

# Effect of Intra-row Spacing and Seed Inoculation on Stem Lodging, Yield and Rain-Use Efficiency of Maize under Different Climatic Conditions

Violeta Mandić<sup>1</sup>, Snežana Đorđević<sup>2</sup>, Zorica Bijelić<sup>1</sup>, Vesna Krnjaja<sup>1</sup>, Dragana Ružić Muslić<sup>1</sup>, Maja Petričević<sup>1</sup> and Aleksandar Simić<sup>3</sup>

<sup>1</sup>Institute for Animal Husbandry, Department of Feed Science, Belgrade University, 11080 Belgrade, Republic of Serbia

<sup>2</sup>Agrounik doo, Research and Development Centre, 11000 Beograd, Republic of Serbia

<sup>3</sup>Faculty of Agriculture, Department of Crop Science, Belgrade University, 11080 Belgrade, Republic of Serbia

Author for correspondence; e-mail: violeta\_randjelovic@yahoo.com; Tel.: +381 11 2670 121; Fax: +381 11 2670 164

**The effects of three intra-row spacings (20, 24 and 28 cm) with inter-row spacing of 70 cm and seed inoculation with plant-growth-promoting bacteria (PGPB) [(*Azotobacter chroococum*, *Azotobacter vineland*, *Bacillus megaterium* and *Bacillus licheniformis*)] were investigated on stem diameter (SD), stem lodging (SL), percentage of barren plants (PBP), grain yield (GY) and rain-use efficiency (RUE) in maize hybrid Dijamant 6 in the province of Vojvodina, Northern Serbia in 2006, 2007 and 2008. The highest SD and GY and the lowest SL and PBP were recorded in favorable climatic conditions in 2006. The lowest RUE was recorded in 2008 (unfavorable climatic conditions). The lowest SD and RUE and the highest SL, PBP and GY were obtained at the smallest spacing between plants in a row (20 cm). Seed inoculation significantly increased SD, GY and RUE, and SL and PBP significantly decreased. Generally, in Northern Serbia and similar ecological regions, treatment using 20 cm intra-row spacing (plant density of 71429 plants ha<sup>-1</sup>) and seed inoculation could be recommended to improve stem lodging resistance, decrease the number of barren plants and increase grain yield. To reduce yield losses in high crop densities, farmers should adopt appropriate crop management practices such as inoculation of seed with bio-fertilizer.**

Key Words: intra-row spacing, maize, plant growth promoting bacteria, seed inoculation, stem lodging, grain yield, rain-use efficiency

Abbreviations: GY – grain yield, PGPB – plant-growth-promoting bacteria, RUE – rain use efficiency, SD – stem diameter, SL – stem lodging

## INTRODUCTION

Maize is a very important crop used in livestock and human nutrition and as industrial raw material. Maize (grain and silage) is an important component in the nutrition of all species and categories of livestock. Therefore, the production of maize grain or silage forms the basis of stable and profitable livestock production. Maize grain is used for the preparation of starch, syrup, oil dextrose, flakes, gluten, grain cake, lactic acid and acetone which are used by the textile, foundry, fermentation and food industries. In developing countries, maize is the staple food for 900 million people.

However, many factors influence maize production, especially rainfall and temperature regimes during

summer (Mandić et al. 2013). Therefore, it is important to mitigate the negative effects of unfavorable climatic conditions such as extreme temperature and drought with the application of appropriate adaptive agro-technology. Early research has shown that the proper and timely implementation of agro-technical measures can reduce up to 30% of the adverse effects of drought (Starčević et al. 1995). Integrated agronomic practices are important strategies for improving crop productivity and resource use efficiency. The best solution is irrigation, but in Serbia less than 1% of arable land is irrigated (Mandić et al. 2013). Increase in maize production in the world has risen mainly as a result of an increase in yield per unit area, primarily by an increase in crop density. The number of plants per unit area is the most important component of yield. In Serbia, there is a loss of about 30%

of plants from sowing to harvest with a decrease in yield from 1.5 to 2.2 t ha<sup>-1</sup>. It is a major yield loss. However, the supra-optimal crop density can increase lodging rate, barrenness and cause significant yield loss (Mandić 2011).

