GENETIC CHARACTERIZATION OF SOME LOCAL MAIZE LANDRACES COMING FROM ROMANIA BY RAPD METHOD

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Abstract

The evaluation of morphological differences is a traditional method of evolutionary and pedigree relationship determination. It was particularly useful in maize, where phenotypic differences occur (e.g. color, kernel type and kernel size). However, only molecular markers provide information that is independent of environmental influences or a plant development phase. Therefore, techniques of DNA analysis have become more and more important. Methods based on polymerase chain reaction -PCR- are used widely in research. Ones of the most used methods are the RAPD (Randomly Amplified Polymorphic DNA) method

Key words: : maize local landraces, cold test index, RAPD method

Reconsidering of the evaluation work, documentation and use of maize genetic resources represented by old local landraces, no studied or inadequately studied, represents an actual necessity, at the national and international level. Not incidentally, the work report of the ECPGR Maize Working Group Meeting Rome, Italy (1996) has noted two major needs for collaboration on maize genetic resources:

- Identify of old local populations, valuables for their agronomic characters;
- Establish joint prebreeding programs.

The maize local landraces are distinguished by a high capacity for adaptation and physiological characteristics specific to certain areas, as well as high yield capacity and the its quality attributes (Moşneagă and collab., 1957; Cristea, 2006; Căbulea and collab., 1975; Hallauer and Miranda, 1981; Murariu and collab 1998, 2010).

The Romanian maize local landraces are very different as the ecological conditions in our country under the influence of which were formed and over which were superimposed the effects of empirical selection made by thousands of growers, each in its own way. Although, the maize landraces are very heterogeneous, they are grouped into distinct races, each occupying a certain area (Cristea, 2006).

The fundamental aim of this research is the evaluation of genetic diversity of some Romanian maize landraces stored at the Suceava Genebank.

It makes possible a quick examination of genetic material in a large number of samples at a relatively low cost. In a research conducted in the Agriculture Faculty of Iasi, the modified RAPD technique with a system of primers containing additional DNA sequences partly complementary to the semi-conservative sequences of intron – exon junctions proved to be very useful in a variety of plant species. These primers, known also as semi-random primers, were with success used by Weining and Langridge to target diverse regions of genome in cereals.

This method is used especially for genetic diversity identification and philogenetic studies (Bagheri A. and all, 1995, Hoey B.K. and all, 1996, Iqbal N.J. and all, 1997, Samec and all, 1998, Tinker and all, 1993).

The objective of this research was to determine the genetic variability existing among maize landraces coming from Romania, very resistant to cold temperatures. We hoped to obtain information on the level of intervarietal divergence which is essential for plant breeders. The additional practical goal was to eliminate possible duplicates.

MATERIALS AND METHODS

Sixty one maize landraces taken to the preliminary analysis are maintained in the Suceava Genebank. The selected Maize landraces are very resistant to cold temperatures and coming from different area of Romania (table 1).

All molecular analyses were carrying out in the Agriculture Faculty of Iasi, in frame of molecular markers laboratory.

For genetic characterization of maize germplasm by RAPD method, first, was necessary extracting genomic DNA. Of each variant were taken leaves

Table 1

Local maize landraces coming from Romania, very resistant to cold temperatures, which were characterized from genetic point of view through RAPD method (USAMV, 2011)

