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SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan

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Abstract Information regarding diversity and relationships among breeding material is necessary for hybrid maize (*Zea mays* L.) breeding. Simple-sequence repeat (SSR) analysis of the 60 loci distributed uniformly throughout the maize genome was carried out for 65 inbred lines adapted to cold regions of Japan in order to assess genetic diversity among the inbred lines and to assign them to heterotic groups. The mean value (0.69) of the polymorphic-index content (PIC) for the SSR loci provided sufficient discrimination-ability for the assessment of genetic diversity among the inbred lines. The correlation between the genetic-similarity (GS) estimates and the coancestry coefficient was significant ($r = 0.70$). The average-linkage (UPGMA) cluster analysis and principal-coordinate analysis (PCOA) for a matrix of the GS estimates showed that the Northern flint inbred lines bred in Japan were similar to a Canadian Northern flint inbred line CO12 and a European flint inbred line F283, and that dent inbred lines bred in Japan were similar to BSSS inbred lines such as B73. These associations correspond to the known pedigree records of these inbred lines. The results indicate that SSR analysis is effective for the assessment of genetic diversity among maize inbred lines and for the assignment of inbred lines to heterotic groups.

Keywords Maize · SSR · Genetic similarity · Northern flint

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Introduction

Information regarding genetic diversity and the relationship among breeding materials is indispensable for the development of new maize inbred lines, the assignment of maize inbred lines to heterotic groups, and the choice of testers for trials of hybrid combinations in maize breeding. In addition, a comparison of genetic diversity among representative inbred lines of U.S. [Lancaster Sure Crop (LSC) and Iowa Stiff Stalk Synthetic (BSSS)], European (European flint), Canadian and local inbred lines is useful for the evaluation of local breeding materials, and for the exploitation and the introduction of another germplasm.

The breeding of maize hybrids adapted to cold regions of Japan is based on the use of the heterotic pattern of U.S. dent by Northern flint or European flint. In this breeding system, dent inbred lines have been developed from North American or European hybrids, and flint inbred lines have been developed from local varieties belonging to Northern flint or European hybrids. The local varieties belonging to Northern flint were introduced from North America about 130 years ago (Inoue 1984). The inbred lines developed from the local varieties are superior in respect of low-temperature germination and low-temperature growth (Monma and Okabe 1985). On the other hand, European hybrids are usually a cross of an early maturing dent and a European flint. Therefore, inbred lines developed from European hybrids are a mix of dent and flint germplasm, so it is difficult to assign them to heterotic groups by the conventional method. In addition, there have been few reports of a detailed assessment of genetic diversity among the inbred lines adapted to cold regions of Japan compared with representative inbred lines such as European flint inbred lines, Corn Belt dent inbred lines, and Canadian inbred lines.

Genetic diversity among inbred lines has usually been assessed based on morphological data such as endosperm type, the pedigree record of inbred lines and the amount of heterosis expressed by the hybrid. However, these descriptors present several limitations. The mor-

phological characters often do not reliably portray genetic relationships due to environmental interactions. The Malécot (1948) coancestry coefficient is based on the pedigree records of inbred lines. However, it requires accurate pedigree records, and it cannot evaluate the effects of selection and gene drift (Messmer et al. 1993). Testcross designs requiring several testers are extremely expensive and time-consuming. Therefore, the utilization of molecular markers that directly evaluate genetic differences between inbred lines has been attempted to assess the genetic diversity among maize inbred lines (Melchinger 1999). Senior and Heun (1993) have reported that SSR loci provide a high level of polymorphism in maize. SSR analysis presents the potential advantages of reliability, reproducibility, discrimination, standardization and cost-effectiveness over RFLP analysis (Smith et al. 1997), and Senior et al. (1998) have reported that SSR analysis using high quality agarose gels can conveniently assess the genetic diversity of maize inbred lines.

The objectives of our study were to: (1) evaluate the discrimination ability of the SSR analysis of the 60 loci distributed uniformly throughout the maize genome, (2) assess genetic diversity among the inbred lines adapted to cold regions of Japan and representative inbred lines, and (3) assign the inbred lines developed from hybrids to heterotic groups.

Materials and methods

Plant material

Fifty one maize inbred lines were chosen to represent maize diversity among the breeding materials adapted to cold regions of Japan, and 14 maize inbred lines introduced from the U.S., Canada and Europe were used for comparison (Table 1). Twenty of the 65 inbred lines were developed from flint germplasm and 26 from dent germplasm. Additionally 19 inbred lines which were developed from the European hybrids by crossing an early maturing dent with a European flint were subsequently designated as “miscellaneous”. Among all pairs of inbred lines, those without common parentage were designated as “unrelated”. Genomic DNA was isolated from a bulk of five seedlings from each inbred line using a modified CTAB procedure (Saghai-Maroof et al. 1984).

