

Effect of plant density on leaf area index, anthesis to silking interval, yield and yield components of maize inbreds and their sister-lines

Utjecaj gustoće sklopa na lisnu površinu, interval od prašenja do svilanja, prinos i komponente prinosa linija kukuruza i križanaca srodnih linija

Dario JAREŠ^{1*}, Sanja BALAŠ KRNJIĆ², Marina VRANIĆ¹ and Zlatko SVEČNJAK¹

¹ University of Zagreb, Faculty of Agriculture, Department of Field Crops, Forage and Grassland, Svetošimunska cesta 25, Zagreb, Croatia, *correspondence: svecnjak@agr.hr

² Croatian Agricultural Agency, Poljana Križevačka 185, Križevci, Croatia

Abstract

Yield of maize inbreds is often a limiting factor in hybrid seed production. Thus, the main aim was to evaluate the effect of plant density on leaf area index (LAI), anthesis to silking interval (ASI), grain yield and yield components of inbreds and their sister-lines at two locations over two growing seasons. Inbreds Bc 279 and Bc 64, and their corresponding sister-lines Bc 279026-22 and Bc 822-1064, respectively, were grown at 60,000 and 86,000 plants/ha. When compared to inbreds, grain yields improved for sister-lines by an average of 1,188 kg/ha (21%) for Bc 279026-22 and 4,816 kg/ha (141%) for Bc 822-1064 primarily due to more rows per ear and kernels per ear row. This greater kernel number per ear and per plant was associated with larger LAI for Bc 279026-22, while Bc 822-1064 out-yielded inbred Bc 64 despite of similar LAI and ASI. Inbred Bc 64 and its sister-line Bc 822-1064 increased LAI similarly at higher plant density, but the former showed limited yield increment because of a large increase in barren plants. In contrast, inbred Bc 279 and its sister-line Bc 279026-22 responded similarly for grain yields and LAI across plant densities. A growing season x location x plant density interaction indicated various grain yield responses to plant densities under different environmental conditions. Higher plant density significantly improved grain yields for sister-lines and inbred Bc 279, but had limited advantage for inbred Bc 64; these genotype-specific responses could not be always explained by changes in LAI and ASI.

Keywords: inbred, leaf area index, maize, sister-line, yield, yield components

Sažetak

Nizak prinos sjemena linija kukuruza često je ograničavajući čimbenik u proizvodnji sjemena hibrida. Stoga je glavni cilj istraživanja bio utvrditi utjecaj gustoće sklopa na indeks lisne površine (ILP), interval od prašenja do svilanja (IPS), prinos i komponente prinosa linija i križanaca srodnih linija na dvije lokacije tijekom dvije vegetacijske sezone. Linije Bc 279, Bc 64 i njihovi križanci srodnih linija Bc 279026-22 i Bc 822-1064 uzgajani su u niskoj (60 000 biljaka/ha) i visokoj (86 000 biljaka/ha) gustoći sklopa. U usporedbi s linijama, prinosi križanaca srodnih linija bili su veći za prosječno 1 188 kg/ha (21%) za Bc 279026-22 i 4 816 kg/ha (141%) za Bc 822-1064 prvenstveno zbog većeg broja redova zrna i zrna u redu na klipu. Veći prinos sjemena Bc 279026-22 bio je povezan s većim vrijednostima za ILP, dok je veći prinos Bc 822-1064 u usporedbi s Bc 64 ostvaren unatoč sličnim vrijednostima za ILP i IPS. Linija Bc 64 i njezin križanac srodnih linija Bc 822-1064 slično su povećali ILP u visokoj gustoći sklopa, ali je povećanje prinosa bilo relativno malo za Bc 64 zbog velikog broja neplodnih biljaka. Nasuprot tome, linija Bc 279 i njezin križanac srodnih linija Bc 279026-22 slično su reagirali kako za ILP tako i za prinos sjemena pri povećanju gustoće sklopa. Nadalje, signifikantnost interakcije vegetacijska sezona x lokacija x gustoća sklopa ukazuje da je povećanje prinosa sjemena u visokoj gustoći sklopa ovisilo o okolinskim uvjetima. Povećanje prinosa u visokoj gustoći sklopa bilo je osjetno veće za liniju Bc 279 i oba križanca srodnih linija u usporedbi sa linijom Bc 64, a te specifične reakcije su djelomično objašnjene s promjenama u vrijednostima za ILP i IPS.