Accession number	Accession name	Origin	Accession number	Accession name	Origin
SVGB-8012	METES 1	AB	SVGB-8026	ZLATNA 4	AB
SVGB-8022	ZLATNA	AB	SVGB-1790	PETROSANI 2	HD
SVGB-9800	CIUNGANI	HD	SVGB-3599	MAGURA ILVEI 1	BN
SVGB-9807	CERBAL 2	HD	SVGB-1806	CN-21-84C	CJ
SVGB-845	B-173	SM	SVGB-8043	RACOVITENI 3	BZ
SVGB-7750	VLADESTI	AG	SVGB-9966	SANTMARTIN	HG
SVGB-5880	GLODENI 6	GJ	SVGB-911	DUMBRAVA	CJ
SVGB-5483	POLOVRAGI 4	GJ	SVGB-4813	CALAFAT 2	DJ
SVGB-7900	PADES 2	GJ	SVGB-7820	SARADIS 2	CJ
SVGB-7282	PRUNDU BARGAULUI 13 {A}	BN	SVGB-1423	LUDESTI 2	HD
SVGB-5219	STARCHIOJD 6	PH	SVGB-3764	NEREJU 12	VN
SVGB-3971	CARBUNESTI 480	GJ	SVGB-1357	JITIA DE JOS 3	VN
SVGB-3973	BALCESTI 1	CJ	SVGB-3722	RODNA 10	BN
SVGB-7811	BOLDUT 2	CJ	SVGB-11575	CRISTINESTI	BT
SVGB-7745	DRAGHICI	AG	SVGB-5226	IZVOARELE 3	PH
SVGB-5168	TEREGOVA 5	CS	SVGB-7812	VALEA LUI CATI	CJ
SVGB-11231	POIANA TEIULUI 5	NT	SVGB-7645	PONOARELE 6	MH
SVGB-9591	SATU MARE B 139-84	HR	SVGB-4005	PLENITA II	DJ
SVGB-1399	BALSA 9	HD	SVGB-7624	BEZDEAD 1	DB
SVGB-8865	BRUSTUROASA 10	BC	SVGB-4023	ALMAJ 1	DJ
SVGB-5874	RUNCU 3	GJ	SVGB-9577	SATU MARE B 172-84	HR
SVGB-981	B-159	SM	SVGB-16145	DINTE CAL DE FRASINET	CL
SVGB-499	ILVA MICA 43	BN	SVGB-9887	BAISOARA 5	CJ
SVGB-595	CEAHLAU 154	NT	SVGB-4019	TURNU SEVERIN 1	MH
SVGB-7701	STOENESTI 3	VL	SVGB-1015	CHICHIS	CV
SVGB-952	B-181	HR	SVGB-1179	MIERCUREA	MS
SVGB-5557	PIETRARI	VL	SVGB-1244	ZOLTAN	CV
SVGB-9920	BLAJ CIUGUD	AB	SVGB-1640	GEOAGIU 28	HD
SVGB-5172	TEREGOVA 6	CS	SVGB-11584	GEORGE ENESCU 4	BT
SVGB-7754	VALEA SILISTII	AG	SVGB-14153	JELNA 1	BN
SVGB-9919	BLAJEL	SB			

Table 2

RAPD primers used

Nr crt.	Primer	Secvenţa (5'-3')
1.	ROTH A15	TTC CGA ACC C
2.	ROTH A16	AGC CAG GCA A
3.	ROTH A17	GAC CGC TTG T
4.	ROTH B02	TGA TCC CTG G
5.	ROTH B08	GTC CAC ACT C
6.	ROTH B13	TTC CCC CGC T
7.	ROTH B14	TCC GCT CTG G
8.	ROTH B16	TTT GCC CGG A

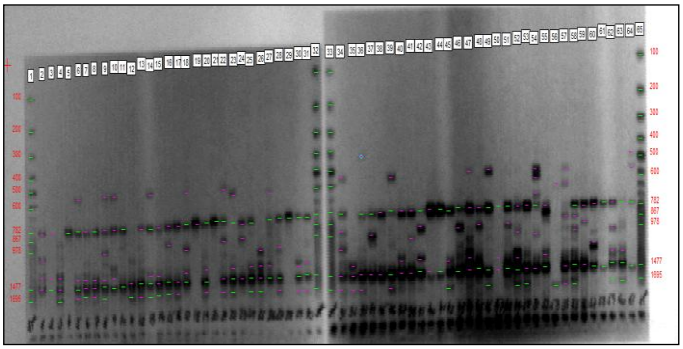

Fig.1. The obtained images with support of B13 primers and analyzed with RFLP Scan 2.1, to those 61 maize local landraces.

of young maize plants (14 days after emergence), which were placed in plastic tubes (Eppendorf tubes) and immediately frozen in liquid nitrogen. The maize samples were kept in a freezer at-70^{0}C.
For DNA extraction CTAB method was used (hexadecyltrimethilammonium bromide) as amended by Doyle and Doyle in 1987. The steps were:

- plant material (100-200 mg) of each variant was grinded in liquid nitrogen in a mini tube (1.5 ml) and introduced again in liquid nitrogen
- at each mini tubes with prepared plant material were added 700 ml of extraction D & D* solution, after that, the mixture was incubated for 20-30 minutes at 650^{0}C

**D & D solution - containing 100 mm Tris-HCl (pH 8.0), 20 mm EDTA (pH 8.0), 1.4 M NaCl, 2% CTAB, 1% Na2S2O5, H2O, all were autoclaved and then 0.2% mercaptoethanol was added.*

- 700 ml CIA* was added and stirred 5 minutes;

* CIA solution (24:1) contains 24 parts chloroform and one part isoamilalcool.

