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Genetic diversity among maize (*Zea mays*, L.) inbred lines in Eastern Croatia

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Abbreviations:
SSR – Simple Sequence Repeats
MRD – Modified Rogers' distance
UPGMA – Unweighted Paired Group Method using Arithmetic Averages

Key words: Maize, Zea mays, L., genetic diversity, SSRs, Eastern Croatia

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Abstract

Background and Purpose: *Assessment of the genetic diversity among inbred lines is important for hybrid maize (Zea mays, L.) breeding. There is no published study reporting genetic diversity and relatedness of maize inbred lines at molecular level among elite inbred lines in major maize production area of Eastern Croatia. The objective of the study was to assess genetic diversity among the inbred lines relevant for maize breeding in Eastern Croatia using simple sequence repeat (SSR) markers.*

Materials and Methods: *SSR analysis of 98 markers distributed uniformly throughout the maize genome was performed for two well-known inbred lines B73 and Mo17, as well as for 13 lines developed at Osijek Agricultural Institute. These lines represent the basic germplasm for maize in Eastern Croatia, which consists of four family groups according to pedigree data.*

Results and Conclusions: *An average of 4.2 alleles per marker was observed with a range from 2 to 8. Expected heterozygosity (allelic diversity) varied from 0.13 to 0.86, with an average of 0.65. Cluster analysis revealed the expected structure within the germplasm, distinguishing conclusively the inbred lines according to their background in two population varieties of Reid Yellow Dent and Lancaster Sure Crop and four subsequent maize families. Inbred lines of Osijek Agricultural Institute were confirmed to be genetically diverse representing a large proportion of the genetic variation occurring in four groups of maize inbred families. This seems to be promising for success in further developments of maize breeding programs.*

INTRODUCTION

Maize (*Zea mays*, L.) exhibits a wide range of shapes and forms, which resulted in several attempts to classify its genetic resources (germplasm). Although not easily understood and not completely refined, a classification using the concept of maize races developed by Anderson and Cutler *(1)* is still in use *(2)*. Over 280 races of maize have been described so far *(3)* yet, for maize breeding programs in temperate areas, the races *per se* have been used sparingly. Currently, the majority of commercial US maize hybrids traces back to two open-pollinated cultivars Reid Yellow Dent and Lancaster Sure Crop, which are complementary heterotic groups of only one maize race (Corn Belt Dent).

Lipman *et al. (4)* compiled six categories of maize genetic resources (or accession types) in order to define the type of germplasm with regard to breeding: inbred lines, landraces, advanced cultivars (open-pol-

TABLE 1

Allele number (A) and Nei's expected heterozygosity (He) of the 98 SSR loci studied.

