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YUGOSLAV LOCAL GERMPLASM IN HYBRID MAIZE BREEDING

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When the spread of hybrid maize began in the mid-20th century, local breeding materials were collected and assembled in gene banks in order to prevent their deterioration and enable their utilization in breeding to develop hybrid varieties. Numerous inbred lines from local populations were selected. The present study includes only those lines from the first cycle of selection that were used for the development of commercial hybrids. The contribution of domestic inbred lines to modern maize breeding consists in the following: 1) Domestic inbred lines possess great variability for most of the agronomically important traits. In nearly every group of lines (lines of the same origin comprise one group), lines with desirable traits have been found, lines that now represent new sources of variability in breeding programs on: grain yield, resistance to lodging, early maturity, ear bareness, grain quality, and other traits. 2) The domestic inbreds differ in their heterotic potential for grain yield when crossed with the inbred testers B73 (BSSS germplasm) and Mo17 (Lancaster Sure Crop germplasm). Lines originating from the populations Vukovarski Žuti Zuban, Šidski Žuti Zuban, Novosadski Zlatni Zuban, and Novosadski Žuti Zuban combine better with the inbred tester Mo17 than with B73, so they belong to the BSSS heterotic group. Lines originating from the populations Bankut Bajsa and Sočice are neutral, i.e. they re-

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spond equally to both types of germplasm (BSSS and Lancaster), so they can be said to belong to a new heterotic group.

Key words: maize germplasm, heterosis, heterotic groups

INTRODUCTION

The identity of maize races that were first grown in southeastern Europe is not quite clear. The first maize genotypes grown in Yugoslavia were the flint maize races from the Caribbean islands and the West Indies — Early Caribbean and Coastal Tropical Flint, which were grown in gardens as a vegetable crop in the 16th century (LANG *et al.*, 1962; PAVLIČIĆ and TRIFUNOVIĆ, 1966). Flint genotypes from Canada and New England arrived in Yugoslavia in the 18th century via France and central Europe (BRANDOLINI, 1968). Then, in the 19th and 20th century, dent maize from the US Maize Belt was introduced, and that was the last great introduction of maize germplasm to Yugoslavia that was of any importance for the formation of a unique dent-type maize (TRIFUNOVIĆ, 1978). The selected US dent varieties Golden Mine and Queen of Prairie were introduced at the begining of 20th century. In parallel with their introduction into production, they were subjected to intensive selection, and some years later the local open pollinated populations Rumski Zlatni Zuban, Vukovarski Žuti Zuban, and Flajšmanov Zuban (FLEISCHMANN, 1918, 1942) as well as Beljski Zlatni Zuban and Novosadski Zlatni Zuban were selected (GIBSMAN, 1956). All Yugoslav maize germplasm has been classified into 16 basic and 2 recently evolved groups (PAVLIČIĆ and TRIFUNOVIĆ, 1966).

During the five centuries of maize evolution in Yugoslavia under various climatic conditions, a wide variety of maize populations have been developed through hybridization, selection, and adaptation. Because of their favourable adaptation to a given set of agroecological conditions, local populations represented valuable selection materials that needed to be collected, assessed, and classified in order to prevent their deterioration and enable their utilization in the development of hybrid varieties that are the foundation stones of modern maize production. This paper will describe the main characteristics of maize inbred lines derived from local populations and their importance for present-day hybrid maize breeding.

MATERIALS AND METHODS

The first domestic inbred lines were developed from the local populations Novosadski Zlatni Zuban, Novosadski Žuti Zuban, Vukovarski Žuti Zuban and others in the mid-20th century using pedigree selection (SARIĆ *et al.*, 1980). Those were the lines from the first cycle of selection. After testing for tolerance to drought, severity of infection by the causal agents of leaf spot and stem, root, and ear rots, as well as hybrid yield potential, 57 inbreds were selected from a set of 1271 inbred lines between 1962 and 1964. For the present study, we chose 35 inbred lines derived from the populations Novosadski Zlatni Zuban (6 lines), Novosadski Žuti Zuban (10 lines), Vukovarski Žuti Zuban (6 lines), Bankut Bajsa (4

lines), Sočice (5 lines), and Šidski Žuti Zuban (4 lines) that were used as parental components in the development of commercial hybrids.