Ključne riječi: indeks lisne površine, komponente prinosa, križanac srodnih linija, kukuruz, linija kukuruza

Introduction

Grain yields of maize inbreds are in the range of 3.8 to 5.4 t/ha (Duvick, 1999), thereby two, three or more times lower comparing to hybrids. Poor inbred seed yield was the main impetus behind using double-cross and three-way hybrids in the past (Jones, 1922; Crow, 1998). Therefore, any increase in grain yield e.g. seed production of maize hybrid parental lines is of great importance. Generally, one of the main factors that affects grain yield of maize is crop solar radiation interception which primarily depends on the value of LAI (Williams et al., 1968; Liu and Tollenaar, 2009). It is well documented that increase in plant density increase LAI of inbred lines and leads to increase in grain yields (Rutger, 1971; Modarres et al., 1998). However, some authors (Hashemi et al., 2005; Liu and Tollenaar, 2009) suggested that there is an optimal LAI value for maximum grain yield and that further increases in LAI will not brought about a greater grain yield. Furthermore, too high plant densities may increase LAI above optimal values and consequently reduce grain yields (Fasoula and Tollenaar, 2005). In addition to the use of higher plant populations, an alternative for an increased seed production of female maize parent is the use of sister-lines (F1 cross between two highly related inbreds) instead of inbred lines (Lee et al., 2006; Castellanos et al., 2009). Maize hybrids produced by using sister-lines as a female parental component are referred as modified single crosses and their performance is

similar to best single cross counterpart (Lee et al., 2006). Greater yield of sister-lines compared to their corresponding inbreds indicates a certain level of heterosis (Lee et al., 2007), but limited information is available about yield components contributing to greater yields of sister lines. Tollenaar et al. (2004) reported that yield heterosis of conventional maize hybrids was primarily obtained through greater number of kernels per ear.

Although maize hybrid responses to plant populations are well documented, there is limited information on sister-lines responses in comparison to their inbred lines. Liu and Tollenaar (2009) stated that maize hybrids are more tolerant to high plant density stress than their parental lines. Authors also found that maize hybrids and inbreds responses to plant density varied on different locations. Under favorable environmental conditions heterosis for yield were similar across various plant densities, while it was less obvious at low- compared to high-plant density under less favorable environment. Plant traits such as earlier flowering have been used to improve maize adaptability to stress. Betrán et al. (2003) reported a negative correlation between grain yields and date of pollination or silking of investigated maize hybrids and inbreds. Parental inbreds generally flower later than their crosses (hybrid). Authors also reported that in stressful conditions, such as severe drought, earlier flowering genotypes may escape the most intensive stress during flowering. However, Modarres et al. (1997) found that inbreds with similar leaf area and silking date may significantly differ in yields. Authors stated that long period from tasseling to silking may cause poor pollination and floret abortion, and consequently, lower yields of inbreds. D'Andrea (2006) also found a negative correlation between the kernel number per plant and anthesis to silking interval (ASI) for inbreds. Borrás et al. (2009) reported that ASI extended by an average of one day when plant density increased from 3 to 9 plants/m for tested maize inbreds.

The main objectives of this study were to determine the effect of plant population density on yield and yield components, LAI and ASI of two inbred lines and their corresponding sister-lines when grown under various environments.

Materials and methods

Field experiments in a maize – soybean – winter wheat crop rotation were conducted at the Faculty of Agriculture Zagreb experimental station Maksimir and at Bc Institut d.d. Zagreb experimental station Rugvica in the growing seasons of 2009 and 2010. At Maksimir maize was grown on silt loam soil (Eutric Cambisol) characterized by high porosity and medium soli water capacity of surface soil layer (Jungić et al., 2015). The soil type at Rugvica is hydroameliorated vertic amphigley with high level (0.6 m to 2.42 m below the soil surface) of groundwater (Petošić et al., 1998). Temperature and rainfall data (Table 1) were recorded daily on meteorological stations located in the vicinity of the experimental fields.

Table 1. Mean monthly air temperature and precipitation at Maksimir and Rugvica over two growing seasons

Month	Mean temperature (°C)				Precipitation (mm)			
	Maksimir		Rugvica		Maksimir		Rugvica	
	2009	2010	2009	2010	2009	2010	2009	2010
April	14.5	12	14.8	12.6	52	63	45	64
May	18.4	16.5	18.6	16.5	49	98	42	124
June	19.8	20.3	19.8	20.1	68	104	65	128
July	22.2	23.3	22.3	23.1	96	53	185	87
August	22.6	20.8	22.6	20.7	79	141	51	150
September	18.9	15.1	18.2	14.9	22	195	39	208
Average/sum	19.4	18	19.4	18	366	653	428	761

At both locations maize crop was grown under high input production system. In October of each year, 500 kg/ha N-P-K 7:20:30 was broadcasted and moldboard ploughed to 30-32 cm. An additional 200 kg/ha of urea was broadcasted before seedbed preparation in spring. Maize crops were planted within the optimum planting date window and each plot consisted of four rows of 6 meter length that were 0.7 m apart. Plots were machine over-planted and thinned to target plant densities at V3 stage (Ritchie et al., 1986). Weeds were controlled by pre-emergence herbicide application and by hand hoeing in early stages of development. Additional 150 kg/ha of ammonium nitrate (27-0-0) was applied in each of two interrow cultivations at V3 and V5 stages.

