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An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays L.*): comparisons with data from RFLPS and pedigree

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Abstract The utility of 131 simple sequence repeat (SSR) loci to characterize and identify maize inbred lines, validate pedigree, and show associations among inbred lines was evaluated using a set of 58 inbred lines and four hybrids. Thirteen sets of inbred parent-progeny triplet pedigrees together with four hybrids and their parental lines were used to quantify incidences of scoring that departed from expectations based upon simple Mendelian inheritance. Results were compared to those obtained using 80 restriction fragment length polymorphism (RFLP) probes. Over all inbred triplets, 2.2% of SSRs and 3.6% of RFLP loci resulted in profiles that were scored as having segregated in a non-Mendelian fashion. Polymorphic index content (PIC, a measure of discrimination ability) values ranged from 0.06 to 0.91 for SSRs and from 0.10 to 0.84 for RFLPs. Mean values for PIC for SSRs and RFLPs were similar, approximately 0.62. However, PIC values for nine SSRs exceeded the maximum PIC for RFLPs. Di-repeats gave the highest mean PIC scores for SSRs but this class of repeats can result in "stutter" bands that complicate accurate genotyping. Associations among inbreds were similar for SSR and RFLP data, closely approximating expectations from known pedigrees. SSR technology presents the potential advantages of reliability, reproducibility, discrimination, standardization and cost effectiveness over RFLPs. SSR profiles can be readily interpreted in terms of alleles at mapped loci across a broad range of maize germ plasm. Consequently, SSRs represent the optimum approach for the identification and pedigree validation of maize genotypes compared to other currently available methods.

Key words Simple sequence repeat · Microsatellite · SSRs · Maize · Variety identification

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Introduction

Microsatellites, or simple sequence repeats (SSRs) are short nucleotide sequences, usually from 2 to 3 bases(b) in length that are repeated in tandem arrays. Amplifiable polymorphisms are revealed because of differences in the numbers of tandem repeats that lie between sequences that are otherwise conserved for each locus. Microsatellite loci have proven to be highly polymorphic and useful as genetic markers in many plant species including *Arabidopsis* (Depeiges et al. 1995), bur oak (Dow et al. 1995), maize (Senior and Heun 1993), seashore paspalum (Liu et al. 1995), rapeseed (Kresovich et al. 1995; Charters et al. 1996), soybean (Akkaya et al. 1992, 1995; Rongwen et al. 1995), sugar beet (Mörchen et al. 1996), sweet potato (Jarret and Bowen 1994) and wheat (Plaschke et al. 1995; Roder et al. 1995).

In this paper, we report the usefulness of SSRs as genetic markers to discriminate between, and to show associations among, inbred lines of maize using a greater number of loci and a broader diversity of maize germ plasm than has been reported previously (Senior and Heun 1993).

Table 1 List and pedigree background of inbred lines used in the present SSR and RFLP profiling study

A632	Pedigree background[a]
A632	BSSS[b] C0 (94%), Minesota 13[c] (6%)
B73	BSSS[b] (100%)
Mo17	Lancaster Sure Crop[e] (50%), Krug[e] (50%)
PH207	Iodent[e] (59%), Long Ear[e] (20%), Minesota 13[e] (11%), Troyer Reid[e] (5%)
B64	BSSS[b] C0 (87.5%), Maiz Amargo[e] (12.5%)
PH595	Midland Yellow Dent[e] (25%), Southern U.S. Landrace Synthetic (19%), Funks G4949 (12.5%), Illinois Long Ear[e] (12.5%), Illinois Two Ear (12.5%)
PH642	BSSS[b] C0 (87.5%), Iodent[e] (9%)
PH814	Lancaster Low Breakage (25%), Southern U.S. Landrace Synthetic (19%), Osterland Yellow Dent[e] (16%), Funks G4949 (13%), Midland Yellow Dent[e] (6%), Tuson B[d] (6%), Brookings 86[e] (5%)
PH848	Minnesota 13[e] (12.5%), Osterland Yellow Dent[e] (12.5%), SRS303[ef] (12.5%), Iodent[e] (12%), Reid Yellow Dent[e] (12%), Lancaster Sure Crop[e] (6%), Longfellow Flint[e] (6%), MHW[d] (6%)
PHB09	BSSS[b] C0 (62.5%), Minnesota 13[e] (25%)
PHB46	BSSS[b] C0 (50%), Alberta Flint[e] (25%), Osterland Yellow Dent[e] (25%)
PHB47	BSSS[b] C0 (87.5%), Brookings 86[e] (12.5%)
PHB76	Smith TC[h] (25%), Midland Yellow Dent[e] (12.5%), NW Dent[i] (12.5%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[e] (8%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two Ear[e] (6%), Osterland Yellow Dent[e] (6%)
PHB89	Coker 616 (25%), Lancaster Sure Crop[e] (12.5%), Midland Yellow Dent[e] (12.5%), Southern U.S. Landrace Syndrace Synthetic (9%), Minesota 13[e] (8%), Funks G4949 (6%), Funks Yellow Dent[e] (6%), Illinois Long Ear[e] (6%), Illinois Two Ear (6%)
PHBE2	Iodent[e] (18%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[e] (9%), Osterland Yellow Dent[e] (6%), Midland Yellow Dent[e] (6%), Long Ear (6%), Funks G4949 (6%), Lancaster Low Breakage (5%)
PHBG4	Iodent[e] (27%), Minnesota 13[e] (11%), Long Ear (9%), Coker 616 (8%), Midland Yellow Dent[e] (6%), Lancaster Sure Crop[e] (6%), Southern U.S. Landrace Synthetic (6%)
PHG12	BSSS[b] C0 (37.5%), Lancaster Low Breakage (25%), M3204[j] (25%)
PHG29	Iodent[e] (59%), Long Ear (20%), Minnesota 13[e] (13%), Troyer Reid[e] (5%)
PHG31	Iodent[e] (44%), Long Ear (15%), Minnesota 13[e] (11%), Midland Yellow Dent[e] (6%), Southern U.S. Landrace Synthetic (5%)
PHG35	Iodent[e] (29%), Midland Yellow Dent[e] (13%), Minnesota 13[e] (11%), Southern U.S. Landrace Synthetic (9%), Long Ear (9%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two Ear (6%)
PHG39	BSSS[b] C0 (69%), Maiz Amargo[e] (25%)
PHG42	Iodent[e] (30%), Lancaster Low Breakage (10%), Southern U.S. Landrace Synthetic (9%), Osterland Yellow Dent[e] (9%), Minnesota 13[e] (7%), Funks G4949 (6%)
PHG45	Iodent[e] (59%), Long Ear (20%), Minnesota 13[e] (13%), Troyer Ried[e] (5%)
PHG50	Iodent[e] (35%), Long Ear (12%), Minnesota 13[e] (12%), Osterland Yellow Dent[e] (7%), SRS 303[e] (6%), Reid[e] (6%)
PHG53	BSSS[b] C0 (91%), Maiz Amargo[e] (6%)
PHG55	PROCOMP[k] (50%), Minnesota 13[e] (6%), Osterland Yellow Dent[e] (6%), SRS 303[e] (6%), Iodent[e] (6%), Reid[e] (6%)
PHG69	BASS[b] (50%), BSSS[b] (50%) BSSS[b] C0 (25%), Alberta Flint (13%), Osterland Yellow Dent[e] (13%)
PHG71	BSSS[1]C0 (47%), Iodent[e] (30%), Long Ear (10%), Minnesota 13[e] (9%)
PHG74	BSSS[b] C0 (89%), Minnesota 13[e] (5%)
PHG80	Dockendorf 101[e] (50%), BSSS[b] C0 (38%)
PHG81	BSSS[b] (50%), Iodent[e] (30%), Long Ear (10%), Minnesota 13[e] (6%)
PHG83	Iodent[e] (30%), Lancaster Low Breakage (13%), Long Ear (10%), Southern U.S. Landrace Synthetic (9%), Osterland Yellow Dent[e] (9%), Minnesota 13[e] (7%), Funks G 4949 (6%)
PHG84	Midland Yellow Dent[e] (13%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[e] (8%), Funks G4949 (6%), Illinois Low Ear (6%), Illinois Two Ear (6%), Qsterland Yellow Dent[e] (6%), SRS 303[j] (6%), Iodent[e] (6%), Reid[e] (6%)
PHG86	BSSS[b] (50%), BSSS[b] C0 (44%), Maiz Amargo[e] (6%)
PHJ76	BSSS[b] (50%), BSSS[b] C0 (38%)
PHK29	BSSS[b] C0 (63%), BSSS[b] (25%), Brookings 86[e] (6%)
PHK42	Iodent[e] (59%), Long Ear (20%), Minnesota 13[e] (13%), Troyer Reid[e] (5%)
PHMK0	BSSS[b] C0 (38%), Southern U.S. Landrace Synthetic (21%), BSSS[b] (13%), Dockendorf 101[e] (13%)
PHMM9	BSSS[b] C0 (53%), Dockendorf 101[e] (25%), Maiz Amargo[e] (13%)
PHN46	Southern U.S. Landrace Synthetic (12%), Iodent[e] (10%), Lancaster Low Breakage (9%), Osterland Yellow Dent[e] (9%), Funks G4949 (8%), Minnesota 13[e] (6%), Midland Yellow Dent[e] (6%)
PHN65	BSSS[b] (50%), Minesota 13[e] (6%), Osterland Yellow Dent[e] (6%), SRS 303[e] (6%), Iodent[e] (6%), Reid[e] (6%)
PHP38	BSSS[b] C0 (66%), Maiz Amargo[e] (13%), BSSS[b] (13%)
PHP85	BSSS[b] C0 (48%), BSSS[b] (38%), Maiz Amargo[e] (6%)
PHPE5	Iodent[e] (22%), Southern U.S. Landrace Synthetic (9%), Midland Yellow Dent[e] (9%), Minnesota 13[e] (8%), Long Ear (8%), Coker 616 (6%), Funks G4949 (6%), Illinois Long Ear (5%), Illinois Two Ear (5%)
PHR03	Iodent[e] (25%), Minnesota 13[e] (11%), Long Ear (8%), Southern U.S. Landrace Synthetic (6%), Midland Yellow Dent[e] (6%), Lancaster Sure Crop[e] (6%)
PHR63	Iodent[e] (29%), Coker 616 (13%), Minnesota 13[e] (10%), Long Ear (10%), Lancaster Sure Crop[e] (6%), Midland Yellow Dent[e] (6%), Southern U.S. Landrace Synthetic (5%)
PHR92	BSSS[b] C0 (69%), Maiz Amargo[e] (25%)
PHT11	BSSS[b] C0 (47%), BSSS[b] (25%), Maiz Amargo[e] (13%), Alberta Flint (6%), Osterland Yellow Dent[e] (6%)
PHT55	BSSS[b] C0 (69%), Maiz Amargo[e] (25%)
PHV25	Iodent[e] (30%), Midland Yellow Dent[e] (13%), Long Ear (10%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[e] (7%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two ear (6%)

