Genetic diversity in the U.S. hard red winter wheat cultivars as revealed by microsatellite markers

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Abstract. Knowledge of the genetic diversity existing in previously released hard red winter wheat (HRWW, *Triticum aestivum* L.) cultivars in the Great Plains region, United States, is essential for effective utilisation of these genetic resources in the various HRWW breeding programs. To ascertain a measure of the genetic diversity of the existing US HRWW, 60 cultivars were analysed with 62 microsatellite markers distributed throughout the wheat genome. Marker data were subjected to distance-based analysis and analysis of molecular variances. In total, 341 polymorphic alleles were scored with a range of 2–12 alleles per locus. Genetic diversity gradually increased in cultivars released after the 1970s. Cultivars released in the 1990s had the highest allelic richness (4.79), gene diversity (0.60), and polymorphic information content (0.56). Levels of genetic diversity were similar between the major HRWW breeding programs. Cluster analysis resulted in eight clusters. Cluster grouping gave close matches with pedigrees and with regional distribution of the cultivars. Using decadal information, cultivars released from 1900–1969 were grouped into one cluster, cultivars from 1990–2005 were grouped into a separate cluster, whereas cultivars from the 1980s did not group with any other decades. Analysis of molecular variance revealed a significant variation among the clusters, signifying that a true genetic variation existed among the clusters. The higher proportion of genetic variation explained by cultivars within clusters compared with among clusters indicates greater genetic diversity among cultivars within clusters. Our results indicate that genetic diversity of Great Plains HRWW cultivars has increased in the past century, and the trend is continuing.

Additional keywords: molecular markers, cluster analysis.

Introduction

Genetic diversity is the foundation for crop genetic improvement, and thus the most important consideration in any plant breeding program. Information related to genetic diversity among adapted lines helps breeders in selecting parents for hybrid production with maximum heterosis and combining useful genes in an adapted genetic background. In contrast to wide genetic diversity, a narrow genetic base is a big limitation to breeding for adaptation to different biotic and abiotic stresses. A major concern for many modern plant breeding programs is the narrow genetic base of their germplasm (Velle 1993; Clunies-Ross 1995). Several authors have argued that the narrowness in genetic diversity could lead to an increased vulnerability to diseases and pests, as well as the ability of plants to respond to changing environmental conditions (Clunies-Ross 1995; Tripp 1996). Thus, quantifying genetic diversity among existing germplasm helps to address this concern. Also, the knowledge of genetic relationships among different genetic materials allows breeders to eliminate unwanted duplication in germplasm and increase the efficiency of breeding programs.

The Great Plains region is the largest winter wheat (*Triticum aestivum* L.) growing area in the USA, and hard red winter wheat (HRWW) is the major market class of wheat produced. Each of the states in the Great Plains region has at least one major winter wheat breeding program, plus the two private breeding programs, AgriPro and Pioneer, have been active in the region. The western part of the southern and central Great Plains is characterised as having low or relatively low rainfall, low incidence of fungal diseases, moderate incidence of viral diseases, and moderate to high incidence of aphids, including Greenbugs and the Russian Wheat Aphid. The breeding programs at the University of Nebraska (UNL), Colorado State University (CSU), Kansas State University (KSU) at Hays, Oklahoma State University (OSU), Texas A & M University (TAMU) at Amarillo, and the two private breeding programs (Pioneer and AgriPro) have developed cultivars with good drought tolerance, good quality, and resistance to these biotic stresses. The eastern and central parts of the Great Plains have higher rainfall and much higher incidence of fungal diseases, especially leaf rust and stripe rust. High yield potential, good industrial quality, and high levels of

