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Research Article



## Impact of row spacing and seed rate on the production characteristics of the perennial ryegrass (*Lolium perenne* L.) and their valorisation

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### Abstract

In this paper are shown the results of the production characteristics of the perennial ryegrass (*Lolium perenne* L.) cv. 'Naki', which grown in rows with different row spacing and seed rate in the agroecological conditions of central Serbia in three successive analysed years. Four levels of two observed factors were used in the experiment: row spacing (12.5; 25; 37.5 and 50 cm) and seed rate (9, 16, 23 and 30 kg ha<sup>-1</sup>). Due to the analyses, the height of the tiller, the length of the spike and the number of spikelets per spike gave better results by sowing in wider rows (37.5 and 50 cm) using lower seed rate (9 and 16 kg ha<sup>-1</sup>). Seed yield and harvest index responded favourably to sowing in rows at a wider row spacing (37.5 and 50 cm) in combination with a lower seed rate (9 and 16 kg ha<sup>-1</sup>), while shoot dry weight gave better results by sowing in narrower rows (12.5 cm) with lower seed rate (9 and 16 kg ha<sup>-1</sup>). Using the appropriate row spacing in sowing and the optimum of seed rate provides the highest results of the production characteristics of the perennial ryegrass that can be applied to further production.

**Keywords:** harvest index; perennial ryegrass; row spacing; seed rate; seed yield

### Introduction

Perennial ryegrass (*Lolium perenne* L.), from an agronomic point of view, represent the most important grassland type for pasture in temperate climates and it is the most important type in the genus *Lolium* (Bolaric *et al.*, 2005). It is a native plant type in Europe from where is widespread to North America, Africa and Australia. In Serbia, it is known as one of the best forage species (Tomić *et al.*, 2007), characterized by high nutritional value and therefore can be used for grazing as well as for hay or silage. It is reported that Perennial ryegrass has been cultivated as a forage species since the 17th century (Grime *et al.*, 1988). Additionally, Perennial ryegrass shows a high degree of genetic variation in the population (Kubik *et al.*, 2001), which is very

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important considering diploid varieties are still the majority of seed sown in the world (Dalton, 1998). Signification of perennial ryegrass as an animal feed derives primarily from its high digestibility and dry matter quality (Frame, 1989), it is well tolerated in stepping and responds well to nitrogen fertilization. In our country, Perennial ryegrass breeders are driven by market needs to produce genotypes with high, stable yields and excellent dry matter quality with improved impedance as well as drought impedance (Sokolović *et al.*, 2012). One of the most important factors of the cultivation process, in addition to creating frost-resistant varieties and the impact of other environmental factors, is the yield and quality of the seed. Important characteristics for cultivation are flowering date, the length of the spike, the number of spikelets per spike, due to their high variability and heritability.

The agro-ecological conditions of the locality are very important factor defining seed production (Gatarić, 2005; Koeritz, 2012; Jamil *et al.*, 2017; Janković *et al.*, 2018; Lakić *et al.*, 2018; 2019; Bojović *et al.*, 2019). Research shows that row spacing has a significant impact on seed yield. The highest seed yield can be achieved by sowing seeds at a distance of 20 cm (Vučković *et al.*, 1998), as well as by sowing at distances of 25 cm (Fišakov, 1984) or 45-60 cm distances in some very dry areas of Turkey (Uzun and Acikgoz, 1998). Interacting in sowing with a different seed rate from 2.8 to 8.4 kg ha<sup>-1</sup> with a wider spacing between rows (30.5 cm) and a greater amount of nitrogen (157 kg ha<sup>-1</sup>), the Perennial ryegrass responded by increasing the seed yield (Koeritz, 2012). In some parts of Canada, sowing seeds of 8-10 kg ha<sup>-1</sup> at a row spacing of 15 to 30 cm has produced great results (Cattani, 2007).

The subject of this paper is using the results of previous research and the results of this research to improve the potential of grass seed production in the agro ecological conditions of central Serbia. The potential for development is based on the proper combination of row spacing and seed rate in a particular locality and in a particular environment which impact on the correlation between stages during the vegetative development of the flowering plants and following the seed yields depends on many factors.

