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Allelic reduction and genetic shift in the Canadian hard red spring wheat germplasm released from 1845 to 2004

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Abstract Analysis of genetic diversity changes in existing gene pools of cultivated crops is important for understanding the impact of plant breeding on crop genetic diversity and developing effective indicators for genetic diversity of cultivated plants. The objective of this study was to assess genetic diversity changes in 75 Canadian hard red wheat (*Triticum aestivum* L.) cultivars released from 1845 to 2004 using 31 simple sequence repeats (SSRs) markers. A total of 267 SSR alleles were detected, and their allelic frequencies ranged from 0.01 to 0.97, with an average of 0.14. Significant allelic reduction was observed at only four SSR loci for the cultivars released from 1970 onwards. However, 51 alleles (about 19%) present in pre-1910 cultivars were undetected in cultivars released after 1990 and were spread over 27 SSR loci. The proportion of SSR variation accounted for by six breeding periods was 12.5%, by four ancestral families, 16.5%, and by eight breeding programs, 8.4%. The average genetic diversity measured by three different band-sharing methods did not change significantly among cultivars released from different breeding periods, breeding programs, and ancestral families. However, genetic shift was obvious in the cultivars released over the six breeding periods, reflecting well the various breeding efforts over years. These results clearly show the allelic reduction and genetic shift in the Canadian hard red spring wheat germplasm released over time. Consequently, more effort needs to be made to broaden the wheat breeding base and conserve wheat germplasm.

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Introduction

Analysis of genetic diversity changes in existing gene pools of cultivated crops is important for understanding the impact of plant breeding on crop genetic diversity and developing effective indicators for the genetic diversity of cultivated plants. Little attention, however, has been paid to such analysis, particularly through the application of molecular markers. A few marker-based studies on diversity changes in cultivated gene pools found that the genome-wide loss of genetic diversity accompanying plant improvement was negligible (Donini et al. 2000; Christiansen et al. 2002; Fu et al. 2002, 2003b; Koebner et al. 2003) but that a significant allelic loss could occur at specific loci (Russell et al. 2000; Lu and Bernardo 2001; Fu et al. 2003a; Roussel et al. 2004). Such findings appear to offer little help for assessing the common assertion that modern crop breeding reduces genetic diversity (Duvick 1984; Allard 1996; Hoisington et al. 1999) and, consequently, provide little justification for the need for broadening plant breeding materials in a breeding program and conserving elite germplasm (Swanson 1996; Tripp 1996). While some of these findings may be true for a number of the existing gene pools of cultivated crops, biases may also exist due to the use of less representative cultivars in these studies or from the application of different molecular markers and diversity measurements. Thus, further effort is warranted to assess the diversity changes in existing gene pools of cultivated plants.

Wheat (*Triticum aestivum* L.) breeding in Canada began in 1886 with the goal to decrease the time to

maturity and improve the adaptation and quality of early introductions such as Red Fife (Neatby 1942; DePauw et al. 1995). By 1901, this breeding effort had produced many early-maturing cultivars, including Stanley, Preston, Huron, and Percy (Neatby 1942). Continuous selection and hybridization generated the high-quality and productive cultivar Marquis in 1909, and Marquis dominated the Canadian wheat production for two decades (Morrison 1960). With the devastating stem rust epidemics of 1928 and 1935, breeding was directed more toward selection for stem rust resistance, which resulted in several high-yielding cultivars with rust resistance, such as Renown, Apex, and Regent, by 1940 (Neatby 1942). Because the stem rust pathogen continually changes, breeding for stem rust resistance was shifted to develop multigenic, multi-race resistant cultivars (Green and Campbell 1979). Since the 1950s, backcrossing of stem rust-resistant genes into the Thatcher lines has produced several highly successful cultivars, such as Canthatch in 1959, Manitou in 1965, and Neepawa in 1969 (DePauw et al. 1995). Following the stabilization of stem rust resistance, around 1970, researchers shifted to backcrossing newly identified leaf and stem rust resistance genes to Neepawa (Knott 1982), which further enhanced the rust resistance of newly developed cultivars in the 1990s, such as Pasqua, AC Minto, AC Domain, and AC Cora (DePauw et al. 1995). Pasqua, released in 1990, carried five effective leaf rust resistance genes (Townley-Smith et al. 1993). In the early 1940s, an additional breeding effort was made to select for resistance to wheat stem sawfly (caused by *Cephus cinctus* Nort.; Fletcher 1897), which resulted in the generation of several sawfly-resistant cultivars such as Rescue in 1946 and Chinook in 1952. Over the last two decades, selection has been aimed more at the improvement of productivity, resistance to biotic and abiotic stresses, and end-use quality such as increased grain protein.

Early breeding efforts were largely carried out at research stations of Agriculture Canada and in the agricultural colleges of several universities. Currently, there are ten institutions across Canada with about 12 wheat breeders devoted to wheat improvement in ten different market classes, each with unique end-use suitability parameters. To date, breeding programs have developed and released over 100 spring wheat cultivars, most of which have had significant impacts on the economy of western Canada (Statistics Canada 2004). In spite of impressive achievements in grain yield (Hucl and Baker 1987), water-use efficiency, disease resistance, grain protein, and concentration and end-use suitability, there is concern about the narrowing of the wheat gene pool because selection has been based on fewer than 15 parental lines over the century of breeding. Up to now, no comprehensive study of the genetic diversity in Canadian wheat cultivars has ever been conducted (van Beuningen and Busch 1997).

Microsatellite (or simple sequence repeat; SSR) markers have proven to be important tools in wheat genetics and germplasm research (Devos et al. 1995; Plaschke et al. 1995; Röder et al. 1995; Donini et al. 1998; Bohn et al 1999). In recent years, these markers have also been applied to analyze diversity changes in wheat germplasm released over time. Donini et al. (2000) assessed the genetic diversity in 55 winter wheat cultivars grown in the UK from 1934 to 1994 and observed a qualitative shift in genetic diversity over time, probably due to the use of semi-dwarf wheat germplasm and the introgression of novel disease resistance genes. In an analysis of 75 Nordic spring wheat cultivars released from 1901 to 1993, Christiansen et al. (2002) found an increase in genetic diversity from 1901 to 1940, followed by a decrease from 1940 to 1960, and a second increase again from 1960 onwards. Roussel et al. (2004) made a comprehensive assessment of 559 French bread wheat accessions released from 1800 to 2000 which revealed a 25% decrease of allele richness from landraces to cultivars and a continuous reduction in allele richness for cultivars released since 1930. These findings, along with those of gliadin-based diversity observed in French and Spanish common wheats (Metakovksy and Branlard 1998; Metakovsky et al. 2000), appear to provide inconsistent information for understanding the impact of plant breeding on the genetic diversity of wheat cultivars.