No.	SSR locus	A	He	No.	SSR locus	A	He
1	bnlg149	6	0.793	50	bnlg1287	3	0.681
2	bnlg1014	6	0.775	51	bnlg1237	3	0.248
3	bnlg1429	4	0.706	52	phi087	3	0.579
4	bnlg1866	3	0.662	53	bnlg118	4	0.579
5	phi109275	6	0.780	54	phi128	2	0.330
6	bnlg2238	4	0.671	55	phi126	8	0.853
7	bnlg2086	3	0.577	56	bnlg1538	3	0.543
8	bnlg615	4	0.754	57	bnlg426	4	0.717
9	phi037	4	0.720	58	bnlg1371	5	0.747
10	bnlg1720	4	0.670	59	umc1887	3	0.618
11	phi064	4	0.696	60	phi031	2	0.287
12	phi120	3	0.627	61	bnlg1732	5	0.677
13	phi96100	5	0.664	62	phi078	5	0.708
14	bnlg1302	2	0.464	63	bnlg1740	4	0.641
15	phi083	2	0.515	64	phi089	2	0.460
16	bnlg1831	5	0.763	65	umc1545	2	0.239
17	bnlg1138	5	0.641	66	bnlg1094	4	0.724
18	bnlg1329	6	0.726	67	bnlg1808	8	0.864
19	bnlg1662	4	0.614	68	bnlg1070	6	0.794
20	bnlg1940	8	0.858	69	bnlg572	3	0.600
21	bnlg1520	3	0.508	70	bnlg2259	3	0.600
22	phi099	3	0.349	71	phi051	3	0.653
23	bnlg1325	7	0.864	72	phi116	2	0.331
24	bnlg1523	4	0.644	73	bnlg1194	5	0.545
25	bnlg1904	5	0.763	74	phi119	5	0.639
26	bnlg2047	3	0.432	75	bnlg1834	6	0.683
27	bnlg1456	3	0.662	76	bnlg1176	5	0.745
28	phi053	3	0.545	77	bnlg1782	6	0.660
29	bnlg1449	3	0.540	78	phi100175	3	0.660
30	bnlg1605	2	0.331	79	phi015	4	0.653
31	bnlg1931	3	0.653	80	bnlg1131	4	0.756
32	bnlg1108	3	0.685	81	bnlg2122	4	0.734
33	bnlg1182	6	0.795	82	phi033	2	0.460
34	bnlg1257	6	0.812	83	bnlg244	5	0.775
35	nc004	2	0.515	84	phi017	3	0.662
36	phi021	2	0.515	85	phi061	2	0.129
37	bnlg1265	6	0.777	86	phi065	3	0.600
38	umc2027	3	0.681	87	bnlg1209	2	0.369
39	bnlg1189	5	0.701	88	bnlg1129	4	0.644
40	bnlg1784	4	0.708	89	bnlg128	6	0.834
41	dup28	6	0.736	90	umc1675	2	0.435
42	phi093	2	0.481	91	bnlg619	6	0.619
43	bnlg1917	5	0.536	92	umc1152	3	0.577
44	bnlg1890	4	0.490	93	phi050	2	0.508
45	bnlg589	5	0.745	94	bnlg1074	3	0.561
46	phi024	2	0.515	95	bnlg1526	2	0.409
47	bnlg105	6	0.675	96	bnlg1028	3	0.255
48	bnlg1046	7	0.788	97	bnlg1360	6	0.828
49	bnlg1208	4	0.513	98	bnlg1839	5	0.798

linated varieties), composites, synthetics, and wild or related species. Inbred lines as parental components for hybridization *(5)* are the most valuable source in maize genetics and breeding. They have been crucial for diverse genetic studies including the development of linkage maps *(6),* quantitative trait locus mapping *(7),* molecular evolution *(8)* and developmental genetics *(9, 10)*.

Microsatellites or simple sequence repeats (SSRs) *(11)* composed of a DNA sequence motif of 2–6 bases in length that is repeated tandemly usually five or more times are markers that have become widely used in genetic studies in maize. 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 *(12)*. Besides for measuring diversity, SSRs are a very useful tool for assigning lines to heterotic groups and for genetic fingerprinting *(13)*.

Many studies have reported genetic diversity and relatedness of maize inbred lines at molecular level, but none was conducted to assess genetic variability among elite inbred lines in major maize production area of Croatia. Like in U.S. Corn Belt, the majority of maize breeding material in Eastern Croatia traces back to the maize race of Corn Belt Dent, although there was a wide range of maize genetic sources described and used in greater Pannonian plain *(14, 15, 16)*. The objective of the study was to assess the genetic diversity among inbred lines relevant for maize breeding in Eastern Croatia using SSR markers.