## Materials and Methods

### *Plant material*

The research was conducted over three following years, between 2012-2014 in the terrain and in the laboratory. The terrain experiment was located near Arandjelovac (44° 21'N, 20° 27'E), Serbia. In experimental conditions, the soil on which the experiment was set is cambisol with low percentage of humus - 2.27%. The main characteristics of the soil at a depth of 0 - 30 cm were: texture: clay; CaCO<sub>3</sub>: 0.83%; pH in KCl: 6.1; K<sub>2</sub>O: 14.7 mg / 100 g; P<sub>2</sub>O<sub>5</sub>: 1.6 mg / 100 g.

Before the terrain experiment was set up, the fertilization of commercial seeds of the 'Naki' variety was tested in laboratory conditions and the results obtained were in accordance with the ≈ 87% declaration. The weight of 1000 seeds of commercial seed of diploid variety 'Naki' was 2.14 g. The terrain experiment was conducted according to a random block system in four repetitions on 10 m<sup>2</sup> (5 x 2 m<sup>2</sup>) elementary plots. The diploid variety of perennial ryegrass used in the experiment is cv. 'Naki' and it was sown at the beginning of the experiment (2011) and seeds were collected and examined after each harvest for the next three years (2012, 2013 and 2014). The Perennial ryegrass was sown at four rows spacing (12.5; 25; 37.5 and 50 cm) in autumn of 2011. The sowing rate used in the experiment was equivalent to 9, 16, 23, 30 kg ha<sup>-1</sup> with the following amount of nitrogen fertilizer - 0, 30, 60, 90 kg ha<sup>-1</sup>. The observed factors were combined with each other and it was measured the impact of the factors on the production characteristics of perennial ryegrass.

Prior to the seed harvest, some biometric measurements were made such as the generative the height of the tiller, the length of the spike and the number of spikelets per spike based on 10 randomly sampled plants from each plot. The perennial ryegrass was harvested by sickle in the third decade of June and the first decade of July. Harvesting of the perennial ryegrass for all three years began when the gentle hand rubbing of the spines resulted in a visible breakage of the seeds. The tillers of perennial ryegrass were put in bunches and dried

outdoors in a well-ventilated place. After free drying, the seeds were ground, cleaned and weighed. The straw was weighed as a dry mass.

### Statistical analysis

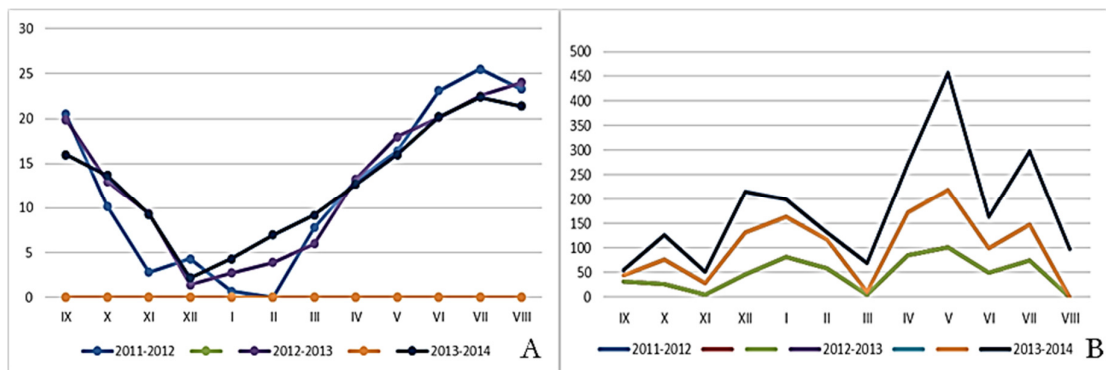
Hence the harvest index was calculated using the formula  $HI = [SI / (SI + SDM)] \times 100$ , where HI is the harvest index (%), SI is the seed yield ( $kg\ ha^{-1}$ ), and SDM is the dry mass (straw yield) ( $kg\ ha^{-1}$ ). The data collected were sublimated, categorized by year and parameter and analysed using accurate statistical analyses (e.g., ANOVA) using the Statistical Package for the Social Sciences (SPSS) and Microsoft Excel 2016.