Experiment 1 - After harvesting in 1997, ear length (cm), cob percentage, 100-grain mass (gr), grain type, and grain color were analyzed in 10 randomly selected ears per inbred line. The stem lodging and barren plant percentages were determined, based on the number of plants broken below the uppermost ear and the number of plants without ears, respectively. The experimental plot was 20 m^2 in size and plant density 57,100 plants per hectare. ((The number of broken or barren plants per plot/the total number of plants per experimental) x 100). The significance of the mean values was estimated using the t-test for P=95% and 29 degrees of freedom.

Experiment 2 - The analyzed maize inbreds obtained from different local populations are presented on the basis of polymorphism of seven enzyme systems: Glu, ACP, MDH, PHI, PGM, PGD and IHD and their isozymes, the direct products of 11 mapped genes and their allelic variants according to STUBER *et al.* (1988). Several-day-old seedlings were analyzed and by electrophoresis and the isoenzyme expression of genes and their alleles was obtained. A single linkage between inbred lines and cluster analysis was determined for the alleles found on the basis of Euclidian distance.

Experiment 3 - In 1996, two separate spatial isolations were used to cross inbred lines with two testers. The grain yield of SC hybrids from crosses between 35 inbreds and two inbred testers (B73 and Mo17) was studied in trials carried out at Rimski Šančevi during 1997 and 1998. The trials were conducted on chernozem soil according to a randomized block design with three replicates. The experiments were set up on 19 April, 1997 and 25 April, 1998. The experimental plot included two rows of 20 hills with two kernels per hill. The rows were spaced 0.7 m apart and the hills 0.25 m apart. The hills were thinned to one plant after emergence for a final plant density of approximately 57,000 plants ha^{-1}. Data were collected for grain yield (kg ha^{-1} at 14% moisture). The standard maize growing technology was used and both sowing and harvesting were done by hand. All analyses were made using the MSTAT package.

RESULTS

Mean values and variability of traits. - Less than 1% of the total number of lines of domestic origin from the first cycle of selection were chosen for this study. Even in such a small sample (35 lines), however, great variability was found for most of the agronomic traits. In every group of lines (which consisted of lines of the same origin), there were lines with one or more desirable traits.

The lowest and highest numbers of lodged plants were found in the inbred lines originating from the local populations Šidski Žuti Zuban and Sočice (2.0 and 11.5%, respectively) (Table 1 and 2). Significant differences were also found among the lines originating from the populations Novosadski Zlatni Zuban and Sočice (Table 2). Inbred lines with 0.0% of lodged plants were selected from the populations Vukovarski Žuti Zuban, Šidski Žuti Zuban, and Novosadski Žuti

Zuban, showing that they contain desirable genes for this trait that can be incorporated into active breeding materials using the appropriate breeding methods.

Table 1. Mean values of traits of inbred lines originating from local populations

Characters	The origin of inbred lines					
	Vukovarski žuti zuban	Bankut bajša	Šidski Žuti zuban	Sočice	Novasadski zlatni zuban	Novosadski žuti zuban
Lodging (%)	5.1	7.4	2.0	11.5	6.3	4.8
Grain moisture (%)	21.4	23.6	25.2	25.4	26.9	24.9
Ear length (cm)	16.3	11.9	15.7	18.6	13.7	16.5
100 grain mass (gr)	25.6	26.1	35.3	28.2	32.9	29.0

The lowest and highest values of average grain moisture content were recorded in lines from Vukovarski Žuti Zuban, and Novosadski Zlatni Zuban (21,4 and 26,9 % respectively) (Table 1). The proportion of water in the grain significantly differed between the lines from Vukovarski Žuti Zuban and those from Šidski Žuti Zuban, Sočice, Novosadski Zlatni Zuban and Novosadski Žuti Zuban as well as between lines from Bankut Bajsa and Sočice and from Novosadski Zlatni Zuban and Novosadski Žuti Zuban (Table 2).