Table 1 Derivation and group of 65 inbred lines used in SSR analysis

Inbred line	Derivation	Group[a]
Flint inbred lines bred in cold regions of Japan		
Ho11	Mutu	N. flint
Ho20	N206×N28	N. flint
Ho22	Mutu	N. flint
N19	Sakashita	N. flint
N21	Sakashita	N. flint
N85	Iwanai zairai A	N. flint
N138	Kiwase	N. flint
N146	Iwanai zairai B	N. flint
To15	Kiwase×(Yamamotoshu, Sakashita)	N. flint
To38	Kiwase×(Yamamotoshu, Sakashita)	N. flint
To39	Kiwase×(Yamamotoshu, Sakashita)	N. flint
To77	To15×To31	N. flint
To79	To15×To31	N. flint
To82	To15×To36	N. flint
To85	To15×To31	N. flint
To88	To9×To15	N. flint
To90	To15×To36	N. flint
Dent inbred lines bred in cold regions of Japan		
Ho32	Pioneer 3747	Dent
Ho40	Pioneer 3790	Dent
Ho43	Pioneer 3732	Dent
Ho45	Pioneer 3747	Dent
Ho47	Pioneer 3747	Dent
Ho52	Pioneer 3732	Dent
Ho54	Pioneer 3790	Dent
Ho57	Pioneer 3389	Dent
Ho60	Pioneer 3790	Dent
Ho68	DK403	Dent
Ho72	4332	Dent
Ho77	Pioneer 3897	Dent
Ho78	X9232	Dent
Ho79	N4545	Dent
To92	Pioneer 3747	Dent
Representative flint inbred lines		
CO12	Unknown, Canada-Ottawa	N. flint
F2	Lacaune population	E. flint
F283	F227×F228 (50%F7)	E. flint
Representative dent inbred lines		
A619	(A171×Oh43)×Oh43	LSC (Oh43)
A679	B73×A662	BSSS
B73	Iowa SSS C5 Sel.	BSSS
C103	Lancaster Surecrop	LSC (C103)
CM37	KE3	LSC (Canada)
CMV3	A21×W185	LSC (Canada)
CO158	Dekalb 46	Dent
H99	Illinois Syn. 60C	LSC
Mo17	CI187.2×C103	LSC (C103)
Oh43	Oh40B×W8	LSC (Oh43)
W79A	Minn. 13	M13
Miscellaneous origin		
Ho3	INRA258	
Ho4	INRA258	
Ho34	(N85×Ho11)×Oh545	
Ho36	(Ho4×Ho3)×N204	
Ho37	(Ho4×Ho3)×W452	
Ho42	(Ho4×Ho3)×N204	
Ho49	N85×Ho4	
Ho50	N85×Ho4	
Ho59	PH1407	
Ho63	EXP736	
Ho64	EXP736	
Ho65	SH6061	
Ho66	SH7329	
Ho67	SH7329	
Ho73	Astrid	
Ho81	LG2080	
To113	DEA	
To132	LG2266	
To133	LG2266	

[a] N. flint, E. flint and dent stand for Northern flint, European flint, and the inbred line developed from the dent hybrid, respectively

SSR primer selection

Based on their chromosome loci, we chose 100 SSR primers from Maize DB and assayed their preliminary discriminatory power using a sample of 16 inbred lines. Primers were excluded from the study if they did not show different band sizes or consistently failed to amplify in 16 inbred lines. A final set of 60 SSR primers was selected for further analysis (Table 2). Among this set of SSR loci, 31 (51%) were di-repeats, 7 (12%) tri-repeats, 8 (13%) tetra-repeats, 3 (5%) penta-repeats, 1 (2%) a hexa-repeat and 10 (17%) were unknown. Each chromosome had at least five SSR loci, and the mean map distance between neighboring SSR loci was approximately 21.1 cM.

