Published May, 2002

SSR Variation in Important U.S. Maize Inbred Lines

James G. Gethi, Joanne A. Labate, Kendall R. Lamkey, Margaret E. Smith, and Stephen Kresovich*

ABSTRACT

Historically important public inbred lines continue to play an important role in maize (*Zea mays* L.) improvement in many different breeding programs. Their continued use means they have undergone numerous seed increases in diverse programs since their original release. Our objective was to estimate the level of genetic diversity among and within inbred lines from different sources using SSR markers. We sampled six inbred lines (B73, CM105, Mo17, Oh43, W153R, and Wf9) obtained from 14 sources (breeding programs). The data were analyzed by analysis of molecular variance (AMOVA), genetic diversity statistics, and genetic distance (Dice's coefficient). Of the total variation observed in gene frequency, 87.8% was found among inbred lines, 7.6% among sources within inbred lines, and 4.6% within sources. Genotypes of identically named inbred lines from eight different sources differed slightly on the basis of 44 SSR loci. The mean genetic similarity between sources of the same inbred was greater than 85%. It can be concluded that although more diversity exists among these six inbred lines than within them, a small but significant amount of variation exists among seed sources within inbreds. This variation may have arisen through differences in seed maintenance, since we found no evidence to suggest high mutation rates or extensive outcrossing. The small but statistically significant level of variation raises concerns in germplasm conservation, mapping studies, marker development, and long-term recombinant inbred line development, especially when high resolution is desired.

Hybrid maize production in the USA is based on development and crossing of inbred lines. Since the 1920s, over 600 public inbred lines have been developed, some of which are now extinct (Zuber and Darrah, 1980; Gerdes et al., 1994). Most of the modern inbreds being used in public breeding programs are second or third cycle lines that were developed from other inbred lines or from synthetic populations derived from crossing inbreds (Baker, 1984). Although the older generation inbred lines have been retired from hybrid seed production in the USA, they are still widely used in inbred line development, genetic studies, and as testers in many breeding programs (Smith and Gracen, 1993; Nedev et al., 2000; Marcon et al., 1999; Bing et al., 1992; Hallauer et al., 2000). The availability of different inbred lines from different sources, both within and outside the USA, indicates their continued importance (Mauria et al., 2000; Livini et al., 1992). Surveys conducted in the late 1970s and mid 1980s on inbred lines showed that some of the inbreds continued to contribute substantially to hybrids marketed in the USA. For example, B73 and Mo17 were used in about 28% of all seed planted in the USA in 1979. This fell to 12.8% of the total seed requirements in 1985 (Zuber and Darrah, 1980; Darrah and Zuber, 1985). This may be attributed to a shift in resource allocation from public to private breeding efforts (Frey 1996). Mo17 was released from the University of Missouri in 1964 and B73 was released from Iowa State University in 1972 (Troyer, 1999). Other lines such as Wf9, released in 1936 at Purdue University, and Oh43, released from the Ohio Agricultural Research and Development Center at Wooster in 1949, have been used in maize improvement for over 50 yr.

The most widely recognized and utilized inbreds in the USA fall into one of three heterotic groups. The most commonly used heterotic group is Reid Yellow Dent, of which the most utilized source population is Iowa Stiff Stalk Synthetic (BSSS). The Lancaster Surecrop group consists of material that is more flinty (harder starch in the endosperm) in nature than Reid Yellow Dent (Gerdes and Tracy; 1993; Baker, 1984; Mumm and Dudley, 1994). The other miscellaneous heterotic category comprises inbred lines developed from crosses between the two major heterotic groups, between adapted and exotic germplasm, or derived from distantly related material such as Minnesota 13.

Once released, inbred lines have been maintained for decades through periodic seed increases in breeding programs and at germplasm repositories. Effects of artificial selection regimes, natural selection in maintenance environments, drift, migration (contamination), and mutations could lead to genetic changes (Ajmone-Marsan et al., 1998; Senior et al., 1998; Mumm and Dudley, 1994). Such genetic changes would be influenced by the frequency of regeneration, methods used for regeneration, unintentional outcrossing, and addition of newer versions of the same inbred from other sources.

Variation can be investigated by means of phenotypic and genotypic measures. Quantitative character studies of long-time inbred lines detected genetic changes larger than those expected by breeders (Russell et al., 1963; Fleming et al., 1964; Russell and Vega, 1973). These changes could affect yields of hybrid combinations after several cycles of regeneration. Genetic changes were not constant across inbred lines and sources, and were thought to have resulted from residual heterozygosity, mutation, or a combination of both (Fleming et al., 1964). Busch and Russell (1964) studied 31 sublines from two inbred lines (Os420 and M14) and reported biological changes in more than one character in a subline. They attributed such changes either to pleiotropism or mutations at more than one locus. Russell and Vega (1973) reported that genetic changes occurring in inbred

J.G. Gethi and M.E. Smith, Dep. of Plant Breeding, 252 Emerson Hall, Cornell Univ., Ithaca, NY 14853-1902; J.A. Labate, USDA-ARS, Plant Genetic Resources Unit, Cornell Univ., Geneva, NY 14456-0462; K.R. Lamkey, USDA-ARS, Corn Insects and Crop Genetics Research Unit, Dep. of Agronomy, Iowa State Univ., Ames, IA 50011-1010; S. Kresovich, Institute for Genomic Diversity, Biotechnology Building, Cornell Univ., Ithaca, NY 14853-2703. KRL, JAL, and SK acknowledge funding from the USDA-NRI plant genome program (CSREES Award No. 99-35300-7757). Received 28 Feb. 2001. *Corresponding author (sk20@cornell.edu).

