

***Lolium perenne* L. Production Effect on Row Spacing, Sowing Rate, and Nitrogen Fertilization**

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ABSTRACT

Perennial ryegrass (*Lolium perenne* L.) is one of most dominant perennial grasses in temperate climate. Temperature is a key factor influencing plant development, and under most climate change scenarios, it will be increasingly important to cultivate *L. perenne* varieties adapted to new temperature regimes. Understanding the variability in plant responses to temperature and nutritional inputs is therefore essential for breeding improvement. This study examined the effects of spring nitrogen nutrition, sowing rate, and inter-row spacing on the seed yield of the diploid perennial ryegrass variety 'Naki' over a three-year period in the central region of the Republic of Serbia. Seed yield in second year is significantly higher (648.9 kg ha⁻¹) than yield obtained in the third year of research. Sowing rate, application of nitrogen fertilizers and inter-row spacing had significant influence on the yield of perennial ryegrass. The year had the significant influence on the production. The third year of research was the most unfavorable for seed yield (208.4 kg ha⁻¹). Across all three years, the highest seed yield was accomplished by sowing in distance on 50 cm in row, with consumption of 9 kg ha⁻¹ of seed in sowing and by application of 90 kg ha⁻¹ of nitrogen. Analysis of variance indicates that there are very significant differences between the sowing rate and the amount of nitrogen, $F_{\text{ekp}}=5.176$ in second year and $F_{\text{ekp}}=14.507$ in third year of research. Breeders should use this revealed diversity to develop future perennial ryegrass cultivars better adapted to evolving climatic conditions.

Keywords: perennial ryegrass, seed yield, amount of nitrogen, sowing rate, inter-row spacing, environmental.

INTRODUCTION

The Perennial ryegrass (*Lolium perenne* L.) belongs the group of the perennial grasses, high quality and a wide area of distribution and represents one of the most dominant grass species for turfs in moderate climatic conditions (Bolaric et al., 2005; Baskin and Baskin, 2014). Perennial ryegrass has little or no juvenility and can be vernalized in the seed (Heineck et al., 2020). It is adapted to areas with a cold and humid climate, where between 457-635 mm of rain is recorded annually (Thorogood, 2003). The best results are achieved on fertile and well-drained soils. It tolerates acidic and alkaline

soils very well; while the maximum production is realized at pH 5.5-7.5 (Hannaway et al., 1999; Anon, 2008).

Thanks to its characteristics: easy establishment, long growing season and the achievement of a stable yield of high-quality seeds, the economic importance of perennial ryegrass in lawns is justified. Perennial ryegrass is important in ruminant nutrition in temperate climates; also is part of natural and sown pastures (Stošić, 2005; Zhang et al., 2013; Zejak et al., 2022) and natural seeded meadows (Lazarević et al., 2001). In addition to seed production, perennial ryegrass is used as hay and silage by cutting (Dinić et al., 2003; Stošić, 2005; Georgieva et al., 2025).

Can be used for special horticultural purposes (Jovanović et al., 2012), as well as in the fight against erosion, for soil stabilization; as well as in mixes with other grasses and legumes (Sokolović et al., 2007). Due to high seed production and effective dispersal capacity, perennial ryegrass has the ability to rapidly colonize new areas where conditions are favorable (Wang et al., 2023).

The largest producers of perennial ryegrass seeds (according to data from 2020) are: Denmark (76,883.2 t); The Netherlands (15,332 t) and Germany (7,041 t); while the lowest yield was recorded in the Czech Republic (592 t) (Delran et al., 2009). In the Republic of Serbia, breeders are guided by the needs of the market for the production of genotypes with a high, stable yield and excellent dry mass quality, as well as improved drought resistance (Sokolović et al., 2012).

The seed yield of perennial ryegrass depends on the interaction of row spacing and seed quantity (Kelner, 1975; Brown, 1979; Schoberlein, 1980). Longer sowing spacing combined with higher amounts of nitrogen fertilizers and greater spacing affect later seed yield (Koeritz et al., 2015; Todorović et al., 2020).

