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Source: Canadian Journal of Plant Science, 102(2) : 347-355

Published By: Canadian Science Publishing

URL: <https://doi.org/10.1139/CJPS-2021-0109>

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# Introgression of pre-harvest sprouting resistance from hexaploid wheat into high yielding durum wheat germplasm

Guillermo Gerard, Pierre Hucl, Curtis Pozniak, and Connie Briggs

**Abstract:** Pre-harvest sprouting (PHS) is a critical factor affecting wheat producing regions characterized by rainfall and high humidity combined with cool temperatures at harvest. This phenomenon is particularly important in durum wheat (*Triticum durum* L.), which is generally susceptible to PHS, in contrast to common wheat (*Triticum aestivum* L.) which expresses better resistance. The transfer of PHS from hexaploid common wheat into tetraploid durum wheat germplasm is one option for genetic improvement, because the two species share two related sub-genomes. In the present study, through interspecific hybridization and modified backcross approaches, we developed experimental durum lines that showed greater PHS resistance than the recurrent durum wheat parent, with some expressing better PHS performance than the resistant common wheat donor. The introgression of PHS resistance did not negatively impact additional traits in the durum background and several backcross derived experimental lines expressed superior grain yield related and quality traits when compared with the recurrent parent. These lines represent a promising genetic resource for the development of new sprouting resistant durum wheat cultivars. Our results demonstrate that PHS resistance can be transferred from common wheat to improve PHS of durum wheat germplasm.

**Key words:** wheat, pre-harvest sprouting, seed dormancy, genetic resistance, interspecific introgression.

**Résumé :** La germination sur pied (GP) est un problème majeur dans les régions où l'on cultive le blé caractérisées par la pluie et un taux d'humidité élevé associés à des températures fraîches à la récolte. Le phénomène revêt une importance particulière pour le blé dur (*Triticum durum* L.), plante généralement sensible à la GP, contrairement au blé ordinaire (*Triticum aestivum* L.) qui y résiste davantage. Transférer la résistance à la GP du blé ordinaire, hexaploïde, au blé dur, tétraploïde, est une solution possible sur le plan de l'amélioration génétique, car les deux espèces ont des sous-génomes apparentés. Recourant à l'hybridation entre espèces et à une forme de rétrocroisement, les auteurs ont conçu des lignées expérimentales de blé dur qui affichent une meilleure résistance à la GP que la lignée parentale de blé dur récurrente. Certaines exprimaient même un rendement plus élevé que celui du blé ordinaire donneur en présence de la GP. L'introgression de la résistance à la GP n'a eu aucun impact négatif sur les autres caractères du blé dur et plusieurs lignées expérimentales obtenues par rétrocroisement ont donné un rendement grainier supérieur et un grain de meilleure qualité que le parent récurrent. Ces lignées forment un réservoir prometteur de gènes pour le développement de nouvelles variétés de blé dur qui résisteront à la germination sur pied. Les résultats des chercheurs prouvent qu'on peut transférer la résistance à la GP du blé ordinaire au plasma germinal du blé dur en vue de l'améliorer. [Traduit par la Rédaction]

**Mots-clés :** blé, germination sur pied, dormance des graines, résistance génétique, introgression interspécifique.

## Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is an important food crop worldwide, used in the preparation

of diverse food products including bread, couscous, bulgur, and pasta. The world harvested durum area is approximately 16 million hectares, with an average

Received 4 May 2021. Accepted 6 August 2021.

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annual production of 40 million tons, accounting for 5% of global wheat production (Sall et al. 2019; International Grains Council [IGC]. 2020). Canada is the second largest durum wheat producer in the world, where it is grown on approximately 2 million hectares, mainly in the western prairie provinces of Saskatchewan and Alberta, comprising about 25% of the total planted wheat area (Canada 2018).

Pre-harvest sprouting (PHS), a phenomenon where seeds germinate on the plant before harvest, is an important production issue in many wheat-producing areas, especially in environments characterized by rainfall and high humidity combined with cool temperatures near physiological maturity. Pre-harvest sprouting is characterized by an elevated level of starch hydrolytic enzyme activity (primarily of alpha-amylases) that catalyzes the breakdown of endosperm starch, providing the initial energy needed for seed germination but also leading to marked alterations in grain quality and yield (Olaerts et al. 2016). Thus, PHS damage often results in down-grading of premium milling quality wheat, limiting end-use applications with reduced revenue to farmers and food processors. The falling number test (Hagberg 1961) measures the impact of pre-harvest sprouting, and low falling numbers typical of severely sprouted samples are becoming a grading factor for producers at the point of sale in Canada (Canadian Grain Commission [CGC]. 2019). In western Canada, where harvest can be associated with excess rainfall and humidity combined with cool temperatures, the reduction in wheat value has exceeded \$100 million in years with significant sprouting damage (DePauw et al. 2012). In this context, planting PHS resistant varieties is an effective strategy to reduce the losses from sprouted grains in wheat production (Barrero et al. 2015). Therefore, PHS improvement is critical and remains a major focus in wheat breeding programs.