Published in Crop Sci. 42:951–957 (2002).

lines were independent and occurred continuously. The studies by Busch and Russell (1964) and Russell and Vega (1973) revealed that mutations had little practical importance, particularly for expressed characteristics like yield, but were biologically significant and may have negative implications for usage after years of regeneration. Higgs and Russell (1968) showed that inbred lines from six different sources had significant differences in traits such as plant height, silking date, ear height, and grain yield. This was attributed to maintenance, residual heterozygosity, or mutation. Self-pollination in ear-to-row progenies resulted in less genetic change (thus reduced vigor) compared with other methods of maintenance such as selfing and then bulking and sib mating.

Smith and Smith (1987; 1988) studied associations among 18 Lancaster and BSSS-derived inbred lines using electrophoretic, allozymic, and pedigree data. They were able to identify uniquely 79% of the Lancaster lines. When material was not closely related, the allozyme data from 22 loci could discriminate 93% of the lines. The allozyme data for 33 loci uniquely discriminated 15 of the 17 BSSS-derived lines they studied. When the inbred lines were closely related, allozyme data could not distinguish among them (e.g., inbred pairs Oh43 and 247 and Oh43 and A619 sharing approximately 50 to 75% pedigree relationship had similar profiles).

In a study involving 148 U.S. maize inbred lines, Mumm and Dudley (1994) used 46 restriction fragment length polymorphisms (RFLP) markers to cluster all the inbred lines into the two major heterotic groups. They were also able to identify subgroups within the major heterotic groups. Although some discrepancies were observed, their data tended to correlate with pedigree data. Dillmann et al. (1997) used RFLPs and morphological distances to study 145 maize inbreds released in France. They concluded that RFLP markers could serve as tools to discriminate between closely related individuals from different breeding sources (thus different combinations of genes) or close similarity due to high relatedness.

More recently, random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and amplified fragment length polymorphism (AFLP) analyses have been used in maize diversity studies (Pejic et al., 1998; Senior et al., 1998). In a study of 33 inbred lines, SSRs produced twice as much information as AFLPs and RAPDs, and 40% more than RFLPs in terms of numbers of alleles per locus (Pejic et al., 1998).

Many studies have reported genetic diversity and relatedness of maize inbred lines at the molecular level (Dubreuil et al., 1996; Dubreuil and Charcosset, 1998, 1999; Ajmone-Marsan et al., 1998; Melchinger et al., 1991); however, none were conducted to assess genetic variation at this level within and among identically named inbred lines maintained by different programs. This approach is complementary to phenotypic measures in quantifying genetic changes because it may show variations in DNA that may not be phenotypically expressed. The objective of this study was to estimate the level of genetic diversity both among and within inbred lines from different sources by means of SSR markers.

MATERIALS AND METHODS

Seeds of six maize inbred lines were provided by 15 different public and private breeding programs (Table 1). These programs represented different maize maturity zones in nine U.S. states and one province in Canada. The generation year of the materials varied among sources, and spanned from 1975 to 1999 (for samples where dates were provided). The inbreds chosen represent the two major heterotic groups and the miscellaneous category noted previously. Eight different seed sources were evaluated for each inbred. This resulted in 48 inbred-source combinations (Table 1). The inbred lines chosen for genotyping were ones that had not been reportedly improved or modified (for example, Oh43 was chosen instead of Oh43Ht).

Leaf tissue samples were obtained from two plants per source per inbred. The plants were grown in the greenhouse at 28°C under 14 h of light and 10 h of darkness. At 9 to 12 d after planting, 50 mg of leaf tissue was harvested and stored at −80°C until DNA was extracted. DNA was extracted by a CTAB miniprep method (Mitchell et al., 1997). DNA was quantified with the PicoGreen dsDNA quantification kit (Molecular Probes, Eugene, OR). Forty-four fluorescently labeled SSR primer pairs (acquired from the Iowa State University DNA Sequencing and Synthesis Facility) were used for genotyping (Table 2). Primer pairs were chosen on the basis of their properties of detecting single loci, their broad coverage of the genome, and their high levels of polymorphism when applied to a broad range of maize germplasm (Sharon E. Mitchell, personal communication, 2000). The sequences of the 44 primer pairs are available from the maize database project, MaizeDB at the University of Missouri (http://www.agron.missouri.edu; verified 2 Jan. 2002). PCR reactions were prepared by a Genesis RSP 200 robot (TECAN, Research Triangle Park, NC) and amplified by a thermocycler. Each 20-μL PCR reaction consisted of 1× PCR buffer, 0.4 m*M* dNTPs, 1.2 m*M* $MgCl_2$, 0.2 unit of Taq polymerase, 1 μL

Table 1. Maize inbred lines, including seed sources and release information, used to study genetic diversity among and within inbred lines from different sources using SSR markers.

