EVALUATION OF ROMANIAN MAIZE LOCAL LANDRACES FOR INCREASING THE EFFICIENCY OF THEIR USE IN BREEDING PROGRAMS

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Abstract

At present there can be noticed a main interest of plant genetic resources users for accessing information related to characterization and evaluation descriptors. Maize genetic resources represented by local populations originating from different areas, represent important useful genes sources for improving species. Their e is possible due to studies and comprehensive measures which can lead to the maintenance of biodiversity and increase its efficiency. The paper presents the results of characterization and evaluation of a total of 61 local landraces with cold test index >80%, selected from a total of 300 studied local landraces. These local landraces can be useful genes sources for maize breeding at low temperature, being a main trait for maize cultivation in wetter and colder areas in Romania.
The study shows a high diversity for most of the morphological characterization descriptors of the plant, ear and kernel, physiological evaluation at low temperature resistance of the plantlets, precocity and biochemical evaluation for kernels quality.
In order to obtain information, at intervariety level, for maize local landraces studied, molecular characterization was performed by RAPD method (random amplified polymorphic DNA).
Many maize local populations with values of characterization and evaluation descriptors of real interest were emphasized. Utilization of these local landraces as starting material can lead to the identification of useful genes sources for improvement of important agronomic characters of maize (yield capacity, precocity, resistance to low temperatures, quality and genetic integrity).

Key words: maize local landraces, cold test index, protein content, 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) have noted two major needs for collaboration on maize genetic resources:

- Identify of old local populations, valuables for their agronomic characters;
- Establish a 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; Ulinici, 1961; Gologan, 1965; Mureşan, 1972; Cristea, 1972 b, 2006; Suba, 1973; Căbulea and collab., 1975; Hallauer and Miranda, 1981; Murariu and collab 1999, 2001, 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).

In the breeding programs the maize local populations could have a main interest, especially as sources of useful genes for environmental adapting, agronomic, physiological traits and valuable qualities.

At present, the unanimous opinion of the specialists is that genetic resources represented by the local maize populations, coming from different areal, represents important reserves of useful genes

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for breeding of the species. The exploitation of these reserves becomes possible through studies and complex measures that can lead to the keeping of biodiversity and the increase of efficiency of using it .

Important collections of maize old landraces are kept in the gene banks. Thus, the Suceava Genebank, holds a rich collection of over 4300 samples collected from sub mountain and mountain areas of Romania.

A comprehensive assessment of these genetic resources could be achieved through morphological, physiological, biochemical and molecular characterization (Karp and collab.,1995, 1997, 1998, Welsh and collab., 1990, Williams and collab.,1990, Pejic 1998), subordinate of an important purpose, namely, the highlight of the compelling value of maize local populations in the genetic background with breeding value and practical use of these genetic resources for promoting of sustainable agriculture This opportunity results as a consequence of realizing the use, especially that of local resources, is highly reduced. That is why there is a great need of reconsidering the attitude towards this situation, especially through complex studies that could highlight the useful genetic potential of these materials

In all breeding programs the germplasm play an important role both in creating of hybrids and lines, and in maize landraces improving. A valuable germplasm has high genetic variability and its performance.

All these are scientific reasons that were the basis for a complex evaluation system achievement, able to reveal genetic variability and lead to the identification of some valuable genotypes in the main breeding directions.

MATERIAL AND METHOD

A comprehensive morpho-physiological, biochemical and molecular assessment of important germplasm fund, represented by a total of 300 maize local landraces, was made possible through a national project with title ”Increase of the Use Efficiency of a Main Local Maize Germplasm Fond from Romania”, grant no 52127/2008 (2008-2011).

Characterization of local maize landraces was done in an appropriate experiment system, based on morpho-physiological descriptors edited by International Plant Genetic Resources Institute (BIOVERSITY).

We have analyzed 12 morphological descriptors of plant architecture: plant height, insertion height of the main ear, total numbers of leaves per plant, number of leaves above the uppermost ear, leaf length, leaf width above the uppermost ear, architectural elements of pannicle, maximum and minimum diameter of the stem, and 11 morphological descriptors for ear and grain: ear length, maximum and minimum diameter of the ear, number of kernel rows, no of kernel per row, length, width and thickness of grains, grain weight/ear and 1000 kernel weights.

