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Genetic Diversity Analysis of Maize Inbred Lines from SCDA Turda - Romania Revealed by RAPD Molecular Markers

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Abstract. Knowledge of genetic diversity and relationships among maize inbred lines is indispensable to a breeding program. Our objective was to investigate the level of genetic diversity among maize inbred lines. Eighty-three maize inbred lines obtained from SCDA Turda were genotyped using 20 decamer primers. These primers generated, among the studied genotypes, a number of polymorphic bands comprised between 17 bands (OPA 03) and 7 bands (OPAB 11). The highest numbers of polymorphic bands were obtained with primer OPA 03, respectively 17 bands, followed by OPA 01, OPB 08 (16 polymorphic bands) and OPX 03 and OPAL 20 (13 polymorphic bands). Genetic distances were established using Nei Li/Dice coefficent and an UPGMA dendrogram was constructed with FreeTree software. The built dendrogram shows phylogenetic relationships between the analysed biological material.

Keywords: RAPD, maize, inbred line, heterotic groups.

INTRODUCTION

Corn is a major field crop in the Romanian agriculture, even in these difficult times, which this sector crosses today, because many of the mechanical works involved in the technology of this species can be replaced by human activities and, also, maize is the crop that responds best to fertilization with organic fertilizers. Of course, agricultural research objectives tend to a different type of technology in corn, ones to best use climate and soil conditions of Romania and to highlight the great diversity of the existent biological material.

In addition, the last fifteen years have brought to attention the industrial uses of corn that highlights the high level of efficiency of this culture.

A large number of local and synthetic corn populations were developed and evolved in Transylvania, due to pedo-climatic conditions favourable to this crop.

Since 1957, SCDA Turda maintains a collection of local populations originating from Transylvania and the Western part of the country, cultivars, hybrids and makes exchanges with research institutes and universities abroad.

Maize is an excellent representative of open-pollinated plant species with present polymorphism of enzyme systems, allelic variability of isoenzymatic loci, which is the crucial parameter in identification of material in the final stage of plant improvement process.

New maize lines are created or improved by reciprocal recurrent selection. Crosses between inbreds from different heterotic groups result in vigorous F1 hybrids with significantly more heterosis than F1 hybrids from inbreds within the same heterotic group or pattern.

Biotechnology of corn includes biochemical-genetic-molecular markers at the level of protein, RNA, and DNA respectively. They are used for gene mapping for desired traits and the ultimate goal is genetic identification of genotype (Zlokolica *et al*., 2002). Usage of molecular markers is being introduced into a basis of genetic researches by which all components of breeding are connected and have a key role in genetic, biochemical, physiological and molecular basis of heterosis (Smith and Beavis, 1996). The PCR-based markers require small amount of DNA, permitting to conduct many reactions from a single leaf. In maize, numerous studies yielded a significant correlation between genetic distances obtained by molecular markers and the coefficient of coancestry (Bernardo *et al*., 2000). RAPD markers are commonly used because they are quick and simple to obtain, enabling genetic diversity analysis in several types of plant materials, such as natural populations, populations in breeding programs and germplasm collections (Ferreira and Grattapaglia, 1996). In maize, RAPD markers have been used in the analysis of genetic distance among segregant lines (Marsan *et al*., 1993) to predict the best crosses among lines for hybrid development (Lanza *et al*., 1997) and to assess genetic diversity among collections of native maize (Moeller and Schall, 1999). The goal of this research was to investigate the level of genetic diversity in 83 inbred lines of maize populations and cultivars. This will contribute to identifying efficient strategies for the sustainable management of the genetic resources of the germplasm used in this study.

MATERIALS AND METHODS

The 83 maize lines used in this study (Tab. 1) were obtained from the maize collection maintained at SCDA Turda.

Tab. 1

List comprising the analyzed maize lines

No.	Line	Field no.	No.	Line	Field no.
1	T 248 Nrf C	7115	43	TD 305	7627
2	T 291	7135	44	TD 364	7689
3	TC 208 Nrf C	7145	45	TD 368	7700
4	TC 209	7152	46	TE 362	7817
5	TC 344	7525	47	TA 426	7845
6	K 1080	8374	48	TA 427	7848
7	T 169a	7071	49	TA 428	7851
8	TC 109A	7083	50	F 135	7896
9	TC 184	7089	51	LC Sv 952	7962
10	T 243	7109	52	LC Sv 954	7964
11	T 251	7127	53	Bucovina 66	7970
12	TC 243 Nrf C	7172	54	PI 187	7984
13	TD 233	7194	55	MV 492 CRF	8217
14	TD 234	7196	56	MV 540	8218
15	TD 235	7198	57	D-Be 16	8357
16	TD 236	7200	58	K 1077	8370
17	TD 237	7202	59	K 1653	8394
18	TD 238	7204	60	K 2308	8422
19	TD 239	7206	61	A 344	8736
20	TD 270	7242	62	A 495 Rf	8738
21	TE 202 A	7290	63	A 619	8740
22	TE 202 B	7292	64	A 632 Nrf T	8744
23	TE 202 C	7294	65	A 635	8746
24	TE 203 A	7298	66	A 665	8760

