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Developing standardized methods for breeding preharvest sprouting resistant wheat, challenges and successes in Canadian wheat

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Abstract Preharvest sprouting (PHS) in spring wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. var *durum*) causes significant economic losses due to a reduction in grain yield, grain functionality and viability of seed for planting. Average annual estimated losses in Canada are about $100 million. Genetic resistance to PHS reduces these losses. Development of PHS resistant cultivars is complicated by the effects of factors under genetic control, such as spike morphology, seed dormancy, environment, and kernel diseases. Resistance to PHS has been a breeding priority since the late 1960s. Development of RL4137, which is the primary source of PHS resistance in the Canada Western Red Spring market class, has led to cultivar improvements. A white-seeded derivative of RL4137 is the primary source of PHS in the Canada Prairie Spring White and Canada Western Hard White Spring wheat market classes. Procedures to select for PHS resistance vary among breeding programs, market classes and by degree of inbreeding. Methods include artificial sprouting of intact spikes, germination tests, natural weathering in field trials, artificial weathering trials, and indirect assessment of sprouting by measuring Hagberg falling number. Although many genetic loci have been attributed to preharvest sprouting resistance, application of molecular markers is currently limited due to the complex inheritance of the trait. In Canada, cultivars are characterized for their relative level of PHS resistance and the information is made available to producers.

Keywords Dormancy · Pre-harvest sprouting · Genetic resistance · Breeding methods · *Triticum*

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Introduction

Germination of wheat (*Triticum* spp.) kernels, including initiation of germination and the induction of alpha-amylase without any visible evidence of embryo growth, causes significant economic losses in grain yield, grain functionality and viability of seed for planting (Derera 1989). In Canada, losses amounting to several hundred millions of dollars are experienced due to reduction in market grade in about three out of ten years (Derera 1989; Clarke et al. 2005). Economic losses due to preharvest sprouting (PHS) can be reduced by developing cultivars with increased

dormancy and preharvest sprouting resistance (Czarnecki et al. 1986; McCaig and DePauw 1992) and by direct harvesting procedures (Clarke et al. 1984).

Procedures used to study and or incorporate genetic resistance differ by science project objectives and by breeding objectives. Various methods have been developed to measure seed dormancy and to assess preharvest sprouting tolerance (Derera 1989). DePauw and McCaig (1991) compared various assays that measure sprouting tolerance, seed dormancy, and alpha-amylase activity. The phenotypic correlations among the various assays were significant and positive. Genotypic effects accounted for 44–90% of the phenotypic variation. Heritability expressed on a genotype mean basis ranged from 0.59 to 0.93 and was highly significant. In genetic analyses and cultivar development procedures, it is important to measure a trait such that it minimizes the error variation relative to the genetic variation. Because preharvest sprouting tolerance is only one trait of a very large suite of traits to be integrated into a cultivar, the methods used in cultivar development have to be moderately to highly heritable, reliable and yet low cost, especially in the early generations.

Canadian wheat is segregated into market classes based on end-use suitability parameters of grain protein concentration, gluten strength, and kernel color (DePauw and Hunt 2001) (Table 1). Producers choose to grow cultivars of a market class based on economic considerations, market opportunities, and cultivar response to local abiotic and biotic stresses. Breeding programs target release of cultivars to meet the agronomic performance, resistance to biotic factors, and the necessary quality attributes that define each market class.

Table 1 Percentage of total wheat area seeded to market classes of spring and winter hexaploid wheat and durum wheat in Canada from 2005 to 2010

Wheat market class[a]	2005–2010[b]
Canada Western Red Spring (CWRS)	64.8
Canada Western Amber Durum (CWAD)	21.9
Canada Western Red Winter (CWRW)	4.0
Canada Prairie Spring Red (CPSR)	2.3
Canada Western Soft White Spring (CWSWS)	1.1
Canada Western Hard White Spring (CWHWS)	1.7
Canada Prairie Spring White (CPSW)	0.2
Canada Western Extra Strong (CWES)	0.1
Canada Western General Purpose (CWGP)	0.1
Canada Eastern Winter Wheat	3.8
Total	100.0

[a] Details of market classes is in DePauw and Hunt (2001)

[b] Adapted from: M. Grenier Canadian Wheat Board

This paper reviews the breeding methods employed by Canadian breeders to incorporated genetic resistance to preharvest sprouting in various wheat market classes. This information can serve as a guide to future Canadian breeders and to breeders establishing programs in other nations.