A key trait for the prevention of PHS is seed dormancy, which is the suppression of germination during otherwise favorable conditions (Finkelstein et al. 2008; Rodríguez et al. 2015). Although the different types of dormancy usually cause a delay in germination, the underlying causes may vary. The seed can be prevented from completing germination because the embryos themselves are dormant (embryo dormancy), due to physiological and physical constraints caused by the presence of a hard seed coat (coat-enhanced dormancy), due to inhibitory chemicals that interfere with embryo growth or a combination of such factors (Finch-Savage and Leubner-Metzger 2006). Dormancy is a quantitative trait regulated by multiple genes and strongly influenced by environmental conditions (Knox et al. 2012; Rodríguez et al. 2015). In common wheat (*Triticum aestivum* L.) more than one hundred quantitative trait loci (QTL) linked to seed dormancy have been localized to all 21 chromosomes. The most frequently detected QTL are those on the group three chromosomes (Kulwal et al. 2004;

Mori et al. 2005; Liu et al. 2017), 4A (Mares et al. 2005; Chen et al. 2008; Singh et al. 2010; Cabral et al. 2014), and 5A (Groos et al. 2002; Singh et al. 2010). Durum wheat varieties, in general, exhibit greater sprouting susceptibility than hard red spring bread wheat cultivars grown in Western Canada, despite significant efforts by breeders. The lack of progress in addressing sprouting issues in durum wheat is due both to limited genetic sources of resistance relative to common wheat, and the limited availability of molecular markers to support selection for improved sprouting resistance. To date, only a few PHS resistant QTL have been identified in tetraploid durum wheat (Gelin et al. 2006; Nakamura et al. 2011; Knox et al. 2012; Singh et al. 2014), and most provide only marginal improvements.

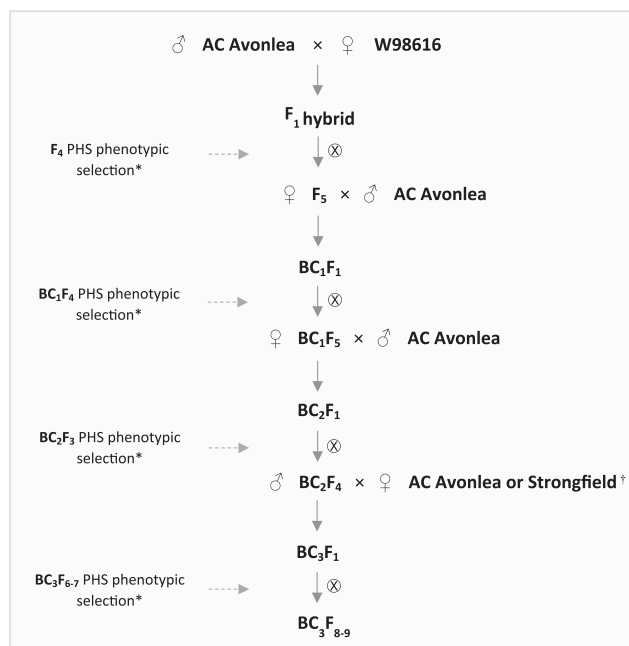
When there is limited genetic variation within a gene pool, the use of related species as a source of genes is one option for crop improvement (Han et al. 2014). Interspecific hybridization between durum and common wheat has proven to be a useful approach in the breeding of both species by using backcross breeding strategies (Rajaram et al. 1993; Palve and Raghavaiah 2002). For example, the  $Al_3^+$  and  $Na^+$  tolerances of durum wheat have been improved by introgression of common wheat chromosome fragments (Dvorak and Gorham 1992; Dvorak et al. 1994; Dubcovsky et al. 1996; Han et al. 2014). Similarly, the introgression of genes encoding gluten proteins from hexaploid wheat has been used to improve durum bread making traits, while maintaining pasta quality (Rao et al. 2010; Sissons et al. 2014). In the present study, we introduced PHS resistance from common wheat into high yielding durum wheat germplasm using a backcross breeding approach and then assessed associated effects on durum grain yield and quality traits.

## Material and Methods

### Plant material and experimental design

We used the common wheat cultivar W98616 (Hucl and Matus-Cádiz 2002; Singh et al. 2010) as the resistance donor and the durum wheat cultivar AC Avonlea (Clarke et al. 1998), which is PHS susceptible, as the recurrent parent. W98616 is a white-grained, dormant line obtained from the cross AUS1408/RL4137, where RL4137 is the source of pre-harvest sprouting resistance in many Canada Western Red Spring cultivars (Noll et al. 1982). The first cross was made using the donor parent W98616 as the female and AC Avonlea as the pollen parent. The  $F_1$  was self-pollinated to  $F_4$  seed, which were evaluated for PHS resistance. Twelve  $F_5$  lines with similar or higher PHS resistance levels than W98616 were then backcrossed to the recurrent parent AC Avonlea and advanced to the  $BC_1F_4$  generation by self-pollination. The cycle of backcrossing, self-pollination, and phenotypic evaluation of PHS resistance was repeated three times, but in the last backcross AC Avonlea was used as the female parent to recover the durum cytoplasm.