MATERIAL AND METHODS

All inbred lines used in the present study trace back to the racial complex Corn Belt Dents either from the population varieties Reid Yellow Dent or Lancaster Sure Crop, which present the basic germplasm for maize in Croatia. They also represent a various array of germplasm used in breeding programs at Osijek Agricultural Institute. As part of a greater investigation, we analyzed a total of 15 maize inbred lines consisting of four groups according to pedigree data. The grouping was made according to families within the two populations of Reid Yellow Dent and Lancaster Sure Crop. According to the MBS Genetics Handbook *(17),* an inbred belongs to a family (or subfamily) if that (sub)family contributes a significant portion to the inbred's derivation. The most important Lancaster families are Mo17 and Oh43 (Ohio), named after well-known public inbreds. Thus, Group 1 and Group 2 comprised eight inbred lines including original Mo17, L1, L2, L3 of the Group 1 (Mo17 Group), and O1, O2, O3 O4 lines of the Group 2 (Ohio group). Group 3 and Group 4 comprised seven inbred lines including original B73 and B1 (B73 group) belonging to Iowa Stiff Stalk Synthetic (BSSS) and five lines I1, I2, I3, I4 and I5 belonging to Iodent group, a strain of Reid Yellow Dent. Mikel and Dudley *(18)* described a detailed evolution of North American dents utilizing pedigree in-

formation for a greater understanding of relations among maize germplasm. With the exception of the two best known public inbred lines Mo17 and B73, all other lines in this study were derived and developed at Osijek Agricultural Institute.

Fifteen inbred lines are genotyped at Eurofins/Agrogene laboratories, France utilizing the technology of capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer equipped with GeneScan software. Hundred SSRs had been selected based on their broad coverage of the maize genome, robust single-locus amplification, high degree of polymorphism and high reproducibility of results. A list of 100 SSR loci with their chromosomal locations has been presented *(19)*. The sequences of the primer pairs are available from the MaizeGDB database (http://www.maizedgdb.org). Eventually, 98 SSR loci were chosen for this study (Figure 1) since two loci were monomorphic.

For each SSR locus, we calculated the number of alleles (A) and expected heterozygosity (He) on the basis of Nei's unbiased estimate *(20)* as a measure of allelic diversity at a locus. The term »heterozygosity«is somewhat misleading in this case because all analyzed individuals were homozygotes. It is directly comparable to the polymorphic information content (PIC) *(21),* or even referred to as PIC *(22)*. We use He instead of PIC because it is more widely used in the literature *(23)*

We calculated the modified Roger's distance (MRD) between two inbred lines *(24)*. Average linkage (UPGMA, Unweighted Paired Group Method using Arithmetic Averages) clustering was calculated based on MRD estimates between pairs of inbred lines. The cluster analysis