Inbred line	Source†	Release information‡	Heterotic Group	Reference
B73	CU, ISU, UWM, UMC2, OSU, UMC, Pioneer, Garst	1972, Ames, IA	Reid Yellow Dent	Baker, 1984; Troyer, 1999
CM105	AgCanada, Garst, Pioneer, PSU, UWM, OSU, Holden, Syngenta	1970, Morden, MB	Reid Yellow Dent	Henderson, 1984; Gerdes et al., 1994
Mo17	CU, Garst, USDA, PSU, Pioneer, UMC, UMC2, UWM	1964, Columbia, MO	Lancaster Surecrop	Zuber, 1973
Oh43	PU, PSU, AgCanada, CU, ISU, NCSU, UMC, UWM	1948, Wooster, OH	Lancaster Surecrop	Henderson, 1984
W153R	PU, CU, Garst, Holden, PSU, Pioneer, UWM, OSU	1952, Madison, WI	Miscellaneous	Huber, 1958; Henderson, 1984
Wf9	Garst, ISU, Pioneer, PSU, UMC, USDA, NCSU, OSU	1937, West Lafayette, IN	Miscellaneous	Anonymous, 1938

† Sources of seed lots for an inbred: CU = Cornell Univ., NY; ISU = Iowa State Univ., IA; UWM = Univ. of Wisconsin-Madison, WI; OSU = Ohio State Univ., OH; UMC = Univ. of Missouri-Columbia, MO; UMC2 = Univ. of Missouri-Columbia (1973), MO; PSU = Penn State Univ., PA; NCSU = North Carolina State Univ., NC; PU = Purdue Univ., IN; USDA = United States Department of Agriculture; AgCanada = Agriculture Canada, ON.
‡ Year and location of release.

Table 2. SSR loci used to genotype eight different seed sources from the US and Canada for each of six inbred maize lines.

Multiplex set	Marker†	No. of alleles	Size range (bp)‡		Map location	Percentage data missing§
			Min	Max		
1	phi127	3	112.06	126.52	2.07	0.0868
	phi051	4	134.41	143.23	7.06	0.0217
	phi115	3	291.87	311.52	8.03	0.1953
	phi015	3	86.14	104.21	8.08	0.0434
	phi033	3	236.29	251.72	9.02	0.0434
2	phi053	3	169.37	194.74	3.05	0.1302
	phi072	3	134.39	155.31	4.01	0.0434
	phi085	2	237.00	261.53	5.06	0.0434
	phi034	6	117.65	144.90	7.02	0.0651
	phi121	2	97.44	101.54	8.04	0.0434
3	phi056	3	256.13	262.08	1.01	0.0868
	phi064	5	78.27	98.48	1.11	0.0434
	phi083	4	124.46	138.26	2.04	0.0000
	phi050	5	80.27	96.12	10.03	0.3689
4	phi96100	3	278.43	296.49	2.00	0.0868
	phi101249	2	122.04	145.22	Unknown	0.1302
	phi109188	3	164.07	170.37	5.00	0.1736
5	phi029	4	139.00	160.99	3.04	0.1736
	phi073	2	188.55	196.43	3.05	0.4123
	phi96342	2	241.51	250.00	10.02	0.0651
	phi109275	3	122.22	139.92	1.00	0.0217
6	bng1653	4	144.91	158.37	5.04	0.1736
7	phi059	2	147.48	157.64	10.02	0.0217
8	phi427913	3	122.98	131.62	1.01	0.0217
	phi265454	3	221.06	236.66	1.10	0.1302
	phi402893	4	210.15	234.90	2.00	0.2170
9	phi427434	3	127.35	140.43	2.08	0.0868
	phi389203	1	307.05	308.37	6.03	0.0868
10	phi453121	4	208.82	226.70	8.00	0.2821
	phi233376	3	139.89	152.33	8.03	0.0000
11	phi346482	2	121.62	128.43	Unknown	0.0000
	phi308090	2	220.69	223.87	4.04	0.0000
	phi330507	3	134.48	145.35	5.02	0.0000
12	phi213984	4	286.24	316.18	4.01	0.0217
	phi339017	2	147.67	157.17	1.01	0.0217
	phi159819	5	123.11	138.87	6.00-6.08	0.0000
13	phi193225	3	134.18	141.14	3.02	0.0868
	phi452693	2	125.12	134.58	6.04	0.2170
14	phi328189	4	117.24	125.33	1.11	0.1085
15	phi438301	2	210.94	215.10	4.05	0.0651
	phi236654	2	119.94	126.27	9.05	0.0217
	phi331888	3	130.69	136.18	5.02	0.0434
16	phi308707	4	118.71	133.61	1.09	0.0217
17	phi299852	4	110.98	132.56	6.07	0.0000

† Marker details can be obtained from the maize database at http://www.agron.missouri.edu.
‡ An allele was considered different if the size difference between alleles at a locus was ≥2.0 bp.
§ Expressed as a percentage of missing data points with respect to the total data set. Missing data for the entire data set was 4.3%.