A two-factorial experiment was arranged as strip plot design with five replications where maize genotypes were horizontal and plant densities vertically oriented plots. Tested maize genotypes were inbreds Bc 279 and Bc 64 and their corresponding sister-lines Bc 279026-22 and Bc 822-1064, respectively. Inbreds and their corresponding sister lines were chosen based on the preliminary research indicating differences in yield components and maturity. Plant density consisted of 60,000 (low density) and 85,000 (high density) plants/ha.

In each plot, 5-th and 10-th leaf were marked on 10 representative plants for further leaf measurements during vegetative growth. When first signs of flowering were visible, the onset of anthesis and silking date were observed daily on two inside rows. Anthesis for plot was taken when 75% of plants began to shed pollen, while silking was registered when 75% of plants exposed silks at least 2 cm long. Number of days from sowing to anthesis and silking was counted for every plot and anthesis-silking interval (ASI) was computed. When silking finished, the total leaf number, first green leaf position and ear position on stalk were recorded on 10 representative plants and area of all green leaflets was measured. Leaf was considered green when 75% or more

of its area was green (Binford and Blackmer, 1993). Lamina length (L) and maximum lamina width (W) of leaflets were measured and recorded values used to calculate the individual leaf area (A) as in Montgomery (1911; Eq. $A = 0.75 \cdot L \cdot W$). Leaf area index (LAI) was calculated as a product of average plant leaf area and number of plants per land unit area. Total plant leaf area was separated into leaf area of leaves below ear, leaf area of ear leaf and leaf area of leaves above ear.

Before harvest plots were evaluated for final plant stand, stalk lodging, barren and two ears bearing plants. Very few lodged plants occurred in experiments and because of that data are omitted from results. After physiological maturity ears from the two central rows of each plot were harvested by hand and transferred to laboratory to dry before yield component analysis. Ears were sorted by size from the smallest to the largest and 10 of them selected (every fifth to seventh ear depending on plant density) for yield component determination. Row number per ear was determined by counting kernel rows on central part of ear. Kernels in two opposite rows on ear were counted and their average represented kernel number per row. Ears were then hand-shelled and kernel number and grain weight per ear was determined. A 1,000-kernel weight was calculated as grain weight per ear/kernel number per ear*1,000. The remainder of ears from each plot was hand-shelled and grain yield was calculated including kernels weight from 10 ears analyzed for yield components. Grain samples were weighted and dried at 105 °C until constant weight to determine moisture content. Grain yield and 1,000-kernel weight are presented with 14% moisture content. Grain yield was divided by number of ear bearing (fertile) plants per hectare to calculate kernel weight per fertile plant. Kernel weight per fertile plant was divided by kernel weight to determinate number of kernels per plant.

All collected data were analyzed with analysis of variance (ANOVA), where plant densities, genotypes, growing seasons and locations were considered fixed factors and replications random factor. When significant F-tests ($P < 0.05$) were observed mean separation was obtained using a LSD test at the 0.05 probability level.

Results and discussion

The lowest average grain yields of investigated maize genotypes were produced at Maksimir in the growing season of 2009 (Table 2). Location Maksimir is characterized by porous surface soil layer and medium soil water capacity, which combined with the drought conditions of 2009 (Table 1) most likely resulted in severe soil water deficit. This water shortage could not be compensated by 96 mm of rain in first decade of July. Small rainfall during the vegetative stages of maize (April to June) at Maksimir in 2009 brought about the smallest number of leaves per plant (Table 3), the lowest plant leaf area and the greatest number of barren plants per ha (Table 2) across all locations/growing seasons. In 2009, the silking of tested genotypes at Maksimir was observed during the last decade of July (data not presented) with visible signs of water deficit on plants. It is well-known that water deficit during silking period has deleterious effect on maize grain yield (Grant et al., 1989; Otegui et al., 1995). The smallest grain yield at Maksimir in 2009 was associated with the lowest kernel number per ear due to the smallest rows per ear number as well as the lowest kernel number per row (Table 2).

Table 2. Effect of plant density on average grain yield, barren plants and yield components of maize genotypes at Maksimir and Rugvica over two growing seasons

Growing season	Location	Plant density	Grain yield	Barren plants per hectare	Grain weight per plant	1,000-kernel weight	Kernels per ear	Rows per ear	Kernels per row
		plants/ha	kg/ha	No.	g	g	No.	No.	No.
2009	Maksimir	60,000	4,780	1,349	82.5	290	288	13.7	22
	Maksimir	85,000	5,100	6,495	72.5	286	267	13.3	21
	Rugvica	60,000	5,478	833	93.2	294	331	14.3	23.5
	Rugvica	85,000	6,412	3,274	80.8	281	313	14.3	22.3
2010	Maksimir	60,000	6,579	417	111.8	326	346	15	25.4
	Maksimir	85,000	7,833	1,310	90.2	311	303	14.5	22.8
	Rugvica	60,000	5,222	1,149	90	290	314	14.6	23.6
	Rugvica	85,000	6,357	3,072	78.5	282	291	14.5	22
LSD (0.05) ¹			609	1,286	6.94	8.8			
LSD (0.05) ³			423	1,099	3.95	7.2	NS ²	NS	NS

¹ LSD values for comparing means within growing season. ² Non significant for growing season x location x plant density interaction at P<0.05. ³ LSD values for comparing means across growing seasons.