- It was centrifuged at 10,000 rpm (4 0C) for 10 minutes;
- the liquid phase was extracted, was added 600µl CIA and stirred 5 minutes;
- it was centrifuged at 10,000 rpm (4 0C) for 10 minutes;
- the liquid phase was transferred in new mini tubes adding 50 ml NH4OAc (10 M) and 60µl NaOaC (3M, pH 5.5) and was stirred gently;
- 500µl 2-propanol was added and stirred gently;
- It was centrifuged at 4000 rpm, 4 minutes;
- the liquid was removed using a pipette;
- it added (for washing for 10 minutes) 70% ethanol solution 10mM NH_4OAc;
- it was centrifuged at 4000 rpm, for 4 minutes;
- the liquid was removed with a pipette;
- the mini tubes were dried (open) in a thermostat at 37°C until complete evaporation of alcohol;
- in each mini tube was added 100 ml TE solution, containing 10mM Tris-HCl (pH 8.0), 1mM EDTA (pH 8.0);
- the mini tubes were kept in a freezer at –200^{0}C.

For establishing DNA concentration fluoro spectofotometer NanoDrop 2200 type, was performed. The appropriate dilutions for PCR mixture (5 ng / ml) were performed with TE solution.
In order to determine the genetic diversity of 61 maize landraces, 8 decameric RAPD primers (Table 2.) were selected, after making an initial screening with 20 primers. It was chose only those primers that generated polymorphic fragments.
For PCR mixture in a volume of 20µl was performed, that were pipetted: 5 ngADN genomic, 10 mm of dNTP, 25 mM MgCl2, 5pmol / ml decamer primer (ROTH), 0.1 units Taq DNA polymerase (Go Taq Polymerase - Promega) and 10x buffer.
Amplification with an Eppendorf termocicler was performed. The conditions in which amplification was performed were: initial denaturing for 3 min at 95^{0}C after which followed a number of 45 cycles of amplification, each amplification, having the following steps:

- Distortion: 1 min at 93^{0}C;
- Primers attaching: 1 min at 34^{0}C;
- Extension: 1 min at 72^{0}C.

The last phase was the final extension, 10 min at 72^{0}C.
Electrophoresis separation of the amplification products was carried out in agarose gel with a concentration of 2%.
The fragments visualizing by staining with ethidium bromide was achieved, at a concentration of 0.5 ml/ml.

RESULTS AND DISCUSSIONS

The genetic classification on related genetic groups was performed using similarity coefficient Lei, Ni and UPGMA (unweighted pair-group method arithmetic average). In order to determine the similarity of 61 maize populations was used RAPD (Random Amplified Polymorphic DNA), resulting in a total of 91 bands with sizes between 74 and 1687 bp, of which 86 were polymorphic.
The lowest number of amplified fragments was 6 (A15) and the highest number was 17 (ROTH B13 and B14). As shown in table 3, the polymorphic bands level, in the 8 primers used for RAPD analysis, ranged between 83% (ROTH A15) and 100% (ROTH A16, A17 and B08).

Table 3.

The amplified fragment numbers, polymorphic bands numbers and polymorphism percentage for each used primer to the RAPD analyses

Primer	Număr fragmente amplificate	Fragmente polimorfice	Mărime fragmente (bp)	Procent polimorfism (%)
ROTH A15	6	5	373-972	83%
ROTH A16	10	10	376-1058	100%
ROTH A17	7	7	204-873	100%
ROTH B02	10	9	253-964	90%
ROTH B08	14	14	74-1281	100%
ROTH B13	17	16	413-1678	90%
ROTH B14	17	16	283-1397	94%
ROTH B16	10	9	351-1207	90%

With support of SPSS software program, it calculated genetic similarity between genotypes analyzed, resulting dendrogram in Figure 2. and

DNA bands frequency chart in Figure 3. In the first chart is noted that from point of similarity degree view, the samples were grouped into three clusters (C1-.. C3), and in the second graph (Fig. 3), we see that there are not duplicates in the 61 maize local populations analyzed; the matrix values are very close but not similar.

