Theor Appl Genet (2001) 103:613–617

H. Lu · R. Bernardo

Molecular marker diversity among current and historical maize inbreds

Received: 21 August 2000 / Accepted: 5 January 2001

Abstract Advanced-cycle pedigree breeding has caused maize (*Zea mays* L.) inbreds to become more-elite but more-narrow genetically. Our objectives were to evaluate the genetic distance among current and historical maize inbreds, and to estimate how much genetic diversity has been lost among current inbreds. We selected eight maize inbreds (B14, B37, B73, B84, Mo17, C103, Oh43 and H99) that largely represented the genetic background of current elite inbreds in the U.S. seed industry. A total of 32 other inbreds represented historical inbreds that were once important in maize breeding. Cluster analysis of the inbreds, using data for 83 SSR marker loci, agreed well with pedigree information. Inbreds from Iowa Stiff Stalk Synthetic (BSSS), Reid Yellow Dent, and Lancaster clustered into separate groups with only few exceptions. The average number of alleles per locus was 4.9 among all 40 inbreds and 3.2 among the eight current inbreds. The reduction in the number of alleles per locus was not solely due to sample size. The average genetic distance (D_{ij}) was 0.65 among the eight current inbreds, 0.67 among the 32 historical inbreds, and 0.67 among all 40 inbreds. These differences were statistically insignificant. We conclude that genetic diversity among current inbreds has been reduced at the gene level but not at the population level. Hybrid breeding in maize maintained, rather than decreased, genetic diversity, at least during the initial subdivision of inbreds into BSSS and non-BSSS heterotic groups. We speculate, however, that exploiting other germplasm sources is necessary for sustaining long-term breeding progress in maize.

Keywords Genetic diversity · Maize · SSR

Communicated by M.A. Saghai Maroof

H. Lu
Department of Agronomy, Purdue University,
West Lafayette, IN 47907-1150, USA

R. Bernardo (✉)
Department of Agronomy and Plant Genetics,
University of Minnesota, 411 Borlaug Hall,
1991 Upper Buford Circle, St. Paul, MN 55108-6026, USA

Introduction

Maize (*Zea mays* L.) inbreds were once commonly selfed from open-pollinated populations. For example, the best inbreds in the 1930s were selfed from nearly 100 different open-pollinated cultivars (Jenkins 1936). But with the development of the maize hybrid-seed industry, crosses among elite inbreds became the preferred source of new inbreds (Hallauer et al. 1988; Troyer 1990). This inbred re-cycling approach, which has become known as advanced-cycle pedigree breeding, often involves crosses between related inbreds as source populations. Consequently, most of the current elite maize inbreds are derived from only a few progenitor inbreds (Darrah and Zuber 1985; Smith et al. 1999). For example, among inbreds available from U.S. foundation seed companies in 1999, only 82 out of 381 inbreds (22%) had genetic backgrounds other than eight widely used inbreds: B14, B37, B73, B84, Mo17, C103, Oh43 and H99 (MBS Inc. 1999). The use of only a few inbred families in advanced-cycle breeding has raised concerns regarding the loss of genetic diversity in maize (Troyer 1999).

An issue confronting maize breeders is whether the genetic base of current maize breeding germplasm has become too narrow, not only for sustaining genetic improvement but also for reducing genetic vulnerability to biotic and abiotic stresses. Smith et al. (1985a, b) found, from isozyme data, that historical maize inbreds comprised a wide range of genetic diversity. How genetically different are current inbreds from historical inbreds that are no longer used in maize breeding? How much, if any, genetic diversity has been lost because of advanced-cycle pedigree breeding? Our objectives in this study were to (1) evaluate the genetic distance among current and historical maize inbreds, and (2) estimate how much genetic diversity has been lost by advanced-cycle pedigree breeding. We used methods that have been applied, and some inbreds that have been investigated, in previous studies of maize diversity (e.g., Smith et al. 1985a,b). The novel aspect of our methodology is that we investigated genetic diversity in current inbreds independently

of reduced sample size. Our novel finding is that hybrid breeding maintains genetic diversity at the population level, at least during the initial subdivision of inbreds into heterotic groups.