(4 pmol/μL) of each primer, and 5 μL (25–50 ng) of DNA. To reduce the number of PCR amplifications, up to seven primer pairs were combined to form 17 multiplex sets of primers (Table 2). The amplification conditions were 95°C for 1 min; 25 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min; and a terminal extension step at 72°C for 1 h. To prepare the PCR products for detection, 0.5 μL of the amplified DNA was mixed with 0.1 μL Genescan 500XL Rox Standard (Applied Biosystems, Foster City, CA) and 1 μL of 50% formamide loading buffer and the DNA was denatured by heating at 95°C for 5 min.

The samples were loaded and electrophoresed on 5% (w/v) denaturing Long Ranger (FMC) 36-cm well-to-read gels by means of an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA). Data were collected by the GeneScan (Applied Biosystems, Foster City, CA) and Genotyper (Applied Biosystems, Foster City, CA) software and stored as gel images for counterchecking.

Statistical Analyses

The number of alleles per locus was determined and coded with a number ranging from 1 to n (number of alleles) (Table 2). Descriptive statistics H_o and H_e were estimated by Genetic Data Analysis (GDA) software (Lewis and Zaykin, 2001). Observed heterozygosity, H_o was estimated as:

$$\hat{H}_{ol} = \sum_i \sum_{i \neq j} n_{lij}/n$$

for locus l, alleles i and j, sample size n, and observed count of heterozygotes n_{lij} where $i \neq j$ (Weir, 1990). Expected heterozygosity, H_e, was estimated for a locus l as:

$$\hat{H}_{el} = 2n(1 - \sum \hat{x}_i^2)/(2n - 1)$$

for n individuals, where $\hat{x}_i^2$ is the frequency of the ith allele and $\hat{x}_i = \tilde{x}_{ii} + \Sigma_{i \neq j} \tilde{x}_{ij}/2$ (Nei, 1987).

Data were transformed to binary code to obtain a full design matrix of presence versus absence of an allele with missing values represented by 9. This format was used to perform cluster analysis on the basis of the average linkage method, known as the Unweighted Pair Group Method using Arithmetic averages (UPGMA) by NTSYSpc 2.02i software (Rohlf, 1998). A dendrogram was constructed for the 48 inbred-source combinations on the basis of Dice similarity coefficients (Dice, 1945). Dice's coefficient is a matching coefficient for binary data. In this case, the binary observations consisted of presence versus absence of an allele within a plant, over all alleles. To compare the inbred lines and sources, H_o and H_e means were

Table 3. Descriptive statistics (over all loci) for six inbred maize lines from the US and Canada.

Population	*n*†	*A*‡	H_e§	H_o¶
B73	15.3409	1.2045	0.0317	0.0043
CM105	15.7273	1.3409	0.0563	0.0043
Mo17	15.5227	1.3864	0.0431	0.0086
W153R	15.6136	1.3864	0.0722	0.0085
Oh43	14.0233	1.4419	0.0860	0.0114
Wf9	15.6818	1.5455	0.1139	0.0115
Mean	15.3183	1.3842	0.0672	0.0081
SE			0.0123	0.0013

† Sample size corrected for missing data; perfect sample size was 16 (two plants from each of eight sources for each inbred).
‡ Mean number of alleles per locus.
§ Mean expected heterozygosity values for the eight sources of each inbred.
¶ Mean observed heterozygosity values for the eight sources of each inbred.

tested for equality by the *t*-test function of the MINITAB software (Minitab 1998).

Analysis of molecular variance (AMOVA) was performed by Arlequin ver 2.0 software (Schneider et al., 2000). This procedure analyzes variance of gene frequencies taking into account the number of molecular haplotypes. The total variance was partitioned into various components due to intra- and inter-individual differences and/or inter-population differences (Arlequin, 2000). Allele frequencies for all loci were estimated by GENEPOP version 1.2 software (Raymond and Rousset, 1995).

RESULTS AND DISCUSSION

Descriptive Statistics

A total of 137 alleles were detected across all loci in the inbreds. The number of alleles per locus ranged from 1 to 6. Missing data, which included true nulls and missing data arising from failed PCR amplifications, amounted to 4.3% of the total. An allele was categorized as null if it was not detected at a locus in all 16 plants per source.

H_o within inbreds was low (Table 3) as would be expected from the inbred nature of the materials studied. H_o was lower in more recently released inbreds such as B73 and CM105 compared to older inbreds. Use of a *t*-test to compare means (Sokal and Rohlf, 1981) revealed that inbreds differed significantly for H_o. The H_e or allelic diversity also was significantly different among inbreds. The lowest H_e was in B73 (0.0317) and highest in Wf9 (0.1139). Russell and Vega (1973) reported variable rates of genetic change in quantitative characters in the long-time inbred lines they studied. Wf9, released in 1936, has been in use for breeding over 60 yr (Nedev et al., 2000), although it was retired as a hybrid parent decades ago, compared with B73, which was released in 1972. This difference in release dates may have introduced more opportunities for change through inadvertent contamination during regeneration or genetic drift and this may have increased differentiation among sources of Wf9. Other older inbreds (Oh43 and W153R) also had high H_e levels. Inbred lines released after 1960 (Mo17, B73, and CM105) had relatively lower H_e values when compared to inbred lines released before 1960 (W153R, Oh43, Wf9) (Table 3).

