CHARACTERIZATION OF THE NATIONAL LOCAL MAIZE LANDRACES

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Abstract

Maize is one of the most important crops in Romania. In the mountainous and sub-mountainous areas there has been a great variety of local landraces, which have been replaced over time by maize hybrids. The local maize landraces preserved in the Suceava Genebank are the result of collecting from all country areas, both through the care of RICTP Fundulea and the Agricultural Research Stations: Suceava, Turda, Șimnic, Lovrin, Albota, Podu Iloaiei and Geoagiu (1957-1975), as well as of the collecting laboratory within Suceava Genebank (1990-2021). The paper presents the results of the characterization of 1358 local maize landraces obtained within 30 years. Morpho-physiological observations, biochemical and genetic analyzes were performed, both at Suceava Genebank and at the other research institutions. The characterization of local landraces has led to the identification of gene sources useful for maize breeding of the important characteristics (yield capacity, precocity and quality, resistance to low temperatures and to Fusarium infection). A significant number of populations have shown good agronomic stability, and can be considered potential sources of genes for improving the tolerance of maize to biotic and abiotic stress factors and the quality of new created hybrids, which in addition to high yield capacity to have a high protein content, to be resistant to low temperatures and to Fusarium infection.

Key words: maize locale landraces, morpho - physiological characterization, secondary evaluation, molecular biology

The maize, a particularly generous plant species, due to its production potential, through its wide use as a source of food for humans and animals and raw material for industry, extended in cultivation on large areas throughout the world, as well as in Romania, is placed on a priority place in the development of a modern and performing agriculture.

Due to the importance and role of maize in the Romanian economy, over time a series of technical and organizational rules were organized and applied for the continued growth of maize production. Important in this regard are the works of breeder's maize, starting with the improvement of local populations, in order to improve some of their deficient characteristics: poor resistance to root and stalk lodging, low production level, etc.

The long journey taken by maize germplasm from the wild form to the domesticated form and to its spread throughout the world proves the validity of the principle that germplasm is alive and therefore moves and transforms, being transmitted over time from one generation to another, and its mobility allows it to spread easily. The domestication of maize taking place on the American continent respectively in Mexico, with 4250 years B.C., after a long period of growth in this new state, in the area of origin, for 6000 years, arrives in the year 1412 in Europe, first in the west of the old continent and from here directly or through intermediate countries, throughout the region (Murariu M. *et al*, 2012; Murariu D., Cristea M., 2019).

Between 1631 and 1679 maize arrived on the territory of Romanian regions in the form of a germplasm, somewhat transformed, several forms can be distinguished. Among these, a more obvious differentiation was found in the flints forms with the "hard grain", probably as a result of the introduction of this type of maize, earlier in Romania compared to the dent one, but we appreciate that the main motivation found was the Romanian peasant's preference for this type of maize. Once it arrived on the territory of Romania, maize was received with great interest and joy, constituting a special event for the Romanian peasant, who got closer to maize, perhaps more than any other species (Andronescu D., 1938; Antonescu S., 1932).

The entire cultivated area was represented by a huge volume of germplasm, made up of local populations and varieties, which later formed the basis for the creation of improved varieties and hybrids, obtained from inbred lines.

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The varieties and local populations were collected from almost all the maize growing areas in our country, through the care of the National Agricultural Research Institute, Fundulea (eng. Cozma Octavian) and the Agricultural Research Stations, Turda (Dr. Ing. Lucia Roman), Lovrin County Timiș (dr. Șuba Ritus), Albota, Jud. Argeș (dr. Ștefan Ion), Șimnic, Jud. Olt (dr. eng. Ilicievici S.) Podu Iloaiei, Jud. Iași (dr. eng. Tamara Petrovici), Geoagiu, Hunedoara County (dr. eng. Homorodeanu V.) and Suceava, Suceava County (Mihai D. Cristea). Part of the research material collected from the whole country was maintained, studied and analyzed in the period 1957-1973, under the conditions of the Suceava Agricultural Research Station, by the team of researchers from the maize breeding laboratory led in that time by Mihai D. Cristea (Cristea M., 1977).

