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ASSESSING GENETIC DIVERSITY IN ROMANIAN MAIZE LANDRACES, USING MOLECULAR MARKERS

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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. colour, kernel type and kernel size) (Murariu et al., 2002). However, only molecular markers provide information that is independent of environmental influences or of 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. One of the used methods is the RAPD method (Randomly Amplified Polymorphic DNA) (Beebe et al., 2000). Our aim was to determine the genetic variability existing among maize landraces coming from western part of Romania and to adapt a method for their distinguishing. The use of molecular characterization has allowed the study of diversity within these accessions and the investigation of genetic relationships among them, revealing a wide genetic diversity in this set of 60 accessions, having a highest genetic diversity; they belong to different groups and could be used as base populations for future breeding programs.

Keywords: maize landraces, AFLP markers, DNA fragments.

INTRODUCTION

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

Molecular markers make possible a quick examination of genetic material in a large number of samples at a relatively low cost. In an earlier research conducted in the Plant Breeding Agricultural Institute – Radzików, Poland, 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 (Rafalski et al., 1998). These primers, also known as semi-random primers, were used with success by Weining and Langridge (1991) to target diverse regions of genome in cereals.

Other method commonly used in DNA research is the Amplified Fragment Length Polymorphism (AFLP). The AFLP marker technology allows efficient DNA fingerprinting and an analysis of large numbers of polymorphic fragments on polyacrylamide gels. The AFLP technique is based on the detection of DNA restriction fragments amplified by PCR and can be used for DNA of any origin or complexity (Vos et al., 1995).

The AFLP technique has major advantages over other PCR based fingerprinting techniques. It is a fast method, requires no prior sequence knowledge and gives access to a very large range of polymorphisms, because of access to the complete genome (the non-expressed DNA is also subject to analysis).

AFLP analysis has emerged as a popular method for genetic mapping, species identification and phylogenetic analysis (Beebe et al., 2000; Schut et al., 1997; Sharma et al., 1996; Virk et al., 2000).

The objective of this paper was to determine the genetic variability existing among maize landraces coming from western part of Romania and to adapt a method for their distinguishing. 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.

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MATERIAL AND METHODS

Plant material

The maize landraces (60 accessions) taken to the molecular analyses are maintained in the Suceava Genebank. Landraces were collected during expeditions conducted from 1966 to 1994 and they originated from western part of Romania in 8 districts (Table 1).

Table 1. Origin and brief description of the 60 accessions of Romanian maize landraces included in the genetic characterization