Optimization of in-row plant density is a simple procedure with low cost which has a significant influence on maize grain yield (Mandić et al. 2016a). In Serbia, intra-row spacing is used to determine crop density. Sangakkara et al. (2004) and Mandić (2011) reported that intraspecific competition for radiation, water and nutrients becomes more intense with increasing plant density of maize. Plants spaced more uniformly in fact compete minimally for growth factors, primarily light, nutrients and water (Li et al. 2015; Sharratt and McWilliams 2005). At higher plant density, plants will produce ears with smaller values of all traits and lower yield per plant, but the higher number of ear per unit area generally leads to an increase in the final grain yield (Mandić 2011; Testa et al. 2015).

Stem lodging is a very important characteristic in maize because it may result in loss of the ear at harvest. Thus, Mandić (2011) reported that stem lodging results in less leaf photosynthetic activity and grain yield, while Flint-Garcia et al. (2003) pointed that stem lodging (breakage of the stem below the ear) ranges from 5% to 20% annually worldwide. Grain yield losses due to stem lodging were estimated to be 40% (Ransom 2005), even 75% (Van Dyk 2001), while in the United States of America, losses were between 5% and 25% (Nielsen 2006). Generally, many factors can cause environmental stress and increase the potential for stem lodging in maize such as high plant populations, extremes in soil moisture, nutrient deficiencies and/or imbalances, insect damage, cropping sequence and hybrid susceptibility. At higher plant population levels, the amount of light is reduced and maize plants become tall and thin, and that is why the physical strength of the stem is significantly reduced. Also, plant-to-plant competition for light, nutrients, and water enhances the competition for carbohydrates between the stem and ear within the plant, thus reducing the vigor of the cells in the stem and predisposing them to invasion by stem rot. This process is referred to as stem cannibalization and causes disintegration of the pith cells. On the contrary, the lower plant population levels will further reduce stem lodging, but also grain yields due to insufficient number of ears per unit area. Stem lodging in maize may result in loss of the ear at harvest, more difficult harvest and

reduction in crop quality. Economic loss from lodging also depends on the harvest method used in the region. If harvest is by machine, many lodged plants will not be harvested. If the farmer harvests by hand, lodging will increase the time required and the labor costs. Paszkiewicz and Butzen (2007) reported that percent stem lodging increased by 0.5% for each increase of 1,000 plants ha<sup>-1</sup>. Maize grain yield is reduced when crop density is increased beyond the optimal, because the harvest index decreases, and the stem lodging increases (Tollenaar et al. 1997). Many researchers found that increasing plant populations increase the grain yield of maize (Sharifi et al. 2009; Amiri et al. 2014; Mandić et al. 2016a).

Results of Inagaki et al. (2015) showed that bacterial seed inoculation is the method for enhancing stem lodging in maize because it improves the accumulation of soluble solids, especially lignin in the stem and stem diameter. Inoculant biofertilizers contain plant growth-promoting bacteria (PGPB) which flourish in the rhizosphere of plants. There are several mechanisms by which PGPB stimulates plant growth such as production of indole-3-acetic acid, gibberellic acid, cytokinins and ethylene, asymbiotic N<sub>2</sub> fixation, biocontrol of pathogenic microorganisms and solubilization of mineral phosphate and other nutrients (Cunha et al. 2006). Hajnal-Jafari (2012) and Mandić et al. (2016b) observed a significantly higher maize grain yield in variance with seed inoculation with growth-promoting bacteria than in the control.

The aim of this study was to determine the effects of years, intra-row spacing and seed inoculation on stem diameter (SD), stem lodging (SL), percentage of barren plants (PBP), grain yield (GY) and rain-use efficiency (RUE) of maize hybrid Dijamant 6 (FAO maturity group 600).

