Field and laboratory screening of Romanian maize landraces very resistant to low temperatures

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ABSTRACT

Submountain and mountain areas of Romania have a wide variety of maize landraces that have not been characterized, and landraces varieties may be a good source of new allelic diversity for useful traits. There 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 >84%, selected from a total of 300 studied local landraces. They were characterized in the field and laboratory from morphologic, agronomic and physiologic point of view, as a basis for use of this maize accessions in a breeding program for creating of extra earlier hybrids. Landraces maize accessions: SVGB-1790, SVGB-7754, SVGB-8012, SVGB-14453, SVGB-5172 were identified with high yield components traits and extremly resistant to low temperatures (K>0,92).
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 and to *Fusarium* infection).
This accessions have a good agronomic stability, could be potentials donors of genes to improve maize tolerance to low temperature in order to create hybrids for cold and wet areas.

Key words: maize landraces, genetic resources, coldtest index, kernels,

INTRODUCTION

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 necesity, 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 programms.

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 (Cristea, 2006; Hallauer and Miranda, 1981; Murariu et all 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 areals, represents important reserves of useful genes 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 submountain and mountain areas of Romania.

A comprehensive assessment of these genetic resources could be achieved through morphological, physiological, biochemical and molecular characterization (Karp et all,1995, 1997, 1998, Welsh et all, 1990, Williams et all, 1990, Pejic 1998), subordoned 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

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.

MATERIALS AND METHODS

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 submountain and mountain areas of Romania.

The biologic material was represented by 300 accessions belonging to local landraces coming from different areas of Romania (figure 1).

First, it was determined the resistance to low temperature with the coldtest index after Rotari and Comarov (1992) method, such us:

The seeds of the each sample are sown in two physiological vase using 30 grains. Plants are grown at 14h in the light (10-15.000 lx,), 10h dark. After 14-15 days at emergence of the third leaf, one vase from each sample is transferred at 4-5^0 C in dark and 8-9^{0}C in light. The samples are kept in these conditions 7 days. And the end of the stress period, the samples is transferred in the initial conditions. At this temperature, the plants are kept 14-15 days. After this period we cut 20 plants from each vase, and we determined the dry weight. After that we determined Ki index (proportion between the dry weight of the samples stressed and the samples grew in the normal conditions.)

When Ki> 0,85 very resistant plants to the low temperature;

0,80-0,85 resistant plants to the low temperatures;

0,6-0,79 medium resistant plants to the low temperatures;

0,4-0,59 low resistant plants to the low temperature;

< 0,4 weak plants to the low temperatures;

The 61 maize landraces with values of coldtest index >84% (figure 2), were selected from all studied samples (300 accessions), for morphological, biochemical and physiological characterization.

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 destinated 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 vigour and sum of the termic degrees to the silking and tasseling data, as proxy indicators of precocity.

For the morphological and physiological descriptors it were calculeted the following estimators: the arithmetic average (x), the variation amplitude, variance (s^2) and variation coefficient(s%). 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) and a specific formula used by Booth, (1971).

REZULTS AND DISCUSSIONS

In this study, 22 morphological descriptors for characterization of plant architecture, ear and grain were determined (table 1). 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 secundary branches of pannicle and grain weight per ear.

The middle values of variation coeficient 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, peducle 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.

The coldtest index, which was determined in laboratory, attests a different ressistance 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.

In order to emphasize the correlations between coldtest index and two agronomic traits (1000 kernel weight and kernel weight/on ear) it assigned the regression lines**.** Kernel weight/ear was positively correlated to coldtest index (r=0,24*) (figure 3) and also was positively colletated to 1000 kernel weight (r=0,25*) (figure 4).

The correlations between these traits, suggesting that there are local varieties very resistant to low temperatures and with high values of 1000 kernel weight and kernel weight/ear, may be adapted to low temperature environment.

The maize populations show a protein content between 9.67 and 12.08%. We observed that there are many maize local landraces with higher protein content than the other Romanian hybrids (for example, the Romanian hybrids: Montana, Bucovina, Nordic = 10.00-11.12% protein content). 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% (table 2), 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 low to middle attack of *Fusarium moniliforme* and a low attack of *Fusarium graminearum*.

Majority of accessions are infected with *Fusarium moniliforme*, except accession SVGB-7900 which is infected with *Fusarium graminearum* (table 2).