Variant number	Collecting site	Collecting altitude (m)	Ear shape	Kernel type	Collecting year
1	Bereni, Mureş	382	cylindrical	dent	1994
2	Atid, Harghita	491	cylindrical	dent	1994
3	Batoş, Mureş	449	cylindrical	dent	1994
4	Geoagiu, Hunedoara	480	cylindrical	dent	1971
5	Vlaha, Cluj	470	cylindrical	flint	1994
6	Cluj-Napoca, Cluj	516	cylindrical	flint	1989
7	Berghin, Alba	382	cylindrical	flint	1994
8	Băiţa, Bihor	400	cylindrical	dent	1994
9	Buceş, Hunedoara	464	cilyndro-conic	flint	1994
10	Valea Seacă, Satu Mare	450	cylindrical	flint	1994
11	Mălădia, Sălaj	400	cilyndro-conic	flint	1990
12	Negreni, Cluj	498	cylindrical	dent	1990
13	Ciucea, Cluj	640	cylindrical	flint	1990
14	Luncoiu, Hunedoara	454	cylindrical	flint	1994
15	Isla, Mureş	428	cylindrical	dent	1989
16	Răzoare, Cluj	396	cylindrical	dent	1989
17	Luduş, Mureş	321	cylindrical	dent	1969
18	Cluj-Napoca, Cluj	353	cylindrical	flint	1989
19	Geoagiu, Hunedoara	480	cylindrical	dent	1989
20	Geoagiu, Hunedoara	480	cylindrical	dent	1989
21	Geoagiu, Hunedoara	480	cylindrical	dent	1989
22	Geoagiu, Hunedoara	480	cylindrical	dent	1989
23	Cluj-Napoca, Cluj	353	cylindrical	flint	1989
24	Geoagiu, Hunedoara	480	cylindrical	dent	1989
25	Cluj-Napoca, Cluj	353	cylindrical	flint	1989
26	Beriu, Hunedoara	288	cilyndro-conic	flint	1989
27	Certeze, Satu Mare	315	cylindrical	dent	1989
28	Luncanii de Jos, Timiş	500	cilyndro-conic	flint	1994
29	Răzoare, Mureş	399	cylindrical	dent	1994
30	Băiţa, Bihor	400	cilyndro-conic	flint	1994
31	Prăvăleni, Hunedoara	350	cylindrical	dent	1994
32	Geaogiu, Hunedoara	480	cilyndro-conic	dent	1994
33	Mărtineşti, Hunedoara	480	cylindrical	dent	1994
34	Luduş, Mureş	321	cylindrical	dent	1994
35	Mihăieşti, Cluj	469	cylindrical	dent	1994
36	Iclod, Cluj	251	cylindrical	dent	1994
37	Gyula, Cluj	354	cylindrical	flint	1994
38	Bobâlna, Cluj	210	cylindrical	dent	1994
39	Albeşti, Mureş	375	cylindrical	flint	1994
40	Acăţari, Mureş	350	cylindrical	flint	1994
41	Cârneşti, Hunedoara	410	cylindrical	flint	1994
42	Cornești, Mureş	418	cylindrical	flint	1994
43	Valea Dragului, Cluj	646	cylindrical	flint	1990
44	Ciucea, Cluj	640	cylindrical	flint	1990
45	Suatu, Cluj	328	cylindrical	flint	1989
46	Tureni, Cluj	516	cylindrical	flint	1989
47	Berind, Cluj	444	cylindrical	flint	1989
48	Luncoiu, Hunedoara	410	cylindrical	dent	1991
49	Răstoliţa, Mureş	700	cylindrical	flint	1991

50	Deda, Mureş	550	cylindrical	flint	1991
51	Comşeşti, Cluj	539	cylindrical	dent	1992
52	Cârneşti, Hunedoara	410	cylindrical	flint	1967
53	Acăţari, Mureş	350	cylindrical	dent	1969
54	Bobâlna, Cluj	210	cylindrical	flint	1966
55	Geoagiu, Hunedoara	480	cylindrical	flint	1971
56	Boian, Cluj	429	cylindrical	flint	1973
57	Cluj-Napoca, Cluj	476	cylindrical	dent	1984
58	Geoagiu, Hunedoara	480	cylindrical	dent	1971
59	Răzoare, Mureş	399	cylindrical	flint	1994
60	Sarmizegetuza, Hunedoara	600	cylindrical	flint	1994

Most of studied accessions come from mountains and sub-mountains areas (up to 400 m altitude). Accessions were collected during a long time period (28 years; Table 1) from an area between 45º30` and 48º51`N (Figure 1). For each accession, passport data were collected. Also the primary characterization was performed. Generally these local landraces have cylindrical ears and flint kernels (Table 1). The general criteria used to choose these accessions were their origin from western part of Romania.

For each accession, extracting genomic DNA was necessary. Leaves of young maize plants (14 days after emergence), were taken from each variant. They were placed in plastic tubes (Eppendorf tubes) and immediately frozen in liquid nitrogen. The samples were kept in a freezer at -70ºC.

