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GENETIC DIVERSITY AMONG AND WITHIN MAIZE INBREDS AS REVEALED BY 100 SSR MARKERS

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SUMMARY

Many studies have reported genetic diversity and relatedness of maize inbred lines at the molecular level, but none was analyzed genetic variation among elite Croatian inbred lines. Hundred Simple Sequence Repeats (SSRs) based molecular markers have been used to analyze the genetic relationship among nine inbred lines, as well as within two sources of inbreds B73 and Mo17. Genetic dissimilarity ranged from 8 between two sources of Mo17 up to 92 between Mo17 and Os438-95. Mean observed heterozygosity values within samples were relatively low with 2.18% in average across all samples. Considerably higher heterozygosity level was found only in B73 from Agrogene source with 14%. Our results indicate that the relation among samples determined by SSR markers, as well as clustering of inbreds revealed by UPGMA method agreed with the pedigrees of these lines. If single seed source should be used, though, heterozygosity level of different sources should be taken into account to obtain more relevant data for maize breeders.

Key word: maize, inbred lines, genetic diversity, SSR markers

INTRODUCTION

A successful breeding program depends on the complete knowledge and understanding of the genetic diversity of the available germplasm. In maize, several descriptors have been used to characterize germplasm diversity including morphological traits, pedigree data (Duvick 1984; Darrah and Zuber, 1986), inter-inbred heterosis (e.g. Troyer et al., 1988), isozymes (Smith, 1984), zein chromatographic profiles (Smith, 1988), and DNA-based markers.

Although the concept of using genetic markers is not a new idea (Sax, 1923), fairly recent advancement in the development of DNA-based genetic markers now provide for marker saturation of nearly any experimental genome. Restriction fragment length polymorphism (RFLP), random amplified

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

SSRs (microsatellites) (Jacob et al., 1991) are loci which are comprised of highly variable arrays of tandemly repeated, 2 to 6 base pair (bp) long DNA sequences. Besides its high level of polymorphism, SSRs are useful molecular markers because they are abundant, uniformly distributed, codominant, rapidly produced by PCR, relatively simple to interpret and easily accessed by other laboratories via published primer sequences (Saghai-Maroof et al., 1994). Besides for measuring diversity, SSRs are very useful tool for assigning lines to heterotic groups and for genetic fingerprinting (Senior et al., 1998).

Many studies have reported genetic diversity and relatedness of maize inbred lines at the molecular level, but none was conducted to assess genetic variability among elite Croatian inbred lines. Moreover, only one study has assayed genetic divergence within identically named inbred lines maintained by different programs (Gethi et al., 2002). The objective of the study was to estimate the level of genetic diversity among nine inbred lines, as well as within inbreds B73 and Mo17 from two different sources by means of SSR markers.

MATERIALS AND METHODS

All inbred lines used in the present study trace back to the racial complex Corn Belt Dents (either from the population varieties Lancaster Sure Crop or Reid Yellow Dent), which presents the basic germplasm for virtually all of the corn produced in the USA as well as in most other temperate regions. They represent a diverse array of germplasm used in breeding programs at Agricultural institute Osijek (Table 1). Five of them (B73Os, Os84-44, Os438-95, B84Os, Os3-48) belong to the Reid Yellow Dent gene pool, while the other four (Mo17Os, Os6-2, Os135-88, Os163-9) are of Lancaster origin. The lines Os84-44 and Os163-9 came from original synthetic populations made at Agricultural institute Osijek (AIO) designated as Os Synth 32 (Vujević, 1987) and Os Helm. turc. Synth 1964 (Radić and Vekić, 1970), respectively. Additionally, inbreds B73 and Mo17 from Agrogene S.A. depot were added to estimate genetic diversity within these two lines from two different sources (Agrogene and AIO).

Total of eleven inbred samples were genotyped at Agrogene S.A., Moissy Cramayel Cedex, France. Hundred SSR primer pairs were chosen on the basis of their broad coverage of the genome (Table 2). The sequences of the primer pairs are available from the maize database project, Maize DB at the University of Missouri (http://www.agron.missouri.edu). Procedures of genomic DNA isolation as well as molecular marker assays can be obtained by Agrogene, S.A. (http://www.agrogene.com).