Amplification and detection conditions

The reactions were carried out in a DNA Thermal Cycler (Perkin-Elmer, Norwalk, Ga., USA). The reaction consisted of a denaturation step of 1 min at 96 °C, followed by a touchdown procedure as described by Mellersch and Sampson (1993). This procedure began with 1 min at 96 °C, 1 min at 65 °C, and 2 min at 72 °C. The annealing temperature was then reduced at each cycle by 0.5 °C until a final annealing temperature of 55 °C was reached. The last cycle was repeated 20 times and was terminated at 72 °C for 2 min. Then, the reaction was finished with a continuous cycle at 4 °C. The 10-µl reaction mix consisted of 20 ng of each primer, 1 unit of *Taq* DNA polymerase, 200 µM of each dNTP, 1 × reaction buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 100 µg ml^{-1} of gelatin: pH 8.3), 30 ng of template DNA, and distilled de-ionized water. Reactions were stopped with 10 µl of loading-dye (50% de-ionized formamide, 40% glycerol, 20 mM EDTA, 0.6 mg ml^{-1} of bromphenol blue). After the reaction, 20 µl of the reaction mix was heated at 96 °C for 2 min, placed on ice, then loaded onto a 10% polyacrylamide denaturing gel (16 cm × 16 cm) containing 8 M urea. After a migration at 300 V, gels were soaked for 15 min in distilled water to reduce the concentration of urea in the gel, so that denatured DNA would anneal and thus be able to be stained with EtBr.

Statistical analysis

Estimates of genetic similarity (GS) were calculated for all possible pairs of inbred lines according to the following equation (Dice 1945; Nei and Li 1979): GS(i,j)=2N(i,j)/[N(i)+N(j)], where GS(i,j) is the GS estimate between inbred lines i and j, N(i,j) is the total number of bands common to i and j, and N(i) and N(j) is the number of bands for inbred lines i and j, respectively. The standard error [SE(i,j)] of GS(i,j) was estimated by $SE(i,j) = \{GS(i,j)[1–GS(i,j)]/[N(i)+N(j)]^{0.5}\}$ (Dubreuil et al. 1996). This estimator is strictly equivalent to the jackknife estimator used in published studies (e.g. Melchinger et al. 1991; Messmer et al. 1992) and overestimates the actual variances when loci have been chosen to optimize the genome coverage Dubreuil et al. 1996). Thus, in this study, estimated SE values had to be considered as an upper limit for the actual values. Average linkage (UPGMA) cluster analysis and principal coordinate analysis (PCOA) were performed with the matrix of GS estimates using appropriate procedures of the program NTSYS-pc (Rohlf 1989). The mean GS estimates of the inter- and intra-groups were calculated among all possible pairs of the inbred lines belonging to each group. In order to estimate the mean genetic similarity (GS_M) of unrelated pairs of the inbred lines with the highest precision, GS_M was calculated from all GS estimates of 520 unrelated pairs among 26 dent inbred lines and 20 flint inbred lines. The Malecot coancestry coefficient (f) was calculated for all pairs of the inbred lines with known pedigrees. The f value for the unrelated pairs of inbred lines was set to zero. For a given f value of two lines, the expected genetic similarity (GS_{EXP}) was calculated by $GS_{EXP} = f+(1–f)\ GS_M$ (Messmer et al. 1993). The PIC for each SSR locus was determined as described by Smith et al. (1997). PIC is a measure of the allele diver-

Table 2 Allele numbers and PIC values for SSR loci found in 65 maize inbred lines

SSR locus	No. chrom.[a]	cM[a]	Repeat class[a]	No. alleles	PIC value
phi056	1	6.1	3	4	0.69
bnlg1007	1	39.7	2	11	0.86
phi001	1	60.7	2	14	0.90
bnlg1273	1	85.1	2	8	0.75
bnlg1564	1	111.2	2	9	0.84
bnlg1597	1	135.0	2	2	0.42
phi120	1	150.5	3	4	0.64
bnlg1017	2	25.7	2	10	0.84
bnlg469	2	48.9	–	5	0.67
phi083	2	80.9	4	6	0.66
nc003	2	89.3	2	12	0.82
phi127	2	106.1	4	4	0.62
bnlg1520	2	130.0	2	9	0.72
umc1057[b]	3	–	3	2	0.42
bnlg1523[b]	3	27.0	2	8	0.48
phi036	3	38.8	2	7	0.52
phi053	3	51.4	4	4	0.63
bnlg197	3	71.9	–	12	0.89
phi046	3	82.2	4	2	0.41
phi047	3	103.7	3	3	0.62
phi072	4	14.4	4	4	0.47
phi021	4	39.1	2	12	0.76
bnlg252	4	68.3	–	5	0.58
bnlg2291	4	77.4	2	6	0.67
bnlg1444	4	98.0	2	17	0.91
bnlg1565	4	124.7	2	6	0.75
phi024	5	21.6	3	5	0.67
phi008	5	50.2	3	3	0.40
dupssr10	5	71.7	2	15	0.84
bnlg1237	5	95.3	2	5	0.41
bnlg1695	5	122.7	2	12	0.88
bnlg389	5	144.5	–	6	0.62
phi126	6	4.9	2	7	0.81
bnlg249	6	17.0	–	11	0.79
bnlg1371	6	22.8	2	10	0.76
nc010	6	42.2	4	4	0.61
phi070	6	91.3	5	4	0.54
phi123	6	102.5	4	3	0.50
bnlg2132	7	13.1	2	7	0.59
umc1066	7	35.5	6	5	0.49
bnlg657	7	52.7	–	9	0.79
bnlg434	7	60.5	–	7	0.75
dupssr13	7	87.4	2	8	0.67
phi116	7	113.8	–	4	0.71
bnlg2235	8	26.0	2	11	0.84
bnlg125	8	35.9	2	4	0.66
bnlg162	8	55.0	–	6	0.76
bnlg1152	8	66.7	2	10	0.80
bnlg1823	8	85.7	2	9	0.77
phi080	8	112.1	5	5	0.67
phi028	9	19.4	3	3	0.49
bnlg1401	9	33.3	2	9	0.75
phi065	9	45.6	5	4	0.50
bnlg1270	9	69.8	2	6	0.76
bnlg1525	9	88.1	2	11	0.79
phi041	10	0.0	4	5	0.78
bnlg1451	10	30.3	2	12	0.85
bnlg210	10	46.9	–	7	0.57
bnlg1518	10	55.1	2	10	0.83
bnlg1250	10	67.3	2	10	0.83