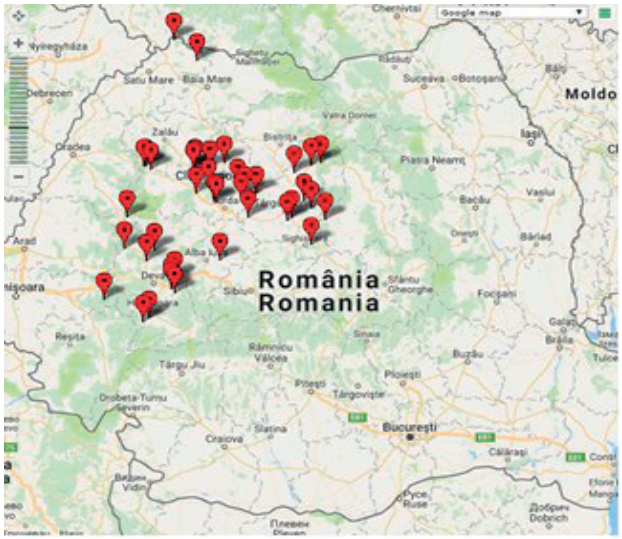

Figure 1. Collecting sites of the analysed maize landraces

Table 1. Selection of AFLP primers combinations for maize local landraces investigation

	MseI-CTT	*MseI*-CAC	*MseI*-CAT	*MseI*-CTG	*MseI*-CAA	*MseI*-CTT
EcoR I- AAG	X	X	X	X	X	
EcoR I- AGG						X

The most polymorphic 6 primers were chosen to the investigation (Table 2).

Minimum of a height of the peak taken to the analysis was 100 points. Range of the analysis was from 50 to 500 bp. Electrophoresis was conducted on 4.5% polyacrylamide denaturing gel 36 cm long, during 4 h, with voltage 2400 V. Pictures of bands were performed using Genescan software. Zero-one template was generated with application of the Genotyper software.

Cluster analysis was based on the matrix of Jaccard distance and UPGMA (Unweighted Pair Group Method Average) method.

RESULTS AND DISCUSSION

A total of 243 polymorphic bands produced, based on combined banding patterns all the 60 accessions were identified. The obtained results were transformed to binary data, taking into consideration presence or lack of DNA fragments. On this basis, using SPSS software, frequencies and similarity phenogram for the examined genotypes was generated.

The 60 accessions were classified in 20 groups (Figure 2) based on Jaccard distance and UPGMA (Unweighted Pair Group Method Average) method. Similarity level ranged from 0 to 25. The clusters were related to geographical origin (Table 3)

The first cluster (C1) comprises 7 maize landraces and come from 3 neighbour districts (Cluj, Hunedoara and Mureş). The second cluster (C2) is formed by 3 accessions coming from the same districts. The third cluster (C3) includes 2 landraces, which come from one district (Hunedoara). The fourth group (C4) contains 4 landraces, coming from Cluj and Alba districts. The two accessions grouped in C6 come from Sălaj and Timiş. These two districts are not bordering. The group eight (C8) comprises 5 populations coming from 4 districts and C9 is formed of two accessions coming from Hunedoara and Cluj. Group C10 also contains two landraces coming from the same districts like populations grouped in C9. Group C11 includes 3 landraces originating from Cluj. The largest group (C12 – 11 accessions) is formed by populations coming from 5 districts (Cluj, Hunedoara, Mureş, Satu Mare and Bihor). This cluster is formed by two sub-groups with six and five accessions, respectively. C14 is another group which is formed of two subgroups with six and two populations, originating from Mureş, Cluj and Hunedoara. The group C18 comprises two populations coming from Mureş and Hunedoara. Groups C5, C7, C13 C15, C16, C17, C19 and C20 include only one population for each group.