The height of perennial ryegrass seed yield reacts positively on application of mineral fertilizers (Stevens et al., 1989; Sokolović et al., 2012; Gatarić et al., 2014), especially on the time and the way of application of the nitrogen fertilizers (Cookson et al., 2000). According this authors, multiple application (fall, winter and spring) causes increasing of the biomass ($\approx 40\%$) in comparison with single application of nitrogen fertilizers in fall ($\approx 18\%$). Therefore, combining of winter and spring fertilization has significant influence of seed yield increasing. Probing of different amounts of nitrogen in spring fertilization in combination with different levels of seed rates and inter-sow spacing can give the results which may influence by

increasing the area under this forage crops. Also, perennial ryegrass silage has the best total nutritional value (166 g kg⁻¹ dry matter of crude protein and 39.1 g kg⁻¹ dry matter of crude fat; and the lowest content of dry matter (321 g kg⁻¹ dry matter) compared to the composition of pure and mixed silage) (Merkevičiūtė Lauksmė et al., 2022). The aim of our research was to determine the influence of the application of nitrogen fertilizers, the sowing rate and the inter-row spacing on the yield of the diploid variety of perennial ryegrass Naki during a three-year study.

MATERIAL AND METHODS

Experimental design

The experimental trial was set up with the diploid variety of perennial ryegrass, Naki (*Lolium perenne* L. cv. Naki) according to the randomized block system in four replicates on elementary plots of 10 m² in a three-year period in the Darosava locality (Progoreoci village), Arandelovac municipality (Central Serbia). In the experiment, the impact of three factors was monitored, which were combined in 64 different treatments (AxBxC). Observed factors had 4 levels each: A - Inter-row spacing: 12.5; 25; 37.5 and 50 cm; B - sowing rate: 9; 16; 23 and 30 kg ha⁻¹; C - amount of nitrogen fertilizer: 0; 30; 60 and 90 kg ha⁻¹. Sowing was done manually, by sowing seeds in long strips 5 m (Figures 1a and 1b). In spring, protective agents were used in the fight against broadleaf weeds (Mustang - active substance Florasulam 6.25 g l⁻¹ + 2.4- D-2-EHE 300 g l⁻¹, in formulation SE- suspoemulsions), as well as manual hoeing of the crop. Two-phase harvesting was done manually. After that, the plant material was dried under natural conditions, as well as the separation of the seeds from the rest of the plant mass (Figures 1b and 1c).