The overall objective of this study was to analyze the patterns of genetic variability in 75 Canadian hard red spring wheat cultivars released from 1845 to 2004 using 31 SSR markers. It is this gene pool from which cultivars are registered to be eligible for grades of the Canada Western Red Spring class. Specifically, the SSR variability was analyzed with respect to breeding period, breeding program, and ancestral family with the aim of assessing the genetic diversity changes in the Canadian hard red spring wheat gene pool.

Materials and methods

Plant materials

Seventy-five Canadian hard red spring wheat cultivars (Table 1) were selected from a collection of 94 cultivars maintained at Plant Gene Resources of Canada (PGRC), Agriculture and Agri-Food Canada. The selection was based on pedigree analyses, agronomic and economic importance, and representation of different eras of hard red spring wheat breeding in Canada. Several Canadian wheat breeders and researchers were also consulted regarding the cultivar selection. The information collected for each cultivar with respect to its release year and originating program was verified by comparison with data available from the literature (Fraser and Whiteside 1956; DePauw et al. 1995; F. Clarke, personal communication) and from the related online information resources available on wheat pedigrees. Each cultivar was assigned to an ancestral family largely based on the coefficients of parentage calculated from the known pedigrees.

Table 1 Seventy-five Canadian hard red spring wheat cultivars chosen for this study with their year of release, origin, and accession number

Cultivar	Year[a]	Program[b]	Ancestry[c]	CN[d]	Cultivar	Year	Program	Ancestry	CN
Red Fife	1845	I.GC	EIR	11425	Neepawa	1969	CRC	NEE	11189
Ladoga	1887	I.RU	EIR	10921	Canuck	1973	SPARC	THA	9741
Stanley	1893	ECORC	EIR	11999	Sinton	1975	SPARC	THA	11968
Preston	1895	ECORC	EIR	11327	Chester	1976	LBRC	MAR	9786
Huron	1900	ECORC	EIR	1922	Benito	1979	CRC	NEE	2796
Percy	1901	ECORC	EIR	33679	Columbus	1980	CRC	NEE	37156
White Fife	1908	ECORC	EIR	12172	Katepwa	1981	CRC	NEE	38927
Marquis	1909	ECORC	MAR	11061	Leader	1981	SPARC	THA	38926
Prelude	1913	ECORC	EIR	11320	Lancer	1984	SPARC	THA	17840
Ruby	1917	ECORC	EIR	11902	Kenyon	1985	CDC	NEE	43842
Early Triumph	1918	REF	EIR	12093	Conway	1986	CDC	NEE	43840
Kota	1921	I.US	EIR	1798	Roblin	1986	CRC	NEE	43847
Supreme	1921	REF	EIR	12011	Laura	1986	SPARC	THA	44167
Renfrew	1924	UOA	MAR	1796	CDC Makwa	1990	CDC	NEE	52587
Garnet	1925	ECORC	EIR	10123	Pasqua	1990	CRC	NEE	106306
Broatch's Whitehead	1925	CDC	MAR	11140	CDC Teal	1991	CDC	NEE	52585
Red Bobs # 222	1926	UOA	EIR	11404	AC Minto	1991	CRC	NEE	106307
Ceres	1928	I.US	MAR	9774	CDC Merlin	1992	CDC	NEE	52586
Reward	1928	ECORC	MAR	11778	AC Michael	1993	LRC	NEE	52557
Reliance	1932	I.US	MAR	11766	AC Eatonia	1993	SPARC	THA	106365
Canus	1935	UOA	MAR	33637	AC Domain	1993	CRC	THA	106358
Thatcher	1935	I.US	THA	12060	Invader	1993	APAU	THA	106366
Coronation	1937	CRC	MAR	9844	AC Barrie	1994	SPARC	NEE	106318
Apex	1937	CDC	MAR	33627	AC Cora	1994	CRC	NEE	106353
Renown	1937	CRC	MAR	11773	Pacific	1994	CRC	NEE	106352
Regent	1939	CRC	MAR	11444	AC Majestic	1995	CRC	NEE	106357
Rescue	1946	SPARC	MAR	45654	Prodigy	1995	SWP	NEE	106329
Redman	1946	CRC	MAR	11428	AC Cadillac	1996	SPARC	NEE	106337
Saunders	1947	ECORC	THA	11943	McKenzie	1997	SWP	NEE	106330
Lee	1950	I.US	MAR	10947	AC Intrepid	1997	SPARC	NEE	106338
Chinook	1952	SPARC	THA	1738	AC Splendor	1997	CRC	NEE	106351
Selkirk	1953	CRC	MAR	11955	AC Abbey	1998	SPARC	THA	106336
Lake	1954	SRF	MAR	10923	Superb	2001	CRC	THA	106382
Canthatch	1959	CRC	THA	9740	Lovitt	2002	SPARC	NEE	106395
Pembina	1959	CRC	THA	11280	Journey	2002	SWP	NEE	106379
Cypress	1962	SPARC	THA	1739	Lillian	2003	SPARC	NEE	106394
Park	1963	LRC	THA	11267	Harvest	2004	CRC	THA	106393
Manitou	1965	CRC	THA	9883					

[a]Year of cultivar release or registration

[b]The code for the origin or breeding program from which a cultivar was developed. APAU, AgriPro and Agricore United joint breeding program; CDC, Crop Development Centre, University of Saskatchewan; CRC, Cereal Research Centre, Winnipeg; ECORC, Eastern Cereal and Oilseed Research Centre, Ottawa; I, Introductions from Australia (I.AU), Galicia region of central Europe (I.GC), Russia (I.RU), and USA (I.US); LRC, Lacombe Research Centre; LBRC, Lethbridge Research Centre; REF, Rosthern Experimental Farm; SPARC, Semiarid Prairie Agricultural Research Centre, Swift Current; SRF, Scott Research Farm; SWP, Saskatchewan Wheat Pool; UOA, University of Alberta

