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Original scientific article

EFFECT OF RECIPROCAL CROSSES ON GRAIN YIELD AND OTHER AGRONOMIC TRAITS IN MAIZE

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The goal of this experiment was to examine a possible influence of reciprocal crosses on grain yield and some morphological traits in maize. Field trials were set up on three locations (Zemun Polje, Pančevo, Bečej) during 2015 and 2017. Five elite inbred lines were selected in order to produce hybrid combinations for the trial, two of them were of Lancaster origin and used as tester lines (ZPT1, ZPT2), while remaining three had a Non Lancaster origin (ZPL1, ZPL2 and ZPL3). By performing reciprocal crossings between Lancaster and non Lancaster inbred lines, twelve single cross hybrids were produced for the trial. Trials were set up on three locations during two years and grain yield, grain moisture, plant height, ear height and mass of 1000 kernels were analyzed. Location, year and reciprocal crosses were significant factors for all examined traits. Reciprocal crosses had statistical significance on two hybrid combinations in terms of grain yield and grain moisture. SNPs molecular markers were used to assess the genetic diversity of the inbred lines involved in this experiment. When it comes to plant height and mass of thousand kernels, a statistically significant difference was observed in one hybrid combination. The largest reciprocal differences between original and reciprocal hybrids were recorded in grain yield, which were statistically significant for the crosses ZPH4/ZPH4R and ZPH2/ZPH2R (20.03% and 19.49% respectively). The ZPH2/ZPH2R hybrid combination is the combination with the statistically significant differences between the original and reciprocal hybrid, for all evaluated traits except for grain moisture. Reciprocal hybrids ZPH5/ZPH5R and ZPH6/ZPH6R with ZPL3 as their maternal inbred line didn't express a statistically significant differences between both variants, e.g. their reciprocal effect was low.
Key words: grain yield, inbred lines, maize, morphological traits, reciprocal effect

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INTRODUCTION

In maize breeding, it is of great importance to know the performance and combining ability of the inbred lines used as parental components of the hybrid. The knowledge of the direction of the reciprocity effects for both characters is only possible if direct and reciprocal hybrids are analyzed (RADANOVIĆ *et al*., 2015; BÓDI *et al.*, 2008).

Reciprocal effect in maize refers to phenotypic difference between reciprocal F_1 hybrids. Reciprocal effect has been recognized by several researchers as one source of genetic variability for various agronomic traits in different types of maize for diverse use (DJORDJEVIĆ *et al.*, 2016; EGESEL *et al.*, 2003; MANN and POLLMER, 1981; SEITZ *et al.*, 1995). Depending on the genotype, the reciprocal effect can be either positive, negative or even statistically insignificant. The choice of the maize inbred line to serve as a maternal or paternal component in any specific maize hybrid combination can have a considerable impact on some phenotype characteristics of the hybrid. The maternal effect is in theory usually defined as the contribution of the maternal parent to the phenotype of its off spring beyond the equal chromosomal contribution expected from both parents (GRČIĆ *et al*, 2018).

Maternal effects and reciprocal effects are important components, especially when traits are maternally determined. Ear and grain quality traits are definitely target traits for this type of analyses (JUMBO and CARENA, 2007).

Because reciprocal differences were generally not consistent across environments, there is little consensus concerning the relative importance and systematic exploitation of these effects in practical maize breeding programs. Current molecular studies may provide some insight into how reciprocal effects are modified by environmental triggers (GONZALO *et al.*, 2007).

In order to overcome the obstacles in the breeding and development of inbred lines, the use of molecular markers for the assessment of genetic distances has been proposed (NDHLELA *et al*., 2015). The advantage of this use is the possibility of assessing only the appropriate crossings of the most distant inbreds. VAN INGHELANDT *et al*. (2010) stated that Single Nucleotide Polymorphism (SNP) molecular markers are a tool used for germplasm characterization and genetic diversity assessment. They are biallelic, highly polymorphic and reproducible.