[a] Loci and repeat class were referred from Maize DB and the SSR Consensus 1998 (Romero-Severson 1998)

[b] umc1057 and bnlg1523 assigned to bin 3.02 and bin 3.03 were referred from Maize DB

sity at a locus and is equal to $1 - \sum h_k^2$, where h_k is the frequency of the kth allele. When calculated in this manner, PIC is synonymous with the term "gene diversity" as described by Weir (1996).

Results and discussion

Sixty SSR primers produced 433 alleles among 65 maize inbred lines, and the allele number for the SSR loci ranged from 2 to 17, with the mean allele being 7.3, and the PIC values for the SSR loci ranged from 0.41 to 0.91, with the mean PIC being 0.69 (Table 2). The mean PIC value in this study was higher than that determined by Smith et al. (1997) (0.62) and that determined by Senior et al. (1998) (0.59). The higher PIC value probably resulted from excluding the SSR primers with low discriminatory power by the preliminary test. Di-repeat SSR loci gave a higher mean allele (9.3) and mean PIC (0.74), and tri-, tetra- and penta-repeat SSR loci gave lower mean alleles (3.4–5.0) and mean PIC (0.56–0.59) (Table 3). The highest mean PIC value of the di-repeat SSR loci is consistent with the results of Smith et al. (1997) and Senior et al. (1998). Smith et al. (1997) have reported that di-repeat SSR loci abound with alleles, but that some di-repeat SSR loci tend to produce additional stutter bands. In this study, because of exclusion by the preliminary test for SSR primers, stutter bands did not appear.

The f and the GS estimates for 56 pairs among inbred lines with a pedigree record ranged from 0.063 to 0.750 and from 0.224 to 0.826, when the unrelated pairs of inbred lines were excluded. The correlation between the GS estimates and f, and the GS_{EXP} for them was significant ($r = 0.70$ and $r = 0.70$, $P < 0.01$ respectively). This significant correlation agrees with the results of Messmer et al. (1993), Dubreuil et al. (1996) and Smith et al. (1997). However, deviations between the GS estimates and the GS_{EXP} averaged 0.10. The deviations increased especially with increasing f, and the mean GS estimate for f = 0.5 pairs (0.548) was lower than the GS_{EXP} (0.637) and ranged widely from 0.311 to 0.826. The differences between the GS estimates and the coancestry coefficient are considered to result from the effects of selection and gene drift.

The GS_M estimate of 520 unrelated pairs among 26 dent inbred lines and 20 flint inbred lines was 0.271. The SE of individual GS estimates had a mean of 0.06 and ranged from 0.04 to 0.06. Bardosa-Neto et al. (1997) have reported that marker loci should be chosen uniformly over an entire genome in genetic-diversity studies, avoiding biases due to sampling, and the precision of GS estimates increases as the number of marker loci increases. However, given the high cost and intensive labor of DNA-marker assays, it is necessary to choose the minimum number of markers required for a given level of precision in GS estimates. We chose 60 SSR primers uniformly over the maize genome from the Maize DB, with the mean SE of the GS estimates in this study being

Table 3 Information score summary statistics by repeat class

Repeat class	Mean no. alleles	Mean PIC value	PIC standard error
2	9.3	0.74	0.02
3	3.4	0.56	0.05
4	4.0	0.59	0.04
5	4.0	0.57	0.05
6	5.0	0.49	–
3–6	3.9	0.57	0.03