[c]The code for cultivar ancestral family. EIR, Early introductions and their relatives; MAR, Marquis family; THA, Thatcher family; NEE, Neepawa family

[d]CN, Canadian National accession number in the PGRC collection

DNA extraction

Approximately 20 seeds of each cultivar were randomly selected from the accession in the PGRC wheat collection and grown in the greenhouse at the Saskatoon Research Centre, Agriculture and Agri-Food Canada. Young leaves were collected from ten 5-day-old seedlings, bulked for each cultivar, freeze-dried with a Labconco Freeze Dry System (Kansas City, Mo.) for 3–5 days, and stored at −80°C. From each bulked sample, dry leaves were finely chopped and ground to a fine power in a 2-ml Eppendorf tube with two 3-mm glass beads on a horizontal shaker. Genomic DNA was extracted using a DNeasy Plant Mini kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer's directions. Extracted DNA was quantified by fluorimetry using Hoechst 33258 stain (Sigma Chemical, St. Louis, Mo.), followed by dilution to 25 ng/μl for SSR analysis.

SSR analysis

Based on reported polymorphism and genome coverage (Röder et al. 1998), 28 SSR primer pairs were selected for this study (Table 2). The left primer (4 μ*M*) was labeled with 74 kBq/μl γ-[^{33}P]-dATP (Perkin Elmer, Boston, Mass.) in 1× kinase forward reaction buffer, 0.8 U/μl T4 polynucleotide kinase (Invitrogen, Burlington, ON) in a final volume of 25 μl per 100 reactions, at

Table 2 Numbers of microsatellite alleles at individual loci for Canadian hard red spring wheat cultivars of the six breeding periods and significances of the permutation test for the difference in allelic count between cultivars before 1910 and those released from the other breeding periods

Locus	Chromosome arm	Total allele count[a]	PIC	Allele count for cultivars of various breeding periods[b]					
				pre-1910 (8)	1910–1929 (11)	1930–1949 (10)	1950–1969 (10)	1970–1989 (12)	1990–2004 (24)
gwm135	1A	7	0.93	2	3	3	3	3	1
gwm164	1A	6	0.65	4	4	4	5	3	4
gwm357	1A	5	0.51	2	4	3	3	2	3
gwm497	1A	8	0.66	1	2	2	2	2	1
gwm296	2A	18	0.48	5	8	4	3	2	4*
gwm448	2A	11	0.33	7	8	6	5	7	8
gwm497	2A	8	0.64	1	2	2	1	2	1
gwm636	2A	10	0.65	2	6	7	5	4	3
gwm102	2D	5	0.66	3	5	2	4	3	3
gwm210	2D	5	0.18	3	3	4	4	3	3
gwm261	2D	5	0.24	4	4	3	2	3	3
gwm296	2D	18	0.69	1	5	4	4	3	5
Gwm455	2D	7	0.34	4	5	2	3	3	4
Gwm484	2D	10	0.57	2	5	2	2	2	3
Gwm247	3B	10	0.47	3	6	4	4	3	2
Gwm003	3D	5	0.46	2	5	2	2	3	2
Gwm314	3D	3	0.54	1	2	2	1	1	1
Gwm497	3D	8	0.61	2	1	2	3	2	2
Gwm006	4B	13	0.35	6	6	7	6	9	6*
Gwm194	4D	6	0.46	3	3	4	4	1	5
Gwm213	5B	13	0.79	4	8	5	5	3	3*
Gwm544	5B	7	0.57	4	5	5	2	3	4
Gwm174	5D	22	0.37	8	13	5	6	6	10
Gwm190	5D	5	0.72	3	4	2	4	4	3
Gwm088	6B	16	0.44	7	9	9	10	8	12
Gwm276	7A	15	0.02	4	7	4	6	7	10
Gwm046	7B	11	0.70	3	7	3	6	3	6
Gwm302	7B	12	0.50	4	7	5	6	4	6
Gwm577	7B	9	0.80	2	5	7	6	5	6
Gwm611	7B	12	0.92	5	7	4	5	4	6
Gwm437	7D	13	0.64	5	7	4	4	3	4*

*Significance (0.05 level) of the permutation test for the difference in allele count between cultivars before 1910 and those released from the breeding period of interest

[a]According to the sizes of alleles mapped by Röder et al. (1998)

[b]The number in parenthesis below the breeding period is the number of cultivars assayed for the period

37°C for 1 h and 70°C for 10 min. The PCR contained 125 ng DNA, 1× buffer (Promega, Madison, Wis.), 1.5 m*M* $MgCl_2$, 200 µ*M* each of dNTP, 400 n*M* right primer, 360 n*M* unlabeled left primer, 40 n*M* γ-[^{33}P]-labeled left primer, and 1 U *Taq* polymerase (New England Biolabs, Pickering, ON) in a final volume of 25 µl per reaction. Different "Touchdown" PCR programs were used for different primer pairs depending on their melting temperatures (Röder et al. 1998). The PCR products were separated on a sequencing gel (Bio-Rad sequencing system; Hercules, Calif.) containing 6% polyacrylamide, 7 *M* urea, and 1× TBE at 90 W constant power for 2–3 h, blotted onto Whatman 3MM paper, vacuum dried for 2 h at 80°C, and exposed to Kodak BIOMAX film at −80°C for about 6 days depending on signal intensity.

Data analysis

To generate a dataset of SSR allele counts for each cultivar, DNA fragments amplified by SSR primer pairs were identified based on their sizes in base pairs as measured with a 10-bp DNA ladder (Invitrogen, Carlsbad, Calif.) and compared with the fragment sizes reported in the literature (Röder et al. 1998). Frequencies of the scored alleles were calculated with respect to primer, breeding period, breeding program, and ancestral family. To assess the informativeness of each marker, we calculated the polymorphic information content (PIC) for each locus, as described in Roussel et al. (2004). In this study, six decadal breeding periods (Table 2) were defined to reflect the major breeding efforts. To assess the patterns of allelic changes for each locus, the numbers of alleles detected in the cultivars of the different breeding periods were calculated. To analyze the overall allelic changes for the alleles detected by all the primer pairs, the numbers of alleles detected in the cultivars of various groupings (breeding period, breeding program, and ancestral family) were calculated.

