

**MAIZE GENE BANK COLLECTIONS AS POTENTIALLY  
VALUABLE BREEDING MATERIAL**

Jelena VANČETOVIC, Snežana MLADENOVIC DRINIĆ, Milosav BABIĆ,  
Dragana IGNJATOVIĆ-MICIĆ and Violeta ANDELKOVIĆ

Maize Research Institute, Belgrade, Serbia

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Characterization and evaluation of the genetic resources provide breeders with valuable information on an effective utilization of the genetic resources in breeding programs. In this paper we present the results of different research programs aimed at identification of superior genotypes among MRI gene bank accessions, regarding stress tolerance (drought and herbicides), better nutritional quality (phosphorus) and specific traits (cytoplasmic male sterility - CMS). Fifty-two genotypes were identified as a potential source for drought tolerance. Considering herbicide tolerance only genotypes with resistance to the Pivot were found. Within 100 sources of

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*Corresponding author:* Jelena Vančetović, Maize Research Institute, S. Bajica 1,  
11185 Belgrade, Serbia, e-mail: vjelena@mrizp.rs

CMS in the collection S cytoplasm was identified as the predominant type. Phytate analysis of 60 maize populations identified three groups of populations – with low (8), intermediate (25) and high (27) phytate content. The results of these researches, which are a part of pre-breeding activities, will be included in MRI breeding programs, with the aim of developing new genotypes with improved traits important in commercial maize breeding and seed production.

*Key words:* CMS, drought, genetic resources, maize, phytate

#### INTRODUCTION

Introduction and germplasm exchange, collection, characterization, evaluation, documentation and conservation are essential steps that cannot be overemphasized. An appropriate synchronism among these activities is required for the bank to be effective in maintaining genetic variability and to assure germplasm utilization. Activities in germplasm banks demand qualified researchers in several areas of expertise. Besides the conservation of genetic variability for the future, the actual utilization of available accessions is another important goal. (NASS and PATERNIANI, 2000). The main factors responsible for the low utilization of plant genetic resources are lack of documentation and adequate description of collections, lack of the desired information by breeders, accessions with restricted adaptability, and lack of collections evaluations.

The search for superior genotypes regarding yielding ability, disease and pest resistance, stress tolerance or better nutritional quality is very hard, competitive and expensive. This is why breeders tend to concentrate to adapted and improved materials, avoiding wild parents, landraces and exotics, available in germplasm banks which would require long time, high financial support besides the difficulty to identify potentially useful genes. MARSHALL (1989) emphasized that the difficulty to identify useful genes is the main factor responsible for the low utilization of these accessions. Evidently, there is a gap between available genetic resources and breeding program activities. While germplasm banks try to preserve as much as possible the genetic variability to be used by breeders, breeding programs do not explore efficiently the available diversity, relying almost exclusively on their working collection.

Hybrid maize breeding is a very competitive enterprise and the trend is that this competition is likely to increase. Breeders are almost exclusively using their working collections, and recently they have started to use F1 hybrids to develop new populations and inbred lines (PARKS, 1993). In that way, a genetic base is narrowed and all disadvantages of a uniform material are increasing.

In a short time, available commercial hybrids probably will be very similar since they partake in the same genetic background. Genetic vulnerability must be a constant concern in plant breeding for all species. Theoretical expectations and actual experience show that the use of uniform materials can be dangerous, as occurred with cytoplasm T, susceptible to *Helminthosporium maydis* race T in the United States in 1970 and next year in other regions of the world.

Pre-breeding is the most promising alternative to link genetic resources and breeding programs. This concept refers to all activities designed to identify desirable

characteristics and/or genes from unadapted (exotic or semi-exotic) materials, including those that, although adapted have been subjected to any kind of selection for improvement. As pre-breeding is being carried out, the resulting materials are expected to have merit to be included in ordinary breeding programs.

The need to keep the amount of accessions in genetic banks in a manageable number is becoming more evident. In this way, an additional advantage of well succeeded pre-breeding programs is the establishment of core collections. The main goal of the core is to represent the genetic diversity of a crop species and its relatives with a minimum of repetitiveness. BROWN (1989a) suggested that the core should consist of about 10% of the collection, which represents nearly 70% of the genetic variability. It is important to consider that a core collection is a dynamic rather than a static set of accessions. It is possible to introduce new accessions into the core and also replacements due to changes of breeder's needs.

