World Journal of Agricultural Sciences 2 (3): 239-248, 2006
ISSN 1817-3047

Genetic Diversity of Canadian Wheat Cultivars as Revealed by Simple Sequence Repeat Markers

[1]Yong-Bi Fu, [2]John M. Clarke, [1]Ken W. Richards and [3]R.S. Sadasivaiah

[1]Plant Gene Resources of Canada, Saskatoon Research Centre,
Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, Canada S7N 0X2
[2]Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada,
P.O. Box 1030, Swift Current, SK, Canada S9H 3X2
[3]Deceased, formerly at Lethbridge Research Centre, Agriculture and Agri-Food Canada,
P.O. Box 3000, Lethbridge, AB, Canada T1J 4B1

Abstract: Genetic diversity assessment of improved crop germplasm can facilitate the expansion of the genetic base in a plant breeding program, but little effort has been made to assess the Canadian wheat gene pool established over the past century. Simple sequence repeat (SSR) markers were applied to assess the genetic diversity of 75 Canadian hard red spring (HRS), 5 soft white spring (SWS), 5 winter bread wheat and 5 durum wheat cultivars released from 1845 to 2004. Twenty-eight SSR primer pairs were applied and 319 polymorphic bands were scored for each cultivar. The frequencies of the scored bands ranged from 0.01 to 0.99 and averaged 0.12. The proportion of total SSR variation residing among four market classes (and type) of wheat was 27.8% and among four ancestral families of HRS wheat (early introductions and their relatives, Marquis, Thatcher and Neepawa families) was 16.5%. Durum, winter and SWS wheat cultivars were genetically distinct from HRS wheat, but were also genetically narrow within their own classes. The introduced HRS wheat cultivars were the most distinct, followed by the members of Marquis, Thatcher and Neepawa families. Five most distinct cultivars were Norstar, Ceres, Prelude, CDC Kestrel and Stewart 63. Clustering of the 90 wheat cultivars revealed separate groups for four market classes and two major sub-groups representing the Marquis family and the mixed Thatcher-Neepawa family of HRS wheat. High congruency to ancestral HRS families based on parental contributions was observed. These findings are useful for the selection of genetically distinct or less related wheat materials, particularly within the HRS wheat class, to improve the genetic background of the wheat breeding gene pool.

Key words: Simple sequence repeat (SSR) · wheat · *Triticum aestivum* · *Triticum turgidum* · genetic distinctness · genetic relationship · genetic structure

INTRODUCTION

Wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) breeding in Canada began in 1886 with the goal to decrease the time to maturity and improve adaptation and quality of early introductions such as Red Fife [1, 2]. Since then, great improvements have been made in grain yield [3], water use efficiency, disease resistance, grain protein and concentration and end-use suitability [4]. Hundreds of wheat cultivars have been developed and released [2, 5], many of which have had significant impacts on the economy of western Canada [6]. However, concerns exist about narrowing of the wheat gene pool because selection has been based on less than 15 parental lines over the century of breeding due to stringent requirements of seed quality and disease resistance and about the sustainability of long-term wheat improvement in Canada [7]. Interestingly, little effort has been made to assess the genetic base of Canadian wheat cultivars.

Molecular characterization of crop germplasm established over years of breeding efforts can not only allow for a better understanding of the selective impacts of breeding practices on the genetic base of the improved crop gene pools [8], but can also facilitate the

Corresponding Author: Dr. Yong-Bi Fu, Plant Gene Resources of Canada, Saskatoon Research Centre,
Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, Canada S7N 0X2

selection of genetically diverse and/or distinct germplasm from the existing gene pool to widen the genetic base of breeding materials. Traditional pedigree analyses can also enhance such selection by assessing the genetic relatedness of the existing cultivars, but these are not always informative because the assumption of equal parental contributions is rarely met [9]. DNA microsatellite (or simple sequence repeat; SSR) markers have proven to be important tools in wheat genetics [10-11] and germplasm research [12-14]. In recent years, several studies have analyzed genetic diversity changes in wheat germplasm released over time [15-17]. These analyses provided useful information for understanding the impact of regional plant breeding on the genetic diversity of wheat cultivars, but not directly for the improvement of the genetic background of breeding materials.