## Results

Weather conditions were monitored from the nearest meteorological station in Smederevska Palanka (SP) in Serbia. They are shown by average values of monthly temperatures and monthly rainfall from September to August next year, which coincides with sowing and harvesting of seeds. Average monthly temperatures did not deviate significantly from the annual average between March and August (vegetative period). The exception was May, when in all three growing seasons the average was less than the average for the period 2005-2014. The rainfall regime during the 2011/2012 season has two noticeable periods: November - March; within the month with the highest rainfall was January (81.6 mm) and the other period is April-June, with the highest rainfall being recorded in May (117 mm). The period between June and July is a period of drought, with rainfall and the temperature rising (23.1 and 25.5 °C, respectively). This indicates an unfavourable impact on seed maturation and seed expansion, which if prevented by intensive harvesting.

During the second experimental year (2012/2013), 117 mm of water sediment was registered in May, which causes intensification of weeds. The most important months for maturation and seed harvesting (June - 49.6 mm and July - 74.1 mm) were dry but also favourable for safe seed production. Temperature regime throughout the growing season was favourable, with temperatures increasing from March (18.0 °C) to June (20.1 °C) and July (22.5 °C) which means that year was very good for seed production. The last vegetative season of 2013/2014. is characterized by the sum of rainfall during the vegetation period (March-August) which is twice as enormous than the average for ten years (2005-2014). In May, 238.2 mm of rainfall was recorded (Figure 1a; 1b).

In the last decade, research on grassland populations has focused on quantified production per unit area. Some plants have been observed as a minimal physiological unit in grassland components (Simić *et al.*, 2009; Janković *et al.*, 2018). The tillers are the basic unit of production and durability of the lawn (Matthéy *et al.*, 2011), so that to further improvement of production, the characteristics of the tillers are important for some species.



**Figure 1.** Description of average monthly temperatures (A) and Description of total monthly precipitation; (B) in Smederevska Palanka, Serbia [2011-2014].

**Table 1.** Effect of inter-row spacing and seeding rate on tiller characteristics, 2012-2014

Parameter	Tiller length (cm)	Spike length (cm)	Spikelets per spike (no. tiller <sup>-1</sup> )
Inter-row spacing, cm, A	2012		
12.5	62.8 <sup>c</sup>	20.9 <sup>c</sup>	19.7 <sup>c</sup>
25.0	66.3 <sup>b</sup>	21.9 <sup>b</sup>	22.0 <sup>b</sup>
37.5	70.2 <sup>a</sup>	25.7 <sup>a</sup>	24.3 <sup>a</sup>
50.0	75.0 <sup>a</sup>	27.9 <sup>a</sup>	27.0 <sup>a</sup>
Seeding rate, kg ha <sup>-1</sup> , B	2012		
9	72.1 <sup>a</sup>	25.6 <sup>a</sup>	24.7 <sup>a</sup>
16	71.3 <sup>a</sup>	25.4 <sup>a</sup>	24.3 <sup>a</sup>
23	65.6 <sup>b</sup>	22.8 <sup>b</sup>	22.2 <sup>b</sup>
30	65.2 <sup>b</sup>	22.6 <sup>b</sup>	21.8 <sup>b</sup>
Average	68.6	24.1	23.3
Inter-row spacing, cm, A	2013		
12.5	67.7 <sup>c</sup>	25.2 <sup>c</sup>	20.5 <sup>c</sup>
25.0	70.1 <sup>b</sup>	29.0 <sup>b</sup>	23.5 <sup>b</sup>
37.5	77.0 <sup>a</sup>	29.9 <sup>a</sup>	26.8 <sup>a</sup>
50.0	83.8 <sup>a</sup>	35.4 <sup>a</sup>	28.9 <sup>a</sup>
Seeding rate, kg ha <sup>-1</sup> , B	2013		
9	78.1 <sup>a</sup>	31.2 <sup>a</sup>	26.1 <sup>a</sup>
16	77.2 <sup>a</sup>	31.0 <sup>a</sup>	25.7 <sup>a</sup>
23	71.8 <sup>b</sup>	28.8 <sup>b</sup>	24.0 <sup>b</sup>
30	71.5 <sup>b</sup>	28.5 <sup>b</sup>	23.8 <sup>b</sup>
Average	74.7	29.9	24.9
Inter-row spacing, cm, A	2014		
12.5 cm	60.8 <sup>b</sup>	22.3 <sup>b</sup>	16.9 <sup>c</sup>
25.0 cm	60.0 <sup>b</sup>	22.6 <sup>b</sup>	18.6 <sup>b</sup>
37.5 cm	64.5 <sup>a</sup>	24.0 <sup>a</sup>	21.5 <sup>a</sup>
50.0 cm	68.7 <sup>a</sup>	24.7 <sup>a</sup>	22.4 <sup>a</sup>
Seeding rate, kg ha <sup>-1</sup> , B	2014		
9	68.8 <sup>a</sup>	24.7 <sup>a</sup>	20.6 <sup>a</sup>
16	67.6 <sup>a</sup>	24.6 <sup>a</sup>	20.5 <sup>a</sup>
23	59.2 <sup>b</sup>	22.3 <sup>b</sup>	19.2 <sup>b</sup>
30	58.4 <sup>c</sup>	21.9 <sup>c</sup>	19.1 <sup>b</sup>
Average	63.5	23.4	19.8