Methods for selection of preharvest sprouting resistance used at Cereal Research Centre

The methods used to incorporate PHS resistance, at Cereal Research Centre Agriculture and Agri-Food Canada, Winnipeg, to meet cultivar registration requirements are similar for the Canada Western Red Spring (CWRS) and Canada Western Hard White Spring (CWHWS) market classes. Crosses aim to include at least one parent that has a high level of PHS resistance (i.e. AC Domain, AC Majestic, Harvest, Snowbird, Snowstar or derivative genotype Table 2). All of these parents are derivatives of RL4137 which has a proven high level of dormancy (DePauw et al. 2009). DePauw and McCaig (1983) provided evidence that RL4137 has two mechanisms for dormancy: one associated with red seed coat color and another mechanism independent of seed coat color. For crosses with parentage that suggests PHS resistance is likely inadequate, F_2 spikes are selected without stratification for maturity and stored at −20°C until phenotyping. The F_2 spikes are subjected to a wetting treatment in a rain simulator and only those spikes with no sprouted seeds are selected, threshed, and kernels examined. The F_2 spike-F_3 progeny are grown out in greenhouses over the winter months so that thorough screening is conducted and not rushed to make contra-season nursery seeding dates.

Per population, 400 to 500 F_2 spikes (Table 3) are exposed to 3–5 days of artificial weathering. Spikes are rated for evidence of visible germination: spikes with more than two seeds germinated (visible coleoptile or roots) are discarded. The remaining spikes are dried, threshed, and seed rated for appearance: seed rated "good" generally has no visible sprouting;

Table 2 Examples of cultivars with preharvest sprouting resistance, parentage, and market class

Name	Parentage	Class[a]	Reference	Source PHS
Columbus	Neepawa*6/RL4137	CWRS	Campbell and Czarnecki 1981	RL4137_derivative
AC Domain	ND499/RL4137//ND585	CWRS	Townley-Smith and Czarnecki 2008	RL4137_derivative
Pasqua	BW63*2/Columbus	CWRS	Townley-Smith et al. 1993	RL4137_derivative
AC Vista	HY344/Losprout 'S'//HY358*3/Bt10	CPS_white	DePauw et al. 1998	W_RL4137[b]
AC Majestic[c]	Columbus*2//Saric 70/Neepawa/3/Columbus*5// Saric 70/Neepawa	CWRS	Townley-Smith and Czarnecki 1995	RL4137_derivative
Waskada	AC_Domain*2/Sumai_3//2*Superb (Superb = Grandin*2/AC Domain)	CWRS	Fox et al. 2009	RL4137_derivative
Harvest	AC Domain*2/ND 640	CWRS	Fox et al. 2010	RL4137_derivative
Snowbird	RL4137*6//Thatcher/Poso48/3/AC Domain	CWHWS	Humphreys et al. 2007	W_RL4137[b]
Snowstar	94B46*G22/McKenzie	CWHWS	DePauw et al. 2006	W_RL4137

[a] CWRS is Canada Western Hard Red Spring. CPS_white is Canada Prairie Spring_White. CWHWS is Canada Western Hard White Spring

[b] White seeded derivative of RL4137 as a source of preharvest sprouting resistance

[c] AC Majestic tested as BW173

Table 3 Summary of response of F_2 intact spikes using an artificial rain simulator to identify preharvest sprouting resistant lines

Cross	Parentage	Year	Spikes		
			# 'good'	Total	% rated 'good'
BG30	BA83-EC-8/BW841	2008	89	434	21
BG33	99B26-AK3B/BW391	2008	65	407	16
BG48	BB07A*A637/BW342	2008	33	385	9
BG51	BA21-CM-9/BW342	2008	97	408	24
BG57	99B28-EK4F/BA21-CD-20	2008	53	414	13
BH03	ND739/BW431//BW342	2009	143	507	28
BH07	BW415/BW342	2009	156	501	31
BH20	SD3948/BW430	2009	171	500	34
BH32 + BH33	K2619 = HF15*A0084/K2626&2628	2009	217	500	43
BJ11	ACS 54608/Waskada	2010	30	512	6
BJ19	3 × 1-134*FA0067/BW357	2010	69	550	13
BJ25	ND04/3-21/BW874	2010	44	561	8
BJ05	HW341/Waskada	2010	69	511	14

"fair" seed is somewhat shriveled with little or no visible sprouting; "poor" seed is shriveled with many seeds sprouted. Seed shriveling may be due to seed pathogens which may have a tendency to reduce dormancy (Fox et al. 2003). The CWRS cultivars AC Majestic, AC Domain and Harvest all derive from F_2 populations that were subjected to artificial weathering and have parentage related to RL4137 (Table 2).

