SEED QUALITY OF RECIPROCAL MAIZE SINGLE CROSSES AND THEIR PARENTAL INBRED LINES

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Five direct and reciprocal early hybrids, five direct and reciprocal late hybrids and a total of their 12 parental inbred lines were used in order to test reciprocal effects among hybrids in early germination phase and seed quality of their parental inbred lines. Five hybrids were of early maturity FAO 300-400 group, and five of late maturity FAO 500-600 group. In the early group one line of Lancaster origin (L1) was in common, and in direct crosses used as male parent, while in reciprocal crosses as female parent. The same case was with late hybrids where another Lancaster line (L2) was in common. In 2015 and 2016 all the material was multiplied in hand pollination and after six months of storage three tests according to ISTA standards were performed: standard germination test (SGT), cold-test (CT) and vigor radicle test (RE). According to the Analysis of variance, a strong reciprocal effect was found among early hybrids for four out of five parameters in RE and SGT, while it was found only for RE late hybrids. However, in CT none of the parameters had significant reciprocal effect. Two tested Lancaster inbreds were superior in the hybrids over non-Lancaster lines when used as maternal components, while as lines *per se* they did not display such superiority. Mid-parent heterosis (MPH) ranged from -62.32% to 211.11% in RE test, with an average of 44.26%; from -10.15% to 17.93% in SGT test, with an average of 6.27%, and from -5.31% to 4.49% in CT, with an

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average of 0.64%. Significant differences of MPH were found for nine pairs of reciprocal hybrids for RE, three pairs for SGT and seven pairs for CT. This shows that reciprocal differences in MPH were higher as the applied level of stress during early seed growth raised. All the results showed very complicated mode of inheritance of tested traits in the early developmental stages of young plants, including the phenomenon of heterosis which is expressed already at the germination stage.

Keywords: heterosis, ISTA, reciprocal effects, seed quality testing, *Zea mays* L.

INTRODUCTION

Seed quality is one of the most important factors affecting early performance and latter productivity in maize (SABRY, 2018), the same as in other crops. It can be defined as the sum of all physical, physiological, genetic and health traits that affect seed ability for vital functioning - good longevity, germination, and vigor (GOGGI *et al.*, 2008). Good quality of maize hybrid seed is essential for good stands, field performance and it improves final grain yield. For producers, it is very important that improvement of grain yield and quality of maize hybrids is not at the expense of seed quality. Lower quality seed can cause poor field emergence, slower vegetative growth, poor stands, delay during reproduction phase (especially the sensitive period) and finally reduced grain yield. Physical traits of maize seed are mostly genetically controled (seed size, thickness of the pericarp, permeability to gases and moister, resistance to pests), but they also largely depend on the growing conditions during seed production. The main rule is to produce seed of a genotype in the environments it is adapted to (MUNAMAVA *et al.*, 2004). The problem arises when maize hybrid seed is produced at one site, but grown in a completely different environment. Seed storage conditions are also essentially important, since maize seed quality can drop during storage and make it unacceptable for planting (SEBETHA *et al.*, 2015). Finally, maize seed germination in the field largely depends on soil temperature (SILVA-NETA *et al.*, 2015). The authors claim that at the 15°C germination period can last up to 14 days and with decreased temperature the germination period increases. Thus, seed stays longer in the soil and can be attacked by different insects and pests.

International Seed Testing Association (ISTA) defined international standards to test the quality of each seed lot that should be sold on market. The most widely used test for seed quality is standard germination test (SGT) which is declared by law (ISTA, 2015). It presents the seed germination and early growth under optimal growing conditions. Another well established and most widely used is cold-test (CT). It combines stress effects of low temperatures and highly moisture-saturated soil, simulating conditions when planting seed in wet, cold soil. This is very important not only in northern growing maize regions, but also in southern regions, like Serbia. In several previous years in Serbia and neighboring countries maize producers often faced cold and humid springs, followed by extremely hot and dry summers (www.hidmet.gov.rs). On the other hand, to mitigate global climate changes and global warming consequences, a new strategy for avoiding drought stress during flowering time in maize is an early sowing, while soil is still cold and humid (BONELLI *et al.*, 2016). In MUNAMAVA *et al.* (2004) seeds produced in cooler regions had better results of a CT, and *vice versa*, thus maize breeders are interested for improvement of this trait (FRASCAROLI and LANDI, 2013; STRIGENS *et al.*, 2013; GRZYBOWSKI *et al.*, 2019).

In addition to the mentioned above, a series of vigor tests are defined. Since 2001, seed vigor was first introduced into ISTA (POWELL, 2022). High seed vigor influences high germination, resistance to stresses, and finally high crop yield. Seed vigor is a complex trait influenced by genetic and physical purity of the seed, its mechanical damage and physiological conditions. MILIVOJEVIĆ *et al.* (2021) have found that differences in seed quality among 15 maize inbred lines were higher when established by vigor tests than by SGT test. HAN *et al.* (2014) have found out 23 candidate genes associated with seed vigor traits which functions are in the glycolytic pathway and protein metabolism.

In maize hybrid seed production, a good synchronization between silking of mother line and pollination of father line is very important. If one of the components has poor seed quality it will have delayed emergence, and consequently be late in flowering, causing disproportion in silking of maternal and pollination of paternal component. This can lead to decrease in hybrid maize seed production and cause great economic losses.

The endosperm in maize, like in other cereals, is the primary nutrient source for embryo and seed enlargement. Its development largely depends on the capacity and assimilates of mother plant, implying strongly maternal effect on its growth. Uniformity of seed size is also important, since it allows more uniform emergence and competition among young plants. Seed size and weight are under strong maternal control (ZHANG *et al.*, 2016). Endosperm area, starchy endosperm cell size and kernel dry mass accumulation displayed positive correlation with patterns of grain filling of maternal parent. These authors have postulated four patterns by which mother plant influences seed size: via seed coat which represents the maternal tissue, maternal provision of chemicals during seed development, maternal influence of seed plasticity in response to the changing environment, and the effect of triploid endosperm in which gene imprinting most often occurs. Seed size, on the other hand, strongly influences seed quality parameters. In the mentioned research, inbred lines with larger kernels had larger endosperms with smaller cells and *vice versa*, and hybrids followed the developmental rate of their mother parents.