\*Notes (legend): Meanings in the columns followed by the same letter in the index are not significantly different according to Fisher's protected LSD values (LSD test,  $p < 0.05$ ).

The highest and smallest height of tillers were recorded in 2013 (74.7 cm) and in 2014 (63.5 cm), respectively. Due to very wet conditions in 2014 (rainfall in the vegetative period was 710.9 mm and in 2013 it was 332.5 mm), these two years were climatic extremes for the height of the tillers (Table 1).

Both the spacing and the rate of sowing had a greater effect on all components of seed yield than their interaction (Tables 2 and 3). The highest height of tiller was achieved by sowing the seed at wider row spacing in all three years linking with a higher sowing rate (seed rate); which without clear regularity is the result of greater use of nutrients and characteristics of location. Sowing in rows with narrower spacing had a greater impact on length of the spike in all three years, so the longest spike was measured in 2013 (29.9 cm), while the shortest spike was achieved in 2014 (23.4 cm), Table 1.

**Table 2.** Effect of inter-row spacing and seeding rate on harvest characteristics, 2012-2014

Parameter	Seed yield (kg ha <sup>-1</sup> )	Shoot DM (kg ha <sup>-1</sup> )	Harvest index (%)
Inter-row spacing, cm, A	2012		
12.5	443.2 <sup>c</sup>	844.2 <sup>a</sup>	34.3 <sup>c</sup>
25.0	454.3 <sup>b</sup>	808.0 <sup>a</sup>	36.0 <sup>b</sup>
37.5	465.4 <sup>a</sup>	775.2 <sup>b</sup>	37.4 <sup>a</sup>
50.0	485.2 <sup>a</sup>	729.7 <sup>c</sup>	39.8 <sup>a</sup>
Seeding rate, kg ha <sup>-1</sup> , B			
9	471.0 <sup>a</sup>	804.0 <sup>a</sup>	36.9 <sup>a</sup>
16	466.3 <sup>a</sup>	799.8 <sup>a</sup>	36.7 <sup>b</sup>
23	455.8 <sup>b</sup>	776.8 <sup>b</sup>	36.9 <sup>a</sup>
30	455.0 <sup>b</sup>	776.3 <sup>b</sup>	36.9 <sup>a</sup>
Average	462.0	789.2	36.9
Inter-row spacing, cm, A	2013		
12.5	461.7 <sup>c</sup>	881.4 <sup>a</sup>	34.4 <sup>c</sup>
25.0	539.2 <sup>c</sup>	840.5 <sup>a</sup>	39.1 <sup>b</sup>
37.5	594.5 <sup>a</sup>	807.2 <sup>c</sup>	42.4 <sup>a</sup>
50.0	648.9 <sup>a</sup>	789.4 <sup>c</sup>	45.1 <sup>a</sup>
Seeding rate, kg ha <sup>-1</sup> , B			
9	572.1 <sup>a</sup>	839.9 <sup>a</sup>	40.4 <sup>a</sup>
16	569.4 <sup>a</sup>	838.1 <sup>a</sup>	40.4 <sup>a</sup>
23	551.7 <sup>c</sup>	818.2 <sup>b</sup>	40.2 <sup>a</sup>
30	551.1 <sup>c</sup>	822.4 <sup>b</sup>	40.0 <sup>b</sup>
Average	561.1	829.4	40.2
Inter-row spacing, cm, A	2014		
12.5	208.4 <sup>c</sup>	972.2 <sup>a</sup>	17.5 <sup>b</sup>
25.0	257.7 <sup>c</sup>	953.3 <sup>a</sup>	21.4 <sup>b</sup>
37.5	309.0 <sup>a</sup>	896.5 <sup>c</sup>	25.7 <sup>a</sup>
50.0	343.7 <sup>a</sup>	878.1 <sup>c</sup>	28.2 <sup>a</sup>
Seeding rate, kg ha <sup>-1</sup> , B			
9	293.9 <sup>a</sup>	955.0 <sup>a</sup>	23.6 <sup>a</sup>
16	288.6 <sup>a</sup>	951.9 <sup>a</sup>	23.3 <sup>a</sup>
23	268.5 <sup>b</sup>	897.3 <sup>c</sup>	22.9 <sup>b</sup>
30	267.8 <sup>b</sup>	895.9 <sup>c</sup>	22.9 <sup>b</sup>
Average	279.7	925.0	23.2