Materials and methods

Maize inbreds

We selected eight inbreds, B84, B37, B73, B14, Mo17, C103, Oh43 and H99, that largely represented the genetic background of current inbreds in the U.S. seed industry (MBS Inc. 1999). For brevity we refer to these as current inbreds; they are no longer used in the U.S. seed industry but are the progenitors of current elite inbreds (Smith et al. 1999). We selected 32 historical inbreds that were once important in maize breeding (Troyer 1999; see Table 1). These historical inbreds were included in an unpublished list of inbreds, compiled by Dr. Paul L. Crane at Purdue University, that were parents of old double-cross hybrids or that were once used extensively as parents for developing new inbreds. The 40 inbreds were derivatives of Iowa Stiff Stalk Synthetic (BSSS), different strains of Reid Yellow Dent (i.e., Funk, Krug, Osterland, Troyer, and Iodent strains), Richey Lancaster, Lancaster, Leaming, and miscellaneous populations.

SSR analysis

Ten to fifteen seedlings of each inbred were grown in a greenhouse. Leaf tissue of 10 day old plants was harvested, freeze dried, and ground into fine powder. The DNA was extracted using a CTAB procedure (Saghai-Maroof et al. 1984). The SSR primers were synthesized by Research Genetics, Inc. (Huntsville, Alabama, USA; Table 2). The SSR marker procedures were described by Kantety (1997). The reaction constituents were: 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; 0.01% Gelatin; 0.01% Triton-X-100; 125 M each of dCTP, dTTP, dATP, and dGTP; 2.5 units of *Taq* DNA polymerase (Promega Biotech); 0.1 mM cresol red dye; 0.5 µM of primer; and 50 ng of genomic DNA. The total reaction volume was 25 l. The PCR reaction was carried out in a touchdown fashion with a first denaturation at 94°C for 120 s, followed by 16 cycles of: (1) denaturation at 94°C for 30 s, (2) annealing at 70°C for 30 s, and (3) extension at 72 C for 90 s, with the annealing temperature being reduced by 1°C per cycle. This procedure was followed by 30 cycles of (1) denaturation at 94°C for 30 s, (2) annealing at 55 C for 45 s, and (3) extension at 72°C for 90 s, and a final extension at 72°C for 15 min. Reaction products were electrophoresed on 4% agarose gels and were visualized by staining with ethidium bromide. Out of 98 SSR marker loci we originally used, we selected 83 loci that amplified single, distinguishable bands for each inbred. A total of 77 loci had been mapped onto the ten maize chromosomes (http://www.agron.missouri.edu/coop/ssr_probes/ssr1.html; see Table 2). Six loci had unknown map locations.

Genetic diversity of current and historical inbreds

We used two criteria to assess the genetic diversity among current inbreds and among historical inbreds: (1) the average number of alleles per SSR locus, and (2) the average genetic distance (D_{ij}) among inbreds. With a single band corresponding to a single allele among inbreds, D_{ij} was an estimate of the expected heterozygosity in the F_1 between inbreds *i* and *j* (Nei and Li 1979):

$$D_{ij}=1-(2\,N_{ij}/T_{ij}),$$

where: N_{ij} was the number of bands common to *i* and *j*, and T_{ij} was the sum of the number of bands in *i* and the number of bands in *j*. Cluster analysis of the 40 inbreds was performed through PC SAS (SAS Institute 1987). The average linkage method was applied to non-normalized data in clustering. To assess the validity of the cluster diagram, we calculated the cophenetic coefficient as the correlation between the D_{ij} values and the distances among inbreds as indicated by the cluster diagram.

At least two factors contributed to the difference in genetic diversity between current and historical inbreds: (1) the number of inbreds in each group (i.e., sample size), and (2) the amount of genetic diversity per se given a constant sample size. We used a resampling procedure to eliminate the effect of sample size on genetic diversity. Specifically, we selected a random set of eight inbreds out of the 40 inbreds and calculated the average number of marker alleles and the average D_{ij} in the sample. We repeated this procedure 5000 times to generate an empirical distribution for the average number of marker alleles and for the average D_{ij}, given a sample size of eight inbreds. We then used these empirical distributions in evaluating the loss in genetic diversity due to advanced-cycle pedigree breeding.