Table 3. Collecting sites and clustering groups of analysed accessions

Variant number	Collecting site	Cluster number	Variant number	Collecting site	Cluster number
3	Batoş, Mureş	C1	5	Vlaha, Cluj	C11
12	Negreni, Cluj	C1	18	Cluj-Napoca, Cluj	C11
17	Luduş, Mureş	C1	25	Cluj-Napoca, Cluj	C11
22	Geoagiu, Hunedoara	C1	2	Atid, Harghita	C12
24	Geoagiu, Hunedoara	C1	4	Geoagiu, Hunedoara	C12
35	Mihăieşti, Cluj	C1	8	Băiţa, Bihor	C12
43	Valea Dragului, Cluj	C1	15	Isla, Mureş	C12
37	Gyula, Cluj	C2	20	Geoagiu, Hunedoara	C12
40	Acăţari, Mureş	C2	27	Certeze, Satu Mare	C12
45	Suatu, Cluj	C2	29	Răzoare, Mureş	C12
52	Cârneşti, Hunedoara	C2	21	Geoagiu, Hunedoara	C12
9	Buceş, Hunedoara	C3	31	Prăvăleni, Hunedoara	C12
26	Beriu, Hunedoara	C3	34	Luduş, Mureş	C12
6	Cluj-Napoca, Cluj	C4	36	Iclod, Cluj	C12
7	Berghin, Alba	C4	30	Băiţa, Bihor	C13
13	Ciucea, Cluj	C4	46	Tureni, Cluj	C14
23	Cluj-Napoca, Cluj	C4	49	Răstoliţa, Mureş	C14
33	Mărtineşti, Hunedoara	C5	50	Deda, Mureş	C14
11	Mălădia, Sălaj	C6	51	Comşeşti, Cluj	C14
28	Luncanii de Jos, Timiş	C6	54	Bobâlna, Cluj	C14
42	Corneşti, Mureş	C7	55	Geoagiu, Hunedoara	C14
10	Valea Seacă, Satu Mare	C8	56	Boian, Cluj	C14
39	Albeşti, Mureş	C8	59	Răzoare, Mureş	C14
44	Ciucea, Cluj	C8	48	Luncoiu, Hunedoara	C15
47	Berind, Cluj	C8	41	Cârneşti, Hunedoara	C16
60	Sarmizegetuza, Hunedoara	C8	57	Cluj-Napoca, Cluj	C17
19	Geoagiu, Hunedoara	C9	53	Acăţari, Mureş	C18
38	Bobâlna, Cluj	C9	58	Geoagiu, Hunedoara	C18
14	Luncoiu, Hunedoara	C10	1	Bereni, Mureş	C19
16	Răzoare, Cluj	C10	32	Geaogiu, Hunedoara	C20