No.	Line	Field no.	No.	Line	Field no.
25	TE 210 A	7304	67	B 73	8764
26	TE 229	7328	68	N4	8766
27	TC 314	7469	69	TA 419	8892
28	TC 316	7471	70	Mo 17	8822
29	TC 317	7475	71	D 105	8794
30	TC 318	7477	72	TB 329	8850
31	TC 322	7481	73	Mont	8856
32	TC 335	7503	74	F2 Nrf C	8456
33	TC 344 A	7528	75	Fv 7	8466
34	TC 344 RfC	7530	76	F 1852 Nrf T	8518
35	TC 365	7564	77	F 564	8558
36	TC 384A	7588	78	K 2051	8400
37	TC 385 A	7592	79	LO 3 Berg.	8300
38	TC 385 A Nrf C	7594	80	P 131	8069
39	TC 393	7611	81	P 1931	8071
40	TC 399	7621	82	P 1932	8072
41	TD 301	7623	83	W 153R	8688
42	TD 302	7625			

DNA was isolated from young leaves, collected at SCDA Turda and immediately stored at -80 ºC prior to extraction, using the protocol developed by Lodhi *et al.* (1994) and modified by Pop *et al.* (2003). Leaf tissues were ground to fine powder in liquid nitrogen in an Eppendorf tube. 700 µL of 65ºC preheated extraction buffer [100 mM Tris-HCl, 20 mM sodium EDTA, pH=8.0, 1.4 M NaCl, 2% (w/v) CTAB, 2% PVP, 5 mM ascorbic acid and 4 mM DIECA, the last three components being added to the extraction buffer just before the heating at 65ºC on the water bath] were then added to the tube and the mixture was incubated at 65ºC for 25 min. The lysate was extracted with 700 µL of chloroform/isoamyl alcohol (24:1) and centrifuged for 15 min at 11000 rpm in a microcentrifuge. In order to precipitate the nucleic acids, the aqueous fraction was mixed with an equal volume of 5 M NaCl and then with 600 µL of ice cold 96% ethanol. The nucleic acid precipitate was washed two times in 76% ethanol and air dried before being resuspension in 50 µL TE buffer (10 mM Tris-HCl, pH=8.0, 1 mM disodium EDTA). The concentration and purity of extracted DNA were determined using a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific).

PCR amplification (Williams, 1990) was performed with 25 µl reaction volume containing 50 ng of template, 200 µM dNTP, 0,2 µM primer, 2,5 mM MgCl2, 2,5 mM 10 x buffer, 1 U Taq DNA Polymerase (Promega) and 2% PVP in a 96 Well Gradient Palm-Cycler CG1-96 (Corbett 229 Research) programmed for 1 cycle of 95ºC for 3 minutes for initial denaturation, followed by 45 cycles of 1 minute at 93ºC, primer annealing step at 34ºC for 1 minute and primer extension at 72ºC for 1 minute. After a final extension cycle for 10 min. at 72ºC the samples were stored at 4ºC prior to analysis. For the visualization of amplified products the samples were analyzed by agarose gel electrophoresis.

The PCR amplicons along with the 100bp DNA Step Ladder - Promega (used as molecular marker) were separated on 1,4% agarose gel in 1 x TBE at 57 volts for 2,5 hours. Gels were visualized on a UV light Biospectrum AC Imaging System (UVP BioImaging Systems) after staining with 0.5 µg/µl ethidium bromide, for 25 min. Gel images were analyzed using TL120 software (Nonlinear Dynamics).

Bands resulted after RAPD amplification were scored as present (1) or absent (0) and data entered into a binary matrix. Genetic distance between accessions was calculated using Nei and Li/Dice coefficient of similarity (Nei and Li, 1979). Cluster analysis was conducted with FreeTree software using an UPGMA algorithm (Hampl *et al*., 2001) and the dendrogram

was visualized using TreeView software (Page, 1996). A synthetic outgroup was used for dendrogram rooting.