resistance to biotic stresses are the priority breeding objectives. The programs at KSU (Manhattan), OSU, TAMU (Vernon), UNL, and the two private breeding programs have developed successful cultivars for these regions. Collectively, these programs have released most of the cultivars that were included in this study. The first widely cultivated genotype in the region was 'Turkey', a landrace from southern Russia, and this cultivar was dominant until the end of the 1920s. Since then, considerable progress has been achieved by conventional plant breeding programs. From 1930 to 1950, the dominated cultivars were Blackhull, Pawnee, Wichita, Triumph, and Comanche, and all were derivatives of Turkey (Cox *et al.* 1986). Genetic diversity was first introduced to incorporate better rust resistance, primarily from US spring and winter wheat materials. Subsequently, in the late 1960s and 1970s, CIMMYT germplasm was used as a source of the semi-dwarf character and for better stem rust resistance (A. R. Klatt, unpublished 2007). The first widely grown HRWW semi-dwarf variety was 'Newton' and it was obtained by crossing a CIMMYT spring wheat semi-dwarf advanced line to Scout. In the 1970s, breeding programs focussed primarily on incorporating shorter straw and stem rust resistant genes into adapted, high-yielding lines. During the 1980s and 1990s, the main breeding objectives were to incorporate resistance to other diseases, especially leaf rust, and to improve industrial quality traits. Thus, during the last four decades, breeding programs have focussed on a few major objectives. Therefore, it is important to know the extent of genetic diversity that has been achieved and that currently exists among released cultivars in the Great Plains. Twenty years ago, Cox *et al.* (1986) made a landmark study on the genetic diversity in the commercially cultivated HRWW and soft red winter wheat (SRWW) varieties grown between 1919 and 1984. That study revealed that genetic diversity had increased in HRWW, but decreased in SRWW. According to their results, up to the 1950s there was a high degree of genetic uniformity, most probably due to the widespread use of Turkey and its derivatives, but uniformity had decreased somewhat from 1919 to 1949. After 1950, diversity increased, maybe due to the introduction of different disease resistance and quality genes from a variety of germplasm. In the past two decades, many new cultivars have been released. Also, in the last 2–3 decades there has been increased collaboration between breeding programs, many use similar parents in their crossing program, and much sharing of germplasm (gene pool) has occurred between different breeding programs. There has not been a recent study to provide information on how genetic diversity has changed in modern HRWW cultivars except Fufa *et al.* (2005), who reported a comparison between phenotypic and molecular marker-based approaches of estimating genetic diversity, using a set of 30 HRWW cultivars only from the Northern Great Plains of the USA. As a result, there is a need to quantify the genetic diversity existing among the different parts of the Great Plains HRWW cultivars released in the last several decades.

The use of molecular markers for the evaluation of genetic diversity of wheat has recently received a great deal of attention from molecular geneticists and wheat breeders. With the help of molecular markers, geneticists can monitor subtle changes in DNA sequences in genotypes released at different times (Ortiz 2001). This approach would help breeders to assess the

allelic combinations selected generation after generation, and thus provide a genetic ideotype for future marker-assisted selection (Christiansen *et al.* 2002). This information would help breeders to incorporate useful genetic variation into adopted gene pools by selecting for marker alleles linked to loci controlling important agronomic or quality traits (Tanksley and McCouch 1997). Several authors have studied genetic diversity in wheat by using different molecular markers, such as microsatellite (Devos *et al.* 1995; Bohn *et al.* 1999; Donini *et al.* 2000; Manifesto *et al.* 2001; Christiansen *et al.* 2002; Huang *et al.* 2002; Dreisigacker *et al.* 2004; Fufa *et al.* 2005), amplified DNA fragment polymorphism markers (Schut *et al.* 1997; Barrett *et al.* 1998; Manifesto *et al.* 2001), and random amplified polymorphic DNA markers (Joshi and Nguyen 1993). Microsatellite markers, also known as simple sequence repeats (SSR), have proved to be the most suitable molecular markers for studying genetic diversity in wheat because of its multi-allelic nature, chromosome specificity, high polymorphism, and distribution throughout the genomes (Röder *et al.* 1998*a*, 1998*b*). Most of the genetic diversity studies in wheat have been conducted by using genetic materials from different geographic locations or from different mega-environments or from different market classes. So far, genetic diversity in the HRWW cultivars released in different eras in the Great Plains has not been well documented. The objective of this study was to assess the extent and trend for genetic diversity among HRWW cultivars released from different USA Great Plains breeding programs in different eras.

Materials and methods

Genetic materials and DNA extraction

In total, 60 wheat cultivars representing the Great Plains HRWW regions of the USA, released from 1900 to 2005, were used in this study (Table 1). The cultivars were grouped into six decadal periods based on their year of release, namely <1960 (1900–59), 1960s (1960–69), 1970s (1970–79), 1980s (1980–89), 1990s (1990–99), and 2000s (2000–05). Comparatively higher numbers of cultivars were selected from the 1990s because a significant number of cultivars were released during the 1990s in the Great Plains. Also, many of these cultivars were still under commercial cultivation and were sown on a significant proportion of the total wheat area in the region at the time of this study. The cultivars were also grouped into 8 different classes based on the breeding programs, namely Oklahoma, Kansas, Texas, Nebraska, Colorado, AgriPro, Pioneer, and other (Table 1). In each breeding group, the cultivars were selected from different decades of release, and listed along with their known pedigrees. Pure seeds of the cultivars were collected from the National Plant Germplasm System (NPGS, USDA-ARS), and from the wheat breeders of Oklahoma, Kansas, Texas, Colorado, and Nebraska. Leaves were collected from 6–10 seedlings of each cultivar at the 2-leaf stage for DNA isolation using Qiagen DNeasy Plant Mini Kit (Qiagen Inc.; Valencia, CA, USA).

