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ORIGINAL PAPER

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The use of microsatellite markers for the detection of genetic similarity among winter bread wheat lines for chromosome 3A

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Abstract Previous studies with chromosome substitution and recombinant inbred chromosome lines identified that chromosome 3A of wheat cv. Wichita contains alleles that influence grain yield, yield components and agronomic performance traits relative to alleles on chromosome 3A of Cheyenne, a cultivar believed to be the founder parent of many Nebraska developed cultivars. This study was carried out to examine the genetic similarity among wheat cultivars based on the variation in chromosome 3A. Forty-eight cultivars, two promising lines and four substitution lines (in duplicate) were included in the study. Thirty-six chromosome 3A-specific and 12 group-3 barley simple sequence repeat (SSR) primer pairs were used. A total of 106 polymorphic bands were scored. Transferability of barley microsatellite markers to wheat was 73%. The coefficient of genetic distance (*D*) among the genotypes ranged from 0.40 to 0.91 and averaged *D*=0.66. Cluster analysis by the unweighted pair-group method with arithmetic averages showed one large and one small cluster with eight minor clusters in the large cluster. Several known pedigree relationships largely corresponded with the results of SSR clusters and principal coordinate analysis. Cluster analysis was also carried out by using 22 alleles that separate Wichita 3A from Cheyenne 3A, and three clusters were identified (a small cluster related to Cheyenne of mainly western Nebraska wheat cultivars; a larger, intermediate cluster with many modern Nebraska wheat cultivars; a large cluster related to Wichita with many modern high-yielding or Kansas wheat cultivars). Using three SSR markers that identify known agronomically important quantitative trait loci (QTL) regions, we again separated the cultivars into three main clusters that were related to Cheyenne or Wichita, or had a different 3A lineage. These results suggest that SSR markers linked to agronomically important QTLs are a valuable asset for estimating both genetic similarity for chromosome 3A and how the chromosome has been used in cultivar improvement.

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Introduction

In crop improvement programs, information on genetic diversity and the relationships among elite experimental lines and cultivars is useful for the development of new cultivars. This knowledge may speed up the breeding procedure and identify lines that need to be crossed to combine desirable characters. To understand and use genetic diversity, molecular markers that are highly polymorphic and easy to use are beneficial. The degree of genetic variation in wheat (*Triticum aestivum* L.) has been assessed with different types of DNA molecular markers (Koebner et al. 2001). Microsatellites (syn. simple sequence repeats, SSRs) are commonly used to study genetic relationships among lines of a species because of their high level of polymorphism (Bowcock et al. 1994; Röder et al. 1995). In addition, microsatellites exhibit codominant inheritance (Hernandez et al. 2002), which is essential for effective discrimination between closely related lines (Akkaya et al. 1992). Microsatellite markers are currently used to identify genotypes, quantitative trait loci (QTLs) and genetic diversity (Gupta and Varshney 2000; Budak et al. 2003).

Recent investigations in comparative genetics have revealed that gene content and order are highly conserved among closely related species (Devos and Gale 2000; Gaut 2002). Colinearity of common markers illustrated by comparative maps suggests that a marker of one genus/

Table 1 Pedigree and origin of the wheat cultivars or advanced lines used for the genetic similarity assessment