Physiological descriptors are particularly important for maize destined for cultivation in wetter and colder areas. For this reason, it was considered appropriate to highlight the following physiological descriptors: the resistance of maize plantlets at low temperatures, plant growth vigor and sum of the temperatures degrees to the silking data, as a proxy indicator of precocity.

In the laboratory we determined the resistance of maize plantlets at low temperatures in 300 maize landraces. Assessment was done by the method Debbert cold test index determination (1988), cited by Rotari and Comarov (1992). The 61 maize landraces with values of cold test index > 80%, were selected for analyzing of protein content, presence on the kernels of *Fusarium spp.* and molecular characterization.

For the morphological and physiological descriptors were calculated the following estimators: the arithmetic average (x), the variation amplitude, variance (s^2) and variation coefficient(s%) (Ceapoiu, 1968). The dispersion of the results concerning the morpho-physiological descriptors of studied maize local landraces, gives a conclusive analysis on the existing genetic diversity within this germplasm, insufficiently exploited. It was considered appropriate determining the corn resistance to infection with mycotoxins produced by *Fusarium moniliforme* and *Fusarium graminearum*. This study was achieved by using a scoring system described by Naumova (1972), after that, the attack degree, using a specific formula (Booth, 1971) was determined.

Biochemical characterization refers to the determination of sugars content in maize plantlets which were treated with low temperatures (Buyse and Merckk, method) and protein content of kernels (Kjeldahl method).

Molecular characterization of the 61 maize landraces, very resistant to low temperatures was realized by RAPD method At the beginning, it was necessary the genomic DNA extraction. For this purpose we used young plants of maize accessions. Leaves collected of each variant were placed in plastic tubes (previously marked) and immediately frozen in liquid nitrogen. The samples were stocked in the freezer at-20^0C. For DNA extraction CTAB method was used (hexadecyltrimethilammonium bromide) modified by Doyle and Doyle in 1987. Determination of concentrations using NanoDrop fluorospectofotometer 3300 type, was performed. After reading the concentration of DNA, necessary dilutions PCR mix, were made. For RAPD method, DNA solution concentration should be 5 ng / µl.

For determination the genetic diversity of 61 maize landraces, a total of eight RAPD primers was used, (table 1) which were selected after initial screening achievement on 20 primers. Thus, we chose only those primers which gave polymorphic fragments in maize landraces. PCR reaction in a volume of 20 µl, was performed, in which were pipetted: 5 ng genomic ADN, 10 µM of dNTP, 25 mM $MgCl_2$, 5pmol/ml decamer primer (Roth), 0.1 units Taq DNA polymerase (Go Taq polymerase - Promega) and 10 x reaction buffer. Amplification was performed in two thermocyclere: Corbett and Eppendorf. The amplification was performed in the following conditions: initial denaturing for 3 minutes at 95^0C, followed by 45 amplification cycles, each of them having the following steps: denaturing: 1 min at 93^0C, attaching of the primers on 1 min at 34^0C, and extension on 1 min at 72^0C. The last step was the final extension on 10 min at72^0C.

Table 1

Primers used in the PCR reaction generating reproducible polymorphisms

No.	Primer	Sequence (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

The amplification products in agarose gel electrophoresis were separated at concentration of 2%, and a visualization of fragments was performed by coloring with ethidium bromide, concentration of 0.5 µl / ml.

The analyses of images derived from the RAPD analysis was performed using the program RFLPScan 2. We selected, marked and used in the calculation only those bands that were clear and did not raise any question about the presence or absence of them. Data provided by the program RFLPscan were processed using a program NTSYS pc. 7.

Grouping of the genetic variants on related groups, was performed with program, which use as variables the similarity coefficient Lei and Ni and UPGMA (unweighted pair-group average method arithmetric).

RESULTS AND DISCUSSIONS

Results on the morphological, physiological and biochemical characterization of the 300 maize landraces studied, can be found in the *online* database at the web address:

- ***www.biomaize.ro***, which includes information concerning the value of biological material with useful genes in the main directions of improvement.