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

Tablica 1. Inbred linije kukuruza i podaci o izvoru sjemena i pedigreu za analizu genetske različitosti između i unutar inbred linija pomoću SSR markera

Inbred	Source†	Genetic background	Pool	Developed by†
B73	Agrogene	Iowa SSS C5	Reid-BSSS	ISU
B73Os	AIO	Iowa SSS C5	Reid-BSSS	ISU
Mo17	Agrogene	(187-2×C103)	Lancaster	UMC
Mo17Os	AIO	(187-2×C103)	Lancaster	UMC
Os84-44	AIO	Os Synth 32	Reid-BSSS	AIO
Os438-95	AIO	(B73×HARK46)	Reid-BSSS	AIO
B84Os	AIO	BS13(S2)C0	Reid-BSSS	ISU
Os6-2	AIO	Lancaster Sure Crop	Lancaster	AIO
Os135-88	AIO	(Mo17×Polj17)	Lancaster	AIO
Os163-9	AIO	Os Helm. turc. Synth 1964	Lancaster	AIO
Os3-48	AIO	Iodent	Reid-Iodent	AIO

† AIO – Agricultural institute Osijek, UMC – University of Missouri Columbia, ISU – Iowa State University

Genetic similarities (GS) coefficients were calculated from SSR data for all possible pairs of inbreds (Nei and Li, 1979), and converted into genetic distance (dissimilarity) (GD): GD = 1-GS. Genotypes were clustered based on the matrix of genetic dissimilarities using the unweighted pair-group average (UPGMA) clustering algorithm performing by STATISTICA program package (StatSoft Inc. 1984-2000).

RESULTS AND DISCUSSION

The 100 SSR primers have been mapped to regions that were dispersed throughout the maize genome (Table 2). Bins are named by the chromosome number, followed by a decimal, and a numeric identifier, e.g. 1.00 is the most distal (left or top-most) bin on the short arm of chromosome. Bin boundaries are defined by a set of Core Markers (for deitails see at http://www.agron.missouri.edu). SSR primers produced total of 396 alleles among eleven maize inbred samples (i.e. mean number of alleles per locus is 3.96). The number of alleles per locus ranged from 1 to 11. Mean observed heterozygosity values within samples were relatively low, but considerably higher in B73 from Agrogene source (data not shown). In average, heterozygosity level was 2.18%, ranging from 0% in Os135-88 and Os438-95 up to 14% in B73.

Table 2. Allele numbers, size range (in base pairs) for SSR markers found in 11 genotypes

Tablica 2. Broj alela i raspon veličine (u parovima baza) za SSR markere nađene u 11 genotipova

Marker	Locus	No. of alleles	Size range (bp)	Bin number
1	bnlg149	4	150 - 177	1.0
2	bnlg1014	4	145 - 161	1.01
3	bnlg1429	3	182 - 201	1.02
4	bnlg1866	3	109 - 123	1.03
5	phi109275	4	125 - 143	1.03
6	bnlg2238	4	174 - 205	1.04
7	bnlg2086	11	231 - 238	1.04
8	bnlg615	5	185 - 223	1.07
9	phi037	4	128 - 152	1.08
10	bnlg1720	3	232 - 245	1.09-1.10
11	phi064	4	71 - 95	1.11
12	phi120	2	62 - 65	1.11
13	phi96100	5	265 - 293	2.01
14	bnlg1302	3	135 - 146	2.02
15	phi083	3	128 - 134	2.04
16	bnlg1831	4	174 - 192	2.06
17	bnlg1138	3	176 - 191	2.06
18	bnlg1329	5	82 - 98	2.08
19	bnlg1662	5	122 - 165	2.08
20	bnlg1940	8	200 - 244	2.08
21	bnlg1520	3	171 - 197	2.09
22	phi099	3	141 - 145	3.04
23	bnlg1325	6	157 - 193	3.03
24	bnlg1523	4	178 - 239	3.03
25	bnlg1904	4	164 - 185	3.04
26	bnlg2047	3	134 - 145	3.04
27	bnlg1456	3	180 - 187	3.05
28	phi053	3	173 - 199	3.05
29	bnlg1449	3	86 - 152	3.06
30	bnlg1605	2	100 - 118	3.07
31	bnlg1931	2	162 - 166	3.07
32	bnlg1108	2	111 - 135	3.08
33	bnlg1182	4	71 - 97	3.09
34	bnlg1257	3	170 - 219	3.09

Table 2. Allele numbers, size range (in base pairs) for SSR markers found in 11 genotypes (cont'd)

Tablica 2. Broj alela i raspon veličine (u parovima baza) za SSR markere nađene u 11 genotipova (nastavak)