The number of accessions for each group varied greatly, so a random permutation procedure was developed to assess the significance of the observed difference in allelic counts detected by all of the primer pairs between two groups of cultivars (Fu et al. 2003a). Specifically, an allele was chosen and, based on its

observed frequency of occurrence in the 75 cultivars, it was randomly allocated to the 75 cultivars without replacement regardless of cultivar origin or release year. This step was repeated for the other alleles identified in this study, followed by a count of the number of alleles for the "artificial" cultivars from a known group of a given size. The difference in allelic counts between two groups of "artificial" cultivars was calculated and compared with the actual observed difference. This random permutation of alleles was repeated 10,000 times. The numbers of alleles in these "artificial" cultivars was averaged over 10,000 runs to generate the expected and standard deviation of number of alleles for the cultivars in each group of interest. The proportion of the 10,000 runs in which the difference in allelic counts was larger than the observed allelic difference gave the probability of detecting the allelic difference between two cultivar groups. The simulation was done using a SAS program written in SAS IML (SAS Institute 2004) with respect to cultivars of various groupings (breeding period, ancestral family, and breeding program). To assess the significance of the observed difference in allelic counts per locus over the six breeding periods, we also performed the simulation for the alleles detected by each primer pair with respect to six decadal groups. The SAS program is available from the senior author.

An analysis of molecular variance (AMOVA) was performed using ARLEQUIN VER. 2.001 (Schneider et al. 2002) to partition the SSR variation within and among various groups of cultivars (breeding period, breeding program, and ancestral family). As the AMOVA takes into account not only the allelic richness but also allelic abundance (Excoffier et al. 1992), this analysis should offer more information to assess the genetic diversity changes over the breeding periods than those assessments based on allelic richness alone.

The overall diversity changes among various groups of the wheat cultivars was assessed by also estimating the average genetic similarity using three commonly employed similarity (or band-sharing) methods. The simple matching method, first described by Sokal and Michener (1958) and later applied by Apostol et al. (1993), defines the similarity as: $S_{ij}=(a+d)/(a+b+c+d)$, where S_{ij} is the similarity between two individuals i and j, a is the number of bands present in both i and j, b is the number of bands present in i and absent in j, c is the number of bands present in j and absent in i, and d is the number of bands absent from both i and j. The second method, proposed by Dice (1945) and later applied by Nei and Li (1979), calculates the similarity as: $S_{ij}=(2\ a)/(2\ a+b+c)$. The third method, described by Jaccard (1908), estimates the similarity as: $S_{ij}=\ a/(a+b+c)$. Note that a, b, and c used in the last two methods are the same as in the first method. In this study, dissimilarity (i.e., 1−similarity) was calculated using a SAS program written in SAS IML. To assess the associations of the individual cultivars, a principal coordinate (PCO) analysis was made based on their similarity matrix generated with Dice's method using the NTSYS-PC program (Rohlf 1997), and plots were made of the first three resulting principal coordinate scores. To assess the associations among decadal groups of the wheat cultivars, the groupwise similarity matrix was generated following Leonard et al. (1999) and clustered using NTSYS-PC with the algorithm of unweighted pair-group methods using arithmetic averages.

Results

SSR polymorphism

The 28 SSR primer pairs used revealed a total of 31 loci on 13 chromosomes (Röder et al. 1998; Table 2), representing all seven wheat homologous chromosome groups. Most primer pairs detected only one locus, but primer pair gwm296 detected two loci on chromosome arms 2AS and 2DS, and gwm496 revealed three loci on chromosome arms 1AL, 2AS, and 3DL, based on the fragment sizes reported by Röder et al. (1998). Overall, six loci located on chromosome arm 2DS (gwm102, gwm210, gwm261, gwm296, gwm455, and gwm484) and four loci on chromosome arm 1AL (gwm135, gwm164, gwm357, and gwm497) and chromosome arm 2AS (gwm296, gwm448, gwm497, and gwm636) represent the three largest clusters of loci in this chromosomal survey. A total of 267 SSR alleles was detected, but these could include some null alleles, as it was difficult to separate non-amplification due to experimental errors from null alleles. The number of detected alleles per primer ranged from 2 by gwm497 to 22 by gwm174, with an average of 9.5 alleles per primer pair. Values of each marker PIC ranged from 0.18 to 0.93, with an average of 0.54, but such variation was not significantly associated with the number of alleles detected. The two most informative loci were gwm135 detected on chromosome arm 1A and gwm611 on chromosome arm 7B. The frequency distribution of the 267 alleles is shown in Fig. 1A. The observed allelic frequencies ranged from 0.01 to 0.97, with an average of 0.14. There was one allele with an occurrence frequency of 0.97 in the cultivars and 205 alleles with frequencies of 0.15 or less. Among the 205 infrequent alleles, there were 60 with frequencies of 0.02 or less and 125 with frequencies of 0.05 or less. Thus, the detected SSR variation was widely spread over the wheat genome.

Changes in allelic richness

Based on the test with 10,000 random permutations, significant allelic reductions over the six breeding periods were observed at only four of the 31 SSR loci identified by gwm296 on chromosome arm 2AS, gwm006 on 4BL, gwm213 on 5BL, and gwm437 on 7DL (Table 2). Also, two SSR loci (gwm135 on 1AL and gwm247 on 3BL) displayed a marginal significance of allelic reduction ($0.05 < P < 0.07$). These reductions were