We initiated in 2003 an assessment on the genetic diversity of 90 Canadian wheat cultivars representing the four market classes of breed wheat [hard red spring (HRS), soft white spring (SWS), winter] and durum wheat (*Triticum turgidum* L. var. *durum*, 2n=4x=28, AABB) using 31 genomic SSR markers. In a companion paper, we focused on the determination of genetic narrowing in the Canadian wheat gene pool by analyzing genetic diversity changes only in the HRS wheat class [18]. Here we assessed the genetic distinctness, relationships and structures of four different market classes of Canadian wheat, with the aim to facilitate the selection of genetically diverse and distinct wheat germplasm in genetic improvements of Canadian wheat.

MATERIALS AND METHODS

Plant materials: Ninety Canadian wheat cultivars (Table 1) were selected from the wheat germplasm collection maintained at Plant Gene Resources of Canada, Agriculture and Agri-Food Canada, Saskatoon. The selected cultivars consisted of four major market classes of bread wheat [75 hard red spring (HRS), 5 soft white spring (SWS), 5 winter] and 5 durum wheat cultivars. Selection of more HRS wheat cultivars allows not only for an informative assessment of genetic diversity changes, but also a reliable identification of genetically distinct genotypes, in the HRS wheat class, as the HRS wheat breeding reflected the major effort of Canadian wheat breeding over the past century. Cultivar selection was made based on pedigree analyses, agronomic and economic importance and representation of the four wheat market classes. Several Canadian wheat breeders and researchers were 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 [2, 5; F. Clarke 2004, personal communication] and from the related online information resources on wheat pedigrees. All the HRS wheat cultivars were classified as four ancestral families (Early introductions and their relatives, Marquis, Thatcher and Neepawa families) based on the coefficients of parentage calculated from known pedigrees by Dr. Ron DePauw in a companion study [18].

DNA extraction: Approximately 20 seeds of each cultivar were randomly selected from the PGRC wheat collection and grown in the greenhouse at the Saskatoon Research Centre. Young leaves were collected from ten 5-day-old seedlings of each cultivar, bulked, freeze-dried (in a Labconco Freeze Dry System for 3 to 5 d) and stored at -80°C. Dry leaves from each bulked sample were finely chopped and ground to a fine powder in a 2 ml microcentrifuge tube with two 3 mm glass beads on a horizontal shaker to a fine powder. Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc., Mississauga, ON, Canada) according to the manufacturer's directions. Extracted DNA was quantified by fluorimetry using Hoechst 33258 stain (Sigma Chemical Co., St. Louis, MO, USA), followed by dilution to 25 ng μL^{-1} for SSR analysis.

SSR analysis: Based on reported polymorphism and genome coverage [19], 28 SSR primer pairs were selected for this study (Table 2). Left primer (4 μM) was labeled with 74 kBq/μL [γ^{33}P] dATP (PerkinElmer, Boston, MA, USA) in 1X kinase forward reaction buffer, 0.8 unit/μL T4 polynucleotide kinase (Invitrogen, Burlington, ON, Canada) in a final volume of 25 μL per 100 reactions at 37°C for 1 h and 70°C for 10 min. The polymerase chain reaction (PCR) contained 125 ng DNA, 1X buffer (Promega, Madison, WI, USA), 1.5 mM $MgCl_2$, 200 μM each of dNTP, 400 nM right primer, 360 nM unlabeled left primer, 40 nM [γ^{33}P] labeled left primer and 1 unit of *Taq* polymerase (New England Biolabs, Pickering, ON, Canada) in a final volume of 25 μL per reaction. Different "Touchdown" PCR programs were used for different primer pairs depending on their melting temperatures [19]. The PCR products were separated on a sequencing gel (BioRad sequencing system, Hercules, CA, USA) containing 6% polyacrylamide, 7 M urea and 1X TBE at 90 W constant power for 2 to 3 h, blotted onto Whatman 3 MM 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.

Table 1: Ninety Canadian wheat cultivars chosen for this study with their year of release, origin and average dissimilarity