Results and discussion

Groupings of inbreds

Groupings of inbreds based on their SSR data agreed well with pedigree information (Fig. 1). The cophenetic correlation coefficient of 0.63 was significant at $P<0.01$. The BSSS inbreds B14, B37, B73 and N28 were tightly clustered together. The current BSSS inbreds B14, B37 and B73 were more similar to each other than to B84. Three WF9 derivatives (W64a, Pa91 and WF9 per se)

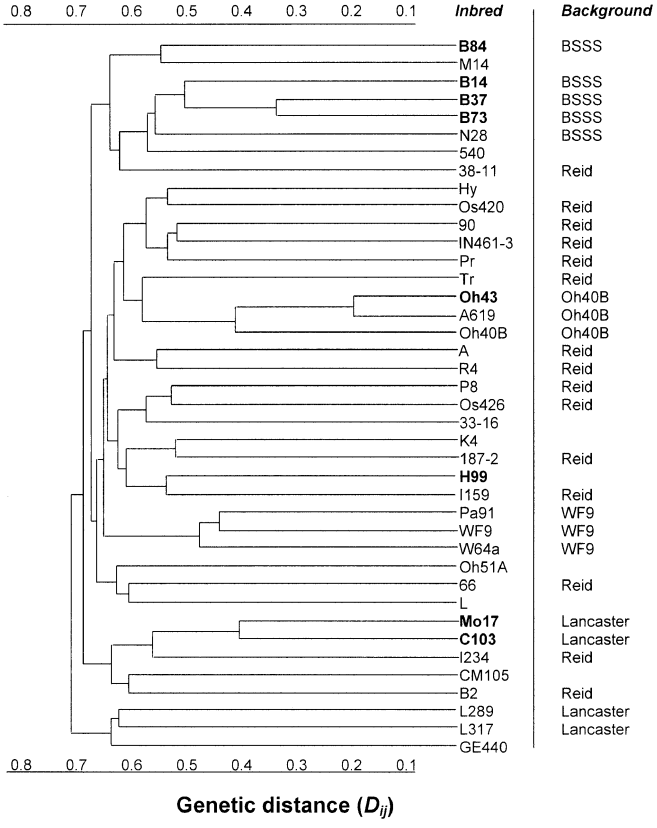

Fig. 1 Groupings of 40 maize inbreds on the basis of the genetic distance (D_{ij}) at 83 SSR marker loci. The eight current inbreds are in *bold*

Table 1 Genetic background of current and historical maize inbreds

Population	Inbred[a]
BSSS	B84 B37 B73 N28 B14
Funk Yellow Dent Reid	A R4 38–11 (Funk 176 A) 90 (Funk 90 Day)
Krug Reid	187–2
Troyer Reid	Tr IND461–3 B2
Osterland Reid	Os420 Os426
Other Reids	P8 (Palin Reid)[b] 66 WF9 (Wilson Farm Reid) Pr (Proudfit Reid Yellow Dent)
Iodent	I234 I159
Richey Lancaster	L289 Oh43 L317 Oh40B A619 [(A17×Oh43)×Oh43][c]
Lancaster	C103
Leaming	L
Miscellaneous	Mo17 (187–2×C103)[b] Hy (Illinois High Yield) 33–16 (Johnson County White) K4 (Kansas Sunflower) H99 (Illinois Synthetic 60 C) M14 (BR10×R8) Oh51 A [(OH51×Oh17)×Oh51][c] GE440 (Hastings Prolific) Pa91 {[WF9×Oh40B]S_4× [(38–11×L317)×38–11]S_4}[c] CM105 (V3×B14)[c] W64a (WF9×187–2)[c] 540 (Illinois Two-Ear Synthetic)

[a] Unless otherwise stated, pedigrees or genetic backgrounds are from Henderson (1976)
[b] From Troyer (1999)
[c] From MBS Inc. (1999)

were tightly grouped, and three Oh40B derivatives (Oh43, A619 and Oh40B per se) were also tightly clustered in a separate subgroup. C103 and Mo17 (a derivative of C103) were in the same subgroup. Two Richey Lancaster inbreds (L289 and L317) were grouped together. The Reid Yellow Dent inbreds did not form a single distinct cluster. The diversity among Reid Yellow Dent inbreds confirmed that "You can get anything out of Reid" (Wallace and Brown 1956).