Cultivar/line name	Accession no.	Origin	Release date	Pedigree
Turkey	PI11610	USA	1874	Selection from Crimean
Cheyenne	PI192268	Nebraska	1933	Selection from Crimean; selection from Turkey-Red
Wichita	CI11952	Kansas	1944	Early-Blackhull/Tenmarq
Warrior	CI13190	Nebraska	1960	Pawnee/Cheyenne
Scout 66	CI13996	Nebraska	1967	Selection from Scout
Eagle	CI15068	Kansas	1970	Selection from Scout
Scoutland	CI14075	Nebraska	1970	Selection from Scout
Centurk	CI15075	Nebraska	1971	Kenya-58//Newthatch/3/Hope/2[a]Turkey/4/Cheyenne/5/Parker
Baca	CI15891	Colorado	1972	Selection from Scout
Buckskin	CI17263	Nebraska	1973	Scout/3/Quivira/Tenmarq//Marquillo/Oro
Sage	CI 17277	Kansas	1973	Agenta/4*Scout
Hiplains	CI17262	Nebraska	1973	Gage/Lancer
Homestead	CI17264	Nebraska	1973	Scout/4/Kenya-58/Newthatch/3/Cheyenne/Tenmarq//Mediterranean/ Hope/3/Pawnee/ Cheyenne
Sentinel	CI17265	Nebraska	1973	Scout/5/Kenya-58/Newthatch/3/Cheyene/Tenmarq//Mediterranean/ Hope/3/Pawnee/ Cheyenne
Lancota	CI17389	Nebraska	1975	Atlas-66/Comanche//Lancer
Agate	CI17463	Nebraska	1975	Ponca/3*Cheyenne/4/Kenya-58/Newthatch/3/2[a]CheyenneE/Tenmarq// Mediterranean/ Hope/5/Scout
Bennett	CI17723	Nebraska	1978	Scout/3/Quivira/Tenmarq//Marquillo/ORO/4/Homestead
Capitan	CI17591	Nebraska	1978	Pawnee/Cheyenne/3/Pawnee/Kenya-58//Cheyenne
Centurk 78	CI17724	Nebraska	1978	Selection from Centurk
Brule	PI466739	Nebraska	1981	(Ponca/3*Cheyenne//Selkirk/2[a]Cheyenne)//(Seu-Seun-27/3/CI12500// Mediterranean/ Hope/4/Pawnee/5/Cheyenne/Ponca//Turkey-Red/Cheyenne)/Gage
Colt	PI476975	Nebraska	1983	(Ponca/3*Cheyenne/4/Kenya-58/Newthatch/3/2[a]2Cheyenne/Tenmarq// Mediterranean/ Hope/5/Scout)/(Sturdy(SIB)/KAW)
Siouxland	PI483469	Nebraska	1984	(Warrior*5/Agent)*2/Kavkaz
Cody	PI486212	Nebraska	1986	(Warrior*5/Agent)/Centurk-78
Arapahoe	PI518591	Nebraska	1988	Brule/3/Parker*4/Agent//Belotserkovskaya-198/Lancer
Rawhide	PI543893	Nebraska	1990	Warrior*5/Agent//Kavkaz/4/Parker*4/Agent//Belotserkovskaya-198/ Lancer/3/Vona
Vista	PI562653	Nebraska	1992	(Warrior//Atlas-66/Comanche/3/Comanche/Ottawa)/(Spring*2/TR-TI/4/ Cheyenne/Tenmarq//Mediterranean/Hope/3/Sando-60)//Centurk/3/Brule
Arlin	PI564246	Kansas	1992	Selection from an unspecified buld population on intercrossed HRW and HRS
Alliance	PI573096	Nebraska	1993	Arkan/Colt//Chisholm
Nekota	PI584997	Nebraska	1994	Benett/TAM-107
Niobrara	PI584996	Nebraska	1994	TAM-105*4/Amigo//Selection from Brule
Akron	PI584504	Colorado	1994	TAM-107/HAIL
Jagger	PI593688	Kansas	1994	KS-82-W-418/Stephens
Windstar	PI597379	Nebraska	1996	(TAM-103/KS-73167)//Caldwell/Brule/3/Siouxland
Pronghorn	PI593047	Nebraska	1996	Centura/Dawn//(SIB)Colt
Wesley	PI605742	Nebraska	1998	(Plainsman-V/Odesskaya-51)/(Colt/Cody)
Betty	PI612578	Kansas	1998	Selection from Jagger
Heyne	PI612577	Kansas	1998	Pitic-62/(SIB)Chris//2*Sonora-64/3/Klein-Rendidor/4/Scout
Culver	PI606726	Nebraska	1999	(Trapper//Comanche/Ottawa/3/CIMMYT/Scout/4/Buckshin/Homestead/Arapahoe
Cougar	PI613098	Nebraska	1999	(Warrior,USA*5/Agent//Kavkaz/4/NE-63218/Kenya-58/3/NTH/2*CMTH//Ponca/ 2*-Cheyenne)/Thunderbird
Millennium	PI613099	Nebraska	1999	Arapahoe/Abilene//(Colt/3/Warrior,USA*5/Agent//Kavkaz)
Nuplains		Nebraska	1999	Abilene/(Plainsman-V//Newton/Arthur-71)
Trego	PI612576	Kansas	1999	(Lr16/Lr17//LEE/3/Cumhuriyet/LEE/5/TAM-107)/Rio Blanco

Table 1 (continued)

Cultivar/line name	Accession no.	Origin	Release date	Pedigree
Wahoo		Nebraska	2001	Arapahoe/Abile//Arapahoe
Harry		Nebraska	2002	NE 90614/NE 87612
Goodstreak		Nebraska	2002	SD 3055/KS 88H164//NE 89646
Jagalene			2002	Abilene/Jagger
Hondo[a]				Not known
Alsen[a](Spring)		DNK		ND674//ND2710/ND688
NE 96623[a]		Nebraska		Victory//Colt/Cody/3/Arapahoe
NE96628[a]		Nebraska		NE 87613/SB-360
CNN(WI 3A)		Nebraska		Substitution line
CNN(WI 6A)		Nebraska		Substitution line
WI(CNN 3A)		Nebraska		Substitution line
WI(CNN 6A)		Nebraska		Substitution line

[a]Fusarium head blight (incited by *Fusarium* spp.)-resistant lines

species will be present in another related genus/species (Van Deynze et al. 1998; Tikhonov et al. 1999). Sequence data obtained from several crop plants indicate homology exists between genomes in the regions flanked by SSR loci (Brown et al. 1996; Peakall et al. 1998; Van Deynze et al. 1998; Tikhonov et al. 1999) . Thus, primer pairs designed for one species could be used to detect SSRs in related species. The transfer of SSRs has been proposed and successfully demonstrated in many genera, including *Glycine* (Peakall et al. 1998), *Prunus* (Dirlewanger et al. 2002), between *Aegilops* and *Triticum* (Sourdille et al. 2001) and wheat, rye (*Secale cereale* L.) and Triticale (×*Triticosecale* Wittmack) (Kuleung et al. 2004).

Scientists conducting QTL experiments in wheat have access to unique genetic resources, including chromosome substitution lines. The evaluation of reciprocal chromosome substitution lines between two parental lines for complex traits enables the identification of single chromosomes containing QTLs for those characters. Since cv. Cheyenne (CNN) is believed to be the founder parent of many Nebraska developed cultivars, a complete set of chromosome substitution lines between CNN and some other cultivars were developed at Nebraska by Dr. M. R. Morris to study the effect of individual chromosomes (Berke et al. 1992a, b; Campbell et al. 2003).