Table 1 Continued

A632	Pedigree background[a]
PHV35	BSSS[b] (50%), BSSS[b] C0 (34%), Maiz Amargo[c] (13%)
PHV78	Iodent[c] (15%), Southern U.S. Landrace Synthetic (14%), Midland Yellow Dent[c] (13%), Funks G4949 (9%), Illinois Long Ear (6%), Illinois Two Ear (6%), Lancaster Low Breakage (6%), Long Ear (5%), Minnesota 13[c] (5%), Tuson B[d] (5%)
PHV94	BSSS[b] C0 (53%), Dockendorf 101[e] (25%), Maiz Amargo[c] (13%)
PHW52	BSSS[b] (50%), BSSS[b] C0 (34%), Maiz Amargo[c] (13%)
PHW53	Iodent[c] (21%), Osterland Yellow Dent[c] (11%), Minnesota 13[c] (10%), Long Ear (7%), Lancaster Low Breakage (6%), SRS 303[f] (6%), Reid[c] (6%), Southern U.S. Landrace Synthetic (5%)
PHWK9	Maiz Amargo[c] (50%), BSSS[b] C0 (50%)
PHZ38	BSSS[b] (50%), BSSS[b] C0 (41%)
PHZ51	Osterland Yellow Dent[c] (14%), Lancaster Low Breakage (13%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[c] (8%), Funks G4949 (6%), SRS 303[c] (6%), Iodent[c] (6%), Reid[c] (6%)

[a] Contributions of 5% or greater by pedigree are provided
[b] Iowa Stiff Stalk Synthetic
[c] Open-pollinated variety
[d] Derived from Tuson, an open-pollinated variety from the West Indies
[e] Population derived from Minnesota 13 open-pollinated variety
[f] Stiff Root and Stalk or Stalk Rot Synthetic selection from Krug
[g] Dawes open-pollinated variety from Nebraska most likely from Reid obtained from Mount Haleb, Wisconsin
[h] Smith top-cross derived from HAT0 fling synthetic
[i] Northwest Dent, open-pollinated variety once grown in northwest and north central U.S.
[j] Synthetic from Mississippi
[k] Composite of Southern U.S. prolific germplasm and Corn Belt lines made by W. L. Brown in the 1960's; known as "BS11" at Iowa State Univeristy
[l] Hybrid once sold by Dockendorf

Materials and methods

DNA was extracted from 58 maize inbred lines (Table 1) and from four maize hybrids (Pioneer hybrids 3183, 3377, 3732, and 3747). The 58 inbreds encompass a broad range of genetic diversity for Corn Belt materials, including pairs of lines that span pedigree relationships from unrelated to highly related. Among these inbred lines were 13 sets of triplets (a progeny line and both its parents) that provided opportunities for tests of inheritance and/or reliable band scoring. In addition, four hybrids were also profiled, providing additional opportunities to check the scoring and inheritance of polymorphisms. Initial DNA extractions were made using the CTAB procedure (Saghai-Maroof et al. 1984). Subsequent DNA extractions were performed using a proprietary method for which patent protection is being sought. Both methods provide DNA suitable for amplification by these SSRs and gave equivalent results. SSR loci were individually amplified using DNA of each inbred and hybrid using protocols described by Chin et al. (1996), except that fluorescent-labeled primers were used. Samples containing 0.5 µl of the PCR products, 0.5 µl of GENESCAN 500 internal lane standard labeled with N, N, N′, N′-tetramethyl-6-carboxyrhodamine (TAMARA) (Perkin Elmer-Applied Biosystems), and 50% formamide were heated at 92°C for 2 min, placed on ice, then loaded on 6% denaturing acrylamide gels. DNA samples were electrophoresed (29 W) for 7 h on an ABI Model 373A automative DNA sequencer/fragment analyzer equipped with GENESCAN 672 software v. 1.2 (Perkin Elmer-Applied Biosystems). DNA fragments were sized automatically using the "local Southern" sizing algorithm (Elder and Southern 1987). PCR products from individual samples were assigned to specific alleles at each locus based on "binning" of a range of sizes ($\pm$0.5 bp) as determined by ABI GeneScan™ and GENOTYPER™ software using the "local Southern" algorithm. Primer pairs for 200 potentially useful SSR loci were identified from the sequence data of maize that were published in Genbank, from di-repeat libraries made by Ben Burr (Brookhaven National Laboratory) and Lynn Senior (North Carolina State University), and from additional sequences available within Pioneer Hi-Bred International, Inc. An initial screen of nine inbred lines was used to evaluate utility (Chin et al. 1996). Sequence data for primers to amplify these SSRs are available via the electronic maize database (Maize DB, Polacco 1996). Attempts were made to profile all of the 58 inbred lines and four hybrids with these SSRs. It was possible to obtain profiles for all of the inbreds and hybrids included in this survey for 131 SSRs (see Table 2). Genomic locations for most SSRs are provided according to the nomenclature used in Coe (1996). Among this set of SSRs, 59 (45%) were di-repeats, 36 (27%) were tri-repeats, 21 (16%) were tetra-repeats, 7 (5%) were penta-repeats, 5 (4%) were hexa-repeats, 2 (2%) were septa-repeats, and 1 (1%) was an octa-repeat.