Stage two testing involves collection of spikes from single row plots 5 m long of F_9 and F_{10} lines, and entries into pre-registration and registration trials. Stratification for maturity is achieved by sampling spikes from a plot when the majority of the ultimate stem nodes have turned brown and collapsed. Spikes are air dried for 2 days prior to storage at −20°C until phenotyping. All lines are evaluated for sprouting

scores on a 1–9 scale (Rasul et al. 2009). F_{10} lines and entries into pre-registration and registration trials are also evaluated for Hagberg falling number using artificial weathering as described by Rasul et al. (2009). Lines that demonstrate poor PHS resistance relative to standard cultivars are dropped from further testing.

Methods for selection of preharvest sprouting resistance used at SemiArid Prairie Agricultural Research Centre (SPARC)

At SPARC, Agriculture and Agri-Food Canada, Swift Current, similar methods are used to incorporate PHS resistance to meet cultivar registration requirements in CWRS, CWHWS, and Canada Western Amber Durum (CWAD) market classes. Crosses are planned with at least one parent that has a high level of PHS resistance. To ensure dormancy genes are not lost unintentionally from the non-germination of dormant seed during rapid generation advancement or use of contra season nurseries, dormancy breaking methods are applied to the seed such as hydrogen peroxide (10 g l^{-1}), potassium nitrate (2 g l^{-1}), gibberellic acid (0.1 g l^{-1}), heat treatment, scarification, and or temperature cycling (DePauw and Clarke 1976; Matus-Cádiz and Hucl 2003). The rigor of testing increases as lines advance through the breeding program with three stages of testing.

In the first stage to characterize early generation breeding lines for preharvest sprouting response in targeted populations, a sprouting index is determined based on both the number of spikes out of a ten spike sample with visible evidence of sprouting and on the intensity of the sprouting (DePauw et al. 2009). Each population is stratified for maturity by collecting a sample of 10 spikes when the ultimate stem node collapse and turn brown (equivalent to about 16% moisture on a wet weight base), air dried for 24 h, and stored at −20°C until phenotyping. The ten spikes are bound tightly into a bundle by a water-resistant sticky label upon which the appropriate experimental unit information is printed. The response to sprouting of intact spikes is assayed by subjecting the ten-spike bundles to a uniform wetting treatment in a rain simulation chamber. The ten-spike bundles are placed upright on a tray fitted with wire mesh on a 0.5 cm grid. An initial wetting treatment of about 135 mm in 5 h is followed by ½ h misting every 12 h. Temperature is maintained at 18°C and relative humidity greater than 95%. If a population is segregating for seed coat color, the experimental lines are classified for seed color using a sodium hydroxide test (DePauw and McCaig 1988). Selection is based on sprouting index within groups based on seed color. A selection cut-off is established relative to the sprouting index of a set of check cultivars with known response. Selection for preharvest sprouting response is performed on the F_4 and F_6 breeding lines in conjunction with selection for a multitude of diseases, agronomic response traits, and end-use quality. A typical starting population of about 10,000 single F_2 plants per cross rapidly declines until either nothing remains or an experimental line meets sprouting resistance criteria of check cultivars and all criteria to be registered as a cultivar such as AC Vista (Table 2).

Stage two testing is more detailed than stage one and involves an assessment of the length of the dormancy period by using two sampling periods about 10 days apart as well as two or more replications (DePauw et al. 2009). The protocol involves stratifying for maturity and collecting a sample of ten spikes as described above. Following the artificial rain simulation treatment and scoring for number of heads with visible evidence of sprouting, the samples are dried down, threshed and percentage of kernels sprouted is determined following the methods described by DePauw et al. (2009).

Stage three testing is performed on experimental lines in the final year of registration trials and recently registered cultivars. Stage three testing includes the two aforementioned treatments and provision for natural weathering without stratification for maturity (DePauw et al. 2009). In the natural weathering test, the initial harvest occurs when the latest maturing genotypes have attained about 16% moisture content on a wet weight basis. The second harvest date occurs after about 10 mm rainfall and within an interval of 10–25 days. The third harvest date involves similar criteria but before permanent snow cover. Variables measured on the natural weathered samples are Hagberg falling number, grain volume weight and polyphenyl oxidase. After 3 years of testing, the cultivars are classified for resistance to preharvest sprouting, and the information is made available producers and the public (e.g. http://www.agriculture.gov.sk.ca/Varieties_Grain_Crops).