Plant density significantly affected the grain yield of investigated maize genotypes (Table 2). At increased plant density all yield components were significantly reduced, while the number of barren plants increased when compared to low plant density. In spite of this, a 42% increase in plant population resulted in a 18% greater average grain yield. Larger grain yields of inbreds at increased plant populations were also reported by others (Rutger, 1971; Modarres et al., 1998; Jovin et al., 2005). However, a significant growing season x location x plant density interaction for grain yield indicated that grain yield responses to plant densities were affected by locations and growing seasons (Table 2). Grain yield significantly increased at the high compared to the low plant density in both growing seasons at Rugvica because the majority of yield components including barren plants were similarly influenced by plant populations over two growing seasons. Similar response was also found at Maksimir under favorable growing conditions of 2010. However, grain yield increases at high compared to low plant density were the smallest under the lowest yielding environment (location Maksimir in the growing season of 2009) due to previously mentioned dry conditions (Table 1). This is in accordance with Tollenaar et al. (2006) who showed that the impact of crowding stress on grain yield except by plant population density is also influenced by climatic conditions (precipitation, temperature and solar radiation). Relatively low response in grain yield to increased plant density

at Maksimir in 2009 is mainly explained by a large increase in the number of barren plants at high plant density (Table 2). This highest number of barren plants at Maksimir was also associated with long ASI (Table 3). Betrán et al. (2003) reported that average ASI increased in stress conditions, such as drought, what resulted in a reduced yield. Other authors (Monneveux et al., 2005; Borrás et al., 2009) also found that increased plant density extends average ASI for maize inbreds.

Table 3. Effect of plant density on average anthesis to silking interval (ASI), leaf area index (LAI) and other leaf traits of maize genotypes at Maksimir and Rugvica over two growing seasons

GS ¹	Location	Plant density	LAI	Plant leaf area	Leaf area below ear	Ear leaf area	Leaf area above ear	Leaf number	Ear leaf number	ASI
		plants/ha		cm ² /plant	cm ² /plant	cm ² /plant	cm ² /plant	no./plant	no./plant	Days
2009	Maksimir	60,000	1.84	3,107	1,249	376	1,490	17.1	12.2	4.4
	Maksimir	85,000	2.38	3,088	1,267	364	1,409	17.1	12.3	5.6
	Rugvica	60,000	2.33	3,938	1,806	443	1,655	18.1	12.8	5.1
	Rugvica	85,000	3.13	3,815	1,835	428	1,526	18.2	12.9	6.2
2010	Maksimir	60,000	2.79	4,735	2,255	538	1,941	18.8	13.5	2.9
	Maksimir	85,000	3.9	4,442	2,187	510	1,756	18.6	13.4	3.6
	Rugvica	60,000	2.1	3,555	1,536	443	1,576	18.9	13.8	3.6
	Rugvica	85,000	2.87	3,439	1,541	430	1,469	18.8	13.7	4.2
LSD (0.05) ²		0.151								
LSD (0.05) ⁴		0.16		NS ³	NS	NS	NS	NS	NS	NS

¹ GS - growing season. ² LSD values for comparing means within growing season. ³ Non significant for growing season x location x plant density interaction at P<0.05. ⁴ LSD values for comparing means across growing seasons.

Although ASI increased similarly under higher plant density in all environments (Table 3) it is likely that drought conditions during flowering stage at Maksimir in 2009 caused shortage of viable pollen for the late developing silks or even resulted in abortion of late fertilized ovaries. Ribaut et al. (1997) noted that ASI was not correlated with grain yield under well-watered conditions while correlation became highly significant under water stress conditions. Other authors (Edmeades and Daynard, 1979; Fasoula and Tollenaar, 2005; Liu and Tollenaar, 2009) also found that increased plant densities led to increased plant to plant variability because competition between plants for sources intensified, which in turn, reduced kernel set

per plant. Furthermore, it is well documented that drought and shading stress reduce plant net photosynthesis and substantially decrease kernel set or even essentially eliminate kernel formation (Schussler and Westgate, 1991a, b; Echarte and Tollenaar, 2006). It can be assumed that grain yield of maize inbreds and their sister-lines could not be increased by using higher plant population densities when crop is exposed to stressful conditions, such as drought during silking period. Increase of LAI at higher plant density brought about greater competition among plants, which in turn, reduced plant leaf area (Table 3). In addition, the average ear leaf area and total leaf area above ear was reduced at higher plant density. However, leaf area below ear and total leaf number was not affected by plant density, which indicated similar interplant competition at the low and high plant densities during early vegetative growth stages. Maddonni et al. (2001) also found negligible impact of plant population density on the total plant leaf number and leaf area below ear. It is interesting that LAI was greater by an average of 29% at the high compared to the low plant density even under dry growing conditions of 2009 at Maksimir (Table 3).