MATERIAL AND METHODS

Five elite inbred lines belonging to Maize Research Institute “Zemun Polje” were selected in order to produce hybrid combinations for the trial, two of which were used as tester lines (ZPT1, ZPT2). Selected inbred lines are of different genetic origins and belong to maturity groups ranging from FAO 400 to FAO 700.

Table 1. Heterotic group and FAO maturity groups of inbred lines

Inbred lines	Heterotic group	FAO maturity groups
ZPT1	Lancaster Sure Crop	400
ZPT2	Lancaster Sure Crop	600
ZPL1	BSSS (Iowa Stiff Stalk Synthetic)	400
ZPL2	BSSS (Iowa Stiff Stalk Synthetic)	600
ZPL3	BSSS (Iowa Stiff Stalk Synthetic)	700

Table 2. Original and reciprocal variants of investigated inbred lines

Hybrid	Parent components
ZPH1	ZPL1 × ZPT1
ZPH1R	ZPT1 × ZPL1
ZPH2	ZPL1 × ZPT2
ZPH2R	ZPT2 × ZPL1
ZPH3	ZPL2 × ZPT1
ZPH3R	ZPT1 × ZPL2
ZPH4	ZPL2 × ZPT2
ZPH4R	ZPT2 × ZPL2
ZPH5	ZPL3 × ZPT1
ZPH5R	ZPT1 × ZPL3
ZPH6	ZPL3 × ZPT2
ZPH6R	ZPT2 × ZPL3

Following traits were recorded in the trial:

- Grain yield adjusted at 14% grain moisture (kg ha^{-1}),
- Grain moisture (%),
- Plant height (cm),
- Ear height (cm), and
- Mass of 1000 kernels (g)

Each plot consisted of two 4-meters rows, with 75 cm between rows and a plant-to-plant distance of 20 cm. The data for morphological traits were recorded on ten average plants for each replication after flowering. Grain yield was recorded for each plot and then calculated for yield per hectare at 14% grain moisture. Additionally, ten ears from each genotype were selected for the measurement mass of 1000 kernels.

Investigated inbred lines were genotyped by Maize 25K XT Illumina Infinium array consisted of 23908 markers evenly distributed on the maize genome. All steps of the standard protocols for DNA extraction and analysis were conducted by TraitGenetics GmbH, Germany. Custom R script was used for quality control of raw genotyping data and unsuccessfully called SNPs (miss rate > 5%) were excluded leaving 22439 SNPs for further analysis. TASSEL software (BRADBURY *et al*., 2007) version 5.2.64 was used to calculate the distance matrix using IBS (Identity By State) similarity, with IBS defined as the probability that alleles drawn at random from two individuals at the same locus are the same. Using the distance matrix as input data, a neighbour-joining tree was constructed to graphically present inbred lines clusters.

Statistical data processing included analysis of variance - ANOVA for the random block design (RCB). The software package used for the analysis of variance was MSTAT (MSTAT Development Team, 1989). The least significant difference test (LSD) was used to compare the significance of the difference in yield and other agronomic traits between the original and corresponding reciprocal crosses tested.

RESULTS AND DISCUSSION

The results in table 3 show that years, locations, and genotypes were significant factors for most of the examined traits. Years and genotypes affected all traits at 1% level of significance. This is in accordance to results of GRČIĆ *et al.* (2018) and VARADARAJU and JOEL (2017). Different locations didn't affect variation in mass of thousand kernels only. Both Year × Location and Year × Genotype interactions were significant for all examined traits, while Location × Genotype interaction influenced yield, moisture and ear height. The only trait which was affected by all three mentioned factors was grain yield. The coefficient of variation (C_v) for examined traits ranged from 3.17% for plant height to 11.31% for grain yield.