Marker	Locus	No. of alleles	Size range (bp)	Bin number
35	phi072	3	139 - 160	4.01
36	nc004	3	137 - 175	4.02
37	phi021	3	84 - 120	4.02
38	bnlg1265	5	205 - 238	4.05
39	phi096	2	236 - 238	4.05
40	umc2027	3	103 - 113	4.06
41	bnlg1189	5	118 - 218	4.07
42	bnlg1784	5	231 - 245	4.07
43	dup28	7	111 - 137	4.08
44	phi093	3	281 - 287	4.08
45	bnlg1917	5	99 - 152	4.10
46	bnlg1890	5	124 - 179	4.11
47	bnlg589	5	148 - 222	4.10
48	phi024	2	160 - 166	5.01
49	bnlg105	5	62 - 96	5.02
50	bnlg1046	6	172 - 207	5.03
51	bnlg1208	2	102 - 118	5.04
52	bnlg1287	3	146 - 150	5.04
53	bnlg1237	2	150 - 152	5.05
54	phi087	4	146 - 170	5.06
55	bnlg118	4	100 - 120	5.08
56	phi128	3	102 - 112	5.07
57	phi126	7	142 - 177	6.00
58	bnlg1538	4	204 - 231	6.01
59	bnlg426	3	104 - 116	6.01
60	bnlg1371	3	113 - 123	6.01
61	umc1887	2	89 - 95	6.03
62	phi031	3	184 - 219	6.04
63	bnlg1732	4	95 - 107	6.03
64	phi078	3	119 - 163	6.07
65	bnlg1740	4	111 - 167	6.07
66	phi089	2	81 - 82	6.08
67	umc1545	4	75 - 88	7.00

Table 2. Allele numbers, size range (in base pairs) for SSR markers found in 11 genotypes (cont'd)

Tablica 2. Broj alela i raspon veličine (u parovima baza) za SSR markere nađene u 11 genotipova (nastavak)

Marker	Locus	No. of alleles	Size range (bp)	Bin number
68	bnlg1094	4	161 - 201	7.02
69	bnlg1808	5	120 - 140	7.02
70	bnlg1070	6	211 - 246	7.03
71	bnlg572	2	94 - 110	7.03
72	bnlg2259	4	165 - 195	7.04
73	phi051	2	135 - 139	7.05
74	phi116	3	159 - 169	7.06
75	bnlg1194	4	133 - 197	8.02
76	phi119	4	159 - 167	8.02
77	bnlg1834	5	190 - 206	8.03
78	bnlg1176	5	186 - 246	8.05
79	bnlg1782	6	217 - 266	8.05
80	phi10075	4	136 - 146	8.06
81	phi015	4	88 - 106	8.08
82	bnlg1131	6	100 - 118	8.09
83	bnlg2122	3	230 - 246	9.01
84	phi033	1	248 - 248	9.01
85	bnlg244	6	127 - 182	9.02
86	phi017	2	96 - 101	9.02
87	phi061	2	74 - 83	9.03
88	phi065	4	129 - 149	9.03
89	bnlg1209	3	169 - 175	9.04
90	bnlg1129	5	180 - 197	9.08
91	bnlg128	4	174 - 203	9.07
92	umc1675	3	157 - 163	9.07
93	bnlg619	7	226 - 266	9.07
94	umc1152	4	161 - 176	10.02
95	phi050	2	76 - 83	10.03
96	bnlg1074	6	154 - 177	10.05
97	bnlg1526	2	106 - 116	10.03
98	bnlg1028	2	149 - 152	10.06
99	bnlg1360	5	119 - 140	10.07
100	bnlg1839	3	176 - 188	10.07

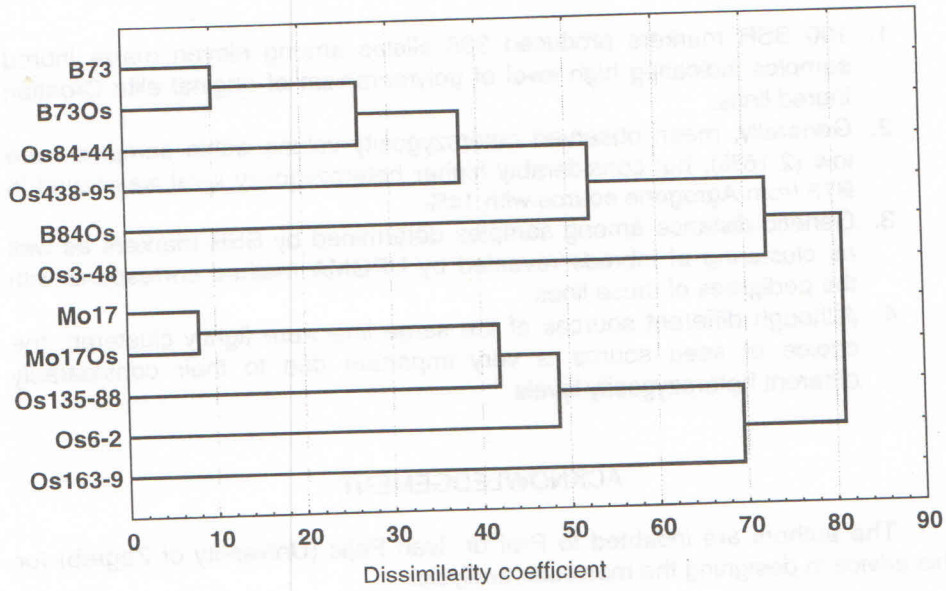
Table 3. Genetic distance among 11 maize genotypes based on SSR data