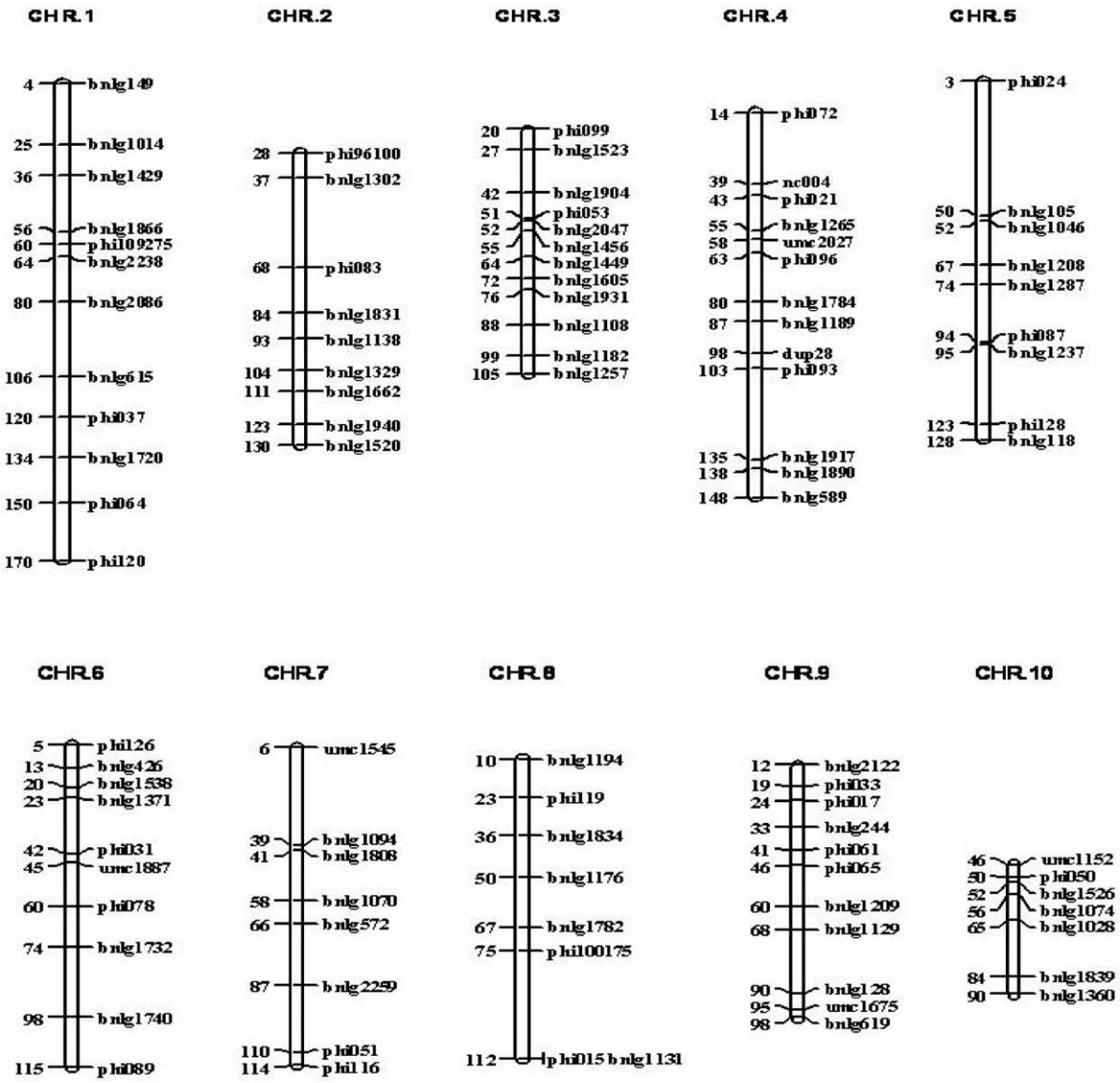

Figure 1. *Linkage map based on 98 SSR marker loci. Right of the bars are names of SSR markers. Left of the bars are their absolute positions in centi Morgan (cM). Graphs were made with Map Chart* (34).

was performed with the distance matrix using UPGMA method, since the purpose of the analysis of inbreds was to study the overall pattern of genetic diversity in a set of inbreds, and not to maximize the distance between Operational Taxonomic Units *(25)*. The reliability of the cluster was assessed by applying a bootstrap procedure *(26)*.

RESULTS

Only 4.5% of all marker data points were missing due to amplification failure or null alleles. The number of alleles per SSR marker varied from 2 to 8, with an average of 4.18, and a total of 205 different alleles were detected (Table 1). Expected heterozygosity (He) values varied from 0.129 to 0.864, with an average of 0.646. These values were significantly correlated with the number of alleles (r = 0.768), though the highest He values had two loci (bnlg1325 and bnlg1808) having 7 and 8 alleles, respectively.

UPGMA ordered the inbreds in two broad groups (Figure 2). As expected, one group consisted of inbreds from Reid Yellow Dent, and the other included all Lancaster inbred lines, with bootstrap values for the two trees of 30% and 24%, respectively. There was a clear further separation in four groups of inbreds making a total of four main clusters. The first main cluster consisted of only Iodent lines, where the lines I2 and I3 were clustered together, while the inbred I1 was positioned apart from all other Iodent lines. The second main cluster consisted of two BSSS lines. Each of the two other main clusters constituted two clear subclusters. The line Mo17 was clustered together only with the line L1 with the MRD value of 0.63, while other three subclusters had lower MRD values. The most strongly supported nodes by bootstrap analysis were the clusterings within the BSSS and Ohio groups. The low bootstrap values of the tree for Mo17 group indicated no evidence of higher-order groups within the group.