Table 3. Factor and their interactions significance according to analysis of variance for examined traits

	Factors and Interactions	Df	Yield	Moisture	Plant height	Ear height	1000 kernels weight
1	Replication	1	0.728^{ns}	0.146^{ns}	358.471*	49.585^{ns}	711.111^{ns}
2	Year	1	202.445**	129.713**	72684.161**	7780.71**	78166.84**
3	Location	2	8.849**	22.677**	7497.093**	1479.769**	2138.064^{ns}
4	Year × Location	2	31.895**	47.371**	252.452*	1055.631**	10281.554**
5	Genotype	11	6.077**	17.986**	313.049**	152.594**	9251.168**
6	Year × Genotype	11	7.046**	17.343**	151.619**	92.823**	3349.89**
7	Location × Genotype	22	1.538**	2.732*	73.558^{ns}	75.957**	989.201^{ns}
8	Year × Location × Genotype	22	2.489**	1.357^{ns}	59.518^{ns}	30.015^{ns}	1224.262^{ns}
9	Error	71	0.647	1.479	51.447	22.767	855.213
	C_v(%)		11.31%	7.72%	3.17%	5.13%	9.09%

Table 4. Grain yield mean values for original and reciprocal hybrids and their difference (kg ha^{-1})

Yield (*kg ha^{-1}*)	ZPH 1	ZPH 1R	ZPH 2	ZPH 2R	ZPH 3	ZPH 3R	ZPH 4	ZPH 4R	ZPH 5	ZPH 5R	ZPH 6	ZPH 6R
x	7073	7722	7202	5798	7368	7476	8132	6503	7514	7813	6264	6421
Δ	-649		1404**		-108		1629**		-299		-157	
%	-9.18		19.49		-1.47		20.03		-3.98		-2.51	

*,**; Significant at the p< 0,05 and p <0.01 level, respectively

The data in Table 4 shows mean values for grain yield and the differences between original and reciprocal variants of the same hybrids. The highest yielding hybrids were original ZPH4 (8132 kg ha^{-1}), and reciprocal hybrids ZPH5R (7813 kg ha^{-1}) and ZPH1R (7722 kg ha^{-1}). The smallest differences between original and reciprocal hybrids were recorded for ZPH3/ZPH3R and ZPH6/ZPH6R, 1.47% and 2.51% respectively. The highest reciprocal differences between original and reciprocal hybrids which were statistically significant were recorded for ZPH4/ZPH4R (20.03%) and ZPH2/ZPH2R (19.49%). In both of these cases, original hybrids were higher yielding than their reciprocal variants. Both of these combinations had ZPT2 tester line as the paternal component in original hybrids. Also, all of the hybrids that had ZPT1 tester line as the maternal component (ZPH1R, ZPH3R, ZPH5R) obtained higher yields than their original combinations.

Mean values for grain moisture are presented in table 5. Hybrids with the lowest grain moisture were ZPH1 (13.55%), ZPH2R (14.20%) and ZPH2 (14.27%) all of which had line ZPL1 as one of the components. On the other side, hybrids which had line ZPL2 as the maternal component, ZPH3 and ZPH4 were the genotypes with the highest values for grain moisture.

Table 5. Mean Grain moisture values for original and reciprocal hybrids and their difference (%)

Moisture (%)	ZPH 1	ZPH 1R	ZPH 2	ZPH 2R	ZPH 3	ZPH 3R	ZPH 4	ZPH 4R	ZPH 5	ZPH 5R	ZPH 6	ZPH 6R
Σ	13.55	16.78	14.27	14.2	17.07	15.96	17.55	16.54	15.47	16.22	15.85	15.59
Δ	-3.23**		0.07		1.11		1.01*		-0.75		0.26	
%	-23.84		0.49		6.50		5.75		-4.85		1.64	

*,**; Significant at the p< 0,05 and p <0.01 level, respectively

The smallest differences in grain moisture between original and reciprocal hybrids were obtained for ZPH2/ZPH2R and ZPH6/ZPH6R. The highest difference was recorded for combination ZPH1/ZPH1R where reciprocal cross had grain moisture higher by 23.84% in comparison to the original hybrid. Coefficient of variation for this trait was 7.72%. JUMBO and CARENA (2008) in their study also found significantly different among crosses for grain yield and grain moisture.