Cultivar	Year[a]	Program[b]	Anc/Class[c]	AD[d]	Cultivar	Year	Program	Anc/Class	AD
Red Fife	1845	I.GC	EIR	0.140	Leader	1981	SPARC	THA	0.135
Ladoga	1887	I.RU	EIR	0.165	Lancer	1984	SPARC	THA	0.124
Stanley	1893	ECORC	EIR	0.156	Kenyon	1985	CDC	NEE	0.119
Preston	1895	ECORC	EIR	0.164	Conway	1986	CDC	NEE	0.116
Huron	1900	ECORC	EIR	0.143	Roblin	1986	CRC	NEE	0.142
Percy	1901	ECORC	EIR	0.155	Laura	1986	SPARC	THA	0.129
White Fife	1908	ECORC	EIR	0.128	CDC Makwa	1990	CDC	NEE	0.119
Marquis	1909	ECORC	MAR	0.123	Pasqua	1990	CRC	NEE	0.117
Prelude	1913	ECORC	EIR	0.186	CDC Teal	1991	CDC	NEE	0.134
Ruby	1917	ECORC	EIR	0.169	AC Minto	1991	CRC	NEE	0.138
Early Triumph	1918	REF	EIR	0.161	CDC Merlin	1992	CDC	NEE	0.136
Kota	1921	I.US	EIR	0.170	AC Michael	1993	LRC	NEE	0.121
Supreme	1921	REF	EIR	0.152	AC Eatonia	1993	SPARC	THA	0.127
Renfrew	1924	UOA	MAR	0.130	AC Domain	1993	CRC	THA	0.149
Garnet	1925	ECORC	EIR	0.167	Invader	1993	APAU	THA	0.136
Broatch's Whitehead	1925	CDC	MAR	0.147	AC Barrie	1994	SPARC	NEE	0.139
Red Bobs # 222	1926	UOA	EIR	0.138	AC Cora	1994	CRC	NEE	0.118
Ceres	1928	I.US	MAR	0.187	Pacific	1994	CRC	NEE	0.146
Reward	1928	ECORC	MAR	0.150	AC Majestic	1995	CRC	NEE	0.150
Reliance	1932	I.US	MAR	0.158	Prodigy	1995	SWP	NEE	0.127
Canus	1935	UOA	MAR	0.161	AC Cadillac	1996	SPARC	NEE	0.138
Thatcher	1935	I.US	THA	0.113	McKenzie	1997	SWP	NEE	0.139
Coronation	1937	CRC	MAR	0.135	AC Intrepid	1997	SPARC	NEE	0.142
Apex	1937	CDC	MAR	0.129	AC Splendor	1997	CRC	NEE	0.137
Renown	1937	CRC	MAR	0.136	AC Abbey	1998	SPARC	THA	0.158
Regent	1939	CRC	MAR	0.160	Superb	2001	CRC	THA	0.151
Rescue	1946	SPARC	MAR	0.150	Lovitt	2002	SPARC	NEE	0.119
Redman	1946	CRC	MAR	0.141	Journey	2002	SWP	NEE	0.146
Saunders	1947	ECORC	THA	0.128	Lillian	2003	SPARC	NEE	0.145
Lee	1950	I.US	MAR	0.164	Harvest	2004	CRC	THA	0.141
Chinook	1952	SPARC	THA	0.149	Fielder	1976	I.US	SWS	0.156
Selkirk	1953	CRC	MAR	0.147	AC Reed	1991	LBRC	SWS	0.156
Lake	1954	SRF	MAR	0.141	AC Nanda	1998	LBRC	SWS	0.162
Canthatch	1959	CRC	THA	0.136	AC Andrew	2001	LBRC	SWS	0.169
Pembina	1959	CRC	THA	0.121	AC Meena	2001	LBRC	SWS	0.151
Cypress	1962	SPARC	THA	0.162	Kharhov 22 MC	1912	MC	WIN	0.158
Park	1963	LRC	THA	0.144	Winalta	1961	LBRC	WIN	0.162
Manitou	1965	CRC	THA	0.120	Sundance	1971	LBRC	WIN	0.175
Neepawa	1969	CRC	NEE	0.115	Norstar	1977	LBRC	WIN	0.192
Canuck	1973	SPARC	THA	0.149	CDC Kestrel	1991	CDC	WIN	0.186
Sinton	1975	SPARC	THA	0.120	Pelissier	1920	I.AG	DUR	0.182
Chester	1976	LBRC	MAR	0.141	Stewart 63	1963	CDC	DUR	0.185
Benito	1979	CRC	NEE	0.117	Hercules	1969	CRC	DUR	0.174
Columbus	1980	CRC	NEE	0.140	Kyle	1984	SPARC	DUR	0.169
Katepwa	1981	CRC	NEE	0.120	AC Morse	1996	CRC	DUR	0.179

[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, Winnipeg; CDC=Crop Development Centre, Univ. of Saskatchewan; CRC=Cereal Research Centre, Winnipeg; ECORC=Eastern Cereal and Oilseed Research Centre, Ottawa; I=Introductions from Australia (I.AU), from Algeria (I.AG), Galicia region of central Europe (I.GC), Russia (I.RU) and USA (I.US); LRC=Lacombe Research Centre; LBRC=Lethbridge Research Centre; MC=MacDonald College, McGill University; REF=Rosthern Experimental Farm; SPARC=Semiarid Prairie Agricultural Research Centre, Swift Current; SRF=Scott Research Farm; SWP=Saskatchewan Wheat Pool; UOA=Univ. of Alberta, [c] The code for cultivar ancestral family or class. EIR=Early introductions and their relatives; MAR=Marquis family; THA=Thatcher family; NEE=Neepawa family; SWS=Soft white spring wheat; WIN=Winter wheat; DUR=Durum wheat, [d] AD=average dissimilarity of the cultivar against the remaining cultivars assayed

