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ORIGINAL PAPER

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Impact of plant breeding on genetic diversity of the Canadian hard red spring wheat germplasm as revealed by EST-derived SSR markers

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Abstract Genetic diversity changes in wheat germplasm have been studied using different molecular markers, but little is known about the impact of plant breeding on the transcribed segments of the wheat genome. The objective of this study was to assess diversity changes in 75 Canadian hard red wheat cultivars released from 1845 to 2004 using 37 EST-derived microsatellite (eSSR) markers. These markers were derived from at least 19 transcribed sequences with putative functions assigned and sampled 17 wheat chromosomes. A total of 138 eSSR alleles was detected, and their allelic frequencies ranged from 0.01 to 0.99 with an average of 0.41. Allelic counts were significantly reduced at three loci for cultivars released after 1990. Sixteen alleles at 14 loci in pre-1910 cultivars were lost in cultivars released after 1990. The lost alleles had frequencies ranging from 0.03 to 0.17 and averaging 0.07. Partitioning the eSSR variation showed the four ancestral families accounted for 14.7% of the variation, followed by the six breeding periods with 12.8% and the eight breeding programs with 5.8%. A genetic shift was observed in the cultivars released over the six breeding periods, reflecting the various breeding efforts. These results illustrate the impact of the Canadian wheat breeding on the transcriptional segments of the wheat genome. These findings, along with those from genomic SSR markers, suggest the Canadian wheat breeding programs have reduced genetic diversity in the hard red spring wheat.

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Introduction

Genetic diversity changes in wheat (*Triticum aestivum* L.) germplasm have been studied using several different kinds of molecular markers, but the impact of modern plant breeding on wheat genetic diversity in regional breeding programs has been poorly understood. Some studies have suggested the diversity reduction accompanying plant improvement has been limited (Donini et al. 2000; Christiansen et al. 2002; Khan et al. 2005; Reif et al. 2005). For example, Donini et al. (2000) assessed the genetic diversity in 55 winter wheat cultivars grown in the UK from 1934 to 1994 and observed just a qualitative shift in genetic diversity over time. Other studies have demonstrated the reduction of allelic counts in some improved gene pools of wheat (Roussel et al. 2004, 2005; Fu et al. 2005). Roussel et al. (2004) assessed 559 French bread wheat accessions released from 1800 to 2000 and revealed a 25% decrease of allele counts from landraces to cultivars, and a continuous reduction in allelic diversity for cultivars released since 1930. Such a discrepancy can be partly explained, as breeding methods and selective pressures may differ in various breeding programs and significant diversity reduction should not be expected to occur in every improved gene pool of wheat. Also, all the studies used different markers of unequal quality (Bohn et al. 1999) and diversity measurements of variable accuracy (Mohammadi and Prasanna 2003), thus making the generalization of the findings difficult. Moreover, bias may exist in the diversity comparison of unequally sized groups and in the selection of less representative cultivars for different breeding periods (Fu et al. 2003). Thus,

it is important to recognize these limitations for an informative study of genetic diversity changes.

In 2003, we initiated an assessment on the genetic diversity of 75 Canadian hard red wheat cultivars released since 1845 using 31 genomic simple sequence repeat (gSSR) markers. This study revealed a significant reduction in allelic count at four (13%) gSSR loci for the cultivars released from 1970 onwards (Fu et al. 2005). Little is known if such a selective impact has also affected the transcribed segments of the wheat genome, as gSSR markers presumably are neutral (or frequently non-coding) and may represent different regions of the wheat genome. Publications in 2004 of many mapped wheat EST (expressed sequence tag)-derived microsatellite (eSSR) markers (Gao et al. 2004; Nicot et al. 2004; Yu et al. 2004) inspired a repeat of the initial assessment with the hope of determining the generality of the selective impact of plant breeding, particularly on the transcribed segments of the wheat genome. A loss of function-associated alleles is of most concern, as narrowing the base of functional genes may reduce the allelic diversity contributing to an adaptive or economic value.