\*Notes (legend): Meanings in the columns followed by the same letter in the index are not significantly different according to Fisher's protected LSD values (LSD test,  $p < 0.05$ ).

The number of spikelets per spike was also significantly influenced by the spacing between rows and sowing speeds, so that the best results are achieved at a wider spacing using the lowest seed rate. It is observed the same effect of humidity on the number of spikelets per spike - the highest in 2013 (25 spikelets) and the lowest in 2014 (20 spikelets).

Dry weight (straw) accumulation was influenced by the way it was determined in the experiment. During all three years, it was a more constant parameter in relation to seed yield. The highest amount of dry weight was achieved in the last year - 2014 (925.0 kg ha<sup>-1</sup>).

At the harvest, as an indicator of seed production efficiency, were influenced by all treatments applied.

**Table 3.** Statistical summary of changes in tiller and harvest characteristics according to stand density (LSD test)

Parameter	2012	2013	2014
Inter-row spacing A	**	Tiller length	**
Seeding rate B	**	**	**
AxB	ns	ns	ns
Inter-row spacing A	**	Spike length	**
Seeding rate B	**	**	**
AxB	ns	**	ns
Inter-row spacing A	**	Spikelets per spike	**
Seeding rate B	**	**	**
AxB	ns	ns	ns
Inter-row spacing A	**	Seed yield	**
Seeding rate B	**	**	**
AxB	ns	ns	ns
Inter-row spacing A	**	Shoot DM	**
Seeding rate B	**	**	**
AxB	*	ns	ns
Inter-row spacing A	**	Harvest index	**
Seeding rate B	ns	**	**
AxB	ns	**	**

The highest harvest index was recorded in 2013 (40.2%), which was an unexpected result. To explain the harvest components more objectively, they can be compared to the yield components using correlation analysis. As follows, a very significant correlation was noticed between the height of the tiller and seed rate ( $r = 0.839$ ), the height of the tiller and harvesting tiller ( $r = 0.721$ ). The length of the spike and number of spikelets per spike have lower impact on seed yield and dry weight. The harvest index negatively affected the correlation of dry weight in all three years, while the seeding rate on yield increased.

## Discussion

During the period 2012-2014, the level of impact of row spacing and sowing norm on seed yield in the main role is determined by agro ecological conditions, temperature and rainfall.