Several studies have found evidence of the importance of chromosome 3A in wheat. Berke et al. (1992a, b) evaluated a full set of reciprocal chromosome substitution lines between cv. CNN and cv. Wichita (WI) and found that WI 3A carried allele(s) that significantly increased grain yield, 1,000 kernel weight and grain volume weight while decreasing plant height and anthesis date when placed in the CNN background. They also found that CNN 3A carried allele(s) that significantly decreased grain yield, tillers per square meter and grain volume weight when placed in the WI background. In order to identify and locate these genes on WI 3A, a set of chromosome 3A recombinant inbred chromosome lines (RICLs-3A) was derived from a cross between CNN and CNN (WI 3A). Shah et al. (1999a, b) evaluated 50 RICLs-3A. Anthesis date was mapped as a single gene on the short arm of chromosome 3A and explained a significant variation for 1,000 kernel weight, kernel per spike and plant height. Additional QTLs were detected for yield components and plant height elsewhere on the chromosome. Campbell et al. (2003), using a larger population of RICLs and more replications, detected QTLs for seven agronomic traits and mapped the QTLs to three regions of chromosome 3A. Major QTLs for kernels per square meter and grain yield were located within a 5-cM interval and were inherited as single QTL with pleiotropic effects. These studies indicate that wheat cultivars containing chromosome 3A from high-yielding cultivars could contribute to grain-yield enhancement and agronomic improvement. Therefore, a comparison of genetic diversity and similarity among cultivars of bread wheat for chromosome 3A is useful for the evaluation of local breeding material and for the exploitation and introduction of these QTLs in other germplasm.

To date, molecular markers, including SSRs, have been used to assay diversity of wheat lines or cultivars (Mercado et al. 1996; Kim and Ward 1997; Barrett and Kidwell 1998; Huang et al. 2002; Sun et al. 2003). The purpose of the investigation reported here was to address these three hypotheses: (1) current SSR markers that target 3A loci are robust tools for breeders that can effectively distinguish genotypes and measure relationships in lines and cultivars that represent important central plains winter bread wheat germplasm; (2) chromosome 3A alleles can be identified that mark cvs. WI and CNN chromosomes and allow breeders to track these chromosomal segments in cultivars adapted for high-yielding or high-stress environments; (3) chromosome 3A alleles from markers linked to agronomically important QTLs can be used to determine if cultivars have marker genotypes associated with high yield versus high-stress tolerance.

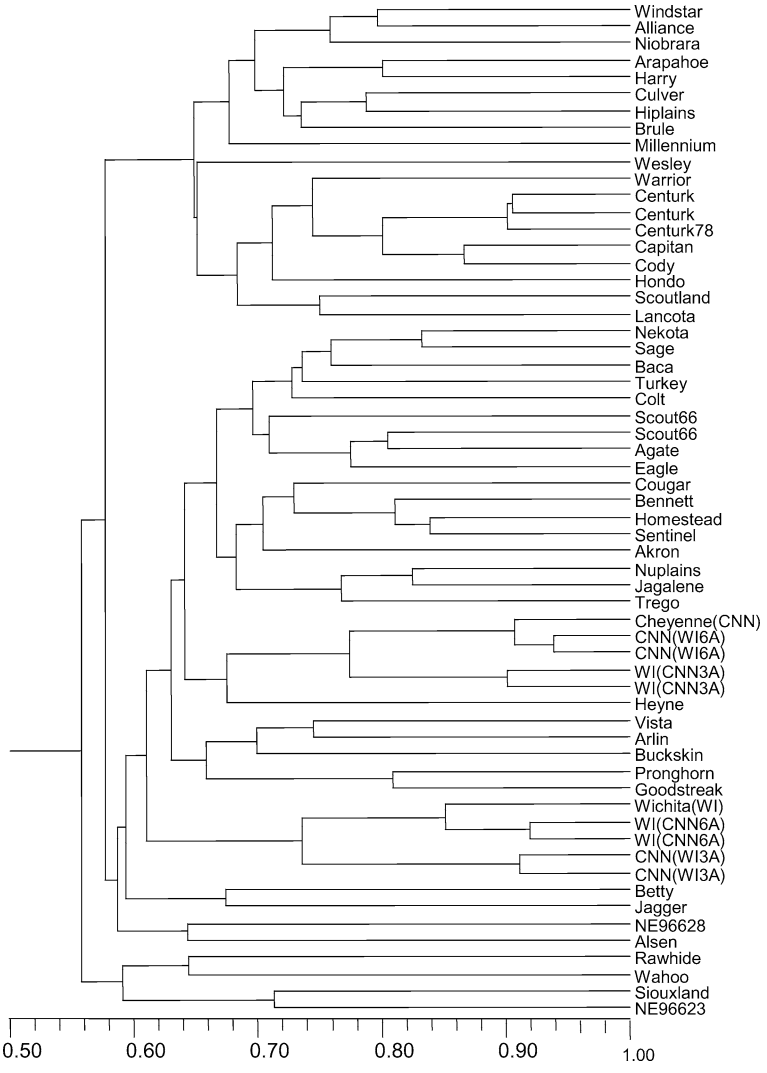

Fig. 1 Dendrogram of 60 wheat cultivars/lines based on the unweighted pair-group method with arithmetic averages analysis using the similarity matrix generated by the Nei and Li (1979) coefficient after amplification with 48 pairs of microsatellite primers

Materials and methods

Plant material

Forty-eight elite winter wheat cultivars including WI and CNN, two advanced lines and four substitution lines in duplicate [CNN(WI 3A), CNN(WI 6A), WI(CNN 3A) and WI(CNN 6A)] were included in the study (Table 1). Two cultivars (Scout 66 and Centurk) were included twice because seed from different sources was obtained. The two sets of the four substitution lines were included because they were independently developed, and we wanted to confirm their genetics. Of the genotypes, 38 were elite winter wheat cultivars and two advanced lines developed by Nebraska, eight cultivars were developed by Kansas and two cultivars were developed by Colorado. These genotypes were selected for this study because CNN or WI are founder parents of many cultivars released in the Great Plains. Seed were obtained from the breeders or the USDA National Small Seed Grain Collection, Aberdeen, Idaho.

