance index was calculated to characterize dormancy. Dormancy appeared to be complexly inherited in this cross. Lines were observed that were significantly ($P < 0.05$) more dormant than the parents. The lines transgressive for dormancy expressed in different combinations of the three environments, indicating an environmental interaction. DNA of lines and parents was tested with simple sequence repeat primers and AFLPs that were used in quantitative trait loci (QTL) analysis of dormancy. Significant QTLs for dormancy were found, with the most notable being on chromosome 1A, where other QTLs for pre-harvest sprouting resistance have been reported in common wheat.

Introduction

More than two million hectares of durum wheat are grown annually in western Canada. In most years, at least a portion of the crop is downgraded due to weather-related factors such as pre-harvest sprouting. In the period 1978–1988, for example, pre-harvest sprouting damage occurred in four of the years, and was estimated to cost C $25,000,000 in each of those years. Improved pre-harvest sprouting resistance is thus a breeding objective for Canadian durum.

Pre-harvest sprouting has less severe effects on pasta quality than on bread-making (Dexter & Edwards, 1999). Milling quality is relatively unaffected by sprouting (Dexter et al., 1990). Elevated alpha-amylase activity does not affect pasta processing (Dexter et al., 1990), and the cooked texture of cooked pasta is not affected by sprout damage (Dick et al., 1974). However, pasta processors tend to require stringent falling number standards for the durum that they purchase. Consequently, sprout damage is an important criterion in determination of market grade of durum wheat. In Canada, for example, a maximum of 0.5% kernels with visible sprouting are permitted in the top grade (Canadian Grain Commission, 2003).

There is genetic variation for pre-harvest sprouting resistance in durum. Limited surveys of commercial durum cultivars have shown intermediate sprouting resistance relative to the range available in common wheat (*T. aestivum* L.) (Mares, 1987; Hare et al., 1988; McCaig & DePauw, 1992). Gordon (1983) found that the durum cultivar 'Stewart' showed the lowest-amylase activity and best grain appearance following weathering treatments in a study of 43 white seed coat *Triticum* spp. genotypes. Clarke et al. (1994) reported that the best sources of resistance in a set of

185 durum accessions approached that of sprouting resistant common wheats.

Little is known about the genetics of the dormancy component of pre-harvest sprouting resistance in durum. Our objective was to conduct a preliminary investigation of inheritance of pre-harvest sprouting in a durum wheat cross, and to look for associated DNA polymorphisms.

Materials and methods

Ninety-eight random inbred lines from the cross CI13102/Kyle and the parent cultivars were grown in field tests in a three replicate randomized complete block design near Swift Current in 1996, 1997 and 1998. Plots were single 3 m long rows with approximately 200 plants/row. Spikes were collected from each plot when 50% or more of the primary tillers within that plot had collapsed stem nodes, at which time grain moisture content is about 16%. Samples of five to ten spikes were collected, depending on testing requirements. The harvested spikes were stored at −23 °C until sampling was completed.

Heads were threshed by hand to ensure that the seed coat was not damaged. Seed was bulked from five spikes and sampled for the germination tests. Thirty-three seeds were germinated in 1996 and 30 seeds in 1997 and 1998. Seeds were germinated in Petri dishes in an incubator at 15 °C and 50% RH following surface sterilization with 'No Damp'. The seeds were placed crease downward on two 12.5 cm discs of moistened filter paper.

Each dish was examined daily for 21 days. Embryos of seeds ranging from white radicle or plumule protruding with a visible tear in the seed coat to the plumule or radicle 2 mm long were counted as germinated and were removed from the Petri dishes. On day 21 the ungerminated seeds in each dish were treated with 4.0–6.0 ml of 0.0005 M GA_3 (made by dissolving the salt in double distilled water) solution for 1 h. The excess solution was poured or blotted out, and the Petri dishes containing GA_3 solution-treated seed samples were placed in a low temperature environment of approximately 2–5 °C for 12 h or overnight, and returned to the germinator. Daily counts were continued for another 3 days; any seed that was not germinated was considered to be 'non-viable' and was excluded from the total seed count.

A germination resistance index was used to calculate number of days to 50% germination of the germinable seeds (Gordon, 1971):

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

where $d_1, d_2, \ldots, d_i$ are the $1st$, $2nd$ to ith day of the germination counts, $n_1, n_2, n_3, \ldots, n_d$ are the number of seeds germinated on 1st, 2nd, 3rd to dth day, and N is the total number of seeds germinated.

Heritabilities were estimated from variance components (Baker, 1986) with corresponding confidence intervals (Knapp et al., 1985), and number of effective factors was estimated as described by Snape et al. (1984).