RFLP data were obtained by Linkage Genetics (Salt Lake City, Utah) using DNA extraction and other protocols described by Helentjaris et al. (1985). Eighty single-locus probes that collectively sampled every chromosome arm were used.

PIC values were calculated using the algorithm:

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} f_i^2$$

where f_i^2 is the frequency of the i^{th} allele.

PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies). For example, a marker locus that reveals five alleles, but where one allele is found in very high frequency (e.g., freq. = 0.9), has overall less discriminatory capability than a locus that also has five alleles, but in which those alleles are found in more equal frequencies.

Genetic distances between pairs of inbred lines from SSR and RFLP data were calculated from comparisons of the band scores using a modified Nei's distance (Nei and Li 1979). Pedigree distances between pairs of inbreds were calculated from 1-Malecot's Coefficient of relatedness (Malecot 1948). Associations among inbreds from SSR, RFLP and pedigree data were revealed using average linkage cluster analysis.

Results

SSRs that failed to amplify against the majority of inbreds or which gave amplified products that could not be clearly resolved were re-amplified and electrophoresed a second time. If results were still poor, then primers were re-designed (designated with '−2' following the SSR locus name) for further evaluation. If amplified products still failed to yield clearly scorable profiles for less than 95% of the inbred lines, then those SSRs were discarded from this study. This exercise resulted in scorable data being obtained for the 58 inbreds and four hybrids from 131 SSRs (Table 2). Primers with different sequences for loci already published (Coe 1996) may result in amplification products with different molecular weights from those obtained using the initial primer sequences.

Thirteen parent-progeny triplets were available for the examination of inheritance and scoring accuracy. For SSRs, non-Mendelian scores (where an amplified product was scored in a progeny inbred that had not been scored in one or both parental inbreds) ranged from 0 to 7 of the SSRs (0–5.3% of SSRs) per triplet. The mean was 2.85 incidences of non-Mendelian scoring (2.2% of all SSRs) per triplet. For RFLPs the range of non-Mendelian scores was from 0 to 7 RFLPs per triplet (0–8.8% of RFLPs per triplet). The mean for RFLPs was 2.85 (3.6% of RFLPs) incidences of non-Mendelian scoring per triplet.

Twenty five of the 131 SSRs were associated with one or more incidences of non-Mendelian scoring in the triplets. One SSR (bngl 619), a di-repeat, was so detected in four triplets; phi 011, a tri-repeat resulted in non-Mendelian scores for three triplets; six SSRs gave rise to non-Mendelian scores in each of two triplets; the remaining 17 SSRs that gave rise to non-Mendelian scores did so in only single triplets. Of all the SSRs implicated in non-Mendelian scoring, ten were di-repeats (16% of all di-repeats), eight were tri-repeats (24% of all tri-repeats), five were tetra-repeats (24% of all tetra-repeats), and two penta-repeats (33% of all penta-repeats).

Incidences of non-Mendelian scoring (absence of a parental band in a hybrid or presence of a non-parental band in a hybrid) expressed as a percentage of the 131 SSR loci for each hybrid were 3% for Pioneer brand hybrids 3183 and 3377 and 1.5% for Pioneer brand hybrids 3732 and 3747. The mean was 2.3% per triplet. Of the 12 instances of non-Mendelian scoring that were found, 11 were due to the absence of one of the inbred parental bands in the hybrid and one resulted from the presence of a band in the hybrid that was scored in neither parent.

PIC values for SSRs are presented in Table 3. PIC values for SSRs ranged from 0.06 to 0.91; the mean PIC for SSRs was 0.62. Summary data for numbers of bands and PIC values for each repeat class are presented in Table 4. Di-repeats gave high PIC values (0.70). Other frequently used classes (tri- and tetra-repeats) resulted in PIC values of 0.53 and 0.59, respectively.

Associations among inbreds on the basis of pedigree, RFLP and SSR data are presented in Figs. 1, 2 and 3, respectively. Associations of inbreds on the basis of pedigree (Fig. 1) were similar to that which could be expected on the basis of either marker method (Figs. 2 and 3). Very similar associations of inbreds were revealed from analyses of the RFLP and the SSR data (Figs. 2 and 3). The correlations of pairwise distances

Table 2 a SSR markers and map locations; primer sequences are given by Coe (1996)

SSR Locus	Genomic Location	SSR Locus	Genomic Location
phi056	1.01	bngl249	6.01
phi097	1.01	bngl107	6.02
bngl182	1.03	bngl480	6.03
bngl439	1.03	phi031	6.03
phi001	1.04	bngl176	6.04
bngl421	1.05	phi070	6.06
bngl615	1.07	phi025	6.07
bngl100	1.08	phi078	6.07
phi011	1.10	phi057	7.01
phi055	1.10	phi112	7.01
phi094	1.10	phi114	7.02
bngl504	1.11	bngl657	7.03
phi064	1.11	bngl434	7.03
bngl108	2.04	bngl155	7.04
bngl166	2.04	phi082	7.06
bngl420	2.04	bngl669	8.03
phi083	2.04	phi115	8.03
bngl602	3.04	phi119	8.03
nc030	3.04	bngl240	8.04
phi029	3.04	phi014	8.05
phi073	3.05	phi060	8.05
bngl197	3.07	phi015	8.08
phi072	4.01	phi080	8.08
phi021	4.02	phi017	9.02
bngl490	4.04	phi028	9.02
bngl667	4.04	phi033	9.02
bngl252	4.05	phi044	9.02
phi096	4.05	bngl127	9.03
phi092	4.08	bngl244	9.03
phi093	4.08	bngl430	9.03
bngl589	4.10	phi022	9.03
phi006	4.10	phi027	9.03
phi019	4.10	phi061	9.03
phi076	4.10	phi065	9.03
phi024	5.00	phi016	9.04
bngl143	5.01	phi042	9.04
bngl105	5.02	bngl128	9.07
phi113	5.02	bngl619	9.07
phi008	5.03	phi059	10.02
bngl653	5.04	phi063	10.02
bngl278	5.06	bngl640	10.03
bngl609	5.06	phi071	10.04
phi085	5.06	phi084	10.04
bngl386	5.09	bngl236	10.06
bngl238	6.00	bngl594	10.06
phi075	6.00		

