

VARIATION IN NITROGEN USE EFFICIENCY OF WINTER WHEAT

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Mirosavljević M., V. Aćin, V. Sabadoš, D. Dorotić (2019): *Variation in nitrogen use efficiency of winter wheat.*- Genetika, Vol 51, No.3, 1165-1174.

Optimization of nitrogen fertilization to specific cultivar requirements is a major objective for improvement of trade-offs between grain yield, environmental sustainability and maximum profitable production. The aim of this study was to assess the effects of nitrogen fertilization on grain yield, and nitrogen use efficiency of modern wheat cultivars in different growing seasons. The trials with eight winter wheat cultivars and seven top-dressing nitrogen treatments were carried out in three successive growing seasons under rain-fed conditions of the southern Pannonian plain. The results from our study showed a significant variation in grain yield and nitrogen use efficiency among winter wheat cultivars when grown under different environmental and soil nitrogen level conditions. On average, grain yield ranged from 4961 to 6375 kg ha⁻¹ among winter wheat cultivars. Increase of N soil level resulted in significant grain yield increase and nitrogen use efficiency decrease compared to the control. In 2015/16, 2016/17, and 2017/18 growing seasons grain yield of winter wheat cultivars reached plateau at 156, 175, and 128 nitrogen soil level, respectively. Significant influence of cultivar by nitrogen fertilization interaction indicated that it is necessary to adjust nitrogen fertilization to each cultivar. Moreover, notable weather variability between different growing seasons is a major limiting factor for optimal nitrogen fertilizer application in winter wheat production under conditions of the Pannonian plain.

Keywords: cultivar, grain yield, growing season, NUE, *Triticum aestivum* L.

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INTRODUCTION

Wheat is one of the most important and widely grown crops in the world, cultivated at more than 220 million of hectares with total production near 750 million tonnes. India, Russian Federation, European Union, China, United States of America are five most important wheat-growing regions in the world with 30, 27, 27, 24 and 18 million hectares, respectively (FAOSTAT, 2018). Recently, countries of the Black Sea region (Russia, Ukraine, Kazakhstan, Romania, Bulgaria, Hungary and Serbia) have emerged as one of the most important producers and exporters of wheat in Europe. Also, there has been a significant increase in the wheat grain yield (GY) and total grain production in Serbia in the current decade.

Constant wheat GY increase was a result of more intensive agricultural practice management and introduction of high yielding cultivars (PELTONEN-SAINIO *et al.*, 2009). Nitrogen (N) is widely considered as the critical factor for obtaining high GY and protein content in different cereal crops. Number of humans supported per hectare of arable land has increased from 1.9 to 4.3 persons between 1908 and 2008, mainly due to N fertilizer application (ERISMAN *et al.*, 2008). Since the mid-20th century as a result of the Green Revolution influence, the application of N fertilizers in production of major cereal crops has notably increased. During the last 50 years, N fertilization rate in wheat increased by more than 60 kg ha⁻¹ (LADHA *et al.*, 2016).

In the Pannonian countries, such as Serbia, N is used in high doses both prior to sowing and in spring growing period (topdressing) before the stem elongation phase. Generally, if a higher level of N fertilizer is applied in spring, N dose is often split in two treatments to enhance nutrient efficiency. However, approximately 33% of N fertilizer applied to cereal crops are removed by grain (RAUN and JOHNSON, 1999). The remaining N is lost from the soil by denitrification, volatilization, and surface runoff and leaching, having negative influence on the environment (CAMERON *et al.*, 2013). Moreover, N loss is additional cost for the farmers. Therefore, N application in wheat production should be optimized in order to increase GY. Also, simultaneous genetic improvement in nitrogen use efficiency (NUE) while maintaining high GY potential under lower N application should be an important goal in small grain cereal breeding programs.