Among sources, H_o and H_e means were highly significant when compared by the *t*-test. No one inbred–source combination had consistently large H_o and H_e values for all inbreds, implying the changes occurring could be random and independent of the source. For example, H_o values for B73 from Cornell University were 5-fold higher and those from Ohio State University were 2.5-fold higher than the mean H_o value for B73 across all sources. CM105 from Syngenta, Holden, and Agriculture Canada had H_o values 2.5-fold higher than average H_o values for CM105. Mo17 from Pennsylvania State University had a H_o value 3.5-fold higher than the mean of Mo17 across all sources. Other inbred lines from these sources had H_o values close to the specific inbred mean across all other sources. When the same inbred line is grown in different environments, some of the loci that have undergone genetic changes may contain alleles that are latent in one environment but expressed in the other environment, subjecting them to selective pressure in that environment (Fleming et al., 1964).

Analysis of Molecular Variance (AMOVA)

Of the 44 loci, 27 (61%) were polymorphic in at least one inbred-source combination. The number of loci with more than one allele within an inbred-source combination ranged from one to eight with an average of 1.58 alleles per locus per inbred-source combination. When data from the eight sources of each inbred were pooled, the number of loci showing polymorphism varied from 7 to 11 per inbred. No single inbred had a disproportionate number of polymorphic loci. This suggests that the inbred lines studied are relatively stable at the loci examined. Some lines however have been reported to have high levels of instability when characterized using quantitative traits (Russell and Vega, 1973; Russell et al., 1963). In 14 (29%) out of the 48 inbred–source combinations examined, all 44 loci were fixed for a single allele. The two plants representing the inbred CM105 from Holden were polymorphic at eight loci, which was higher than the average of 1.58 across the other sources. On closer examination, one plant within this source had unique alleles, absent in all of the other CM105 accessions. The unique alleles were homozygous, suggesting an earlier outcrossing event that has since been genetically fixed. One locus (phi328189) was polymorphic within plants in 47.9% of the 48 inbred–source combinations. This was relatively high compared with the mean of 2.6% at the other 43 loci. Four alleles were found at this locus compared to a mean of 3.1 alleles per locus at the other 43 loci, which does not suggest that phi328189 is affected by a relatively higher mutation rate on the basis of the number of alleles at the other loci. H_o at this locus was 13.2%, as compared to an overall average of 0.8% across all the other loci. The increased heterozygosity may be conferring increased fitness to the inbred lines (Wendel et al., 1987; Lamy et al., 1994).

Highly significant differences in gene frequencies were observed among the six inbred populations. Among inbred variation accounted for 87.8% of the total, implying that 12.2% of the variation was among sources

Table 4. Analysis of molecular variance (AMOVA) of gene frequencies in six inbred maize lines from different sources in the US and Canada.

Source†	df	SS‡	Variance components	Percentage of variation	*P*-value
Inbred lines	5	1655.29	10.2182	87.8	0.00000
Sources (Inbred lines)	42	171.19	0.8844	7.6	0.00000
Plants (Sources*Inbred lines)	144§	77.50	0.5382	4.6	0.00000

† Sources of inbred seed are listed in Table 1.
‡ Sums of squares.
§ AMOVA tests haplotypes within plants therefore for 96 plants, df = (96 × 2) = 192, df for plants/sources/inbreds, (192 − 48) = 144.

and among plants within sources (Table 4). A significant amount of variation (7.6%) was contributed by the different sources within inbreds. This level of variation could have arisen through different methods used in maintenance of the inbred lines. In breeding programs, inbred lines are variously maintained through self-pollination with or without bulking (pooling seed from different plants) and sibbing. Bogenschutz and Russell (1986) compared sib-mating and self-pollination as methods of maintenance for 11 generations using 10 maize inbred lines differing in origin and in the number of regenerations before the study. Although differences between the selfed and sib-mated generations were small and significant, 26% more significant changes were found for lines under selfing than for lines under full-sib mating, suggesting that selfing causes more alteration of line integrity than sibbing.

Even within a source, the two plants analyzed showed significant genetic differences on average, accounting for 4.6% of the total variation. This small but significant amount of variation indicates that complete homozygosity across all loci should not be assumed in any inbred line seed source.

It is necessary to take into consideration the source of the inbred lines if a high level of homozygosity is desired. From this study, combining seed from various sources of an inbred line would give materials having an average of 87.8% homozygosity across loci. If an