In the spring of 2012, it was the optimum rainfall, the main monthly temperature was about average, but temperatures above average have a significant impact. Also, the wider row spacing (37.5 cm and 50 cm) and the lower seed rate in the sowing ( $9 \text{ kg ha}^{-1}$  and  $16 \text{ kg ha}^{-1}$ ) had a significant impact on the length of the tiller. A significant impact of the treatment was also recorded in the length of spike and in the number of spikelets per spike. The treatments had an impact on seed yield and harvest index (wider sowing and lower seed rate in sowing), but have a significant impact of narrow sowing (12.5 cm and 25 cm) on the dry weight of tillers.

During 2013, it was achieved the highest length of the tiller from all of three observed years (74.7 cm). It was recorded a significant effect of wider row spacing (37.5 cm and 50 cm) and lower seed rate ( $9 \text{ kg ha}^{-1}$  and  $16 \text{ kg ha}^{-1}$ ) were observed. The dry weight tiller is decreasing with increasing spacing between rows as well as increasing seed yield.

Recommended sowing norms (seed rate in sowing) and row spacing in Perennial ryegrass production indicate that the agro ecological state of the locality is an important factor in seed production (Vuckovic *et al.*, 1999). The same authors assume that a lower sowing rate can have a positive impact on seed yield compared to a high sowing rate. Sowing in narrower rows has a positive impact on weed control (Paul-Praat, 1995). In some other research, it has been noticed that optimal seed yield is obtained by sowing seeds at mid-spacing (20-25 cm) (Gatarić, 2005); but also, there are good results achieved with wide sowing. Perennial grass seed responds favorably to autumn sowing, but the amount of seed obtained was lower than the results obtained from sowing in the mix (Szczepanek *et al.*, 2004). Also, in the interaction between five sowing norms (1.4; 2.8; 5.6; 8.4; and 11.2 kg ha<sup>-1</sup>) and three rows spacing (10.2; 20.3; and 30, 5 cm) placed in a random block with 4 repetitions, it was observed that a larger seed rate with sowing a wider row gives better results (Koeritz, 2012).

The results show that rainfall and the main monthly sum of temperatures in the last year (2014) have a significant impact on all treatment components. High rainfall over two successive years in months important for seed production led to abundant biomass accumulation (Simić *et al.*, 2009) in the third year. It has also been noted that there was no interaction between the impact on seed yield and dry weight (except in 2012). Also, it has been observed that the seed rate lost crops in combination with climatic conditions.

Climate change has the potential to both positively and negatively affect the location, timing, and productivity of crop at local, national, and global scales (Popović, 2015; Mihailović *et al.*, 2020). Climate and climate change are a particular challenge to the law, because the global characteristic of the impact of climate on the life of the entire planet creates the need for cooperation and commitment at international, regional and national levels. The right to a healthy environment belongs to the modern human rights corps. Environmental law as a whole, and in particular the section on climate change, presents a complex challenge in various professional and scientific fields. The right of its instruments should help and improve solutions for overcoming the negative consequences of the complex problem of contemporary civilization in the field of climate change (Jovanović and Mihailović, 2020).

Heavy rains in the spring of 2014 reduced the stock of photo-assimilates for seed development (Griffith, 2000). Growth of dry weight of tillers followed by a decreasing in seed yield and harvest index results in differences between 2013 and 2014 as a consequence of environmental tolerance.

## Conclusions

During the tested period, the level of impact of row spacing and sowing norm on seed yield in the main role is determined by temperature and precipitation. Therefore, it can be concluded that the length of the tiller, the length of the spike and the number of spikelets per spike give better results by sowing in wider rows at 37.5 and 50 cm apart, using lower seed rate in the sowing (9 and 16 kg ha<sup>-1</sup>). Seed yield, the harvest index, also responds favourably to sowing in wider rows (37.5 and 50 cm) with lower seed rate (9 and 16 kg ha<sup>-1</sup>). The resulting amount of straw (dry weight) was larger by sowing in narrow rows (12.5 cm), with lower seed rate (9 and 16 kg ha<sup>-1</sup>).

## Authors' Contributions

In this research, all authors contributed effectively. Conceptualization, resources, methodology; Formal analysis and writing original draft: MJT, SV, MI and VP; Designed the experiments; Supervision; Writing - review and editing: SJ, VS, VL and AM. All authors read and approved the final manuscript.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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