Tablica 3. Genetska udaljenost između 11 genotipova kukuruza na osnovi SSR podataka

Genotype	B73	B73Os	Mo17	Mo17Os	Os84-44	Os438-95	B84Os	Os6-2	Os135-88	Os163-9
B73										
B73Os	10									
Mo17	88	90								
Mo17Os	88	89	8							
Os84-44	30	23	84	85						
Os438-95	38	32	92	91	44					
B84Os	49	47	81	84	58	56				
Os6-2	83	83	43	46	81	86	79			
Os135-88	79	81	43	41	79	85	77	57		
Os163-9	74	73	69	69	79	76	78	72	68	
Os3-48	72	74	74	73	74	74	68	72	73	71

Figure 1. UPGMA dendrogram for eleven genotypes (inbreds B73 and Mo17 are in duplicate due to different seed sources) determined on the basis of genetic dissimilarity by means of 100 SSR markers.

Slika 1. UPGMA dendrogram za jedanaest genotipova (linije B73 i Mo17 su iz dvostrukog izvora sjemena) izračunatih na osnovi genetske udaljenosti pomoću 100 SSR markera.



Genetic dissimilarity calculated for 55 combinations of 11 samples ranged from a low of 8 between the pair Mo17 vs. Mo17Os, and 10 between B73 vs. B3Os up to 91 between the pairs Os438-95 vs. Mo17Os and Os438-95 vs. Mo17Os (Table 3). This result indicates that the relation among samples determined by SSR markers correspond with pedigree data.

UPGMA ordered the inbreds in two broad groups (Figure 1). One group consisted of inbreds from Reid Yellow Dent, and the other included all Lancaster inbred lines. As expected, the Iodent line OS3-48 was clearly separated from other Reid lines. The same is true for the line Os163-9 in the Lancaster group. Within the groups, different sources of the same line were tightly clustered. Grouping of inbreds revealed by the present analysis generally agreed with the pedigrees of these lines and clusters were representative of heterotic groups.

This study showed that different seed sources of the same inbred did not vary considerably. However, Gethi et al., (2002) pointed out that the low level of heterozygosity is necessary if single seed source should be used. Heterozygosity of B73 from Agrogene with 14% is much higher than suggested by Gethi et al., (2002). They proposed if an inbred was obtained from a single source, a heterozygosity level should be at most 4.6%. Therefore, establishing the level of homozygosity in seed is very important because of usefulness of data to other breeders using same designated materials.

CONCLUSIONS

1. 100 SSR markers produced 396 alleles among eleven maize inbred samples indicating high level of polymorphism of original elite Croatian inbred lines.
2. Generally, mean observed heterozygosity values within samples were low (2.18%), but considerably higher heterozygosity level was found in B73 from Agrogene source with 14%.
3. Genetic distance among samples determined by SSR markers as well as clustering of inbreds revealed by UPGMA method correspond with the pedigrees of these lines.
4. Although different sources of the same line were tightly clustered, the choice of seed source is very important due to their considerably different heterozygosity levels.

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GENETSKA RAZLIČITOST IZMEĐU I UNUTAR INBRED LINIJA KUKURUZA OTKRIVENA SA 100 SSR MARKERA

SAŽETAK

U mnogim je istraživanjima proučavana genetska različitost i srodstvo inbred linija kukuruza na molekularnoj razini, ali niti u jednome radu dosada nije analizirana genetska varijabilnost između elitnih hrvatskih inbred linija. Stotinu molekularnih markera na osnovi tehnike Simple Sequence Repeats (SSR) korišteni su za analizu genetskih suodnosa između devet inbred linija, ali i između dva izvora sjemena linija B73 i Mo17. Genetska različitost bila je od 8 za razliku između dva izvora sjemena linije Mo17, do 92 za razliku između Mo17 i linije Os438-95. Srednja vrijednost udjela heterozigotnosti unutar uzoraka linija bila je relativno niska i iznosila je 2.18% u prosjeku svih uzoraka. Značajno viši stupanj od 14% heterozigotnosti nađen je kod linije B73 iz partije sjemena tvrtke Agrogene (Francuska). Naši rezultati ukazuju da genetske udaljenosti između uzoraka inbred linija kukuruza utvrđene SSR markerima, kao i odgovarajuće grupiranje linija pomoću UPGMA metode odgovaraju pedigreima proučavanih linija. Međutim ako se u istraživanjima koristi samo jedan izvor sjemena inbred linija, stupanj heterozigotnosti se mora uzeti u obzir kako bi se dobili relevantniji podaci za oplemenjivače kukuruza.

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