SSR analysis

The experiment was conducted in the USDA-ARS Small Grain Genotyping Laboratory in Kansas State University, Manhattan, KS, and Wheat Genetic Laboratory in Oklahoma State

Table 1.   Pedigrees, origins and year of release for the 60 hard red winter wheat cultivars used in the study

Cultivar	Breeding program	Year of release	Pedigree
Triumph 64	Oklahoma	1964	Danne Beardless Blackhull/3/Kanred/Blackhull//Florence/4/Kanred/Blackhull//Triumph
Agent	Oklahoma	1967	Triumph/KS464708
Chisholm	Oklahoma	1983	Sturdy sib/Nicoma
Cimarron	Oklahoma	1990	Payne*2/CO725052
Custer	Oklahoma	1994	F29-76/TAM 105//Chisholm
Tonkawa	Oklahoma	1994	F29-76/TAM 105//Chisholm
OK101	Oklahoma	2001	OK87W663/Mesa//2180
OK102	Oklahoma	2002	2174/Cimarron
Deliver	Oklahoma	2005	OK91724/Karl
Endurance	Oklahoma	2005	HBY756A/Siouxland//2180
Early Blackhull	Kansas	1928	Selection from Blackhull
Wichita	Kansas	1944	Early Blackhull/Tenmarq
Kaw61	Kansas	1960	Kaw Oro//Mediterranean/Hope/3/Early Blackhull/Tenmarq
Larned	Kansas	1976	Ottawa/5* Scout
Newton	Kansas	1978	Bluebird/Scout
Karl92	Kansas	1992	PlainsmanV/3/Kaw/Atlas 50//Parker*5/Agent
Jagger	Kansas	1994	KS82W418/Stephens
Overley	Kansas	2004	U1275-1-4-2-2/Heyne'S'//Jagger
Sturdy	Texas	1966	Sinvalocho/Wichita//Hope/Cheyenne/3/2*Wichita/4/Seu Seun 27
TAM 101	Texas	1971	Norin 10/3/Nebraska 60//Mediterranean/Hope/4/Bison
TAM 107	Texas	1984	TAM 105*4/Amigo
TAM 200	Texas	1986	(TX391-56-D8/Tascosa//Centurk)*3/Amigo
TAM 202	Texas	1992	Siouxland outcross
TAM 110	Texas	1996	(TAM 105*4/Amigo)*5//Largo
Lockett	Texas	1998	TX86V1540/TX78V2430-4
TAM 302	Texas	1998	Probrand 812/Caldwell//TX86D1310
TAM 111	Texas	2002	TAM 107//TX78V3630/Centurk78/3/TX87V1233
Pawnee	Nebraska	1942	Kawvale/Tenmarq
Warrior	Nebraska	1960	Pawnee/Cheyenne
Scout66	Nebraska	1967	Composite of 85 selections from Scout
Siouxland	Nebraska	1984	(Warrior*5/Agent)*2/Kavkaz
Arapahoe	Nebraska	1988	Brule/3/Parker*4/Agent//Beloterkovskaia 198/Lancer
Alliance	Nebraska	1993	Arkan/Colt//Chisholm sib
Cheyenne	Nebraska	1993	Selection from Crimean
Niobrara	Nebraska	1994	TAM 105*4/Amigo//Brule
Millennium	Nebraska	2000	Arapahoe/Abilene/4/Colt/3/Warrior*5/Agent//Kavkaz
Vona	Colorado	1976	II 21183/CO 652363//Lancer/KS 62136
Hawk	Colorado	1982	Jaral66/3/Trapper/4/CO 652643/5/Baca
Akron	Colorado	1994	TAM 107/Hail
Prairie Red	Colorado	1998	CO850034/PI 372129//5*TAM 107
Yumar	Colorado	1998	Yuma/PI 372129//CO850034/3/4*Yuma
Powers 99	Colorado	1999	CO850060/PI372129//5*Lamar
Venango	Colorado	2000	HBE 1066-105/HBF0551-131
Above	Colorado	2001	TAM 110*4/FS2
Avalanche	Colorado	2001	KS87H325/Rio Blanco
Longhorn	AgriPro	1990	NS2630-1/Thunderbird
Tomahawk	AgriPro	1991	Ironstraw S4
Ogalalla	AgriPro	1993	TX81V6187//OK711252/W76-1226
Coronado	AgriPro	1996	Mustang/W80-425//COMP76B-1-84-1/SW74-8A-47
Hondo	AgriPro	1998	W84-179/W81-171/3/Sturdy/Hawk//Vona/W76-1141
Thunderbolt	AgriPro	1999	OK711252A/W76-1226//KS90WGRC10
Cutter	AgriPro	2001	KS84063-9-39-3//TAM 200/W81-296
Jagalene	AgriPro	2001	Jagger/Abilene
2157	Pioneer	1984	Caprock/B 86//Sc 3212
2163	Pioneer	1989	Pioneer line W558/5/Etoile de Choise//Thorne/Clarkan/3/CI15342/4/Pur 4946A4-18-2
2180	Pioneer	1989	TAM W-101/Pioneer W603//Pioneer W558
2137	Pioneer	1995	W2440/W9488A//2163
2174	Pioneer	1997	IL71-5662/PL145//2165
Kharkof	Ukraine	1900	LV-UKR, Staroblesk
Winalta	Canada	1962	Minter/Wichita