There are different equations to calculate relation between N and GY in major cereal crops (HAWKESFORD, 2014). One of the most widely applied equations (also used in our study) was developed by MOLL *et al.* (1982), where NUE is calculated as ratio between GY and N recovery. According to different studies, NUE in winter wheat varied significantly due to the influence of genotype, environment, management practices and their interaction (BARRACLOUGH *et al.*, 2010; GUTTIERI *et al.*, 2017; YIN *et al.*, 2018). Information about genotypic difference in NUE could be used by wheat producers, in order to grow high yielding cultivars that require low nitrogen input.

Due to the negative influence of changing climate, production of winter wheat in the Pannonian plain shows high year-to-year variability. Understanding responses of new wheat cultivars to different N treatments and genotypic variation in NUE is important so as to achieve optimal N fertilization rates and high GY. Therefore, the main objective of this study was to analyse the effect of nitrogen fertilization on GY, NUE of eight modern wheat cultivars under rain fed conditions of the southern Pannonian Plain.

MATERIALS AND METHODS

Plant material and experimental setup

This study included eight winter wheat cultivars widely grown in Serbia and other surrounding countries in the southern Pannonian plain. The cultivars were “NS Azra”, “NS Iliina”, “NS Mila”, “NS 40S”, “NS Obala”, “NS Vljajna”, “Simonida” and “Zvezdana”, released by the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Selection of the cultivars for the study was based on their variability in plant height, GY and grain quality potential. The cultivars were grown under field conditions under seven top-dressed N fertilization levels. During the tillering phase (end of February), mineral nitrogen in the soil was determined by Nmin analysis (WEHRMANN and SCHARPF, 1979). According to the results of this analysis, treatments included an unfertilized control and N fertilization in order reach N level of 105, 125, 145, 165, 185, and 205 kg ha⁻¹. Treatments were arranged in a split-plot design with three replications. Main plots were assigned to the nitrogen levels and sub-plots to cultivars.

Growing conditions and measurements

The trial was set up in three successive growing seasons on Agricultural Extension Service fields in Sombor (SO - 2015/16) and Gakovo (GA - 2016/17 and 2017/18) under rain fed conditions on non-carbonate chernozem. Maize was the preceding crop in each growing season. Crops were sown on recommended sowing date for southern Pannonian plain (mid-October) with target density of 500 plants per square meter. A fertilizer combination (N, P and K – 15:15:15; the average applied dose was ca. 38 kg/ha N, 38 kg/ha P₂O₅ and 38 kg/ha K₂O) was applied before ploughing to avoid N, P and K deficit according to soil agrochemical analysis. The soil was prepared by ploughing along with two harrowing procedures. Each plot consisted of 10 rows, with row spacing of 0.12 m and length of 4 m. Pests, weeds and diseases were prevented or controlled by applying the recommended insecticides, herbicides and fungicides. No additional irrigation was applied.

Grain yield was determined from combine-harvested plots in each of the four replications. Moisture content was determined using grain analysis computer (Model GAC2100, Dickey-John, Auburn, IL) and GY was corrected to 140 g moisture kg⁻¹. NUE was calculated according to MOLL *et al.* (1982), as GY divided to amount of available N for each plot (soil mineral N + applied N). Relative reduction (RR) in GY under low N was calculated as: $RR = 1 - (GY_{LN}/GY_{HN})$ where GY_{LN} was GY under unfertilized control conditions and GY_{HN} was GY under 205 kg ha⁻¹ available N.

Analysis of variance (ANOVA) was performed using INFOSTAT (student version), while regression analysis was performed using SEGREG program. Treatment means were compared using the Tukey test.

Weather conditions

Weather data were collected from the local meteorological station located near the experimental fields (Figure 1). Weather conditions, especially the amount and distribution of precipitation and average daily temperature, varied significantly across the growing seasons. Air temperature, level and distribution of precipitation in autumn enabled fast emergence and establishment of good plant population in each growing season. Winter of 2016/17 was characterized by low temperature (especially in January), while winters of 2015/16 and 2017/18 were moderately cold. Temperature in spring growing period was similar in 2015/16 and

2016/17, while in 2017/18 there were lower temperatures in February and March, and notably higher temperatures in April and May. Precipitation level in June of 2017/18 growing season was extremely high, having negative influence on harvest, grain weight and GY.