Table 5 Mean genetic similarities (GS) between the representative inbred lines and the inbred lines bred in Japan calculated from 60 SSR data[a]

Representative inbred line	Group	SSR-based mean GS with	
		Dent inbred lines bred in Japan (n = 15)	Northern flint inbred lines bred in Japan (n = 17)
C103	LSC (C103)	0.321	0.266
Mo17	LSC (C103)	0.307	0.341
Oh43	LSC (Oh43)	0.283	0.289
A619	LSC (Oh43)	0.228	0.242
CM37	LSC (Canada)	0.256	0.323
CMV3	LSC (Canada)	0.249	0.279
H99	LSC	0.313	0.305
W79A	M13	0.291	0.272
B73	BSSS	0.348	0.257
A679	BSSS	0.342	0.294
CO158	dent	0.311	0.251
F2	European flint	0.232	0.298
F283	European flint	0.195	0.381
CO12	Northern flint	0.262	0.452

[a] The mean genetic similarity estimates of 520 unrelated pairs among 26 dent and 20 flint inbred lines (GS_M) was 0.271

Table 4 Mean genetic similarities (GS) within and among groups calculated from 60 SSRs[a]

Group	SSR-based mean GS with		
	Representative dent inbred lines (n = 11)	Dent inbred lines bred in Japan (n = 15)	Northern flint inbred lines bred in Japan (n = 17)
Representative dent inbred lines	0.289	0.295	0.284
Dent inbred lines bred in Japan		0.405	0.273
Northern flint inbred lines bred in Japan			0.424

[a] The mean genetic similarity estimates of 520 unrelated pairs among 26 dent and 20 flint inbred lines (GS_M) was 0.271

almost equal to the mean SE in Dubreuil et al. (1996) (0.06). When the genome was uniformly covered with marker loci, an estimate of SE overestimates the actual variance of the GS estimates (Dubreuil et al. 1996), making the presicion of this study sufficient for estimating GS among maize inbred lines. Therefore, the SSR analysis of the 60 loci provided sufficient precision for the GS estimates, and this analysis system appears to be effective for the assessment of genetic diversity among maize inbred lines.

The mean GS estimate for unrelated pairs among the dent inbred lines bred in Japan (0.405), and the mean GS estimate for unrelated pairs among the Northern flint inbred lines bred in Japan (0.424), were higher than the mean GS for unrelated pairs among the representative dent inbred lines (0.289) (Table 4). Each value was also greater than GS_M. Therefore, the dent and Northern flint inbred lines bred in Japan were considered to have a narrower genetic diversity than the representative dent inbred lines respectively. However, the mean GS estimate between the dent and the Northern flint inbred lines bred in Japan (0.273) is almost equal to GS_M. This result supports the observation of heterosis in crosses of the dent inbred lines bred in Japan with the Northern flint inbred lines bred in Japan.

The Canadian flint inbred line CO12 had the greatest mean GS estimate (0.452), and the European flint inbred line F283 had the second greatest (0.381), with the Northern flint inbred lines bred in Japan (Table 5). This result agrees with the origin of the local varieties in cold regions of Japan, which were introduced from North America. However, another European flint inbred line F2 had a smaller mean GS estimate with the Northern flint inbred lines bred in Japan (0.298). European flint inbred lines were selected from European open-pollinated populations, which presumably trace back to tropical flints from the West Indies and Caribbean islands (Wallace and Brown 1956). Therefore, their progenitors seem to be different from the Northern flint. However, the result suggests that some of their progenitors received the effects of the Northern flint. Two LSC inbred lines, Mo17 and CM37, had the greatest mean GS estimate with the Northern flint inbred lines bred in Japan (0.341 and 0.323, respectively) among the representative dent inbred lines, and the mean GS estimates were higher than GS_M. The Corn Belt dents were developed from mixing the Southern dents with the Northern flints (Goodman and Brown 1988). These inbred lines are considered to have inherited more genes from the Northern flint than other dent inbred lines.