University, Stillwater, OK. Sixty-two pairs of SSR primers comprising 19 *XGWM* (Röder *et al*. 1998*a*), 20 *BARC* (Song *et al*. 2005), and 23 *WMC* (Gupta *et al*. 2002) primers were used in the study. The primers were selected from all 21 chromosomes of hexaploid wheat. The PCR was performed in a volume of 10 µL containing 2 µL of DNA (20 ng/µL) and 8 µL of the master mix that consisted of 3.2 µL of ddH$_2$0, 1 µL of 10 × PCR buffer, 1.1 µL of 25 mM MgCl$_2$, 0.5 µL of 5 mM dNTPs, 0.5 µL of each forward and reverse primer (1 p.m./µL), 1 µL of tailed fluorescence-labelled (IRD-700 or IRD-800) M13 primer (Oetting *et al*. 1995), and 0.2 µL of *Taq* polymerase (5 U/µL). The PCR was performed by using a touchdown universal program consisting of 5 cycles of denaturing at 95°C for 45 s, annealing at 68°C for 5 min decreasing by 2°C in each subsequent cycle, and extension at 72°C for 1 min. In the next 5 cycles, the annealing temperature was started at 58°C for 2 min and decreased by 2°C in each subsequent cycle. An additional 25 cycles consisted of 45 s of denaturing at 95°C, 1 min of annealing at 50°C, and 1 min of extension at 72°C with a final extension cycle at 72°C for 5 min. The PCR product was denatured at 95°C for 5 min before it was separated in a 6.5% polyacrylamide gel on Li-Cor IR-4200 or IR-4300 DNA sequencer (Li-Cor Inc.; Lincoln, NE, USA).

Data analyses

Marker data were scored as presence or absence of an allele corresponding to each SSR locus using the software GeneImageIR ver. 4.05 (BD Biosciences, USA). Nei's (1972) genetic distance algorithm was used to calculate the pair-wise genetic distances among the cultivars, and clusters were shown in a neighbour joining tree. This is a hierarchical algorithm for clustering genotypes into different groups. Another neighbour joining tree was created using the decadal information (among the six defined decades). PowerMarker software (Liu and Muse 2005) was used to calculate the genetic distance among the 60 cultivars as well as among the decadal groups. Frequency and number of alleles, gene diversity, and polymorphic information content (PIC) were calculated for each SSR locus, chromosome, and genome for the entire set of cultivars, six decadal groups, and the regions/breeding programs using the PowereMarker software. Gene diversity was defined by Weir (1996) as the probability of two randomly chosen alleles from a population being different, and PIC value was defined by Botstein *et al*. (1980) as the value of a marker for detecting polymorphism within a population which depends on the number of detectable alleles and their frequency distribution. Tree view program MEGA ver.4.0 (Tamura *et al*. 2007) was used to construct the cluster trees. Levels of genetic variation within and among clusters as identified from the cluster analysis were estimated from the allelic frequencies using the analysis of molecular variance (AMOVA) (Weir and Cockerham 1984), and were implemented by using the software Arlequin ver. 3.0 (Excoffier *et al*. 2005). The AMOVA analyses were also tested using the groups designated by the decades of cultivar release and breeding programs. These analyses separate the total molecular variance into components of inter and intra groups and test the level of significance among the groups. Wright's fixation index (F_{ST}) was used to estimate the variance within the groups, and statistical significance of the index was evaluated using a 1000-times permutation of the genotypes among and within the

groups. Genetic diversity among the clusters was evaluated based on the pair-wise F_{ST} comparison that measures the fixation of alleles in the population.