Table 2 b SSR markers, their map locations (where known) and primer sequences (5′ → 3′); forward primers listed first, followed by the reverse primers. (SSR loci ending in ' – 2' were amplified using primer sequences different from those previously reported)

SSR locus	Genomic location	Primer sequence	
phi002	1.08	CATGCAATCAATAACGATGGCGAGT	TTAGCGTAACCCTTCTCCAGTCAGC
phi037	1.08	CCCAGCTCCTGTTGTCGGCTCAGAC	TCCAGATCCGCCGCACCTCACGTCA
phi038	1.08	TCAGACTCCGCCCAGCAATCATCTG	AGCCTAGTGCTTATCTTGAAGGCTT
phi039	1.08	ACCGTGTCTAATGTGTCCATACGG	CGTTAGGAGCTGGCTAGTCTCA
phi120	1.11	GACTCTCACGGCGAGGTATGA	TGATGTCCCAGCTCTGAACTGAC
bngl339	1S	CCAACCGTATCAGCATCAGC	GCAGAGCTCTCATCGTCTTCTT
phi098	2.02	GAGATCACCGGCTAGTTAGAGGA	GTATGGTTGGGTACCCGTCTTTCTA
bngl125-2	2.03	AAGCAGAGGCTGCTCTCACTGA	AAATCAATGGCAAGGGACCTCGTAG
bngl381-2	2.03	TGGCGGCCGCTCTAGTAACT	AGGGTTTCCATGGGCAGGTGT
nc003	2.06	ACCCTTGCCTTTACTGAAACAACAGG	GCACACCGTGTGGCTGGTTC
phi127-2	2.07	ATATGCATTGCCTGGAACTGGAAGGA	AATTCAAACACGCCTCCCGAGTGT
bngl198-2	2.08	CTGAAAAATAAAATCATGGTTTGTGCAAGTGTCA	ATGCACTGTGCACTGGCATTCACA
phi090	2.08	CTACCTATCCAAGCGATGGGGA	CGTGCAAATAATTCCCCGTGGGA
bngl371-2	2L	ATCTAATCGCAACGCGAAGCAGAGA	TATCGACCGTAGCTCCGACTGT
phi099	3.02	TACAAAAATCAGGACTGCGAAAAACCCAA	GTCGGTGTGTGATCCTTCCAC
phi053	3.05	CTGCCTCTCAGATTCAGAGATTGAC	AACCCAACGTACTCCGGCAG
phi046	3.08	GATCTTGCCCGGAACTCTGAC	ATCTCGCGAACGTGTGCAGATTC
phi047	3.09	GGAGATGCTCGCACTGTTCTC	CTCCACCCTCTTTGACATGGTATG
phi026	4.04	TAATTCCTCGCTCCCGGATTCAGC	GTGCATGAGGGAGCAGCAGGTAGTG
phi074	4.04	CCCAATTGCAACAACAATCCTTGGCA	GTGGCTCAGTGATGGCAGAAACT
phi079	4.04	TGGTGCTCGTTGCCAAATCTACGA	GCAGTGGTGGTTTCGAACAGACAA
phi066	4.08	CCATCCTTGAGGTGGTGTGAC	GAAGGAGCAGTAGCACTTGGTG
phi086	4.08	TACGTCGACGAGATCACTGGTC	CCACCATGATGCACCCACACT
bngl219	5.02	TGTTCCTGTACGGAGGCACTTCAA	TTCCAAGGTAATCCTCGCCTCAG
bngl557-2	5.02	TTCCTCCAAGGTCGCGTTTCAC	AGGAAAGGGATGGGAAGAACCGAA
bngl603-2	5.04	AGCTGGCCCCTGTGAATGGT	GCAACGTCCCTGGTTAGTTGAG
phi069	5.04	AGACACCGCCGTGGTCGTC	AGTCCGGCTCCACCTCCTTC
phi087	5.06	GAGAGGAGGTGTTGTTTGACACAC	ACAACCGGACAAGTCAGCAGATTG
phi101	5.06	TGTTCGCCGTCTAGCCTGGATT	TCATCAGCAACGACGACTACTCC
phi058	5.07	AGGTGCTGGACACAGACTTCAAC	ACTGAGATCCAGGCTCCTCTTC
phi128-2	5.07	TTGCYCGGTATGAAGAAAATAGTCTTTCC	ATCTTGCAACTAGACTGAGGCAACCA
bngl118-2	5.08	GCCTTCCAGCCGCAACCCT	CACTGCATGCAAAGGCAACCAAC
bngl389-2	5.08	CGTCGGCCAACAGGGTATC	CTCGCACGCGGTCTTCTTC
bngl150-2	5S	AGTAGAAAGAAAAACCCCCTCCCC	AAATCTGGGATCTCTGCCAATGGC
phi036	6.00	CCGTGGAGAGACGTTTGACGT	TCCATCACCACTCAGAATGTCAGTGA
phi077	6.01	GAGAAGAGGATCAGGTTCGTTCCA	CGCGTTGTACATCTTGCCTGCTT
phi126-2	6.01	TCCTGCTTATTGCTTTCGTCAT	GAGCTTGCATATTTCTTGTGGACA
bngl391-2	6.02	GATAGAACCAGATATCACAGCATCAGAAG	ACGCAGCTCTCCTTCGTTTGTTC
phi129	6.03	GTCGCCATACAAGCAGAAGTCCA	TCCAGGATGGGTGTCTCATAAAACTC
nc013	6.04	AATGGTTTTGAGGATGCAGCGTGG	CCCCGTGATTCCCTTCAACTTTC
phi102	6.05	TGAATCTAAACATAACTTATGTCTAGGTACATAGCAAA	CCTCGGATTCCGGATTGTAAGTCA
phi123-2	6.06	GGAGACGAGGTGCTACTTCTTCTTCAA	TGTGGCTGAGGCTAGGAATCTC
phi081	6.07	AAGGAACTGGTGAGAGGGTCCTT	AGCCCGATGCTCGCCATCTC
bngl161	6S	GCTTTCGTCATACACACACATTCA	ATGGAGCATGAGCTTGCATATTT
bngl147-2	7.01	TATGACCTTCTTTGGACGCTGACAC	ATTTGTTGTGCTAGCTTCGCCCAAG
phi034	7.02	TAGCGACAGGATGGCCTCTTCT	GGGGAGCACGCCTTCGTTCT
phi091	7.03	ATCTTGCTTCCATAAGATGCACTGCTCT	CTCAGCTTCGGTTCCTACACAGT
phi051	7.06	GGCGAAAGCGAACGACAACAATCTT	CGACATCGTCAGATTATATTGCAGACCA
phi116	7.06	GCATACGGCCATGGATGGATGGGA	TCCCTGCCGGGACTCCTG
phi049	7.07	GTNTGGCCATACCGTACTGCTTCT	TCCAGTTCTTCCGAAACGAAAGGG
phi125	8.03	ACCGCCGGTGCGAGTTGAAG	CTTGGGATTGCCCTCATCCAC
phi121	8.04	AGGAAAATGGAGCCGGTGAACCA	TTGGTCTGGACCAAGCACATACAC
bngl162	8.06	ACTAGCAGCAGTAAAACCTAATAAAGGGA	CAAGTAGCTAGCATGCATTTGCAGTGT
bngl666	8.06	AAAAGGCAAGTAGCTAGCATGCATTTGCAG	GGCTCACGTCCGTATCCAAACCAACA
phi067-2	9.01	CTGCAAAGGTAAGCACTAGGATGCT	CATCATTGATCCGGGTGTCGCTTT
phi068	9.01	GTACACACGCTCCGACGATTAC	TCTTCTCCACCAGAGCCTTGTAAG
phi043	9.02	AGCTGTACCGCTACATTTGCGATACCAA	TCACAGTCAGGCCGAACGCTTCGTAG
phi032	9.04	CTCCAGCAAGTGATGCGTGAC	GACACCCGGATCAATGATGGAAC
phi040	9.05	GGGATATATGTCCCCCACAATCGT	GGCCCTAAGCGAAAATCTATGCTGA
phi118-2	10.00	ATCGGATCGGCTGCCGTCAAA	AGACACGACGGTGTGTCCATC
phi041	10.00	TTGGCTCCCAGCGCCGCAAA	GATCCAGAGCGATTTGACGGCA
phi052	10.02	CAGAATGGGACGACAAGGTCATC	GGGACACTTCTAGCAGGATCTGTTT
bngl275	10.03	AGAAAAGAGAGTGTGCAATTGTGATAGAG	AATGGGTGCCTCGCACCAAG
phi050	10.03	TAACATGCCAGACACATACGGACAG	ATGGCTCTAGCGAAGCGTAGAG