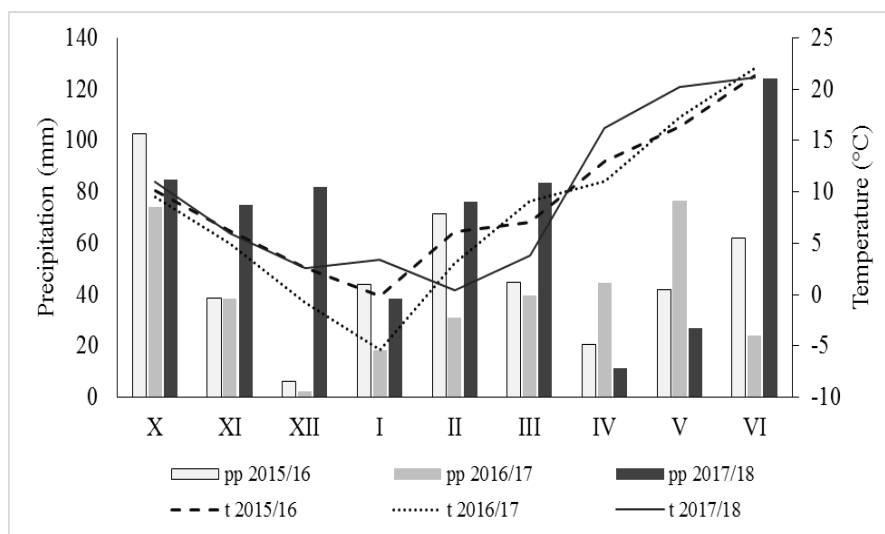


Figure 1. Monthly average daily temperature and precipitation in 2015/16, 2016/17 and 2017/18

RESULTS

Results from our study showed significant influence of the environment, nitrogen fertilization, cultivar and their interaction on GY of winter wheat cultivars (Table 1). Overall average GY of the wheat cultivars was 5857 t ha^{-1} . N fertilization resulted in GY increase when compared to the untreated control. On average, highest GY was observed at 185N (6407 kg ha^{-1}) and 205N (6336 kg ha^{-1}). On the other hand, absence of N application (control treatment) resulted in the lowest average GY of 4751 kg ha^{-1} . Moreover, cultivars differed significantly in GY, and the average GY among cultivars ranged from 4961 to 6375 kg ha^{-1} . Also, there was a significant influence of cultivar by nitrogen interaction on GY, indicating different cultivar responses to N application. For example, by increasing N level from unfertilized treatment to 205 kg N ha^{-1} , GY of cultivars NS Mila and NS 40S increased by almost 2000 kg ha^{-1} , while in cultivar Simonida GY increase was less than 1000 kg ha^{-1} . Moreover, the studied cultivars reached the highest GY at different nitrogen levels. For example, NS Mila achieved the highest GY at 205 kg ha^{-1} , and NS Obala at 165 kg N ha^{-1} . There was a clear difference in mean GY between the environments (7635 kg ha^{-1} , 5346 kg ha^{-1} and 4596 kg ha^{-1} for 2015/16, 2016/17 and 2017/18, respectively). A significant $N \times$ environment interaction on wheat grain was recorded, and on average wheat cultivars achieved highest GY at 205N, 185N and 185N in 2015/16, 2016/17 and 2017/N, respectively.

Table 1. Grain yields and relative yield reduction (RR) of eight winter wheat cultivars grown in seven nitrogen (N) soil levels (Cont.-205N) in three growing seasons