RESULTS AND DISCUSSION

A number of 20 decamer primers was used to amplify DNA extracted from the 83 maize samples used in this study. The total number of polymorphic bands obtained was 234, with a mean of 11.7 polymorphic bands per primer. The highest numbers of polymorphic bands were obtained with primer OPA 03, respectively 17 bands, following OPA 01, OPB 08 (16 polymorphic bands), OPX 03 and OPAL 20 (13 polymorphic bands). The lowest number of polymorphic bands was obtained with primer OPAB 11, respectively 7 polymorphic bands (Tab. 2). The high obtained polymorphism is characteristic for a germplasm collection that includes genotypes from different countries of origin.

Tab. 2

RAPD primers used to analyze genetic variation in maize genotypes

Primer	Total no. of bands	No. of polymorphic bands	No. of monomorphic bands	Percent of polymorphism (%)
OPA 01	16	16	0	100,0
OPA 03	17	17	0	100,0
OPAB 18	14	13	1	92,85
OPAB 11	13	13	0	100,0
OPAL 20	13	13	0	100,0
OPB 10	12	12	0	100,0
OPB 08	16	16	0	100,0
OPB17	12	11	1	91,66
OPE 14	13	13	0	100,0
OPAB 11	8	7	1	87,5
OPA 04	11	10	1	90,9
OPD20	10	10	0	100,0
OPA 17	9	9	0	100,0
AB 11	9	8	1	88,8
OPF 02	10	10	0	100,0
OPA 02	9	9	0	100,0
OPO 14	11	10	1	90,9
OPA 03	12	12	0	100,0
OPX 03	14	13	1	92,85
OPC 2	14	12	2	85,7