**Fig. 1.** Common/durum wheat crossing schematic to obtain interspecific introgression durum lines. \*Lines with PHS resistant levels similar or better than W98616 were retained. †In BC3 we use either AC Avonlea or Strongfield as a female parent.



During the final backcross, we also generated populations with the durum wheat cultivar Strongfield (Clarke et al. 2005). After completion of backcrossing and self-pollination, 96 BC<sub>3</sub>F<sub>8/9</sub> experimental durum lines were generated (Fig. 1; Supplementary Table S1<sup>1</sup>).

The ninety-six experimental durum lines together with the check cultivars W98616, AC Avonlea, Strongfield, and Kyle, were grown under field conditions in replicated ( $n = 4$ ) rows during 2013 and 2014 at the University of Saskatchewan's Seed Farm, Saskatoon (52.2° N, 106.6° W). At Zadoks' Growth Stage 92 (Zadoks et al. 1974), 50 spikes per line were collected and allowed to dry at room temperature for one week. The spikes from each line were bulk threshed together using a rubber belt thresher and seeds were stored in a freezer at -20 °C to minimize metabolic activity that would result in a loss of dormancy due to after-ripening (Knox et al. 2012). The sixteen most dormant lines, based on the PG performance in the first 2 yr of field testing, were grown in 2015 in replicated yield trials at the Kernen and Goodale Research Farms, Saskatoon (52.1° N, 106.5° W and 52.0° N, 106.5° W), using a randomized complete block design with two replications and evaluated for PHS resistance, agronomic and end-use quality related traits. Plots consisted of five 3.7 m long rows spaced 20 cm apart, with a seeding rate of 330 seeds m<sup>-2</sup>.

The durum wheat cultivars AC Avonlea, Strongfield, and Kyle together with two common wheat varieties RL4137 and W98616 were included as checks.

## Phenotypic traits evaluations

### Preharvest sprouting

Fifty seeds on a plot basis were placed in Petri dishes containing a Whatman #1 filter paper soaked with 3.0 mL of distilled water. 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. The Petri dishes were incubated at 20 °C and relative humidity level of 90% and germination count was performed after 3 and 7 d. Based on the germination data, we calculated three common measurements used in the characterization of PHS resistance; percentage of germination (PG; Belderok 1961), germination index (GI; Reddy et al. 1985) and germination resistance (GR; Gordon 1971), as a follow:

$$PG = (\text{seeds germinated} / \text{total seeds}) \times 100$$

$$GI = [(d \times n_1) + (d - 1 \times n_2) + \dots + (1 \times n_d) / (d \times \text{total sds})] \times 100$$

where  $d$  is the total number of days for which the seed was counted;  $n_1, n_2, \dots, n_d$  are the number of seeds germinated on 1st, 2nd to  $d$ th day in which the germination count was carried out.

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

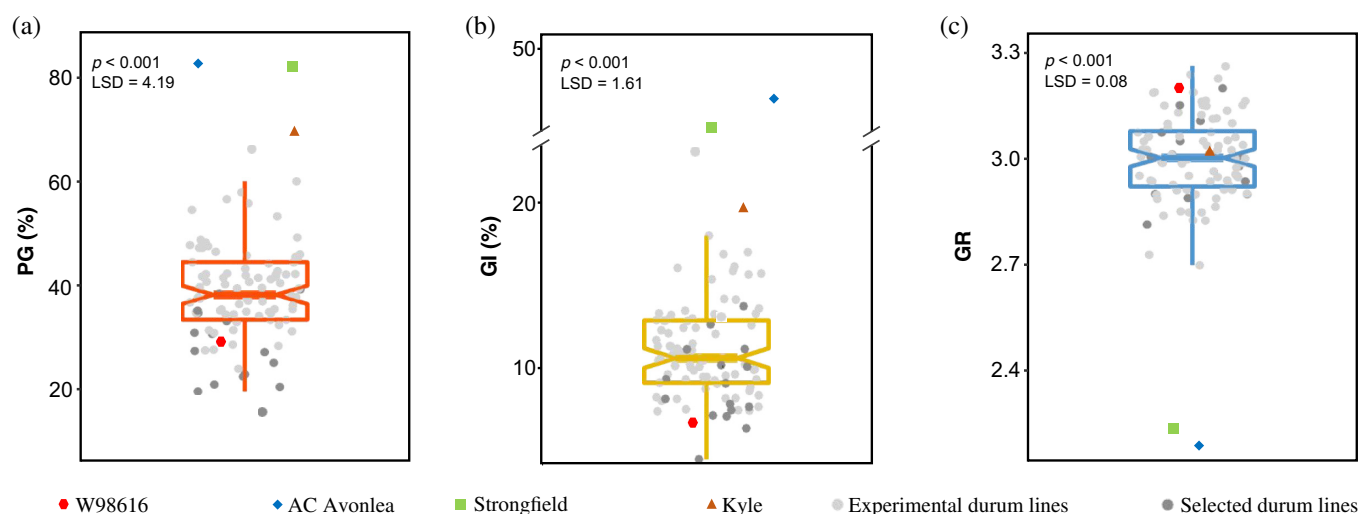
where  $d_1, d_2, \dots, d_i$  are the 1st, 2nd to  $i$ th day in which the germination count was carried out;  $n_1, n_2, n_3, \dots, n_i$  are the number of seeds germinated on 1st, 2nd to  $i$ th day and  $N$  is the total number of seeds germinated.