DNA extraction

Genomic DNA of each line was isolated from 100 mg fresh tissue using a sap extraction method. Leaves of 2-week-old seedlings grown in greenhouse were placed between the two rollers of a sap extraction apparatus (Ravenel Specialities, Seneca, S.C.), and 1 ml of extraction buffer (50 m*M* Tris-HCl, 25 m*M* EDTA, 1 *M* NaCl, 1% CTAB, 1 m*M* 1,10-phenathroline and 0.15% 2-mercaptoethanol) was slowly added to the rollers, immediately mixing with the sap for collection in 1.5-ml microcentrifuge tubes. The extract was incubated at 60°C for 1 h then mixed with equal volume of chloroform-isoamyl alcohol (24:1). After centrifugation at 12,000 rpm the supernatant was transferred to a new tube and isopropanol added for a 30-min incubation to precipitate the DNA. The pellet was dried, resuspended in 200 µl TE buffer (10 m*M* Tris-HCl, 0.1 m*M* EDTA, pH 8.0) plus 20 µg of RNase, then incubated at room temperature overnight. The DNA solution was mixed with 20 µl 8 *M* ammonium acetate and 400 µl of cold absolute ethanol for 30 min, centrifuged for 10 min and air-dried at room temperature. The DNA was then resuspended in 200 µl TE

buffer and the DNA concentration quantified by spectrophotometry (TKO100 Fluorometer, Hoefer Scientific Instruments, San Francisco, Calif.).

PCR amplification

Fifty-two wheat chromosome 3A (kindly provided by Dr. P. Cregan, USDA-ARS, Beltsville, Md.; Röder et al. 1998) and 37 barley (*Hordeum vulgare* L.) group-3 (Liu et al. 1996) SSR markers were screened for amplification and polymorphism in 60 genotypes of wheat. The PCR reaction mixture (25 µl total) consisted of 50 m*M* KCl and 10 m*M* Tris-HCl, pH 8.8), 2 m*M* $MgCl_2$, 125 µ*M* of dNTP, 50 ng of each primer, 1.0 U *Taq* polymerase (Promega, Madison, Wis.) and 20 ng of genomic DNA. Amplification was carried out in a MJ Research PTC_100 (programmable thermal controller; MJ Research, Waltham, Mass.) using a program that consisted of initial denaturation for 1 min at 94°C, followed by 32 cycles of 30 s at 94°C, 50 s at 53 and 72°C, and final extension for 5 min at 72°C. The amplified PCR products were gel-fractionated on a 12% polyacrylamide gel (37:1, acrylamide:bis-acrylamide) in a TAE buffer (40 m*M* Tris-HCl, 20 m*M* sodium acetate, 1 m*M* EDTA) using a Hoefer vertical gel apparatus (SE600) at 300 V for 3 h at 20°C and a circulating bath to maintain temperature. The gels were stained in ethidium bromide (1 µg/ml) for 20 min, destained in deionized water for 1 h and photographed under the Gel Doc2000 (Bio-Rad, Hercules, Calif.).

Data analysis

Presence or absence of each SSR fragment was coded as "1" and "0", where "1" indicated the presence of a specific allele and "0" indicated its absence. Genetic diversity for each locus was then calculated by Weir's (1996) gene diversity (D_l)=1−ΣP_{lu}^2 and average gene diversity (D)=1 −(1/m)$\Sigma_l\Sigma_u P_{lu}^2$, where P_{lu} is the frequency of the uth allele at lth locus and m is the number of loci. All analyses were performed using the NTSYSPC ver. 2.1 software package (Exeter Software, Setanket, N.Y.) (Rohlf 2000). Genetic similarities between pairs of accessions were measured by the DICE similarity coefficient based on the proportion of shared alleles (Dice 1945; Nei and Li 1979) with the SIMQUAL module. The Dice similarity coefficient=2a/(2a+b+c), where a is the number of positive matches (presence of a band in both accessions), and b+c is the number of no matches (presence of a band either in one accession but absent in the other accession). The accessions were clustered based on a similarity matrix using an unweighted pair group method with arithmetic average (UPGMA) algorithm with the SAHN module. The result was used to construct a dendrogram with the TREE module. A principal coordinate analysis was performed with the DCENTER and EIGEN modules and illustrated by the 3D PLOT module.

Results and discussion

Transferability of barley group-3 SSR markers to wheat

Of the 37 barley group-3 SSR markers, 27 (73%) amplified in wheat. Of the 27 amplified markers, 15 (56%) were monomorphic and 12 (44%) were polymorphic. The barley SSR-amplified fragments were usually weaker than those of the wheat marker fragments. Several reports indicate that microsatellite primer pairs developed from one genus or species can amplify DNA of close relatives. Korzun et al. (2001) reported that approximately 27% of wheat SSR markers were amplified in rye, whereas Röder et al. (1995) observed a high level of transferability of the wheat SSR markers to rye (60%), although most amplified products were weak. While the transferability of the plant SSR markers is usually less effective than that of restriction fragment length polymorphism (RFLP) markers (Korzun et al. 1998; Van Deynze et al. 1998), this is not always the case, Dirlewanger et al. (2002) reported a high transferability of SSR markers in peach (*Prunus* spp.).