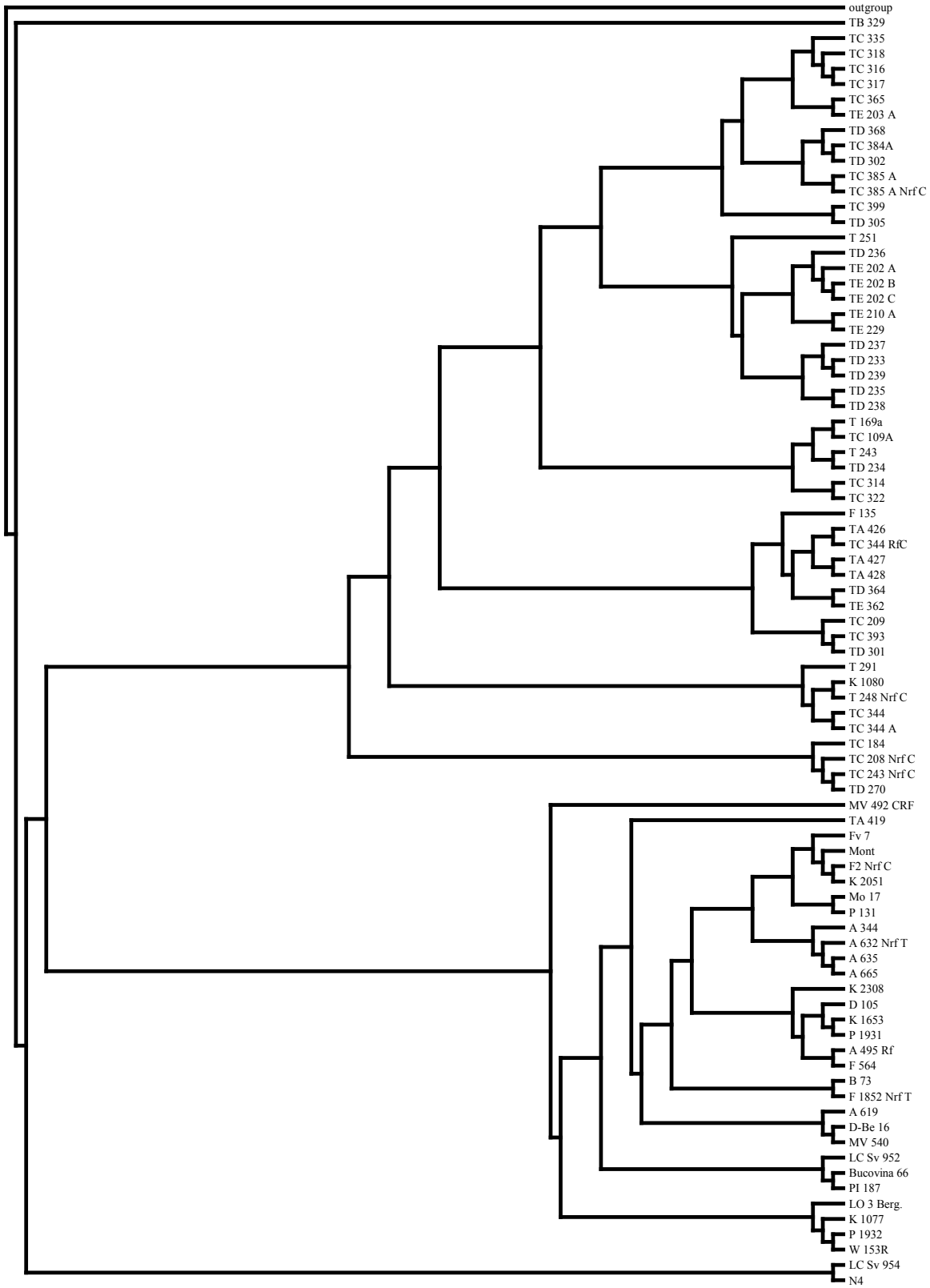

Fig. 1. Dendrogram built using UPGMA analysis of Nei Li/Dice coefficient of similarity between maize lines

The lowest value of genetic distance (0.078) was recorded between lines TC 316 and TC 317, which suggests a high genetic similarity, while the highest value of genetic distance was 0.77 (between TD 302 and LC Sv 954). The average value of genetic distance was 0.45.

The UPGMA dendrogram is presented in Figure 1. The used RAPD primers clustered the genotypes according to their origin, into three main groups. The first two groups are formed from other subgroups comprising inbred lines closely related to each other. The third group includes only two inbred lines, Sv 954 LC and N4, which are the most distant from the others, in terms of genetic similarity. Inbred lines with common parents are found in the same group, such as TD 233, TD 234, TD 235, TD 236, TD 237, TD 238, TD 239, confirming the results of field data.

CONCLUSIONS

The used markers allowed us to unequivocally distinguish all the maize lines analyzed. The ability to identify the closely related inbred lines demonstrates that RAPD markers are suitable for sure, easy, quick and inexpensive identification of maize germplasm.

RAPD markers were able to group maize lines according to their origin and variety and determine genetic similarities between them.

There is a fairly high concordance between the degree of kinship/genetic divergence obtained using RAPD markers and the known genealogy of the studied inbred lines.

Regarding the inbred lines obtained from commercial hybrids, the dendrogram and the genetic distances allow their inclusion in heterotic groups.

RAPD is a reliable and a relatively simple procedure to study genetic relationships among maize lines, which can be useful in current maize breeding programs, allowing the identification of new lines as well as the assessment of the genetic similarity among different genotypes.

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REFERENCES

1. Bernardo, R., J. Romero-Severson, J. Ziegle Hauser, L. Joe, G. Hookstra and R.W. Doerge (2000). Parental contribution and coefficient of coancestry among maize inbreds: pedigree, RFLP, and SSR datal. Theor Appl Genet. 100:552–556.
2. Ferreira, M. E. and D. Grattapaglia (1996). Introdução ao uso de marcadores moleculares em análise genética. 2nd ed. Ed. Embrapa-Cenargen : 121-130.
3. Hampl, V., A. Pavlícek and J. Flegr (2001). Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with a freeware program FreeTree: Application to trichomonad parasites. International Journal of Systematic and Evolutionary Microbiology. 51: 731-735.
4. Lanza, L. L. B., Jr. C. L. De Souza, L. M. M. Ottoboni, L. M. C. Vieira and A. P. De Souza (1997). Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. Theor. and Appl. Genet. 94:1023-1030.
5. Lodhi, M. A., Y. Guang-Ning., F. Norman Weeden and B. I. Reisch (1994). A simple and efficient method for DNA extraction from grapevine cultivars. Vitis species and Ampelopsis. Plant Molecular Biology Reporter 12(1): 6-13.
6. Marsan, P. A., G. Egidy, G. Monfredini, S. Disilvestro and M. Motto (1993). RAPD markers in maize genetic-analysis. Maydica. 38:259-264.
7. Moeller, D. A. and B. A. Schaal (1999). Genetics relationships among native American maize accessions of the Great Plains assessed by RAPDs. Theor. and Appl. Genet. 99:1061-1067.

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

9. Page, R. D. M. (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.

10. Pop, R., M. Ardelean, D. Pamfil and I. M. Gaboreanu. (2003). The efficiency of different DNA isolation and purification protocols in ten cultivars of *Vitis vinifera*. Bul. USAMV-CN. 59:259-261.

11. Smith, S. and W. Beavis (1996). Molecular markers assisted breeding in a company environment. The Impact of Plant Molecular Genetics. BWS Sobral. Ed. Boston. 259-272.

12. Williams J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, Nucleic Acids Research 18: 6531–6535.

13. Zlokolica, M. Y., M. B. Milosevia, Z. T. Nikolia and V. O. Galovia (2002). Identification of genetic characteristics of maize (Zea mays) using genetic markers. Proceedings for Natural Sciences. Matica Srpska Novi Sad. 103: 47-56.