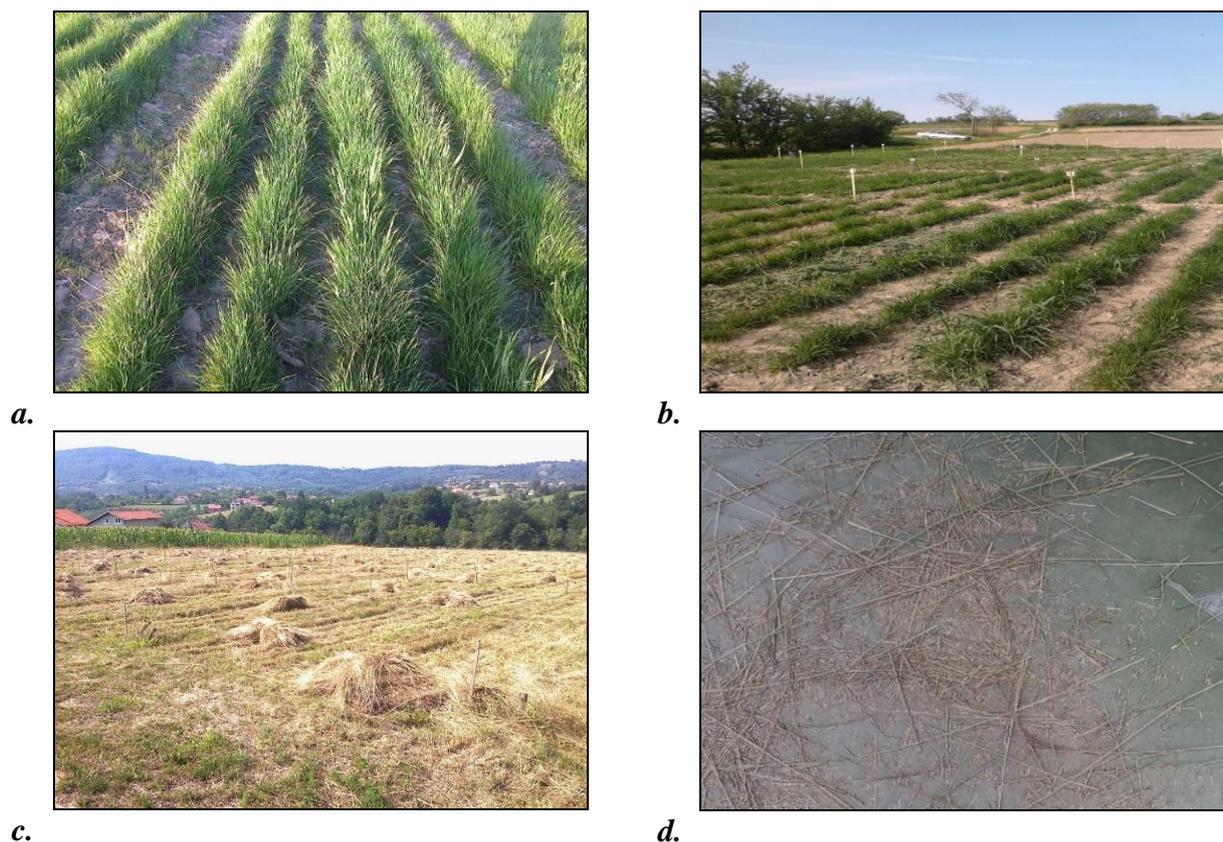


Figure 1. Experimental fields (a.), Spring hoeing and nitrogen fertilization (b.), Harvest of *Perennial ryegrass L.* (c.), Separating seeds from straw (d.)

Soil characteristics

Chemical characteristics of the soil:

Agrochemical analysis of the soil before setting up the experiment (Table 1) shows that the soil had a medium acidic reaction [pH (u KCl) - 6.1]; it was poorly provided

with humus (2.7%); good provided with nitrogen (0.2%), while the content is CaCO_3 was low - 0.83%; very poor in phosphorus, P_2O_5 - 1.6 mg kg^{-1} and moderately supplied with potassium, K_2O - 14.7 mg kg^{-1} .

Table 1. Chemical composition of the soil before the establishment of the experimental trial *Lolium perenne*

Parameter						
Depth, cm	pH in KCl	Humus, %	Total N, %	CaCO_3 , %	P_2O_5 mg/100 g	K_2O mg/100 g
0-30	6.10	2.7	0.2	0.83	1.6	14.7

Statistical analysis

The experimental data were analyzed with descriptive and analytical statistics using the statistics module Statistics 12. All significance assessments were made based on the ANOVA test at the 0.5% and 0.1% significance levels.

Meteorological conditions

The experiment was set up near Arandelovac (Republic of Serbia). Weather conditions are shown in Figures 1-3. The average monthly temperatures do not deviate

significantly from the multi-year average for the observed period. An exception was observed in third season, when the measured temperature was 2-4 degrees lower during May. The average sum of monthly precipitation in the observed period was lower than the multi-year average, and in third season, twice the average sum of precipitation was recorded, which had a significant positive impact on the average yield of perennial ryegrass seeds obtained (Figures 2-4).