As expected, grain yields of inbreds Bc 279 and Bc 64 (Table 4) were significantly lower than for their corresponding sister-lines Bc 279026-22 and Bc 822-1064, respectively. These findings support the opportunity for increased seed production of female maize hybrid component by the use of sister-lines instead of inbred lines, as also reported by Lee et al. (2007) and Castellanos et al. (2009). Average grain yield for sister-line Bc 822-1064 was by 141% higher than its related inbred line Bc 64. This grain yield increment was primarily due to a 134% higher kernel weight per fertile plants and 71% less barren plants per unit area when compared to its related inbred line Bc 64. This large difference in average kernel weight per fertile plant was a result of 135% greater kernel number per plant for Bc 822-1064 compared to Bc 64, while both genotypes were characterized by similar 1,000-kernel weight. Tollenaar et al. (2004) reported that heterosis for hybrid grain yield was mostly contributed by greater kernel number per unit area. The higher average kernel number per ear for sister-line Bc 822-1064 was achieved by more rows per ear and kernels per row in comparison to inbred line Bc 64 (Table 4). Inbred Bc 64 and its corresponding sister-line Bc 822-1064 had similar ASI (Table 5). In addition, average LAI for these two genotypes were also similar and consequently, grain yield advantage of sister-line Bc 822-1064 over inbred Bc 64 could not be associated with LAI. In spite of similar average leaf area and leaf number full anthesis of sister-line Bc 822-1064 occurred 4.1 days earlier than for inbred Bc 64. Betrán et al. (2003) found that inbred lines are characterized by longer ASI compared to their hybrids. Tollenaar et al. (2004) reported LAI increments for maize hybrids due to their greater leaf dimensions compared to inbreds, while leaf number per plant only slightly increased. In their later study, Liu and Tollenaar (2009) grew maize genotypes under the same LAI (2.7) and hybrids still out-yielded both parental inbreds. Authors argued that less yielding genotypes have more barren plants because they have lower dry matter accumulation and higher threshold plant growth rate during the critical period for kernel establishment.

Table 4. Effect of plant density on average grain yield, barren plants and yield components of maize inbreds Bc 279 and Bc 64 and their corresponding sister-lines Bc 279026-22 and Bc 822-1064, respectively

Genotype	Plant density	Grain yield	Barren plants per hectare	Ears per plant	Grain weight per plant	1,000-kernel weight	Kernels per ear	Rows per ear	Kernels per row
	plants/ha	kg/ha	No.	No.	g	g	No.	No.	No.
Bc 279	60,000	5,032	779	1.01	88.1	336	266	13.1	21.8
	85,000	6,027	2,665	1	76.7	331	242	13.1	19.9
	Mean	5,530c ¹	1,722b	1.01b	82.4c	334a	254c	13.1c	20.8c
Bc 279026-22	60,000	6,159	539	1.01	102.6	323	322	13.9	24.7
	85,000	7,277	1,745	1	86.8	310	294	13.9	22.7
	Mean	6,718b	1,142b	1.01b	94.7b	316b	308b	13.9b	23.7b
Bc 64	60,000	3,222	2,024	1.04	56.8	275	207	13.6	17.5
	85,000	3,594	7,417	1	46.6	261	189	13.1	16.4
	Mean	3,408d	4,720a	1.02a	51.7d	268c	198d	13.3c	16.9d
Bc 822-1064	60,000	7,645	406	1.03	130.2	267	483	17	30.6
	85,000	8,803	2,323	1.01	111.8	257	449	16.7	29.1
	Mean	8,224a	1,365b	1.02a	121a	262c	466a	16.8a	29.9a
LSD (0.05) ²		339.3	929.8	0.01					
LSD (0.05) ⁴		437.7	1,050.3	0.009	NS ³	NS	NS	NS	NS

¹ Means within a column with different letters are significantly different at the 0.05 probability level. ² LSD values for comparing means within genotype. ³ Non significant for genotype x plant density interaction at P<0.05. ⁴ LSD values for comparing means within plant density.

Sister-line Bc 279026-22 produced higher grain yield by an average of 1,188 kg/ha (21%) than its corresponding inbred Bc 279 (Table 4). This yield increment was associated with heavier kernel weight per fertile plant, while there was no difference between these two related genotypes for barren plants. Larger kernel weight per plant for sister-line Bc 279026-22 may be explained by 22% more kernels per plant whereas 1,000-kernel weight was by 6% lower compared to inbred Bc 279. It is interesting that sister-line Bc 279026-22 had higher average kernel number per plants despite of significantly longer ASI (Table 5). Consequently, yield increment for sister-line Bc 279026-22 was associated with greater LAI (16% on average) compared to its related inbred Bc 279, which is an opposite response to sister-line Bc 822-1064 and its related inbred line Bc 64. This indicate that higher yield increment was found for sister-line produced from low yielding inbred line, as also reported by Lee et al. (2007).