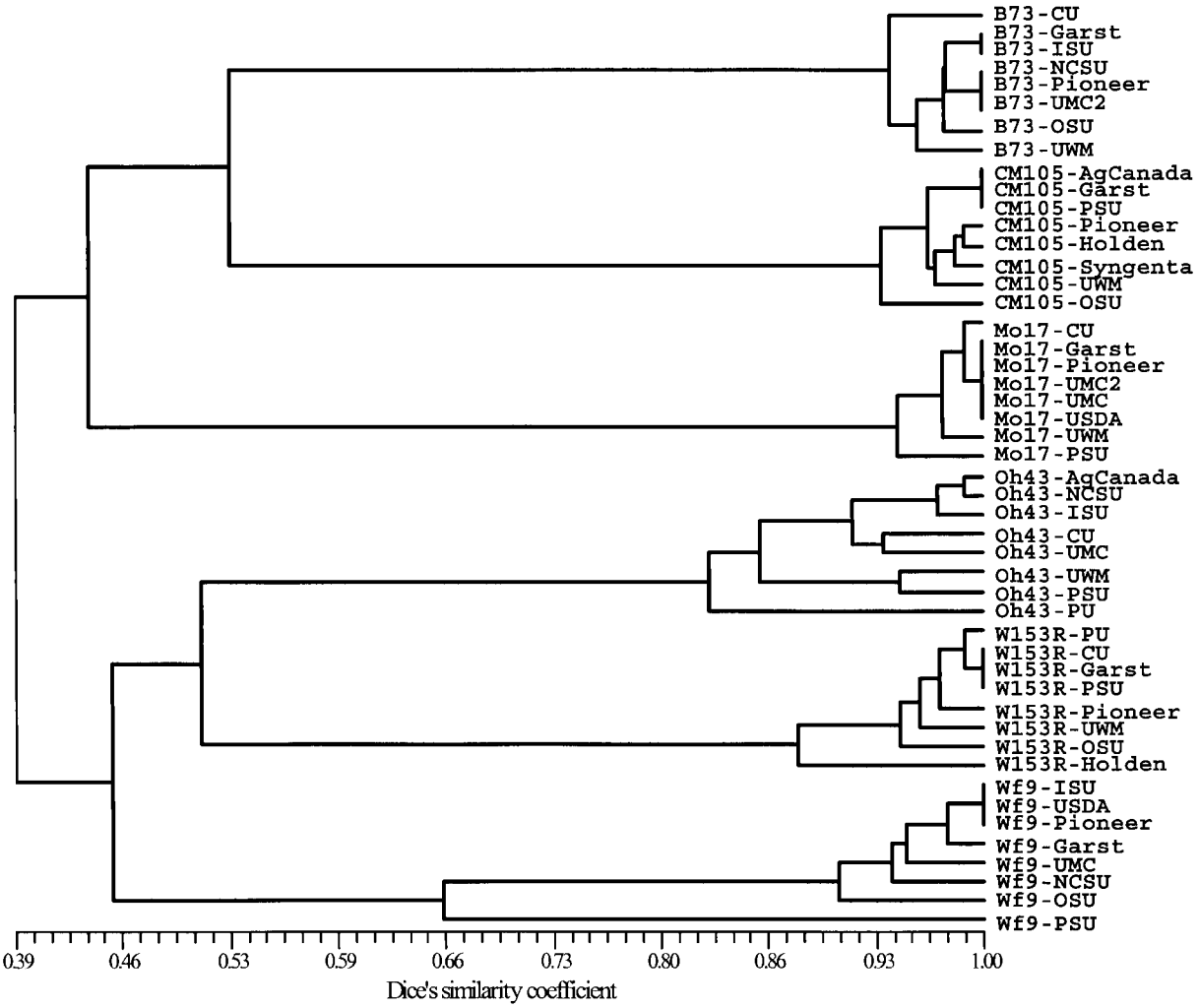

Fig. 1. UPGMA dendrogram for 48 maize inbred–source combinations determined on the basis of genetic similarity by means of 44 SSR markers.

inbred were obtained from a single source, a homozygosity level of 95.4% would be expected. When a high degree of uniformity is required, such as in conservation, mapping, sequencing, and gene frequency studies, a single source for the seeds should be utilized.

Genetic Distance

UPGMA ordered the populations of inbred lines first into two broad groups (Fig. 1). One group consisted of B73, CM105, and Mo17. The other group consisted of Oh43, W153R, and Wf9. Within the two groups, different seed sources of the same inbred grouped together (Fig. 1). Dice's similarity coefficient ranged from a high of 1.00 to a low of 0.66 in Wf9 from Pennsylvania State University. Comparison of the inbred lines within a source revealed that most of the inbred lines had similarity coefficients of over 93%. Wf9 from Pennsylvania State University and Oh43 from Purdue University were the only inbreds that had similarity coefficients of less than 85% relative to the rest of the seed sources of the same inbred line.

Among seed sources within inbred lines variability was low, consistent with Dubreuil et al. (1996) who reported diversity index values of up to 0.85. Our results showed that small but significant variation exists among and within sources. Stuber and Goodman (1983) analyzed allozyme data for popular and historically important maize inbreds and reported that they could differentiate three of the 10 lines coming from the same source, but the others were identical. They were also able to detect within-source variation in 12 of 15 lines that had double entries.

CONCLUSIONS

This study showed that different seed sources of the same inbred contribute a potentially important source of genetic variation. Establishing the level of heterozygosity in seed is critical because it improves the usefulness of data to other breeders using identically named materials. It also ensures uniformity and stability of any materials developed from them. The similarity level in maize inbred lines based on AMOVA and Dice's similarity coefficient, although sufficient for general identification and in testing programs, may not be sufficient in studies that require higher resolution, such as fine mapping of quantitative trait loci (QTLs) and development of marker such as single nucleotide polymorphisms (SNPs).