Genetic similarity between cultivars for chromosome 3A

Of the 52 SSR primer pairs specific for wheat chromosome 3A, 36 (69%) were polymorphic. Hence, 36 wheat chromosome-3A and 12 barley group-3H SSRs (total of 48) were used for estimating genetic diversity. About one-half of the samples were repeated to test for reproducibility, and only reproducible and unambiguous bands were used for the analysis.

Based on the 106 shared alleles of the 48 SSRs, we calculated a similarity coefficient for each pair of the 60 wheat genotypes. This similarity coefficient ranged from 0.40 to 0.91 and averaged 0.66 (data not shown). The greatest diversity was observed between Siouxland and Alliance, while Centurk and Bennett were most closely related cultivars. Three of the most closely related cultivars to CNN (WI 3A) were Nekota, Homestead and Trego, each with similarity coefficients of 0.70 or more. Nekota and Trego shared a common ancestor, Bennett, and Homestead is one of the parents of Bennett. Wichita is related to Bennett, as both share a common parent, Tenmarq.

The UPGMA cluster analysis established from the similarity data revealed two main cluster groups of individuals within the population of 60 cultivars/lines (Fig. 1). Windstar and NE 96623 (both developed by the University Nebraska breeding effort) were found to span the extremes of the dendrogram, with all other germplasm distributed between the maximum genetic distance of 0.56 U. The clustering generally agreed with the pedigree information. One of the two main groups consisted of four cultivars/lines (Rawhide, Wahoo, Siouxland and NE 96623) released from the University of Nebraska; all except Siouxland share four common ancestors (Parker,

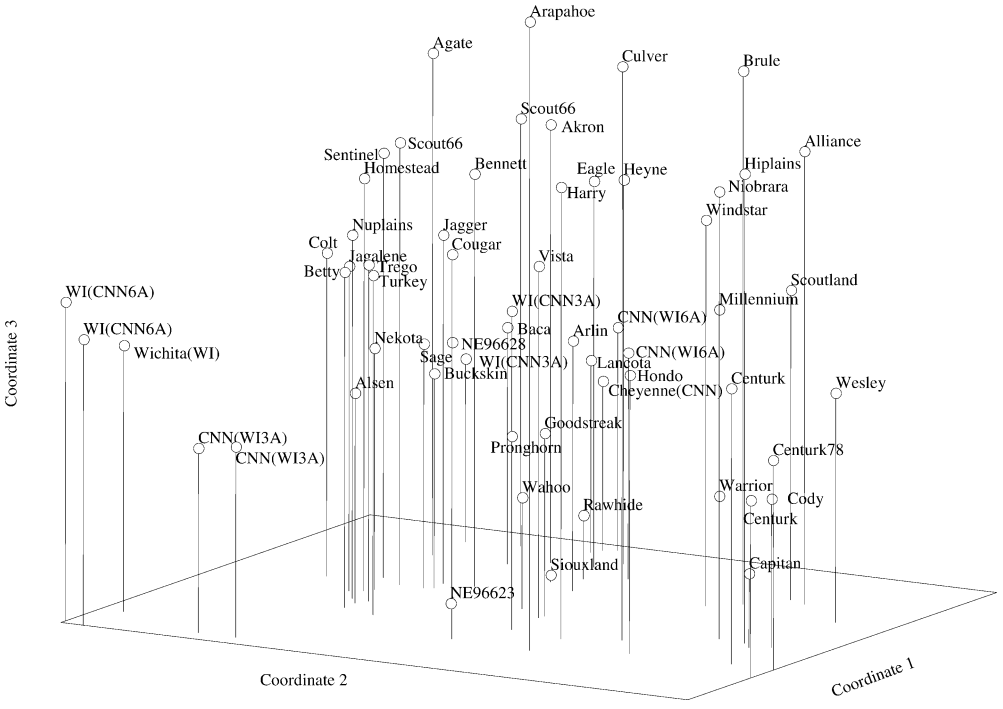

Fig. 2 Relationships among the 60 wheat genotypes visualized by PCA of SSR-based genetic similarities. Coordinates *1*, *2*, and *3* are indicated

Agent, Beloterkovskaya 198 and Lancer). It is unlikely that Agent was the reason for this cluster, as it was used as the donor parent with five backcrosses to the recurrent parent in many cultivars in addition to the four listed above, and the selection was for the important disease resistance genes (*Sr24*, *Lr24*) from an alien species translocated to chromosome 3DL. Only if our primers amplified an allele on the homoeologous group-3 chromosome would alleles from Agent be visualized.

The other main group was subdivided into eight subgroups. One of the subgroups, which consisted of nine lines, including seven recently released cultivars developed at the University of Nebraska after 1988 (Windstar, Alliance, Niobrara, Arapahoe, Harry, Culver and Millennium), and two older cultivars developed before 1988 (Hiplains and Brule), grouped together at a genetic distance value of 0.67 U. Brule and Hiplains were extensively used as parents of the seven new cultivars of this group. Another subgroup consisted of eight older Nebraska cultivars, a newer Nebraska cultivar, Wesley and a cultivar developed by AgriPro (Hondo). All cultivars shared the common parent, CNN. In this group, Nebraska cv. Centurk was repeated twice as seed was received from two different sources. Centurk was developed in 1971 and was a highly heterogeneous cultivar as can be seen by Centurk 78 being selected from Centurk. The genetic similarity between two sources of Centurk and Centurk 78 (a selection from Centurk) was 0.89 U. The differences between the two sources of Centurk may be due to a small number of plants being sampled for DNA extraction in a known heterogeneous cultivar or possibly due to mutation or outcrossing in one source. Seventeen cultivars were clustered together, all of which, except for Turkey, were either selected from Scout or shared Scout as an ancestral parent. Cultivars Cheyenne and Heyne grouped with the