In maize, reciprocal effects imply the phenotypic difference among traits between reciprocal F_1 hybrids. Besides reciprocal effect on morphological and grain traits of mature hybrids, this effect was found even at the first stages of embryogenic development (KOLLIPARA *et al.*, 2002; HOECKER *et al.*, 2006; MOTERLE *et al.*, 2011; ZHANG *et al.*, 2016; SANTOS *et al.*, 2017). According to HOECKER *et al.* (2006) enlarged primary roots of hybrids can mainly be due to the elongated cortical cells. These authors concluded that heterosis is manifested already during very early root development, a few days after germination.

The goal of this research was to study reciprocal effects of ten maize F_1 hybrids and their reciprocals, their mid-parent heterosis on seed quality traits, as well as seed parameters of their parental inbred lines. Results could serve as recommendation for the seed production.

MATERIALS AND METHODS

Plant material

Ten single cross maize hybrids, their reciprocals and parental inbred lines were chosen for this research. Five hybrids belong to FAO 300-400 maturity group, and the rest belong to FAO 500-600. In total, 12 inbred lines were tested, since five early hybrids have L1 of Lancaster

origin in common, while five late hybrids have L2 line in common (also Lancaster in origin). Another components of five early hybrids were denoted L3, L4, L5, L6 and L7, of non-Lancaster origin, while components of late hybrids were denoted L8, L9, L10, L11 and L12 (also non-Lancaster). List of single crosses and their corresponding reciprocals is given in Table 1. In direct crosses Lancaster lines were used as paternal components, while in reciprocal crosses they were used as maternal components.

Table 1. Single cross hybrids and their reciprocal crosses

	FAO 300-400		FAO 500-600	
Type of hybrid	Hybrid	Components	Hybrid	Components
	H3_1	L3×L1	H8_2	L8×L2
	H4_1	L4×L1	H9_2	L9×L2
Direct hybrids	H5_1	L5×L1	H10_2	L10×L2
	H6_1	L6×L1	H11_2	L11×L2
	H7_1	L7×L1	H12_2	L12×L2
	H1_3	L1×L3	H2_8	L2×L8
	H1_4	L1×L4	H2_9	L2×L9
Reciprocal hybrids	H1_5	L1×L5	H2_10	L2×L10
	H1_6	L1×L6	H2_11	L2×L11
	H1_7	L1×L7	H2_12	L2×L12

Production of the genotypes, selfing of inbred lines and their crossing to obtain hybrids, was done in Zemun Polje (Serbia), in 2015 and 2016 in two replications according to Completely Randomized Design (CRD). Usual agronomic practices for maize production were applied. At physiological maturity seed was harvested, dried, and put into storage for six months.

Seed quality parameters

In both years, standard germination test (SGT) and cold-test (CT) were performed on untreated seed according to ISTA protocols in ISTA accredited Seed Testing Laboratory of Maize Research Institute Zemun Polje. For SGT, the method between the filter paper was used. Two hundred seeds per sample were tested in four replicates of 50 seeds each. They were placed between two wet filter papers, wrapped in a roll, and put at 20ºC with photoperiod of 16 h/ darkness and 8 h/ light. After five days germination energy (GE; % of normal seedlings) was calculated. According to ISTA standards this is done at fourth day, but this experimental material was more developed and thus more appropriate for the test on the fifth day. At seventh day, final percentage of normal seedlings (NS) was estimated. CT was done using the method in a rolled filter paper. Fifty seeds per four replications (200 seeds per sample) were placed on a wet filter paper covered with a thin layer of soil, and covered with another wet filter paper and wrapped in a roll. The rolls were put in a germinator for seven days at 10ºC, followed by another seven days at 20ºC. Subsequently, the emerged seedlings (NS) were counted and cold germination

percentage was calculated. Percentages of abnormal (AN) and dead (D) seeds were also counted and turned into percents for SGT and CT. Another vigor test was done, namely the radicle emergence (RE) test. After 66 h at the same conditions as for SGT, seedlings with primary roots longer than 2 mm were counted and expressed in per cent of total seeds tested.

Statistical analysis

Two-way factorial Analysis of variance (ANOVA) for CRD including reciprocal effect was done for hybrids. Simple two-way ANOVA was also done separately for hybrids and lines in order to perform LSD test at significance level of 0.05. Mid-parent heterosis (MPH) was calculated for each F_1 hybrid and its reciprocal as a deviation of hybrid trait from the mid-parent value given in per cent. Statistical differences between MPH of each single cross and its reciprocal were tested by two-sided t-test. It was equally applied to positive and negative values. All statistical analyses were done using SPSS 16.0 software, for hybrids separately for early and late ones since these two groups each have one hybrid component in common.

RESULTS AND DISCUSSION

Two-way ANOVA for hybrids including reciprocal effect showed that year was significant at *p<0.01* for RE, NS and GE for early hybrids and for RE and AN for late hybrids. It was also significant at *p<0.001* for NS and GE for late hybrids (data not shown). Differences between genotypes for early hybrids were significant for RE, NS and D (*p<0.01*) and also for GE (*p<0.05*), while among late hybrids significant differences were determined for NS (*p<0.05*) and for GE (*p<0.01*). Genotype × year interaction was significant for RE and GE (*p<0.01*) in early hybrids and for late hybrids for RE, NS and AN (*p<0.05*) and GE (*p<0.01*). This is in accordance with MUNAMAVA *et al.* (2004), where genotype × location interactions of several seed quality parameters were found to be significant. According to these authors, growing conditions like type of soil, temperature and moisture could largely affect seed quality. In MILIVOJEVIĆ *et al.* (2021) genotype and storage conditions were statistically significant among 15 maize inbred lines tested for several seed parameters, as well as the interaction between these two factors in all except two cases.