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

Table 1 Numbers of EST-derived microsatellite alleles at individual loci, significances of permutation test for the difference in allelic count between cultivars released before 1910 and after 1990, and putative functions assigned

Primer/locus[a]	Chromosome arm[b]	Total allele count	Period-specific allele count[c]			Function (accession number)[d]
			1	6	Loss	
cwm435	1A	3	3	3		Gamma-gliadin (AAK84773)
cwm261	1AL	5	2	3	L1	
cwm75	1AS	5	3	3	L1	LMW glutenin precursor (AA017157)
ksum104	1AS	3	2	3		LMW glutenin subunit (AJ293097)
cwm109	1AS	3	2	3		LMW glutenin subunit (T06982)
ksum117	1AS/1BS	3	3	3		
ksum157	1BS/6AS	4	4	4		
cwmB	1D	3	2	2		Gamma-gliadin (AAK84779)
cwm170	1DS	4	1	2		LMW glutenin (CAB41921)
cwm214	2AS	3	3	3		LTS responsive protein (BAC16385)
cnl127	2AS	2	2	1	L1*	
cwm568	2BL	4	3	3	L1	HL zipper protein (AA072559)
ksum174	2DS	4	2	4		
ksum244	2DS/4BL	2	1	1		
cwm325	3AL	5	3	3	L1	Hypothetical protein (AAM65538)
cwm93	3AS	4	3	2	L1	PEP carboxykinase (AJ250829)
cnl062	3BL	3	2	3		Inorganic pyrophosphatase (AF093629)
cwm502	3BS	3	3	2	L1	
ksum134	4AL	2	2	1	L1*	
ksum8	4AL	5	5	3	L2**	
ksum130	4AL^	4	3	4		
ksum24	4AL/5AL	3	1	2		
ksum154	4BL	2	2	2		
ksum62	4BL	8	5	5	L1	
cnl123	4BL/4D	4	4	4		Zinc finger protein (AB001882)
ksum180	4D	4	4	3	L1	Alpha-/beta-gliadin A-II (M10092)
cwm232	5BL	3	2	3		
ksum69	5BS^/5D	3	1	3		
cwmA	5D	4	2	2		Small GTP-binding protein (AF112964)
cwm94	5DL	5	4	4	L1	ABAiP membrane protein (U80037)
cwm162	6A	2	2	1		Alpha-gliadin precursor (T06282)
Cnl64	6BS	4	2	4	L1	Phenylalanine ammonia-lyase (Z49146)
cnl113	6BS	2	2	2		
cwm48	7A^	10	4	8		Vacuolar invertase (AF069309/T06226)
cwm334	7AS	2	2	2		GAMYB-binding protein (AY167561)
cwm267	7AS	3	3	3		CSC subunit-like protein (T51546)
cwm206	7BL	5	3	3	L2	

[a]The primer/locus designation follows Yu et al. (2004) and Gao et al. (2004). Primer pairs cwmA have left primer 5-AGGAGTTTCTCCCCGGATT-3, right primer 5-TAGAAGAACCAGCTTGGCGT-3, and motif (ccggcg)[3]. Primer pairs cwmB have left primer 5-AAAGCGATTGCCAAGTGATGC-3, right primer 5-GCTGGTTGTTGTGGTTGGATG-3, and motif (aac)[6]

[b]The chromosome arm was cited following Yu et al. (2004) and Gao et al. (2004). Possible multiple loci were given with ^ and loci with uncertain (short or long) chromosomal arms were given with the chromosomes only

[c]Allele count was made for cultivars of two breeding periods: 1 (pre-1910) and 6 (1990–2004). The loss column shows the number of alleles lost in the cultivars after 1990 relative to alleles detected in the pre-1910 cultivars. L1 or L2 = one or two alleles lost. Significant loss was shown with * or ** for $P < 0.05$ or $P < 0.01$, respectively

[d]The putative function was cited from the related accession available in Genbank

37 eSSR markers. Specifically, the eSSR variability was analyzed with respect to breeding period, breeding program and ancestral family with the aim to determine the impact of plant breeding on the transcriptional segments of the wheat genome.