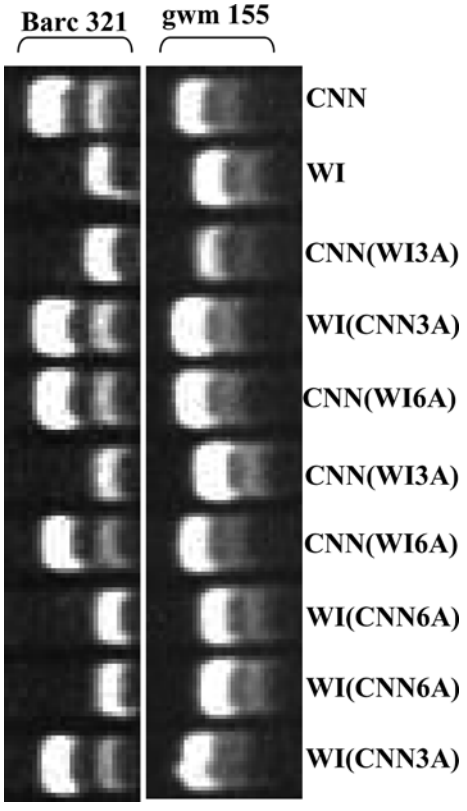

Fig. 3 PCR amplification of genomic DNA of ten wheat genotypes using SSR markers: **a***Xgwm 155*, **b***Xbarc 321*. The amplification products were fractioned on a 12% non-denaturing polyacrylamide gel and the gel subsequently stained with ethidium bromide. *WI* Wichita, *CNN* Cheyenne, *CNN*(*WI 3A*) Wichita chromosome 3A substitution in CNN, *WI*(*CNN 3A*) Cheyenne 3A substitution in WI, *CNN*(*WI 6A*) Wichita chromosome 6A substitution in CNN, *WI* (*CNN 6A*) Cheyenne chromosome 6A substitution in WI

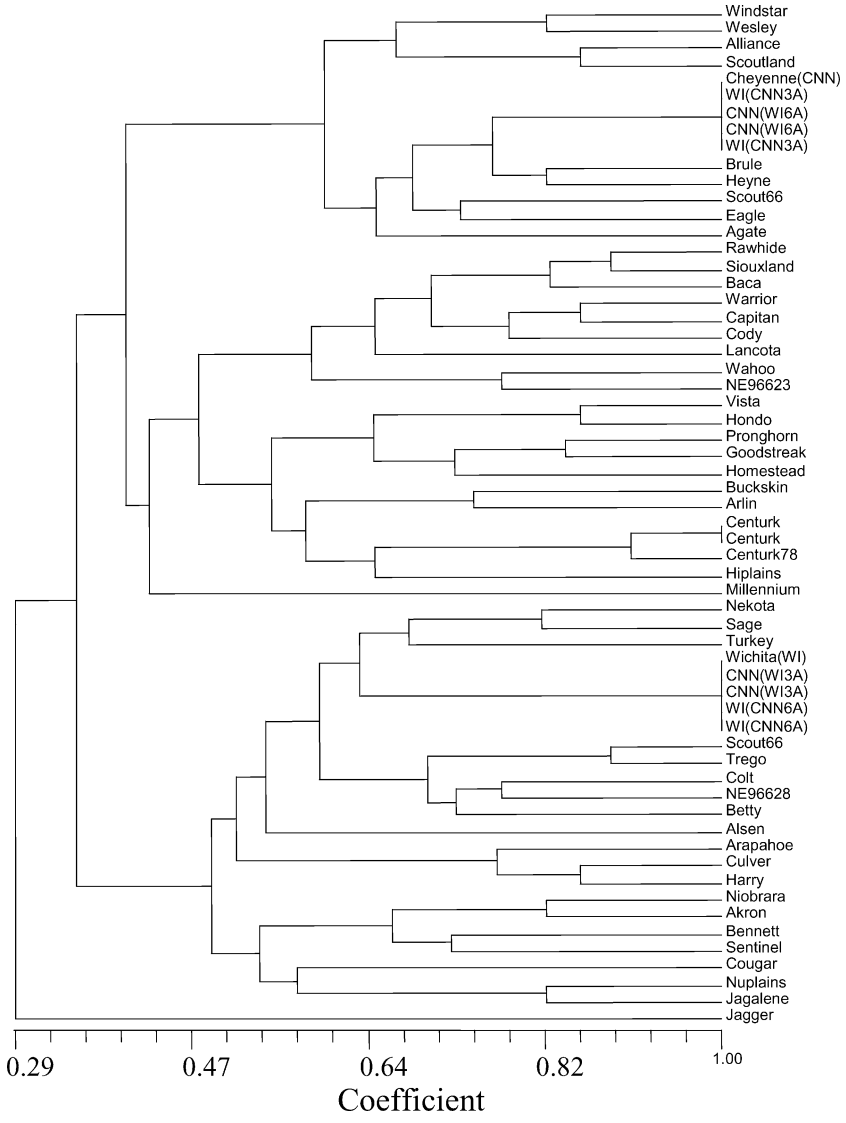

Fig. 4 Dendrogram of 60 wheat cultivars/lines revealed by 22 microsatellite alleles specific to chromosome 3A of Wichita or 3A of Cheyenne