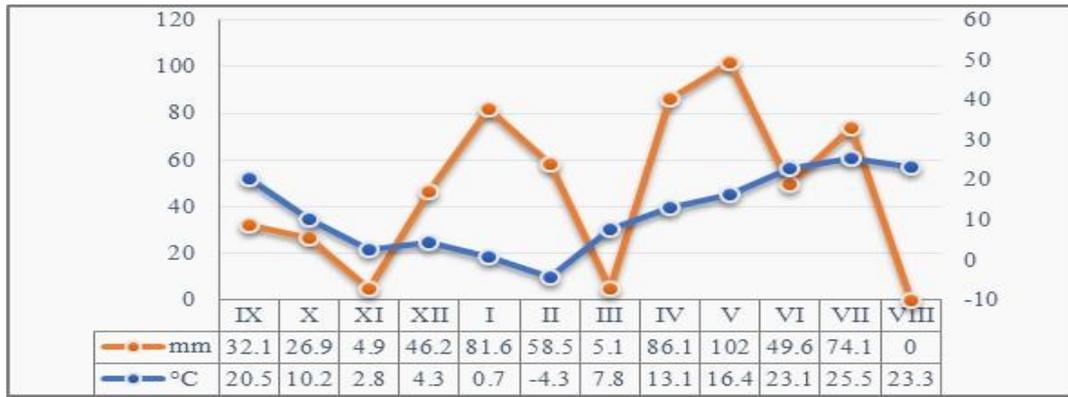


Figure 2. Temperatures (°C) and precipitation (mm) in Arandjelovac, Serbia, in first season

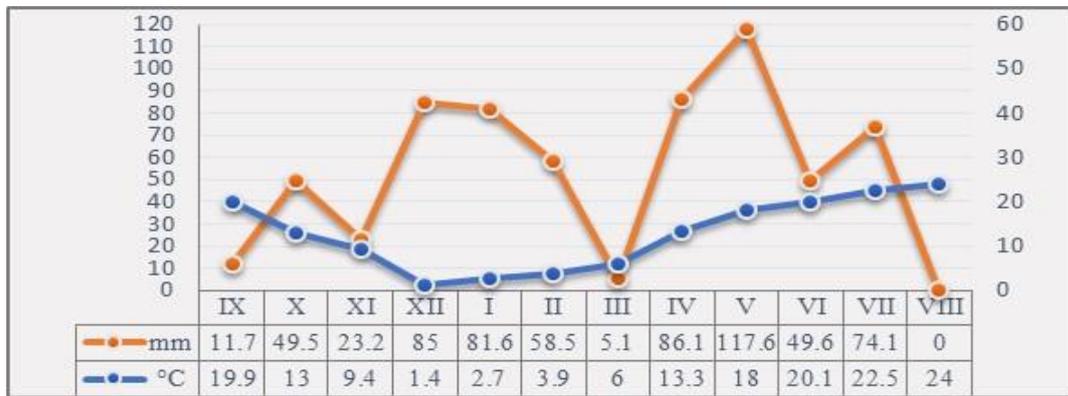


Figure 3. Temperatures (°C) and precipitation (mm) in Arandjelovac, Serbia, in second season



Figure 4. Temperatures (°C) and precipitation (mm) in Arandjelovac, Serbia, in third season

Seed yield is a production trait and was processed using the statistical method of three-factor analysis of variance using the SPSS statistical package. The obtained results serve as a recommendation for the future improvement of perennial ryegrass seed production.

RESULTS AND DISCUSSION

The results of the research indicate that the obtained average values of seed yield in the

first year of the research varied from $344.3 \pm 10.4 \text{ kg ha}^{-1}$ to $576.3 \pm 12.7 \text{ kg ha}^{-1}$ depending on the influence of all three examined factors. By sowing perennial ryegrass seeds at a greater inter-row distance, the yield of the obtained seeds also increased. By sowing seeds at a distance of 12.5 cm, an average seed yield of $443.2 \pm 63.4 \text{ kg ha}^{-1}$, while at the maximum inter-row spacing (50 cm) an average seed yield $485.2 \pm 62.8 \text{ kg ha}^{-1}$.

The seeding rate had a significant statistical effect on the values of the seed

yield of perennial ryegrass. In the experimental plots where it was 9 kg ha⁻¹ of seed, the yield of 471.0±62.7 kg ha⁻¹ is achieved, while the average seed yield of 455.0±63.8 kg ha⁻¹ was obtained by sowing 30.0 kg ha⁻¹ of seed.

Supplementation with nitrogen fertilizers in the first year of the experiment had a

statistically significant effect on the seed yield of perennial ryegrass. By using the largest amount of fertilizer (90 kg ha⁻¹), the highest yield has been achieved (541.3±27.6 kg ha⁻¹), while it is in the control variant, on the plots without fertilization (0 kg ha⁻¹), the amount of seed obtained was statistically significantly smaller (377.0±23.2 kg ha⁻¹).