Table 2 b Continued

SSR locus	Genomic location	Primer sequence	
phi054	10.04	AGAAAAGAGAGTGTGCAATTGTGATAGAG	AATGGGTGCCTCGCACCAAG
phi062	10.04	CCAACCCGCTAGGCTACTTCAA	ATGCCATGCGTTCGCTCTGTATC
6754/5		AAAAGGCCGTCAGAGCAGAACTGA	GTGACCGTGCCGTTGTATCACAA
7676/7		TGCTGCGCGCTCCACCAC	AGCTGCAGCTCGTCAATCAGG
7680/1		CTGGGCCACCAGCTTTGACC	CCGATGTGGGGTACGACCTC
7684/5		GCGGGCGACGCTTTCAAAC	CGCGGCCAAAACGCTTTCCC
7768/9		CCATGCCCATGGATGTTATTGCC	GCGGACCATGCATCCATCAAAC
10168/9		GAATTGGGAACCAGACCACCCAA	ATTTCCATGGACCATGCCTCGTG
bngl149		CATCCTCCAAAAGCACTACGT	CAGCTGTCCGACACTTATTCTTGTA
bngl468		AGGGTGTACAGGTCCAAGTCCAA	AATGTGGGTCGTCAGCCATCAG
phi111		ATCTCGCGAACGTGTGCAGATTCT	TCGATCTTTCCCGGAACTCTGAC

Table 3 Polymorphic index content (PIC), number of bands and repeat class for SSR markers

PIC	Marker	Number of bands	Repeat class
0.91	bngl619	11	2
	zct155	12	2
0.89	zca381	9	2
0.87	bngl176	10	2
	phi001	10	2
0.86	bngl105	8	2
0.85	bngl127	8	2
	bngl609	7	2
	phi026	9	2
0.84	bngl238	8	2
	phi015F	7	4
	phi119	5	2
0.83	zct161	8	2
	zct166	8	2
0.82	nc003	8	2
	phi064	7	4
	zca391_2	7	2
0.81	bngl128	7	2
	phi054	8	2
0.80	phi043	6	2
0.79	zag557	5	2
0.78	bngl615	7	2
	phi034	4	3
	phi079	3	5
0.77	zag249	6	2
	zca468	4	2
0.76	bngl182	5	2
	phi042	4	4
	phi127	4	4
	zct197	6	2
0.75	phi049	7	3
	phi083	4	4
	phi085	3	5
0.74	bngl421	6	2
0.74	bngl602	4	2
	phi037	5	2
	phi120	5	3
	zct118_2	5	3
0.73	bngl108	5	2
	zct434	6	2
0.72	phi021	4	2
	phi113	3	4
	zca150_2	3	2
0.71	bngl504	3	2
	phi056	5	3

Table 3 Continued

PIC	Marker	Number of bands	Repeat class
	phi061	3	8
	phi073	3	3
	zag240	4	2
	zcal147_2	3	2
0.70	bngl278	4	2
	phi075	3	2
	zct420_2	7	2
0.69	bngl653	3	2
	nc013	5	2
	phi047	4	3
	phi093	3	4
0.68	phi070a	4	5
0.67	_76623	2	2
	phi029	3	2
	phi116	5	7
0.66	bngl439	4	2
	phi036	4	2
	zct339	7	2
0.65	_67623	4	4
	bngl666	7	2
0.64	phi068	6	2
0.63	bngl1430	4	2
	bngl589	5	2
	phi078	2	4
0.62	_7676	2	3
	phi008	4	3
0.62	phi011	4	3
	phi025	2	2
	phi101	3	3
0.61	bngl162	4	2
	phi027	3	5
	phi046	3	4
	phi057	4	3
0.60	bngl657	5	2
	phi006	4	3
	phi031	4	4
	phi041	5	4
	phi091	3	5
	phi099	5	2
0.59	phi017	3	3
	phi019	2	3
	phi128_2	4	3
0.58	phi032	2	4
	phi076	3	6
	phi121	2	3
	phi123_2	2	4

Table 3 Continued

PIC	Marker	Number of bands	Repeat class
0.57	phi112	5	2
0.56	phi058	2	3
	phi129	5	4
0.55	phi024	2	3
0.54	bngl252	4	2
	phi069	4	3
	phi115	2	6
0.53	phi038	2	2
	phi050	3	4
	phi074	2	3
0.52	phi016	3	3
	phi066	2	3
	phi081	3	6
	phi084	2	3
0.51	phi071	2	3
	phi072	5	4
0.48	_76801	4	3
	phi062	2	3
0.47	phi118_2	4	3
0.46	phi102	2	2
	zag389	3	2
0.45	phi022	2	4
	phi055	3	3
	phi059	2	3
0.44	_76845	2	4
0.43	phi051	5	7
0.41	_101689	2	4
0.36	phi097	2	3
0.35	phi096	2	5
0.34	phi040	3	3
0.31	phi094	2	3
0.28	phi090	2	5
0.26	phi028	3	3
	phi060	3	6
0.24	phi082	4	2
0.23	phi033	4	3
0.20	phi014	2	3
0.18	phi052	3	6
0.09	phi098	3	2
0.06	phi044	2	4

Table 4 Information score summary statistics by repeat class

Repeat class	Band # mean	PIC mean	PIC standard error
2	5.56	0.70	0.02
3	3.19	0.53	0.02
4	3.57	0.59	0.04
5	2.86	0.57	0.07
6	2.80	0.41	0.08
7	5.00	0.54	0.12
8	3.00	0.70	

among all pairs of inbred lines for SSRs compared to RFLPs was $r = 0.85$. The correlation for pairwise distances between all pairs of inbreds for RFLPs compared to pedigree was $r = 0.80$; the correlation for SSRs with pedigree data was $r = 0.81$.