Materials and methods

Plant materials

The same 75 Canadian hard red spring wheat cultivars described in detail in Table 1 of Fu et al. (2005) were studied here. Briefly, the wheat accessions consisted of cultivars introduced since 1845 from four regions (Australia, Central Europe, Russia, and USA) and developed from seven major groups of the Canadian wheat breeding program from 1893 to 2004. The major breeding groups were Crop Development Centre (CDC), Saskatoon; Cereal Research Centre (CRC), Winnipeg; Eastern Cereal and Oilseed Research Centre (ECORC), Ottawa; Semiarid Prairie Agricultural Research Centre (SPARC), Swift Current; Lacombe Research Centre (LRC), Lethbridge Research Centre (LBRC), Scott Research Farm (SRC); Univ of Alberta, Edmonton; and AgriPro and Agricore United joint breeding program (APAU), Saskatchewan Wheat Pool (SWP), Rosthern Experimental Farm (REF). Cultivars released from six different periods (pre-1910, 1911–1929, 1930–1949, 1950–1969, 1970–1989, and 1990–2004) were grouped to facilitate the analysis of diversity changes. Based on the coefficients of parentage calculated from known pedigrees, four ancestral families of cultivars were established (Introductions and relatives, Marquis family, Thatcher family, and Neepawa family) to reflect the breeding efforts aimed at improving productivity, disease resistance, resistance to abiotic stress, and end-use quality (DePauw et al. 1995).

DNA extraction and eSSR analysis

Seed selection, greenhouse planting, bulk sampling of young leaves for each cultivar, DNA extraction, and quantification were described in Fu et al. (2005). The same extracted DNA was used for this analysis. Based on reported polymorphism, 169 eSSR primer pairs were chosen for a preliminary screening on eight diverse wheat cultivars: 21 Cornell eSSR (cnl), 64 Kansas State University eSSR (ksum) (Yu et al. 2004), and 84 Chinese wheat eSSR (cwm) (Gao et al. 2004) primers. The polymerase chain reaction (PCR) solution for the cnl and ksum primers from Yu et al. (2004) was applied using 50 ng of DNA template in a final volume

Table 2 Numbers of EST-derived 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]	Cultivarcount	Observed allele count[b]			Adjusted alleles (%)[c]			Prob($E > O$)[d]
		Total	Lost	New	Total	Lost	New	
Breeding period								
Pre-1910	8	98			95			
1910–1929	11	111	4	17	101	33	88	0.8647
1930–1949	10	101	17	20	94	131	110	0.3788
1950–1969	10	102	15	19	95	115	104	0.4471
1970–1989	12	100	20	22	89	182	108	0.1041
1990–2004	24	108	16	26	85	348	91	0.0037
Ancestral family								
Introductions and relatives	14	110			95			
Marquis family	17	119	8	17	99	73	113	0.8579
Thatcher family	19	116	18	24	95	189	150	0.5005
Neepawa family	25	102	30	22	80	457	121	0.0001
Breeding program								
Introductions	7	107			107			
CDC (Saskatoon)	7	80	33	6	80	207	38	0.0001
CRC (Winnipeg)	22	116	15	24	93	314	80	0.0015
ECORC (Ottawa)	11	109	12	14	99	110	66	0.0760
SPARC (Swift Current)	15	112	15	20	96	192	79	0.0132
LRC, LBRC, SRF	4	84	29	6	96	127	57	0.0343
UOA (Edmonton)	3	84	27	4	104	101	49	0.2440
APAU, SWP, REF	6	101	19	13	105	107	91	0.3609

[a]See the text 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]Percent of adjusted alleles = the observed allele count adjusted by 100 over the expected allele count under random scenario with a given group size. The expected allele count was obtained from 10,000 random permutations

[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

of 25 μl. Touchdown PCR cycling conditions were modified by reducing the cycle times to 30 s denaturation, 30 s primer annealing, and 1 min synthesis. For the cwm primers, the PCR solution of Röder et al. (1998) was used. Different touchdown PCR programs based on those of Röder et al. (1998), with cycle times reduced to 40 s denaturation, 40 s primer annealing, and 1 min synthesis, were used for different primers depending on their melting temperatures (Gao et al. 2003). All PCR reactions were performed in an MJ Research DYAD thermocycler (BioRad, Mississauga, ON, Canada). The PCR products were separated on 3% 7:6 Metaphor (Cambrex, Rockland ME, USA): Type 1-A Low EEO (Sigma Chemical Co., St. Louis, MO, USA) agarose in 1× TAE buffer for 5 h at 100 V. Gels were stained post-separation with 0.5 mg/l ethidium bromide for 20 min and recorded on a digital gel documentation system.

Based on the preliminary screening and the coverage of the wheat genome, 37 of the most informative primer pairs (Table 1) were selected to genotype 75 Canadian wheat cultivars. Samples were re-amplified using the protocols described above. The PCR products were separated on a 6% (w/v) non-denaturing acrylamide:bis-acrylamide (19:1) (BioRad, Mississauga, ON, Canada) gel in 1× TBE buffer with 0.5 mg/l ethidium bromide for 2–2.5 h (Wang et al. 2003).