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 measured with a 10 bp DNA ladder (Invitrogen, Carlsbad, CA, USA) and compared with the fragment sizes reported in the literature [19]. Frequencies of the scored alleles were calculated with respect to primer, ancestral family and market class. To assess the informativeness of

each marker, the polymorphic information content (PIC) was calculated for each locus, as described in Roussel *et al.* [17].

To assess the genetic distinctness of wheat cultivar, the similarities of each cultivar with the remaining cultivars assayed were calculated using the simple matching coefficient [20] as: $S_{ij}=(a+d)/(a+b+c+d)$, where S_{ij} is the SSR similarity between the cultivar i (i=1 to n) and the other cultivar j [j=1 to (n-1)], 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 SSR dissimilarity for each pair of cultivars can be defined as $1-S_{ij}$. The average SSR dissimilarity for the cultivar i can be obtained by averaging all of the n-1 SSR dissimilarities that the cultivar was associated with. This average dissimilarity measures the overall genetic difference present between the cultivar (i) of interest and the remaining cultivars assayed. A higher average dissimilarity means that the cultivar has a genetic background more distinct from the other cultivars [21]. This assessment was done using a specific SAS program written in SAS IML [22].

To assess the genetic relationships of wheat cultivars, the dissimilarity matrix of pairwise cultivars was calculated using simple matching coefficient and clustered using TREECON software [23] with the algorithm of unweighted pair-group methods using arithmetic averages. The support for clustering was assessed using 100 bootstrapped replicates. A principal component analysis of 90 cultivars was conducted using NTSYS-PC 2.01 [24] based on the similarity matrix of 319 SSR bands and plots of the first three resulting principal components were made to assess the cultivar associations and to identify genetically distinct cultivars.

To compare the SSR variation among various wheat classes, the numbers of alleles detected at each locus were calculated. To assess the significance of the difference in allelic count between any two market classes of unequal numbers of wheat cultivars, the same random permutation procedure described in Fu *et al.* [18] was applied. An analysis of molecular variance (AMOVA) was also performed using Arlequin version 2.001 [25]. This analysis not only allows the partition of the total SSR variation into within- and among- class variation components, but also provides a measure of inter-class genetic distance as the proportion of the total SSR variation residing between any two classes (Φ_{st} - Phi statistic [26-27]). Significance of resulting variance components and inter-class genetic distances was tested with 10,100 random permutations.

RESULTS AND DISCUSSION

SSR variation: The 28 SSR primer pairs used detected a total of 31 loci on 13 chromosomes representing all seven wheat homologous chromosome groups [19] (Table 2). Most primer pairs detected only one locus, but the primer pair gwm296 detected two loci on chromosomes 2A and 2D and gwm497 revealed three loci on chromosomes 1A, 2A and 3D, according to the fragment sizes reported by Röder *et al.* [19]. Overall, six loci located on chromosome 2D (gwm102, gwm210, gwm261, gwm296, gwm455 and gwm484) and four loci on chromosome 1A (gwm135, gwm164, gwm357 and gwm497) and chromosome 2A (gwm296, gwm448, gwm497 and gwm636) represent the three largest clusters of loci in this chromosomal survey. Interestingly, most of D genome specific SSR primers also amplified alleles in durum wheat (Table 2) and clearly, further verification of these durum wheat alleles in wheat chromosomes is needed.

A total of 319 SSR alleles were detected in this study, but they could include some null alleles, because 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 23 by gwm174, with an average of 11.4 alleles per primer pair. Values of each marker PIC ranged from 0.01 for the marker detected by gwm276 to 0.89 for the marker by gwm611 with an average of 0.54, but this variation was not significantly associated with the number of alleles detected at each locus. The three most informative loci were gwm611 and gwm577 detected on chromosome 7B and gwm213 on chromosome 5B. The observed occurrence frequencies of the 319 alleles ranged from 0.01 to 0.99 with an average of 0.12. There were only five alleles with an occurrence frequency of 0.80 or larger in the cultivars and 247 alleles with frequencies of $\leq$0.12. Among the 247 infrequent alleles there were 114 of $\leq$0.02 and 169 of $\leq$0.05. Some of these infrequent alleles may be useful as diagnostic markers for some of the assayed wheat cultivars.