Cheyenne substitution lines or WI(CNN 3A), and Heyne has Cheyenne as an ancestor. Three new Nebraska cultivars (Vista, Pronghorn and Goodstreak), one older Nebraska cultivar, Buckskin, and one Kansas cultivar, Arlin, were grouped together. All cultivars of this group have all or many of the lines Ponca, CNN, Tenmarq and Hope as ancestors. This group was remarkably diverse in that Vista and Arlen are high-yielding semidwarf cultivars, while Buckskin, Pronghorn, and Goodstreak are cultivars of conventional height that yield best in low-yielding, drought-prone environments. The diversity among these cultivars presumably is due to genes on other chromosomes. As was expected, cv. Wichita grouped with its substitution lines having the WI 3A chromosome, although the CNN(WI 3A) was not as similar as WI (CNN 6A), which would have the WI 3A indicating our SSR makers may amplify DNA from homoeologous chromosomes. Two cultivars released from Kansas, Betty and Jagger were grouped together as Betty was developed as a selection from Jagger. NE 96628 and Alsen grouped together. Very little is known about the pedigree of Alsen, but it includes Wheaton, Grandin and Sumai 3, which is known to have a QTL for Fusarium head blight on chromosome 3BS (Liu and Anderson 2003). Both lines were included because they are reported to be tolerant to Fusarium head blight (incited by *Fusarium* spp.), and a QTL for Fusarium head blight has been found on chromosome 3A (Shen et al. 2003). Therefore, these lines possibly share a common ancestor.

Duplicate sets of each substitution line were used, and each set had a small diversity compared with that of the other. As these lines were developed by the backcrossing method, the differences may be due to the number of backcrosses made with the recurrent parent during preparation of the lines, or some scorable bands may be from chromosome(s) other than 3A. In this study, two samples of Scout 66 and Centurk were used to represent seed from two different sources. Both sources of Centurk were similar, however two sources of Scout 66 showed some dissimilarity. Scout 66 was a derivative of Scout, a highly heterogeneous cultivar as indicated by the fact that Eagle, Scoutland and Baca were selected from Scout. Scout 66

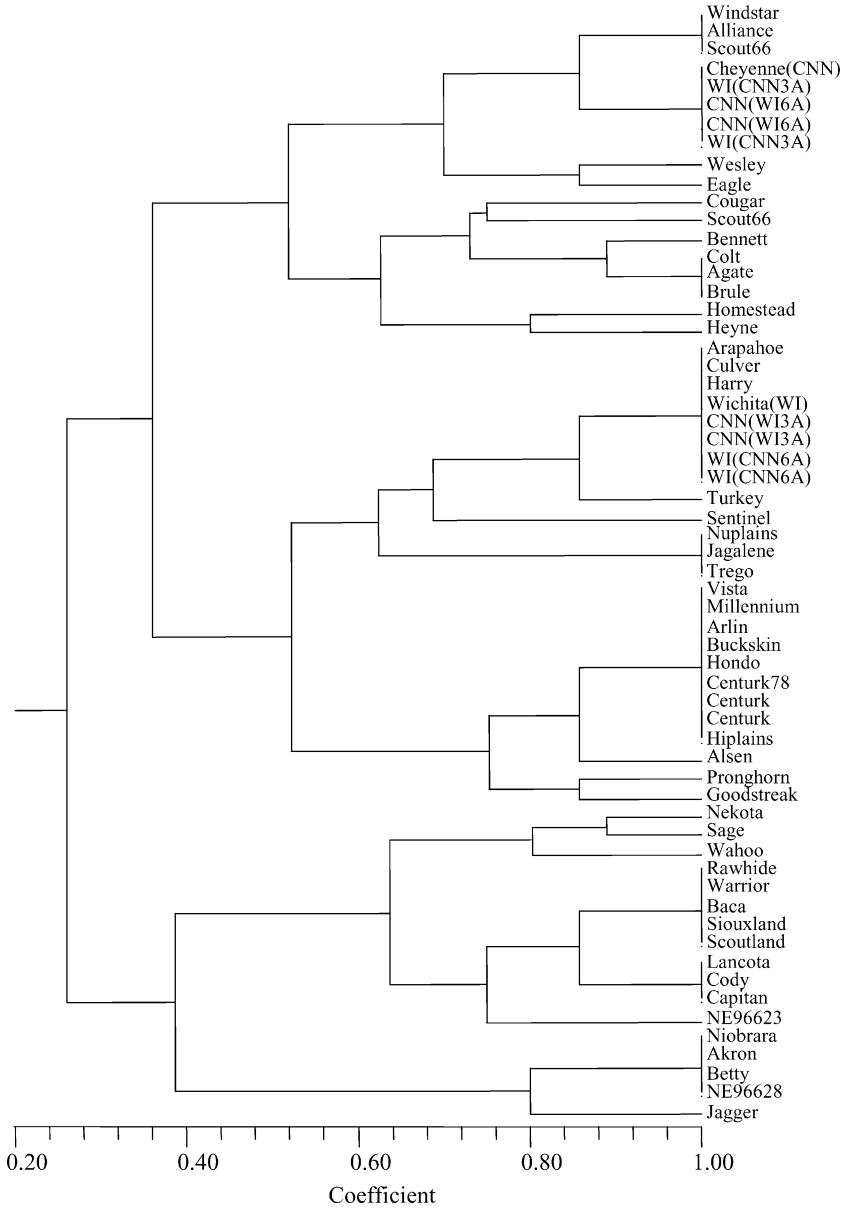

Fig. 5 Dendrogram of 60 wheat cultivars/lines constructed by using the data of three primer pairs, *Xbarc 12*, *Xbarc 57* and *Xbarc 67*, linked to agronomically important traits

may also be heterogeneous, and this dissimilarity may be the result of sampling a few plants from a heterogeneous cultivar.