Two BSSS inbred lines, B73 and A679, had the greatest mean GS estimate with the dent inbred lines bred in Japan (0.348 and 0.342, respectively) among the representative dent inbred lines. C103-related inbred lines (C103 and Mo17) had a comparatively greater mean GS estimate with the dent inbred lines bred in Japan (0.314) than GS_M. However, Oh43-related inbred lines (Oh43 and A619) and the Canadian dent inbred lines (CM37 and CMV3) had low mean GS estimates with the dent inbred lines bred in Japan (0.256 and 0.253, respectively). The dent inbred lines bred in Japan were developed from U.S. and European hybrids. From isozyme and chromatographic data, the U.S. varieties appear to be heavily dependent on the usage of B73, A632, Oh43 and Mo17, or their close derivatives (Smith 1988). And, in the LSC inbred lines, C103-related inbred lines are not comparatively similar to Oh43-related inbred lines based on RFLP and SSR analysis (Mumm and Dudley 1994; Senior et al. 1998). The GS estimates among the dent inbred lines bred in Japan and the representative dent inbred lines indicate that the dent inbred lines bred in Japan are comparatively similar to the BSSS and the

Table 6 Mean, minimum, and maximum of genetic similarities (GS) for unrelated pairs between the miscellaneous inbred lines and the dent or flint inbred lines calculated from 60 SSRs data[a, b]

Inbred line	Derivation	SSR-based GS with					
		Dent inbred lines (n = 26			Flint inbred lines (n = 20		
		Mean	Min	Max	Mean	Min	Max
Ho3	INRA258	0.257	0.131	0.361	0.247	0.163	0.361
Ho4	INRA258	0.227	0.131	0.311	0.238	0.179	0.325
Ho34	(N85×Ho11)×Oh545	0.257	0.182	0.364	0.383	0.217	0.529
Ho36	(Ho4×Ho3)×N204	0.284	0.197	0.377	0.343	0.295	0.407
Ho37	(Ho4×Ho3)×W452	0.280	0.180	0.377	0.240	0.197	0.295
Ho42	(Ho4×Ho3)×N204	0.239	0.163	0.341	0.261	0.179	0.339
Ho49	N85×Ho4	0.269	0.161	0.368	0.262	0.161	0.387
Ho50	N85×Ho4	0.298	0.180	0.423	0.271	0.195	0.358
Ho59	PH1407	0.351	0.228	0.488	0.285	0.210	0.348
Ho63	EXP736	0.333	0.210	0.455	0.280	0.211	0.371
Ho64	EXP736	0.356	0.222	0.496	0.329	0.270	0.416
Ho65	SH6061	0.298	0.149	0.430	0.304	0.180	0.430
Ho66	SH7329	0.296	0.198	0.430	0.354	0.262	0.430
Ho67	SH7329	0.303	0.195	0.410	0.360	0.309	0.426
Ho73	Astrid	0.283	0.148	0.393	0.333	0.246	0.426
Ho81	LG2080	0.312	0.224	0.406	0.377	0.301	0.621
To113	DEA	0.294	0.213	0.397	0.329	0.231	0.628
To132	LG2266	0.312	0.228	0.439	0.280	0.230	0.359
To133	LG2266	0.332	0.240	0.468	0.306	0.226	0.372

[a] Standard error of estimated GS ranged from 0.04 and 0.06

[b] The mean genetic similarity estimates of 520 unrelated pairs among 26 dent and 20 flint inbred lines (GS_M) was 0.271

C103-related inbred lines, and are not similar to the Oh43-related and the Canadian dent inbred lines. The U.S. hybrids used as source of the dent inbred lines bred in Japan are considered to have originated from BSSS inbred lines crossed with the C103-related inbred lines.

Nineteen miscellaneous inbred lines were grouped based on the relative magnitude of the mean GS estimates with the dent and flint inbred lines (Table 6). Six miscellaneous inbred lines (Ho34, Ho36, Ho65, Ho66, Ho73 and To113) had greater mean GS estimate with the flint inbred lines than GS_M, and are considered to be similar to the flint inbred lines. Three miscellaneous inbred lines (Ho59, Ho63 and To132) had greater mean GS estimate with the dent inbred lines than GS_M, and are considered to be similar to the dent inbred lines. Four miscellaneous inbred lines (Ho64, Ho67, Ho81 and To133) had a greater mean GS estimates with the dent and flint inbred lines than GS_M, and are considered to be intermediate with the dent and flint inbred lines. However, six miscellaneous inbred lines (Ho3, Ho4, Ho37, Ho42, Ho49 and Ho50) did not have greater GS estimates with the dent and flint inbred lines than GS_M, and are considered not to be similar to the dent and flint inbred lines.