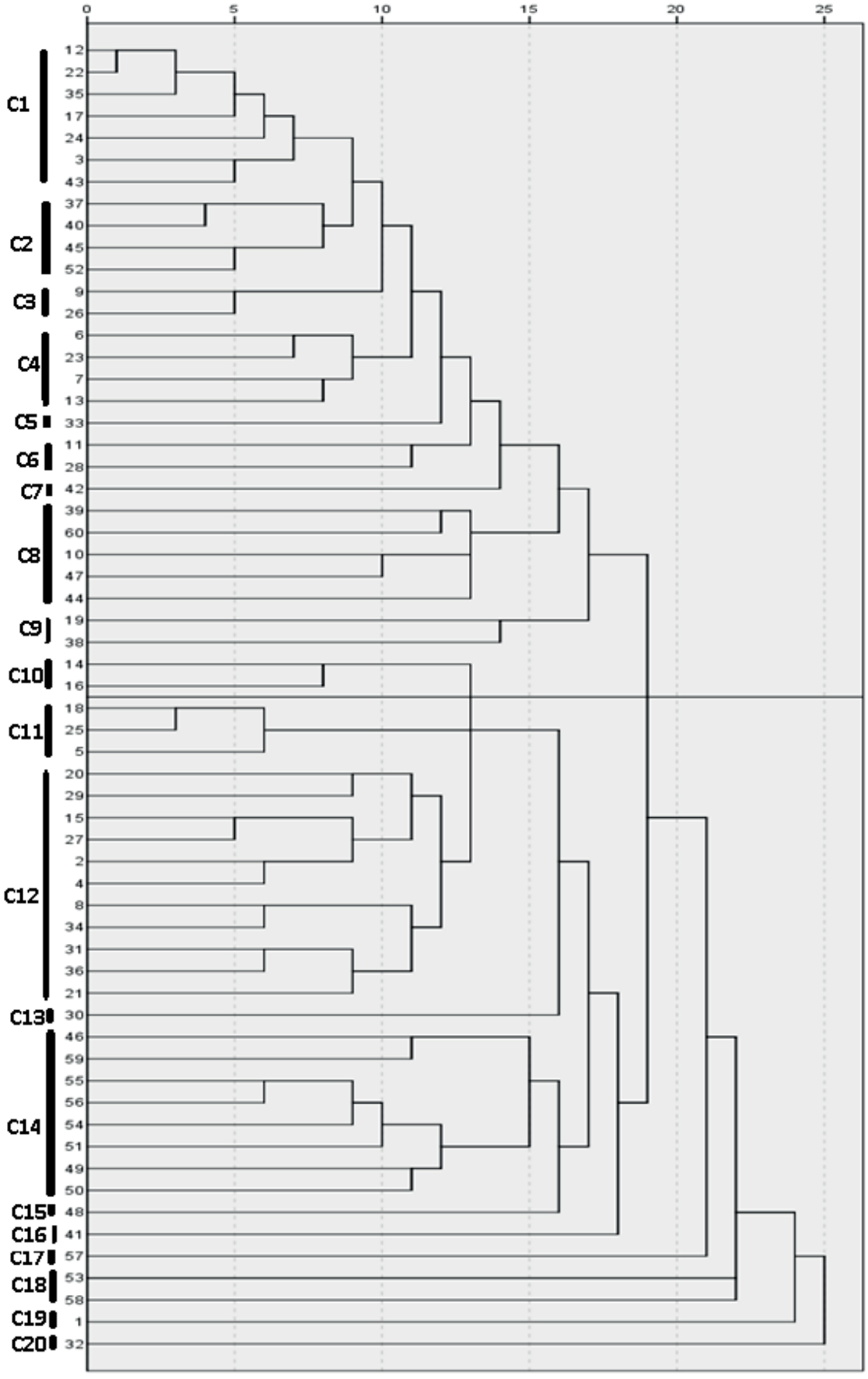

Figure 2. Cluster of the 60 maize accessions characterized with AFLP, by using the Jaccard distance and UPGMA (Unweighted Pair Group Method Average) method

Concerning the frequencies of the gene pools we noticed that many maize landraces are identical, 41 variants being gathered in 16 groups with two, three or four variants/group and 19 variants are unique (Table 4).

These results are a first approach to study genetic diversity and structure of maize landraces from the western part of Romania. These open-pollinated populations have good variability and a high potential value for breeding as a result of adaptation to cold conditions. Presumably, natural selection has concentrated favourable genes for tolerance to cold stress in order to allow the adaptation to variable biotic and abiotic stressful and unpredictable environmental conditions. Maize from the mountain and sub-mountain areas could derive from early introductions that suffered selection for adaptation through their migration from the north part of Romania. Such selection should have reduced variability; however, most of the variability is located in the northern part, suggesting that the north part could have behaved as secondary centres of diversification.