In this study, 23 morphological descriptors for characterization of plant architecture, ear and grain were determined. For emphasizing of the genetic variability of maize local populations the following dispersal indexes were calculate: mean value, amplitude of variation, variance and coefficient of variation (*table 2*). Interpretation of the results in this regard is based on the coefficient of variation as an expression of diversity of the analyzed biological material. There were high coefficients of variation for: insertion height of the main ear, number of primary and secondary branches of panicle and grain weight per ear.

The middle values of variation coefficient were recorded for descriptors: plant height, the total number of the leaves per plant, number of leaves above the uppermost ear, leaf width above the uppermost ear, peduncle length of the panicle, the maximum and minimum diameter of the stem, ear length, minimum and maximum diameter of the ear, number of kernel rows, no of kernel per row and 1000 kernel weights.

Table 2

Morphological and physiological descriptors estimators values on 61 maize local landraces characterized in Suceava in year 2009

Estimators	Plant height (cm)	Insertion height of the main ear (cm)	Total numbers of leaves per plant	Number of leaves above the uppermost ear	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	Peduncle length (cm)	Number of first order branches	Number of second order branches	Maximum diameter of the stem (mm)	Minimum Diameter of the stem (mm)
Plant descriptors												
X	194,78	64,60	10,53	6,02	70,64	8,37	61,54	24,37	13,00	2,11	18,31	9,72
Max.	260,00	109,00	14	8,00	89,00	11,00	95,00	67,00	29,00	11,00	32,00	14,60
Min.	129,00	27,00	7	3,00	10,00	0,00	44,00	13,00	2,00	0,00	12,00	6,80
S^2	447,03	220,97	1,65	1,11	75,20	1,18	35,57	21,97	19,58	2,69	5,10	2,0
S%	10,85	23,02	12,16	17,44	12,27	13,02	9,68	19,24	34,00	77,73	12,34	14,51
Estimators	Ear length (cm)	Maximum diameter of the ear (mm)	Minimum diameter of the ear (mm)	No of kernel rows	No of kernel/row	Kernel length (mm)	Kernel width (mm)	Kernel thickness (mm)	Kernel weight/on ear	1000 kernel weight (g)	Coldtest index (%)	Protein content (%)
Ear and kernel descriptors												
X	17,01	42,40	34,42	11,87	34,57	9,85	8,87	4,53	121,9	313,90	86,2	10,77
Max.	22,30	52,00	43,70	18,00	45,00	11,40	11,20	5,50	221,0	492,00	94	12,08
Min.	9,70	26,10	24,50	8,00	26,00	7,90	5,60	3,40	52,0	144,00	81	9,67
S^2	5,58	18,49	14,24	3,67	19,7	0,62	0,99	0,2	1017,4	3347,07	12,9	0,60
S%	13,87	10,14	10,95	16,18	12,84	7,99	11,22	9,87	26,2	18,43	4,16	7.15

Sum of the temperatures degrees as a proxy indicator of precocity, emphasizes important differences between local landraces. Very early, early, middle and tardives maize populations were distinguished.

The cold test index, which was determined in laboratory, attests a different resistance to low-temperature of maize plantlets. The coefficient of variation for this trait is lower in the 61 populations as a result of their selection of the total number of 300. However, the determinations made at all germplasm, a very large differentiation of maize local populations were observed. These populations can be grouped in resistant, middle resistant and susceptible maize populations to low temperatures (***www. biomaize.ro***).

The maize populations shows a protein content between 9,67 and 12,08%. We observed that there are many maize local landraces with higher protein content than the Romanian hybrid Montana (11,12%). The variation coefficient of this trait is low because, generally the protein content from the kernels varied in the reduced limits.

The estimation of the kernels resistance to the infection with mycotoxins produced by *Fusarium spp.* shows a high variability of accessions, having the percentages of infection with values between 0 and 20% (*tab. 3*), Estimation of *Fusarium attack* according Booth, 1971, shows the following attack levels: 1-2% - negligible attack, 2-5% - low attack, 5-10% -low to middle attack, 10-20%- middle attack, 20-30% - middle to intense attack, 30-50% -intense attach and 50% - very intense attack. In this study there is a middle attack of *Fusarium moniliforme* and a low attack of *Fusarium graminearum*.