Data analysis

To generate a dataset of eSSR allele counts for each cultivar, DNA fragments amplified by eSSR primer pairs were identified based on their sizes in base pairs measured with a 10 bp DNA ladder (Invitrogen, Carlsbad, CA, USA) and compared with the fragment sizes reported in the literature (Gao et al. 2003, 2004; Yu et al. 2004). In this study, single locus was considered for the possible multiple loci generated by the three primer pairs (ksum130, ksum69, and cwm48), as there was not enough information available to distinguish among alleles of different loci. Frequencies of the scored alleles were calculated with respect to primer, breeding period, breeding program, and ancestral family, and plotted for visualization. To assess the informativeness of each marker, the polymorphic information content (PIC) was calculated for each locus, as described in Roussel et al. (2004). Further analyses of eSSR data were made following Fu et al. (2005) to assess (1) the allelic changes for single locus for the six breeding periods, (2) the allelic changes for all the loci with respect to breeding period, ancestral family, and breeding program, (3) the molecular variances for breeding period, ancestral family, and breeding program, and (4) the genetic associations of the individual and grouped cultivars.

The same random permutation procedure described in Fu et al. (2005) was applied to (1) assess the significance of the difference in allelic count between two groups of unequal numbers of cultivars and to (2) generate the expected allele counts for groups of unbalanced cultivar numbers. 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 counting the number of alleles for the "artificial" cultivars from a known group of 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. These random permutations were 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).

Results and discussion

SSR polymorphism

The 37 eSSR primer pairs consisted of five SSR types (7 di-, 24 tri-, 2 tetra-, 1 penta-, and 3 hexa-nucleotide repeats) and revealed 37 loci on 17 chromosomes (Table 1, Gao et al. 2004; Yu et al. 2004), representing all seven wheat homologous chromosome groups. The chromosome with the most loci detected was 1A (with six possible loci), followed by 4A (with four possible loci). A total of 138 eSSR alleles was detected, but they could include some null alleles, as it was difficult to separate non-amplification from null alleles. The number of alleles detected per primer ranged from two for ksum134 to 10 for cwm48, with an average of 3.7 alleles per primer pair. PIC values of each marker ranged from 0.03 to 0.98 with an average of 0.38, but such variation was not significantly associated with the number of alleles detected. The two loci with the most discriminatory power were ksum154 on chromosome arm 4BL and cwm206 on chromosome arm 7BL. The frequency distribution of the 138 alleles is shown in Fig. 1a. The observed allelic frequencies ranged from 0.01 to 0.99 with an average of 0.41. Most of the alleles appeared to be either frequently or infrequently present in the assayed cultivars. Forty-six alleles (33%) were present in a small proportion of the cultivars (with frequencies of 0.10 or lower) and 22 alleles (16%) were present in most of the accessions (with frequencies of 0.90 or higher). Among the 46 rare al-