Results and discussion

Diversity of the SSR markers

Allele frequency, number of alleles/locus, gene diversity, and PIC values for each SSR marker were calculated, and a total of 341 polymorphic alleles were scored with a mean of 5.5 alleles per primer pair (data not shown). Number of alleles for each primer pair varied greatly, ranging from 2 (*BARC* 1097; *GWM* 210) to 12 (*GWM* 261). Studies conducted earlier suggested that around 350 alleles were sufficient to assess the relationship between wheat accessions (Zhang *et al*. 2002). Major allelic frequency ranged from 0.23 (*XGWM* 604) to 0.98 (*WMC* 477), with a mean of 0.51. Gene diversity ranged from 0.03 (*WMC* 477) to 0.86 (*WMC* 707), with an average of 0.62 (data not shown). Among the detected alleles, 97 (28.4%) alleles were considered as rare alleles that occurred at a frequency of less than 5%. Estimated gene diversity in our study was 0.62, which was a little higher than the estimate of 0.57 from 68 advanced CIMMYT lines (Dreisigacker *et al*. 2004) and the estimate of 0.55 from 134 durum wheat accessions (Maccaferri *et al*. 2005), but lower than the estimates of 0.66 in 43 bread wheat cultivars from 7 USA market classes (Chao *et al*. 2007) and 0.66 in 559 French wheat accessions (Roussel *et al*. 2004), and 0.77 in 998 accessions from 68 countries (Huang *et al*. 2002). Studies reported earlier with higher gene diversity can be attributed to the use of genetic materials from well defined market classes or landraces, or genotypes from gene banks, or cultivars from different geographic locations (Huang *et al*. 2002; Roussel *et al*. 2004; Chao *et al*. 2007). Although we have used only one market class of wheat, the moderately high gene diversity indicates that the breeders in the Great Plains wisely used genetic materials for different sources of genes. The PIC values ranged from 0.03 (*WMC* 477) to 0.85 (*BARC* 184), with an average of 0.58 per primer pair (data not shown). Allelic richness (6.1), gene diversity (0.69), and PIC values (0.65) were the highest for chromosome 4, which suggests that most genetic variations were incorporated into chromosome 4 in the HRWW cultivars studied. Among the three genomes, gene diversity (0.63) and PIC value (0.63) were comparatively higher for the B genome, which means that more genetic diversity among the cultivars used in the study may reside in the B genome (data not shown). Hai *et al*. (2007) and Huang *et al*. (2002) also reported the highest gene diversity and PIC values in the B genome.

Major allelic frequency, alleles detected, gene diversity, and PIC values of the cultivars were estimated for the decade of cultivar release, and for the regions/breeding programs where the cultivars were released (Table 2). Mean allele number (4.79), gene diversity (0.60), and PIC (0.56) were the highest for the cultivars released during the 1990s. Cultivars from the 1980s and 2000s had similar gene diversity and PIC values. Cultivars from the 1970s showed a decrease in mean allele number, gene diversity, and PIC values compared with the other decadal groups (Table 2). In general, diversity measurements were higher in the cultivars released after the 1980s than in those

Table 2.   Summary statistics of the microsatellite marker diversity of the decadal groups, and regions of release/breeding programs of the 60 hard red winter wheat cultivars

Decades/ regions of release	No. of cultivars	Major allelic freq.	Mean allele no.	Gene diversity	Polymorphic info. content
Decades					
<1960	4	0.57	2.55	0.50	0.43
1960s	7	0.58	3.18	0.53	0.48
1970s	4	0.65	2.19	0.41	0.35
1980s	9	0.54	3.50	0.57	0.52
1990s	24	0.53	4.79	0.60	0.56
2000s	12	0.55	3.78	0.57	0.52
Regions/breeding programs					
Oklahoma	10	0.55	3.55	0.57	0.52
Kansas	8	0.53	3.37	0.57	0.52
Texas	9	0.57	3.35	0.54	0.49
Nebraska	9	0.59	3.42	0.53	0.48
Colorado	9	0.57	3.31	0.54	0.49
AgriPro	8	0.54	3.47	0.56	0.51
Pioneer	5	0.55	2.89	0.53	0.47
Others	2	0.70	1.55	0.23	0.23

released before the 1980s. However, cultivars released from 2000 to 2005 showed a decrease in allelic richness compared with those released in the 1990s. This might be due to the fact that, during the 1990s, a significant number of cultivars were released from different breeding programs, and no single cultivar dominated the commercial cultivation as in some of the previous decades. As a result, a comparatively larger number of cultivars from the 1990s have been used in this study. Also during the 1990s, the two private breeding programs released many cultivars that might have represented different gene pools or new diversity. The cultivars released during the 2000s represent only a 5-year period instead of a 10-year period, and thus may result in decreased diversity measurements. However, the reduction of allelic richness in the 1970s and 2000s could be due to the elimination of deleterious genes rather than the erosion of useful genetic resources in those years. No substantial differences were observed among the major HRWW breeding programs of the Great Plains in terms of allelic richness, gene diversity, and PIC values, except a comparatively low allelic richness in the Pioneer genetic materials (Table 2). These observations indicate that the major HRWW breeding programs of the Great Plains are consistent in maintaining genetic diversity in their released cultivars for the last several decades.

The increase in gene diversity and allelic richness after the 1970s reflects the use of diverse sets of germplasm and the introduction of new genetic variation. The breeders in the southern Great Plains introduced considerable spring wheat into the HRWW gene pool in the late 1960s and early 1970s. Mexican semi-dwarf wheat was the major source of spring wheat diversity added to the HRWW germplasm pool. 'Newton', released in 1978, had a CIMMYT spring wheat background, which created a truly new diversity in the wheat germplasm from the southern Great Plains (A. K. Fritz, pers. comm., 2007). Moreover, integration and selection for several important yield

and adaptive traits also led to an increase in average gene diversity and allelic richness.