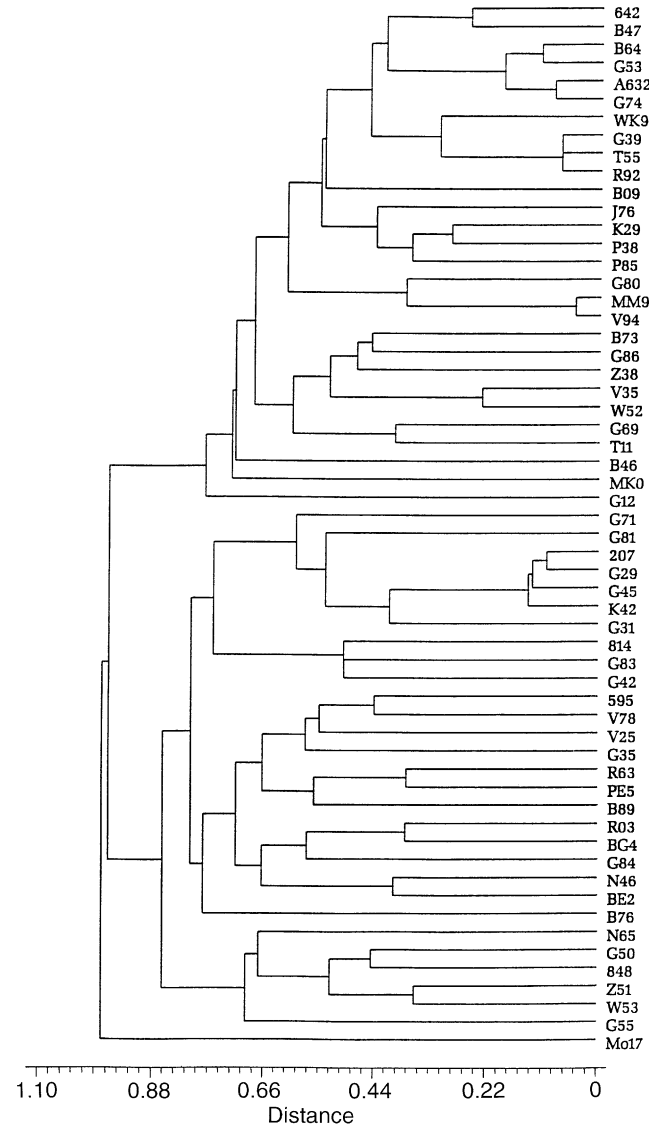

Fig. 1 Associations among maize inbred lines revealed by cluster analysis of pedigree distance data

Discussion

Scoring of data

Scoring of bands as being present in progeny that were not scored in either parent does not necessarily indicate non-Mendelian inheritance. Instead, bands could have been mis-scored and given the wrong allelic designation. The discriminative ability of gel-separation technology, effective use of molecular-weight-marker ladders, use of internal genomic-standard-check inbreds or hybrids, visual checking of scoring and manual-data entry are factors that all determine capabilities to score bands accurately and repeatedly. Artifactual "stutter" bands, that are especially prone to occur from di-repeat SSRs, can also cause incorrect genetic scoring

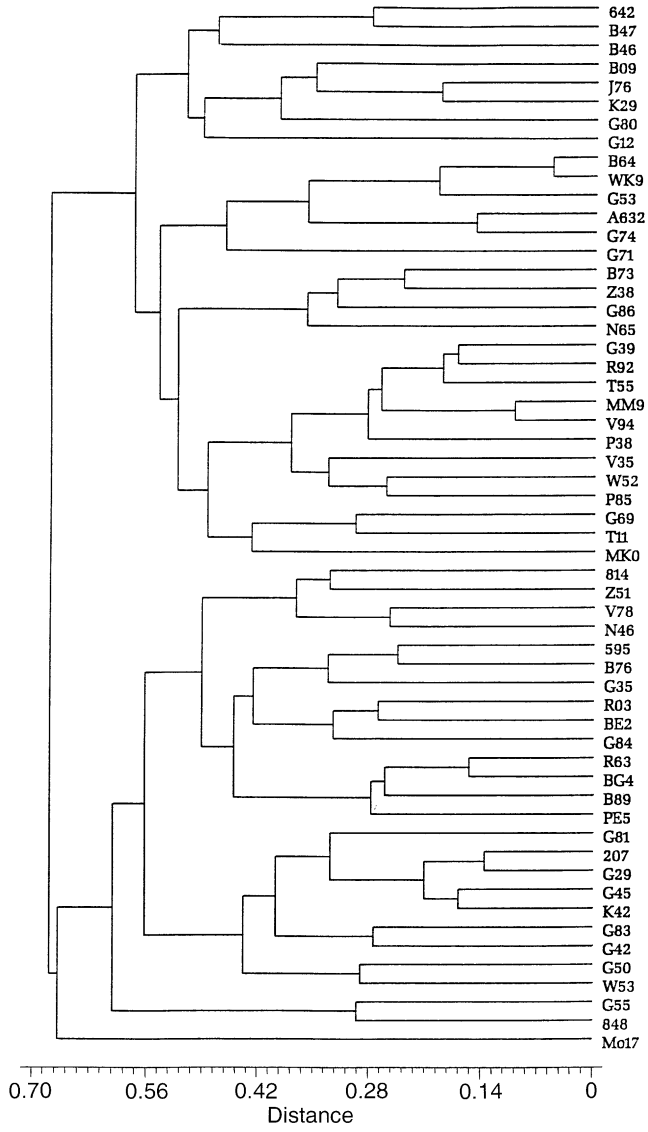

Fig. 2 Associations among maize inbred lines revealed by cluster analysis of RFLP distance data

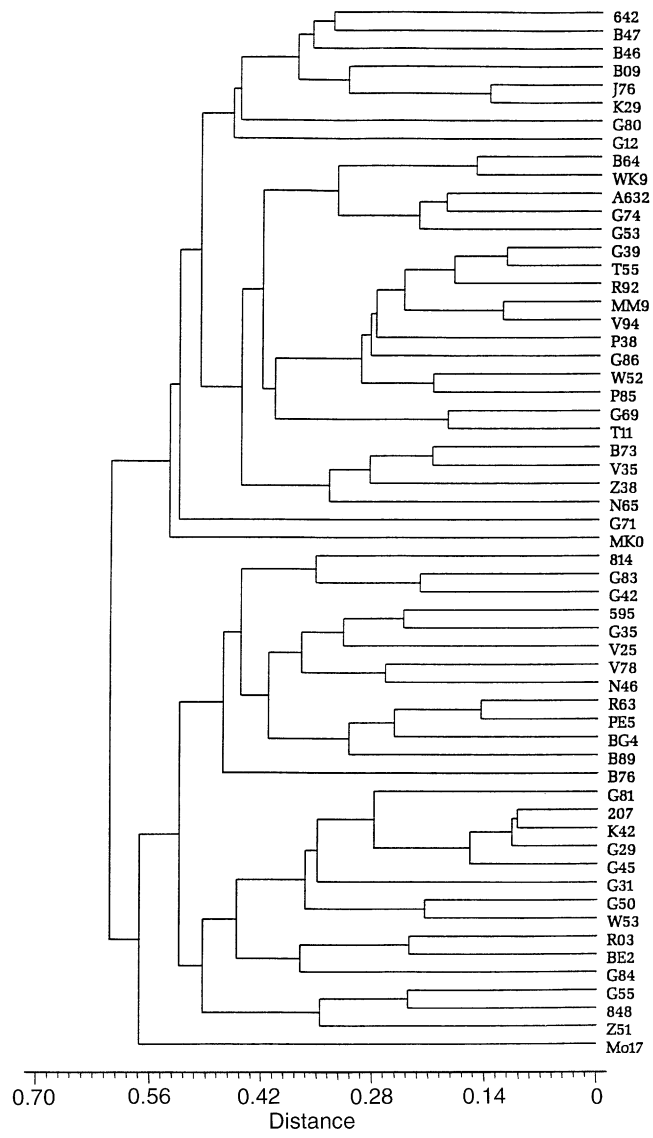

Fig. 3 Associations among maize inbred lines revealed by cluster analysis of SSR distance data