A few discrepancies existed between the groupings based on SSR data and based on pedigree information (Table 1 and Fig. 1). Inbred 540, a non-BSSS inbred, was grouped loosely with the BSSS inbreds. Hy (Illinois High Yield) was in the same subgroup as four Reid Yellow Dent inbreds. It was previously unknown whether Hy was more similar to Reid Yellow Dent or to Lancaster germplasm. Tr, a Troyer Reid inbred, clustered with three Oh40B derivatives.

Maize hybrid germplasm is organized into heterotic groups (Hallauer et al. 1988). The major heterotic groups in the U.S. are BSSS and non-BSSS. New maize inbreds are usually selfed from a cross between two inbreds from the same heterotic group, e.g., BSSS×BSSS. In contrast, single-cross cultivars are made between two elite inbreds from complementary heterotic groups, i.e., BSSS× non-BSSS. The SSR results (Fig. 1) confirm that the current BSSS inbreds (B14, B37, B73 and B84) are less diverse than the current non-BSSS inbreds (Mo17, C103, Oh43 and H99). But despite the greater diversity among non-BSSS inbreds, breeders have generally paid more attention to improving BSSS inbreds than non-BSSS inbreds (Sprague 1984).

Genetic diversity of current and historical inbreds

The number of alleles per locus measures genetic diversity at the gene level. In contrast, the average D_{ij} among a set of inbreds measures genetic diversity at the population level. There were 404 SSR alleles across 83 loci among the 40 maize inbreds. Among all 40 inbreds, the average number of alleles per locus was 4.9, with maximum of 12 alleles at locus DUPSSR23 (Table 2). Among the eight current inbreds, the average number of alleles per locus was 3.2, with a maximum of six. The 35% reduction (significant at $P<0.01$) in the average number of alleles per locus indicated a substantial loss of genetic diversity, at the gene level, among the current inbreds. At each locus, the number of alleles in the current inbreds was less than or equal to the number of alleles in the historical inbreds (Table 2). Furthermore, we found that the current inbreds did not have any alleles that were absent in the historical inbreds.

Among 5000 sets of eight inbreds, sampled at random from the 32 inbreds, the average number of alleles per locus was 3.4. This average was significantly higher ($P<0.01$) than the average of 3.2 among the eight current inbreds. The 0.2 difference in the number of alleles per locus was small, but nevertheless indicated that the reduced genetic diversity among the current inbreds was therefore not solely due to the reduced number of inbreds. In other words, the D_{ij} among the eight current inbreds was lower than the D_{ij} that would have been obtained if the eight inbreds had been chosen at random.

Our results indicated, however, that genetic diversity at the population level was not substantially different between the current inbreds and the historical inbreds. The average D_{ij} among pairs of inbreds was 0.65 for the eight current inbreds, 0.67 for the 32 historical inbreds, and 0.67 for all 40 inbreds. Among 5000 sets of eight inb-

Table 2 Number of alleles at each SSR locus in current and historical maize inbreds