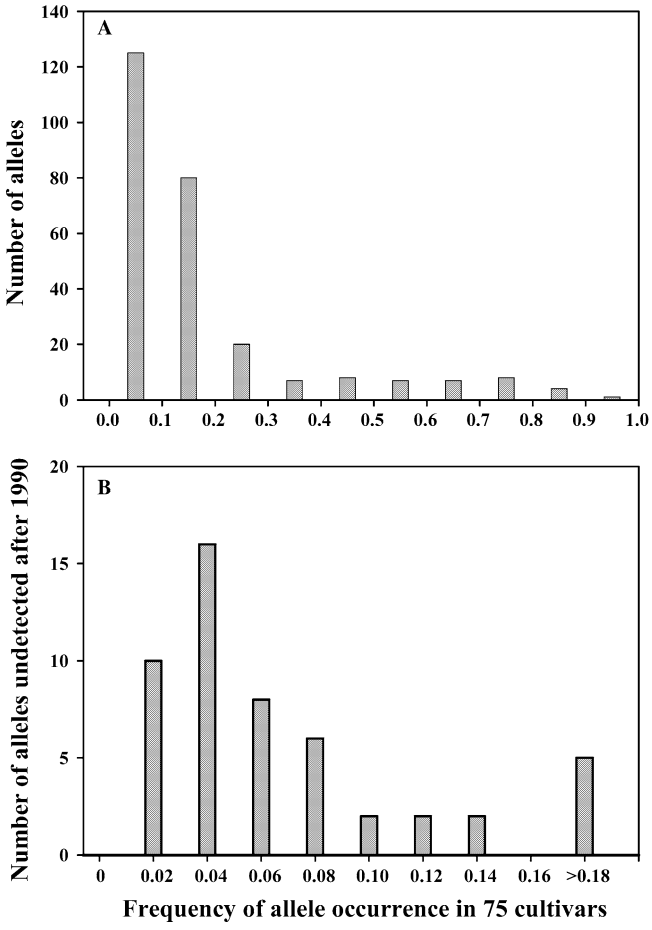

Fig. 1 Distributions of 267 microsatellite alleles (**A**) and 51 alleles undetected in the wheat cultivars released after 1990 (**B**) with respect to their occurrence frequencies in all 75 wheat cultivars. Note that different scales are presented in the axes of **A** and **B**

widely spread over six chromosome arms, indicating that the artificial selection had a significant impact on the wheat genome. Overall, a significant allelic reduction relative to those alleles detected in the cultivars released before 1910 was found for those cultivars released from 1970 onwards (Table 3). To reduce the effect of unequal group sizes on the comparisons of allelic changes among different periods, the observed allelic counts for each period were adjusted by the expected allelic count for a given number of cultivars for the period and standardized by 100. Clearly, up to 18% of the alleles present in the pre-1910 cultivars were undetected in cultivars released after 1990 (Table 3). Specifically, the percentages of adjusted alleles detected before 1930 ranged from 88% to 118%, but after 1970 they ranged from 76% to 70% (Table 3). These changes were due to the increased allelic disappearance associated with the decreased allelic introduction to the later cultivars over the six breeding periods. For example, the percentages of alleles lost relative to those cultivars before 1910 increased from 78% to 257%, while the percentages of alleles introduced decreased from 160% to 86%. From 1910 to 1929, more new alleles (160%) were introduced into the cultivars than the disappearance of the old alleles (78%). In contrast, from 1990 to 2004, only 86% alleles were introduced, but 257% disappeared.

Compared with the cultivars released before 1910, 51 alleles (19%) were undetected in the cultivars released after 1990, and their allele frequencies in all cultivars ranged from 0.01 to 0.37, with an average of 0.07 (Fig. 1B). There were 26 alleles with frequencies of less than 0.05, and five alleles with frequencies ranging from 0.20 to 0.37, indicating more rare alleles were lost than frequent alleles. These undetected alleles came from 27 (out of 31) SSR loci, suggesting that the allelic reduction was widely spread over the wheat genome. There were seven alleles undetected from the primer pair gwm006 on chromosome arm 4BL, six alleles from gwm611 on 7BL, and five from gwm296 on 2AS, and none from gwm497 on 2AS and 3DL, gwm314 on 3DL and gwm284 on 2DS.

Significant allelic reductions were also found with respect to ancestral family and breeding program. When cultivars were combined into three groups based on their pedigree and then compared with those introduced and their relatives, the percentages of alleles present in an ancestral family ranged from 61% to 99% and were significantly lower than 110% observed in the introductions and their relatives (Table 3). The percentages of allelic loss for an ancestral family ranged from 162% to 330%, while the percentages of alleles introduced into an ancestral family ranged from 115% to 70% (Table 3). The percentages of allelic disappearance were always larger than the percentages of allelic introduction into an ancestral family (Table 3). Similarly, when cultivars were grouped by breeding programs and compared with eight introductions, the percentages of alleles present in a breeding program ranged from 74% to 102% and were significantly lower than the 125% found in the introductions (Table 3). The percentages of allelic disappearance for a breeding program ranged from 135% to 319%, while the percentages of alleles introduced to a breeding program ranged from 48% to 99% (Table 3). The percentages of allelic disappearance in a breeding program were always larger than the percentages of allelic introduction into a breeding program (Table 3).

Changes in molecular variance

Based on the analyses of molecular variance of 267 SSR alleles, significant SSR differences existed among various groups of the wheat cultivars (Table 4). The proportion of SSR variation accounted for by decadal grouping was 12.5%, by ancestral family, 16.5%, and by breeding program, 8.4%. The proportions of SSR variation residing between the earliest (i.e., pre-1910) and the later decadal groups showed a gradual increase from 3.6% to 25.4% over the six breeding periods, but the increase was statistically significant only for those cultivars released from 1930 onwards (Table 5). This level of increase was compatible with the difference in the

Table 3 Numbers of microsatellite alleles observed in Canadian hard red spring wheat cultivars of various groups (breeding period, ancestral family, and breeding program) and percentages of alleles lost and newly detected in various groups relative to the earliest released group

Group[a]	Cultivar count	Observed allele count[b]			Percentage of adjusted alleles[c]			Prob(E > O)[d]
		Total	Lost	New	Total	Lost	New	
Breeding period								
Pre-1910	8	107			88			
1910–1929	11	166	28	87	118	78	160	1.0000
1930–1949	10	122	48	63	91	127	124	0.6065
1950–1969	10	126	48	67	94	127	132	0.7516
1970–1989	12	111	56	60	76	167	103	0.0167
1990–2004	24	134	51	78	70	257	86	0.0001
Ancestral family								
Introductions and relatives	14	171			110			
Marquis family	17	166	67	62	99	162	115	0.0402
Thatcher family	19	146	77	52	83	203	89	0.0001
Neepawa family	25	119	101	49	61	330	70	0.0001
Breeding program								
Introductions	7	142			125			
CDC (Saskatoon)	7	85	77	20	75	184	48	0.0001
CRC (Winnipeg)	22	148	61	67	80	319	73	0.0001
ECORC (Ottawa)	11	142	58	58	101	181	99	0.0020
SPARC (Swift Current)	15	119	71	48	74	274	67	0.0001
LRC, LBRC, SRF	4	83	79	20	97	146	76	0.0001
UOA (Edmonton)	3	72	82	12	97	135	58	0.0001
APAU, SWP, REF	6	108	68	34	102	151	92	0.0025

[a]See Table 1 for the cultivar grouping for ancestral family and breeding program

[b]Total, The total number of alleles detected in the cultivars of a specific group; Lost, the total number of alleles undetected in the cultivars of a specific group relative to those present in the earliest released group; New, the total number of new alleles detected in the cultivars of a specific group relative to those present in the earliest released group

[c]Percentage of adjusted alleles, The observed allele count adjusted by 100 over the expected allele count under a random scenario with a given group size. The expected allele count was obtained from 10,000 random permutations, as described in the text

[d]Prob(E > O), The proportion of the 10,000 random permutations showing that the simulated difference in the number of alleles between the earliest released group and the cultivars of a specific group was larger than the observed difference

percentages of adjusted alleles for the earliest and latest decadal groups (18% = 88% − 70%; Table 3).