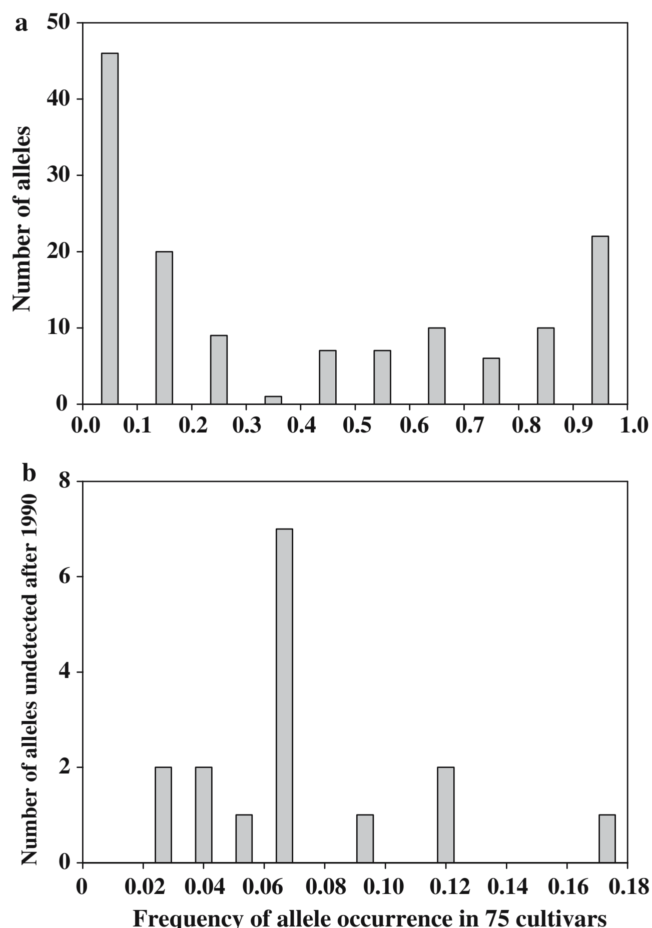

Fig. 1 Frequency distributions of 138 EST-derived microsatellite alleles (**a**) and 16 alleles lost in the wheat cultivars released after 1990 (**b**) with respect to their occurrence frequencies in all 75 wheat cultivars. Note that different scales are presented in the axes of (**a**–**b**). The interval of occurrence frequency is shown in *X*-axis for **a**, but the exact occurrence frequency for (**b**)

leles there were 21 with frequencies less than 0.05 and 25 with frequencies from 0.05 to 0.1. Clearly, the eSSR variation detected was widely distributed over the wheat genome.

Changes in allele number

Significant reductions in allelic count occurred over the six breeding periods at only three out of 37 eSSR loci. Those loci were identified by ksum8 and ksum134 on chromosome arm 4AL and cnl127 on 2AS (Table 1), but it was not clear what functional genes these loci were associated. Also, allelic counts at three other eSSR loci (cwm93 on 3AS, cwm502 on 3BS, and cwm162 on 6A) were marginally reduced ($0.05 < P < 0.08$). These reductions were distributed on five chromosome arms, indicating the artificial selection had significant impact on the wheat genome. Overall, a significant reduction in allelic count relative to the cultivars released before 1910 was found for those cultivars released after 1990 (Table 2). 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 10% of alleles present in the pre-1910 cultivars were undetected in the cultivars released after 1990 (Table 2). Specifically, the percentages of adjusted alleles detected before 1930 ranged from 95 to 101% of those relative alleles expected for the breeding periods of given sample sizes, followed by a reduction from 94 to 85% from 1930 onwards (Table 2). These changes reflected the loss of alleles over the six breeding periods, while the rate of introduction of new alleles was largely unchanged (Table 2). For example, the percentages of alleles lost relative to those cultivars before 1910 increased from 33 to 348% of those relative alleles expected for the breeding periods of given sample sizes. The percentages of introduced alleles increased from 88 to 110% before 1950, and decreased to 91% after 1990.

Compared with the cultivars released before 1910, 16 (12%) alleles were undetected in the cultivars released after 1990. The frequencies of these undetected alleles in all cultivars ranged from 0.03 to 0.17 with an average of 0.07 (Fig. 1b). Thirteen of the 16 alleles lost had frequencies of < 0.10, indicating the rare alleles were most frequently lost. These undetected alleles

Table 3 Results for the analysis of molecular variance of 138 EST-derived microsatellite alleles 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	2.07***	12.8
Within breeding periods	69	14.06***	87.2
Total	74	16.13	
Ancestral family			
Among ancestral families	3	2.41***	14.7
Within ancestral families	71	13.95***	85.3
Total	74	16.36	
Breeding program			
Among breeding programs	7	0.93***	5.8
Within breeding programs	67	14.97***	94.2
Total	74	15.90	

***Significant at $P < 0.001$

Table 4 Results for the analysis of molecular variance of 138 EST-derived microsatellite alleles over six breeding periods of Canadian hard red spring wheat cultivars

Source	Pre-1910	1910–1929	1930–1949	1950–1969	1970–1989	1990–2004
Pre-1910	27.5	3.6 $P < 0.083$	12.5 $P < 0.001$	13.4 $P < 0.001$	19.1 $P < 0.001$	24.6 $P < 0.001$
1910–1929		34.4	7.8 $P < 0.001$	11.2 $P < 0.001$	16.7 $P < 0.001$	20.7 $P < 0.001$
1930–1949			26.9	4.7 $P < 0.041$	9.3 $P < 0.002$	13.9 $P < 0.001$
1950–1969				29.2	3.7 $P < 0.055$	11.7 $P < 0.001$
1970–1989					26.9	4.2 $P < 0.017$
1990–2004						26.1

The average pairwise difference among cultivars of a breeding period is given on the diagonal. The percentage of variation residing between breeding periods of the wheat cultivars and the level of significance test by 10,000 random permutations are given above the diagonal

came from 14 (out of 37) eSSR loci with two loci losing two alleles (Table 1), suggesting the allelic reduction was widely spread over the wheat genome. Seven of the loci with allelic loss were detected by primers designed from transcribed sequences with putative functions assigned (Table 1). The loci with allelic loss apparently were not associated with the SSR types of various nucleotide repeats and their PIC values.