## MATERIALS AND METHODS

### Experimental Details and Treatments

The experiments were carried out in dry land farming in the Autonomous Province of Vojvodina, in the Srem district (Northern Serbia), at the village of Putinci in the vicinity of Ruma (latitude 44° 59' 19" N; longitude 19° 58' 11" E). The commercial maize hybrid Dijamant 6 (FAO maturity group 600) was grown during three growing seasons (2006–2008) at three intra-row spacings and two seed inoculation treatments. The effects of three intra-

row spacings (20, 24 and 28 cm) with inter-row spacing of 70 cm were tested. Thus, three plant densities were formed (71429 plants ha<sup>-1</sup>, 59524 plants ha<sup>-1</sup> and 51020 plants ha<sup>-1</sup>, respectively). Also, two seed inoculation treatments were tested: un-inoculated control and seed inoculation with plant growth-promoting bacteria (PGPB). Seed inoculation was done before sowing (20 mL of inoculant per 100 g seeds). Inoculant consisted of the following bacteria titre: *Azotobacter chroococum* – 10<sup>8</sup> (CFU/mL), *Azotobacter vinelandi* – 10<sup>8</sup> CFU/mL, *Bacillus megaterium* – 10<sup>9</sup> CFU/mL and *Bacillus licheniformis* – 10<sup>9</sup> CFU/mL. The preceding crop was winter wheat in all seasons. Maize was planted during the second half of April. Sowing was carried out manually with two seeds. Land rolling was applied after sowing and thinning seedlings after germination. Plot size was 16.8 m<sup>2</sup> and the sub-plot was 4.2 m<sup>2</sup>. The plot was set up in a randomized block design with four replicates. A standard cultivation practice was applied. In autumn, plots were fertilized with 300 kg ha<sup>-1</sup> and with 10:30:20 NPK fertilizer. Plots were fertilized with 90 kg ha<sup>-1</sup> with calcium ammonium nitrate in two doses (1/2 at the three-leaf stage and 2/2 at the 7- to 9-leaf stage).

#### Soil Characteristics and Climatic Conditions

The soil type was a Chernozem with a pH in H<sub>2</sub>O of 7.52, pH in n/1KCl of 7.31, CaCO<sub>3</sub> of 13.44%, humus of 3.2%, total N of 0.18%, P<sub>2</sub>O<sub>5</sub> of 19.5 mg 100 g<sup>-1</sup> soil and K<sub>2</sub>O of 25.2 mg 100 g<sup>-1</sup> soil.

The amount of rainfall, rainy days and monthly air temperature from April to September were 398.4 mm, 87 d and 18.0 °C in 2006; 358.8 mm, 40 d and 18.8 °C in 2007; and 313.3 mm, 106 d and 18.6 °C in 2008, respectively (Table 1). Generally, the best rainfall distribution was in 2006. The average monthly temperatures were lower in 2006 than in 2007 and 2008.

#### Data Collection

Maize harvest was performed manually in October in all years. The central two rows from each plot were used to determine stem lodging (SL), percentage of barren plants (PBP) and grain yield (GY). GY is calculated on a 14% moisture basis. Ten plants from each plot were taken for measuring stem diameter (SD). Rain-use efficiency (RUE) (t ha<sup>-1</sup> mm<sup>-1</sup>) was

**Table 1.** Optimal monthly rainfall (OMR) and monthly total rainfall (MTR), rainy days (RD) and average temperature (T) from April to September during three growing seasons (2006–2008).

Month	OMR	2006			2007			2008		
		MTR (mm)	RD	T (°C)	MTR (mm)	RD	T (°C)	MTR (mm)	RD	T (°C)
April	50.0	63.9	13	12.5	0	0	13.0	52.4	16	12.9
May	75.0	31.4	31	16.4	79	7	18.5	42.4	21	18.3
June	90.0	92.3	2	19.6	85.2	5	22.0	58.1	18	21.7
July	100.0	39.0	15	22.8	38.7	5	22.6	61.0	27	21.7
August	95.0	156.2	6	19.1	62.5	18	22.3	22.7	9	21.5
September	80.0	15.6	20	17.5	93.4	5	14.3	76.7	15	15.4
Σ / x̄	490.0	398.4	87	18.0	358.8	40	18.8	313.3	106	18.6

calculated according to the formula grain yield/total seasonal rainfall.