An assessment of the within-group SSR variation measured as average pairwise difference among cultivars of each breeding period revealed a large increase—from 37.2 to 50.8—for the cultivars released from 1910 to 1929, followed by a sharp decrease to 38.7 for those released from 1930 to 1949 (Table 5). Again, a slight increase to 41.6 was observed for the cultivars released from 1950 to 1969, followed by a large decrease to 31.0 for those released from 1970 to 1989. In the last decadal group, the within-group variation showed some increase to 35.6. Overall, the within-group variation was relatively reduced over the six breeding periods (Table 5). Such changes in SSR variance appeared to be consistent with the net reductions in SSR alleles observed over the first five breeding periods (Table 3). However, significance testing of allelic reduction occurred only for the cultivars released after 1970, rather than after 1930 as for SSR variance. Higher sensitivity in AMOVA testing was expected as AMOVA took into account both allelic richness and abundance.

Table 4 Results for the analysis of molecular variance for Canadian hard red spring wheat cultivars released in different breeding periods, ancestral families, and breeding programs

Group/source	*df*	Variance component	Variation accounted for (%)
Breeding period			
Among breeding periods	5	2.75***	12.5
Within breeding periods	69	19.21***	87.5
Total	74	21.96	
Ancestral family			
Among ancestral families	3	3.69***	16.5
Within ancestral families	71	18.69***	83.5
Total	74	22.38	
Breeding program			
Among breeding programs	7	1.83***	8.4
Within breeding programs	67	19.92***	91.6
Total	74	21.75	

***Significant at $P < 0.001$

Changes in genetic similarity

When the genetic diversity was measured by the similarity (or dissimilarity) of 267 SSR alleles, the changes in average diversity relative to those cultivars in the earliest released group were not statistically significant over

Table 5 Results for the analysis of molecular variance of 267 microsatellite alleles over six decadal groups of Canadian hard red spring wheat cultivars. The average pairwise difference among cultivars of a breeding period is given on the diagonal. The percentage of variation residing between decadal groups of the wheat cultivars and the level of significance test by 10,000 random permutations are given above the diagonal

Source	Pre-1910	1910–1929	1930–1949	1950–1969	1970–1989	1990–2004
Pre-1910	37.2	3.6 $P < 0.063$	11.1 $P < 0.001$	13.0 $P < 0.001$	26.0 $P < 0.001$	25.4 $P < 0.001$
1910–1929		50.8	7.4 $P < 0.004$	6.2 $P < 0.005$	17.4 $P < 0.001$	18.1 $P < 0.001$
1930–1949			38.7	2.7 $P < 0.409$	14.1 $P < 0.001$	16.8 $P < 0.001$
1950–1969				41.6	5.6 $P < 0.035$	8.8 $P < 0.001$
1970–1989					31.0	1.8 $P < 0.115$
1990–2004						35.6

Table 6 Average genetic diversity of Canadian hard red spring wheat cultivars released in different breeding periods, ancestral families, and breeding programs calculated using Dice's method, simple matching coefficient, and Jaccard's method

Group[a]	Cultivar count	Average dissimilarity (standard deviation)[b]		
		Dice	SMC	Jaccard
Breeding period				
Pre-1910	8	0.486 (0.157)	0.136 (0.042)	0.638 (0.149)
1910–1929	11	0.650 (0.132)	0.189 (0.038)	0.780 (0.105)
1930–1949	10	0.509 (0.137)	0.144 (0.042)	0.663 (0.128)
1950–1969	10	0.567 (0.135)	0.154 (0.035)	0.713 (0.119)
1970–1989	12	0.427 (0.135)	0.115 (0.039)	0.585 (0.149)
1990–2004	24	0.498 (0.134)	0.132 (0.036)	0.653 (0.129)
Ancestral family				
Introductions and relatives	14	0.596 (0.136)	0.170 (0.038)	0.737 (0.115)
Marquis family	17	0.543 (0.152)	0.156 (0.047)	0.690 (0.139)
Thatcher family	19	0.528 (0.136)	0.143 (0.038)	0.680 (0.128)
Neepawa family	25	0.401 (0.144)	0.109 (0.040)	0.557 (0.155)
Breeding program				
Introductions	7	0.679 (0.098)	0.198 (0.030)	0.804 (0.077)
CDC (Saskatoon)	7	0.404 (0.149)	0.112 (0.044)	0.558 (0.171)
CRC (Winnipeg)	22	0.519 (0.161)	0.138 (0.042)	0.667 (0.151)
ECORC (Ottawa)	11	0.579 (0.138)	0.164 (0.038)	0.724 (0.117)
SPARC (Swift Current)	15	0.537 (0.134)	0.142 (0.037)	0.688 (0.125)
LRC, LBRC, SRF	4	0.517 (0.063)	0.147 (0.015)	0.680 (0.056)
UOA (Edmonton)	3	0.553 (0.049)	0.156 (0.026)	0.711 (0.041)
APAU, SWP, REF	6	0.545 (0.107)	0.161 (0.036)	0.699 (0.094)

[a]See Table 1 for the cultivar grouping for breeding program and ancestral family

[b]The three band-sharing methods used in this study were Dice's method (Dice 1945), simple matching coefficient (SMC, Sokal and Michener 1958), and Jaccard's method (Jaccard 1908)