On the basis of our results, studies done with the same inbred line should be comparable regardless of the seed source used in about 92.4% of the cases (Table 4). Although this study was done with public inbred lines which are no longer used in hybrid seed production in the USA, breeders need to be aware of the variation arising from seed sources as they continue to use these inbreds for genetic studies and as testers. The detection of 4.6% variation within an inbred from one source should be taken into account, especially when sampling earlier released materials which showed higher H_e values. No evidence was found to support high mutation rates at the loci examined because no unique alleles were detected and the number of alleles did not vary greatly among inbred–seed source combinations studied.

ACKNOWLEDGMENTS

We gratefully acknowledge Drs. J. Ayers, R. Bernardo, J. Coors, L. Darrah, J. Deutsch, M.M. Goodman, A.R. Hallauer, D. Harper, S. Openshaw, R. Pratt, L. Reid, S. Smith, and N. Widstrom for supplying us with inbred line seeds, and Chris Miller and Xiaoyun Wang for laboratory assistance.

REFERENCES

Ajmone-Marsan, P., P. Castiglioni, F. Fusari, M. Kuiper, and M. Motto. 1998. Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor. Appl. Genet. 96:219–227.

Anonymous. 1938. Report of the Corn Improvement Conference. Chicago, IL. 3 Dec. 1937. U.S. Gov. Print. Office, Washington, DC.

Arlequin. 2000. Arlequin users manual. Ver. 2.0. Population genetic structure inferred by analysis of variance (AMOVA) p. 90–96. Available online at http://lgb.unige.ch/arlequin/software/2.000/manual/Arlequin.pdf (verified 20 Dec. 2001).

Baker, R. 1984. Some of the open pollinated varieties that contributed the most to modern hybrid corn. p. 1–19. *In* Proc. of the 20th Annual Illinois Corn Breeders School. 6–8 May 1984. Univ. of Illinois, Urbana-Champaign.

Bing, J.W., W.D. Guthrie, and F.F. Dicke. 1992. Genetics of resistance to the corn leaf aphid (Homoptera: Aphididae). J. Econ. Entomol. 85:1476–1479.

Bogenschutz, T.G., and W.A. Russell. 1986. An evaluation for genetic variation within maize inbred lines maintained by sib-mating and self-pollination. Euphytica 35:403–412.

Busch, R.H., and W.A. Russell. 1964. Hybrid expression of mutations affecting quantitative characters in inbred lines of *Zea mays* L. Crop Sci. 4:400–402.

Darrah, L.L., and M.S. Zuber. 1985. 1985 United States farm maize germplasm base and commercial breeding strategies. Crop Sci. 26: 1109–1113.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26:297–302.

Dillmann, C., A. Bar-Hen, D. Guerin, A. Charcosset, and A. Murigneux. 1997. Comparison of RFLP and morphological distances between maize *Zea mays* L. inbred lines. Consequences for germplasm protection purposes. Theor. Appl. Genet. 95:92–102.

Dubreuil, P., and A. Charcosset. 1998. Genetic diversity within and among maize populations: A comparison between isozyme and nuclear RFLP loci. Theor. Appl. Genet. 96:577–587.

Dubreuil, P., and A. Charcosset. 1999. Relationships among maize inbred lines and populations from European and North-American origins as estimated using RFLP markers. Theor. Appl. Genet. 99:473–480.

Dubreuil, P., P. Dufour, E. Krejci, M. Causse, D. de Vienne, A. Gallais, and A. Charcosset. 1996. Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Sci. 36:790–799.

Fleming, A.A., G.M. Kozelnicky, and E.B. Browne. 1964. Variations between stocks within long-time inbred lines of maize (*Zea mays* L.). Crop Sci. 4:291–295.

Frey, K.J. 1996. National Plant Breeding Study I. Human and financial resources devoted to plant breeding research and development in the United States in 1994. Special Report 98. 45 pages. Iowa Agriculture and Home Economics Experiment Station. Iowa State University.

Gerdes, J.T., C.F. Behr, J.G. Coors, and W.F. Tracy. 1994. Compilation of North American maize breeding germplasm. CSSA, Madison, WI.

Gerdes, J.T., and W.F. Tracy. 1993. Pedigree diversity within the Lancaster Surecrop heterotic group of maize. Crop Sci. 33:334–337.