Table 2. Analysis of variance of treatments on seed yield of perennial ryegrass in the first year tested

Parameter	SS- Sum of square	MS - Middle of square	F test	Signif.
Corrected Model	1001508.859b	15896.966	85.379	0.000
Intercept	54647208.141	54647208.141	293498.111	0.000
Inter-row distance - IRD	61726.297	20575.432	110.506	0.000**
Sowing density - SD	11969.203	3989.734	21.428	0.000**
Quantity of nitrogen-N	916010.703	305336.901	1639.897	0.000**
IRD×SD	1966.359	218.484	1.173	0.314 ^{ns}
IRD×N	4286.609	476.290	2.558	0.008 ^{ns}
SD×N	487.203	54.134	0.291	0.977 ^{ns}
IRD×SD×N	5062.484	187.499	1.007	0.461 ^{ns}
Error	35749.000	186.193		
Total	55684466.000			

Based on the analysis of variance in the first year of testing, it can be concluded that there are very significant differences between the tested treatments of fertilization and English ryegrass seed. The interaction between the examined factors (IRD×SD; IRD×N; SD×N; IRD×SD×N) had no significant effect on the seed yield of perennial ryegrass (Table 2).

Average seed yields in the second year varied from 426.0±0.74 kg ha⁻¹ to 714.0±16.3 kg ha⁻¹ (Table 3).

Average seed yields were statistically significantly dependent on the influence of all three investigated factors. Sowing in wide

rows of perennial ryegrass seeds (50 cm) resulted in the highest yield of seeds - 648.9±41.9 kg ha⁻¹. With close seeding (12.5 cm), a significantly lower seed yield was achieved - 461.7±25.7 kg ha⁻¹ (a decrease of 29%). At the lowest sowing rate (9 kg ha⁻¹), the average seed yield was 572.1±80.4 kg ha⁻¹, while at the maximum sowing rate (30 kg ha⁻¹), the lowest seed yield was achieved - 551.0±75.8 kg ha⁻¹. In the control variant (variants without nitrogen fertilization), the seed yield of perennial ryegrass was 522.3±66.6 kg ha⁻¹, while the highest seed yield was obtained with 90 kg ha⁻¹ of N fertilizer (606.3±78.8 kg ha⁻¹).

Table 3. Analysis of variance of treatments on seed yield of perennial ryegrass in the second year tested

Parameter	SS - Sum of square	MS - Middle of square	F test	Signif.
Corrected Model	1512094.734b	24001.504	122.436	0.000
Intercept	80584284.766	80584284.766	411073.307	0.000
Inter-row distance - IRD	1228438.266	409479.422	2088.820	0.000**
Sowing density - SD	24151.297	8050.432	41.067	0.000**
Quantity of nitrogen - N	244447.922	81482.641	415.656	0.000**
IRD×SD	1929.797	214.422	1.094	0.369 ^{ns}
IRD×N	9132.047	1014.672	5.176	0.000**
SD×N	378.391	42.043	0.214	0.992 ^{ns}
IRD×SD×N	3617.016	133.964	0.683	0.879 ^{ns}
Error	37638.500	196.034		
Total	82134018.000			

Based on the analysis of variance in the second year of the trial, row spacing, seeding rate and nitrogen amount had a very significant effect on the yield of perennial ryegrass seeds. The interaction between the investigated factors (IRD×N) had a very significant effect on yield ($F_{ekp}=5.176$), $p=0.005$. Other second-level interactions and third-level interactions did not show statistical significance for the observed parameter (Table 3).

The average seed yield in the third year of the study varied from $117.3 \pm 25.7 \text{ kg ha}^{-1}$ to $391.0 \pm 8.6 \text{ kg ha}^{-1}$ and showed dependence on the influence of all three tested factors. As the row spacing increased, the average seed yield also increased. Seed yield in narrow-row sowing (12.5 cm) was $208.4 \pm 48.5 \text{ kg ha}^{-1}$, while in wide-row sowing (50 cm) it was $343.7 \pm 31.7 \text{ kg ha}^{-1}$. With the sowing rate, the opposite trend was recorded in relation to the row spacing. The largest amount of seeds was obtained by sowing the minimum amount of seeds (9 kg ha^{-1}) - $293.9 \pm 54.9 \text{ kg ha}^{-1}$, while the smallest amount of seeds obtained by sowing the maximum amount of seeds (30 kg ha^{-1}), $267.8 \pm 64.9 \text{ kg ha}^{-1}$. In the variants without fertilization, the lowest seed yield was achieved - $245.2 \pm 66.2 \text{ kg ha}^{-1}$, while the highest yield was achieved in plots with the most intensive fertilization - $315.2 \pm 49.0 \text{ kg ha}^{-1}$.