### Grain yield and quality related traits

After planting, field trials plots were regularly inspected to determine days to heading (DH) and days to physiological maturity (DM), estimated when 50% of the spikes reached those stages (anthers extruded and peduncle yellowish-golden in color, respectively). Plant height (PH) was determined by measuring the total length of the plant, excluding awns, on two measurements per plot. Plots were harvested to determine grain yield (GY), the grain was dried to a moisture content of 10% ± 1% in forced air driers. Thousand kernel weight (TKW) was determined from a 250-kernel subsample, and test weight (TW) measured by using a 0.5 hL cup. Kernel hardness index (KHI) was determined according to the AACC International Approved Method 55–31.01 with a Perten SKCS 4100 single-kernel characterization system (Perten Instruments North America, Springfield,

<sup>1</sup>Supplementary data are available with the article at <https://doi.org/10.1139/cjps-2021-0109>.

**Fig. 2.** PHS phenotypic distribution of 96 experimental durum lines and checks tested in field trials. (a) PG, germination percentage, (b) GI, germination index, (c) GR, germination resistance. [Colour online.]



IL, USA.). Samples were ground on a UDY cyclone mill (Udy Corp, Fort Collins, CO). Grain protein concentration (GPC) and yellow pigment content (YP) were measured on the ground meal by near-infrared reflectance (NIR) spectroscopy using a Foss NIR56500 (Foss North America, Inc.) analyzer. All calibration equations for the NIR56500 were developed in-house at the Grains Innovation Laboratory (University of Saskatchewan) and were validated with a known sample set (AACCI Approved Method 39–00.01). For the validation set, the correlation coefficient ( $r$ -value) between predicted and actual values for GPC and YP were 0.99 and 0.97, respectively. Finally, each sample was evaluated for starch viscosity (as noted above, an indirect measure of sprout damage) using the falling number (FN) test according to the American Association of Cereal Chemists International (AACCI) Approved Method 56–81.03. The FN test was carried out 2 m postharvest using a sample taken from the combine-harvested plots.

#### Data analysis

The phenotypic data collected was analyzed using Statistical Analysis Software (SAS) version 9.4 (SAS Institute Inc., Cary, NC). Mixed linear models using PROC MIXED and the Satterthwaite method to determine degrees of freedom were used to calculate least squares (LS)-means. Experimental durum lines were considered as fixed effect and environment (year/location) and replications as random effects. The phenotypic similarity between experimental durum lines and the recurrent durum wheat parent was tested through a two-way hierarchical clustering analysis using the pheatmap R package (Kolde 2018). Finally, to determine potential linkage drag between PHS resistance and grain yield and quality related traits, Pearson's correlation

coefficients were estimated using the ggcorrplot R package (Kassambara 2016).

## Results

### Preharvest sprouting

The phenotypic distribution of LS-means showed considerable variation across ninety-six experimental durum lines in the three measurements PG, GI and GR used to characterize PHS resistance (Fig. 2). For the three measurements (PG, GI and GR), the statistical analysis revealed significant differences ( $p < 0.001$ ) among the ninety-six experimental durum lines. As expected, the PHS resistance levels of the three durum checks (Strongfield, AC Avonlea, and Kyle) included in the field trials were significantly lower than those of the common wheat line W98616 for all measurements. In addition, all the experimental durum lines had greater PHS resistance levels than AC Avonlea, while some of them appeared to have even higher dormancy levels than W98616 (Fig. 2). The PG and GI phenotypic values from the experimental durum lines exhibited a high and significant correlation between each other ( $r_{(PG, GI)} = 0.72$ ;  $p < 0.001$ ). In addition, a moderate but still significant correlation was found between GI and GR values ( $r_{(GI, GR)} = -0.31$ ;  $p < 0.001$ ), while PG and GR had a weak and not significant association ( $r_{(PG, GR)} = -0.02$ ;  $p < 0.197$ ).

### Grain yield and quality related traits

With the exception of GY, cultivars differed significantly ( $p < 0.001$ ) in terms of grain yield related and quality traits (Table 1). The recurrent parent AC Avonlea had significantly higher expression than W98616 for GPC, KHI, YP, DM, and TKW, while the common wheat cultivar exhibited higher PH and FN values. For GY, DH, and TW traits, both parents did not differ significantly averaged over two environments. Transgressive segregation



**Table 1.** Fixed-effect *F*-tests and least square means for grain yield and quality traits of 16 experimental durum lines (EDL) and five checks, averaged over two environments (Kernen and Goodale).