A significant plant density x genotype interaction indicated that tested maize genotypes had various responses to plant densities (Table 4). Inbred Bc 64 was characterized by the lowest grain yield increase (382 kg/ha) at higher plant density compared to other genotypes. This relatively small yield increment at higher plant density was mainly due to large increase in barren plants compared to other genotypes. The highest number of barren plants for inbred line Bc 64 may be associated with the longest sowing to anthesis period and one of the longest ASI (Table 5), which in turn, might lead to the lack of pollen for late appearing silks in crop. Echarte and Tollenaar (2006) stated that some inbreds are characterized by high threshold plant growth rate for kernel set and that high plant density leads to low plant growth rate, which results in a low kernel number per ear or increased frequency of barren plants. It may be assumed that there is a mechanism behind limited yield increase at the higher plant density for the lowest yielding inbred line (Bc 64), which is not expressed by its sister-line Bc 822-1064 or by other tested genotypes. Furthermore, LAI averaged 3.49 for inbred line Bc 64 at higher plant density, while Liu and Tollenaar (2009) reported than the optimal LAI value for inbreds in their study was 2.7. Therefore, inbred Bc 64 might have achieved near-optimal LAI (2.51 on average) already at the low plant density and further grain yield increase through higher LAI values at higher plant density was limited. Although an increase in plant population density resulted in similarly improved LAI for inbred Bc 64 and its corresponding sister-line Bc 822-1064, average grain yield increment was larger for the latter (1,159 kg/ha). These result clearly indicated that grain yield increases were not always associated with LAI values.

Table 5. Effect of plant density on average anthesis to silking interval (ASI), leaf area index (LAI) and other leaf traits of maize inbreds Bc 279 and Bc 64 and their corresponding sister-lines Bc 279026-22 and Bc 822-1064, respectively

Genotype	Plant density	Leaf area index	Plant leaf area	Leaf area below ear	Ear leaf area	Leaf area above ear	Leaf number	Sowing to anthesis period	ASI
	plants/ha		cm ² /plant	cm ² /plant	cm ² /plant	cm ² /plant	no./plant	Days	Days
Bc 279	60,000	1.83	3,160	1,446	403	1,306	18.3	84	3.4
	85,000	2.44	3,000	1,397	386	1,217	18.1	83.8	4.1
	Mean	2.13c ¹	3,080d	1,422c	394d	1,261c	18.2a	83.9c	3.7c
Bc 279026-22	60,000	2.09	3,442	1,467	425	1,487	18.1	83.1	3.8
	85,000	2.86	3,321	1,439	415	1,411	18.1	83.1	4.7
	Mean	2.47b	3,382c	1,453c	420c	1,449b	18.1a	83.1d	4.3b
Bc 64	60,000	2.51	4,282	1,928	483	1,914	18.2	93.1	4.1
	85,000	3.49	4,163	1,942	459	1,748	18.2	93.1	5.5
	Mean	3a	4,223b	1,935b	471b	1,831a	18.2a	93.1a	4.8a
Bc 822-1064	60,000	2.63	4,450	2,005	490	1,955	18.3	88.5	4.6
	85,000	3.51	4,300	2,042	473	1,783	18.3	88.8	5.2
	Mean	3.07a	4,375a	2,024a	482a	1,869a	18.3a	88.7b	4.9a
LSD (0.05) ²		0.146							
LSD (0.05) ⁴		0.142	NS ³	NS	NS	NS	NS	NS	NS

¹ Means within a column with different letters are significantly different at the 0.05 probability level. ² LSD values for comparing means within genotype. ³ Non significant for genotype x plant density interaction at P<0.05. ⁴ LSD values for comparing means within plant density.

Conclusions

Inbreds Bc 279 and Bc 64 produced significantly lower grain yields than their corresponding sister-lines Bc 279026-22 and Bc 822-1064, respectively, supporting the opportunity for increased seed production of maize hybrid parental components by the use of sister-lines. Grain yield increments for sister-lines were primarily associated with more kernels per ear and per plant, while 1,000-kernel weights were similar or slightly lower when compared to corresponding inbreds. However, tested sister-lines differed in their responses for grain yields. When compared to related inbred, grain yield increments averaged 21% for sister-line Bc 279026-22. Much larger grain yield increment (141% on average) was found for sister-line Bc 822-1064, which out-yielded its related inbred Bc 64 despite of similar LAI and ASI. All tested genotypes increased grain yields at higher plant density mainly due to an increment in LAI. Grain yield responses across two plant densities were smallest for the lowest-yielding inbred Bc 64, and these genotype-specific response could not be explained by LAI values. Inbred Bc 64 and its sister-line Bc 822-1064 similarly increased LAI at higher plant density, but the former also had large increase in barren plants. Thus, low yielding genotypes might have some other physiological mechanism limiting their yield increase at higher plant density. In contrast, inbred Bc 279 responded similarly to its sister-line Bc 279026-22 across plant densities. A growing season x location x plant density interaction indicated that grain yield responses to plant densities varied under different environmental (growing season/location) conditions. In conclusion, the use of sister-line Bc 822-1064 rather than inbred Bc 64 greatly increased its seed production, especially at higher plant density, while yield advantage of sister-line cross Bc 279026-22 over inbred Bc 279 may hardly overcome costs derived from sister-line production.