Genetic relationships among the HRWW cultivars

Cluster analysis using the Nei's genetic distance algorithm separated the 60 HRWW cultivars into 8 clusters (Fig. 1). A wide range of genetic distances, from 0.33 to 1.79, was observed among the cultivars. The furthest genetic distance (1.79) was between cv. TAM 200 and 2174, and the shortest distance (0.33) was between TAM 107 and TAM 110. The cv. TAM 110 was developed using TAM 107 as a backcross parent (Table 1). The clusters (Fig. 1) generally agreed with the pedigree or genetic background of the cultivars. The cultivars did not cluster according to the defined decades of release, but in several cases cultivars released from a specific breeding program were grouped together. For example, cultivars released in different states from Pioneer lines, such as 2137, 2157, 2163, 2174, and 2180, were grouped together. Several cultivars released from Nebraska, such as Scout66, Niobrara, Warrior, Alliance, and Cheyenne, grouped together. Two historical cultivars, Early Blackhull and Kaw61 from Kansas, formed a unique cluster. The Texas-released cvv. TAM 101, TAM 200, and TAM 202 grouped together. Jagger, the most widely grown cultivar from the Great Plains in 2000–05, grouped with Jagalene and Overley, which was as expected because Jagger is one of the parents of Jagalene and Overley. All clusters contained cultivars from multiple states, except the smallest cluster with only two Kansas cultivars (Early Blackhull and Kaw61), suggesting that frequent exchange and sharing of breeding materials occurred across HRWW breeding programs from different states in different time periods. The fact that the predominant cluster was based on regional adaptation (Fig. 1) within different parts of the Great Plains indicates the likeliness of widespread sharing of related ancestral lines within the various breeding programs.

To evaluate the temporal variation and relationship among different decades, marker data were used to group different decades. The analysis identified 3 major decadal groups, i.e. 1980s decadal group, pre-1980 decadal group, and post-1980s decadal group (Fig. 2). Breeding objectives before 1980 mainly focussed on incorporating semi-dwarfing genes, especially Rht1, and stem rust resistance genes. In the 1980s, breeding objectives shifted mainly to leaf rust resistance, which continues to be a very important objective in all the HRWW breeding programs along with end-use quality. Cox *et al.* (1986) reported a low genetic diversity among the cultivars released before 1950 in the Great Plains HRWW region due to the widespread and continuous use of Turkey, a land race, and its derivatives Pawnee, Wichita, Triumph, and Comanche. They also reported an increase in genetic diversity among the cultivars from 1950 to the 1980s, due to the extensive use of semi-dwarfing genes from soft red spring wheat in the 1960s and 1970s (Cox *et al.* 1986). Our results indicate that although the breeding objectives for HRWW were relatively narrow across decades and a lot of cultivars shared a common ancestry, genetic diversity was maintained among the commercial cultivars to date by the introduction of new genes from different gene pools. Breeders always selected for cultivars that were well adapted to the variable regional environments while introducing genetic diversity from
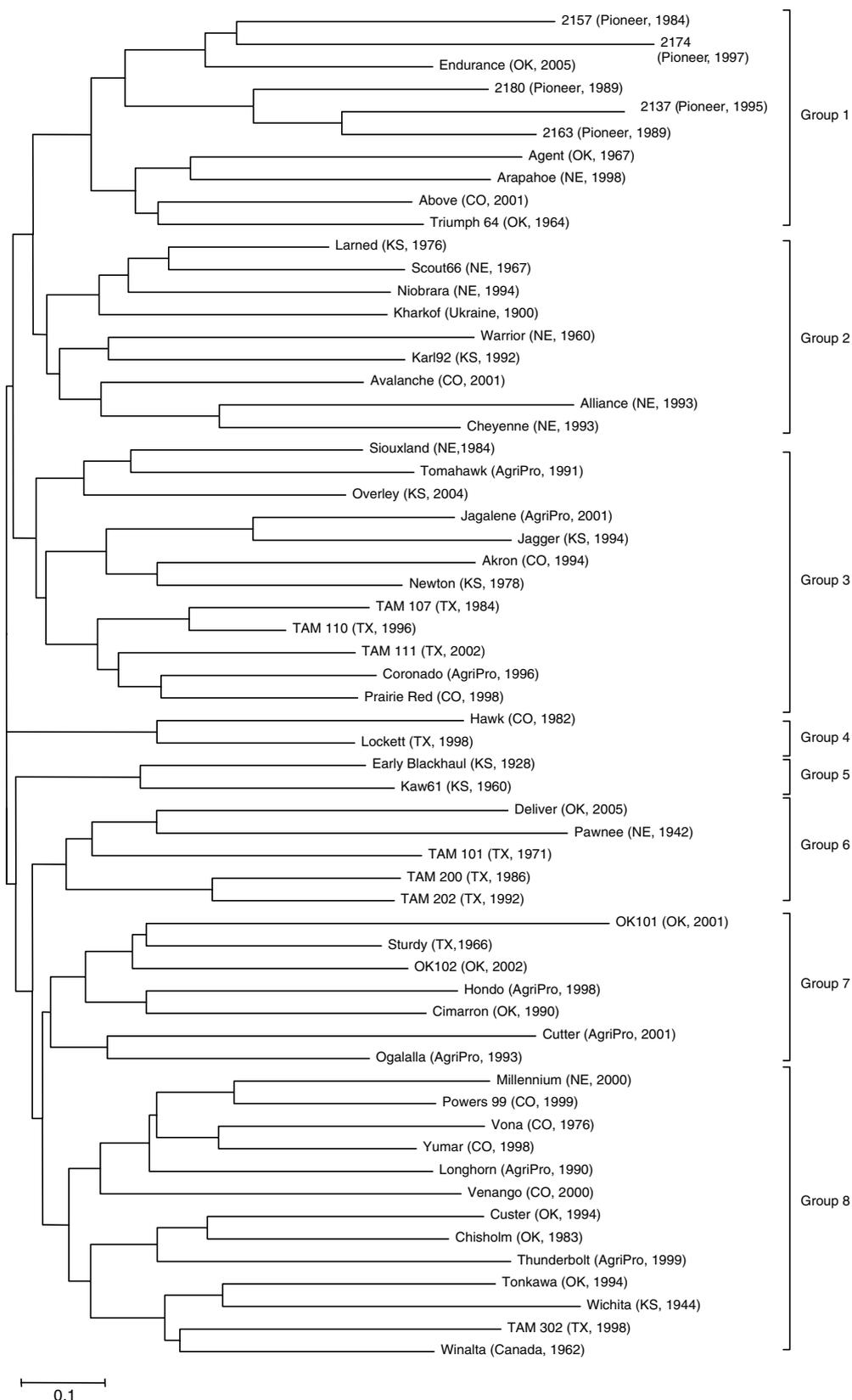
Fig. 1. Clusters showing the relationship of 60 hard red winter wheat cultivars based on 62 polymorphic SSR markers using Nei's genetic distance algorithm.
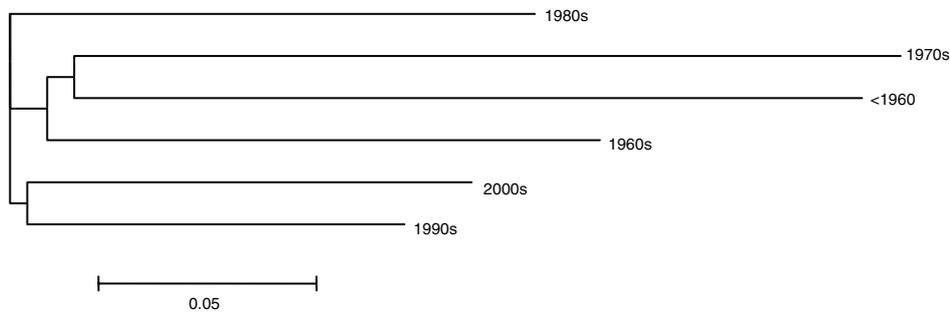
Fig. 2.   Neighbour joining tree of the 6 decadal groups of 60 hard red winter wheat cultivars based on Nei's genetic distance algorithm.