In these expeditions, 1880 samples were collected from 32 counties. Researchers from the other institutions stored the samples in uncontrolled environmental conditions, reproduced them in the field, but over time, some of them were lost, others were donated to agricultural universities, and the smallest part was sent to Plant Genetic Resources Bank "Mihai Cristea" Suceava.

Since 1967, the Suceava Agricultural Research Station, at the initiative of M. Cristea, inventoried all the collected samples and stored them at a temperature of +7°C in metal containers, until 1990, when the Suceava Plant Genetic Resources Bank was established, all the populations were transferred to the Bank, being stored in medium and long-term controlled atmosphere conditions. In addition to the local populations received from the Suceava Agricultural Research, the researchers from the Suceava Genebank collected maize local populations from all Romanian geographic areas. During the 32 years, many of them, have being characterized, morpho - physiologically, biochemically and molecularly, point of view.

MATERIAL AND METHOD

The field and laboratory evaluation of local maize populations, stored in the Suceava Gene Bank, was carried out by:

-Morpho-physiological characterization in the field – 1358 accessions;

- Cold resistance testing, under laboratory conditions – 346 accessions;

-Biochemical characterization - 890 accessions (protein, starch and fats);

-Molecular characterization -615 accessions.

Morphological - physiological characterization in the field. The morphological characterization of the local maize populations, originating from different areas of Romania (*figure 1*) was carried out on the basis of the international morpho-physiological descriptors edited by the International Institute of Plant Genetic Resources (BIOVERSITY) based in Rome. 12 morphological descriptors regarding plant architecture were analyzed: plant height, insertion height of the main spike, total number of leaves, number of leaves to the main spike, length and width of the leaf from the main spike, elements of panicle architecture, diameters maximum and minimum of the stem and 11 morphological descriptors for ear and kernel: length of ear, maximum diameter and minimum diameter of ear, number of rows of grains on ear, number of grains per row, length, width, thickness and shape of grain, weight of grains on ear and the weight of 1000 grains.

It was also considered appropriate to determine the resistance of local maize populations to the root and stalk lodging and infection with mycotoxins produced by *Fusarium verticillioides* and *Fusarium graminearum*, using the score system described by Naumova (1972) and Booth's formula (1971).

Cold resistance testing under laboratory conditions. After it was accomplished the morphological descriptors, the resistance to low temperatures of the maize populations was determined, under laboratory conditions, by performing the coldest index, according to the method developed by Debert (1988) and adapted by Rotari and Comarov (1992).

Biochemical characterization – it was carried out in the biochemical laboratory, where chemical analyzes were carried out on the grain, determining the protein (Kieldahl Method, % d.m.), fat (Soxhlet Method) and starch contents (Ewers Method).

Molecular characterization. The main objective of this study was to evaluate the genetic diversity of some local maize populations, preserved ex situ at the Suceava Gene Bank and to identify possible duplicates. It was used two methods: RAPD (Random Amplified Polymorphic DNA and SNP (Single Nucleotide Polymorphism).

RESULTS AND DISCUSSIONS

Morphological - physiological characterization in the field. The following estimators were calculated for the morphological and physiological descriptors: average ($\bar{x}$), amplitude of variation, variance (s^2), coefficients of variation (s%) and correlation coefficients (r).

The 23 morphological descriptors regarding the architecture of the plant, ear and kernel in the characterized maize populations are presented in *table 1*.