of bands, although Perlin et al. (1995) describe how 'stutter' bands can aid in automated genotyping. A second contributing factor could be residual heterozygosity remaining within an inbred at the time it was originally used to make the parental cross for subsequent progeny development by successive self-pollination and selection. Subsequent self-pollinations of the parent stock would then reduce or eliminate that heterozygosity so that later sources of the parental line (which would then be the representative sources of that line for profiling) would not carry all of the alleles that were still in a heterozygous condition when the line was used as a parent in a breeding cross. Third, the progeny line could have been contaminated by out-sourced pollen due to poor pollen control during its development (i.e., the pedigree could be incorrect). Fourth, a parental stock could have changed genetically after the time it was used to make the parental cross from which the progeny line was subsequently derived, either by mutation, contamination by an out-source of pollen, or by physical mixing of seed from another genotype. High mutation rates have been reported for microsatellites (Levinson and Gutman 1987; Jeffreys et al. 1988; Kelly et al. 1991; Wierdl et al. 1996).

Incidences of non-Mendelian scoring were identified in this study whenever: (1) the progeny line was scored with a band that was not scored in at least one of the parental lines, and (2) whenever the parental lines were scored as both having the same band, but that band was then not scored as being present in the progeny. Therefore, we did not consider instances as non-Mendelian scoring where a parental line was heterozygous

but the progeny did not receive both bands; those were more likely to represent occurrences of residual heterozygosity in the parent and, therefore, did not indicate any problems in either inheritance or in band scoring. As a result, we were unable to distinguish between incidences of non-Mendelian scoring that result from mutation, residual heterozygosity, outcrossing, and mis-scoring. We found no "null" alleles among the triplets, although "null" microsatellite alleles can occur (Callen et al. 1993). Additional study would be needed to determine precisely any level of true non-Mendelian inheritance for SSR data. However, because outcrossing, mislabelling, or physical mixing of seed for either parental or progeny lines would usually be expected to change alleles at many loci, and since no such gross differences in genotypes were identified, these potential causes of non-Mendelian scoring were not considered to contribute to the profiles obtained in this study. Nevertheless, incidences of non-Mendelian scoring, including instances of true non-Mendelian inheritance plus contributions of residual heterozygosity, outcrossing, and mis-scoring of bands, were very low for SSRs; lower than for RFLPs. Therefore, this study provides no evidence that previously reported hypervariability of SSRs (Levinson and Gutman 1987; Jeffreys et al. 1988; Kelly et al. 1991; Wierdl et al. 1996) will cause them to yield data that will be unreliable in characterizing maize inbred lines and hybrids, at least in respect of contemporary and parental germ plasm. A more thorough investigation of mutation rates will be necessary before SSR data can be used to provide reliable measures of phylogeny among germ plasms that are unrelated or very distantly related by pedigree (Nauta and Weissing 1996).

The incidences of non-Mendelian scoring among parent-progeny inbred lines were lower for this set of SSRs (2.2% of SSRs) than for RFLPs (3.6% of RFLPs). The incidence of non-Mendelian scoring for inbred parent-hybrid triplets was also low (2.3% of SSRs). The level of non-Mendelian scoring for SSRs can be improved. For example, the omission of eight SSRs that were involved in non-Mendelian scores for two or more of the triplets would then result in 1.3% of SSRs being associated with problematic scoring. It is advisable to eliminate markers that cannot be scored reliably as one of the activities that must be accomplished in the selection of a standard set of SSRs that can be used in the future. Similarly, many probe/restriction combinations that cannot reliably be scored have been omitted from standard profiling activities utilizing RFLPs.

SSR technology can be more reliable and repeatable than RFLP technology because the methodology that is available to separate amplified bands, to determine molecular weights, and to translate those molecular weights into discrete alleles is very precise and accurate (Schwengel et al. 1994; Mitchell et al. 1997). SSRs can be amplified under high stringency conditions, thereby reducing the chances that non-allelic bands will be amplified. SSRs can be separated on acrylamide sequencing gels in contrast to the less discriminative agarose gels that are used for RFLPs. SSRs can be co-electrophoresed with comprehensive molecular-weight standard ladders in each sample lane, whereas RFLP data are scored with the aid of comprehensive genomic ladders in flanking lanes and/or one or two co-migrating molecular-weight standards. Finally, available technology facilitates optical scoring of SSRs as an integral component of the electrophoretic procedure, thereby promoting the use of procedures that can further eliminate human error.

Discrimination ability

Mean PIC values for the SSRs and RFLPs used in this study were essentially identical. However, the maximum PIC value for SSR loci was 0.91 and the PIC values of nine (7%) SSR loci exceeded the maximum PIC value of 0.84 that was shown by RFLP probes. Consequently, a subset of these SSR loci will have a higher mean PIC value than would an equivalent number of RFLP loci.

Di-repeats gave the highest PIC values. However, di-repeats can present scoring problems because of a tendency to produce additional "stutter" bands. Most of the 69 SSRs that were not carried forward into this profiling set of 131 SSRs were di-repeats that presented this and other problems. However, within this set of 131 SSRs, incidences of non-Mendelian scoring, which would have been inflated by mis-scoring of stutter bands as alleles, were usually not apparent for SSRs that were di-repeats. An exception was bngl 619 that was scored in a non-Mendelian manner for 4 of 13 triplets.

The increase in PIC value shown by these SSRs over single-copy RFLPs for maize is much less than that shown by SSRs compared to single-copy RFLPs in soybean or wheat. That is because this class of RFLPs reveal many more polymorphisms among elite Corn Belt Dent and Flint germ plasm of *Zea mays* than is the case for *Glycine max*, *Triticum aestivum* and *Triticum durum* (Plaschke et al. 1995; Röder et al. 1995; Rongwen et al. 1995; Smith 1995). Consequently, SSRs do not provide the same degree of increase in discrimination power in maize as has been reported for soybean, wheat, or tomato. SSRs can, nonetheless, provide a useful increase in discriminatory power over RFLPs in maize.

Associations among inbred lines

Both SSR and RFLP data provide associations of inbred lines that largely concur with expectations based upon pedigree data. There is a major split between Stiff Stalk and non-Stiff Stalk pedigreed inbreds with

subdivisions that further break out very largely according to pedigree background (compare Fig. 1 with Figs. 2 and 3). However, within the Stiff Stalk lines both SSR and RFLP data cluster PHB46, PHB09, PHJ76, PHG12, PHK29 and PHG80 with PH001 and PHB47, groupings that do not concur precisely with expectations based upon pedigrees. Consequently, the data from SSRs provide further evidence that molecular markers, because they allow the genome to be physically tracked, are, therefore, able to show associations among inbreds that more realistically portray genetic relationships than can pedigree data. In contrast, associations based upon pedigree data are affected by the inaccuracies stemming from the underlying assumptions of no selection, no mutation, and a zero and equal relationship for inbreds that are not connected by pedigree breeding.