Table 2. Significance differences between means for inbred lines originating from local populations according to the t-test

Populations	Ear length	Grain mass	Lodging	Grain moisture
Vukovarski žuti zuban - Bankut bajša	S	NS	NS	NS
Vukovarski žuti zuban - Šidski žuti zuban	NS	S	NS	S
Vukovarski žuti zuban - Sočice	NS	NS	NS	S
Vukovarski žuti zuban - Novosadski zlatni zuban	NS	S	NS	S
Vukovarski žuti zuban - Novosadski žuti zuban	NS	NS	NS	S
Bankut bajša - Šidski žuti zuban	S	S	NS	NS
Bankut bajša - Sočice	S	NS	NS	NS
Bankut bajša - Novosadski zlatni zuban	NS	S	NS	S
Bankut bajša - Novosadski žuti zuban	S	NS	NS	NS
Šidski žuti zuban - Sočice	NS	S	S	NS
Šidski žuti zuban - Novosadski zlatni zuban	NS	NS	NS	NS
Šidski žuti zuban - Novosadski žuti zuban	NS	S	NS	NS
Sočice - Novosadski zlatni zuban	S	NS	NS	NS
Sočice - Novosadski žuti zuban	NS	NS	S	NS
Novosadski zlatni zuban - Novosadski žuti zuban	S	NS	NS	S

S ($p<0.05$); NS (non significant)

The largest mean values of the 100-grain mass were recorded in Šidski Žuti Zuban (35.3 grams), followed by lines from Novosadski Zlatni Zuban (32,9 grams) (Table 1). In contrast to this, the 100-grain mass of lines from Vukovarski Žuti Zuban was only 25.6 grams. Significant differences in 100-grain mass were found between some groups of lines (Table 2).

The average ear length varied from 11.9 cm to 18.6 cm (Table 1). Lines derived from the population Bankut Bajsa had a significantly lower ear length than lines originating from Vukovarski Žuti Zuban, Šidski Žuti Zuban, Sočice, Novosadski Zlatni Zuban, and so did lines originated from Novosadski Zlatni Zuban relative to those from Sočice and Novosadski Žuti Zuban (Table 2).

The agroecological conditions under which the local populations had been developed and grown influenced the characteristics of lines derived from them. Inbred lines developed from Vukovarski Žuti Zuban and Novosadski Žuti Zuban contain desirable alleles for resistance to lodging, ear barrenness, early maturity, and ear length (Table 3). Lines derived from the population Bankut Bajsa, in addition to having some negative traits (stem susceptible to lodging, low grain mass), were also characterized by a shorter growing season and grain of the semi-flint type. Among inbreds originating from Šidski Žuti Zuban lines were found that had a large number of grain rows per ear (16-18) and a large 100-grain mass (over 30 g). Among inbred lines originating from Sočice, there were those that had a firm stem, a low barren plant percentage, and a long stem. Although yellow dent is the dominant type of grain, semiflint and red color were also found.

Table 3. Minimum and maximum values of traits of lines originating from local populations