Reciprocal effect for early hybrids (with L1 as a common parent) was significant for RE, NS, GE and D (*p<0.01*), while for late hybrids only for RE (*p<0.01*). Radicle test, especially while using image analysis, has a great potential for further application in various of species (POWELL, 2022). Year × reciprocal effect interaction was significant only for RE and GE among late hybrids (*p<0.01* and *p<0.001*, respectively). Genotype × reciprocal effect interaction was significant for RE, NS and D in early and for RE and NS in late hybrids at 0.01 level, while in late hybrids significance was also found for GE at 0.001 probability level (data not shown). Also, year × genotype × reciprocal effect (Y × G × R) interaction was significant among early hybrids for RE and GE at 0.01 and 0.05 probability level, respectively, and among late hybrids for RE (*p<0.01*), NS, GE and D (*p<0.001*).

In MOTERLE *et al.* (2011) in a complete diallel cross among nine tropical maize inbreds significant reciprocal effect (*p<0.01*) was revealed for five of the seven studied seed quality parameters. The authors have also found higher significance of non-additive than additive

genetic effects for seed quality traits. Reciprocal effects in their research were even higher than specific combining ability (SCA), indicating different effects when using inbred lines as males or females in production of F_1 hybrids. Similar to our research, the authors emphasize that there is greater recombination of genes in the combinations of inbred lines from different heterotic groups, indicating that maternal effect can be used to determine if line performs better as male or female in hybrid cross.

Table 2a. Average and LSD values (p<0.05) for hybrids and their reciprocals from two-way ANOVA for RE and SGT parameters

Early hybrids					
Hybrid	RE	GE	NS	AN	D
H3_1	71.75c[1]	2.00bcd	94.75abc	1,00ab	2.25b
H1_3	75.00bc	1.50cd	97.75ab	0.25b	0.50b
H4_1	**29.00e**	**7.75a**	**82.00d**	2.25a	**8.00a**
H1_4	**73.00c**	**3.50bc**	**94.50abc**	1.00ab	**1.00b**
H5_1	**9.75f**	3.50bc	94.75abc	1.25ab	0.50b
H1_5	**80.50b**	1.00cd	97.00abc	0.50b	1.50b
H6_1	**47.00d**	**4.75b**	**92.75c**	1.25ab	1.25b
H1_6	**87.75a**	**0.25d**	**98.25a**	0.75ab	0.75b
H7_1	**43.75d**	2.75bcd	93.50bc	1.50ab	2.25b
H1_7	**76.00bc**	2.50bcd	94.50abc	1.50ab	1.50b
$LSD_{0.05}$	6.061	2.94	4.36	1.549	1.846
Average	59.35	2.95	93.98	1.125	1.95
MSG	5238.47***	36.64***	167.61***	2.58	39.31***
MSY	3276,801***	480.19***	594.05***	4.05	0.20
MSG × Y	1144.13***	48.42***	58.49***	3.38	5.76
CV	10.20	99.53	4.63	137.50	94.56
SD	28.08	4.67	6.86	1.61	2.77

[1] - values in the same columns with all different letters are significantly different at 0.05 probability level; MSG, MSY and MSY x G - genotype, year and genotype × year interaction mean square from two-way ANOVA, respectively; CV - coefficient of variation; SD - standard deviation in units of measurement; **,*** - statistically significant at 0.01 and 0.001 level, respectively; bolded are reciprocal hybrids statistically mutually different (*p<0.05*) for the particular parameter

Table 2b. Average and LSD values (p<0.05) for hybrids and their reciprocals from two-way ANOVA for RE and SGT parameters

Hybrid	RE	GE	NS	AN	D
			Late hybrids		
H8_2	**30.25e**	**6.25ab**	**91.75cd**	0.50b	1.50a
H2_8	**61.00ab**	**2.00ef**	**95.50ab**	1.00b	1.50a
H9_2	**33.00e**	**7.50a**	**88.25d**	2.00ab	2.25a
H2_9	**61.50ab**	**3.75cde**	**92.00bc**	1.75ab	2.50a
H10_2	53.00c	**5.25abcd**	90.75cd	**1.00b**	3.00a
H2_10	56.50bc	**2.75ef**	93.00abc	**2.75a**	1.50a
H11_2	**30.75e**	3.00def	94.25abc	1.25ab	1.50a
H2_11	**66.50a**	4.00bcde	92.50abc	1.00b	2.50a
H12_2	**60.00abc**	**0.75f**	**95.75a**	1.75ab	1.75a
H2_12	**42.25d**	**5.50abc**	**91.75cd**	1.25ab	1.50a
LSD$_{0.05}$	7.556	2.392	3.566	1.688	1.852
Average	49.48	4.08	92.55	1.43	1.95
MSG	1585.49***	34.01***	40.31**	3.34	2.53
MSY	6301.25***	344.45***	288.80***	0.05	1.80
MSG × Y	733.58***	51.78***	90.24***	8.05**	10.13**
CV	15.25	58.62	3.85	118.32	94.85
SD	19.51	4.31	5.26	1.82	1.97

[1] - values in the same columns with all different letters are significantly different at 0.05 probability level; MSG, MSY and MSY x G - genotype, year and genotype × year interaction mean square from two-way ANOVA, respectively; CV - coefficient of variation; SD - standard deviation in units of measurement; **,*** - statistically significant at 0.01 and 0.001 level, respectively; bolded are reciprocal hybrids statistically mutually different (*p<0.05*) for the particular parameter

In Tables 2a and 2b average and LSD values (*p<0.05*) for hybrids and their reciprocals from two-way ANOVA for RE and SGT parameters are shown. Among early hybrids (Table 2a), RE was significantly higher for reciprocal hybrids in four and NS in two out of five cases, while GE and D had statistically lower values in two hybrids and one hybrid, respectively, out of five hybrids. It is interesting that hybrids H4_1 and H1_4 showed significant differences for four traits, and H6_1 and H1_6 for three traits, indicating strongest reciprocal effect in the research.

RE values were significantly higher in four out of five early reciprocal hybrids, and in three out of five late hybrids. Surprisingly, in all these cases the Lancaster line performed better as female parent than the non-Lancaster line, which is in contrast with MILIVOJEVIĆ *et al.* (2021)

who have found that Lancaster originated inbred lines had lower seed quality in comparison with BSSS and Iowa Dent inbred lines.