Genetic distinctness of wheat cultivars: The genetic distinctness of a cultivar was measured by the average dissimilarity of a cultivar against the remaining cultivars assayed. A higher average dissimilarity for a cultivar means the genetic background of the cultivar is more distinct. The average dissimilarity of a cultivar ranged from 0.113 for Thatcher to 0.192 for Norstar with a mean of 0.146 for all the cultivars assayed (Table 1). The five most distinct cultivars were Norstar, Ceres, Prelude, CDC Kestrel and Stewart 63. For specific market classes, the

Table 2: Microsatellite allele counts and polymorphic information contents (PIC) at individual loci for Canadian wheat cultivars of four market classes

Locus	Chromosome[b]	Total allelic count	PIC	Allelic count for four wheat classes[a]			
				HRS	SWS	Winter	Durum
gwm135	1A	10	0.05	8	2	5	0
gwm164	1A	6	0.69	6	2	2	2
gwm357	1A	5	0.58	5	2	2	2
gwm497	1A	2	0.65	2	0**	1	1
gwm296	2A	15	0.62	13	3	5	0
gwm448	2A	12	0.33	11	2*	4	5
gwm497	2A	3	0.61	3	0**	2	1
gwm636	2A	13	0.74	9	3	4	2
gwm102	2D	6	0.68	5	2	2	3
gwm210	2D	7	0.06	5	2	4	2
gwm261	2D	7	0.39	5	1	2	4
gwm296	2D	7	0.77	5	1	3	0
gwm455	2D	7	0.46	7	3	2	1
gwm484	2D	12	0.66	10	2	4	0
gwm247	3B	11	0.51	10	1	2	3
gwm003	3D	5	0.56	5	3	3	0***
gwm314	3D	4	0.31	4	1**	1**	1**
gwm497	3D	3	0.60	3	1	1	1
gwm006	4B	17	0.38	14	4	6	3
gwm194	4D	6	0.53	6	1	1	4
gwm213	5B	14	0.80	13	1	2	3
gwm544	5B	10	0.65	7	1	3	3
gwm174	5D	23	0.45	22	4	8	6
gwm190	5D	6	0.73	5	2	2	3
gwm088	6B	19	0.26	16	4	6	4
gwm276	7A	17	0.01	15	3	4	3
gwm046	7B	16	0.76	11	3	4	2
gwm302	7B	15	0.45	12	3	4	5
gwm577	7B	13	0.83	8	2	3	5
gwm611	7B	14	0.89	12	4	3	5
gwm437	7D	14	0.70	13	2	3	1
Total	13	319	270	65	98	75	

[a]HRS=Hard red spring wheat; SWS=Soft white spring wheat; Winter=Winter wheat; Durum=Durum wheat, *,**,*** Significance (at $p < 0.05$, 0.01, 0.001, respectively) of the permutation test for the difference in allele count between the wheat cultivars of a given class and HRS wheat cultivars, [b]According to the sizes of alleles mapped by Röder *et al.* [19]

range and mean of the average dissimilarities were 0.113-0.187 and 0.141, respectively, for HRS wheat; 0.151-0.169 and 0.159 for SWS wheat; 0.158-0.192 and 0.175 for winter wheat and 0.169-0.185 and 0.178 for durum wheat (Table 1). Clearly, durum wheat cultivars were genetically more distinct than the other wheat cultivars assayed, followed by winter wheat cultivars. Within the HRS wheat class, the five most distinct cultivars were Ceres, Prelude, Kota, Ruby and Garnet, largely reflecting the introductions and early developed cultivars. Three important HRS wheat cultivars Marquis, Thatcher and Neepawa had relatively low average dissimilarities (0.123, 0.113 and 0.115, respectively), because many of the HRS wheat cultivars assayed were their descendents with similar genotypes, which added more weight in the averaging of dissimilarities. These HRS wheat results were essentially the same as those from a separate estimation of genetic distinction from the HRS wheat class data alone (i.e., without SWS, winter and durum wheat data; results not shown).

The average dissimilarities given in Table 1 are limited to only those cultivars assessed here and it would be more informative if all the wheat cultivars released in Canada were assessed. Also, this method can recognize the distinctness, but not necessarily the relatedness, of cultivars. For example, two closely related cultivars that were quite distinct from the remaining cultivars would have the similar higher levels of average dissimilarity than the others and both cultivars would have been identified as genetically distinct cultivars. In spite of these limitations, the relative measure of genetic distinctness reported here should provide a guide for selecting specific cultivars with distinct genetic backgrounds for wheat breeding.