Table 3

Infection percentage of the *Fusarium spp.* at the studied accessions

No. crt	Accession number	Infection percentage (%)	No. crt	Accession number	Infection percentage (%)	No. crt	Accession number	Infection percentage (%)
1	SVGB-1357	0	22	SVGB-1399	2	43	SVGB-7800	4,2
2	SVGB-1790	0	23	SVGB-3599	2	44	SVGB-5226	4,2
3	SVGB-5483	0	24	SVGB-5172	2	45	SVGB-3991	4,2
4	SVGB-8012	0	25	SVGB-1015	2,2	46	SVGB-7754	4,2
5	SVGB-9577	0	26	SVGB-1640	2,5	47	SVGB-7820	4,2
6	SVGB-9591	0	27	SVGB-3973	2,5	48	SVGB-5354	4,5
7	SVGB-9920	0	28	SVGB-9887	2,5	49	SVGB-12488	4,5
8	SVGB-7624	0,2	29	SVGB-11584	2,5	**50**	**SVGB-7900**	**5**
9	SVGB-7701	0,7	30	SVGB-4813	3	51	SVGB-1806	5,2
10	SVGB-8022	0,7	31	SVGB-9807	3	52	SVGB-8865	5,7
11	SVGB-3764	1	32	SVGB-11575	3	53	SVGB-5188	5,7
12	SVGB-4005	1	33	SVGB-1244	3,2	54	SVGB-9800	6,2
13	SVGB-5219	1	34	SVGB-1176	3,5	55	SVGB-11231	6,5
14	SVGB-595	1,2	35	SVGB-5226	3,5	56	SVGB-3722	6,7
15	SVGB-3971	1,2	36	SVGB-8026	3,5	57	SVGB-7750	7,5
16	SVGB-7745	1,2	37	SVGB-952	3,7	58	SVGB-4784	17
17	SVGB-9919	1,5	38	SVGB-981	3,7	59	SVGB-1423	17
18	SVGB-9966	1,5	39	SVGB-5168	3,7	60	SVGB-8043	20
19	SVGB-7645	1,7	40	SVGB-7811	4	61	SVGB-7783	20
20	SVGB-499	2	41	SVGB-16145	4			
21	SVGB-845	2	42	SVGB-7282	4,2			

Majority of accessions are infected with *Fusarium moniliforme*, except accession SVGB-7900 which is infected with *Fusarium graminearum.*

For determination of the genetic similarity on 61 local landraces, 8 RAPD primers were used. It results 91 bands with dimensions between 74 and 1687 bp, from which, 86 were polymorphic.

The smallest number of fragments amplified was 6 (ROTH A15) and the largest number of fragments was 17 (ROTH B13 and B14). As can be seen in table 4, the polymorphic bands level at the 8 used primers in the RAPD analysis ranged from 83% (ROTH A15) and 100% (ROTH A16, A17 and B08).

Table 4

Numbers of amplified fragments, numbers of polymorphic bands and polymorphism percentage for each used primer of RAPD analyses

Primer	Number of amplified fragments	Polymorphic fragments	The dimensions of fragments(bp)	Polymorphism percentage (%)
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%