Principal coordinate analysis (PCA) was performed based on the genetic similarity matrix in order to gain a better understanding of the relationship among the cultivars, lines and substitution lines (Fig. 2). The PCA revealed a grouping that was almost similar to that of the UPGMA analysis. As was observed in the dendrogram, substitution lines having Wichita chromosome 3A grouped with cv. Wichita, while substitution lines without WI chromosome 3A grouped with cv. Cheyenne. The four most divergent cultivars of the distinct group in UPGMA were also well separated from the other cultivars. All other cultivars were largely separated into the same groups as they were in the cluster analysis.

Microsatellite analysis of the WI 3A and CNN 3A chromosomes

Of the 48 pairs of primers, 20 primer pairs revealed 22 polymorphic bands specific to the WI 3A or CNN 3A chromosomes such that bands were only present for cv. Wichita (or where the WI 3A chromosome was present in the substitution lines) and absent in Cheyenne (or where the CNN 3A chromosome was present in the substitution lines) or vice versa (Fig. 3). Data on the 22 alleles were used for determining similarity coefficients and performing a clustering analysis of 60 lines/cultivars to determine similarity among lines specific to the WI 3A and CNN 3A chromosome. For each locus, a screen of the wheat cultivars was performed to identify the alleles corresponding in size to the WI 3A or CNN 3A chromosome. we found three main clusters in an UPGMA dendrogram based on these 22 alleles that separated WI and CNN (Fig. 4).

No one cultivar had all 22 alleles from either WI or CNN. The first cluster was small and contained CNN and substitution lines having CNN 3A and predominantly wheat cultivars adapted to western Nebraska (e.g. Alliance, Windstar, Brule, Scoutland) with two exceptions, Wesley and Heyne. The second cluster was a large cluster that was more closely related to CNN than WI and contained many of the Nebraska-bred modern cultivars (e.g. Rawhide, Siouxland, Wahoo, Pronghorn, Millennium). The third cluster was related to WI and contained Turkey, the first important hard wheat in the Great Plains (hence potentially the source of many WI alleles), and some of the Kansas cultivars (e.g. Trego, Sage, Betty). This cluster also included modern Nebraska cultivars that tend to be adapted to higher yielding environments. Twenty-two alleles provided sufficient information for similarity studies of the cultivars with a WI 3A or CNN 3A chromosome. Previous genotype studies found that seven microsatellites were enough for the identification of soybean (*Glysine max* L.) genotypes (Rongwen et al. 1995). The genetic distance and dendrogram was constructed for rice (*Oryza sativa* L.) using ten microsatellites (Garland et al. 1999) and 15 microsatellites in barley (*Hordeum vulgare* L.) (Struss and Plieske 1998). Twelve microsatellite loci were used for chromosomal inheritance studies of 59 wheat cultivars (Pestsova and Röder 2002), and a set of 12 primer pairs were able to distinguish 48 wheat genotypes (Prasad et al. 2000).

Similarity analysis using agronomically important microsatellites

In a previous study (Campbell et al. 2003) comparing CNN and CNN (WI 3A) and their derivatives, QTLs were detected for grain yield, 1,000 kernel weight, kernels per square meter and kernel number per spike linked with *Xbarc 12*, *Xbarc 57* and *Xbarc 67*. Data for only these three primer pairs were analyzed in the present investigation. UPGMA cluster analysis separated the lines into three broad groups (Fig. 5). One group consisted of cultivars related to CNN and its substitution lines, the second group consisted of WI and its substitution lines, while the third group consisted of cultivars apparently not related to any of the two above-mentioned historically important cultivars. We believe these three groups represent: (1) cultivars that generally do well in western Nebraska where the CNN alleles are not deleterious (e.g. Windstar, Alliance, Scout 66), (2) cultivars that tend to do better in eastern Nebraska or are high yielding in western Nebraska where the WI alleles may be beneficial (e.g. Arapahoe, Harry, Culver) and (3) cultivars where the genes on chromosome 3A have been replaced by new alleles not present in either WI or CNN (e.g. Jagger, Lancota, Wahoo). For these three loci, Arapahoe, Culver and Harry had the same genotypes as CNN (WI 3A). These three lines are modern semidwarf cultivars that are or were known to have a very high yield potential in regions where the QTLs on 3A would be beneficial. In contrast, no cultivar had a similarity coefficient of 1.00 with Cheyenne. The most closely related cultivars to Cheyenne for these three loci were Windstar, Alliance, and Scout 66, all western Nebraska cultivars that are grown where the CNN and WI alleles are not beneficial or, in fact, are deleterious (Campbell et al. 2003). Wesley is an interesting cultivar in that it does extremely well in high-yielding conditions, yet has mainly unfavorable alleles from CNN. Wesley would be a good candidate for transferring the alleles from either WI-related lines (e.g. Harry) or new alleles from the unrelated lines (e.g. Jagger).

In conclusion, chromosome 3A is known to be an important chromosome that contains useful QTLs for agronomic performance (Berke et al. 1992a, b; Campbell et al. 2003). Using microsatellites, it is possible to detect polymorphisms specific for chromosome 3A among wheat cultivars, thus allowing them to be grouped on either on the basis of using markers specific to WI 3A or CNN 3A (important historical wheat cultivars and parents of many modern cultivars) or on the basis of known QTL regions on 3A. In all cases, microsatellite-based genetic analysis suggests a hierarchical pattern of genetic similarity among wheat lines. These similar clusters provide insight into how genes and QTLs in ancestral parents have been used in breeding improved cultivars. Also, these results provide useful information for appropriate parent selection that can be used in crossing schemes for elite cultivar development.

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