Based on the analysis of variance in the third year of testing, it can be concluded that

the inter-row distance, the seeding rate and the amount of nitrogen had a very significant impact on the yield of perennial ryegrass seeds. The interaction of the investigated factors (IRD×N) had a significant effect on the seed yield of perennial ryegrass ($F_{ekp}=14.507$), $p=0.005$. Other second-level interactions and third-level interactions did not show statistical significance on the observed parameter (Table 4).

The average values of the seed yield in the three-year period record the dependence on all three observed factors. The second year tested had the most favorable conditions for the production of perennial seed, while the third year tested was the least favorable for production.

The third year had poor weather conditions, heavy rainfall during the sowing period, followed by a sudden rise in temperature, which led to increased seed shedding. In the three-year period, the highest average seed yield was recorded in sowing with the largest row spacing (50 cm), when in the second tested year the yield was 648.9 kg ha^{-1} , while the lowest seed yield at the same row spacing was recorded in the third year tested (208.4 kg ha^{-1}). When sowing 30 kg ha^{-1} , the lowest seed yield was achieved in all three years of the experiment. The lowest yield was achieved in third season and amounted to 267.8 kg ha^{-1} (30 kg ha^{-1}). The highest average seed yields were achieved by sowing the least amount of seed (9 kg ha^{-1}) in all three years.

Table 4. Analysis of variance of treatments on seed yield of perennial ryegrass in third year tested

Parameter	SS - Sum of square	MS - Middle of square	F test	Signif.
Corrected Model	926792.000b	14710.984	69.798	0.000
Intercept	20025625.000	20025625.000	95013.715	0.000
Inter-row distance - IRD	673609.812	224536.604	1065.338	0.000**
Sowing density - SD	35123.531	11707.844	55.549	0.000**
Quantity of nitrogen - N	177180.625	59060.208	280.217	0.000**
IRD×SD	4433.781	492.642	2.337	0.016 ^{ns}
IRD×N	27518.563	3057.618	14.507	0.000**
SD×N	2765.344	307.260	1.458	0.166 ^{ns}
IRD×SD×N	6160.344	228.161	1.083	0.364 ^{ns}
Error	40467.000	210.766		
Total	20992884.000			

Comparing all three years, the highest average yield was recorded in third year and was 572.1 kg ha^{-1} of perennial ryegrass seeds.

Spring nitrogen fertilization had a significant effect on the seed yield of perennial ryegrass. In all three years, an increase in seed yield

was recorded with an increase in the amount of nitrogen: 541.3 kg ha⁻¹, 606.3 kg ha⁻¹ and 315.2 kg ha⁻¹ seeds (at 90 kg ha⁻¹ nitrogen). The lowest seed yield of perennial ryegrass

(245.2 kg ha⁻¹) was achieved in the third year in the control variant, without nitrogen application, 0 kg ha⁻¹ (Figure 5).