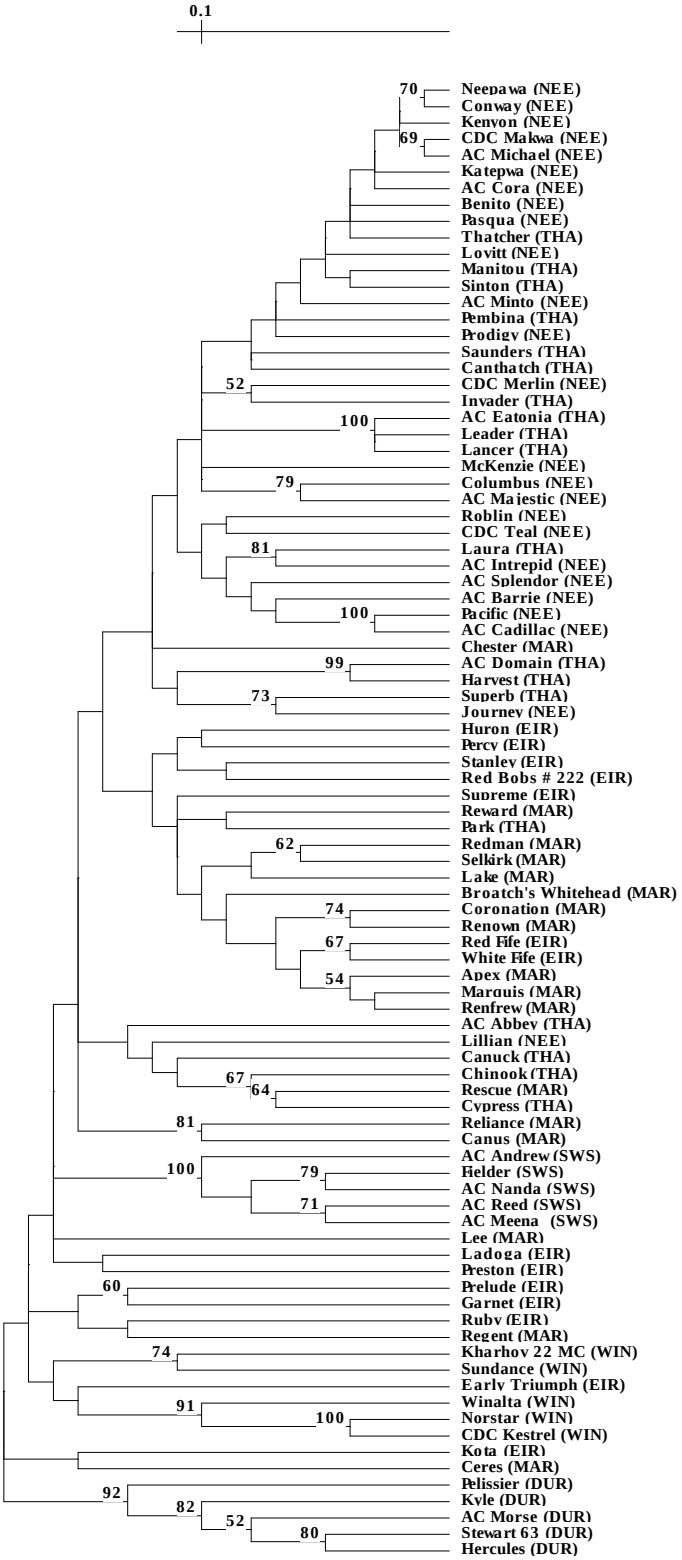

Fig. 1: UPGMA dendrogram reflecting genetic relationships of 90 Canadian wheat cultivars based on their SSR dissimilarities. Bootstrap values higher than 50 (out of 100 replicates) are indicated above the nodes. The label in parenthesis following each cultivar is either the ancestry or wheat class (Table 1)