MRI gene bank is one of the largest maize collections in the world. The entire collection (6371 accessions) is consisted of the local and the introduced collection. The local collection (2217) comprises of local landraces collected from all agroecological regions of former Yugoslavia. The accessions are classified according to their morphological traits, origin and evolution into 18 agroecological groups (PAVLIČIĆ and TRIFUNOVIĆ, 1966; RADOVIĆ *et al.*, 2000). The collection of the introduced maize germplasm is made of accessions (2254 introduced inbred lines and 1335 introduced heterozygous accessions) received from approximately 40 different countries.

In this paper we present results of the search for superior genotypes regarding stress tolerance (drought and herbicides), better nutritional quality (phosphorus) and specific traits (CMS). The search as a part of the pre-breeding activities will be included in MRI breeding programs, with the aim of developing new genotypes with improved traits important in commercial maize breeding and seed production.

#### *Drought tolerance*

During the last four decades July was the month with highest temperatures and with the greatest precipitation deficit in Serbia. This is the most critical period for the grain yield formation due to flowering stage and early grain filling in maize. Breeding drought tolerant maize hybrids include testing of significant number of genotypes and their characterisation under conditions of appropriate environments.

New sources of drought tolerance were searched among the gene bank accessions. The entire material of the MRI gene bank, as well as, some commercial inbred lines and hybrids, was subjected to water stress in Egypt, at the Setz station of the Agricultural Research Centre. This material was divided into five groups, according to the duration of the growing season: extra early, early, medium late and late. Five groups of the experimental material were sown separately and were irrigated until the appearance of the first tassels (within each group), which was approximately two weeks before flowering of the earliest accessions within groups. After that, no irrigation was applied. The material was scored visually before harvesting and the best accessions were recorded. This was done by scoring stay-green and total appearance of the accession. A total of 672 accessions was selected

on the basis of ASI, % of plants with seed set, % of seed set and % of grain filling for further experiments on three locations - Egypt, Macedonia and Serbia/Zemun Polje (ANDJELOVIC et al., 2010).

The same traits were also observed next year and 51 most tolerant accessions were selected.

The information on combining ability (CA) is also necessary for classification of the selected material into heterotic groups. The drought tolerant material, but without good CA, is not acceptable for selection. Therefore, these 51 accessions were crossed to three elite inbred testers from three heterotic groups that mutually combined well (Lancaster, BSSS and independent source).

Testcrosses were studied in field trials in Zemun Polje. The hybrid ZP 341, as one of the most yielding and most drought tolerant ZP hybrids, was included into the trials as a check. The criterion for the selection of good test-crosses was the yield (not significantly different from the check) or the performance index (based on grain yield and grain moisture at harvest) that was over 100% in comparison to the check. Combining abilities and the supposed genetic relatedness with observed heterotic sources are presented in Table 1.

Table 1. Combining abilities and the supposed genetic relatedness with observed heterotic sources of the most drought tolerant maize accessions from the MRI gene-bank

Supposed genetic relatedness with	No. of investigated acc.	Good CA with
BSSS	1	Lancaster, Independent source
Lancaster	4	BSSS, Independent source
Lancaster, BSSS	3	Independent source
Unknown	7	BSSS, Lancaster, Independent source
Independent source	3	BSSS, Lancaster
Independent source, BSSS	7	Lancaster
Independent source, Lancaster	9	BSSS
Useless	17	None

It is important to underline that seven accessions had good CA with all three observed heterotic sources. They enable broadening of genetic variability and these accessions can make a completely different source of favourable germplasm.

The further work with the 34 accessions (17 did not combine with any of the testers used) could be performed in two directions. The long-term approach for the breeding programme include development of synthetics from accessions *per se*, considering a relatively low frequency of favourable alleles for more important agronomic traits (ear height, resistance to lodging, diseases and pests, etc.), except drought tolerance that had already been determined in them. The short-term approach for breeding programmes includes development of synthetics from the test-crosses of the selected accessions per heterotic groups with related testers. Developed synthetics will already have 50% of favourable alleles for important agronomic traits, originating from testers.

A parallel S1-HS recurrent selection, with the use of inbred testers, is recommended for the improvement of developed synthetics. The programme will broaden the genetic base of the elite breeding material and new elite inbred lines

with improved drought tolerance will be developed. They could be used for new generations of maize ZP hybrids of higher yielding potential and adaptability.

