New Breeding Tools Impact Canadian Commercial Farmer Fields

R.M. DePAUW[1], R.E. KNOX[1], D.G. HUMPHREYS[2], J.B. THOMAS[2], S.L. FOX[2], P.D. BROWN[2], A.K. SINGH[1], C. POZNIAK[3], H.S. RANDHAWA[4], D.B. FOWLER[3], R.J. GRAF[4] and P. HUCL[3]

[1]Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Box 1030, Swift Current, S9H 3X2 Saskatchewan, Canada; [2]Cereal Research Centre, AAFC, Winnipeg, MB, Canada; [3]Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada; [4]Lethbridge Research Centre, AAFC, Lethbridge, AB, Canada; e-mail: ron.depauw@agr.gc.ca

Abstract: The high cost of cultivar development encourages efficiencies to reduce time and costs to develop cultivars. Doubled haploid (DH) technology and marker assisted breeding (MAB) are two such tools that improve efficiencies. Since 1997, twenty five wheat cultivars in seven market classes, developed using DH methods, have been registered by the Canadian Food Inspection Agency. These DH cultivars accounted for more than one third of the Canadian wheat acreage in 2009. The DH cultivar Lillian, eligible for grades of Canada Western Red Spring class and currently the most widely grown wheat cultivar in Canada, was developed using MAB to improve grain protein content with the *Gpc-B1/Yr36* on chromosome 6BS introgressed from *Triticum turgidum* L. (Zhuk.) *dicoccoides* (Körn. Ex Asch. & Graebn). AC Andrew, a Canada Western Soft White spring DH cultivar, was the most widely grown cultivar in its class for the last two years. The new market class, Canada Western Hard White Spring wheat, is based entirely on DH cultivars. Goodeve, one of the first Canada Western Red Spring cultivars released with the gene *Sm1* on chromosome 2BS for resistance to the orange wheat blossom midge (*Sitodiplosis mosellana* (Géhin)) was selected by application of the DNA marker WM1. Glencross, the first cultivar in the Canada Western Extra Strong wheat class with *Sm1*, was selected using the WM1 marker on haploid plants prior to doubling. Development of the durum wheat cultivars CDC Verona and Brigade involved the use of a marker for *Cdu1*, a major gene on chromosome 5B that regulates grain cadmium concentration. Marker technology permits a more strategic and integrated approach to breeding by quantifying the introgression of various key genes into advanced breeding material, identifying targeted loci in parents and following up with MAB in the progeny.

Keywords: doubled haploid; field-ready cultivars; marker assisted breeding; wheat

Each year Canada produces about 25 mil t of wheat, of which about 69% is spring hexaploid wheat, 23% is durum wheat, and 8% is winter wheat (Table 1). Due to a small domestic population, about 70% of hexaploid wheat and about 80% of durum wheat is exported to over 70 countries, generating $3.5 to $5 billion Cdn in annual sales. Canadian wheat is segregated into market classes based on end-use suitability parameters of grain protein concentration, gluten strength, and kernel colour. Western Canada accounts for more than 95% of Canadian wheat production representing nine wheat classes.

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 2009

Wheat market class	2005–2009
Canada Western Red Spring (CWRS)	63.6
Canada Western Amber Durum (CWAD)	23.0
Canada Western Red Winter (CWRW)	4.1
Canada Prairie Spring Red (CPSR)	2.4
Canada Western Soft White Spring (CWSWS)	0.9
Canada Western Hard White Spring (CWHWS)	1.9
Canada Prairie Spring White (CPSW)	0.2
Canada Western Extra Strong (CWES)	0.1
Canada Western General Purpose (CWGP)	0.0
Canada Eastern Winter Wheat	3.8
Total	100.0

Adapted from M. Grenier Canadian Wheat Board

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. To be registered as a cultivar, an experimental line is evaluated for three years in replicated and multi-location trials and its agronomic, disease and end-use suitability are determined relative to standard check cultivars. The Canadian Food Inspection Agency grants a license for the cultivar to be sold in Canada.