Methods for selection of preharvest sprouting resistance used at Crop Development Centre (CDC), University of Saskatchewan

The CDC CWRS program has been selecting for pre-harvest sprouting tolerance since 1990. Parents with RL4137 in their background such as Columbus, Pasqua, AC Domain, and AC Majestic were used routinely in developing populations (Table 2). Progeny were selected on the basis of artificial sprouting of intact spikes in the F_5 or F_6 generation. Spikes were harvested from hills that had not been culled on the basis of leaf or stem rust reaction. Starting in the mid-1990's, the CDC CWRS and CWHW program has set up a natural weathering nursery (single rows 1.3 m long per entry) which is typically seeded each year in early May. This nursery contains 1,500–2,000 entries which represent F_7 and F_8 lines in replicated yield trials. The entries are rated for shattering (and stem solidness if appropriate) and spikes are harvested in late October and stored in an un-heated facility. Lines that are advanced on the basis of agronomic performance, disease reaction and small-scale predictive quality tests are then subjected to a Hagberg falling number test. For a number of years weathered grain samples were objectively evaluated using the Hunter-Lab L* and a* values (McCaig et al. 2006); however, lack of progress using the latter approach suggested that it was ineffective.

Hagberg falling number and cultivar registration

For an experimental line to be eligible for registration it must pass three consecutive years of assessment relative to up to five standard cultivars in the categories of agronomic performance, response to diseases and end-use quality. The Quality Evaluation Team of the Prairie Recommending Committee on Wheat, Rye and Triticale, uses five standard cultivars to represent the acceptable range of milling, rheological and baking properties based on input from domestic and international customers familiar with CWRS quality. The same principles hold true for other market classes. Each year the registration trials are grown at about 13 locations in replicated tests. A subsample of the yield trial grain from locations representative of the top two grades are composited and is intended to be representative of the geographical area and protein content of the crop-year. The composite is used as the basis for measuring the milling, rheological and baking properties of the checks and candidate cultivars.

Based on committee membership expertise and customer feedback on wheat class quality, a level of acceptability is established for each quality parameter. A mean of the checks is constructed for each quality parameter. Each check and candidate cultivar is scored as a deviation from the mean of the checks. Deviations above and below this target of acceptability results in five classes: excellent, improvement, equivalent, flag and poor. The ranges for the five categories for Hagberg falling number are presented in Table 4. Within the CWRS market class, Hagberg falling number is classified as an essential trait. Candidates rated poor are eliminated from further trialing regardless of their agronomic performance or disease resistance. A candidate that successfully undergoes 3 years of registration trialing are summarized and

Table 4 Hagberg falling number of the rating scale, five checks and six candidate cultivars rated for acceptability of Hagberg falling number score in the 2008 Central Bread Wheat registration trial based on a seed composite of 6 of the 11 test locations

Genotype		Hagberg falling number (s)	Deviation from mean of checks (s)	Rating
	Scale	520	79	Excellent
	Scale	476	35	Improvement
Mean of checks	Scale	441	0	Equivalent
	Scale	406	−35	Flag
	Scale	362	−79	Poor
Katepwa	Check	430	−11	Equivalent
McKenzie	Check	430	−11	Equivalent
CDC Teal	Check	435	−6	Equivalent
Unity VB	Check	450	9	Equivalent
5603HR	Check	460	19	Equivalent
BW 394	3rd year	450	9	Equivalent
BW 424	1st year	280	−161	Poor
BW 428	1st year	360	−81	Poor
BW 429	1st year	420	−21	Equivalent
BW 432	1st year	285	−156	Poor
BW 433	1st year	450	9	Equivalent

Adapted from 2008 Central Bread Wheat Coop report Quality Evaluation Team, Prairie Recommending Committee Wheat, Rye and Triticale

Table 5 Hagberg falling number and sprouting scores of BW429, and five checks cultivars from yield tests grown in 2008–2010

Cultivar	Falling number on end-use suitability composites				Falling number–natural weathering[a]			
	2008	2009	2010	Mean	2008	2009[b]	2010	Mean
Katepwa	430	435	370	412	253	–	238	246
McKenzie	430	440	405	425	351	–	304	328
CDC Teal	435	430	390	418	258	–	114	186
Unity VB	450	475	435	453	378	–	386	382
5603HR	460	440	450	450	326	–	268	297
Mean	441	444	410	432	313	–	262	288
BW429	420	430	395	415	312	–	330	321
LSD[c]	–	–	–	29	56	–	106	107