Among late hybrids (Table 2b), three hybrids had significantly higher values for RE as reciprocals, while one had a higher value in direct hybrid (H12_2). The same hybrids had the opposite results for GE. For NS, two hybrids had higher and one (H2_12) had a lower value as reciprocals. Considering AN, only one hybrid had significant reciprocal difference, with direct version being better than reciprocal hybrid. All these results show that seed parameters in F_1 hybrids are not only influenced by the parental genotypes, but also by the way they were crossed. Interestingly, although GE values were significantly smaller for two reciprocal crosses in early and three in late hybrids, NS values of four of these hybrids were statistically higher than for their direct versions. In the research of REVILLA et al. (2000) the authors concluded that additive-dominance genetic model is influencing cold-tolerance traits in maize. The authors also concluded that it is possible to combine high percentage of emergence with vigorous seedling growth.

BARATA et al. (2019) have proposed a new partial diallel model for establishing the significance of reciprocal effect for grain yield in maize. Nine hybrids were included into their research, including F_1s and their reciprocals. According to their results no reciprocal effect was significant for grain yield.

In Table 3, average and LSD values ($p<0.05$) for inbred lines from two-way ANOVA for RE and SGT parameters are given. Inbred line L2 had significantly better values for GE and D than L1. For RE, two early and four late lines had significantly lower values than L1 and L2, respectively. L3 and L4 were better than L1 for the same trait, and L12 was better than L2. For GE, one early and two late lines were better than L1 and L2, respectively, and for NS one early and four late lines were poorer than L1 and L2 Lancaster lines, respectively. For AN, only two late lines had significantly higher values than L2, while for D no significant differences for lines in comparison with L1 and L2 were obtained. Considering seed parameters of inbred lines only, Lancaster lines were not superior comparing to non-Lancasters, as it was the case in hybrids. In our research, like in MILIVOJEVIĆ et al. (2021) larger differences were found for RE than for SGT parameters among inbred lines.

Considering cold-test, ANOVA revealed significant differences among: 1) years for late hybrids for NS ($p<0.01$) and AN ($p<0.001$), 2) genotypes in early hybrids for NS ($p<0.001$) and D ($p<0.01$), and 3) genotypes in late hybrids, for NS and D at 0.01 level and for AN at 0.05 level of significance (data not shown). Genotype by year interaction was significant only for NS ($p<0.05$) among early hybrids, while among late hybrids for NS and D ($p<0.001$), as well as for AN ($p<0.05$). Reciprocal effect was not significant for any of the traits, neither for early nor for late hybrids. This implies that stressful conditions mitigated the differences between direct and reciprocal hybrids. On the other hand, interaction of the year with reciprocal effect was significant in early hybrids for NS and D ($p<0.001$) and AN ($p<0.05$), while among late hybrids for NS ($p<0.001$) and for D ($p<0.01$). Genotype by reciprocal effect was significant for all three traits in early hybrids, and for NS and AN in late hybrids, with different levels of significance. Finally, interaction of year by genotype by reciprocal effect (Y × G × R) was significant at 0.05 level among early hybrids for NS and D, and among late hybrids for the same traits at 0.01 level of significance.

Table 3. Average and LSD values (p<0.05) for inbred lines from two-way ANOVA for RE and SGT parameters

Inbred	RE	GE	NS	AN	D
L1	50.50b	1.25e	93.25ab	1.75cd	3.75a
L3	**62.75a**	0.75e	96.00a	0.75d	2.50abc
L4	**59.25a**	5.25de	89.25bcd	3.75abc	1.75abc
L5	**1.25f**	**24.00a**	**71.25g**	2.50cd	2.25abc
L6	**24.75c**	4.75de	89.50bc	2.75bcd	3.00abc
L7	52.00b	4.50de	90.50abc	1.50cd	3.50ab
L2	46.25b	7.50cd	90.25abc	1.00d	1.25c
L8	**5.00ef**	**20.75a**	**74.00fg**	2.75bcd	2.50abc
L9	**10.00e**	12.25bc	**83.25de**	3.00bcd	1.50bc
L10	**24.75c**	**13.75b**	**77.75ef**	**6.25a**	2.25abc
L11	**17.00d**	9.75bcd	86.75cd	1.50cd	2.00abc
L12	**60.50a**	9.25bcd	**83.25de**	**5.25ab**	2.25abc
LSD$_{0.05}$	6.651	5.423	6.036	2.57	2.01
Average	34.5	9.479	85.42	2.73	2.38
MSG	4261.360***	419.494***	476.30***	22.41***	4.50
MSY	3037.498***	51.043	13.50	35.04*	24.00*
MSG × Y	519.227***	105.223***	118.05***	7.50	3.91
CV	19.33	57.35	7.08	94.39	84.84
SD	24.73	9.16	9.86	2.99	2.05

[1] - values in the same columns with all different letters are significantly different at 0.05 probability level; MSG, MSY and MSY x G - genotype, year and genotype × year interaction mean square from two-way ANOVA, respectively; CV - coefficient of variation; SD - standard deviation in units of measurement; *,*** - statistically significant at 0.05 and 0.001 level, respectively; bolded are the values of lines crossed to L1, i.e. L2 that are statistically (*p<0.05*) different from these two lines for a particular trait, respectively

A similar number of significant differences between direct and reciprocal hybrids as in SGT test have been found in cold-test (Table 4). This is not in line with MILIVOJEVIĆ *et al.* (2021), where larger parameters for SGT were found for lines stored at the room temperature than for the same lines stored at controlled conditions at 5 ºC. LIU *et al.* (2019) obtained comparable results and identified 13 QTLs on five chromosome regions for controlling seed longevity in different maize backgrounds.

MILIVOJEVIĆ *et al.* (2021) have postulated that increased cold tolerance in maize inbreds due to breeding and natural selection requires more stressful conditions in CT. In LOVATO *et al.* (2005), authors have found that 10ºC in CT is favorable for discrimination of seed lots of maize, but lower temperatures of 5ºC and 7.5ºC are better for testing high maize seed cold tolerance.

REVILLA *et al.* (2014) have identified maize genotypes suitable for the early sowing under cold conditions.