Mean values for plant height are shown in Table 6. Hybrids ZPH5R, ZPH5, ZPH3 and ZPH3R had greatest values for this trait (234.3 cm, 233.3 cm, 231.4 cm and 228.2 cm respectively). All of these hybrids had tester line ZPT1 as one of the components (paternal in original, maternal component in reciprocal hybrids).

Table 6. Plant height mean values for original and reciprocal hybrids and their difference (cm)

Plant Height (*cm*)	ZPH 1	ZPH 1R	ZPH 2	ZPH 2R	ZPH 3	ZPH 3R	ZPH 4	ZPH 4R	ZPH 5	ZPH 5R	ZPH 6	ZPH 6R
Σ	219.4	220.8	224.9	218.5	231.4	228.2	225	226.9	233.3	234.3	224.7	225.9
Δ	-1.4		6.4*		3.2		-1.9		-1.0		-1.2	
%	-0.64		2.85		1.38		-0.84		-0.43		-0.53	

*,**; Significant at the p< 0,05 and p <0.01 level, respectively

The lowest values for plant height were recorded for hybrids ZPH2R, ZPH1 and ZPH1R sharing a common inbred line ZPL1 as the maternal component in original and paternal in reciprocal hybrids. The highest reciprocal difference was exhibited in hybrids ZPH2/ZPH2R and ZPH3/ZPH3R (2.85% and 1.38% respectively), while the lowest differences were recorded for hybrids ZPH5/ZPH5R and ZPH6/ZPH6R. This trait had the lowest coefficient of variation among all examined traits. RAHIMI and SADEGHI (2017) also found significant difference for plant height and 1000 kernels mass in their study.

Table 7. Ear height mean values for original and reciprocal hybrids and their difference (cm)

Ear Height (*cm*)	ZPH1	ZPH 1R	ZPH 2	ZPH 2R	ZPH 3	ZPH 3R	ZPH 4	ZPH 4R	ZPH 5	ZPH 5R	ZPH 6	ZPH 6R
Σ	90.3	88.1	94.6	88.6	99.2	91.1	96.7	90.8	93.8	97.5	94.4	91.8
Δ	2.2		6.1**		8.1**		5.9**		-3.7		2.6	
%	2.42		6.40		8.21		6.08		-3.93		2.74	

*,**; Significant at the p< 0,05 and p <0.01 level, respectively

Similar results were obtained for ear height (Table 7). The lowest values for this trait were obtained for hybrids ZPH1R, ZPH2R and ZPH1 which is in compliance with plant height values for which the same hybrids also had the lowest values. The greatest values for ear height were recorded for hybrids ZPH3, ZPH5R and ZPH4. Reciprocal effect was the most prominent in hybrids ZPH3/ZPH3R, ZPH2/ZPH2R and ZPH4/ZPL4R (8.21%, 6.4% and 6.08% respectively), and the least for hybrids ZPH1/ZPH1R and ZPH6/ZPH6R (2.42 % and 2.74%). In comparison to original hybrids, all of the reciprocal ones had lower values for ear height, except for ZPH5R compared to its original counterpart. Coefficient of variation for this trait was 5.13 %. VARADAJU (2017) also report similar results for ear height’s variations in reciprocal maize hybrids.