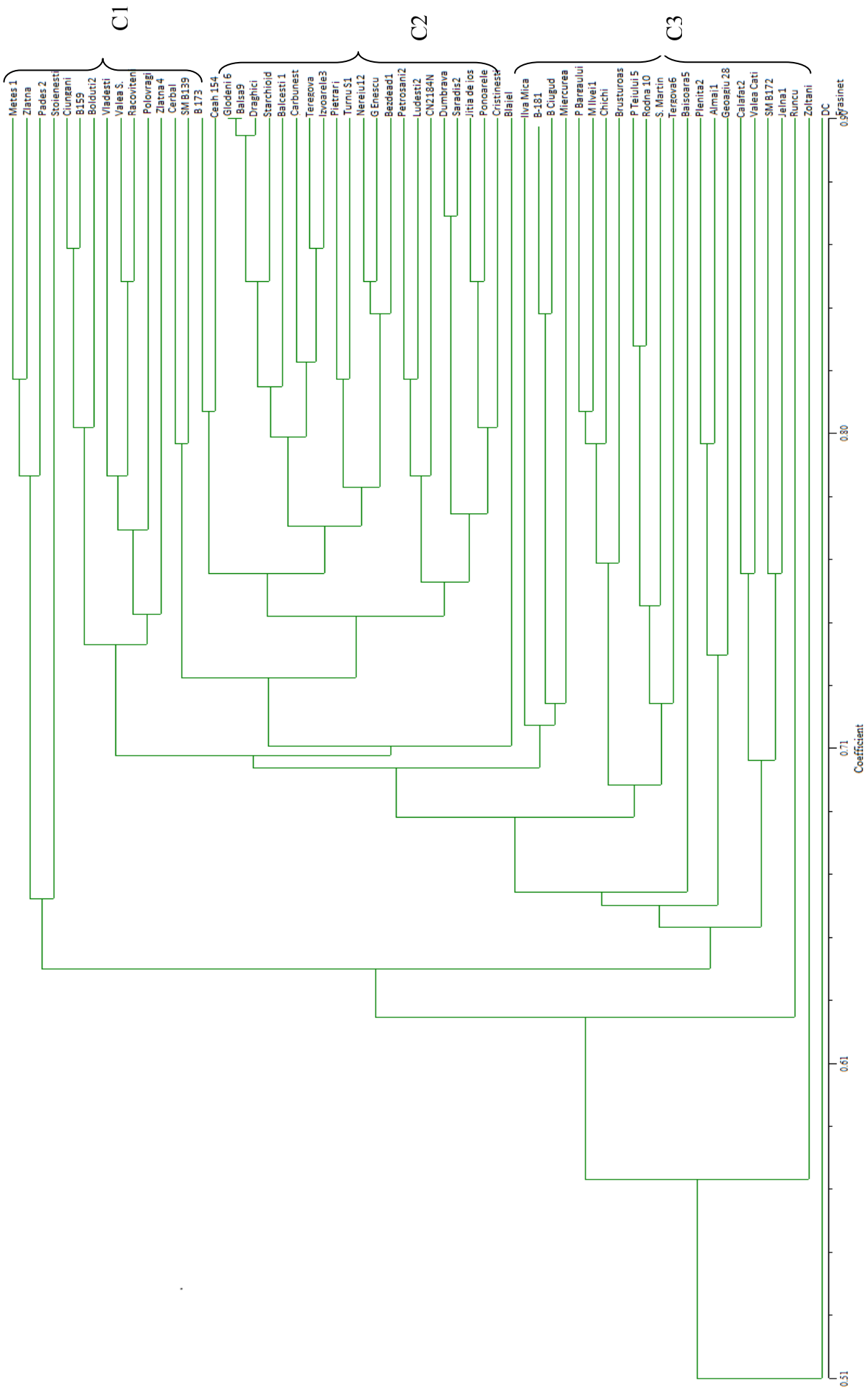

Figura 1 **Dendrogram illustrating genetic diversity of 61 maize local landraces based on 8 RAPD primers**