Significant reductions in allelic count were also found with respect to ancestral family and breeding program. When cultivars were combined into three groups based on their pedigree and compared with those introduced and their relatives, the percentages of alleles present in an ancestral family ranged from 80 to 99% of those relative alleles expected for the families of given sample sizes and were significantly lower than the 95% (of the introductions and their relatives) only for the Neepawa family (Table 2). The percentages of allelic loss for an ancestral family ranged from 73 to 457% of those relative alleles expected for the families of given sample sizes, while the percentages of alleles introduced into an ancestral family ranged from 113 to 150% (Table 2). Similarly, when cultivars were grouped by breeding programs and compared with eight introductions, the percentages of alleles present in a breeding program ranged from 80 to 105% of those relative alleles expected for the breeding programs of given sample sizes and were significantly lower than the 107% (of the introductions) in four of the seven breeding programs (Table 2). The percentages of allelic disappearance for a breeding program ranged from 101 to 314% of those relative alleles expected for the breeding programs of given sample sizes, while the percentages of alleles introduced to a breeding program ranged from 38 to 91% (Table 2). The percentages of allelic disappearance in a breeding program were always larger than the percentages of allelic introduction into a breeding program (Table 2).

Changes in molecular variance

Based on the analyses of molecular variance (AMOVA; Excoffier et al. 1992) of 138 eSSR alleles, significant eSSR variance differences existed among various groups of the wheat cultivars (Table 3). The proportion of eSSR variation accounted for by decadal grouping was 12.8%, by ancestral family 14.7%, and by breeding program 5.8%. The proportions of eSSR variation residing between the earliest (i.e., pre-1910) and the later decadal groups showed a gradual increase from 3.6 to 24.6% over the six breeding periods, but the increase was statistically significant only for the cultivars released from 1930 onwards (Table 4).

An assessment of the within-group eSSR variation measured as average pairwise difference among cultivars of each breeding period revealed an increase from 27.5 to 34.4 for the cultivars released from 1910 to 1929, followed by a sharp decrease to 26.9 from 1930 to 1949 (Table 4). Again, a slight increase to 29.2 was observed for the cultivars released from 1950 to 1969, followed by a decrease to 26.1 for the cultivars released after 1990. Overall, the within-group variation was relatively reduced over the six breeding periods (Table 4). Such changes in eSSR variance appeared to be consistent with the net reductions of eSSR alleles observed over the breeding periods (Table 2). However, significance testing of allelic reduction occurred only for the cultivars released after 1990, rather than after 1930 as for eSSR variance. Higher sensitivity in AMOVA testing was expected as AMOVA took into account both allelic number and frequency.

Shift of genetic background over time

To visualize the genetic associations of various decadal groups of cultivars, groupwise cultivar similarities of 138 alleles were calculated and analyzed. The analysis revealed a shift of genetic background in the cultivars