Table 8. Mean values mass of 1000 kernels for original and reciprocal hybrids and their difference (g)

Mass of 1000 kernels (*g*)	ZPH 1	ZPH 1R	ZPH 2	ZPH 2R	ZPH 3	ZPH 3R	ZPH 4	ZPH 4R	ZPH 5	ZPH 5R	ZPH 6	ZPH 6R
Σ	299.2	320	327.7	354.2	306	291.3	375.4	362.7	302.3	301	304.8	314.6
Δ	-20.8		-26.5*		14.7		12.7		1.3		-9.8	
%	-6.95		-8.09		4.80		3.38		0.43		-3.22	

*,**; Significant at the p< 0,05 and p <0.01 level, respectively

Hybrids with greatest mass of 1000 kernels were ZPH4, ZPH4R and ZPH2R as presented in Table 8. Line ZPL2 was one of the components in all of these hybrids (maternal in original, paternal in reciprocal). Hybrids ZPH3R and ZPH1 had the lowest mass of 1000 kernels. UTTAM *et al*. (2019) used 11 inbred lines and two testers in order to examine combining ability and found significantly differences among crosses for mass of 1000 kernels. The highest reciprocal effect was exhibited in hybrids ZPH2/ZPH2R and ZPH1/ZPH1R (8.09 and 6.95 difference respectively). Both hybrids were crosses of line ZPL1 with different testers (ZPL1 was mother in original and father in reciprocal combinations). Hybrids ZPH5/ZPH5R had the lowest reciprocal effect. Coefficient of variation for this trait was 9.09%.

Hybrid combination ZPH2/ZPH2R was the combination with the most statistically significant differences between original and reciprocal hybrid for all examined traits except for grain moisture. Combinations ZPH5/ZPH5R and ZPH6/ZPH6R had no statistically significant differences between original and reciprocal hybrids, e.g. their reciprocal effect was low. Both of these combinations had line ZPL3 as maternal component in the original hybrids while paternal components were ZPT1 and ZPT2 in ZPH5 and ZPH6 respectively.

The results show that years, locations, and genotypes were significant factors for most of the examined traits. Years and genotypes affected all traits at 1% level of significance. Different locations didn't affect variations in mass of 1000 kernels. Both Year × Location and Year × Genotype interactions were significant for all examined traits, while Location × Genotype interaction influenced yield, moisture and ear height. Of all examined traits, Interaction of all three factors affected yield only. The coefficient of variation (C_v) ranged from 3.17% for plant height to 11.31% for yield.

Table 9. Genetic distance among observed inbred lines

Inbred lines	ZPT1	ZPT2	ZPL1	ZPL2	ZPL3
ZPT1	0				
ZPT2	0.171	0			
ZPL1	0.412	0.414	0		
ZPL2	0.416	0.414	0.361	0	
ZPL3	0.407	0.407	0.367	0.198	0

The genetic distance between pairs of inbred lines ranged from 0.171 (ZPT1 and ZPT2) to 0.416 (ZPT1 and ZPL2), with an average value of 0.30 (Table 9).

The dendrogram was constructed using the UPGMA clustering method (Figure 1). According to the dendrogram, two subclusters were constructed. The first subcluster includes the inbred lines ZPL3 and ZPL2, which belong to the heterotic group BSSS (Iowa Stiff Stalk Synthetic). The second subcluster contains the ZPT1 and ZPT2 lines belonging to the Lancaster Sure Crop heterotic group. The ZPL1 inbred line formed a separate subcluster. Results of the genetic diversity analysis of inbred lines were in accordance with their genetic origin and heterotic pattern. That is in accordance with findings of MLADENOVIC DRINIC *et al*. (2012) and PERIC *et al.* (2021).