#### *Herbicide tolerance*

Control of weed species crops is increasingly based on the cultivation of genotypes tolerant to broad spectrum herbicides, i.e. total herbicides. Methods of recombinant DNA (genetic engineering) can be used in producing genetic modified (GMO), i.e. transgenic plants. Tolerance of plants to herbicides can be also conducted by using somaclonal variability, mutations and conventional breeding methods (DYER, 1996).

Using mutants has a few advantages in comparison to the GMOs: regulations are much clearer, no special isolations are necessary, and there is no danger for their ban. MRI gene bank germplasm was analyzed for the mutants potentially resistant to beneficial broad-spectrum herbicides.

Eleven herbicides potentially useful in maize were chosen for the experiment (Table 2). Table 3 shows the list of the steps of the experiment over progenies and years. Only the resistance to the Pivot herbicide was found (active ingredient imazethapyr), and the genes controlling this trait were dominant. Segregation of resistant versus non-resistant plants to imazethapyr in F2 progenies for each combination of tester x resistant plant is presented in Table 4. Few modes of inheritance were found for these genes (three complementary dominant genes, two complementary dominant genes and one dominant gene). For practical purposes (incorporation in the non-resistant material), the source of one dominant gene is the most appropriate. A large number of so-called unclassified plants could be seen in Table 4. These are plants that showed symptoms of non-resistance in the time of the treatment, but they fully recovered till the pollination time. If genetic control of this resistance is really mediated by three dominant complementary genes, these unclassified plants may be a result of the gene dosage in some genotypes. If this is true for maize, it could also be true for other members of the family *Poaceae*, i.e. weeds, thus some degree of resistance could be expected when using this herbicide.

Table 2. Applied herbicides and their rates on the material of the Gene Bank of MRI, Zemun Polje, Belgrade, in 2001

Herbicide	Active ingredient	Applied dosage
Arsenal	Imazapyr	750-1500g/ha a.T.
Agil 100-EC	propaquizafop	80-150g/ha a.T.
Gallant	haloksifop-etoksi-etil	62.5-187.5g/ha a.T.
Focus ultra	cyklosidim	100-400g/ha a.T.
Fusilade super	fluazifop-p-buthyl	125-500g/ha a.T.
Furore super	fenoxaprop-p-ethyl	5-150g/ha a.T.
Leopard 5-EC	quizalofop-p-ethyl	25-200g/ha a.T.
Pantera 40-EC	quizalofop-p-tefuryl	40-60g/ha a.T.
Pivot 100-E	imazethapyr	80-200g/ha a.T.
Roundap	glyphosate-izopropyl ammonium	960-5760g/ha a.T.
Select super	clethodim	96-240g/ha a.T.

Table 3. Steps of the experiment

Season	Procedure
2000/01	Making 11 bulk samples of accessions from our gene-bank
2001	Bulks treated, and development of testcrosses (F1s) of 5 plants resistant to imazethapyr
2002	Selfing of testcrosses (F1s) and deriving of F2s
2003	Storm destroyed experimental material
2006	F2 segregation analysis, and selfing of resistant F2 plants to produce true-breeding

Table 4. Segregation of resistant versus non-resistant plants to imazethapyr in F2 progenies for each combination of tester x resistant plant

# plants	$\Sigma$ plants	# resistant plants	# non-resist.pl.	$\chi^2$ for 3:1 segreg. <sup>B</sup>	Real segregation ratio	$\chi^2$ for the real ratio of segreg. <sup>B</sup>
2						
T30x2 <sup>A</sup>	269	104	165	189.44**	27:37	1.371 ns
T31x2	366	200	166	80.88**	9:7	0.384 ns
T32x2	501	157	344	509.4**	27:37	24.18**
5						
T31x5	353	215	138	37.4**	9:7	3.1 ns
7						
T26x7	1069	560	509	291.57**	9:7	6.487*
T27x7	244	127	117	68.55**	9:7	1.75 ns
8						
T25x8	1312	721	591	281.17**	9:7	0.895 ns
T26x8	1802	1305	497	6.4*	3:1	6.4*
10						
T30x10	187	136	51	0.515 ns	3:1	0.515 ns
T31x10	323	230	93	2.48 ns	3:1	2.48 ns
T32x10	418	300	118	2.32 ns	3:1	2.32 ns