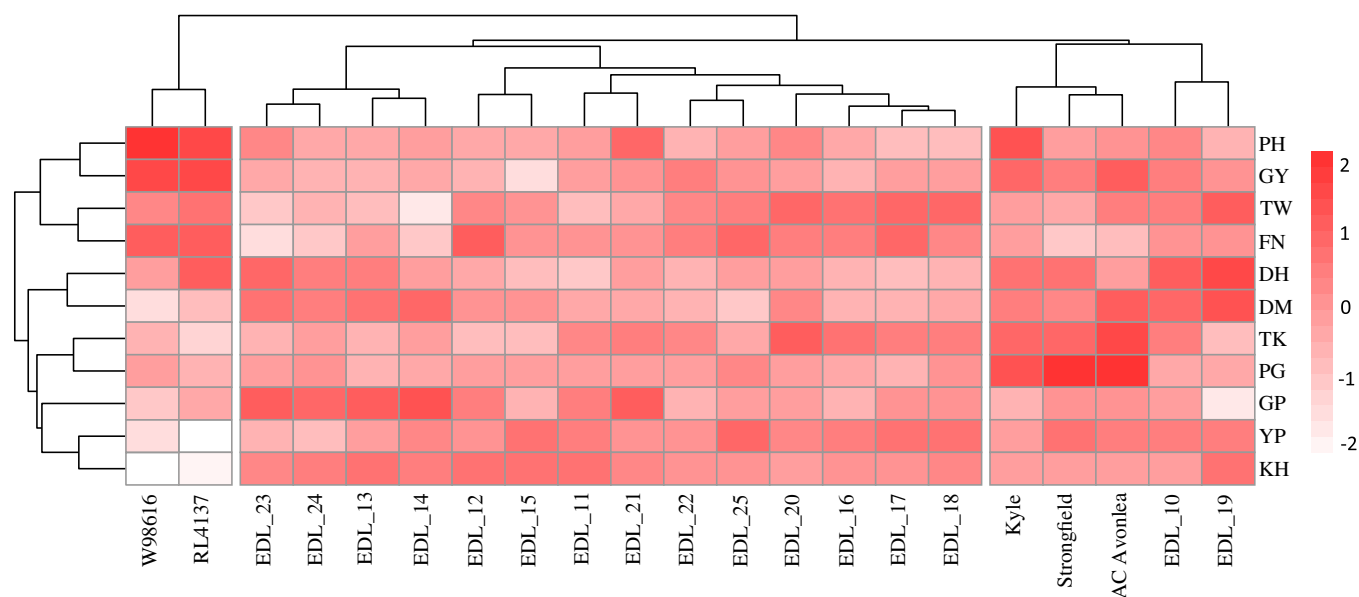
|               | Trait           | PH<br>(cm) | DH<br>(d) | DM<br>(d) | GY<br>(t·ha <sup>-1</sup> ) | TKW<br>(mg) | TW<br>(Kg·hL <sup>-1</sup> ) | FN<br>(sec) | GPC<br>(%) | YP    | KHI  | PG (%) | GI   | GR  |
|---------------|-----------------|------------|-----------|-----------|-----------------------------|-------------|------------------------------|-------------|------------|-------|------|--------|------|-----|
| Line effect   | <i>F</i> value  | 4.64       | 3.6       | 4.9       | 1.2                         | 16.2        | 13.9                         | 4.8         | 7.8        | 139.8 | 20.1 | 30.5   | 31.1 | 2.7 |
|               | <i>P</i> value  | ***        | **        | ***       | ns                          | ***         | ***                          | ***         | ***        | ***   | ***  | ***    | ***  | *** |
| Line LS-means | RL4137          | 88.8       | 54.0      | 89.5      | 3.1                         | 31.9        | 79.8                         | 394.5       | 15.5       | 0.9   | 66.2 | 15.0   | 6.1  | 2.6 |
|               | W98616          | 92.5       | 52.0      | 87.0      | 3.1                         | 34.7        | 78.8                         | 396.3       | 14.8       | 2.3   | 64.0 | 30.5   | 12.9 | 2.5 |
|               | Hexaploid lines | 90.6       | 53.0      | 88.2      | 3.1                         | 33.3        | 79.3                         | 395.4       | 15.1       | 1.6   | 65.1 | 22.8   | 9.5  | 2.6 |
|               | Strongfield     | 76.3       | 53.3      | 93.8      | 2.7                         | 41.4        | 77.7                         | 235.8       | 15.8       | 6.5   | 77.9 | 89.5   | 50.2 | 2.1 |
|               | Kyle            | 88.0       | 53.3      | 94.3      | 2.9                         | 42.1        | 78.0                         | 299.0       | 15.3       | 4.9   | 79.3 | 72.5   | 29.2 | 2.6 |
|               | AC avonlea      | 77.8       | 52.0      | 97.0      | 3.0                         | 44.2        | 79.0                         | 260.0       | 15.8       | 6.0   | 77.8 | 95.0   | 51.3 | 2.1 |
|               | Durum lines     | 80.7       | 52.9      | 95.0      | 2.9                         | 42.6        | 78.2                         | 264.9       | 15.6       | 5.8   | 78.3 | 85.7   | 43.6 | 2.3 |
|               | EDL_10          | 79.5       | 54.0      | 96.3      | 2.7                         | 39.7        | 79.1                         | 321.3       | 15.6       | 6.0   | 79.2 | 25.0   | 7.9  | 3.0 |
|               | EDL_11          | 75.0       | 50.5      | 91.0      | 2.5                         | 38.9        | 76.8                         | 319.5       | 16.3       | 6.0   | 85.7 | 27.5   | 10.2 | 2.7 |
|               | EDL_12          | 73.3       | 51.5      | 92.8      | 2.3                         | 34.2        | 78.7                         | 396.8       | 16.3       | 5.5   | 85.6 | 27.5   | 10.2 | 2.7 |
|               | EDL_13          | 74.0       | 53.0      | 95.0      | 2.3                         | 35.4        | 76.5                         | 297.0       | 17.1       | 5.1   | 85.4 | 17.0   | 7.0  | 2.6 |