Cultivar	Falling number-artificial weathering[d]				Preharvest sprouting score[e]			
	2008	2009	2010	Mean	2008	2009	2010	Mean
Katepwa	96	88	174	119	5.8	5	6.2	5.7
McKenzie	198	172	261	210	2.1	6.3	3.7	4
CDC Teal	73	72	104	83	6.4	6.6	6.8	6.6
Unity VB	266	130	210	202	1.7	3.9	1.7	2.4
5603HR	248	217	303	256	1.9	3	1.4	2.1
Mean	176	136	210	174	3.6	5	4	4.2
BW429	111	161	260	177	4.4	3.5	1.7	3.2
LSD	114	84	74	70	2.0	1.7	2.4	2.1

Quality composite samples were created from grain harvested from 6, 10 and 8 locations for respective years of the Central Bread Wheat registration trial

[a] Field weathered samples are harvested when declines in falling number were observed for the sprouting susceptible cultivar Roblin

[b] Because there was insufficient precipitation to obtain a decline in falling number of Roblin, falling number was not measured on the other cultivars

[c] Least significant difference $P \leq 0.05$ for treatments, protected LSDs

[d] Collected at maturity, this material is placed in a rain simulator at 15°C for 48 h, dried and then seed is ground into meal for falling number determination

[e] ((# spikes with 0 sprouts) × 1 + (# spikes with 1 sprout) × 2 + (# spikes with 2 sprouts) × 3 + (# spikes with 3–5 sprouts) × 5 + (# spikes with >3 sprouts) × 9)/total number of spikes evaluated. Spikes were collected at maturity and stored at −20°C until they were evaluated. The mean was calculated over the 3 years of tests using SAS PROC MIXED procedure

presented to a panel of peers to determine if the candidate has merit for cultivation and use. As a recent example, Table 5 summarizes 3 years of measures of preharvest sprouting response for a recent successful candidate BW429. This protocol to evaluate candidates for registration has successfully minimized candidates which are susceptible to preharvest sprouting from being registered in western Canada.

DNA molecular markers

Characterization of dormancy and response to preharvest sprouting in mapping populations is used to determine inheritance and/or locate QTL's for the trait(s). A range of trait assessment methods have been used such as germination tests and sprouting index on intact spikes (Rasul et al. 2009; Knox et al. 2011). QTLs have been detected and published (Rasul et al. 2009; Knox et al. 2011) and haplotypes have been defined (Ogbonnaya et al. 2007; Fofana et al. 2009). Validation of QTL markers for preharvest sprouting resistance is challenging because of the large effort in phenotyping that is required to confirm desirable alleles are present in the most elite sources of resistance that are preferred as parents. Recently, the CDC hexaploid wheat breeding has been using a molecular marker developed by Singh et al. (2010).

The first two advanced breeding lines from an effort to pyramid *Lr22a* with a major dormancy QTL on chromosome *4A* in a CDC Go (Hucl 2003) plant type were grown in the 2010 Parkland B pre-registration test for very short season northern areas. One of the lines (W09130) was the highest yielding entry in the trial, but was not advanced due to low loaf volume and undesirable FHB score. Currently at CDC, gametic selection in the F_1 is being conducted to backcross the *4A* QTL into CDC Go (backcross 7) as well as selecting parental material for population development. Resources permitting, this may lead to routinely genotype lines in the F_6 and F_7 for both the CWRS and CWHW classes. Generally, dissection of QTL's is required to develop molecular markers suitable for marker assisted breeding purposes.

Conclusions

Cultivars with moderate to high levels of preharvest sprouting resistance based on dormancy primarily from RL4137 have been released in the CWRS market class. White seeded cultivars have been released based on dormancy derived from RL4137, using the dormancy mechanism independent of seed coat color. Snowbird and Snowstar, hard white wheat varieties have high levels for PHS resistance comparable to some cultivars with red seed coat color. Screening protocols generally permit selection for high levels of PHS resistance without unacceptable reduction in population variance for other important traits. Bringing in new genes from non-adapted germplasm for agronomically desirable traits such as high grain yield potential or resistance to diseases such as *Fusarium* head blight while retaining CWRS or CWHWS or CWAD level of PHS resistance continues to be challenging. It is an on-going challenge to identify molecular markers for PHS that are applicable in a breeding program which requires markers to have little linkage drag, relevance across a diversity of genetic backgrounds, low cost and transferable to various laboratories.

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