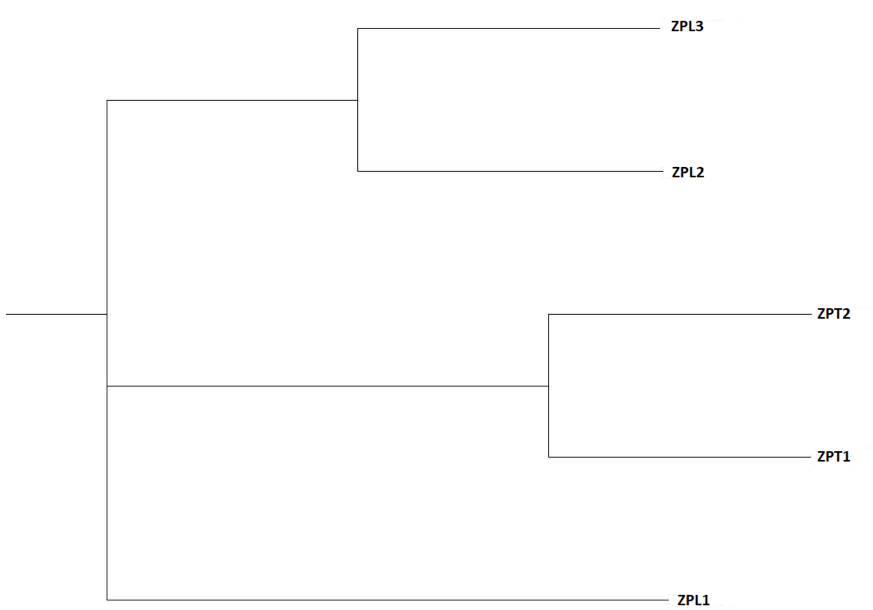

Figure 1. Dendrogram representing genetic distances among observed inbred lines

CONCLUSIONS

Results of the genetic diversity analysis of inbred lines were in accordance with their genetic origin and heterotic pattern providing useful insight into the genetic basis of the breeding material. Significant reciprocal effects were recorded for grain yield, grain moisture, plant height and mass of thousand kernels at least in one examined hybrid combination in the trial.

Hybrids that did not have a recorded reciprocal effect (ZPH5/ZPH5R and ZPH6/ZPH6R) contained the same line (ZPL 3), which indicates the genetic source of this effect. The existence of a statistically significant reciprocal effect for important agronomic traits gives importance to the right choice of which lines will be maternal or paternal component in a certain hybrid combination. For that reason, breeders should take the reciprocal effects in consideration in planning maize crossings strategies.

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UTICAJ RECIPROČNOG UKRŠTANJA NA PRINOS ZRNA I DRUGE AGRONOMSKE OSOBINE U KUKURUZU

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Izvod

Cilj ovog ogleda bio je da ispita moguće uticaje recipročnog ukrštanja na prinos i neke morfološke osobine u kukuruzu. Poljski ogled bio je postavljen na tri lokacije (Zemun Polje, Pančevo, Bečej) u 2015 i 2017 godine. Pet elitnih inbred linija je izabrano za proizvodnju hibridnih kombinacija u ogledu. dve od pet linija su korišćene kao testeri. Izabrane linije su ukrštene sa testerima za vreme 2014 i 2016 godine kada i recipročna ukrštanja. Kao rezultat dobijeno je dvanaest single cross hibrida. Ispitivani su prinos zrna, vlažnost zrna, visina biljaka, visina do klipa i masa 1000 zrna. Lokacije, godine i recipročna ukrštanja bili su signifikantni faktori u svim ispitivanim parcelama. Recipročna ukrštanja imala su statistički značaj na dve hibridne kombinacije za prinos zrna i vlažnost zrna. Kada je u pitanju visina biljaka i masa 1000 zrna, statistički značajna razlika je uočena u jednoj hibridnoj kombinaciji. Najveće recipročne razlike između originalnih i recipročnih hibrida zabeležene su za prinos zrna, koje su bili statistički značajni za ukrštanja ZPH4 / ZPH4R (20.03%) i ZPH2 / ZPH2R (19,49%). Hibridna kombinacija H2 / H2R je kombinacija sa statistički značajnim razlikama između originalnih i recipročnih hibrida za sve ispitivane osobine, osim za vlažnost zrna. Kombinacije ZPH5 / ZPH5R i ZPH6 / ZPH6R nisu imali statističke značajne razlike između originalnih i recipročnih hibrida, njihov recipročni efekat je bio nizak. Obe ove kombinacije sadržale su liniju ZPL3 kao materinsku komponentu.

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