|               | EDL_14          | 76.5       | 51.8      | 96.3      | 2.4                         | 36.6        | 74.8                         | 244.5       | 17.2       | 5.9   | 83.2 | 21.5   | 5.6  | 3.1 |
|               | EDL_15          | 73.8       | 50.8      | 93.0      | 2.0                         | 33.9        | 78.2                         | 321.0       | 15.3       | 6.5   | 84.6 | 30.5   | 9.8  | 2.9 |
|               | EDL_16          | 73.5       | 51.3      | 90.0      | 2.3                         | 40.9        | 79.5                         | 348.5       | 15.3       | 6.1   | 80.0 | 23.5   | 8.8  | 2.7 |
|               | EDL_17          | 71.3       | 50.8      | 90.0      | 2.5                         | 39.8        | 80.2                         | 383.0       | 15.9       | 6.5   | 80.8 | 18.0   | 6.6  | 2.7 |
|               | EDL_18          | 70.3       | 51.3      | 90.8      | 2.5                         | 39.7        | 80.0                         | 342.5       | 15.8       | 6.5   | 81.9 | 34.5   | 11.2 | 2.8 |
|               | EDL_19          | 72.3       | 54.8      | 97.8      | 2.6                         | 34.0        | 80.5                         | 321.0       | 14.2       | 6.3   | 85.5 | 25.5   | 10.8 | 2.5 |
|               | EDL_20          | 79.0       | 51.8      | 93.8      | 2.5                         | 42.4        | 79.9                         | 360.5       | 15.7       | 5.8   | 78.5 | 32.0   | 12.9 | 2.6 |
|               | EDL_21          | 84.5       | 51.8      | 91.3      | 2.6                         | 39.5        | 77.4                         | 313.0       | 17.1       | 5.2   | 81.0 | 30.0   | 12.9 | 2.5 |
|               | EDL_22          | 72.5       | 51.3      | 90.3      | 2.7                         | 38.6        | 78.6                         | 351.3       | 15.3       | 5.4   | 80.6 | 31.5   | 12.2 | 2.7 |
|               | EDL_23          | 79.0       | 53.8      | 95.0      | 2.4                         | 35.4        | 76.1                         | 214.0       | 17.0       | 4.0   | 82.2 | 26.5   | 6.4  | 3.1 |
|               | EDL_24          | 73.8       | 53.0      | 94.0      | 2.3                         | 36.6        | 77.2                         | 235.0       | 16.7       | 3.8   | 84.1 | 36.5   | 12.4 | 2.8 |
|               | EDL_25          | 75.0       | 51.8      | 88.5      | 2.6                         | 36.2        | 79.0                         | 393.5       | 15.7       | 7.0   | 80.4 | 43.5   | 18.8 | 2.5 |
|               | EDL_ $\bar{x}$  | 75.2       | 52.1      | 92.9      | 2.5                         | 37.6        | 78.3                         | 322.7       | 16.0       | 5.7   | 82.4 | 28.2   | 10.2 | 2.7 |
|               | Average         | 77.4       | 52.3      | 92.7      | 2.6                         | 37.9        | 78.4                         | 321.3       | 15.9       | 5.3   | 80.2 | 37.1   | 15.7 | 2.6 |
|               | LSD (0.05)      | 2.8        | 0.6       | 1.3       | 0.3                         | 0.8         | 0.4                          | 25.5        | 0.3        | 0.1   | 1.2  | 10.6   | 6.1  | 0.4 |