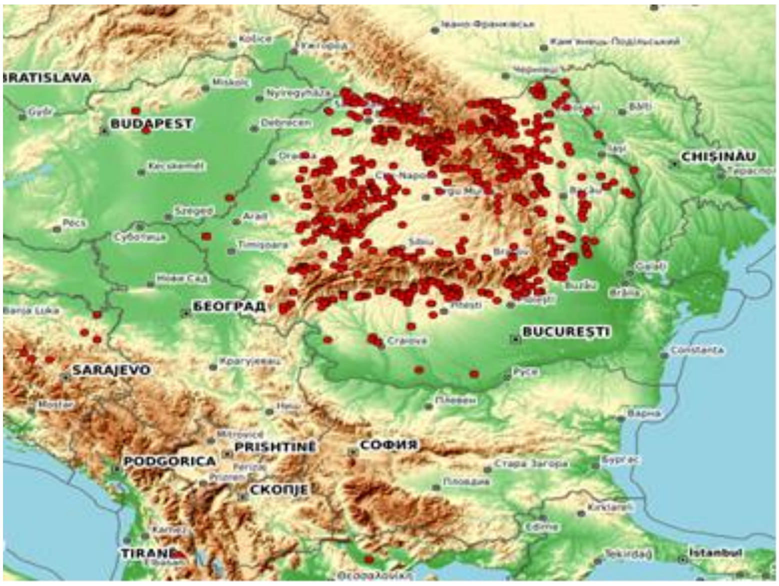

Figure 1 **Geographical distribution of maize local landraces conserved in Suceava Gene Bank**

Table 1

Estimators values of morphological descriptors at plants and ears for maize local landraces characterized in experimental field

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)	Tassel length (cm.)	Peduncle length (cm)	No. of primary branches	No. of secondary branches	Maximum diameter of the stem (mm)	Minimum diameter of the stem (mm)
Average	194,78	64.60	10.53	6.02	70.64	8.37	61.54	24.24	13.00	2.11	18.31	9.72
Max.	260,00	109.00	14	8.00	89.00	11.00	95.00	34.00	29.00	11.00	32.00	14.60
Min.	129,00	27.00	7	3.00	49.00	6.00	44.00	13.00	2.00	0.00	12.00	6.80
s^2	445,93	211.87	1.58	1.07	50.49	0.93	31.15	9.62	18.52	2.44	5.12	1.67
s%	10,84	22.54	11.94	17.18	10.06	11.52	9.07	12.80	33.10	74.03	12.36	13.30
Estimators	**Ear length (cm)**	**Maximum diameter of the ear (mm)**	**Minimum diameter of the ear**	**No of kernel rows**	**No of kernel/row**	**Kernel length (mm)**	**Kernel width (mm)**	**Kernel thickness (mm)**	**Kernel weight/on ear (g)**	**Weight of 1000 kernels**		
Average	16.84	42.15	33.80	12.07	33.95	9.66	8.73	4.56	116.49	302.57		
Max.	30.20	67.00	44.10	18.00	45.00	12.00	11.50	6.10	221.00	504.00		
Min.	8.30	21.10	20.40	8.00	18.00	6.20	5.30	3.20	26.00	124.00		
s^2	6.14	25.88	12.43	3.85	19.69	0.82	1.35	0.21	919.75	4957.07		
s%	14.71	12.07	10.43	16.26	13.07	9.37	13.31	10.05	26.03	23.27		

The interpretation of the results is based on the determination of the coefficient of variation, as an expression of the diversity of the biological material studied. In *table 1*, a high coefficient of variation is observed in the descriptors: insertion height of the main ear, number of primary and secondary panicle branches, weight of kernels/ear, number of kernels row and weight of 1000 kernels.

Medium values of the coefficient of variation were recorded for the descriptors: plant height, total number of leaves/plant, number of leaves to the main ear, length and width of the leaf above the main ear, peduncle length, maximum and minimum stem diameter, ear length, maximum and minimum ear diameter, number of kernels/row.

Minimum values of the coefficient of variation were found for the descriptors: tassel length, grain length, grain thickness.

Regarding the infection levels of maize kernels with *Fusarium*, in the analyses made at the phytosanitary control laboratory on approximately 200 accessions, on the ears it was observed, that the predominant species is *Fusarium verticilliodes*. In the figure 2 it is noticed that approximately 56% of the samples are highly resistant to this pathogen.

Cold resistance testing under laboratory conditions. The results regarding the assessment of resistance to low temperatures (coldest index) in the analyzed maize genotypes are presented in figure 3. The coldest index revealed 132 accessions very resistant to low temperatures out of the 350 tested.