Table 4. Average and LSD values (p<0.05) for hybrids and their reciprocals from two-way ANOVA for cold test parameters

Hybrid	NS	AN	D	Hybrid	NA	AN	D
H3_1	96.00ab[1]	2.00abc	2.00b	H8_2	95.75bcde	1.50abc	2.75ab
H1_3	96.00ab	0.75bc	3.00b	H2_8	98.00ab	0.50c	1.50bc
H4_1	**91.50cd**	2.25abc	**6.00a**	H9_2	**97.50abc**	**0.75c**	1.50bc
H1_4	**97.00ab**	0.75bc	**2.25b**	H2_9	**94.75def**	**2.50ab**	2.75ab
H5_1	**99.00a**	0.25c	0.75b	H10_2	93.50ef	2.75a	3.75a
H1_5	**95.75b**	2.00abc	1.75b	H2_10	93.00f	2.25ab	3.75a
H6_1	96.25ab	1.50abc	2.25b	H11_2	**99.25a**	0.50c	0.25c
H1_6	94.00bc	2.50ab	2.75b	H2_11	**96.50bcd**	1.50abc	1.50bc
H7_1	**95.25b**	1.50abc	**3.25b**	H12_2	95.50cde	1.25bc	3.25ab
H1_7	**89.25d**	3.50a	**7.00a**	H2_12	96.00bcd	1.50abc	2.50ab
LSD$_{0.05}$	3.069	2.059	2.547	LSD$_{0.05}$	2.424	1.421	1.802
Average	95.00	1.70	3.10	Average	95.98	1.50	2.35
MSG	62.67***	7.42	30.02***	MSG	30.38***	5.11**	10.355**
MSY	0.20	1.80	0.00	MSY	110.45***	51.20***	1.80
MSG × Y	52.87***	7.13	27.89***	MSG × Y	45.89***	5.20**	20.36***
CV	3.23	121.00	82.05	CV	2.52	94.61	76.58
SD	4.52	2.17	3.45	SD	3.80	1.80	2.44

[1] - values in the same columns with all different letters are significantly different at 0.05 probability level; MSG, MSY and MSY x G - genotype, year and genotype × year interaction mean square from two-way ANOVA, respectively; CV - coefficient of variation; SD - standard deviation in units of measurement; *,**,*** - statistically significant at 0.05, 0.01 and 0.001 level, respectively; bolded are reciprocal hybrids statistically mutually different (*p<0.05*) for the particular parameter

Inbred lines L5 and L7 were better (*p<0.05*) than L1 for NS and D in cold-test, while L5 was also better for AN (Table 5). Among late lines there were no significant differences for NS, but line L11 was better than L2 for AN, and line L12 was worse for D. Based on these results Lancaster and non-Lancaster lines *per se* did not differ for seed parameters in general.

In our study, like in HOECKER *et al.* (2006), heterosis has been expressed in very early stages of plant development. Mid-parent heterosis (MPH) for NS ranged from -62.32% (H1_5) to 211.11% (H5_1, its reciprocal hybrid) in RE test, with an average of 44.26% (Graph 1); from -10.15% (H1_4) to 17.93% (H5_1) for NS in SGT test, with an average of 6.27% (Graph 2), and from -5.31% (H7_1) to 4.49% (H1_5) for NS in CT, with an average of 0.64% (Graph 3). Significant differences of MPH were found for nine pairs of reciprocal hybrids for RE, three

pairs for SGT and seven pairs for CT. This shows that reciprocal differences in MPH are higher as the applied level of stress during early seed growth raises.

*Table 5. Average and LSD values (p<0.05) for inbred lines from two-way ANOVA for cold* test parameters

Inbred	NS	AN	D
L1	92.50e[1]	1.75ab	5.25a
L3	93.50cde	2.00ab	4.25ab
L4	93.25de	2.50a	4.00ab
L5	**97.00ab**	**0.00c**	**2.00bcd**
L6	95.25abcde	0.50bc	4.00ab
L7	**96.00abcd**	1.25abc	**2.75bcd**
L2	96.25abc	2.00a	1.25cd
L8	95.50abcd	1.75ab	2.00bcd
L9	98.00a	1.00abc	1.00d
L10	93.75cde	2.50a	3.50abc
L11	97.25ab	**0.50bc**	2.00bcd
L12	94.50bcde	1.00abc	**4.00ab**
LSD$_{0.05}$	2.980	1.593	2.458
Average	95.23	1.44	3.00
MSG	24.50**	5.92*	14.55**
MSY	315.38***	40.04***	66.67**
MSG × Y	29.19***	7.04**	13.76*