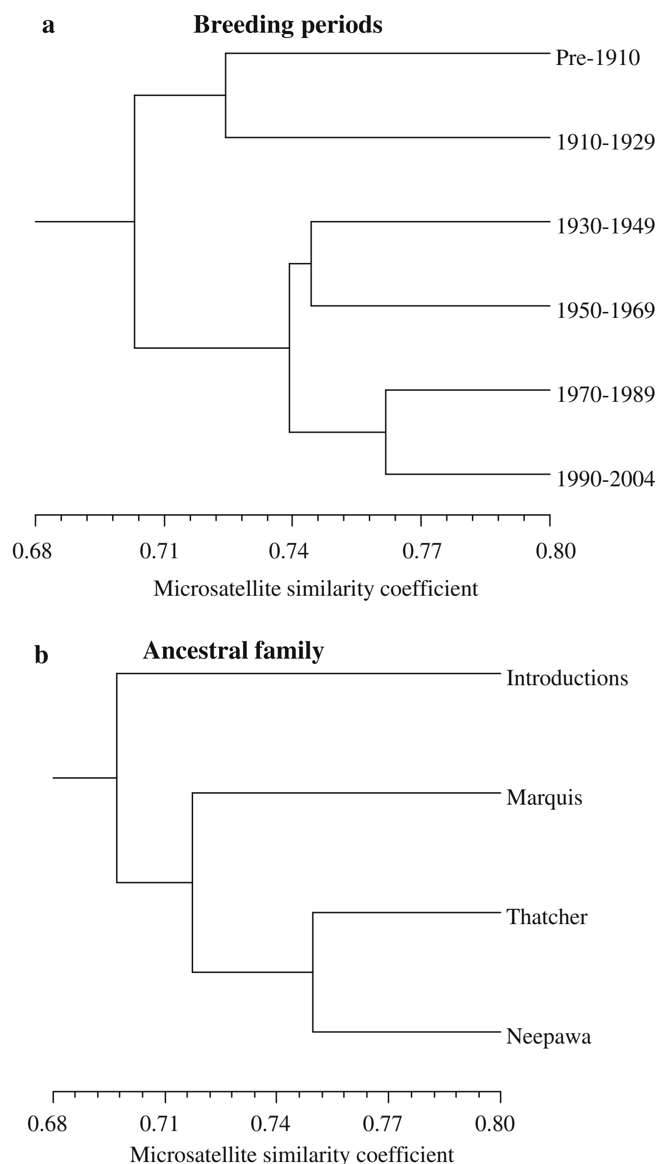

Fig. 2 Associations among six breeding periods (**a**) and four ancestral families (**b**) of Canadian hard red spring wheat cultivars, as revealed by cluster analyses based on the groupwise cultivar similarities of 138 EST-derived microsatellite alleles

released over time. The associations of the cultivar groups representing the six breeding periods are shown in Fig. 2, and three major clusters were found and appeared to be associated with 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. Such association, however, still needs to be empirically determined. Genetically, these efforts increased the similarity (or genetic relatedness) 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 (18.6 and 11.8%, respectively). When the cultivars were labeled according to breeding periods, 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 mainly from 1909 to 1929, and the Neepawa family developed after 1969, but the latter two families were far apart. Overall, the newly established families became genetically more distant away from the early developed families and introductions (Fig. 2b). However, there was no evidence