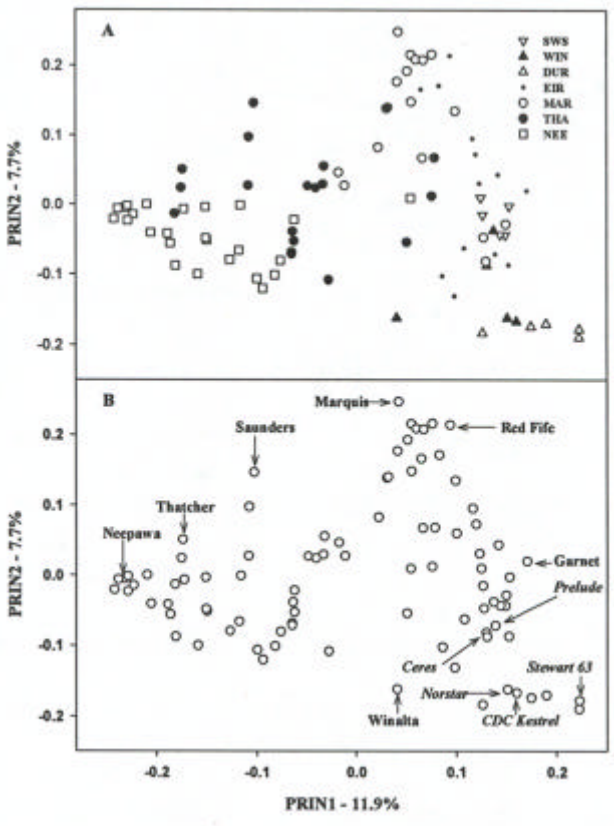

Fig. 2: Plot of the first two principal component scores based on the Euclidean distances converted from the simple matching coefficient matrix of 319 SSR bands for 90 wheat cultivars. These two components accounted for 11.9% and 7.7% of the total SSR variance, respectively. A: Individual cultivars were separately labeled for their ancestries or market classes (Table 1). B: several distinct cultivars identified based on PCA components and by average dissimilarity (in Italics) were labeled. Note that A and B are the same plot with different labeling for clear illustration

Genetic relationships of wheat cultivars: Similarities among the 90 cultivars reflected in the 319 SSR bands were calculated and clustered into several distinct groups (Fig. 1). The four market classes were clearly distinguished and their cultivars were clustered as separate groups. The first distinct group consisted of the five durum wheat cultivars, in which the early released cultivar Pelissier was the most distinct, followed by its derivative Kyle (released in 1984). The winter wheat cultivars and the historic cultivar Early Triumph (released in 1918 from Rosthern Experimental Farm) formed a separate group, which roughly shared the same level of genetic distinctness as many early introduced or developed cultivars. The five SWS wheat cultivars were clustered as a group that was separated from the large group consisting of the HRS wheat cultivars.

Within the large group of HRS wheat, there were two separate sub-groups representing the majority of the Marquis family and the mixed members of Thatcher and Neepawa families, respectively. Clearly, all of the separations within the HRS wheat group were not well supported as reflected in bootstrapped counts, when compared with the grouping of the other wheat market classes. The groupings of Marquis and Neepawa families were highly congruent to the classifications based on parental contributions, but were less so with that for the Thatcher family, because the Thatcher members so classified appeared to be more genetically diverse within the group than the other two families. This may reflect several new wheat lines with various disease resistances introduced into the breeding programs from 1935 to 1969 [2]. While the members of Neepawa family were genetically more related, a few recent cultivars such as Journey and Lillian were genetically more distant from the other family members. Similarly, several recent HRS wheat cultivars released from Winnipeg Research Centre, such

Table 3: Genetic distances among cultivars of four wheat market classes measured by the proportion of the total simple sequence repeat (SSR) variation residing between wheat classes (Φ_{st}; below the diagonal). Significance of each Φ_{st} value is given above the diagonal as the probability obtained by 10,100 random permutations that a random Φ_{st} value was greater than observed value

Class	HRS	SWS	Winter	Durum
Hard red spring wheat (HRS)		***	***	***
Soft white spring wheat (SWS)	0.266		***	**
Winter wheat	0.241	0.412		**
Durum wheat	0.310	0.468	0.383	

, * Significant at $p < 0.01$ and $p < 0.001$, respectively

as Superb, Harvest and AC Domain, showed their genetic distinctness not only from the other Thatcher members, but also from the other HRS wheat cultivars. This is consistent with the diversity of the pedigrees of these cultivars, all of which have parentage including both US and Canadian genotypes. These genetic relationships appear to be consistent with the interpretations of the average dissimilarities obtained for HRS wheat (all above the average for HRS wheat cultivars; Table 1).