[1] - values in the same columns with all different letters are significantly different at 0.05 probability level; MSG, MSY and MSY x G - genotype, year and genotype × year interaction mean square from two-way ANOVA, respectively; CV - coefficient of variation; SD - standard deviation in units of measurement; *,**,*** - statistically significant at 0.05, 0.01 and 0.001 level, respectively; bolded are the values of lines crossed to L1, ie. L2 that are statistically (*p<0.05*) different from these two lines for a particular trait, respectively
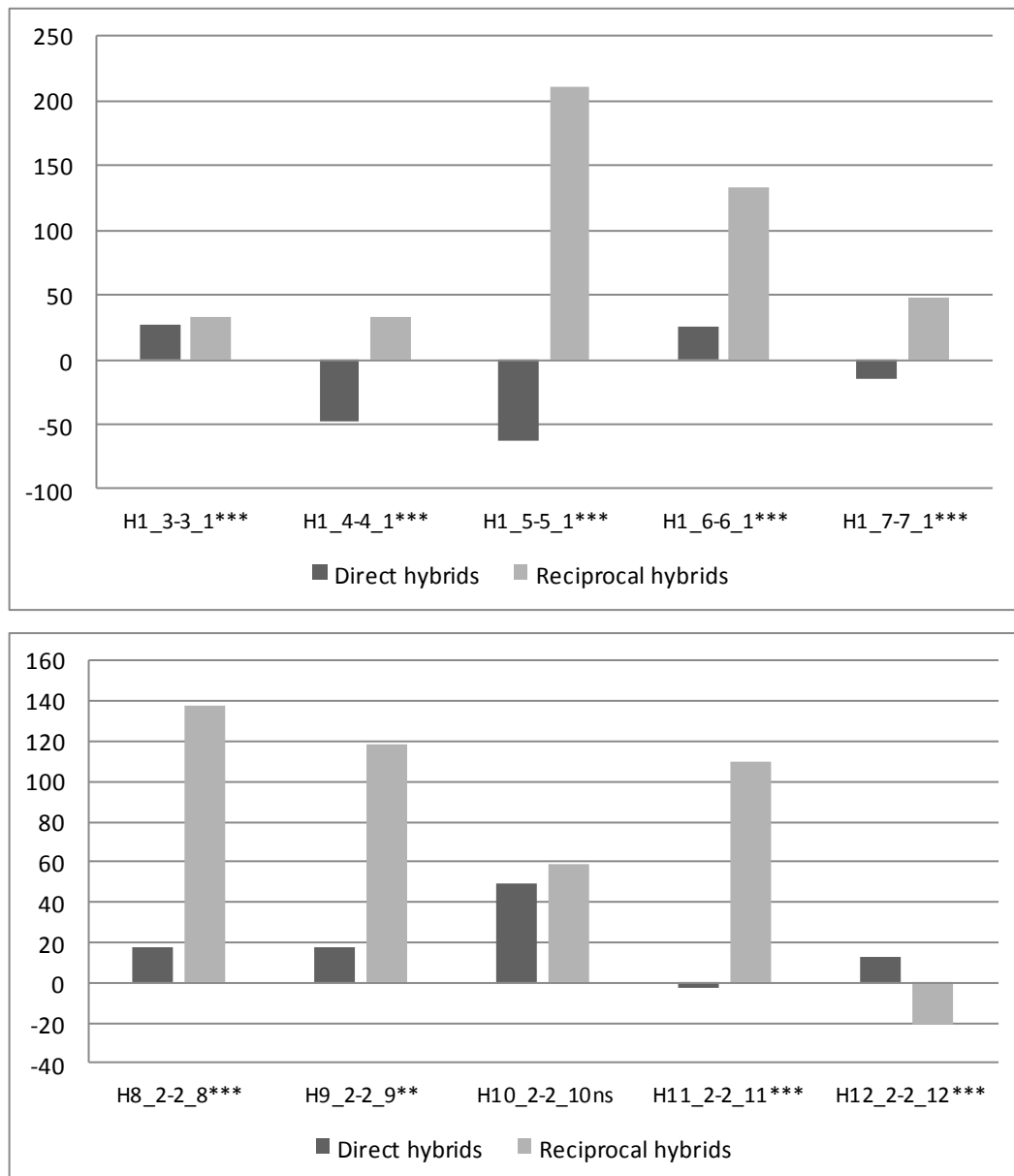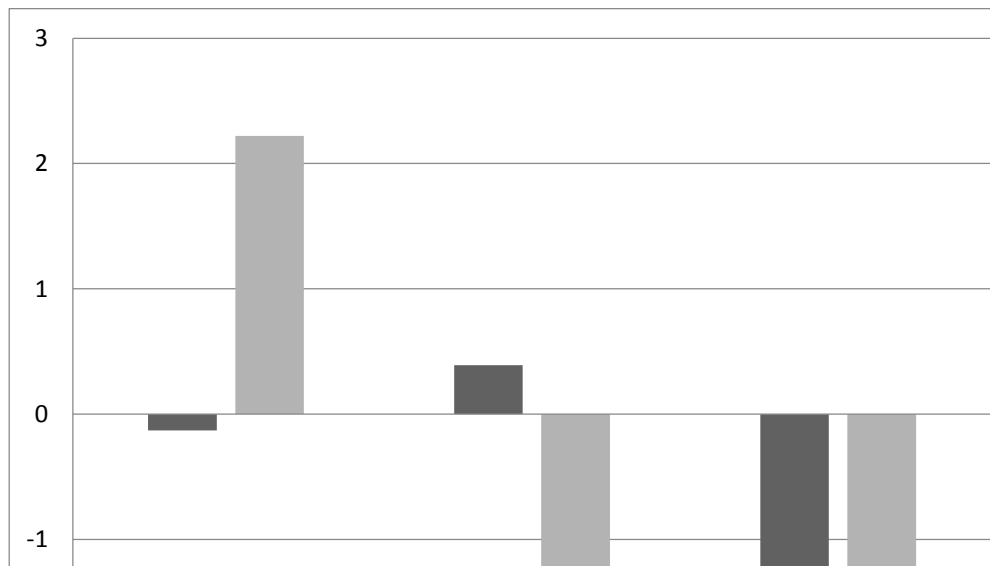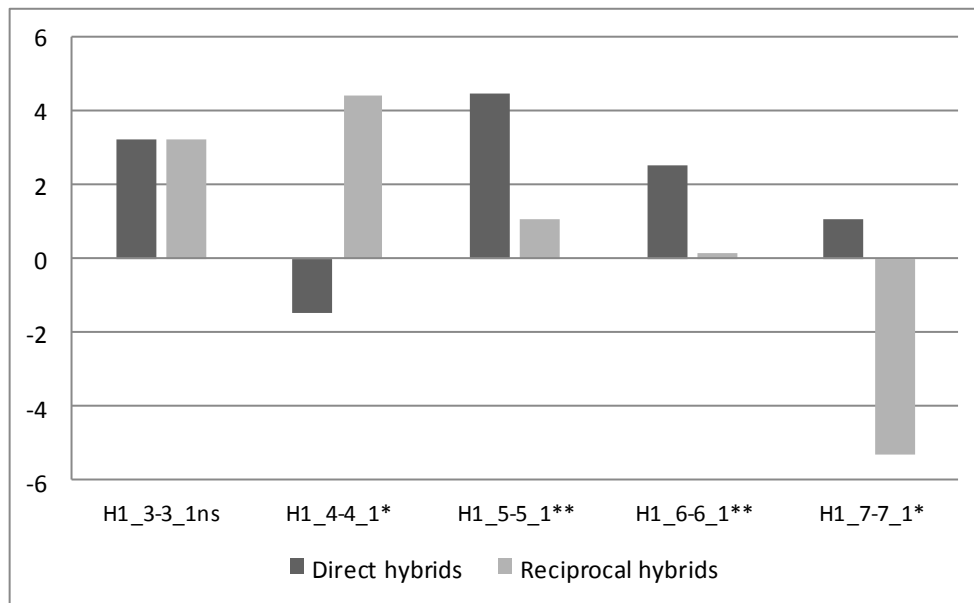
H1_3-3_1 are two reciprocal hybrids, etc.; ***, ** - significantly different MPH at 0.001 and 0.01 level of significance, respectively; ns - statistically non-significant