Table 5

Romanian maize local landraces used for RAPD analysis

Cluster number	Accession number	Accession name	Collecting site	Altitude (m)	Cluster number	Accession number	Accession name	Collecting site	Altitude (m)
C1	SVGB-8012	Metes1	Alba, Metes	490	C2	SVGB-1806	CN-21-84 C	Cluj, Cluj-Napoca	353
C1	SVGB-8022	Zlatna	Alba, Zlatna	600	C2	SVGB-911	Dumbrava	Cluj, Dumbrava	624
C1	SVGB-7900	Pades2	Gorj, Pades	300	C2	SVGB-7820	Saradis2	Cluj, Saradis	656
C1	SVGB-7701	Stoenesti	Valcea, Stoenesti	680	C2	SVGB-1357	Jitia de Jos	Vrancea, Jitia de Jos	480
C1	SVGB-9800	Ciungani	Hunedoara, Ciungani	393	C2	SVGB-7645	Ponoarele	Mehedinti, Ponoarele	480
C1	SVGB-981	B159	Satu Mare, Satu Mare	125	C2	SVGB-11575	Cristinesti	Botosani, Cristinesti	223
C1	SVGB-7811	Boldut2	Cluj, Boldut	405	C2	SVGB-9919	Blajel	Sibiu, Blajel	299
C1	SVGB-7750	Vladesti	Arges, Vladesti	400	C3	SVGB-499	Ilva Mica	Bistrita Nasaud, Ilva Mica	464
C1	SVGB-7754	Valea Silistei	Arges, Valea Silistii	400	C3	SVGB-952	B181	Harghita, Tulghes	935
C1	SVGB-8043	Racoviteni	Buzau, Racoviteni	444	C3	SVGB-9920	Blaj Ciugud	Alba, Blaj, Ciugud	268
C1	SVGB-5483	Polovragi	Gorj, Polovragi	574	C3	SVGB-1179	Miercurea	Mures, Miercurea Nirajului	351
C1	SVGB-8026	Zlatna 4	Alba, Zlatna	600	C3	SVGB-7282	P. Birgaului	Bistrita Nasaud, Prundu Bargaului	637
C1	SVGB-9807	Cerbal	Hunedoara, Cerbal	597	C3	SVGB-3599	M. Ilvei 1	Bistrita Nasaud, Magura Ilvei	838
C1	SVGB-9591	SMB139	Harghita, Satu Mare	628	C3	SVGB-1015	Chichis	Covasna, Chichis	507
C1	SVGB-845	B173	Satu Mare, Satu Mare	125	C3	SVGB-8865	Brusturoasa	Bacau, Brusturoasa	725
C1	SVGB-595	Ceah 154	Neamt, Ceahlau	450	C3	SVGB-11231	P. Teiului 5	Neamt, Poiana Teiului	500
C2	SVGB-5880	Glodeni6	Gorj, Glodeni	282	C3	SVGB-3722	Rodna 10	Bistrita Nasaud, Rodna	601
C2	SVGB-1399	Balsa 9	Hunedoara, Balsa	478	C3	SVGB-9966	Santmartin	Harghita, Sanmartin	628
C2	SVGB-7745	Draghici	Arges, Draghici	500	C3	SVGB-5172	**Teregova 6**	Caras-Severin, Teregova	436
C2	SVGB-5219	Starchiojd	Prahova, Starchiojd	800	C3	SVGB-9887	Baisoara 5	Cluj, Baisoara	551
C2	SVGB-3973	Balcesti 1	Cluj, Balcesti	944	C3	SVGB-4005	Plenita 2	Dolj, Plenita	140
C2	SVGB-3971	Carbunesti	Gorj, Carbunesti	286	C3	SVGB-4023	Almaj 1	Dolj, Almaj	194
C2	SVGB-5168	Teregova	Caras-Severin, Teregova	436	C3	SVGB-1640	Geoagiu 28	Hunedoara, Geoagiu	480
C2	SVGB-5226	Izvoarele 3	Prahova, Izvoarele	550	C3	SVGB-4813	Calafat 2	Dolj, Calafat	43
C2	SVGB-5557	Pietrari	Valcea, Pietrari	343	C3	SVGB-7798	Valea lui Cati	Cluj, Valea lui Coti, Tureni	516
C2	SVGB-4019	Turnu S1	Mehedinti, Drobeta-Turnu Sev	80	C3	SVGB-9577	SM B172	Harghita, Satu Mare	628
C2	SVGB-3764	Nereju 12	Vrancea, Nereju	500	C3	SVGB-14153	Jelna 1	Bistrita Nasaud, Jelna	355
C2	SVGB-11584	G. Enescu	Botosani, George Enescu	153	C3	SVGB-5874	Runcu	Gorj, Runcu	289
C2	SVGB-7624	Bezdead 1	Dambovita, Bezdead	610	C3	SVGB-1244	Zoltan	Covasna, Zoltan	596
C2	SVGB-1790	Petrosani2	Hunedoara, Petrosani	400	C3	SVGB-16145	Frasinet	Calarasi, Frasinet	15
C2	SVGB-1423	Ludesti2	Hunedoara, Ludestii de Sus	600					

Based on analyses of data obtained genetic similarity between the analyzed genotypes was calculated resulting dendrogram (fig. 1).

The 61 genotypes evaluated fell into 3 clusters *(fig.1*). The groups formed by RAPD analysis are not correlated with collecting site and altitude (tab. 5).

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