**Statistical Analysis**

The data were analyzed using factorial ANOVA in STATISTICA (version 10; StatSoft, Tulsa, Oklahoma, USA). Differences between parameter means were assessed using Duncan's Multiple Range Test at  $P \leq 0.05$ . Pearson correlation coefficient was used for statistical relationship between two continuous variables.

**RESULTS AND DISCUSSION**

Results showed that the year had a highly significant effect on all studied parameters (Table 2). Values of SD (2.41 cm), GY (13.86 t ha<sup>-1</sup>) and RUE (0.035 t ha<sup>-1</sup> mm<sup>-1</sup>)

were significantly higher in 2006 than in 2007 (2.31 cm, 12.04 t ha<sup>-1</sup> and 0.034 t ha<sup>-1</sup> mm<sup>-1</sup>, respectively) and 2008 (2.30 cm, 10.21 t ha<sup>-1</sup> and 0.032 t ha<sup>-1</sup> mm<sup>-1</sup>, respectively). On the contrary, values of SL (3.55%) and PBP (0.96%) were significantly lower in 2006 than in 2007 (3.75% and 1.19%, respectively) and 2008 (4.32% and 1.13%, respectively). In 2007, rainfall was not observed at the time of sowing. We spotted slow initial growth seedlings and uneven initial plant growth. It should be emphasized that the experiment fields were rolled after sowing in all years to ensure quick and uniform seed germination.

Land rolling has been proven to be a very important agrotechnical measure, especially in 2007, when all seeds are germinated. We therefore recommend it as a mandatory agricultural measure after sowing of maize,

**Table 2.** Effects of year, intra-row spacing and seed inoculation on stem diameter (SD), stem lodging (SL), percentage of barren plants (PBP), grain yield (GY) and rainfall use efficiency (RUE) of maize.

Factor	SD (cm)	SL (%)	PBP (%)	GY (t ha <sup>-1</sup> )	RUE (t ha <sup>-1</sup> mm <sup>-1</sup> )
<b>Year effects (A)</b>					
2006	2.41 <sup>a</sup>	3.55 <sup>c</sup>	0.96 <sup>c</sup>	13.86 <sup>a</sup>	0.035 <sup>a</sup>
2007	2.31 <sup>b</sup>	3.75 <sup>b</sup>	1.19 <sup>a</sup>	12.04 <sup>b</sup>	0.034 <sup>a</sup>
2008	2.30 <sup>c</sup>	4.32 <sup>a</sup>	1.13 <sup>b</sup>	10.21 <sup>c</sup>	0.032 <sup>b</sup>
F test	**	**	**	**	*
<b>Intra-row spacing (B)</b>					
28 cm	2.36 <sup>a</sup>	3.13 <sup>c</sup>	1.04 <sup>c</sup>	11.46 <sup>b</sup>	0.035 <sup>a</sup>
24 cm	2.35 <sup>a</sup>	3.97 <sup>b</sup>	1.05 <sup>b</sup>	12.20 <sup>a</sup>	0.034 <sup>a</sup>
20 cm	2.30 <sup>b</sup>	4.52 <sup>a</sup>	1.18 <sup>a</sup>	12.46 <sup>a</sup>	0.032 <sup>b</sup>
F test	**	**	**	*	**
<b>Inoculation seed effects (C): Co – Uninoculated control; SI – Seed inoculation</b>					
Co	2.31 <sup>b</sup>	4.22 <sup>a</sup>	1.32 <sup>a</sup>	11.61 <sup>b</sup>	0.032 <sup>a</sup>
SI	2.37 <sup>a</sup>	3.53 <sup>b</sup>	0.87 <sup>b</sup>	12.47 <sup>a</sup>	0.035 <sup>b</sup>
F test	**	**	**	**	**
<b>Interactions (F test)</b>					
AB	*	**	**	ns	ns
AC	**	*	**	ns	ns
BC	**	**	**	ns	ns
ABC	**	**	**	ns	ns
M	2.34	3.87	1.09	12.04	0.034

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ( $p \leq 0.05$ ).  
 \*\* Significant at 1% level of probability  
 \* Significant at 5% level of probability  
 ns - not significant

especially under drought conditions during the sowing of maize. Also, we have planted two seeds per hole and thus ensured the planned spatial distribution of plants.