<sup>A</sup> T – tester<sup>B</sup>  $\chi^2_{0.05}$  for one degree of freedom is 3.84 $\chi^2_{0.01}$  for one degree of freedom is 6.63

\*,\*\* -statistically significant at 0.05 and 0.01 level, respectively

### Cytoplasmic male sterility

Within MRI genebank collection over 100 sources of cytoplasmic male sterility (CMS) were found in field trials, i.e. more than 2% of the total accession numbers (VIDAKOVIĆ et al., 2002). These sources are distributed among Yugoslav open-pollinated varieties, as well as introduced heterozygous genotypes and inbred lines. In order to identify cytoplasm types the gene-bank sources of CMS were screened using a PCR assay with specific primers for C, T and S cytoplasm (LIU et al., 2002). The predominant CMS type within the analyzed accessions was S cytoplasm. Clear presence of some type of CMS was found in 4.56% of all the former Yugoslav varieties, which is a significant percentage and S type makes 84% of all CMS found. S type was also predominant (44%) within introduced

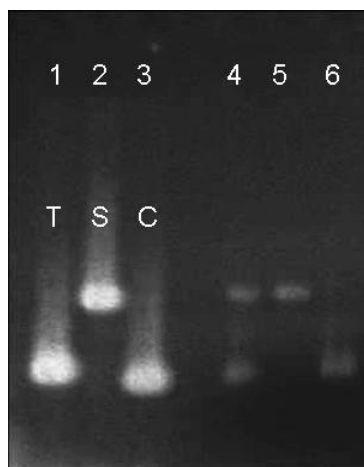
populations. This significantly lesser presence of CMS-S within introduced populations could be the consequence of a smaller sample size analyzed. On the other hand, introduced populations and lines (among which CMS-T was predominant) are of different provenience, coming from different parts of the world and it was shown that, for example, among populations from Argentina the dominant type was CMS-C and from Italy CMS-S (Table 5).

PCR analysis of two former Yugoslav populations showed two bands – one band of 799bp characteristic for CMS-S and another of approximately 400bp size (Pictré 1). The size of this additional band is in between the sizes of PCR products characteristic for C and T cytoplasm. Both bands were of similar intensity. The accessions were analyzed three times and each time the amplification products were the same. These results could indicate the presence of a special sub-type of male sterility, which is still to be checked. One of the possible explanations could be the occurrence of mutations in S cytoplasm genome. Genetic analysis of S-type cytoplasm, which is the largest among the three CMS maize types (WEI *et al.*, 1997) and at the same time the least stable (ESCOTE *et al.*, 1985), has shown that some unstable male sterile plants in CMS-S populations can arise from mutations in cytoplasm genomes.

Table 5. Identified CMS types of the analyzed maize accessions

Provenience	# of accessions analyzed	Identified CMS types					
		S	C	T	No band	Unconclusive results	Two bands
Former Yugoslavia	119	85	12	3*	15	2	2
Argentina	2	-	4	-	-	1	-
Bulgaria	5	2	-	-	-	-	-
Czechoslovakia	3	1	-	4	-	-	-
DDR	4	-	-	3	-	-	-
Greece	2	2	-	2	-	-	-
Iran	12	-	-	1	1	-	-
Italy	1	11	-	1	-	-	-
Jordan	3	-	-	1	-	-	-
Mexico	2	1	-	2	-	-	-
Poland	1	-	-	1	1	-	-
Romania	2	1	-	-	-	-	-
USA	6	-	-	2	-	-	-
Former USSR		3	-	3	-	-	-

\*one of the accessions was analyzed only by classical method of testing with restorers for each known type of CMS



Picture 1 PCR amplification of CMS types with specific primers for C, T and S cytoplasm  
Lanes 1, 2 and 3 – controls for CMS-T, CMS-S and CMS-C; Lane 4: amplification product giving a new profile with two bands; Lane 5: CMS-S; Lane 6: CMS-T.

One more source of CMS was discovered – an accession which carries CMS-T cytoplasm (VANČETOVIĆ et al., 2007). This investigation was done by classical method of testing with restorers for each known type of CMS and lasted three years, much longer than testing of all other accessions by the method of LIU et al. (2002). The multiplex PCR approach enabled a simple, fast and reliable large scale screening of maize cytoplasm within MRI gene bank accessions, reducing time for cytoplasm characterizations from several years to a few weeks (VANČETOVIĆ et al., 2010). Indirectly, this also helps in pre-breeding procedures when presence of specific sterile cytoplasm is required, with the final goal of commercial production of maize hybrids.