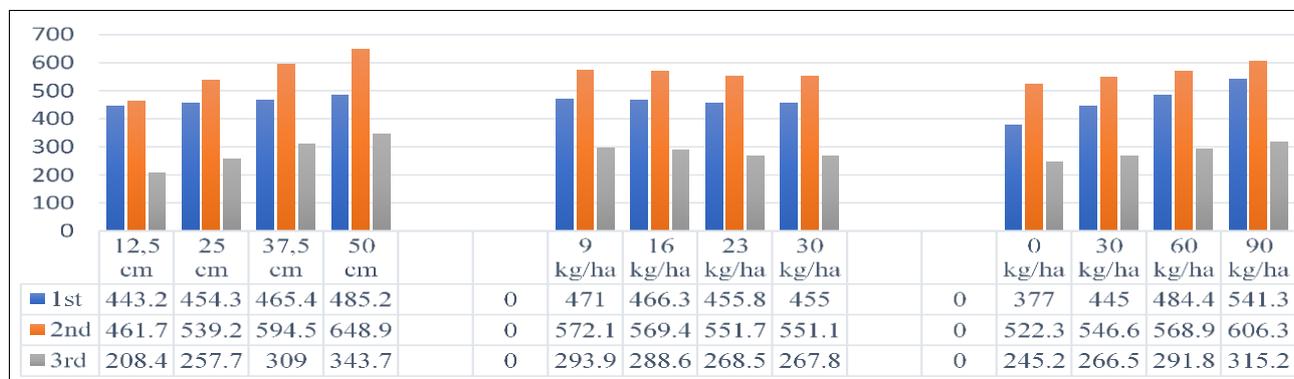


Figure 5. The influence of row spacing, seeding rate and amount of nitrogen on seed yield in the three-year period

Seed yield is a parameter for evaluating the success of seed production, from the aspect of quality and quantity, which directly depends on shoot height, spike length, number of spikelets and fertile flowers per plant. The mentioned parameters indicate the potential, and the seed yield is the utilization of the potential (Busso et al., 1989; Da Silveira Pontes et al., 2015; Fu et al., 2024). The seed yield is significantly influenced by the genotype, the age of the crop, applied agricultural techniques, the presence of plant disease agents in the soil and plant material, as well as the ability to absorb nutrients (Wang et al., 2006; Xu et al., 2010; Popović et al., 2013, 2022; Blair et al., 2014; Huang et al., 2014; Nosalewicz et al., 2018; Hilker and Schmülling; 2019; Lakić et al., 2022; Tan et al., 2022; Bouton, 2023; Dimtirijević et al., 2024; Ding et al., 2024; Ma et al., 2024).

The first year of the study had poor weather conditions which limited the production of perennial ryegrass. In the months of the initial growth of the plant, rainy weather was recorded, then there were months with average temperatures above the long-term average, which contributed to the rapid ripening and shedding of seeds. Seed shedding is prevented by accelerated harvesting. The maximum inter-row distance and the amount of nitrogen had a significant effect on the seed yield (Cattani, 2007), while the sowing rate had the opposite effect (the

highest seed yield was achieved with the smallest amount of seeds).

In the second year of research, the full fertility potential of perennial ryegrass was realized. Weather conditions were satisfactory, with regularly distributed rainy periods. Seed filling and ripening took place in dry June conditions, and seed harvesting was completed in the first half of July and there was no seed shedding. As in the first year, inter-row spacing and top dressing had a significant impact on seed yield, with the same dynamics: with increasing parameter values, seed yield values increased. This can be explained by increased competition and the need for nitrogen (Koeritz et al., 2015). Sowing rate had the opposite effect on seed yield, the highest values of seed yield were achieved with the smallest amount of seed.

The third year of research had bad weather conditions. The highest coefficient of variation was recorded (21.98%). The same trend of the influence of vegetation environment factors on seed yield was confirmed - the highest values of the inter-row distance factor and the amount of nitrogen resulted in the highest number of harvested seeds. The minimum amount of seeds in sowing determined the best yield.

The potential yield of grass seeds is determined by the number of reproductive shoots per unit area, the number of spikelets per inflorescence and the number of flowers

per spikelet (Elgersma, 1990). Seed yield also depends on the proportion of flowers that produce seeds and the weight of individual seeds (Busso and Richards, 1995; Simić and Vučković, 2006; Xu et al., 2009; ESCAA, 2020).

Row spacing affected seed yield only in the diploid cultivar 'Alegro', where 48 cm row spacing reduced seed yield compared to 12 and 24 cm row spacing (Delran et al., 2009).

In the process of plant breeding, in addition to yield as the most important feature, other features are always monitored (Heineck et al., 2020; Todorović et al., 2020; Huber et al., 2021; Javaid et al., 2022).

Knowing the interdependence of studied traits, that is, how and to what extent one trait affects another, enables easier determination of criteria and predicts the course of breeding. By selecting a certain trait in the desired direction, there will be changes in the value of some other traits in size and direction proportional to the strength of the correlation coefficients between the given traits.