Treatments	Cont. **	105N	125N	145N	165N	185N	205N	Aver	RR
Cultivars									
NS Azra	5052 ^{p-t*}	5683 ^{i-o}	6466 ^{a-f}	6434 ^{a-f}	6452 ^{a-f}	6795 ^{a-c}	6744 ^{a-c}	6232 ^A	0.25
NS Ilina	3988 ^{wx}	4981 ^{q-t}	5273 ^{m-s}	5121 ^{o-s}	5986 ^{e-k}	5986 ^{e-k}	6608 ^{a-d}	5420 ^C	0.40
NS Mila	4871 ^{s-u}	5797 ^{h-n}	6547 ^{a-e}	6234 ^{b-i}	6643 ^{a-d}	6650 ^{a-d}	6732 ^{a-c}	6211 ^A	0.28
NS Obala	5564 ^{j-q}	6135 ^{d-j}	6386 ^{a-h}	6478 ^{a-f}	6887 ^a	6752 ^{a-c}	6422 ^{a-g}	6375 ^A	0.13
NS Vlajna	5238 ^{n-s}	5844 ^{g-m}	6382 ^{a-h}	6222 ^{c-i}	6515 ^{a-e}	6819 ^{ab}	6587 ^{a-d}	6230 ^A	0.20
NS 40S	4511 ^{t-w}	4987 ^{q-t}	5479 ^{k-r}	6350 ^{a-h}	5965 ^{e-k}	6414 ^{a-g}	6450 ^{a-f}	5737 ^B	0.30
Simonida	4903 ^{r-u}	5049 ^{p-t}	5841 ^{g-m}	5907 ^{f-l}	6138 ^{d-j}	6243 ^{b-i}	5845 ^{g-m}	5704 ^B	0.16
Zvezdana	3882 ^x	4374 ^{u-x}	5337 ^{l-s}	5027 ^{p-t}	5211 ^{n-s}	5598 ^{j-p}	5297 ^{m-s}	4961 ^D	0.27
Growing seasons									
2015/16	6644 ^e	7435 ^d	7570 ^{cd}	7710 ^{b-d}	7975 ^{ab}	7875 ^{bc}	8234 ^a	7635 ^A	0.19
2016/17	4513 ^l	4825 ^{j-l}	5300 ^{hi}	5486 ^{gh}	5613 ^{fh}	5894 ^f	5788 ^{fg}	5346 ^B	0.22
2017/18	3097 ⁿ	3809 ^m	5022 ^{i-k}	4717 ^{kl}	5087 ^{ji}	5454 ^h	4986 ^{i-k}	4596 ^C	0.38
Aver.	4751 ^E	5356 ^D	5964 ^C	5972 ^C	6225 ^B	6407 ^A	6336 ^{AB}	5857	0.25

* Different letters indicate significant difference at $P < 0.05$ level

** Cont. – unfertilized treatment; 105N, 125N, 145N, 165N, 185N and 205N represent N soil level of 105, 125, 145, 165, 185, and 205 kg ha⁻¹, respectively.

By comparing relative GY reduction (RR), difference in GY between unfertilized control and the highest N soil level (205N) varied between growing seasons and cultivars. Cultivars NS Obala and Simonida showed the lowest GY reduction, while the highest GY reduction was observed in cultivar NS Ilina. The average RR was 0.19%, 0.22%, and 0.38% in 2015/16, 2016/17 and 2017/18, respectively.

Differences in NUE were observed due to the effects of environment, cultivar and soil nitrogen level (Table 2). The grand mean of NUE in our study was 42.8 kg grain kg⁻¹ N ha⁻¹. NUE was largely affected by the change in spring nitrogen top-dressing treatment. As expected, NUE values decreased with increase in nitrogen level in the soil. The average NUE decreased from 56.6 kg grain kg⁻¹ N ha⁻¹ in control to 30.9 kg grain kg⁻¹ N ha⁻¹ in 205N treatment. Significant difference between cultivars was reported for NUE. On average, NS Obala had the highest NUE of all other cultivars, while Zvezdana was characterized by low values of this trait. The cultivar by nitrogen interaction was significant for NUE, as a result of differences among cultivars ranks for NUE at different nitrogen level. NS Obala had the highest NUE in control treatment. The highest value of NUE at 205N was reported in cultivars NS Azra, NS Mila and NS Vlajna, indicating that these cultivars efficiently use N in intensive field production. Moreover, values of NUE varied significantly between the years. On average, wheat cultivars had significantly highest NUE (55.8 kg grain kg⁻¹ N ha⁻¹) in 2015/16, while the following seasons 2016/17 and 2017/18 showed decreased values of NUE. Also, a significant nitrogen × year interaction effect on NUE was recorded, and on average cultivars had the highest value of NUE in control treatment in 2015/16 and 2016/17, while in 2017/18 the highest value of NUE was reported at 125N.