#### *Phosphorus*

The fact that the most of phosphorus is bound in phytate cause many of the problems with phosphorus content in maize grain (RABOY, 2001). Therefore, increasing of amount of available P and reduction the amount of phytate would be desirable. One approach to decrease phytate and increase Pi is through recurrent selection that uses the indigenous quantitative genetic variation. Analysis of 60 maize populations from Maize Research Institute Genebank was conducted to identify genotypes that had either low or high concentration of phytate (DRINIC et al., 2009). Genetic variability in phytate contents was observed, with values ranging from 1,147 to 4,13 and averaged 2,91 g kg<sup>-1</sup>. Pi concentration was between 0,35 and 1,29 and averaged 0,65 g kg<sup>-1</sup> (Table 6). Both phytate and Pi values were within the range of values reported for S1 populations (LORENZ et al., 2008). A larger amount of genetic variation for Pi relative to phytate was found. The low phytate



concentration was measured in eight, intermediate in 25 and high in 27 populations (table 6).

Population 216 was determined to have the lowest phytate concentration of 1,14 g kg<sup>-1</sup>, a Pi concentration 40% greater than Pi mean but lower than average protein content. Population 216 will be used for further breeding genotypes with low phytate content and good agronomic traits.

*Table 6. The phytate, Pi and protein content (Drinic et al, 2009)*

population	Pi gkg <sup>-1</sup>	Phytate gkg <sup>-1</sup>	Protein content gkg <sup>-1</sup>	Level of phytate
4	0,984	2,609	97,16	intermediate
20	0,568	2,564	97,57	intermediate
33	0,690	2,502	89,65	intermediate
79	0,565	2,591	96,26	intermediate
90	0,608	2,681	97,16	intermediate
103	0,752	2,717	98,12	intermediate
121	0,755	2,685	87,03	intermediate
128	0,693	2,690	98,60	intermediate
132	0,727	1,780	98,33	low
138	0,77	1,488	98,12	low
186	0,736	1,698	95,57	low
190	0,963	1,573	104,53	low
216	0,868	1,147	93,78	low
262	1,103	1,497	95,23	low
268	0,678	1,734	96,26	low
280	0,749	1,492	104,80	low
376	0,856	3,686	103,22	high
399	0,486	4,013	121,54	high
408	0,622	3,363	108,04	high
419	0,746	3,291	107,42	high
477	0,831	3,376	108,93	high
485	0,669	3,407	102,87	high
558	0,473	3,492	109,21	high
559	0,626	3,174	113,00	high
588	0,586	3,170	118,85	high
606	0,658	3,520	106,11	high
641	0,348	4,138	114,03	high
674	0,522	3,187	121,68	high
676	0,557	2,640	108,93	intermediate
690	0,806	4,058	112,45	high
692	0,638	3,461	112,79	high
707	0,464	3,403	115,89	high
709	0,484	2,969	96,472	intermediate
710	0,607	2,817	119,75	intermediate

754	0,505	2,754	99,15	intermediate
770	0,273	2,978	87,10	intermediate
785	0,412	2,924	98,88	intermediate
794	0,709	2,893	99,29	intermediate
802	0,692	2,646	102,25	intermediate
807	0,572	3,001	93,09	intermediate
826	0,643	3,288	99,22	high
832	0,598	3,297	113,55	high
850	0,576	3,211	111,28	high
887	0,738	3,086	97,50	intermediate
891	0,450	3,705	112,17	high
900	0,686	3,436	103,91	high
902	0,377	3,195	101,56	high
909	0,610	3,285	100,94	high
914	0,749	3,177	101,08	high
921	0,433	3,060	97,64	intermediate
937	0,570	3,020	89,65	intermediate
965	0,508	3,195	101,77	high
968	0,638	3,334	94,88	high
984	0,366	3,132	98,12	high
5006	0,742	2,760	86,76	intermediate
5011	0,518	2,876	92,34	intermediate
5027	0,867	3,123	99,5	intermediate
5030	1,048	3,002	92,82	intermediate
6739	1,079	2,706	85,72	intermediate
6741	0,949	3,146	83,45	high

Eight populations with low phytine and two populations with high phytate were analysed by 7 RAPD markers. Totally 30 alleles were detected. The number of alleles varied from eight to three, averaged 5. The lowest coefficient of similarity was between populations 132 and 690, one with low and one with high phytate. The highest coefficient of similarity was between populations 132 and 262, both with low phytate. Cluster analysis was done based on RAPD data. Two main clusters were obtained. The first one was composed of two subclusters - A consisting of three populations with low phytate and B of two low phytate populations and population 186 loosely connected with cluster I. The second cluster encompassed four populations - two with low and two with high content of phytate. Genetic dissimilarity of analyzed populations ensure good bases for reduced phytate content of breeding material from different origin.