Cluster analysis classified the 65 inbred lines into four main clusters (Fig. 1). The first main cluster consisted of the entire flint inbred lines and four miscellaneous inbred lines. This cluster subdivided into three subclusters. All Northern flint inbred lines bred in Japan were classified into the subcluster with CO12 and F283. Two miscellaneous inbred lines (Ho34 and Ho81) were included in this subcluster. The F2 were classified into the other subcluster with two miscellaneous inbred lines (Ho65 and To113). The second main cluster consisted of B73, A679, the entire dent inbred lines bred in Japan and eight miscellaneous inbred lines. Therefore, the dent inbred lines bred in Japan appear to be more similar to the BSSS inbred lines than the C103-related inbred lines. In the cold regions of Japan, some cool-weather damage does occur (Monma and Okabe 1985), and the specific genotype suitable for the cold region was prior-selected; as a result the genetic diversity of these dent inbred lines is considered to closely incline toward one side. The cluster was subdivided into two subclusters with one subcluster consisting of the dent inbred lines bred in Japan and three miscellaneous inbred lines (Ho59, Ho63 and Ho64), and the other consisting of five miscellaneous inbred lines (Ho66, Ho67, Ho73, To132 and To133). B73 and A679 were on the edge of this main cluster. The third main cluster consisted of representative LSC inbred lines (Oh43, A619, Mo17 and C103) and INRA258-related miscellaneous inbred lines (Ho3, Ho4, Ho36, Ho37, Ho42, Ho49 and Ho50). The fourth main cluster consisted of five representative dent inbred lines (CM37, CMV3, CO158, H99 and W79A).

PCOA revealed similar groupings of the inbred lines (Fig. 2). The first and second principal coordinates, termed PC1 and PC2, explain 9.0% and 6.2% of the total variation in the SSR data. The representative dent inbred

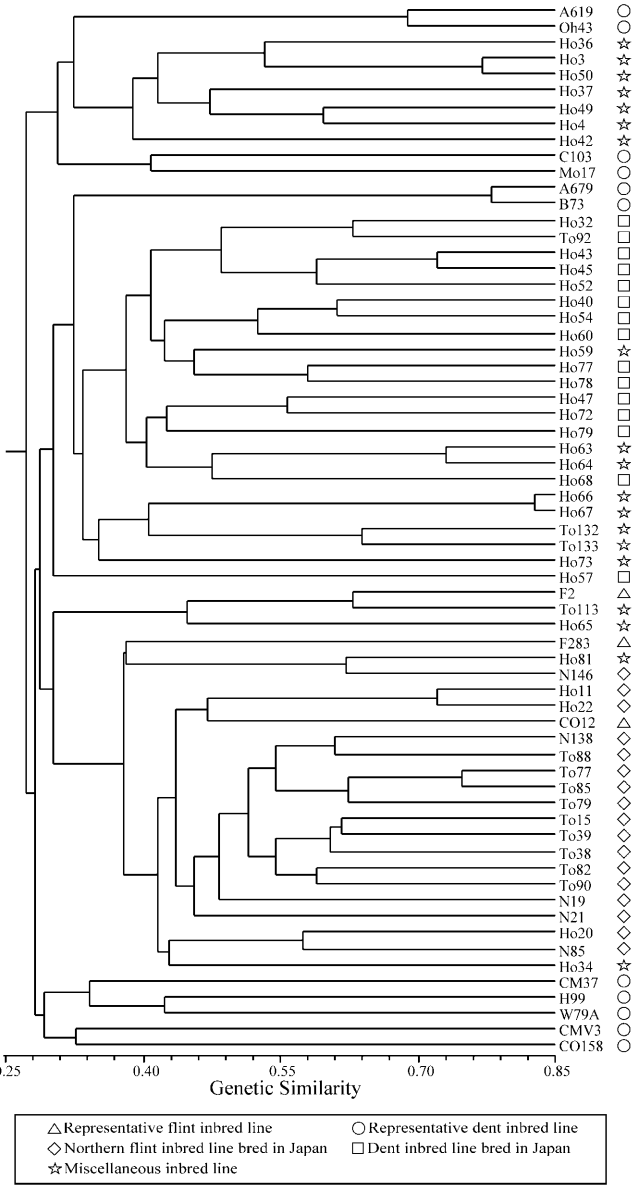

Fig. 1 Dendrogram constructed with a Unweighted Paired Group Method using the Arithmetic Average (UPGMA) clustering algorithm from the pairwise matrix of genetic similarities (GS) among 65 maize inbreds

lines were clearly separated from the representative flint inbred lines with respect to PC1, but were widely spread with respect to PC2. The dent inbred lines were loosely divided into two groups, with the one consisting of B73, A679 and the dent inbred lines bred in Japan, and the other consisting of LSC inbred lines, W79A and CO158.