Doubled haploid technology

Doubled haploid technology has been used to develop populations to study inheritance and develop molecular markers linked to traits (see reviews by Jauhar *et al.* 2009; Somers & Humphreys 2009). DH technology achieves the production of true breeding lines in one generation. DH technology has been integrated into Canadian cultivar development programs for about 20 years. Since 1997, twenty five DH cultivars have been released in seven market classes (Table 2). In Canada, the predominant method to generate doubled haploids has been maize pollination of wheat ovules to generate haploid embryos followed by chromosome doubling (Aung *et al.* 1995; Knox *et al.* 2000). Only two DH cultivars were produced by another culture technique.

Canada Western Red Spring (CWRS) doubled haploid cultivars

The first doubled haploid cultivar, McKenzie, was released in 1997 (Table 2) and was developed using an anther culture protocol (Graf *et al.* 2003). McKenzie had 12.7% higher grain yield than the highest yielding check in the test and similar grain protein concentration and resistance to most diseases at the time of registration. McKenzie has the gene *Lr21* which confers resistance to prevalent races of leaf rust (McCallum & DePauw 2008). Since McKenzie there have been another nine doubled haploid cultivars of the 43 cultivars registered as eligible for grades of CWRS. McKenzie is a parent of five of these 43 recent cultivars. Superb, released in 2001, had slightly higher grain yield than McKenzie (Townley-Smith *et al.* 2010). Lillian was released in 2003 and had 2.4% higher grain yield than the highest yielding check plus 0.3 units more grain protein concentration (DePauw *et al.* 2005). Lillian has a solid stem which confers resistance to wheat stem sawfly (*Cephus cinctus* Nort.).

Historically, solid stem cultivars yield about 8% less than hollow stem cultivars. To address this challenge, a high yielding solid stem wheat line but with lower grain protein concentration was crossed to a breeding line with high grain protein

Table 2. Doubled haploid cultivars in western Canada released by market class, year, breeding institution, and primary traits

Market class[a]	Name	Year registered	Breeding institution	Primary trait
CWRS	McKenzie	1997	Viterra	higher yield, resistance to leaf rust
CWRS	Superb	2001	AAFC, Winnipeg	higher yield
CWRS	Lillian	2003	AAFC, Swift Current & Winnipeg	resistance to wheat stem sawfly, higher yield, *Gpc-B1/Yr36, Lr34/Yr18*
CWRS	Alvena	2006	AAFC, Swift Current	earlier maturity
CWRS	CDC Abound	2007	CDC, University of Saskatchewan	resistance to imidazolinone herbicides
CWRS	Waskada	2007	AAFC, Winnipeg	improved resistance to FHB
CWRS	Stettler	2008	AAFC, Swift Current	higher yield and protein
CWRS	Shaw	2009	AAFC, Winnipeg	resistance to orange wheat blossom midge *Sm1*
CWRS	Carberry	2009	AAFC, Swift Current	improved resistance to FHB, and higher yield and protein
CWRS	Muchmore	2009	AAFC, Swift Current	higher yield
CWSWS	AC Andrew	2001	AAFC, Lethbridge	higher yield
CWSWS	Bhishaj	2002	AAFC, Lethbridge	
CWHWS	Kanata	2000[b]	AAFC, Winnipeg	white seed, preharvest dormancy
CWHWS	Snowbird	2000[b]	AAFC, Winnipeg	white seed, preharvest dormancy
CWHWS	Snowstar	2006	AAFC, Winnipeg	white seed, preharvest dormancy
CWES	Burnside	2004	AAFC, Winnipeg	higher protein, *Gpc-B1*
CWES	Glencross	2008	AAFC, Winnipeg	resistance to orange wheat blossom midge *Sm1*
CWAD	DT801[c]	2010	AAFC, Swift Current	higher yield, greater yellow pigment
CWRW	W434	2010	AAFC, Lethbridge	disease resistance
CWGP winter	Accipiter	2008	University of Saskatchewan	higher yield
CWGP winter	Peregrine	2008	University of Saskatchewan	higher yield
CWGP winter	DH99W18I*45	nr	University of Manitoba	disease resistance
CWGP winter	Sunrise	2009	University of Saskatchewan	disease resistance
CWGP winter	DH99W19H*16	nr	University of Manitoba	disease resistance
CWGP winter	Broadview	2010	AAFC, Lethbridge	higher yield, disease resistance