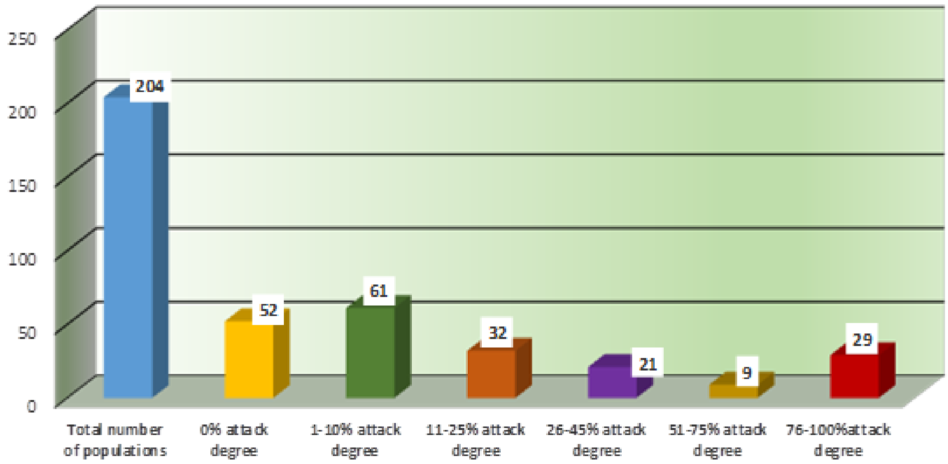

Figure 2 **Genetic sources for resistance to fusarium verticillioides**

Biochemical characterization was carried out over 30 years, and if we refer only to two components: protein and fat, we notice that out of the 890 characterized samples, 261 exceed the average protein content (11.03% d.m.) and 498 exceed the average fat content (4.08% d.m.) (*figure 4*).

From all the characterized local populations, it was selected 10 local populations that presented high values of the yield components, very resistant to low temperatures and to *Fusarium*, having a good agronomic stability and could be considered potential sources of maize improvement intended for growing in humid and cold area of Romania (*table 2*).

Molecular characterization - Starting from 2005, based on collaborations with the Gene Bank, Radzikow from Poland and USAMV Iaşi, was performed on 140 local populations. In order to determine the genetic similarity of the studied populations, RAPD method, was used resulting in a total number of 91 bands with sizes between 74 and 1687 bp, of which 86 were polymorphic *(table 3*).

The grouping of the variants into genetically related groups was carried out with the help of the similarity coefficient Lei and Ni and UPGMA (unweighted pair-group method arithmetic average).

The lowest number of amplified fragments was 6 (A15) and the highest number was 17 (ROTH B13 and B14). As seen in table 4, the band level polymorphic for the 8 primers used in the RAPD analysis ranged between 83% (ROTH A15) and 100% (ROTH A16, A17 and B08).

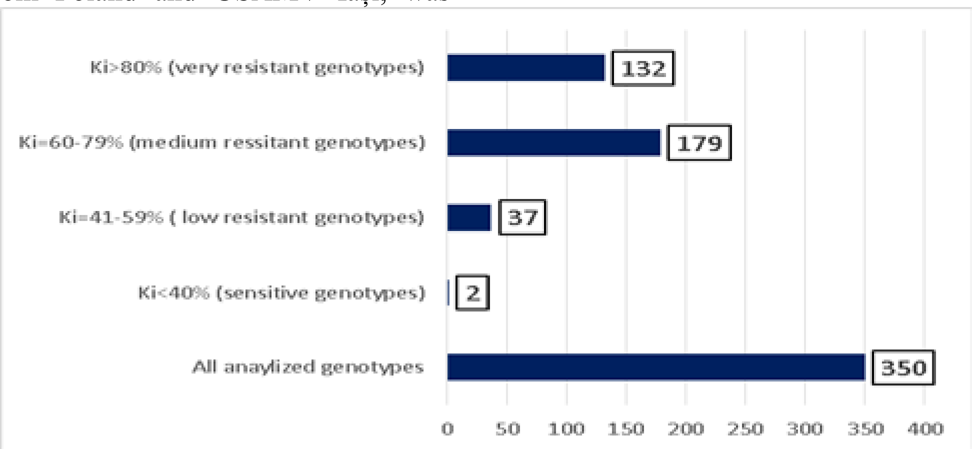

Figure 3 **The low temperatures resistance testing of maize local landraces, in the lab conditions)**