SSR locus	Bin [a]	Number of alleles in:		SSR locus	Bin [a]	Number of alleles in:	
		Current inbreds	Historical inbreds			Current inbreds	Historical inbreds
bngl149	1.00–1.05	3	3	bngl278	5.06	3	4
phi056	1.01	4	4	bngl609	5.06	2	3
bngl109	1.02	2	2	bngl118	5.08	3	4
bngl176	1.02	2	4	bngl386	5.09	3	3
bngl182	1.03	2	5	bngl389	5.09	4	4
bngl439	1.03	3	5	bngl161	6.01	4	7
bngl147	1.03–1.05	3	3	bngl238	6.01	3	6
bngl652	1.04	2	4	bngl249	6.01	3	4
bngl615	1.07	3	5	bngl426	6.01	4	5
DUPSSR12	1.08	4	5	phi034	7.00–7.05	2	3
bngl400	1.10–1.12	3	3	phi112	7.01	2	3
bngl125	2.03	3	4	bngl657	7.02	4	5
bngl381	2.03	6	7	phi114	7.02	2	3
bngl108	2.04	3	5	DUPSSR11	7.03	4	8
bngl121	2.04	3	5	bngl339	7.03–7.06	5	6
bngl166	2.04	3	5	DUPSSR13	7.04	4	6
phi083	2.04	3	3	bngl572	7.04–7.06	3	3
DUPSSR21	2.05–2.06	5	5	bngl669	8.03	3	4
bngl180	2.06	3	4	bngl119	8.04	4	5
bngl198	2.08	3	6	phi014	8.04	2	2
DUPSSR24	2.08	4	11	bngl162	8.05	4	4
DUPSSR25	2.08	4	7	bngl666	8.05	4	5
phi029	3.04	3	3	phi115	8.05	2	3
bngl420	3.05	4	6	bngl240	8.06	3	5
DUPSSR23	3.06	4	12	phi015	8.08	4	6
bngl197	3.07	3	6	phi080	8.08	2	5
phi072	4.00	2	3	DUPSSR14	8.08	3	5
nc004	4.02	2	5	bngl244	9.02	6	8
phi021	4.02	2	5	bngl469a	9.03	3	4
phi079	4.04	3	3	bngl127	9.04	3	3
bngl252	4.05	1	2	bngl128	9.07	4	5
bngl490	4.05	3	7	bngl619	9.07	3	5
phi096	4.05	1	2	bngl640	10.04	2	6
bngl589	4.10	2	3	bngl279	10.07	4	6
bngl143	5.01	1	2	DUPSSR17	10.07	3	6
DUPSSR01	5.01	2	7	DUPSSR05	Unknown	3	6
bngl565	5.02	6	9	MACE01G01	Unknown	3	6
bngl105	5.03	4	7	MACT02B08	Unknown	3	5
bngl557	5.03	5	6	DUPSSR08	Unknown	4	7
bngl150	5.03–5.04	3	3	DUPSSR07	Unknown	5	7
bngl603	5.04	3	4	DUPSSR20	Unknown	2	2
DUPSSR10	5.04	3	7				

[a] From MaizeDB (http://www.agron.missouri.edu/coop/ssr_probes/ssr1.html)

reds, sampled at random from the 40 inbreds, the average expected heterozygosity was also 0.67. Whether or not genetic diversity is lower in the current inbreds than in the historical inbreds therefore depends on whether genetic diversity is evaluated at the gene level (i.e., number of alleles per locus) or at the population level (i.e., D_{ij} among inbreds). For example, locus DUPSSR23, which had the largest number of alleles among all 40 inbreds, had an average D_{ij} of 0.79 among the 32 historical inbreds. Among the current inbreds, this locus had four alleles but a higher average D_{ij} of 0.81. A loss in the number of alleles therefore does not necessarily translate to a loss in D_{ij}, especially if the frequencies of some of the alleles in the original population are low.

Among the eight current inbreds, the average D_{ij} of 0.65 can be partitioned as follows: 0.52 among the four BSSS inbreds (B14, B37, B73 and B84); 0.62 among the four non-BSSS inbreds (Mo17, C103, Oh43 and H99); and 0.71 among the 16 BSSS × non-BSSS crosses. The high D_{ij} among the eight current inbreds was therefore largely due to the D_{ij} between heterotic groups (i.e., BSSS× non-BSSS) rather than the D_{ij} within either heterotic group. This result was consistent with the lower average number of alleles per locus (in parentheses) within the four current BSSS inbreds (2.1) and four current non-BSSS inbreds (2.3), than among the eight current inbreds considered as one group (3.2). Heterosis is due to heterozygosity (i.e., D_{ij}) at loci that exhibit non-additive gene effects (Falconer 1981, pg 232). The BSSS and non-BSSS heterotic groups exploit the superior heterosis in BSSS × non-BSSS crosses (Hallauer et al. 1988). Although advanced-cycle pedigree breeding with

only eight current inbreds has reduced the number of alleles per locus, the choice of inbreds that belong to opposite heterotic groups has therefore been effective in maintaining genetic diversity at the population level.