Characters	The origin of inbred lines					
	Vukovarski žuti zuban	Bankut bajša	Šidski žuti zuban	Sočice	Novasadski zlatni zuban	Novosadski žuti zuban
Lodging (%)	0.0-11.4	2.5-16.3	0.0-8.1	2.5-21.4	1.1-12.5	0.0-13.6
Broken plants (%)	1.1-11.4	1.2-6.3	1.1-4.6	0.0-6.7	1.2-6.2	2.2-8.6
Barren plants (%)	0.0-6.3	2.5-5.8	1.1-2.3	0.0-2.5	0.0-5.0	0.0-4.8
Cob percentage	15.0-16.8	16.6-17.0	17.2-18.4	16.0-20.2	15.8-18.6	15.2-17.8
Grain moisture (%)	19.7-29.5	21.1-25.0	23.5-27.0	24.0-26.5	23.6-29.7	21.1-28.2
Ear length (cm)	13.3-18.3	11.0-14.3	13.5-16.5	13.0-21.3	11.8-15.3	11.0-21.3
Row number	12.0-18.0	14.0-18.0	16.0-18.0	12.0-20.0	16.0-18.0	14.0-20.0
100 grain mass (gr)	22.6-26.9	25.3-26.6	29.9-37.9	23.3-30.6	31.3-34.9	18.6-44.3
Grain type	D	SF	D	D	D	D. SD
Grain color	Y. LY	Y	Y	Y	Y	Y. LY. R

D=Dent; SD=Semi dent; SF=Semi flint; Y=Yellow; LY=Light yellow; R=Red

Divergence of inbred lines on the basis of genetic markers. - In most of the lines, all the analyzed loci were homozygous, which is one of the major factors for lines to be appropriate parent components. However, for several of the lines (1, 3, 4, 5, 6, 26, 27, 34, 35) two different alleles were found (homozygous or heterozygous loci) that proved that self-pollination in this generation had been unsatisfac-

torily uniform. In the analyzed material, polymorphism of all observed loci except Pgm1 and Pgm2 (which were monomorphic) was found. Some alleles were encountered frequently, while others were found only in small numbers or in a single line. The rare alleles were Glu1-N in lines 2 and 24 and Pgd1-N and Idh2-N in line 33, which can also indicate genetic specificity and diversity in relation to the other lines.

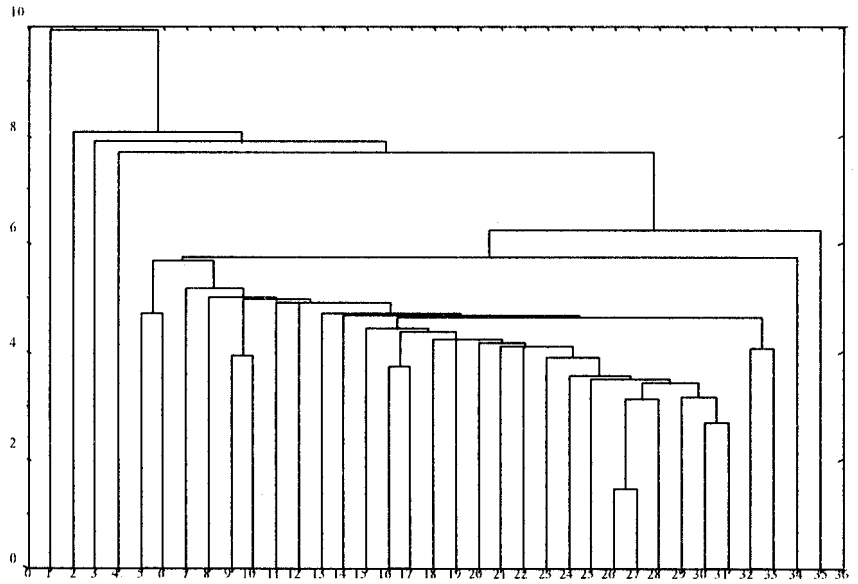

Fig.1. Cluster analysis of 36 inbred lines originating from domestic populations revealed by standard genetic distance

Single linkage based on Euclidian distance between the inbred lines revealed groups of lines, their similarities and differences (Fig 1). The greatest distance was recorded in the group of lines originating from Vukovarski Žuti Zuban (lines 1-6) and the smallest in those derived from Novosadski Zlatni Zuban (lines 22-31). This type of line grouping at the level of genetic markers is related to the origin of the population but rarely reveals the divergence of lines relative to one another. It is also possible on the basis of the chosen marker-loci that the coverage of genomes is not satisfactory and that methods for parent identification for crossing should be broadened at the level of the DNA sequence. According to MOMM and DUDLEY (1994), genetic classification of lines originating from different population groups can be done more efficiently on the basis of molecular markers than on the basis of other types of testing of unknown heterozygous effects.