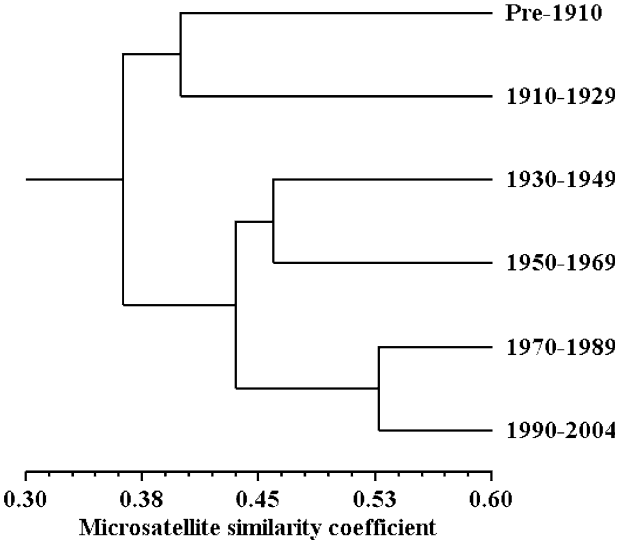

Fig. 2 Associations among six decadal groups of Canadian hard red spring wheat cultivars, as revealed by a cluster analysis based on the groupwise similarities of 267 microsatellite alleles

various breeding periods, breeding programs, and ancestral families (Table 6). For example, the average dissimilarities obtained with the Dice's method for the breeding periods appeared to decrease from 0.650 to 0.498 from 1930 to 2004, implying less diversity was maintained over the later breeding periods. However, the large standard deviations (>0.132) of these estimates made the significance test of the diversity difference less sensitive. Similar patterns were observed for the dissimilarity estimates generated with the simple matching and the Jaccard's methods, although the dissimilarities obtained from the simple matching method appeared to show relatively more variation among the groups with smaller standard deviation than the other two similarity measures. For example, the relative difference in average dissimilarity between cultivars of 1910–1929 and 1970–1989 was 0.39 ($=0.074/0.189$) for the simple matching method, 0.34 ($=0.223/0.650$) for Dice's method, and 0.25 ($=0.195/780$) for Jaccard's method (Table 6). The average diversity in the Neepawa family developed after 1970, although not

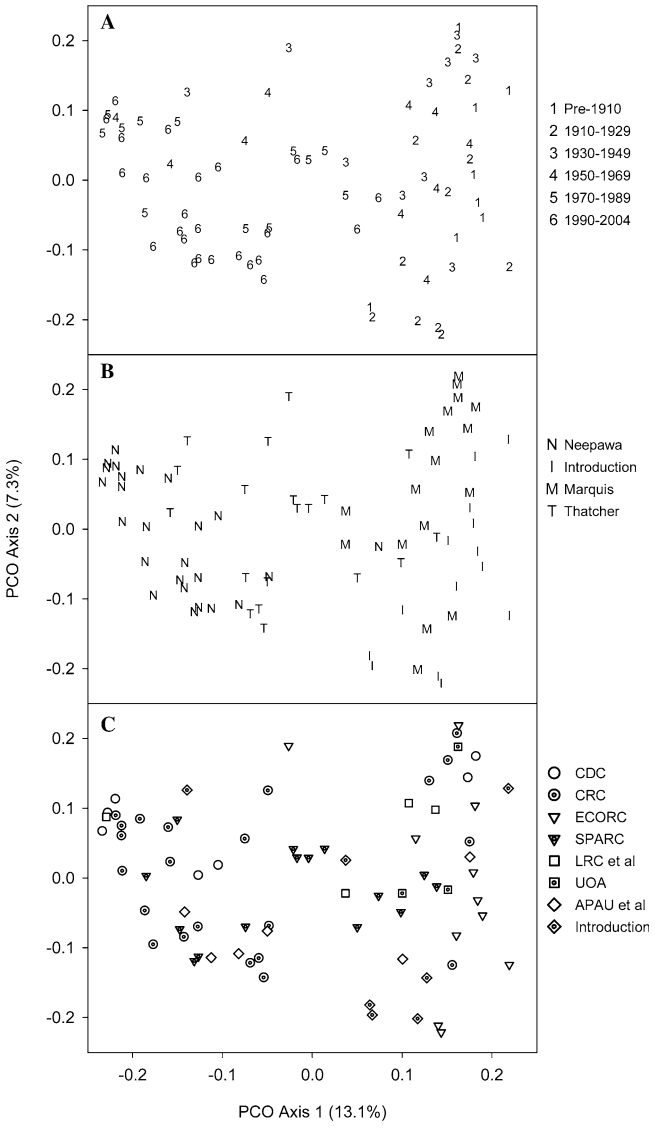

Fig. 3 Associations among 75 Canadian hard red spring wheat cultivars with respect to breeding period (**A**), ancestral family (**B**), and breeding program (**C**), as revealed by a principal coordinate analysis. Note that the three scatter plots (**A–C**) are the same, but individual cultivars were labeled differently with respect to each grouping. See Table 1 for the labels of cultivar grouping for ancestral family (**B**) and breeding program (**C**)

significantly different from the introduced cultivars, was the lowest among the four groups, and had an average dissimilarity estimate of 0.401 obtained from Dice's method. Comparisons of average dissimilarity among seven breeding programs showed that the seven cultivars developed from the Crop Development Centre at Saskatoon appeared to have the lowest average diversity, while the cultivars from the Eastern Cereal and Oilseed Research Centre at Ottawa had the highest average diversity.

While overall genetic diversity did not significantly change, a genetic shift over the six breeding periods was observed (Figs. 2, 3). Figure 2 shows the associations of the cultivar groups representing the six breeding periods. Three major clusters were found and appeared to reflect the three major types of breeding effort over time. Breeding efforts focused on early maturity and yield increase before 1930, disease resistance from 1930 to 1970, and multiple disease resistance and quality improvement after 1970. Genetically, these efforts increased the similarity of cultivars within periods, resulting in a gradual diversity shift. This shift was more obvious in Fig. 3, where associations of individual cultivars were assessed based on the principle coordinate (PCO) analysis. The first two PCO axes explained a reasonable amount of variation (13.1% and 7.3%, respectively). When the cultivars were labeled according to breeding periods, it is clear that the cultivars released later were gradually shifting away from early introductions from the right to the left of Fig. 3A. Such genetic shift reflected well the change in breeding focus over time as identified by ancestral family (Fig. 3B). For example, the Thatcher family, started in 1935, overlapped with the Marquis family established from 1909 to 1929, and the Neepawa family developed after 1969, but the latter two families were far apart. However, there was no evidence of genetic shift in the releases of cultivar from different breeding programs (Fig. 3C).