References

- Betrán, F.J., Beck, D., Bänziger, M., Edmeades, G.O. (2003) Secondary traits in parental inbreds and hybrids under stress and non-stress environments in tropical maize. *Field Crops Research*, 83 (1), 51–65.
DOI: [https://doi.org/10.1016/S0378-4290\(03\)00061-3](https://doi.org/10.1016/S0378-4290(03)00061-3)
- Binford, G.D., Blackmer, A.M. (1993) Visually rating the nitrogen status of corn. *Journal of Production Agriculture*, 6 (1), 41–46.
DOI: <http://dx.doi.org/10.2134/jpa1993.0041>
- Borrás, L., Astini, J.P., Westgate, M.E., Severini, A.D. (2009) Modeling anthesis to silking in maize using a plant biomass framework. *Crop Science*, 49 (3), 937–948. DOI: <http://dx.doi.org/10.2135/cropsci2008.05.0286>
- Castellanos, J.S., Córdova, H.S., Srinivasan, G., Quemé, J.L., Rincón, F., Preciado, R.E., Alvarado, G., López, R. (2009) Exploiting modified single crosses in maize (*Zea mays* L.) To facilitate hybrid use in developing countries. *Maydica*, 54 (1), 77–90.
- Crow, J.F. (1998) 90 years ago: The beginning of hybrid maize. *Genetics*, 148 (3), 923–928.

- D'Andrea, K.E., Otegui, M.E., Cirilo, A.G., Eyhéabide, G. (2006) Genotypic variability in morphological and physiological traits among maize inbred lines - Nitrogen responses. *Crop Science*, 46 (3), 1266-1276.
DOI: <http://dx.doi.org/10.2135/cropsci2005.07-0195>
- Duvick, D.N. (1999) Heterosis: Feeding people and protecting natural resources. In: Coors, J.G and Pandey, S., ed. *The genetics and exploitation of heterosis in crops*. Madison, WI: ASA, CSSA, SSSA, 19-30.
- Echarte, L., Tollenaar, M. (2006) Kernel set in maize hybrids and their lines exposed to stress. *Crop Science*, 46 (2), 870-878.
DOI: <http://dx.doi.org/10.2135/cropsci2005.0204>
- Edmeades, G.O., Daynard, T.B. (1979) The developmental of plant to plant variability in maize at different planting densities. *Canadian Journal of Plant Science*, 59 (3), 561-576. DOI: <https://doi.org/10.4141/cjps79-095>
- Fasoula, V.A, Tollenaar, M. (2005) The impact of plant population density on crop yield and response to selection in maize. *Maydica* 50 (1), 39-48.
- Grant, R.F. Jackson, B.S., Kiniry, J.R., Arkin, G.F. (1989) Water deficit timing effects on yield components in maize. *Agronomy Journal*, 81 (1), 61-65.
DOI: <http://dx.doi.org/10.2134/agronj1989.00021962008100010011x>
- Hashemi, A.M., Herbert, S.J., Putnam, D.H. (2005) Yield response of corn to crowding stress. *Agronomy Journal*, 97 (3), 839–846.
DOI: <http://dx.doi.org/10.2134/agronj2003.0241>
- Jones, D.F. (1922) The productiveness of single and double first regeneration corn hybrids. *Journal of the American Society of Agronomy*, 14, 242–252.
DOI: <http://dx.doi.org/10.2134/agronj1922.00021962001400060006x>
- Jovin, P., Rošulj, M., Tolimir, M., Milenković, J. (2005) Effects of the sowing density on the yield and number of germinated seeds in seed maize. *Genetika*, 37 (1), 87-92. DOI: <https://doi.org/10.2298/GENSR0501087J>
- Jungić, D., Husnjak, S., Ključarić, K. (2015) Soil moisture regime in growing maize and soybean on an experimental field of the Faculty of Agriculture in Maksimir. In: Pospišil, M., *Proceedings of 50th Croatian & 10th International Symposium on Agriculture*, Opatija, Croatia, 16-20 February 2015, University of Zagreb, Faculty of Agriculture, 68-73.
- Lee, E.A., Singh, A., Ash, M.J., Good, B. (2006) Use of sister-lines and the performance of modified single-cross maize hybrids. *Crop Science*, 46 (1), 312-320. DOI: <http://dx.doi.org/10.2135/cropsci2005.0103>
- Lee, E.A., Ash, M.J., Good, B. (2007) Re-examining the relationship between degree of relatedness, genetic effects, and heterosis in maize. *Crop Science*, 47 (2), 629-635. DOI: <http://dx.doi.org/10.2135/cropsci2006.04.0275>
- Liu, W., Tollenaar, M. (2009) Response of yield heterosis to increasing plant density in maize. *Crop Science*, 49 (5), 1807-1816.
DOI: <http://dx.doi.org/10.2135/cropsci2008.07.0422>