Belonging to heterotic groups. - Despite the fact that breeding for heterotic pairs is not conducted systematically anywhere in the world, with the acceptance of the concept of hybrid varieties the selection of opposite groups from

which heterotic inbred lines are to be developed has become the basis of modern maize breeding.

The heterotic potential for grain yield of 35 inbreds was studied in crosses with two inbred testers B73 and Mo17, which belong to different heterotic groups (BSSS and Lancaster Sure Crop, respectively) and together form a heterotic pair due to their outstanding combining abilities. Of the 70 single cross hybrids investigated, 32 had grain yields equal to or higher than those of the standard B73 x Mo17 ($P<0,05$) (Fig 2). The grain yield potential of domestic inbred lines varied according to the type of tester used. When Mo17 was used as the tester, 26 inbreds were selected out of 35, while in the case of B73 the number was 6 out of 35. Since Mo17 and B73 represent one heterotic pair, the domestic inbred lines grouped round them can be crossed not only with the appropriate tester but also among themselves (domestic x domestic) in order to develop heterotic hybrids. Inbred lines originating from Vukovarski Žuti Zuban, Šidski Žuti Zuban, Novosadski Žuti Zuban, and Novosadski Zlatni Zuban belong to the BSSS heterotic group except for line no. 5, which belongs to the Lancaster Sure Crop group (Fig 2). Lines derived from Bankut Bajsa and Sočice do not belong clearly to either of the above two plasm types. What is more, the latter lines respond in the same way to both types of plasm, so it can be assumed that they belong to a new heterotic group, and the existence of a heterotic pair among the lines of domestic origin cannot be excluded, either.

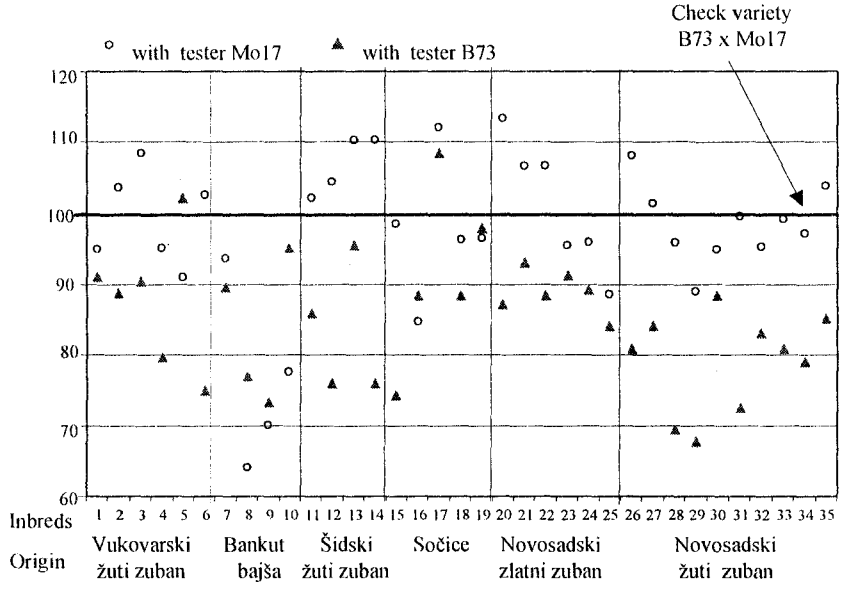

Fig. 2. Percent of grain yield of test crosses between domestic inbred lines and the testers Mo17 and B73 in comparison with the check variety (B73 x Mo17)

Based on their combining abilities, the domestic inbred lines have been divided into groups according to the germplasm type (BSSS, Lancaster, and neutral). Through a recombination of lines within each of the groups, three synthetic populations have been developed that have been included in population improvement programs. After several cycles of reciprocal recurrent selection, these populations will be used in practical breeding work as the initial selection material for the development of new inbred lines.