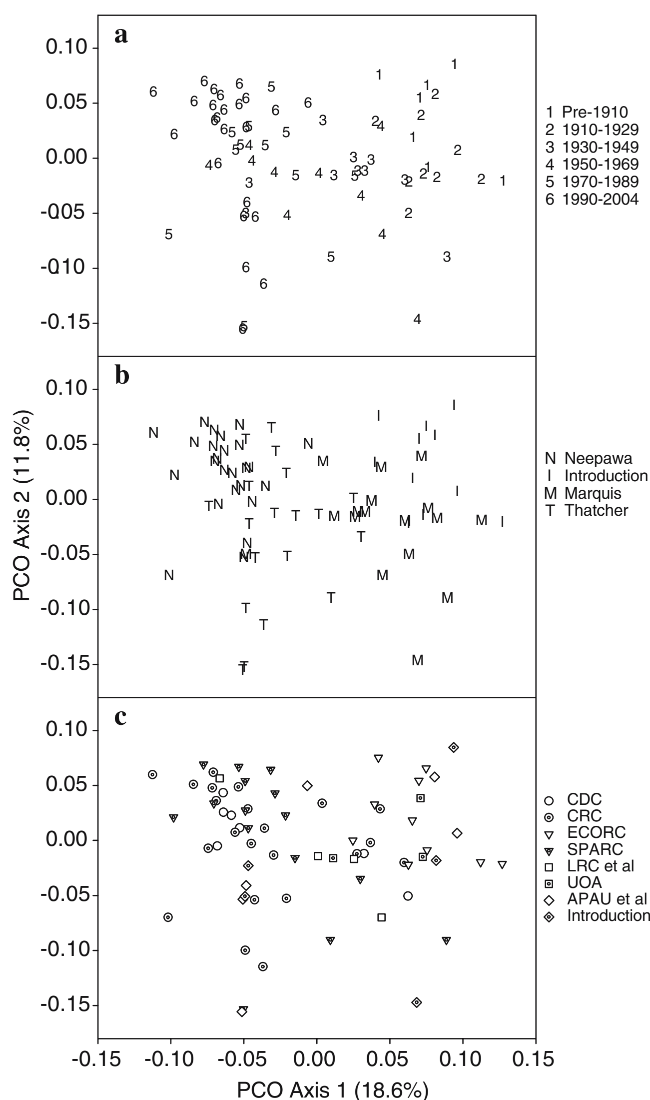

Fig. 3 Associations among 75 Canadian hard red spring wheat cultivars with respect to breeding period (**a**), ancestral family (**b**), and breeding program (**c**), as revealed by a principal coordinate analysis. Note that the three scatter plots (**a**–**c**) are the same, but individual cultivars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

of genetic shift in the releases of cultivar from different breeding programs (Fig. 3c).

Comparison between eSSR and gSSR variations

When the variation observed in this study for 138 alleles at 37 eSSR loci are compared with the variation revealed by 267 alleles at 31 gSSR loci (Fu et al. 2005), several patterns are clear. First, a significant reduction in allelic count was observed at three (8%) eSSR loci for the cultivars released after 1990 and at four (13%) gSSR loci for the cultivars released from 1970 onwards. Thus, more affected loci were observed for gSSR than eSSR markers. Second, 16 (12%) alleles present in the pre-1910 cultivars were undetected in the cultivars released after 1990 and were distributed over 14 (out of 37) eSSR loci assayed, and 51 (19%) gSSR alleles disappeared were spread over 27 (out of 31) gSSR loci. Thus, the disappearance of gSSR alleles was more spread over the wheat genome than that of eSSR alleles. Third, these undetected eSSR alleles occurred at frequencies ranging from 0.03 to 0.17 and averaging 0.07, while the frequencies of the undetected gSSR alleles ranged from 0.01 to 0.37 with an average of 0.07. Thus, most of the disappeared eSSR and gSSR alleles were rare, but some frequent alleles were also lost. Fourth, the patterns of genetic shift over the breeding periods and in the ancestral families were the same for both types of markers, although larger differences were observed for gSSR than eSSR markers. Fifth, while the decadal variations (12.8 and 12.5%) were compatible, the proportions of variation for ancestral family (14.7%) and breeding program (5.8%) were lower for eSSR than gSSR markers (16.5 and 8.4%, respectively). These comparisons demonstrated the century-long wheat breeding in Canada has had considerable impact not only on the non-transcribed, but also transcribed, segments of the wheat genome, although more impact was observed on the non-transcribed chromosomal regions.

Such impact is not surprising, given the degree of linkage disequilibrium in self-pollinating crops such as wheat and the intensive selections made over time within the narrow range of breeding materials (Fu et al. 2005). The demonstrated impact might be more profound if more mapped SSR markers well dispersed over the wheat genome are screened, but could still be biased from the unbalanced representation of the cultivars released over various breeding periods, unless all the released cultivars are assessed.

Concluding remarks

This study represents the first attempt using molecular markers derived from transcribed sequences to assess the genetic diversity changes in an improved gene pool. A significant reduction in allelic count was observed at three (8%) eSSR loci for the cultivars released after 1990. About 12% of the eSSR alleles present in the pre-1910 cultivars were not detected in the cultivars released after 1990 and were distributed over 14 (38%) eSSR loci surveyed. The alleles that were lost were mostly rare, with frequencies ranging from 0.03 to 0.17 and averaging 0.07. These results suggest the Canadian hard red spring wheat breeding has reduced allelic diversity in both transcribed and non-transcribed segments of the wheat genome. Genetic drift, however, might also have contributed to the allelic changes, as the lost alleles were mostly rare. Question remains whether all the lost eSSR alleles are associated with undesirable traits.

The findings presented here, along with those previously reported from genomic microsatellite markers (Fu et al. 2005), support the hypothesis that modern plant breeding is reducing genetic diversity (Fu et al. 2003) in the Canadian hard red spring wheat breeding programs. Conservation of genetically diverse germplasm is justified and useful for long-term breeding efforts (Duvick 1984; Swanson 1996; Tripp 1996). Continuous diversification of plant breeding materials is warranted to ensure that the plant improvement continues to be sustainable in the future (Reif et al. 2005). Developing effective indicators for genetic diversity of cultivated plants not only enhances the monitoring of genetic changes in improved gene pools, but also the effort of germplasm conservation and utilization (Fu et al. 2005).

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