It is anticipated that local yearly precipitation and temperature patterns would be impacted by climate change in Europe. It is starting to pose a serious risk to both cultivated and natural grasslands. According to recent research, local environmental changes will occur more quickly than natural grass populations can respond (Keep et al., 2020). Additionally, it might have an impact on the existing panel of commercial cultivars' performances. Therefore, developing new cultivars that are suited to the climate of the future is one of the major issues facing forage breeders. Therefore, it may be crucial to take advantage of the genetic variety present in natural populations (Blanco-Pastor et al., 2019; Keep et al., 2020).

One of the key elements influencing plant development, including seed germination and plant growth, is temperature. Therefore, in the majority of climate change scenarios, developing perennial ryegrass cultivars adapted to new temperature ranges may be required. However, an explanation of the intraspecific heterogeneity of temperature responses is necessary prior to any breeding efforts in this area (Ghaleb et al., 2021, 2024).

Early indicators for choosing potential material include seed germination (Ahmed, 2015; Ghaleb et al., 2021) and early heterotrophic growth (Escobar-Gutiérrez et al., 1998a, 1998b; Ahmed et al., 2019).

Numerous studies have documented interspecific differences in how each species reacts to temperature during germination. The relationship between temperature and germination in forage grasses has actually been the subject of several research (Baskin and Baskin, 2014). A few of them (Larsen and Bibby, 2005; Anjum et al., 2011; Lin et al., 2018) present comparisons between *L. perenne* and other species. Surprisingly, yet, little is known about the inherent intraspecific diversity of temperature responses, if any, during *L. perenne* germination and early growth. In fact, as far as we are aware, there are just two published (Larsen and Bibby, 2005; Shen et al., 2008).

A major global concern, global climate change has far-reaching effects, particularly in agriculture (Gong et al., 2020; Leisner, 2020).

In the context of crop production, rising temperatures not only make drought issues worse but also make heat waves more frequent and intense (Mittler, 2006; Gornall et al., 2010).

Depending on the crop type, developmental stage, and the type, severity, and duration of the stress, these stresses may cause agricultural production losses of as much as 60% (Farooq et al., 2009; Praba et al., 2009; Jedmowski et al., 2015).

The degree of damage caused by drought is frequently unanticipated and depends on variables including rainfall patterns, soil water retention capacity and evapotranspiration-induced water loss (Huang et al., 2024).

Heat stress causes a number of physiological, biochemical, and morphological alterations that significantly impact plant growth and development (Wahid et al., 2007; Pucciariello et al., 2012; Ahmed, 2015; Zhao et al., 2021) resulting in wilting and delayed growth (Kotak et al., 2007; Wahid et al., 2007; Ozga et al., 2017; Schauburger et al., 2017; Liu et al., 2019; Zhao et al., 2021). Low yields are often the result of heat stress's detrimental

effects (Ozga et al., 2017; Schauburger et al., 2017; Lin et al., 2018).

CONCLUSIONS

The results of this three-year study clearly demonstrate that seed yield of perennial ryegrass (*Lolium perenne* L.) is strongly and consistently affected by inter-row spacing, seeding rate, and nitrogen fertilization, as well as by annual climatic conditions. Across all years, wider row spacing resulted in higher seed yields, indicating that reduced intra-specific competition and improved resource availability per plant play a key role in maximizing reproductive potential. Similarly, lower seeding rates consistently produced higher yields, confirming that excessive plant density negatively affects seed formation and retention. Nitrogen fertilization had a significant and positive effect on seed yield in all years of the study. Increasing nitrogen rates enhanced yield, with the highest values obtained at 90 kg ha⁻¹, highlighting the importance of adequate nitrogen supply for reproductive shoot development and seed filling. However, the magnitude of nitrogen response varied among years, reflecting strong interactions between nutrient availability and weather conditions.

Climatic variability has been identified as a significant factor affecting seed production. The second year of the research presented ideal conditions, enabling the full realization of the yield potential of perennial ryegrass. In contrast, the adverse weather conditions in the third year—marked by excessive rainfall during sowing and elevated temperatures during seed maturation—resulted in increased seed shedding, and a notable decline in yields. These observations highlight the vulnerability of seed production to environmental stress, especially in the context of changing climate conditions.

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