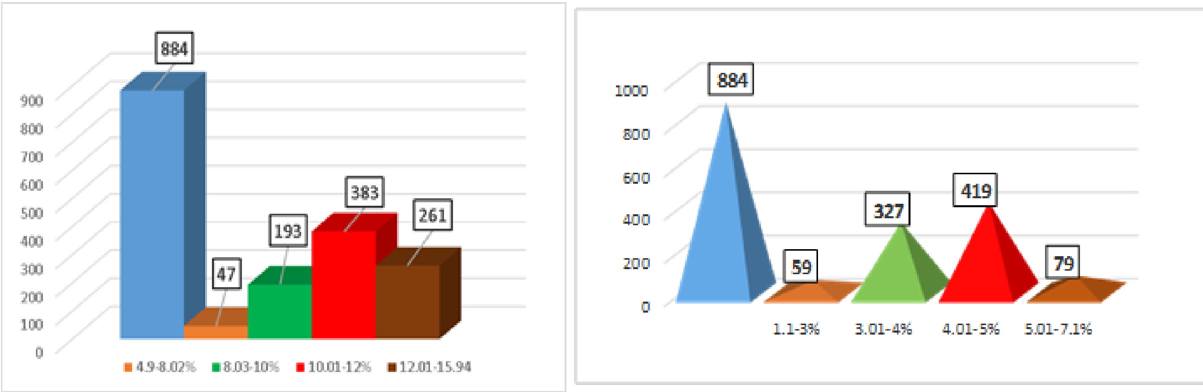

Figure 4 **The protein and fat contents of the maize local landraces analyzed**

Table 2

The productivity components of top ten local landraces, very resistant to low temperatures and the low infection level with Fusarium

Accession number	Collecting site	Elevation (m)	Infection level with *Fusarium* (%)	Coldest index (k)	Weight of 1000 kernels (g)	Kernel weight/ ear (g)	Number of kernels/ ear
SVGB-595	Ceahlău, Neamț	450	1.2	0.86	304.00	0.86	372.00
SVGB-7754	Valea Siliștii Arges	**400**	**4.2**	**0.94**	**368.00**	**0.94**	**310.00**
SVGB-5172	Teregova, Caras-Severin	**436**	**2**	**0.93**	**304.00**	**0.93**	**456.00**
SVGB-1790	**Petroșani, Hunedoara**	**400**	**0**	**0.93**	**392.00**	**0.93**	**350.00**
SVGB-8012	**Meteș, Alba**	**490**	**0**	**0.95**	**352.00**	**0.95**	**396.00**
SVGB-5874	Runcu, Gorj	289	5.7	0.86	492.00	0.86	340.00
SVGB-499	Ilva Mică, Bistrita Nasaud	464	2	0.86	300.00	0.86	462.00
SVGB-3973	Balcești, Vâlcea	944	2.5	0.89	376.00	0.89	400.00
SVGB-14453	Șoimuș, Bistrita Nasaud	**550**	**4.5**	**0.94**	**252.00**	**0.94**	**504.00**
SVGB-9591	Satu Mare, Harghita	628	0	0.89	336.00	0.89	336.00
HS Bucovina	**Standard**	**360**	**3.5**	**0.72**	**368.00**	**0.72**	**574.00**

Table 3

The number of amplified fragments and polymorphic bands and the percentage of polymorphism for each primer used in RAPD analysis

Primer	Number of Amplified fragments	Polymorphic fragments	Fragment size (bp)	Percent Polymorphism (%)
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%

In 2018, a collaboration was initiated with the Institute of Biological Research of Cluj, for the molecular characterization of 475 Romanian local maize populations. The SNP (Single Nucleotide Polymorphism) method was used. SNP data confirm the locations of four genetic poles (*figure 5*). The largest genetic pole being in the north of the country, which extends into three more areas, these being considered reservoirs of the genetic diversity of the local maize germplasm in Romania.