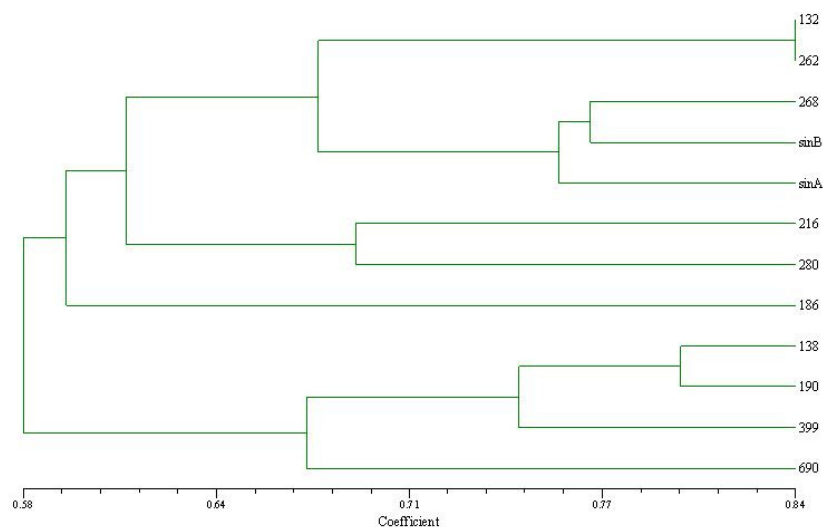


Fig., 1 Cluster analysis of eight low phytate maize population based on RAPD markers sin A and sin B –control sample

### CONCLUSION

Pre-breeding programmes carried out at MRI are a good basis for the development of new useful germplasm. It is well known that breeding today has as a consequence narrowing of genetic base, discovery and usage of new germplasm unrelated to currently used commercial germplasm is of utmost value. Certainly, pre-breeding activities will improve the knowledge about the accessions maintained in germplasm banks and will contribute to reduce the gap between available genetic resources and maize breeding programs.

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**KOLEKCIJE BANKE GENA KUKURUZA KAO  
POTENCIJALNI SELEKSIONI MATERIJAL**

Jelena VANČETOVIĆ, Snežana MLADENOVIĆ DRINIĆ, Milosav BABIĆ,  
Dragana IGNJATOVIĆ-MIČIĆ and Violeta ANDELKOVIĆ

Institut za kukuruz, Beograd, Srbija

**I z v o d**

Karakterizacija i evaluacija genetičkih resursa omogućava njihovo efikasno korišćenje u različitim selekcionim programima. U ovom radu predstavljamo rezultate različitih istraživanja usmerenih na identifikaciju superiornih genotipova iz banke gena Instituta za kukuruz, a u pogledu tolerantnosti na stres (sušu i herbicide), poboljšanih nutritivnih vrednosti (fitin) i specifičnih svojstava (citoplazmatičnu mušku sterilnost – CMS). Kao potencijalni izvor tolerantnosti na sušu identifikovano je 52 genotipa. Među uzorcima ispitivanim na herbicide nađeni su samo genotipovi tolerantni na Pivot. Što se tiče specifičnih svojstava među 100 identifikovanih izvora citoplazmatične muške sterilnosti S tip citoplazme je bio dominantan. Analizom fitina 60 populacija kukuruza identifikovane su tri grupe populacija – sa niskim (8), srednjim (25) i visokim (27) sadržajem fitina. Rezultati ovih istraživanja, koji predstavlja deo *pre-breeding* aktivnosti, će biti uključeni u selekzione programe Instituta za kukuruz, sa ciljem razvijanja novih genotipova sa poboljšanim svojstvima značajnim u oplemenjivanju kukuruza i semenskoj proizvodnji.

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