[a]Abbreviations are listed in Table 1; [b]Interim registered; [c]experimental name; nr – supported for registration but not registered to date by CFIA; AAFC – Agriculture and Agri-Food Canada; CDC – Crop Development Centre

concentration region *Gpc-B1* located on chromosome 6BS. BC_2F_1 plants expressing the *Gpc-B1* marker were selected and DH lines were produced only from these selections. The breeding strategy included simultaneous selection for higher grain yield and protein concentration, and led to the release of Lillian, which has been the most widely grown CWRS cultivar since 2007. It is the first widely grown Canadian cultivar to deploy *Gpc-B1*. From 2006 to 2009, DH cultivars have been grown on over 30% of the area seeded to CWRS cultivars.

The CWRS market class has exceptionally stringent milling and end-use suitability standards, and commands the highest average price of hexaploid wheat traded internationally. Ten of the 25 doubled haploid cultivars are eligible for grades of the CWRS wheat class.

Doubled haploid technology has accelerated introduction of new traits or facilitated enhancement of traits. For example: the DH cultivars, CDC Abound has resistance to the imidazolinone herbicides, Carberry and Waskada have improved resistance to Fusarium head blight, and Shaw has resistance to orange wheat blossom midge based on the *Sm1* gene.

Doubled haploid cultivars in other market classes

The Canada Western Hard White Spring (CWHWS) market class was established in 2001 with registration of Kanata and Snowbird, both doubled haploids (Humphreys *et al.* 2007a, b). This market class has many features of the high milling properties, and strong extensible gluten properties of the CWRS class. The third cultivar in the CWHWS class, Snowstar, is also a DH with McKenzie as a parent (Table 2).

The Canada Western Soft White spring (CWSWS) wheat market class specifies low grain protein concentration and weak gluten suitable for the confectionary markets. AC Andrew, the first DH eligible for grades of CWSWS, had 15% more grain yield than the highest yielding CWSWS check cultivar and enhanced resistance to leaf rust, stem rust, and powdery mildew (Sadasivaiah *et al.* 2004). In 2008, AC Andrew was grown on 99% of the CWSWS acreage. High grain yield, low grain protein concentration and strong straw of AC Andrew has made it suitable for feed and ethanol manufacturing. Bhishaj is a more recent CWSWS DH cultivar. In 2004, the DH cultivar Burnside, was released eligible for grades of Canada Western Extra Strong (CWES) market class, which is known to have very strong gluten properties (Humphreys *et al.* 2010). Burnside had grain yield equal to the highest yielding check, combined with 0.9 units more grain protein and two days earlier maturity. Burnside was grown on 36% of the area seeded to this class in 2009.

Since 2008, there have been four DH cultivars out of five General Purpose winter wheat cultivars supported for registration. In 2010, the doubled haploid W434 received support to register in the Canada Western Red Winter wheat class, which is the first DH in this market class. W434 combines grain yield equal to the highest yielding check with higher grain protein concentration, shorter stronger straw, and improved resistance to leaf rust, stem rust, common bunt, and powdery mildew compared to the highest yielding check.

In 2010, the first DH durum wheat cultivar, DT801 (experimental name), was supported for registration. DT801 had 2.7% more grain yield than the highest yielding check, high grain protein concentration, greater yellow pigment in both the semolina and cooked pasta, and reduced accumulation of grain cadmium. DT801 expressed lower fusarium head blight symptoms and lower deoxynivalenol mycotoxin than the checks. Durum wheat is more recalcitrant than hexaploid wheat to generate doubled haploids, which has been partially overcome by better protocols (Knox *et al.* 2000).

Doubled haploid technology reduces the time to release a spring wheat cultivar by one to three years, while in winter wheat, which lacks the opportunity for a contra-season nursery, the process reduces the time by three to four years. Cultivar purity is easier to achieve in a DH cultivar than a conventionally bred cultivar especially if the final generation of selection is performed before complete homozygosity is achieved.