Hallauer, R., K.R. Lamkey, and P.R. White. 2000. Registration of

B110, B111, B113 and B114 inbred lines of maize. Crop Sci. 40:1518–1519.
Henderson, C. 1984. Maize research and breeders manual no. 10. Inbreds, breeding stocks, maize investigations and academic research personnel. Illinois Foundation Seeds, Inc., Champaign, IL.
Higgs, R.L., and W.A. Russell. 1968. Genetic variation in quantitative characters in maize inbred lines. I. Variation among and within Corn Belt seed sources of six inbreds. Crop Sci. 8:345–348.
Huber, L.L. 1958. Functional characteristics of some inbred lines of corn. Progress Report 196. Pennsylvania State University College of Agriculture, Agricultural Experiment Station, University Park, PA.
Lamy, F., S. Andre, T. Robert, and A. Sarr. 1994. Use of RFLP markers to assess genetic diversity in introgression procedures between adapted and non adapted maize populations (*Zea mays* L.). Hereditas (Landskrona) 120:165–173.
Lewis, P.O., and D. Zaykin. 2001. Genetic data analysis software. Release 1.0 (d16b). Available online at http://lewis.eeb.uconn.edu/lewishome/software.html (verified 26 Jan. 2001).
Livini, C., P. Ajmone-Marsan, A.E. Melchinger, M.M. Messmer, and M. Motto. 1992. Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLPs. Theor. Appl. Genet. 84:17–25.
Marcon, A., S.M. Kaeppler, S.G. Jensen, L. Senior, and C. Stuber. 1999. Loci controlling resistance to high plains virus and wheat streak mosaic virus in a B73 x Mo17 population of maize. Crop Sci. 39:1171–1177.
Mauria, S., N.N. Singh, A.K. Mukherjee, and K.V. Bhatt. 2000. Isozyme characterization of Indian maize inbreds. Euphytica 112:253–259.
Melchinger, A.E., M.M. Messmer, M. Lee, W.L. Woodman, and K.R. Lamkey. 1991. Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms. Crop Sci. 31:669–678.
Minitab. 1998. MINITAB 12. Minitab Inc., State College, PA.
Mitchell, S.E., S. Kresovich, C.A. Jester, C.J. Hernandez, and A.K. Szewc-McFadden. 1997. Application of multiplex PCR and fluorescence-based semi-automated allele sizing technology for genotyping plant genetic resources. Crop Sci. 37:617–624.
Mumm, R.H., and J.W. Dudley. 1994. A classification of 148 U.S. maize inbreds: I. Cluster analysis based on RFLPs. Crop Sci. 34: 842–851.
Nedev, T., M. Kruleva, and B. Krapchev. 2000. Genetic relationship among cytoplasm analogs and morphogenic potential of maize inbreds. Maize Genetics Coop. Newsl. 74:63.
Nei, M. 1987. Molecular evolutionary genetics. p. 178 Columbia University Press, New York.
Pejic, I., P. Ajmone-Marsan, M. Morgante, V. Kozumplick, P. Castiglioni, G. Taramino, and M. Motto. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. Theor. Appl. Genet. 97:1248–1255.
Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenism. J. Hered. 86:248–249.
Rohlf, F.J. 1998. Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.02i). Dep. of Ecology and Evolution, State Univ. of New York, Stony Brook, NY.
Russell, W.A., G.F. Sprague, and L.H. Penny. 1963. Mutations affecting quantitative characters in long-time inbred lines of maize. Crop Sci. 3:175–178.
Russell, W.A., and U.A. Vega. 1973. Genetic stability of quantitative characters in successive generations in maize inbred lines. Euphytica 22:172–180.
Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin, ver. 2.0: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland. Available online at http://lgb.unige.ch/arlequin/software/ (verified 2 Jan. 2002).
Senior, M.L., J.P. Murphy, M.M. Goodman, and C.W. Stuber. 1998. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci. 38:1088–1098.
Smith, J.S.C., and O.S. Smith. 1987. Associations among inbred lines of maize using electrophoretic, chromatographic, and pedigree data. 1. Multivariate and cluster analysis of data from 'Lancaster Sure Crop' derived lines. Theor. Appl. Genet. 73:654–664.
Smith, J.S.C., and O.S. Smith. 1988. Associations among inbred lines of maize using electrophoretic, chromatographic, and pedigree data. 2. Multivariate and cluster analysis of data from Iowa Stiff Stalk Synthetic derived lines. Theor. Appl. Genet. 76:39–44.
Smith, M.E., and V.E. Gracen. 1993. Registration of NYLB31 and NYRD4058 parental lines of maize. Crop Sci. 33:361.
Sokal, R.R., and F.J. Rohlf. 1981. Biometry. 2nd ed. p. 226–229 W.H. Freeman and Co., NY.
Stuber, C.W., and M.M. Goodman. 1983. Allozyme genotypes for popular and historically important inbred lines of corn, *Zea mays* L. USDA Agric. Res. Results, Southern Ser. no. 16. U.S. Gov. Print. Office, Washington, DC.
Troyer, F. 1999. Background of U.S. hybrid corn. Crop Sci. 39:601–626.
Weir, B. 1990. Genetic data analysis. p. 115. Sinauer Associates, Inc., Sunderland, MA.
Wendel, J.F., M.D. Edwards, and C.W. Stuber. 1987. Multilocus genetic control of preferential fertilization in maize. Heredity 58(2): 297–302.
Zuber, M.S. 1973. Registration of 20 maize parental lines. Crop Sci. 13:779–780.
Zuber, M.S., and L.L. Darrah. 1980. 1979 U.S. corn germplasm base. p. 234–249. *In* H.D. Loden and D.W. Wilkinson (ed.) Proc. of the 35th Annual Corn and Sorghum Industry Res. Conf. Chicago, IL. 9–11 Dec. Am. Seed Trade Assoc., Washington, DC.