several germplasm pools, especially from the Mexican spring wheat and European germplasm.

Genetic diversity in the HRWW cultivars

The distribution of molecular variation among and within the identified clusters of 60 HRWW cultivars as indicated in Fig. 1 was estimated by AMOVA procedure (Table 3). The AMOVA revealed that around 10% of the total variation resided among the clusters, whereas around 90% of the total molecular variation existed among the cultivars within the clusters. The higher proportion of genetic variation explained by the cultivars within the clusters indicated a large variation among the cultivars within a cluster as a result of intensive breeding activities within each breeding program, which made use of diverse genetic materials while selecting for local adaptation. Roussel *et al.* (2005) also reported similar trends of larger within-group genetic variation in wheat cultivars from different regions of Europe. Our results suggest that a significant amount of genetic diversity was incorporated into the HRWW cultivars in this study.

To investigate the diversity changes based on the decades and breeding programs, the AMOVA procedure was also carried out using the groups created by decades of cultivar release and

sources of cultivar release (breeding programs) as indicated in Table 2. The AMOVA revealed a significant proportion of genetic variation as explained among the decades as well as among the cultivars within decades (Table 4). The higher proportion of genetic variation was attributed to among cultivars within decades variance (94.68%) compared with the among decades

Table 3.   Analysis of molecular variance (AMOVA) for the 8 clusters of 60 hard red winter wheat cultivars identified in Fig. 1 using Nei's genetic distance

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*P*-value
Among clusters	7	375.2	1.64	9.85	<0.001
Among cultivars within clusters	112	1562.8	15.02	90.15	<0.001
Total	119	1938.0	16.66		

Table 4.   Analysis of molecular variance (AMOVA) for the 6 decadal groups of 60 hard red winter wheat cultivars as listed in Table 2