Both SSR and RFLP data show lines that are the most closely related by pedigree to be those that are also closely related on the basis of marker information. For example, the following pairs of lines are closely related by pedigree, and molecular data provide confirmation: B64, PHWK9, PHMM9-PHV94, PH207-PHG29 and PHJ76-PHK29. However, there are some differences in associations among inbreds according to whether SSR or RFLP data are used. For example, SSR data indicate that the Iodent lines PH207, PHK42 and PHG29 are more closely associated than are the Stiff Stalk lines B64 and PHWK9; RFLP data reveal the opposite. Other subtle differences between the associations shown by SSRs and those shown from RFLP data include: (1) SSRs indicate that PHK42 is more closely associated with PH207 than is PHG29, whereas RFLPs show PH207 and PHG29 to be closest; and (2) SSRs show that PHT55 is more closely associated with PHG39, whereas RFLPs indicate that PHG39 and PHR92 are closer. Pedigree data show each of these Stiff Stalk and Iodent lines to be approximately equally related and, therefore, are essentially moot on the subject.

The high degree of concordance shown for the associations of inbreds on the basis of these SSR data with the associations shown from both the analyses of RFLP and pedigree data indicates that this set of SSRs should provide adequate coverage of the genome for germ plasm identification and pedigree validation. Therefore, it should be possible to select a subset of these SSR loci and still retain fairly complete coverage of the genome. This would result in a subset of SSR loci with a higher mean PIC value than can be obtained from the profiling set of RFLP loci that was used in this study. As a result, it may not be necessary, at least for the purposes of inbred line identification and for many applications that require reliable measures of genetic distance, to have to identify numerous additional SSR loci for maize. Additional SSR loci will be required for more detailed mapping and more genetically precise marker-assisted selection of QTLs.

Conclusions

SSRs exceed the capabilities of RFLPs with regard to the characterization and identification of maize germ plasm for purposes of research, product development, conservation, the measurement and monitoring of genetic diversity in agriculture, and for the support of Intellectual Property Protection. SSRs can be more reliably and repeatedly scored than RFLPs, they can provide greater power of discrimination than RFLPs and can reveal associations among inbred lines that are reflective of pedigree. Further increase in efficiency can be effected through the simultaneous amplification, gel separation, and scoring of more individual SSR loci. These technical developments are underway (Mitchell et al. 1997) and should result in the provision of maize SSR profiling technology that will be faster, more standardizable, and more cost effective than RFLP technology.

SSRs reveal co-dominantly inherited multi-allelic products of loci that can be readily mapped. Therefore, SSR technology presents distinct advantages over most PCR methods that are based upon the amplification of arbitrary sequences, at least with respect to the identification of specific genotypes, because SSR profiles can be interpreted genetically without the need to repeatedly map amplified bands to marker loci in different populations. Commercial products are already available that are instrumental in helping to provide for the highly discriminative and reliable separation of polymorphisms, their scoring and databasing. As a result, it should be anticipated that SSR profiling will replace RFLPs and PCR-based arbitrary primer methods as the method of choice in the identification of maize inbred lines and hybrids for a multitude of applications in research, product development, support of IPP, and in the more effective conservation of maize genetic resources.

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References

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132:1131–1139

Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan PB (1995) Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci 35:1439–1445

Callen DF, Thompson AD, Shen Y, Phillips HA, Richards RI, Mulley JC, Sutherland GR (1993) Incidence and origin of "null" alleles in the (AC)n microsatellite markers. Am J Hum Genet 52:922–927

Charters YM, Robertson A, Wilkinson MJ, Ramsay G (1996) PCR analysis of oilseed rape cultivars (*Brassica napus* L. ssp. *oleifera*) using 5′-anchored simple sequence repeat (SSR) primers. Theor Appl Genet 92:442–447

Chin ECL, Senior ML, Shu H, Smith JSC (1996) Maize simple repetitive DNA sequences: abundance and allele variation. Genome 39 : 866–873

Coe E (1996) Combined table of SSR loci. Maize Genet Coop Newslett 70 : 112–117

Depeiges A, Goubely C, Lenoir A, Cocherel S, Picard G, Raynal M, Grellet F, Delseny M (1995) Identification of the most represented repeated motifs in *Arabidopsis thaliana* microsatellite loci. Theor Appl Genet 91 : 160–168

Dow BD, Ashley MV, Howe HF (1995) Characterization of highly variable GA/CT)n microsatellites in the bur oak, *Quercus macrocarpa*. Theor Appl Genet 91 : 137–141

Elder JK, Southern EM (1987) Computer-aided analysis of one-dimensional restriction fragment gels. Bishop MJ, Rawlings CJ (eds), Nucleic acid and protein sequence analysis – a practical approach, IRL, Press, Oxford, pp 165–172

Helentjars T, King G, Slocum M, Siedenstrang C, Wegman S (1985) Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied breeding. Plant Mol Biol 5 : 109–118

Jarret RL, Bowen N (1994) Simple sequence repeats (SSRs) for sweetpotato germ plasm characterization. Plant Genet Res Newslet 100 : 9–11

Jeffreys AJ, Royle NJ, Wilson V, Wong Z (1988) Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 322 : 278–281

Kelly R, Gibbs M, Collick A, Jeffreys AJ (1991) Spontaneous mutation at the hypervariable mouse ministellite locus Ms6-hm: flanking DNA sequence and analysis of germ line and early somatic events. Proc R Soc Lond B 245 : 235–245

Kresovich S, Szewc-McFadden AK, Blick SM, McFerson JR (1995) Abundance and characterization of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed). Theor Appl Genet 91 : 206–211

Levinson G, Gutman GA (1987) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol 4 : 203–224

Liu Z-W, Jarret RL, Kresovich S, Duncan RR (1995) Characterization and analysis of simple sequence repeat (SSR) loci in seashore paspalum (*Paspalum vaginatum* Swartz). Theor Appl Genet 91 : 47–52

Malécot G (1948) Les mathematiques de l'heredite. Masson and Cie, Paris

Mitchell SE, Kresovich S, Jester CA, Hernandez CJ, Szewc-McFadden AK (1997) Application of multiplex PCR and fluorescence-based, semi-automated allele sizing technology for genotyping plant genetic resources. Crop Sci 37 : 617–624

Mörchen M, Cuguen J, Michaelis G, Hanni C, Saumitou-Laprade P (1996) Abundance and length polymorphism of microsatellite repeats in *Beta vulgaris* L. Theor Appl Genet 92 : 326–333

Nauta MJ, Weissing FJ (1996) Constraints on allele size and microsatellite loci: implications for genetic differentiation studies 143 : 1021–1032

Nei M, Li W (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76 : 5269–5273

Perlin MW, Lancia G, See-Kiong Ng (1995) Toward fully automated genotyping: genotyping microsatellite markers by deconvolution. Am J Hum Genet 57 : 1199–1210

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91 : 1001–1007

Polacco M (1996) Maize geneome database. Maize Genet Coop Newslett 70 : 97

Röder MS, Plaschke J, Konig SU, Borner A, Sorrels ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246 : 327–333

Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90 : 43–48

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

Schwengel DA, Jedlicka AE, Nanthakumar EJ, Weber JL, Levitt RC (1994) Comparison of fluorescence-based semi-automated genotyping of multiple microsatellite loci with autoradiographic techniques. Genomics 22 : 46–54

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36 : 884–889

Smith JSC (1995) Identification of cultivated varieties by nucleotide analysis. In: Wrigley CW (ed) Identification of food-grain varieties. Am Assoc Cereal Chem, St. Paul, Minnesota pp 131–151

Wierdl M, Grene CN, Datta A, Jinks-Robertson S, Petes TD (1996) De-stabilization of simple repetitive DNA sequences by transcription in yeast. Genetics 143 : 713–721