Many researches showed that plants with delayed emergence were responsible for reducing crop growth and grain yield compared with plants characterized by early emergence (Egli and Rucker 2012; Mandić et al. 2016a). The optimal amount of rainfall for maize growth in May is 75 mm. The amount of rainfall in this month in all years of research was less than the optimum. In June, maize is at the stage of intensive stem growth. The amount of rainfall in June in 2006 was higher by 2.3 mm (92.3 mm) compared with the optimum monthly rainfall (90 mm). On the contrary, the amount of rainfall in June in 2007 was lower by 4.8 mm (85.2 mm) and in 2008 by 31.9 mm (58.1 mm) compared with the optimum monthly rainfall.

Our research has shown that the plants have a smaller stem diameter under unfavorable water condition. Generally, drought stress during the vegetative stages results in reduced stem cell expansion (shorter plants). Mandić (2011) found that maize plants have weak and thin stems and lower plant height under unfavorable water condition in June. In July, maize is at the pollination and fertilization stage. In all the investigated years, rainfall amount is lower than the optimal monthly rainfall for maize growth (100 mm) in July. However, the drought period in 2007 affected the poor pollination and reduced kernel development (fewer kernels per ear). In August, maize is at the grain fill stage. The data showed that the amount of rainfall in August in 2006 was higher by 61.2 mm compared with conditional-optimal for grain filling (95 mm), by 93.7 mm than in 2007 (62.5 mm) and by 133.5 mm than in 2008 (22.3 mm). The water stress during grain fill stage in August resulted in shortened grain fill periods, increased lodging and decreased grain size, 1,000 grain weight and grain yield. For this reason, GY was significantly lower in 2008 than in the other years of research. Generally, GY was the highest in 2006 because it was not a critical period for water. On the other hand, drought stress in July in 2007 (the pollination and fertilization stage) and in August in 2008 (grain filling stage) resulted in a drastic reduction in GY.

Analysis of variance showed that intra-row spacing had a highly significant effect on SD, SL, PBP, GY and RUE. The lowest SD (2.30 cm) and RUE (0.032 t ha<sup>-1</sup> mm<sup>-1</sup>) and the highest SL (4.52%), PBP (1.18%) and GY (12.46 t ha<sup>-1</sup>) were obtained at 70 × 20 cm. However, SD and RUE were not significantly different between 70 × 28 cm (2.36 cm and 0.035 t ha<sup>-1</sup> mm<sup>-1</sup>, respectively), and 70 × 24 cm

variants (2.35 cm and 0.034 t ha<sup>-1</sup> mm<sup>-1</sup>, respectively). Also, GY was not significantly different at 70 × 24 cm (12.20 t ha<sup>-1</sup>) and 70 × 20 cm (12.46 t ha<sup>-1</sup>). Generally, the increased plant populations intensified interplant competition for environmental factors and resulted in decreases in SD and GY and increases in SL and PBP. At high density, stems are long and thin and increase the risk of lodging. Higher density planting resulted in taller plant heights, smaller shoot dry weights and stem diameter of plants and made them more susceptible to lodging than the shorter plant heights, bigger shoot dry weights and stem diameters of plants at lesser planting density.

Stem lodging is a major constraint to limit grain yield under increased planting density in modern maize production. The higher planting densities increase plant sterility (Sangoi et al. 2002), SL (Tokatlidis et al. 2010; Brekke et al. 2011; Mandić 2011) and PBP (Mandić 2011), and decrease SD (Mandić 2011; Shi et al. 2016). Also, these authors found that increasing plant density increased GY. In essence, SL and PBP did not result in an increase in grain development with increased plant population and had negative effects on GY per plant. However, GY per unit area was compensated by a greater number of plants and number of ears per unit area.