Among the flint inbred lines, CO12 and the Northern flint inbred lines bred in Japan were firmly grouped. F2 and F283 were slightly separated from this group. Among the 19 miscellaneous inbred lines, INRA 258-related miscellaneous inbred lines were grouped and were independent of the dent and the flint inbred lines, except for Oh43 and A619. The genotype of INRA 258 consisted of Minnesota 13 (M13) and European flint,

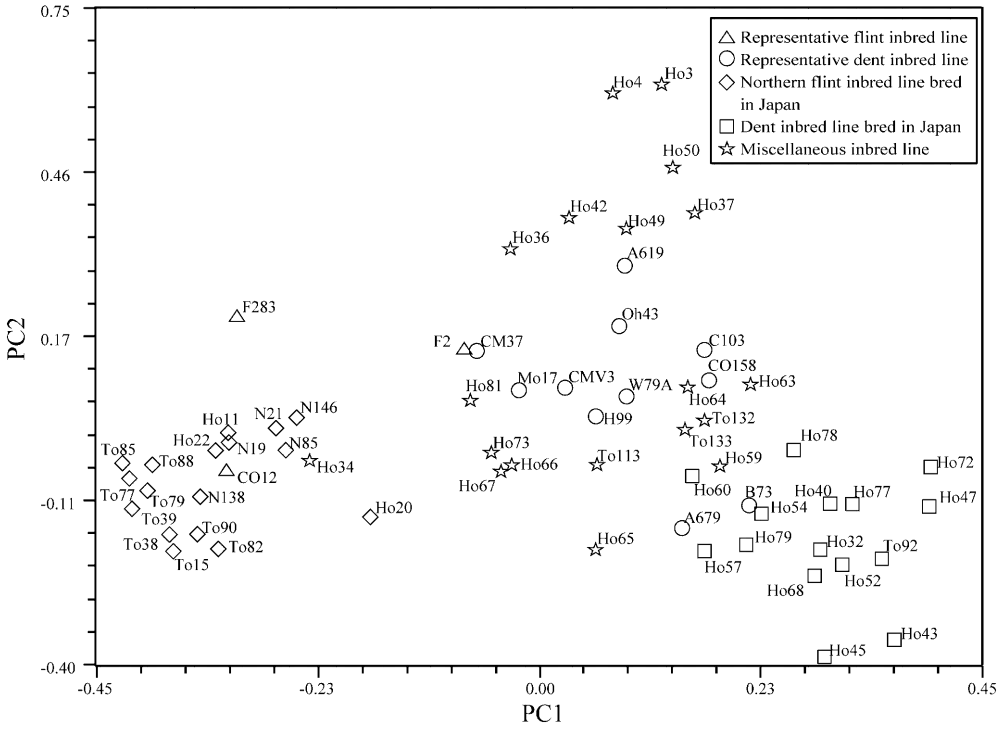

Fig. 2 Associations among the 65 maize inbred lines revealed by principal coordinate analysis (PCOA) performed on genetic similarities (GS) calculated from 60 SSRsrfd

and Oh43 is partially related to M13 (Gerdes and Tracy 1993). Thus, INRA 258-related miscellaneous inbred lines were more similar to the Oh43-related inbred lines than the European flint inbred lines. In addition, INRA 258-related miscellaneous inbred lines had a lower mean GS estimate with the dent inbred lines bred in Japan and the Northern flint inbred lines bred in Japan, and they are considered to be precious breeding material in the cold regions of Japan.

The miscellaneous inbred lines, except for the INRA258-related miscellaneous inbred lines, were spread between the dent and the flint inbred lines with respect to PC1. Among them, Ho34, Ho59, Ho63 and To132 were clearly assigned to the dent or flint groups, and this result agrees with the relative magnitude of mean GS estimates and the results of cluster analysis. The empirical knowledge of heterosis supports this result. On the other hand, the assignment of other miscellaneous inbred lines was different according to the mode of analysis. From PCOA and the relative magnitude of mean GS estimates, they were classified into the intermediate or flint group. However, from the cluster analysis, Ho66, Ho67 and Ho73 were classified into the dent group. The empirical knowledge of heterosis agrees with the results of PCOA and the relative magnitude of mean GS estimates. PCOA is suitable for faithful portrayals of the relationships between larger groups of inbred lines, and cluster analysis is reliable for depicting close relationships between inbred lines (Melchinger 1999). Though the assignment of miscellaneous inbred lines is important in a breeding program, the results may change according to the analysis, so we should assign them with these results and the empirical knowledge of heterosis. The clear assignment of inbred lines developed from the hybrids is difficult based on their pedigree record. However, by comparison with the representative inbred lines, genetic diversity and relationships among the Japanese inbred lines were revealed. These results provide a useful criterion for the exploitation and introduction of breeding materials for hybrids adapted to cold regions of Japan.

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