According to collecting sites altitude of 61 maize local landraces very resistant to low temperatures, it noticed that accessions coming from sites with altitude below 20 hm (Buzau, Calarasi, Dolj etc.) presents middle to intense attack of *Fusarium*.(figure 5)

If we analize some direct components of the grain yield on the top ten maize landraces extremply resistant to low temperatures and the HS Bucovina (table 3) it noticed that there are two maize populations more resistant to low temperatures (k>0,90), and with higher values of the agronomic descriptors: a thousand graing weight, grain individual weight (SVGB-1790, SVGB-7754) then control variety Bucovina.

In concluzion all top ten maize accessions very resistant to low temperatures, were found to have high yield components traits. This accessions have a good agronomic stability and could be potential donors of genes to improve maize tolerance to low temperatures.

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Table 1

Morphological and physiological descriptors estimator's values on 61 maize local landraces characterized

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 (%)**	**Proteine 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

Table 2

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

No. crt.	Accession number	Origin (county)	Infection percentage (%)	No. crt.	Accession number	Origin (county)	Infection percentage (%)
1	SVGB-1357	Vrancea	0	32	SVGB-1244	Covasna	3,2
2	SVGB-1790	Hunedoara	0	33	SVGB-1179	Mures	3,5
3	SVGB-5483	Gorj	0	34	SVGB-5226	Prahova	3,5
4	SVGB-8012	Alba	0	35	SVGB-8026	Alba	3,5
5	SVGB-9577	Harghita	0	36	SVGB-952	Harghita	3,7
6	SVGB-9591	Harghita	0	37	SVGB-981	Satu Mare	3,7
7	SVGB-9920	Alba	0	38	SVGB-5168	Caras Severin	3,7
8	SVGB-7624	Dambovita	0,2	39	SVGB-7811	Cluj	4
9	SVGB-7701	Valcea	0,7	40	SVGB-7812	Cluj	4,2
10	SVGB-8022	Alba	0,7	41	SVGB-4019	Mehedinti	4,2
11	SVGB-3764	Vrancea	1	42	SVGB-7754	Arges	4,2
12	SVGB-4005	Dolj	1	43	SVGB-7820	Cluj	4,2
13	SVGB-5219	Prahova	1	44	SVGB-5557	Valcea	4,5
14	SVGB-595	Neamt	1,2	45	SVGB-14453	Bistrita Nasaud	4,5
15	SVGB-3971	Gorj	1,2	46	SVGB-911	Cluj	4,5
16	SVGB-7745	Arges	1,2	**47**	**SVGB-7900**	**Gorj**	**5**
17	SVGB-9919	Sibiu	1,5	48	SVGB-1806	Cluj	5,2
18	SVGB-5880	Gorj	1,5	49	SVGB-8865	Bacau	5,7
19	SVGB-9966	Harghita	1,5	50	SVGB-5874	Gorj	5,7
20	SVGB-7645	Mehedinti	1,7	51	SVGB-845	Satu Mare	5,7
21	SVGB- 499	Bistrita Nasaud	2	52	SVGB-9800	Hunedoara	6,2
22	SVGB-1399	Hunedoara	2	53	SVGB-11231	Neamt	6,5
23	SVGB-3599	Bistrita Nasaud	2	54	SVGB-3722	Bistrita Nasaud	6,7
24	SVGB-5172	Caras Severin	2	55	SVGB-7750	Arges	7,5
25	SVGB-1015	Covasna	2,2	56	SVGB-16145	Calarasi	10
26	SVGB-1640	Hunedoara	2,5	57	SVGB-11584	Botosani	10
27	SVGB-3973	Cluj	2,5	58	SVGB-4023	Dolj	17
28	SVGB-9887	Cluj	2,5	59	SVGB-1423	Hunedoara	17
29	SVGB-4813	Dolj	3	60	SVGB-8043	Buzau	20
30	SVGB-9807	Hunedoara	3	61	SVGB-7282	Bistrita Nasaud	20
31	SVGB-11575	Botosani	3.2				

Table 3

Maize yield components for the best ten maize landraces very resistant to low temperatures and the control variety HS Bucovina