Graph 1. Mid-parent heterosis for NS in RE test for direct and reciprocal hybrids
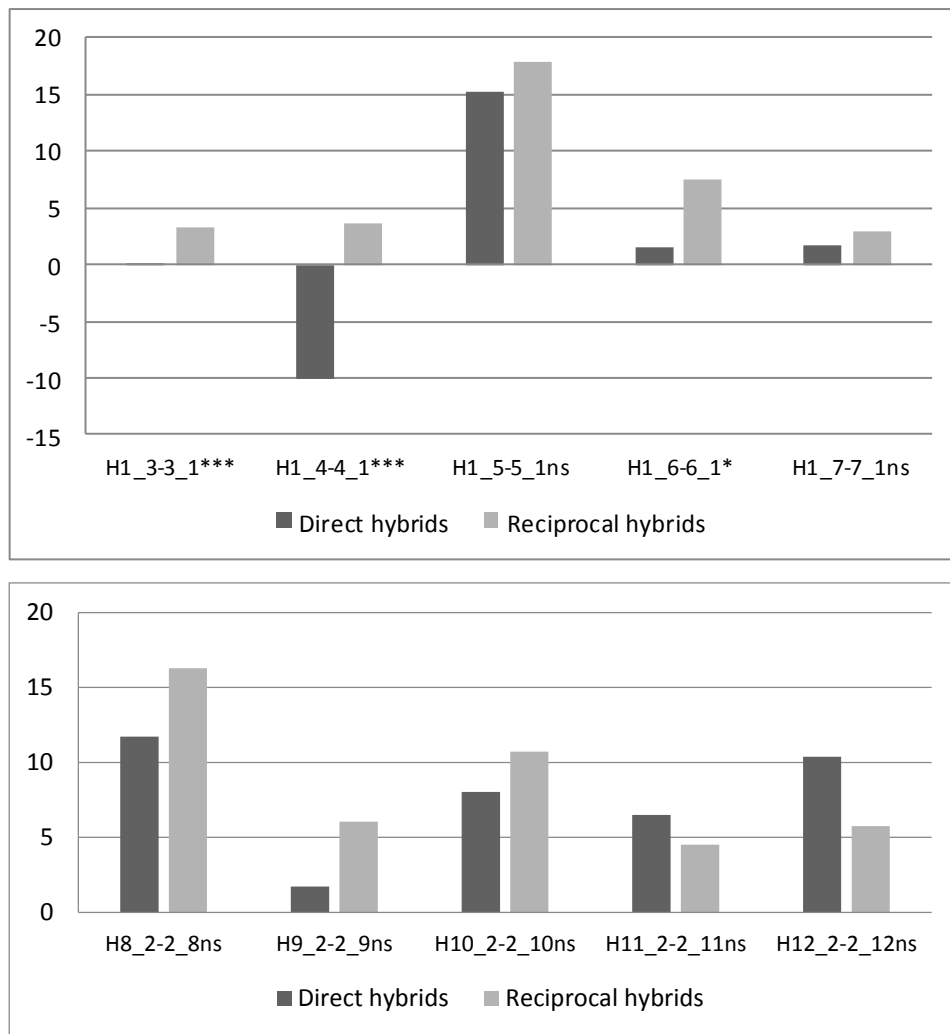
H1_3-3_1 are two reciprocal hybrids, etc.; ***, * - significantly different MPH at 0.001 and 0.05 level of significance, respectively; ns - statistically non-significant

Graph 2. Mid-parent heterosis for NS in SGT test for direct and reciprocal hybrids

H1_3-3_1 are two reciprocal hybrids, etc.; ***, * - significantly different MPH at 0.001 and 0.05 level of significance, respectively; ns - statistically non-significant

Graph 3. Mid-parent heterosis for NS in CT test for direct and reciprocal hybrids

For particular crosses MPH turned out to be positive in direct cross, but negative in reciprocal cross, or *vice versa*. For RE, this is the case for pairs of hybrids H1_4-H4_1, H1_5-H5_1, H1_7-H7_1, H11_2-H2_11 and H12_2-H2_12. In the first three pairs of crosses negative heterosis was obtained when L1 was a maternal parent, while for late hybrids H11_2 and H2_12 had negative heterosis. In SGT test only one pair of hybrids had negative-positive values of MPH, namely H1_4-H4_1 in the same order like in RE. For CT test six pairs of hybrids had this ambivalent values of heterosis: H1_4-H4_1, H1_7-H7_1, H8_2-H2_8, H9_2-H2_9, H11_2-H2_11 and H12_2-H2_12. In the case of H10_2-H2_10 both direct and reciprocal hybrid had negative heterosis. RE and CT tests are more invasive than SGT which is conducted in optimal conditions, and this may be the reason of higher appearance of negative heterosis in RE and CT. Negative heterosis was found in KIANI *et al.* (2015) for grain yield and some of yield components (shelling percentage, kernels per row, kernel rows per ear and 100-grain weight), and in ABEBE *et al.* (2020) for phenology, growth traits and disease parameters in maize.

No obvious rule was perceived for heterotic differences among reciprocal hybrids for NS in this research. Namely, three types of tests gave very different results regarding this parameter. Two rare exceptions were the hybrid pairs H1_4-H4_1, where reciprocal hybrid had lower and negative heterosis in all three tests, and H2_8-H8_2 with the opposite result, namely, the direct hybrid was superior to its reciprocal in all three cases. Largest MPH was obtained for RE test, and poorest for CT test. This was unexpected, since CT was the most invasive test applied in our research and it could have been expected that MPH will be the highest for it.