Seed inoculation had a significant effect on SD, SL, PBP, GY and RUE. Values of SD (2.37 cm), GY (12.47 t ha<sup>-1</sup>) and RUE (0.035 t ha<sup>-1</sup> mm<sup>-1</sup>) were higher, while SL (3.53%) and PBP (0.87%) were lower in bacterial seed inoculation treatment compared with the uninoculated control (2.31 cm, 11.61 t ha<sup>-1</sup>, 0.032 t ha<sup>-1</sup> mm<sup>-1</sup>, 4.22% and 1.32%, respectively). Thus, bacterial inoculation of maize seed can reduce the risk of lodging and obtain high GY at high plant density. Fancelli and Dourado Neto (2000) indicated that bacterial seed inoculation increased tolerance of maize plants to lodging, and improved the accumulation of soluble solids in the stem. Results of Dartora et al. (2013) and Inagaki et al. (2015) showed that seed inoculation with diazotrophic bacteria increased the stem diameter of maize compared with the control. In general, the results involving three intra-row spacings showed agronomic efficiency in improving grain yield of the hybrid Dijamant 6, which received inoculation via seed. Seed inoculation can result in strong flexible stem and high yield of maize at high plant densities. This study indicates that the risk of lodging can be reduced by using appropriate management practices.

The effects of interactions on SD, SL and PBP were significant. Year, intra-row spacing and seed inoculation interactions were not observed for GY and RUE.

A very strong positive correlation was found between GY and RUE ( $r = 0.83^{**}$ ), a strong positive correlation between SL and PBP ( $r = 0.61^{**}$ ) and a moderate positive correlation between SD and GY ( $r = 0.46^{**}$ ), Table 3. Low positive correlation was found between SD and RUE ( $r = 0.14ns$ ) and low negative correlation between SL and RUE ( $r = -0.02ns$ ). Strong negative correlations were found between SD and SL ( $r = -0.67^{**}$ ) and between SD and PBP ( $r = -0.71^{**}$ ). Weak negative correlations were found for GY with PBP ( $r = -0.31^*$ ), GY and SL ( $r = -0.25^*$ ) and PBP with RUE ( $r = -0.23^*$ ). Generally, the coefficient of correlation indicated that PBP and SL are dependent on SD, PBP is dependent on SL, and GY is dependent on RUE. The plants that had lodged were barren. Nzuve et al. (2014) and Silva et al. (2016) found that GY had a significant and negative correlation with lodging, that is, lodging had the most positive direct effect on GY.

### CONCLUSION

Results showed that SD and GY were significantly higher, and SL and PBP were significantly lower in 2006 because there was no water stress during the critical growth periods. The lowest RUE was recorded in 2008. Also, results of the study showed that the decreasing intra-row spacing from 28 cm to 20 cm significantly decreased the SD and RUE and increased SL, PBP and GY. In this case, maize stems became mechanically weaker because of the resulting reduction in individual plant mass. The stems

**Table 3.** Pearson correlation coefficient ( $r$ ) between studied parameters.

Parameters <sup>1</sup>	SD	SL	PBP	GY
SL	-0.67**			
PBP	-0.71**	0.61**		
GY	0.46**	-0.25*	-0.31**	
RUE	0.14ns	-0.02 ns	-0.23*	0.83**

<sup>1</sup>Parameters: SD = Stem diameter; SL = Stem lodging; PBP = Percentage of barren plants; GY = Grain yield  
 \*, \*\*Significant at the 0.05 and 0.01 probability levels, respectively  
 ns: non-significant

are long and thin. Inoculated seed with PGPB (*Azotobacter chroococum*, *Azotobacter vinelandi*, *Bacillus megaterium* and *Bacillus licheniformis*) increased SD by 2.6%, GY by 7.4% and RUE by 9.4%, and decreased SL by 16.4% and PBP by 34.1%. Therefore, the recommended spacing for Dijamant 6 is 70 cm between rows and 20 cm between plants in a row. Also, our results present evidence that should be used in inoculation of maize seed with plant-growth promoting bacteria and that this should be an important strategy to improve stem lodging resistance and increase grain yield. To minimize the risks of lodging and barren plants while sustaining high yield performance, farmers should adopt appropriate crop management practices such as intra-row spacing (plant density) and bio-fertilizer application.

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