Accession number	Collecting site	Collecting altitude (m)	Grain weight/ear (g)	A thousand grain weight (g)	Cold test index (k)	Grain number/ ear	Grain individual weight (g)
SVGB-499	Bistrita Nasaud	464	141.00	300.00	0.86	462.00	0.30
SVGB-14453	Bistrita Nasaud	550	167.00	252.00	0.94	504.00	0.33
SVGB-5172	Caras-Severin	436	109.00	304.00	0.93	456.00	0.24
SVGB-1790	Hunedoara	400	128.00	392.00	0.93	350.00	0.36
SVGB-7754	Arges	400	109.00	368.00	0.94	310.00	0.35
SVGB-8012	Alba	490	136.00	352.00	0.95	396.00	0.34
SVGB-595	Neamt	450	107.00	304.00	0.86	372.00	0.28
SVGB-3973	Cluj	944	156.00	376.00	0.89	400.00	0.39
SVGB-9591	Harghita	628	98.00	336.00	0.89	336.00	0.29
SVGB-5874	Gorj	289	139.00	492.00	0.86	340.00	0.40
HS Bucovina	**control variety**		**190.00**	**368.00**	**0.72**	**574.00**	**0.33**

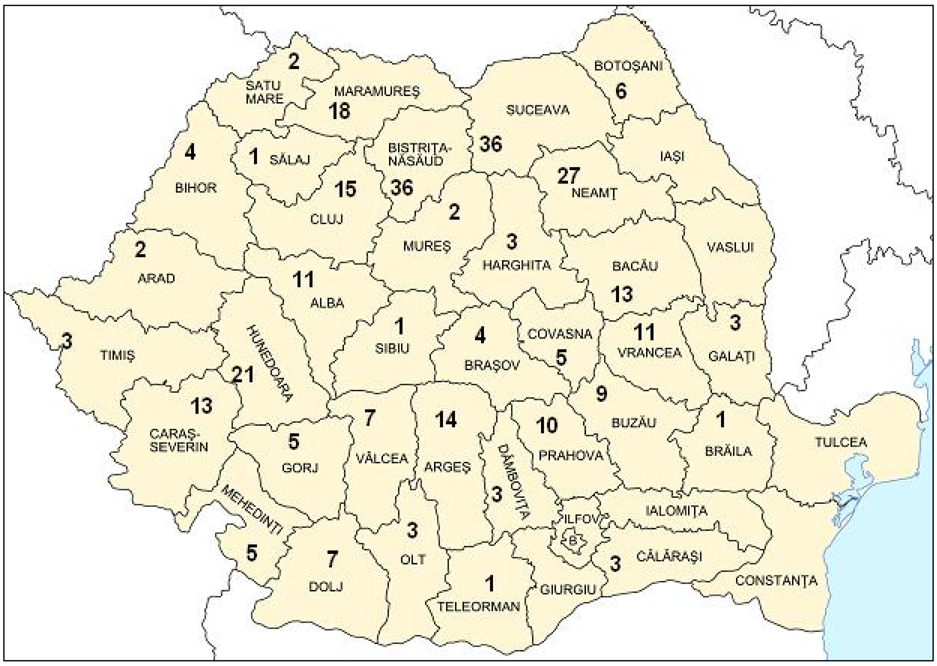

Fig. 1. Collecting sites of 300 accessions coming from different areas of Romania

Fig. 2. Collecting sites of 61 accessions, resistant to low temperatures, coming from different areas of Romania

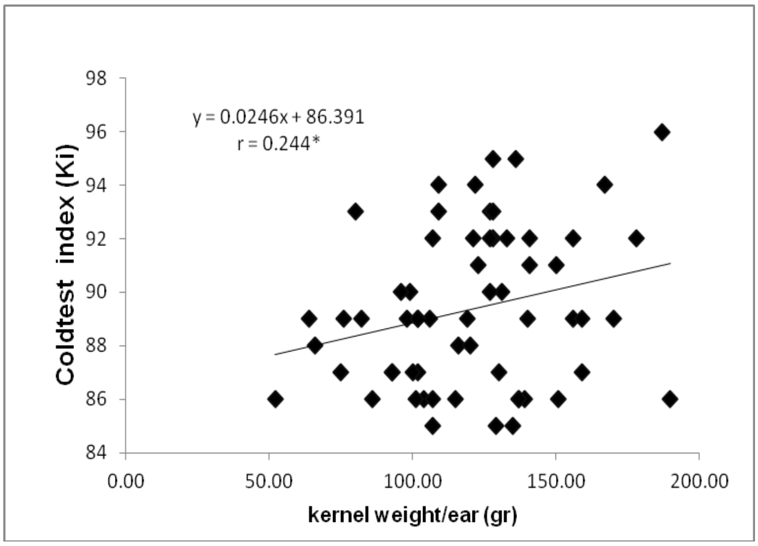

Figure 3. Relationships between kernel weight/ear and coldtest index for 61 maize landraces very resistant to low temperatures.