COSTA SILVA NETA *et al.* (2020) emphasized the importance of knowing an effect of heterosis and reciprocal effects in order to obtain cold tolerant maize genotypes. In their research heterosis for germination at 25ºC ranged from -8.5 to 3%. In the cold-test, heterosis ranged from -1.25 to 81.25% and non-additive genetic effects were more important than additive for germination test.

STUBER (1999) showed that heterosis is the consequence of a combination of a large number of genes. This could also be stated for the research presented herein, since there is a large scope of MPH values for the tested traits, as well as significant differences between reciprocal crosses.

In HOECKER *et al.* (2006) a significantly increased primary root length compared to MPH values was found in 34 of 36 measurements in the period between five and seven days after germination for 12 reciprocal hybrids. Also, in their research 22 out of 30 reciprocal combinations measured three to seven days after germination showed significant reciprocal effect. The authors concluded that the primary root length represents a reliable heterotic trait even during early root development and actually stated that early developmental stages must be studied to identify genes de facto in charge of heterosis. PASCHOLD *et al.* (2010) elucidated this finding. The authors have found organ specific non-aditive gene action in charge of heterosis in maize while studying early maize root system development.

HOECKER *et al.* (2006) found 17-25% MPH for primary root length and 1-7% MPH for primary root width three to seven days after germination in a series of 12 reciprocal hybrids, indicating that heterosis is expressed as early as three days after germination in young seedlings. They have also found that among root traits lateral root density five days after germination had the highest MPH value of 51% on the average, while in some cases MPH gained even 130%. For

this trait 73% of the tested materials showed significant reciprocal effects in their research. Heterosis was obtained even at the early embryogenic development (MEYER *et al.*, 2007).

Several factors cause differences between reciprocal crosses: genomic imprinting (parent-of-origin effect; LAWSON *et al.*, 2013), xenia aka the effects of pollen on the endosperm traits in the year of pollination (BULANT *et al.*, 2000), cytoplasmic effects or so-called maternal effects (BOŽINOVIĆ *et al.*, 2015), and dosage effects in the case of triploid tissues such as endosperm (EGESEL *et al.*, 2003). The third above mentioned effect was reported for seed quality traits in maize (MOTERLE *et al.*, 2011; CABRAL *et al.*, 2013; SANTOS *et al.*, 2017). The most probable cause of the reciprocal effects found in our study is the dosage effect of the endosperm, since it is a triploid tissue (*2n* inherited from a mother and *1n* from a father) and it is the basic organ responsible for early germination, growth and emergence of young maize seedlings.

Our material is composed of lines and hybrids of standard kernel quality, thus kernel chemical composition could not be the cause of the obtained results. According to MUNAMAVA *et al.* (2004) seed composition did not affect SGT parameters among group of lines with different oil and protein content.

In conclusion, a strong reciprocal effect was found among early hybrids for four out of five parameters in RE and SGT tests, and only for RE in late hybrids. However, in CT none of the parameters had significant reciprocal effect, meaning that stressful conditions diminished differences among reciprocal crosses. Two tested Lancaster inbreds were superior in the hybrids over non-Lancaster lines when used as maternal components, while as lines *per se* they did not display superiority over non-Lancaster lines.

No obvious rule for MPH was observed in this research. Largest MPH was obtained for RE test, and poorest for CT test. For particular reciprocal crosses MPH was positive in one case, but negative in another. All of this reflects complicated mode of inheritance of investigated traits, as well as heterosis among them.

Since it was unexpected that Lancaster lines were better performing as the mother parents for seed quality, it would be useful to repeat the experiment after 3-5 additonal years of seed storage and get an insight into the properties of the seeds after longer storage.

CONCLUSIONS

Two tested Lancaster inbreds were superior in the hybrids over non-Lancaster lines when used as maternal components, while as lines *per se* they did not display such superiority. Mid-parent heterosis (MPH) ranged from -62.32% to 211.11% in RE test, with an average of 44.26%; from -10.15% to 17.93% in SGT test, with an average of 6.27%, and from -5.31% to 4.49% in CT, with an average of 0.64%. Significant differences of MPH were found for nine pairs of reciprocal hybrids for RE, three pairs for SGT and seven pairs for CT, mining that reciprocal differences in MPH were higher as the applied level of stress during early seed growth raised. The results of our research showed very complicated mode of inheritance of tested traits in the early developmental stages of young plants, including the phenomenon of heterosis which is expressed already at the germination stage.

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KVALITET SEMENA RECIPROČNIH DVOLINIJSKIH HIBRIDA KUKURUZA I NJIHOVIH RODITELJSKIH KOMPONENTI

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Izvod

Cilj ovog istraživanja bilo je ispitivanje recipročnog efekta kod 10 F1 hibrida kukuruza u fazi klijanja, kao i njihovih roditeljskih inbred linija. Prema ISTA standardima korišćeni su standardni test klijavosti (SGT), cold-test (CT) i test pojave primarnog korena (*radicle emergeny test - RE*). Po pet hibrida imalo je po jednu zajedničku inbred liniju Lancaster osnove i ispitivano je pet ranih i pet kasnih hibrida kukuruza. Značajan recipročni efekat dobijen je za RE i tri od četiri parametra iz SGT za prvih pet ranih hibrida, dok je za kasne hibride samo RE bio značajan. Primentom CT nije bilo značajnih recipročnih razlika. Što se tiče linija, dve Lancaster linije su se pokazale bolje kao materinska komponenta u ispitivanim hibridima u odnosu na ne-Lancaster linije, dok u ispitivanjima *per se* nisu pokazale ovo svojstvo. Heterozis u odnosu na prosek roditelja (MPH) bio je u proseku 44,26% u RE testu, 6,27 % u SGT testu i 0,64% u CT testu. Kod MPH nije bilo pravilnosti u pogledu recipročnih ukrštanja. Svi ovi rezultati ukazuju na kompleksnost nasleđivanja klijavosti u uslovima različitih testova, kao i fenomena heterozisa u ovom ranom uzrastu biljaka kukuruza.