Table 4. Frequencies of DNA fragments on the analysed maize accessions

Variant number	Absence of DNA fragments (%)	Presence of DNA fragments (%)	Variant number	Absence of DNA fragments (%)	Presence of DNA fragments (%)
Var.2	71.6	28.4	Var. 7	82.7	17.3
Var.48	71.6	28.4	Var.37	82.7	17.3
Var.51	71.6	28.4	Var.52	82.7	17.3
Var.15	74.9	25.1	Var.53	82.7	17.3
Var.18	74.9	25.1	Var.3	83.1	16.9
Var.8	75.3	24.7	Var.35	83.1	16.9
Var.59	75.3	24.7	Var.11	84.0	16.0
Var.27	76.5	23.5	Var.17	84.0	16.0
Var.56	76.5	23.5	Var.24	86.0	14.0
Var.16	77.4	22.6	Var.45	86.0	14.0
Var.47	77.4	22.6	Var.32	65.8	34.2
Var.55	77.4	22.6	Var.1	67.5	32.5
Var.41	77.8	22.2	Var.20	70.0	30.0
Var.57	77.8	22.2	Var.49	70.8	29.2
Var.21	79.0	21.0	Var.29	71.2	28.8
Var.36	79.0	21.0	Var.30	72.4	27.6
Var.44	79.0	21.0	Var.4	72.8	27.2
Var.14	79.4	20.6	Var.46	73.7	26.3
Var.26	79.4	20.6	Var.50	74.1	25.9
Var.31	79.4	20.6	Var.34	76.1	23.9
Var.13	79.8	20.2	Var.54	77.0	2.0
Var.43	79.8	20.2	Var.5	78.2	21.8
Var.6	80.2	19.8	Var.33	78.6	21.4
Var.25	80.2	19.8	Var.40	80.7	19.3
Var.58	80.2	19.8	Var.42	81.1	18.9
Var.60	80.2	19.8	Var.38	84.8	15.2
Var.9	81.5	18.5	Var.19	86.4	13.6
Var.28	81.5	18.5	Var.22	87.2	12.8
Var.10	81.9	18.1	Var.12	87.7	12.3
Var.23	81.9	18.1			
Var.39	81.9	18.1			

The use of molecular characterization also allowed the study of diversity within these accessions and the investigation of genetic relationships among them, revealing a wide genetic diversity in this set of 60 accessions. Having a high genetic diversity; they belong to different clusters and could be used as base populations for future breeding programs.

CONCLUSIONS

The use of AFLP analysis for molecular characterization of a set of 60 maize landrace accessions originated from mountains and sub mountains areas of western part of Romania revealed a wide genetic diversity. This is a high genetic diversity; belonging to different clusters suggests that this set of landraces could be used as base populations for future breeding programs.

REFERENCES

Beebe, S., Skroch, P.W., Tohme, J., Duque, M., 2000. *Structure of genetic diversity among common bean landraces of middle American origin based on corespondence analysis of RAPD*. Crop Sci., 40: 264-273.

Hill, M., Witsenboer, H., Zebau, M., Vos, P., 1996. *PCR-based fingerpriting AFLPs as a tool for studying genetic relationships in Latuca spp*. Theor Appl. Genet., 93: 1202-1210.

Murariu, D., Strajeru, S., Avramiuc, M., Popa, M., 2002. *Inventory and Evaluation of the Local Maize Landraces in Romanian Bucovina (NE Carpathian Mountains). Broad Variation and Precise Characterization-Limitation for the Future*. Eucarpia, Section Genetic Resources: 240-244.

Rafalski, A., Wiśniewska, I., Gaweł, M., 1998. *PCR-based system for evaluation of genetic relationship among inbred lines of maize and rye*. Journal of Applied Genetics, 39A: 90.

Schut, J.W., Qi, X., Stam, P., 1997. *Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley*. Theor Appl. Genet., 95: 1161-1168.

Sharma, S.K., Knox, M.R., Ellis, T.H.N., 1996. *AFLP analysis of the diversity and phylogeny of Lens and its comparison with RAPD analysis.* Theor Appl. Genet., 93: 751-758.

Virk, P.S., Newbury, H.J., Jackson, M.T., 2000. *Are mapped markers more useful for assessing genetic diversity?* Theor Appl. Genet., 100: 607-613.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Pelman, J., Kuiper, M., Zabeau, M., 1995. *AFLP: a new technique for DNA fingerprinting.* Nucleic Acids Res., 23: 4407-4414.

Weining, S., Landridge, P., 1991. *Identification and mapping of polymorphism in cereals based on polymerase chain reaction.* Theor. Appl. Genet., 82: 209-216.