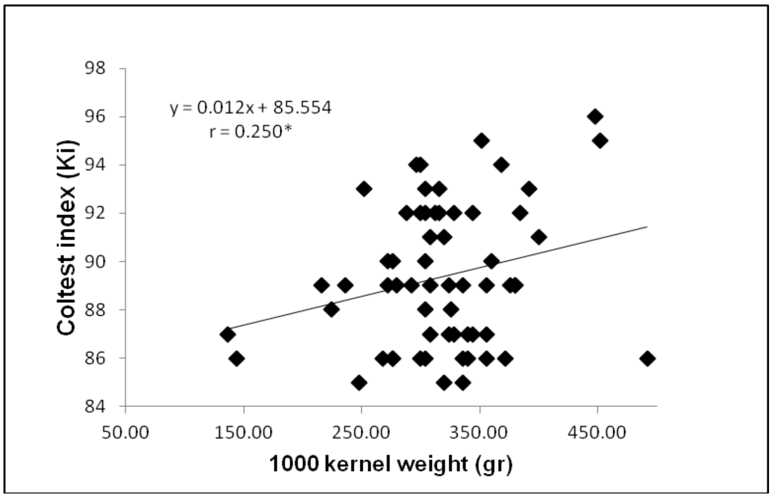

Figure 4. Relationships between 1000 kernel weight and coldtest index for 61 maize landraces very resistant to low temperatures.

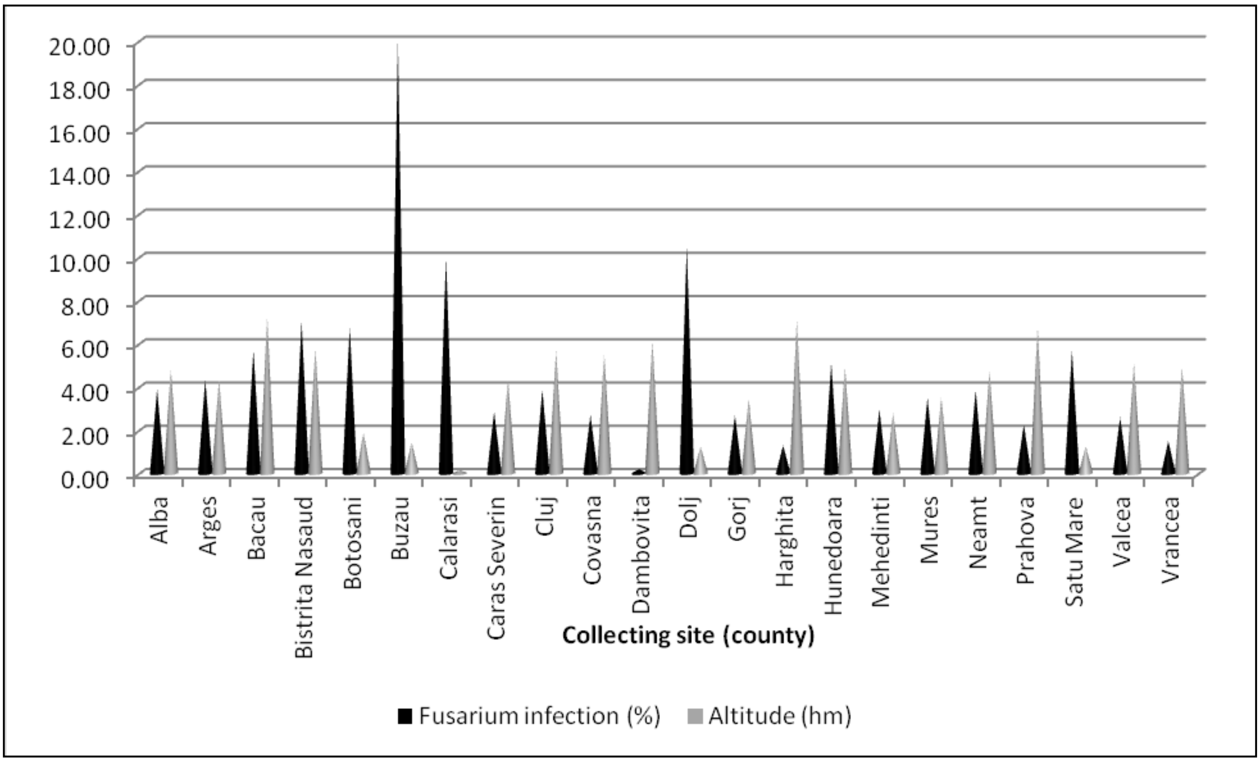

Figure 5. ***Fusarium*** **infection and collecting site altitude on 61 maize local landraces analyzed**

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