**Note:** \*\*, \*\*\* significant at  $p < 0.001$ ,  $p < 0.0001$ , respectively. Abbreviations: PH, plant height; DH, days to heading; DM, days to physiological maturity; GY, grain yield; TKW, thousand kernel weight; TW, test weight; FN, falling number; GPC, grain protein concentration; YP, yellow pigment content; KHI, kernel hardness index; PG, percentage of germination; GI, germination index; GR, germination resistance; ns, not significant; LSD, least significant difference.

**Table 2.** Correlation between the three measurements used to characterize PHS resistance (PG, GI and GR) and grain yield and quality related traits.

|    | PH   | DH    | DM    | GY    | TKW   | TW    | FN    | GPC   | YP    | KHI   | PG    | GI    | GR    |
|----|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| PG | 0.04 | −0.12 | −0.39 | 0.15  | 0.01  | 0.19  | 0.17  | −0.18 | 0.06  | −0.20 | 1.00  | 0.88  | −0.21 |
|    | ns   | ns    | ns    | ns    | ns    | ns    | ns    | ns    | ns    | ns    | —     | ***   | ns    |
| GI | 0.05 | −0.15 | −0.48 | 0.29  | 0.07  | 0.35  | 0.44  | −0.28 | 0.22  | −0.22 | 0.88  | 1.00  | −0.63 |
|    | ns   | ns    | ns    | ns    | ns    | ns    | ns    | ns    | ns    | ns    | ***   | —     | **    |
| GR | 0.03 | 0.14  | 0.40  | −0.28 | −0.12 | −0.41 | −0.60 | 0.26  | −0.25 | −0.01 | −0.21 | −0.63 | 1.00  |
|    | ns   | ns    | ns    | ns    | ns    | ns    | *     | ns    | ns    | ns    | ns    | **    | —     |

**Note:** \*, \*\*, \*\*\* significant at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  respectively. Abbreviations: PH, plant height; DH, days to heading; DM, days to physiological maturity; GY, grain yield; TKW, thousand kernel weight; TW, test weight; FN, falling number; GPC, grain protein concentration; YP, yellow pigment content; KHI, kernel hardness index; PG, percentage of germination; GI, germination index; GR, germination resistance; ns, not significant.

**Fig. 3.** Heat map and dendrogram of experimental durum lines (EDL) and checks based on their phenotypic performance. Branch lengths in dendrogram correspond to the relative degree of similarity between lines. Differential phenotypic expression is represented as a color gradient across all lines from white (lowest) to red (highest). [Colour online.]

in one or both directions was observed for almost all traits. Thus, several experimental durum lines showed better performance than the recurrent parent for traits such as DH, DM, PH, FN, YP, GPC, KHI, and TW. None of the experimental durum lines had higher TKW than AC Avonlea, but for some, TKW was significantly higher than W98616 ( $p = 0.05$ ). Although there were experimental durum lines that were not significantly lower than AC Avonlea for GY, there was a general trend of lower yield (Table 1).

#### Traits relationship and cluster analysis

Pearson's correlation coefficients between the measurements used to characterize PHS resistance (PG, GI and GR) and grain yield and quality related traits are presented in Table 2. Germination percentage and GI, did not exhibit significant correlations with any of the evaluated traits, while GR was only significant

correlated with FN. Both traits showed a moderate and negative association between each other ( $r = -0.60$ ,  $p < 0.05$ ). The cluster analysis separated the 21 genotypes into three main groups (Fig. 3). The first group was formed by the common wheat lines W98616 and RL4137, that had high GY, PH and PHS resistance, but the lowest DM, YP and KHI values. A second group included 14 experimental durum lines, which in general exhibited early DH, low PH and GY. The lines in this group, on average, also had higher GPC, YP and KHI values combined with improved PHS resistance and FN compared with AC Avonlea. The third group was formed by the three durum wheat cultivars (AC Avonlea, Strongfield, and Kyle) together with the experimental durum lines EDL10 and EDL19. The line EDL10, only exhibited a significant reduction in TKW compared with AC Avonlea, but this did not significantly impact on the final grain yield. In addition, EDL10 showed significant

improvements in KHI, FN and PHS resistance. For line EDL20, a more pronounced reduction in TKW did have a significant impact on grain yield relative to AC Avonlea. This line showed significant improvement in KHI, FN, YP and TW. The line EDL17 was the only one that displayed a simultaneous improvement in GPC, YP, KHI and TW in combination with greater PHS resistance related to the recurrent parental.

## Discussion

Pre-harvest sprouting, a phenomenon where seeds germinate on the plant before harvest, is one of the major challenges in wheat production areas with frequent rain and high humidity combined with cool temperatures at harvest time. Seed dormancy, defined as the suppression of germination during otherwise favorable conditions plays a critical role in protection against pre-harvest sprouting. The lack of germination (emergence of the radicle from the seed coat) may be due to the embryos themselves being dormant (embryo dormancy), physiological and physical constraints caused by the presence of a hard seed coat (coat-enhanced dormancy), presence of inhibitory chemicals that interfere with embryo growth or a combination of such factors (Finch-Savage and Leubner-Metzger 2006). In durum wheat, the damage caused by PHS is even more important than for common wheat since it has limited genetic variability for PHS resistance. In this study, through interspecific hybridization and a modified backcross breeding approach, we were able to incorporate the PHS resistance from a common wheat line into adapted durum wheat germplasm. All selected experimental durum lines showed greater PHS resistance than AC Avonlea, while some of them exhibited greater seed dormancy than the donor parent. This suggests that AC Avonlea contributed to genes that resulted in transgressive segregation for seed dormancy. Similar transgressive segregation results in the inheritance of PHS resistance have been previously reported (Knox et al. 2012; Kumar et al. 2015).