The principal component analyses revealed the first three principal components accounted for 24.7% of the total SSR variation (11.9, 7.7 and 5.1%, respectively). A biplot of the first and second components revealed the genetic associations of the 90 cultivars (Fig. 2A) that were largely consistent with the genetic distinctness and relationships described above. For example, all the durum and SWS wheat cultivars are associated closely together. The winter wheat cultivars were diverse as indicated by the spreading on the plot and were more associated with many early introduced or developed cultivars and less with the HRS wheat cultivars. The Marquis family was more associated with the early introduced or developed cultivars than the members of Thatcher and Neepawa families. The Thatcher family was more spread over the space and slightly associated with both the Marquis family and Neepawa family. It appears that there was no direct association among the members of Marquis and Neepawa families. It is also clear that the introductions or early developed cultivars were more diverse as a group than those ancestral families. The most distinct cultivars based on the first two PCA components would be Red Fife, Marquis, Saunders, Thatcher, Neepawa, Winalta, Garnet and Stewart 63 (Fig. 2B). These were different from those identified by the average dissimilarity and shown in Fig. 2B, except for Stewart 63. The PCA analysis was emphasized more by the difference in band frequencies and the average dissimilarity more by the difference in band patterns (or genotypes). Thus, the average dissimilarity should be more informative for assessing genetic backgrounds.

Genetic structures of wheat cultivars: The proportion of the total SSR variation which resided among four market classes of wheat was 27.8%. The largest genetic distance measured by the proportion of the total SSR variation residing between two market classes of wheat was observed between SWS and durum wheats (0.468), followed by between SWS and winter wheats (0.412) and between winter and durum wheats (0.383) (Table 3). These results indicate that winter and durum wheats were genetically most distinct from HRS and SWS wheats and winter wheat was also diverse within the class. Assessment on the average number of pairwise differences within a class obtained by AMOVA showed that HRS wheat had the highest within-class diversity (44.6), followed by winter wheat (43.0), durum wheat (32.8) and SWS wheat (27.4). Comparisons of allelic counts among the four market classes revealed winter wheat maintained a large number of alleles per cultivar (98/5=19.6), followed by durum wheat (75/5=15), SWS wheat (65/5=13) and HRS wheat (270/75=3.6) (Table 2). These comparisons could be biased by the unequal sample sizes of these classes. However, the permutation tests showed that the impact of unequal sample sizes on the observed counts of alleles for different market classes was minimal, as only five (out of 31; 16%) loci displayed allelic differences that can not be explained by the sampling bias and the significant differences were detected largely between SWS and HRS wheat cultivars (Table 2).

For the HRS wheat cultivars, the proportion of the total SSR variation residing among four ancestral families was 16.5%. The largest genetic distance was found between the members of early introductions and the Neepawa family (0.276), followed by between the members of Marquis and Neepawa families (0.248). Assessments of the average number of pairwise differences within a family obtained by AMOVA showed that the introductions and early released cultivars had the highest within-family diversity (46.4), followed by the Marquis family (41.7), Thatcher family (38.2) and Neepawa family (29.0). These results accord well with those of diversity change reported in Fu *et al.* [18] and indicate that SSR variation still existed among the ancestral families and that the introductions and early released cultivars were relatively more diverse than those developed later, particularly for the members of Neepawa family.

Implications for the Canadian wheat breeding: The results presented here are significant for broadening the genetic base of wheat breeding materials in Canadian wheat improvement. Durum, winter and SWS wheat cultivars were genetically distinct from the HRS wheat cultivars, but they were genetically narrow within their own market classes. Substantial genetic variation still existed within the HRS wheat germplasm, particularly for those introduced or developed before 1940. Even for those released in last two decades, some variation still existed as reflected in the average dissimilarities.

Improvements in wheat yields tend to be associated with genetic diversity [28], but in Canada, such yield advances can only be achieved if the end-use quality attributes of the market class are maintained. The genetic relationships obtained for the cultivars in this study should facilitate the selection of less related germplasm for intercrossing. The improvement of genetic background was observed in some recent members of the Thatcher family such as Superb and Harvest. Similarly, several recent cultivars of the Neepawa family such as Journey and Lillian also displayed some distinctness within the group. In spite of this, continuous efforts are still needed to diversify wheat breeding materials from existing Canadian or other wheat gene pools to ensure that breeding programs continue to be sustainable in the future.

ACKNOWLEDGEMENTS

We thank Drs. Ron DePauw, Daryl Somers, Fran Clarke and Pierre Hucl for their stimulating discussions on the project; Mr. Dallas Kessler, Mr. Gregory Peterson and Ms. Angela Taylor for their technical assistance in sampling, planting and genotyping the germplasm using SSR markers and Dr. Van Ripley and Mr. Lasantha Ubayasena for their helpful comments on the early version of the manuscript.

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