- Maddonni, G.A., Otegui, M.E., Cirilo, A.G. (2001) Plant population density, row spacing and hybrid effects on maize canopy architecture and light attenuation. *Field Crops Research*, 71 (3), 183-193.
DOI: [http://dx.doi.org/10.1016/S0378-4290\(01\)00158-7](http://dx.doi.org/10.1016/S0378-4290(01)00158-7)
- Modarres, A.M., Hamilton, R.I., Dwyer, L.M., Stewart, D.W., Dijak, M., Smith, D.L. (1997) Leafy reduced-stature maize for short-season environments: Yield and yield components of inbred lines. *Euphytica*, 97 (2), 129-138.
DOI: <http://dx.doi.org/10.1023/A:1002953906498>
- Modarres, A.M., Hamilton, R.I., Dwyer, L.M., Stewart, D.W., Mather, D.E., Dijak, M., Smith, D.L. (1998) Plant population density effects on maize inbred lines grown in short-season environments. *Crop Science*, 38 (1), 104-108. DOI: <http://dx.doi.org/10.2135/cropsci1998.0011183X003800010018x>
- Monneveux, P., Zaidi, P.H., Sanchez, C. (2005) Population density and low nitrogen affects yield-associated traits in tropical maize. *Crop Science*, 45 (2), 535-545. DOI: <http://dx.doi.org/10.2135/cropsci2005.0535>
- Montgomery, E.G. (1911) Correlation studies in corn. 24th Annual report. Lincoln: Nebraska Agricultural Experiment Station, 108-159.
- Otegui, M.E., Andrade, F.H., Suero, E.E. (1995) Growth, water use, and kernel abortion of maize subjected to drought at silking. *Field Crops Research*, 40 (2), 87-94. DOI: [http://dx.doi.org/10.1016/0378-4290\(94\)00093-R](http://dx.doi.org/10.1016/0378-4290(94)00093-R)
- Petošić, D., Dolanjski, D., Husnjak, S. (1998) Functionality of pipe drainage at the trial field Oborovo in the Sava river valley. *Agriculturae Conspectus Scientificus*, 63 (4), 353-360.
- Ribaut, J.M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G.O., Hoisington, D.A. (1997) Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theoretical and Applied Genetics*, 94 (6-7), 887—896.
DOI: <http://dx.doi.org/10.1007/s001220050492>
- Ritchie, S.W., Hanway, J.J., Benson, G.O. (1986) How a corn plant develops. Special report no. 48. Iowa: Iowa State University.
- Rutger, J.N. (1971) Effect of plant density on yield of inbred lines and single crosses of maize (*Zea mays* L.). *Crop Science*, 11 (4), 475-476.
DOI: <http://dx.doi.org/10.2135/cropsci1971.0011183X001100040003x>
- Schussler, J.R., Westgate, M. E. (1991a) Maize kernel set at low water potential: I. Sensitivity to reduced assimilates during early kernel growth. *Crop Science*, 31 (5), 1189–1195.
DOI: <http://dx.doi.org/10.2135/cropsci1991.0011183X003100050023x>
- Schussler, J.R., Westgate, M.E. (1991b) Maize kernel set at low water potential: II. Sensitivity to reduced assimilates at pollination. *Crop Science*, 31 (5), 1196–1203. DOI: <http://dx.doi.org/10.2135/cropsci1991.0011183X003100050024x>
- Tollenaar, M., Ahmadzadeh, A., Lee, E.A. (2004) Physiological basis of heterosis for grain yield in maize. *Crop Science*, 44 (6), 2086-2094.
DOI: <http://dx.doi.org/10.2135/cropsci2004.2086>

Tollenaar, M., Deen, W., Echarte, L., Liu, W. (2006) Effect of crowding stress on dry matter accumulation and harvest index in maize. *Agronomy Journal*, 98 (4), 930–937. DOI: <http://dx.doi.org/10.2134/agronj2005.0336>

Williams, W.A., Loomis, R.S., Duncan, W.G., Dovrat, A., Nunez, F.A. (1968) Canopy architecture at various population densities and the growth and grain yield of corn. *Crop Science*, 8 (3), 303-308.

DOI: <http://dx.doi.org/10.2135/cropsci1968.0011183X000800030013x>