Table 2. Nitrogen use efficiency (NUE) of eight winter wheat cultivars grown in seven nitrogen (N) soil levels (Cont.-205N) in three growing seasons

Treatments	Cont. **	105N	125N	145N	165N	185N	205N	Aver.
Cultivars								
NS Azra	59.9 ^{BC*}	54.1 ^{E-G}	51.7 ^{F-H}	44.4 ^{J-N}	39.1 ^{O-R}	36.7 ^{P-T}	32.9 ^{T-Y}	45.6 ^B
NS Ilina	47.7 ^{H-K}	47.4 ^{I-K}	42.2 ^{M-O}	35.3 ^{R-X}	36.3 ^{Q-W}	32.4 ^{U-Z}	32.2 ^{U-Z}	39.1 ^D
NS Mila	58.1 ^{C-E}	55.2 ^{D-G}	52.4 ^{FG}	43.0 ^{L-O}	40.3 ^{N-Q}	35.9 ^{R-W}	32.8 ^{T-Y}	45.4 ^B
NS Obala	66.0 ^A	58.4 ^{BCD}	51.1 ^{G-I}	44.7 ^{J-M}	41.7 ^{M-O}	36.5 ^{P-U}	31.3 ^{X-Z}	47.1 ^A
NS Vlajna	62.5 ^{AB}	55.7 ^{D-F}	51.1 ^{G-I}	42.9 ^{LMNO}	39.5 ^{O-R}	36.9 ^{P-T}	32.1 ^{W-Z}	45.8 ^B
NS 40S	53.6 ^{FG}	47.5 ^{H-K}	43.8 ^{J-N}	43.8 ^{K-N}	36.3 ^{Q-W}	34.7 ^{S-X}	31.5 ^{X-Z}	41.6 ^C
Simonida	58.4 ^{B-D}	48.1 ^{H-J}	46.7 ^{J-L}	40.7 ^{M-P}	37.2 ^{P-S}	33.8 ^{S-Y}	28.5 ^{Za}	41.9 ^C
Zvezdana	46.6 ^{J-L}	41.7 ^{MNO}	42.7 ^{L-O}	34.7 ^{S-X}	31.6 ^{X-Z}	30.3 ^{YZ}	25.8 ^a	36.2 ^E
Growing seasons								
2015/16	74.7 ^A	70.8 ^B	60.6 ^C	53.2 ^E	48.3 ^F	42.6 ^H	40.2 ^I	55.8 ^A
2016/17	57.9 ^D	46.0 ^G	42.4 ^{HI}	37.8 ^J	34.0 ^{KL}	31.9 ^{LM}	28.2 ^O	39.7 ^B
2017/18	37.3 ^{IJ}	36.3 ^{JK}	40.2 ^I	32.5 ^{LM}	30.8 ^{MN}	29.5 ^{NO}	24.3 ^P	33.0 ^C
Aver.	56.6 ^A	51.0 ^B	47.7 ^C	41.2 ^D	37.7 ^E	34.6 ^F	30.9 ^G	42.8

* Different letters indicate significant difference at $P < 0.05$ level

** Cont. – unfertilized treatment; 105N, 125N, 145N, 165N, 185N and 205N represent N soil level of 105, 125, 145, 165, 185, and 205 kg ha⁻¹, respectively.