Since 1997 doubled haploid cultivars have been commercialized in seven of ten market classes. There has been no noticeable incompatibility among agronomic, disease resistance and end-use suitability traits, and doubled haploid production techniques. In turn, the doubled haploid cultivars to date have combined well with other parents.

Marker assisted breeding

In the past fifteen years, the development of molecular markers for numerous traits coupled with the evolution of DNA-based marker technologies has made marker assisted breeding (MAB) widely available and increasingly effective. Not only the number of traits amenable to marker assisted breeding has greatly increased but also the number of individuals (i.e. population size) that can be screened has increased (see review Somers & Humphreys 2009). While MAB was initially used mainly for germplasm development, it is now used routinely in most wheat breeding programs in Canada. Early applications of MAB focused on traits controlled by major genes including disease (Gold *et al.* 1999) and insect resistance (Thomas *et al.* 2005). Marker-assisted backcrossing for quality traits such as low cadmium content in durum wheat (Penner *et al.* 1995; Knox *et al.* 2009) and high grain protein content (Khan *et al.* 2000) was also possible. More recently, the fine mapping of genes controlling quantitative traits (Cuthbert *et al.* 2006) and the application of high throughput marker technologies (Somers & Humphreys 2009) have greatly expanded the applicability of MAB in plant breeding. A key to the successful integration of MAB has been development of markers tightly linked to the gene, "perfect" markers, and marker validation (Knox & Clarke 2007).

Since 2003, MAB has aided in the deployment of seven cultivars in three market classes (Table 3). The marker linked to *Gpc-B1*, high grain protein concentration from *Triticum turgidum* L. (Zhuk.) *dicoccoides* (Körn. Ex Asch. & Graebn), has been used to breed three cultivars in two market classes. Our experience has been that the segment of tetraploid wheat linked with elevated protein concentration when introgressed into a hexaploid background often expressed lower test weight but does not have any unduly negative effects on maturity or seed weight (Sherman *et al.* 2008). Using DNA markers, the chromosome segment has been reduced to a small linkage block of *Gpc-B1/Yr36* (DePauw *et al.* 2007). Lillian had time to maturity intermediate to the checks which all lacked the *Gpc-B1* marker, and test weight was similar to the lowest test weight check (DePauw *et al.* 2005). Lillian has expressed resistance to prevalent races of stripe rust in southern Alberta, Canada. The stripe rust resistance is due in part or in whole to *Yr36/Gpc-B1* and *Yr18/Lr34*. Because Lillian has been the most widely grown CWRS cultivar for the past four years, the small region containing *Gpc-B1* does not confer any adaptation disadvantage. Burnside which also carries *Gpc-B1* had grain yield similar to the highest yielding check, 0.9 units more grain protein, test weight similar to the highest yielding check but itself low test weight while maturing two days earlier (Humphreys *et al.* 2010). In 2009, Burnside was grown on 36% of the CWES area. Somerset also has *Gpc-B1*, and expresses higher grain protein concentration than Superb, 8% less grain yield than Superb, the highest yielding check, 2.7 days earlier maturity, but lower test weight (Fox *et al.* 2006).

Resistance to the orange wheat blossom midge *Sitodiplosis mosellana* (Géhin) is conferred by the *Sm1* gene on chromosome 2BS and selectable with the DNA marker WM1 (Thomas *et al.* 2005). The cultivars Goodeve (CWRS) and Burnside (CWES) have resistance to midge based on the marker assisted introgression of *Sm1* (Table 3). Bioassay on the inbred lines confirmed there had been

Table 3. Western Canadian wheat cultivars registered using marker assisted breeding, year of registration and the DNA marker and associated trait

Market class[a]	Name	Year registered	Breeding institution	DNA marker or gene, and trait
CWRS	Lillian	2003	AAFC, Swift Current & Winnipeg	*Gpc-B1/Yr36*, grain protein
CWRS	Somerset	2004	AAFC, Winnipeg	*Gpc-B1*, grain protein
CWRS	Goodeve	2007	AAFC, Swift Current	*Sm1*, midge resistance
CWES	Burnside	2004	AAFC, Winnipeg	*Gpc-B1*, grain protein
CWES	Glencross	2008	AAFC, Winnipeg	*Sm1*, midge resistance
CWAD	Brigade	2008	AAFC, Swift Current	*Cdu1*, grain cadmium uptake
CWAD	Verona	2008	CDC, University of Sask	*ScOpc20, Usw15*, grain cadmium uptake