CONCLUZIONS

1. The sixty one maize local landraces, coming from Romania, very resistant to cold temperature from point genetic molecular of view, by RAPD method were analyzed, resulting a total of 91 bands with sizes between 74 and 1687 bp, of which 86 were polymorphic.
2. There are not duplicates in those 61 analyzed maize local populations; the matrix values are very close but not similar.

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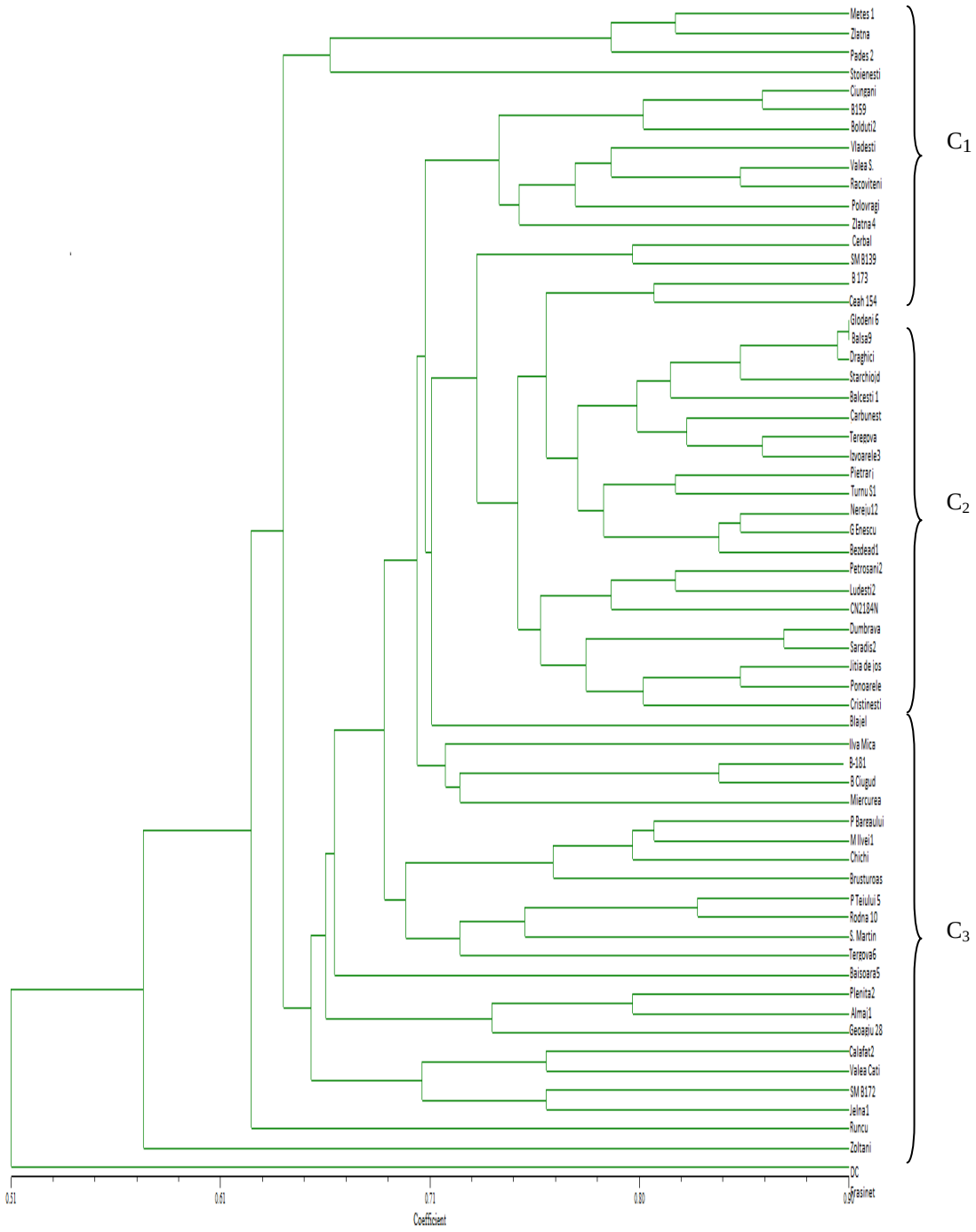