Discussion

This microsatellite analysis revealed several major findings. First, significant allelic reduction was observed at only four out of 31 SSR loci for the cultivars released from 1970 onwards. However, 51 alleles (about 19%) present in pre-1910 cultivars were undetected in cultivars released after 1990 and were spread over 27 of the SSR loci assayed. Second, the proportion of SSR variation accounted for by six breeding periods was 12.5%, by four ancestral families, 16.5%, and by eight breeding programs, 8.4%. Third, the average genetic diversity measured by three different band-sharing methods did not change significantly among cultivars released from different breeding periods, breeding programs, and ancestral families. Fourth, a genetic shift was observed in cultivars released over the six breeding periods which reflected well various breeding efforts over time. These findings clearly demonstrate various natures of the breeding impacts on the wheat genome, not only reducing allelic richness at specific loci, but also shifting genetic background in the Canadian hard red spring wheat germplasm released over years.

A similar genetic shift was also observed in other gene pools of wheat (Donini et al. 2000; Christiansen et al. 2002; Roussel et al. 2004) and barley (Koebner et al. 2003), but it was not as clear-cut as that revealed in the Canadian hard red spring wheat germplasm. This shift was expected, as the genepool was converted on the unique milling and baking properties in Marquis and Red Fife. Only two of nine cultivars from prior to

1910 were retained in the gene pool that led to the later development of unique milling and baking properties of the Marquis-type (Neatby 1942; DePauw et al. 1995). Specifically, the Canadian hard red spring wheat breeding effort has focused mainly on the development of three ancestral families to improve grain yield, water-use efficiency, disease and pest resistance, protein concentration, and quality: Marquis family (1909), Thatcher family (1935), and Neepawa family (1969). Less recognized and appreciated, however, is the importance of genetic-shift analysis in assessing the impact of plant breeding on the constitution of an improved gene pool. As demonstrated in this analysis, the history of ancestry-based crop breeding can map the genetic change in the gene pool of a cultivated crop such as Canadian spring wheat.

Comparisons of the allelic richness, molecular variance, and average genetic diversity over the six breeding periods revealed the same patterns of genetic change (Tables 3, 5, 6), although the change in the average genetic diversity was not statistically significant. The increase in allelic richness, within-group molecular variance, and average genetic diversity of cultivars released from 1910 to 1929 reflected well the consequences of extensive hybridization carried out over the period. This hybridization not only generated impressive improvement in shifting the negative relationship of grain yield and protein concentration, but also simultaneously broadened the genetic background of the released cultivars by employing genetically diverse lines (DePauw et al. 1998). The decrease in genetic diversity of cultivars released from 1930 to 1969 may partly be explained by the large breeding effort since the 1940s to utilize almost all of the known stem rust resistance genes by backcrossing to resistant parental lines such as Thatcher. Continuous backcrossing of newly identified leaf and stem rust resistance genes to Neepawa and intensive selection for quality and grain yield further reduced the genetic diversity in cultivars released after 1970. Such a reduction was more significant, as reflected in the allelic changes at several SSR loci (Table 2).

Comparison of these diversity reductions with those in the Canadian oat gene pool (Fu et al. 2003a; 2004), over approximately the same breeding period, revealed similar patterns of genetic change. Such similarity was not surprising because the breeding methods applied in the Canadian wheat and oat breeding programs were largely the same, particularly with respect to the introgression of disease resistance genes into the gene pools. Thus, reliance on a few ancestral types combined with the introgression of specific genes for biotic and abiotic stresses, and other specific traits had a considerable impact on crop genomes. Undoubtedly, such an impact appeals for more effort in conserving diverse wheat germplasm, particularly when considering the wide distribution of 60 disappeared alleles in the latest cultivars over the wheat genome (i.e., on 27 out of 31 SSR loci) and the significant allelic reduction in four SSR loci. However, such genetic reductions may not pose an immediate genetic threat to the current Canadian hard red spring wheat breeding, as 87% of the SSR loci surveyed displayed non-significant allelic changes. Also, the majority of SSR alleles that disappeared in the cultivars after 1990 were rare, and such rare alleles contributed little to the overall genetic diversity. Moreover, the assayed SSR markers were presumably neutral (or non-functional), and the disappearance of these alleles may not be associated directly with genes that contribute to an adaptive or economic value. To confirm this reasoning requires further assessment on the proportion of the disappeared alleles accounted for by artificial selection, rather than genetic drift, and whether the affected markers were directly "selected" for or indirectly associated with targeted genes in breeding programs. To this end, an attempt was made using 13 functional single-nucleotide polymorphism (SNP) markers derived from hexaploid wheat expressed sequence tags (ESTs; Somers et al. 2003) to assay the same 75 wheat cultivars studied here, but the 26 SNP alleles obtained were not reduced over the six breeding periods (Fu et al. unpublished). This probably is either due to the small number of functional SNP markers used or the biased sampling of functional regions under little breeding pressure.

The findings presented here are consistent with those previously reported in different gene pools of wheat and other crops as introduced above. They help to confirm the minimal impact observed so far by selective improvement on genome-wide diversity, but possible significant loss of alleles at specific loci. Thus, assessment of diversity change in a gene pool would be most informative when allelic diversity itself is the target of study, and multi-allelic markers such as SSRs should be applied to monitor the change in allelic richness. Interestingly, four SSR loci that exhibited significant allelic changes were spread over four chromosomal segments, rather than clustered at one or two chromosome regions. Further analyses of these specific chromosomal segments using mapped SSR markers may shed more insight into the regions of the wheat genome affected by selective improvement.

Allelic reduction and genetic shift observed in the Canadian hard red spring wheat germplasm underscores a need for continuous diversification of wheat breeding materials to ensure that breeding programs continue to be sustainable in the future. To facilitate the diversification of germplasm, conservation of genetically diverse germplasm is a prerequisite and is critical for long-term breeding efforts. Eventually, the introgression of new genes or incorporation of new gene complexes will be needed in wheat breeding programs to overcome a possible "genetic ceiling" in improvement, to avoid genetic vulnerability to biotic and abiotic stresses, and to widen crop adaptation to new environments. Thus, more attention needs to be paid to integrated efforts in the conservation of wheat germplasm and exploration for new sources of desirable alleles.

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