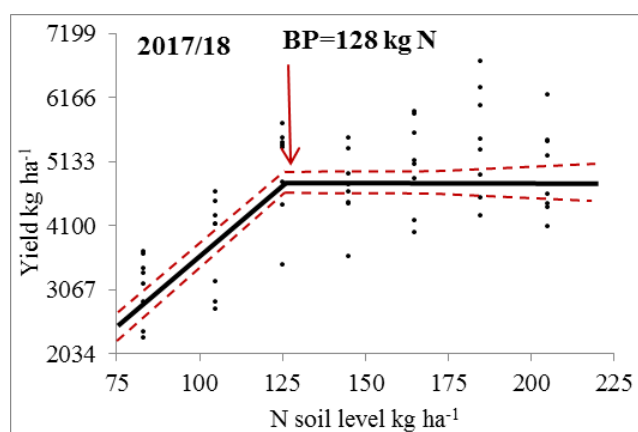


Figure 2. Influence of N soil level on grain yield of winter wheat cultivars in three successive growing seasons (2015/16, 2016/17 and 2017/18). BP – breaking point.

Relationship between N level in soil and GY was almost linear (Figure 2). However, according to Figure 2, GY of winter wheat cultivars reached break point under different N soil levels. In 2015/16 GY of winter wheat cultivars reached the breaking point (stagnation) at 156

kg of available soil N⁻¹, while in 2016/17 and 2017/18 break point in GY was observed at 176 and 126 kg of available soil N ha⁻¹, respectively.

DISCUSSION

In wheat production, management of N fertilization to specific cultivars requirements is a major goal for optimization of trade-offs between GY, environmental sustainability and profit (BARRACLOUGH *et al.*, 2010). According to the results of this study, there was a notable variation in GY and NUE among winter wheat cultivars when grown under different environmental conditions and soil N level. Similarly, many previous studies showed significant variation in small grain cereal GY between growing seasons and locations under conditions of the Pannonian plain (PRŽULJ *et al.*, 2015; MLADENOV *et al.*, 2018; MLADENOV *et al.*, 2019). Generally, influence of the environmental conditions (season/location) is a major source of GY variation in different cereal crops, while effect of the others factors are less pronounced (MIROSAVLJEVIĆ *et al.*, 2018).

Spring application of N fertilizer resulted in significant GY increase compared to control treatment. Positive influence of N fertilization on wheat GY is mainly due to improvement of grain number of per unit area (NEHE *et al.*, 2018). Moreover, N application improves crop above-ground biomass and grain protein content (VELASCO *et al.*, 2012). By increasing N soil level, GY of wheat cultivars raised almost linearly. However, there was different cultivar reaction to increased level of N fertilization, since percent of GY reduction between high N (205N) and un-fertilized control varied remarkably between cultivars (0.13 - 0.40%). The ability of some cultivars (NS Obala and Simonida) to achieve high GY both under high and low N soil level could be a result of a more effective rooting system, as previously reported by NEHE *et al.* (2016), suggesting that these cultivars could be recommended for low input production systems. Although increase of N soil level was followed by increase in wheat GY, this relationship was not linear. According to rising-plateau linear model in 2015/16, 2016/17 and 2017/18 GY of winter wheat cultivars reached plateau at 156, 175 and 128 N soil level, respectively. These results indicate that unfavourable weather conditions (2017/18 characterized by decreased precipitation level and increased temperature during spring) significantly limit NUE and higher GY achievement, since cultivars reached yield plateau earlier. Also, appearance of GY plateau at higher N levels could be a result of increased lodging susceptibility (LU *et al.*, 2014; CHEN *et al.*, 2018) and decrease in NUE (KARROU and NACHIT, 2015). Generally, increased N application improved wheat tillers production and promotes development of crop biomass, and may decrease the potential yield of the main stem (YANG *et al.*, 2019). As a result of limited environmental conditions during generative development, unproductive tillers reduced wheat GY due to competition for nitrogen and solar radiation with fertile tillers (NARUOKA *et al.*, 2011). Furthermore, high N application increased biomass accumulation during pre-anthesis period and reduce harvest index, limiting further GY increase in wheat (UNKOVICH *et al.*, 2010). SUGÁR *et al.*, (2016) reported different trends in harvest index and grain yield components changes under increasing N application. Therefore, wheat cultivars achieved highest GY under different N fertilization levels.