We believe that breeders need to be concerned about further decreases in D_{ij} within each heterotic group, even if the D_{ij} in BSSS × non-BSSS crosses remains high. Suppose that, at a single locus, an allele becomes fixed in all advanced-cycle inbreds developed from B14, B37, B73 and B84. A different allele becomes fixed in all advanced-cycle inbreds developed from Mo17, C103, Oh43 and H99. The D_{ij} at the locus would be equal to 1 in BSSS × non-BSSS crosses but equal to zero among the BSSS inbreds and among the non-BSSS inbreds. The average D_{ij} among all the inbreds would remain high, but no genetic gain can be expected from advanced-cycle breeding within each heterotic group. We therefore speculate that exploiting other germplasm pools (Kauffman et al. 1982), creating new germplasm pools (Bernardo 2001), or utilizing exotic germplasm (Goodman 1985) is necessary for sustaining breeding progress in maize.

References

Bernardo R (2001) Breeding potential of intra- and inter-heterotic group crosses in maize. Crop Sci 41:68–71

Darrah LL, Zuber MS (1985) 1985 United States farm maize germplasm base and commercial breeding strategies. Crop Sci 26:1109–1113

Falconer DS (1981) Introduction to quantitative genetics, 2nd edn. Longman, London

Goodman MM (1985) Exotic maize germplasm: status, prospects, and remedies. Iowa State J Res 59:497–527

Hallauer AR, Russell WA, Lamkey KR (1988) Corn breeding. In: Sprague GF, Dudley JW (eds) Corn and corn improvement, 3rd edn. Agron Monogr 18. Am Soc Agron, Crop Sci Soc Am, Soil Sci Soc Am, Madison, Wisconsin, USA, pp 463–564

Henderson CB (1976) Maize research and breeders manual, no. 8. Illinois Foundation Seeds, Inc., Champaign, Illinois, USA

Jenkins MT (1936) Corn improvement. In: Bressman ES, Hambidge G (eds) 1936 Yearbook of agriculture. US Dept Ag, Washington, D.C., pp 455–522

Kantety RVR (1997) Inter-simple sequence repeats as genetic markers and mapping genes for grain milling quality in maize. PhD dissertation, Purdue University, West Lafayette, Indiana, USA

Kauffman KD, Crum CW, Lindsey MF (1982) Exotic germplasm in a corn breeding program. Illinois Corn Breed School 18:6–39

MBS Inc. (1999) MBS, Inc. genetics handbook, 26th edn. MBS, Inc., Ames, Iowa, USA

Nei M, Li W (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5256–5273

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

SAS Institute (1987) SAS/STAT: guide for personal computers. Version 6. SAS Institute Inc, Cary, North Carolina, USA

Smith, JSC, Goodman MM, Stuber CW (1985a) Genetic variability within U.S. maize germplasm. I. Historically important lines. Crop Sci 25:550–555

Smith, JSC, Goodman MM, Stuber CW (1985b) Genetic variability within U.S. maize germplasm. II. Widely used inbred lines 1970 to 1979. Crop Sci 25:681–685

Smith JSC, Duvick DN, Smith OS, Grunst A, Wall SJ (1999) Effect of hybrid breeding on genetic diversity in maize. In: Coors JG, Pandey S (eds) Genetics and exploitation of heterosis in crops. Mexico City, 17–22 Aug 1997. Am Soc Agron and Crop Sci Soc Am, Madison, Wisconsin, USA, pp 119–126

Sprague GF (1984) Organization of breeding programs. Illinois Corn Breed School 20:20–31

Troyer AF (1990) A retrospective view of corn genetic resources. J Hered 81:17–24

Troyer AF (1999) Background of U.S. hybrid corn. Crop Sci 39:601–626

Wallace HA, Brown WL (1956) Corn and its early fathers. Michigan State Univ Press, East Lansing, Michigan, USA