DISCUSSION

Earlier investigations have shown that maize contains abundant SSRs *(13, 27)* and that these SSRs are highly polymorphic even among small samples of maize inbreds *(28, 29),* which was the case in this study. Most preceding studies of SSR diversity in maize have revealed a similar allelic diversity in inbreds. For example, Lu and Bernardo *(30)* reported that 40 U.S maize inbreds averaged 4.9 alleles for 83 SSR loci, while Senior *et al. (13)* reported on average 5.0 alleles for 94 elite inbreds with 70 markers. The mean He value in this study (0.65) is comparable to those from previous studies of genetic diversity among US maize inbreds with He values of 0.62 *(22)* and 0.59 *(13),* among Portuguese inbreds with He of 0.62 *(31),* and to those among inbred lines adapted to cold regions of Japan (He = 0.73) *(32)*.

The average distance of 0.61 among only 15 inbred lines in this study demonstrated the existence of large variability between the inbred lines of Eastern Croatia, and it is comparable with lower of distances reported by Xia *et al. (33)* and Enoki *et al. (32)*. The lower average MRD in the current study indicates a higher average degree of relatedness among our inbred lines, which is related to sampling effect caused by different criteria used to choose the plant material for a study.

The dendrogram obtained from UPGMA cluster analyses on the basis of MDR estimates confirmed pedigree information, undoubtedly distinguishing lines according to their background in two population varieties

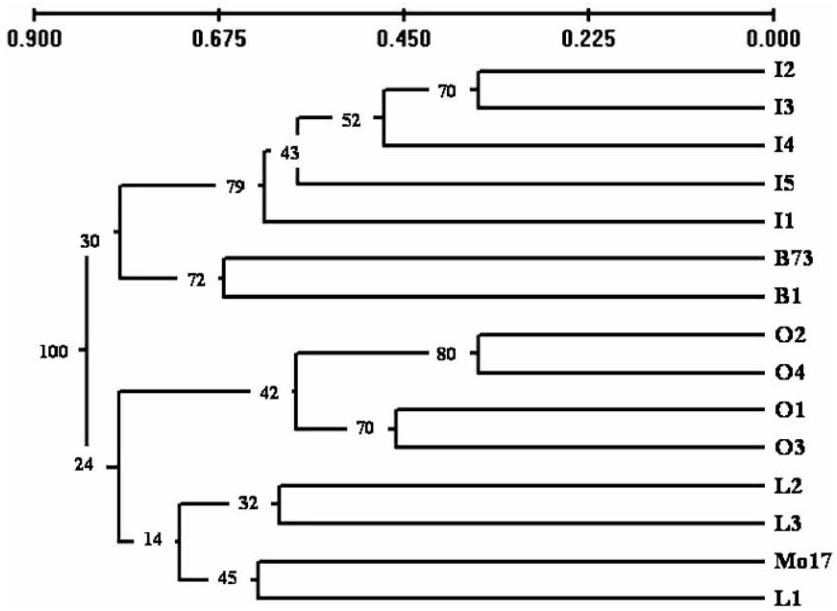

Figure 2. *Associations among 15 samples of maize inbred lines used in breeding programs in Eastern Croatia revealed by UPGMA cluster analysis based on MDR genetic distances* (24) *calculated from SSR data. Values at the nodes indicate percentage of 1000 bootstrap runs supporting a particular node.*

of Reid Yellow Dent and Lancaster Sure Crop. Cluster analysis showed that all of the inbred lines could be distinguished from each other and classified the lines into expected four main clusters. The inbred lines of Osijek Agricultural Institute were confirmed to be genetically diverse, representing a large proportion of the genetic variation occurring in four groups of maize inbred families. Thus, future prospects seem to be promising for further success in finding new superior elite lines through breeding programs.

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