As reported by Knox et al (2012), defining methodologies to measure PHS resistance is a challenge given that there are numerous factors such as harvest environmental conditions, seed storage conditions and time between harvest and testing that can affect the outcome. Here we used three different measurements, PG, GI, and GR which showed different degrees of correlation between each other. Thus, differing number of lines were identified as transgressive segregants depending on the measurement method used. Percentage of germination identified seven lines exhibiting significantly higher PHS resistance levels than W98616 ( $p = 0.05$ ). Germination index detected two lines with greater seed dormancy than W98616, with one having significantly more resistance than the donor, while GR detected four lines exhibiting higher but not statistically significant more PHS resistant values than W98616 (Fig. 2). These

results agreed with an earlier study (Knox et al. 2012), which emphasized that based on the number of transgressive segregants, the different measurement methods quantify different aspects of preharvest sprouting resistance, therefore, for a better understanding of PHS genetic control, the combined use of different measurement methods is recommended. In our study, only one line (EDL\_20) showed significantly higher PHS resistance levels than W98616 for the three measurement methods, while EDL\_22 was significantly more resistant than the donor parent for PG and GI.

Common wheat can serve as a potential source for improving the PHS resistance of durum wheat, but the use of related species carries the risk of genetic drag that may affect additional traits in the durum background. In the present study, beyond the significant variation and transgressive segregation observed in most of the evaluated traits, the three measurements used to characterize PHS resistance (PG, GI and GR) did not exhibit an unfavorable association with any of the evaluated traits. The latter implies that improvement in PHS resistance can be made with no concomitant detrimental effects on grain yield and quality-related traits. In general, the backcross derivatives studied here tended to be lower yielding than the recurrent parent AC Avonlea, but this decrease was not statistically significant (Table 1). Since these lines were derived from only three backcrosses to durum it is not surprising that there were some agronomic deficiencies at this stage of trait introgression. In the case of complex traits, where a greater number of genes are involved, more backcross generations are necessary to recover the genetic background of the recurrent parent (Frisch and Melchinger 2005). On the other hand, we recovered experimental lines combining higher PHS resistance with improved FN, TW, KHI, GPC, YP and DM, which are important traits in defining durum field performance and quality (Troccoli et al. 2000; Borrelli et al. 2008). In the spring wheat growing regions of western Canada, early maturity is an important trait for timely harvest to avoid frost damage, and associated harvest and post-harvest problems (Chen et al. 2015). Thus, early maturing cultivars are less prone to pre-harvest sprouting which is common in years of cold and wet harvest conditions (Hucl and Matus-Cadiz 2002).

Falling Number is the metric used by grain elevators and grain buyers to evaluate the level of sprouting damage and quality of grain (Martinez et al. 2018). When PHS occurs, the FN of wheat grain decreases to a value generally below 300, due to the large quantity of  $\alpha$ -amylase produced during grain germination. In this study, GR was the only one of the three measurements used to characterize PHS resistance that exhibited a significant association with FN. However, the association was negative and only significant at  $p = 0.05$  levels. In line with these results, a weak or non-significant correlation between FN and PHS resistance has been previously reported (Singh et al. 2008; Martinez et al. 2018). Experimental durum lines with



low PHS resistance were screened out during early stages of the study. Consequently, PHS resistance differences among experimental lines finally tested in the field were small, which resulted in non-significant correlations with FN. Thus, many of the developed experimental durum lines had higher FN values (>300) than the three durum cultivars used as checks (<300), with some lines displaying higher values than the donor parent (Table 1). These lines represent a promising genetic resource that can be used to improve the FN values in durum wheat germplasm and therefore avoid reductions in crop grade and commercial value. The lines EDL\_23 and EDL\_24 represent an exception, which despite having good levels of resistance to PHS (PG, GI, and GR) exhibit unacceptable FN values. Interestingly, both lines as well as EDL\_14 also had low TW. Reduced FN associated with low TW has been previously reported (Derera 1988; Kruger 1989). The most promising dormancy lines developed as a result of this study have already been used in further backcrosses to continue with the recovery of the recurrent parent genetic background, as well as in further rounds of introgression with newer durum cultivars in an attempt to incorporate the PHS resistance into locally adapted high-yielding cultivars.

## Conclusion

Our results demonstrate that PHS resistance can be transferred and integrated from hexaploid wheat into a durum wheat background via interspecific hybridization and modified backcross breeding. This strategy allowed us to improve PHS, while recovering lines with agronomic and quality performance typical of the durum wheat checks used in this study. The experimental durum lines developed here represent a promising genetic resource for further use in breeding programs to increase PHS resistance and provide valuable parental material that will lead to the development of new sprouting resistance durum cultivars. The latter will in turn provide a degree of grade protection for wheat producers and exporters.

## Competing Interests

The authors declare there are no competing interests.

## Funding Statement

This research was supported by Agriculture Development Fund (ADF) of Saskatchewan Ministry of Agriculture (ADF Project 20110187) with matching funds from the Western Grains Research Foundation.

## Author Contributions

**Guillermo Gerard:** Formal analysis and Methodology, Writing – Original Draft, Writing – review & editing. **Pierre Hucl:** Project inception and administration, Funding acquisition, Formal analysis and Methodology, Writing – review & editing, Resources and Supervision. **Curtis Pozniak:** Methodology, Writing – review &

editing, Resources and Supervision. **Connie Briggs:** Methodology, Writing – review & editing.

## Acknowledgements

We would like to acknowledge Mike Grieman and Shuhua Zou for their capable technical assistance during the course of this project.

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