Parental DNA was tested with 318 simple sequence repeat primer pairs. The 67 polymorphic markers were tested on the whole population. Marker linkage analysis was performed with MAPMAKER version 3 (Lander et al., 1987). A single marker analysis (Lynch & Walsh 1998) based on a model in Knapp (2001) was performed with SAS PROC MIXED (Littell et al., 1996) on the germination resistance data. Lines grouped by parental marker type were compared with a t-test. Simple interval mapping was performed with MQTL (Tinker & Mather 1995) on least square means using a threshold based on 1500 permutations to maintain a Type I error rate <5%.

Results and discussion

The germination resistance of Kyle was significantly greater ($P < 0.05$) than that of CI13102 in 1996 and 1998, but did not differ in 1997 (Figure 1). There were significant differences among the progeny in all three years. One line had significantly greater germination resistance than Kyle in all three years, while other lines were significantly greater than Kyle in one or two of the years.

Transgressive segregation in the CI13102/Kyle population indicates that both parents contributed dormancy alleles and that these alleles recombined in the progeny to produce dormancy superior to that in Kyle. Different lines expressed transgressive segregation in different years, indicating that environment interacted with expression of germination resistance.

The nature of the distribution of the germination resistance of the lines and the transgressive segregation suggests complex inheritance of the trait. This is substantiated by estimates of the number of effective

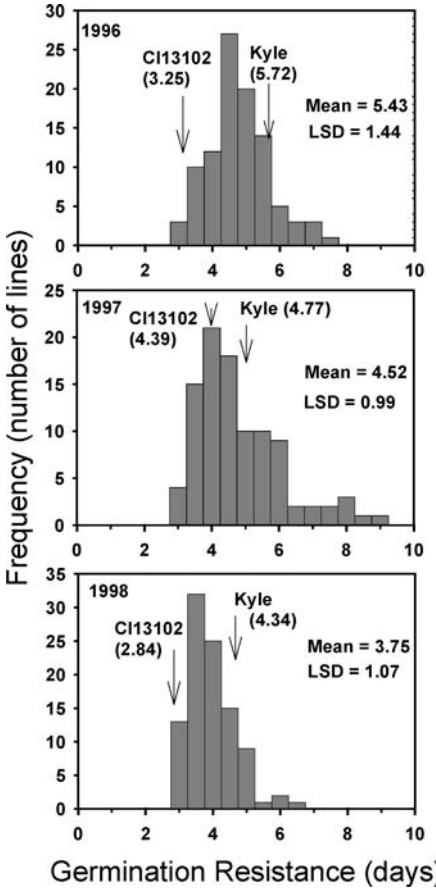

Figure 1. Germination resistance (days to 50% germination) of 98 random inbred lines and parents for the cross CI13102/Kyle grown at Swift Current in 1996–1998.

factors, which ranged from 11–24, depending on environment.

Heritability over the three environments was 0.60, with a 95% confidence interval of 0.46–0.70. This estimate is at the low end of the range (0.58–0.84) reported by Soper et al. (1989) for sprouting score in a cross of a white durum with a red, common wheat-derived durum. In two common wheat crosses, Dyck et al. (1986) reported heritabilities of 0.74 and 0.78 for falling number following rain simulator treatments.

Wheat microsatelite WMC183 showed the strongest association with germination resistance (Table 1). The simple interval mapping test statistic exceeded the threshold in the 1996 data, came close in 1997, but did not show up in 1998. Similarly for the single marker analysis, WMC183 was highly significant in 1996 ($P < 0.001$) and 1997 ($P < 0.01$), but marginally significant in 1998 ($P < 0.05$). Although such results are promising according to guidelines suggested by Lander and Kruglyak (1995), the observed year by marker interaction is an important consideration.

Table 1. Simple interval mapping (SIM) and single marker analysis for wheat microsatelite WMC183 tested on 98 durum wheat lines in three years

	SIM		
Year	Test statistic	Threshold	Single marker
1996	21.5	13.1	***
1997	10.3	13.2	**
1998	–	–	*

Anderson et al. (1993) reported QTLs for preharvest sprouting resistance on chromosome 1A based on RFLP analysis of common wheat, which is consistent with our own data in a common wheat cross (unpublished).

Acknowledgments

We thank C. Barlow, B. Meyer, M. Olfert, I. Piche, K. Richmond, J. Ross and L. Thiessen for their expert technical support and acknowledge the financial support of the Western Grains Foundation, western Canadian wheat producers and the Matching Investment Initiative Fund of Agriculture and Agri-Food Canada.

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