DISCUSSION

Yugoslav maize germplasm has arisen under the conditions of a continental, mountainous climate in the temperate climatic zone. At a wide variety of altitudes, from sea level to 1,200 m above sea level, a rich maize germplasm has been developed. It was then collected and classified into 16 ecotypes and has thus become available to maize breeders across the world (PAVLIČIĆ and TRIFUNOVIĆ, 1966). The dominant maize type in the main maize-growing regions in Yugoslavia (plains and river valleys) is the dent ecotype of the American Maize Belt Dent type. Open pollinated dent populations have thus far been used (and will remain to be used in the future as well) as the starting materials for the development of inbred lines to be employed as parental components of hybrids. Inbred lines from the first cycle of selection originating from the local populations Vukovarski Žuti Zuban, Šidski Žuti Zuban, Bankut Bajsa, Sočice, Novosadski Zlatni Zuban and Novosadski Žuti Zuban are characterized by a great variability of agronomic traits (Tables 1 and 3) and should be used in breeding programs either as sources of new variability for developing new inbred lines or directly as parental components of new hybrids.

Finding out the heterotic potentials of unknown germplasm in combination with an unrelated tester represents a convenient method for classifying unknown germplasm into heterotic groups (LEE *et al.*, 1989; MELCHINGER *et al.*, 1991; RADOVIĆ and JELOVAC, 1995).

A large number of heterotic groups has been discovered thus far, but not all of them are used to the same extent for commercial purposes. In Europe, the usual heterotic pairing is US-type dent by European-type dent or US dent by European-type flint (MIŠEVIĆ, 1989, 1990; ORDAS, 1991; MALVAR *et al.*, 1996). The European flint heterotic group was found between three old lines — EP1, F2 and F7 — and European or American lines and was preserved in the second cycle of selection (CARTEA *et al.*, 1999).

Inbred lines originating from the Yugoslav maize belt are clearly distinguishable when it comes to their heterotic potential for grain yield with Mo17 and B73, which belong to the Lancaster Sure Crop and BSSS heterotic groups, respectively. Inbred lines originating from the populations Vukovarski Žuti Zuban, Šidski Žuti Zuban, Novosadski Zlatni Zuban and Novosadski Žuti Zuban combine better with the inbred tester Mo17 than with B73 and hence belong to the BSSS type of plasm. However, lines have also been discovered in the domestic germplasm that do not belong to either of the these two most commonly used plasm types in

hybrids belonging to the FAO Maturity Groups 600 and 700; instead, they probably form an altogether new heterotic group. Similar results have been obtained by RADOVIĆ and JELOVAC (1995). They studied 125 local populations of Yugoslav origin and found heterosis for grain yield in 70 populations using the inbred tester B73, in 30 populations using Mo17, and in 84 using the local inbred line V395/31. RADOVIĆ and JELOVAC (1995) made use of local populations, whereas in our study inbred lines derived from local populations were used. Given the great variability of the local breeding materials, the heterotic behaviour of the inbred lines derived from local populations should be assessed relative to the major commercial heterotic groups, since it is expected that new heterotic pairs will be found among the domestic lines.

CONCLUSION

We recorded significant variability of phenotypic characteristics among the inbred lines from the first cycle of selection originating from the local populations Vukovarski Žuti Zuban, Bankut Bajsa, Sočice, Šidski Žuti Zuban, Novosadski Zlatni Zuban and Novosadski Žuti Zuban. In almost every group of lines, lines with desirable traits have been found. These lines represent a new source of variability in breeding programs for 100-grain mass, resistance to lodging, early maturity, grain quality, ear length, and number of grain rows.

Domestic inbred lines differ in their heterotic potential for grain yield when crossed with the Mo17 and BSSS inbred testers. The majority of local inbreds combined better with Mo17 than B73 inbred tester, while some of them responded equally to both testers. Based on their hybrids, domestic inbred lines belong to either the Lancaster Sure Crop, BSSS, or a neutral heterotic group.

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