The results were presented by the project coordinator (Miclăuș Mihai) at the International Maize Conference in March 2021, USA, in the paper "Molecular analysis of local maize germplasm from SE Europe".

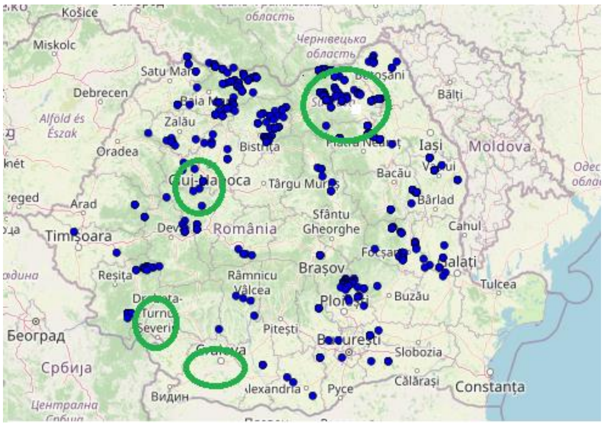

Figure 5 **Geographical distribution of the analyzed populations and the presence of genepools according to SNP molecular analyzes**

CONCLUSIONS

By carrying out these studies and analyses, in the 30 years of morpho-physiological, biochemical and molecular characterization of the local maize populations preserved in Suceava Genebank, results of real interest were obtained, from which the following conclusions can be drawn:

1. The morpho-physiological and biochemical characterization of the local populations, preserved in Suceava Genebank, led to the creation of some useful genetic funds for breeding programs, as follows:
   - High resistance to low temperatures - 132 local populations;
   - High protein and fat content – 245 local populations;
   - High resistance to *Fusarium verticillioides* – 135 local populations;
2. The use of molecular markers allowed the study of genetic diversity within these populations and the investigation of genetic relationships between them, revealing a wide genetic diversity within the analyzed local maize populations.
3. The molecular characterization of local populations using the SNP (Single Nucleotide Polymorphism) method allowed the identification of four genetic diversity centers of the Romanian local maize germplasm.

AKNOWLEGMENTS

The local maize populations preserved in the Bank are the result of collection from almost all areas of the country, both by the Fundulea Cereals and Technical Plant Research Institute and the Suceava, Turda, Șimnic, Lovrin, Albota, Podu Iloaiei and Geoagiu Agricultural Research Stations.

In the period 1957-1975, breeders from the mentioned institutions carried out expeditions in all areas of the country (Cristea M., Cosmin O., Mureșan T., Ilicievici S., Suba T., Gologan I., Rusanovschi V., Căbulea I., etc.). Since 1990, with the establishment of the Suceava Gene Bank, local maize populations were collected by researcher who worked or still work at Suceava Genebank (Lazăr Doina, Murariu Danela, Avramiuc M., Străjeru Silvia, Cherecheș V., Batîr Rusu Diana, Şandru D., Constantinovici Dana, etc).

Morpho-physiological observations, biochemical and genetic analyzes were carried out by researchers who worked at the Suceava Gene Bank (Danela Murariu, Domnica Daniela Plăcintă, Drochioiu Gabi, Popa Mirela, Ibănescu Manuela, Moroșan Petruța) and in other Romanian research institutions: (ARDS Suceava – Murariu Marius; ARDS Turda - Voichița Haș, Ioan Haș), University of Life Sciences, Iași (Simioniuc Dănuț), Center for Biological Research Cluj Napoca (Miclăuș Mihai) and from abroad (Radzikow Gene Bank, Poland – Weslaw Podyma).

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