Fig. 2. Relationships among the investigated maize landraces using RAPD data (Jaccard distance, UPGMA clustering)

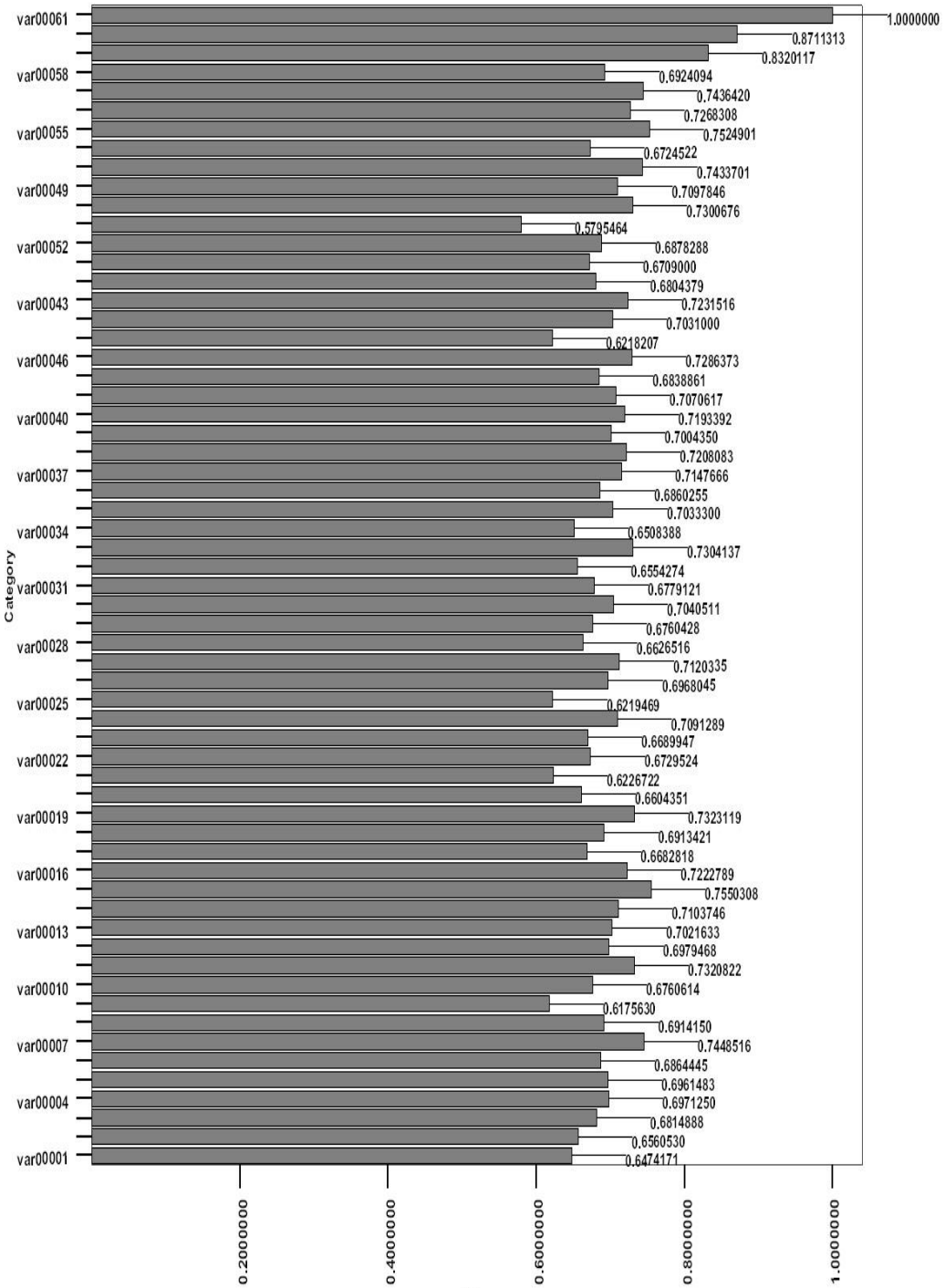

Fig. 3. The DNA bands frequency to those 61 maize local landraces