[a]Abbreviations are listed in Table 1; AAFC – Agriculture and Agri-Food Canada; CDC – Crop Development Centre

no recombination between the marker and *Sm1* (DePauw *et al.* 2009; Humphreys *et al.* 2010).

Parents used in elite crosses are usually selected from advanced breeding lines. Thus, the accelerated production of cultivars also represents the accelerated production of elite parents, which allows plant breeders to deploy improved genetics back into the breeding programs more rapidly. Following the development of Burnside, the pyramiding of both high grain protein content and orange wheat blossom midge resistance was facilitated using MAB, through the registration of the DH CWES cultivar Glencross in 2007.

Grain cadmium (Cd) concentration became a selection criterion in Canadian durum breeding programs in the 1990s because of international concerns of food safety. The European Union, for example, adopted a standard of 200 ng/g for grain on 1 April, 2002. In durum, low grain Cd concentration is controlled by a single dominant gene designated as *Cdu1* (Penner *et al.* 1995; Clarke *et al.* 1997). This gene has been localized to chromosome 5BL (Knox *et al.* 2009), and has recently been fine mapped to a 0.3 cM interval (Wiebe *et al.* 2010). A dominant, random amplified polymorphic DNA (RAPD) marker, OPC-20, is linked in coupling to the high *Cdu1* allele (Penner *et al.* 1995) and has assisted selection for low Cd in the Canadian durum breeding programs. CDC Verona (Pozniak *et al.* 2009) was selected for low grain Cd levels using OPC-20 and expresses a greater than 50% reduction in grain Cd relative to high grain Cd commercial cultivars. The OPC-20 marker was used to evaluate heterogeneous parental lines in the development of Brigade durum wheat (Clarke *et al.* 2009). The marker analysis identified those particular plants within the cultivar which possessed the low Cd allele to be used as the parent in crosses with high Cd uptake parents.

The number of useful markers has increased in wheat, many currently based on simple sequence repeats (SSR). Marker detection, fine mapping leading to more proximal markers, and lab automation are allowing easier and more effective use of MAB. Pyramiding genes with strategic and complex crosses in combination with MAB is now becoming more of a technical matter rather than a research objective. The additional information available with MAB requires information management schemes (for example, International Crop Information Systems) to combine traditional agronomy, disease and end-use quality data with the tide of MAB data that is being generated.

In summary, marker-assisted breeding has proven to be an effective means to deploy traits into new genetic backgrounds. Marker assisted breeding and doubled haploid breeding can be used together to facilitate the rapid production of breeding lines that are fixed for the trait(s) of interest. When combined with modern breeding practices such as off-season nurseries, plant breeders can greatly accelerate the delivery of field-ready cultivars with improved genetics to producers. With the ongoing mapping of agronomically important genes and the development of high throughput marker systems the use of molecular markers in cultivar development will continue to be important for the foreseeable future.

Acknowledgements. Financial support from the producer check-off on wheat collected by the Canadian Wheat Board and administered by the Western Grains Research Foundation is gratefully acknowledged.

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http://www.inspection.gc.ca/english/plaveg/seesem/seeseme.shtml – The Canadian Food Inspection Agency is responsible for the administration of the *Seeds Act* and *Regulations* to help to ensure that seeds sold in, imported into and exported from Canada meet established standards for quality and are labelled so that they are properly represented in the marketplace, and are registered prior to sale in Canada.

http://www.pgdc.ca/committees_wrt.html – The Prairie Recommending Committee for Wheat, Rye and Triticale (PRCWRT) evaluates candidate cultivars of wheat, rye and triticale and makes recommendations to the Variety Registration Office, Canadian Food Inspection Agency regarding the suitability of the candidate for registration.