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*P*-value
Among decades	5	157.6	0.88	5.32	<0.001
Among cultivars within decades	114	1780.4	15.62	94.68	<0.001
Total	119	1938.0	16.50		

Table 5.   Analysis of molecular variance (AMOVA) for the 8 breeding groups of 60 hard red winter wheat cultivars as listed in Table 2

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*P*-value
Among breeding programs	7	331.9	2.24	13.51	<0.001
Among cultivars within breeding programs	112	1606.1	14.34	86.49	<0.001
Total	119	1938.0	16.58		

Table 6.   Pair-wise Wright's fixation index (F_{ST}) values between the 8 clusters identified in Fig. 1 using Nei's genetic distance
*P < 0.05; **P < 0.01

Cluster	1	2	3	4	5	6	7
1	–						
2	0.143**	–					
3	0.170**	0.126**	–				
4	0.302*	0.272 *	0.266*	–			
5	0.276**	0.285**	0.264*	0.501	–		
6	0.188**	0.161**	0.116	0.320	0.280	–	
7	0.169**	0.174**	0.187**	0.273*	0.261*	0.172**	–
8	0.121**	0.119**	0.121**	0.225**	0.242**	0.123**	0.116*

Table 7.   Pair-wise Wright's fixation index (F_{ST}) values between the 6 decadal groups of 60 hard red winter wheat cultivars as indicated in Table 2
*P < 0.05

Decades	<1960	1960s	1970s	1980s	1990s
<1960	–				
1960s	0.069	–			
1970s	0.179*	0.113	–		
1980s	0.102*	0.041	0.112	–	
1990s	0.079*	0.043	0.064	0.027	–
2000s	0.070*	0.050	0.094*	0.046	0.032

Table 8. Pair-wise Wright's fixation index (F_{ST}) values between the 8 breeding programs as indicated in Table 2
$*P < 0.05; **P < 0.01$

Breeding programs	Oklahoma	Kansas	Texas	Nebraska	Colorado	AgriPro	Pioneer
Oklahoma	–						
Kansas	0.106**	–					
Texas	0.138**	0.141**	–				
Nebraska	0.108**	0.105*	0.173**	–			
Colorado	0.118**	0.118**	0.155**	0.093*	–		
AgriPro	0.063	0.074	0.116*	0.100**	0.079	–	
Pioneer	0.131**	0.198**	0.242**	0.161**	0.201**	0.197**	–
Other	0.180	0.209	0.273	0.191	0.216	0.216	0.324*

variance (5.32%). When the different breeding programs were subjected to an AMOVA test (Table 5), the proportion of genetic variation was greater for among cultivars within breeding program variation (86.49%) compared with the among breeding programs variation (13.51%), although both of them were highly significant. When the three AMOVA procedures were compared for among groups variation, the genetic variation was best explained by the among breeding programs variation, which means true genetic variations exist between the different breeding programs in the Great Plains.

Determination of F_{ST} value of the polymorphic loci for all accessions revealed that the F_{ST} values ranged from 0.017 (*WMC* 477) to 0.427 (*BARC* 184), with an average of 0.288 (data not shown). These results suggest that 28.8% of the total variation in allele frequency was due to genetic variation among clusters. It has been reported that markers with higher F_{ST} values have greater power to produce genetic distance estimates (Watkins *et al*. 2003). Genetic variation among the clusters was tested by computing pair-wise F_{ST} values between the clusters (Tables 6–8). Most of the pair-wise F_{ST} values were statistically significant ($P < 0.05$) for groups created by cluster analysis (Table 6) and breeding programs (Table 8). Higher numbers of pair-wise comparisons based on the decadal groups resulted in a non-significant difference among the pairs of decades compared with the other two groups (cluster analysis and breeding programs). This indicates that the enhancement of genetic diversity may not be well classified as true decadal groups compared with the breeding programs or the groups identified in the cluster analysis. The significant F_{ST} values among the clusters (Tables 6 and 8) suggest that a real difference between the clusters exists and a significant amount of genetic diversity is still present among the HRWW cultivars used in this study from different breeding programs of the Great Plains.

These results suggest that there has been no real loss of genetic diversity in the studied HRWW cultivars. In fact, a gradual increase of allelic richness was observed from 1980 to 1999, indicating the incorporation of important alleles for better agronomic, disease resistance, and quality traits until 1999. After 2000, a decrease in allelic richness was due to the qualitative changes in allelic composition. Replacement with alleles favourable for adaptation is a key feature for today's breeding approach, which has led to the apparent loss of allelic richness (Landjeva *et al*. 2007), but the maintenance of gene diversity and high PIC values in the 2000s releases indicate positive changes in the most recent cultivars. Genetic diversity

may not be well assessed using a particular decade because the pattern of changes may not happen in a defined number of years. But the overall results of the study indicate that the wheat breeders in the Great Plains adopted a very effective breeding strategy to introduce new variation in the existing gene pool when needed, and maintained that variation over a long period of time.

Acknowledgments

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