NUE significantly varied between the studied environments. As expected, decreased NUE was recorded in a less favourable growing season (2017/18), characterized by lowest average GY. Generally, as a result of high N level in soil, increased crop biomass in spring is often associated with drought susceptibility and decreased NUE in wheat (MANDIĆ *et al.*, 2014). According to SEMENOV *et al.* (2010), variation in weather conditions between the growing

seasons is a major limiting factor for accurate N fertilization application. Moreover, results of our study showed genetic variability for NUE within modern winter wheat cultivars in the Pannonian plain. Genotypic variability in NUE is the result of the differences in N uptake and N utilization efficiency (YADETA *et al.*, 2009). The studied cultivars were released during the past 15 years, representing a set of new and adapted winter wheat cultivars. Generally, modern wheat cultivars have improved NUE parameters compared to the older ones (CORMIER *et al.*, 2013; GUTTIERI *et al.*, 2017). Breeding progress in NUE is expected since plant breeders select genotypes with higher GY potential under available conditions and indirectly improve NUE. However, the results of our study showed that the cultivars significantly interacted with available N soil level, indicating that cultivars adapted to high N levels were not necessarily performing best at low N treatment. As a result of inadequate nitrogen application, such as high N input for less responsive or low N input for cultivars with high N demands, these cultivars often could not achieve maximum genetic GY potential. For further NUE improvement in small grain cereal crops, especially under low input systems, yield trials should be conducted under suboptimal N conditions.

By increasing soil N level, NUE was reduced almost linearly in each growing season. This supports findings of different authors (KRÖBEL *et al.*, 2012; MAHJOURIMAJD *et al.*, 2016), who previously reported that higher N level decreased NUE in wheat. Decrease of NUE in winter wheat at higher N level could be result of increased N leaching, presence of other yield-limiting factors, poor capture or a lack of sinks to utilize the available N (HAWKESFORD, 2014).

CONCLUSION

Results from our study showed the presence of significant influence of growing season, N soil level, cultivar and their interaction on GY and NUE in winter wheat. Significant influence of cultivar by nitrogen fertilization interaction indicated that it is necessary to adjust nitrogen fertilization to the requirements of each cultivar. However, variation in weather conditions between growing seasons led to the significant change in GY and NUE, representing the most limiting factor for the accurate recommendation of N fertilization in winter wheat under conditions of the Pannonian plain.

Received, September 03th, 2018

Accepted May 18th, 2019

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VARIJACIJA U EFIKASNOSTI UPOTREBE AZOTA KOD OZIME PŠENICE

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Izvod

Usaglašavanje đubrenja azotom prema zahtevima sorte predstavlja glavni cilj u unapređenju balansa između prinosa zrna, održivosti sredine i ostvarivanja maksimalnog profita. Cilj ovog istraživanja je bio utvrđivanje uticaja prihrane azotom na prinos zrna i efikasnost upotrebe azota kod modernih sorti pšenice u različitim sezonama gajenja. Ogled sa devet sorti pšenice i sedam tretmana azotne prihrane je izveden tokom tri sezone u uslovima južne Panonske nizije. Rezultati ovog istraživanja ukazuju na značajnu varijabilnost prinosa zrna i efikasnosti upotrebe azota kod sorti pšenice gajanih u različitim uslovima dostupnosti azota tokom više sezona. U proseku, prinos zrna je varirao od 4961 do 6375 kg ha⁻¹ kod sorti pšenice. Povećanje dostupnosti azota u zemljištu rezultovalo je u značajnom povećanju prinosa zrna i smanjenju efikasnosti upotrebe azota u odnosu na kontrolu. U 2015/16, 2016/17, and 2017/18 sezoni prinos pšenice je dostigao plato pri 156, 175, and 128 kg dostupnog azota u zemljištu. Značajan uticaj interakcije sorte i đubrenja azotom potvrđuje da je neophodno prilagoditi đubrenje azotom zahtevima pojedinačne sorte. Pored toga, značajna varijacija vremenskih uslova između različitih sezona predstavlja glavni ograničavajući faktor za optimalnu preporuku azotne prihrane pri proizvodnji pšenice u Panonskoj niziji.

Primljeno 03.IX 2018.

Odobreno 18. V 2019.