RESEARCH



Nitrogen fertilizer influence on wheat yield and use efficiency under different environmental conditions

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Managing N inputs in wheat production systems is an important issue in order to achieve maximum profitable production, and minimum negative environmental impact. The aim of this investigation carried out in dry land farming in the Vojvodina province (Serbia) was to estimate the effects of different N fertilization levels (0,75, and 150 kg N ha⁻¹) on some quantitative traits, rain-use efficiency (RUE), N agronomic efficiency (NAE), and N use efficiency (NUE) in two Serbian winter wheat (*Triticum aestivum* L.) cultivars 'Pobeda' and 'Renesansa'. 'Pobeda' had higher grain yield (4437 kg ha⁻¹) and RUE (8.32 kg ha⁻¹ mm⁻¹) than 'Renesansa' (4265 kg ha⁻¹ and 8 kg ha⁻¹ mm⁻¹, respectively). Grain yield (4652 kg ha⁻¹) and NUE (31.46 kg kg⁻¹ N) were higher in the 2010-2011 season (favorable weather conditions) than in the 2011-2012 (4050 kg ha⁻¹ and 27.59 kg kg⁻¹ N, respectively). The highly significant effect on grain yield (4396 and 4494 kg ha⁻¹), RUE (8.24 and 8.45 kg ha⁻¹ mm⁻¹), NAE (3.11 and 2.21 kg kg⁻¹ N) and NUE (58.62 and 29.96 kg kg⁻¹ N) had levels of 75 and 150 kg N ha⁻¹. NAE and NUE declined at high N rates. Based on the results of this study, farmers should be advised that the use of large amounts of N increases production costs and reduce the economic benefits. The increase in wheat production is possible by selecting adapted genotypes with improved NUE.

Key words: Nitrogen use efficiency, quantitative traits, *Triticum aestivum*.

INTRODUCTION

Wheat (Triticum aestivum L.) is the most important cereal crop in world and is the staple food for humans. Average harvested area, total production and grain yield of wheat in the Serbia in 2012 was the 480 539 ha, 1.9 million tons, and 4 t ha⁻¹, respectively. In Serbia, the production is dominated by wheat 'Pobeda', 'Evropa 90', 'Renesansa', 'Dragana', 'Ljiljana' and 'Rusija' which occupy over 70% of the total area under wheat (Dencic et al., 2010). The most common variety is 'Pobeda' with 25% of the total area under wheat. Nitrogen is the most limiting nutrient for wheat production that affects the rapid plant growth and improves grain yield. Many researches showed that N application increased grain yield (GY) of wheat (Subedi et al., 2007; Gorjanovic and Kraljevic-Balalic, 2008). Asif et al. (2012) concluded that number of fertile tiller per unit area, number of grain per spike (NGS), and harvest index were significantly increased by increasing N fertilization

levels. Abedi et al. (2011) reported that higher GY (8230 kg ha⁻¹) was produced in treatment receiving 240 kg N ha-1 than in control (3930 kg ha-1), 120 kg N ha-1 (4400 kg ha⁻¹), and 360 kg N ha⁻¹ (6530 kg ha⁻¹). Marino et al. (2009) concluded that increase the N rate increased hulled and unhulled GY, biomass accumulation, number of spike m⁻² (NS m⁻²), kernels m⁻²; decreased 1000 grain weight (TGW) and in some cases no differences were noticed among fertilized treatments for plant height (PH) and number of spikelets per spike (NSS). Iqtidar et al. (2006) reported that increasing the N level from 50 to 200 kg ha⁻¹ significantly increased the PH, total number of plants m⁻², NGS, NS m⁻², spike weight, and GY compared to 0 kg N ha⁻¹. Noureldin et al. (2013) reported that increasing N up to 180 kg ha⁻¹ increased GY and its components (NS m⁻², spike length [SL], NGS, weight of grain per spike [WGS], and TGW). The efficiency of N application in winter wheat is an important indicator for rational fertilization of N-fertilization. The values of N agronomic efficiency (NAE) in grain ranged from 10 to 30 kg grain kg⁻¹ applied N, and values over 30 kg grain kg-1 applied N are encountered in the well-organized systems of growing or at low levels of N fertilization on poor soils (Dobermann, 2005). Raun and Johnson (1999) have reported that globally, N use efficiency (NUE) in grain production is 33%. Sieling et al. (1998) and Li et al. (2013) concluded that the NUE of wheat decreased with

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increasing N fertilization levels. Hatfield and Prueger (2004) and Bertic et al. (2007) have concluded that NUE by the crop depends on the weather conditions, especially rainfall and availability of N to the plants during growing season.

The present study was planned to find optimal N amount to enhance wheat grain yield with the hypothesis that the efficiency of applied N varies with different environment conditions. The goal of this paper was to investigate the effect of three level of N fertilization (0, 75, and 150 kg N ha⁻¹) on the quantitative traits and RUE, NAE, and NUE in Serbian winter wheat cultivars 'Pobeda' and 'Renesansa' in different environmental conditions. This research shows that the N amount affects expression of quantitative traits of wheat and utilization of genetic yield potential, as well as the efficiency of N utilization in years with different weather conditions.

MATERIALS AND METHODS

Experimental details and treatments

The experiments were carried out in dry land farming in the region of Southwest Vojvodina province (Serbia), at experimental field of Institute for Animal Husbandry of Zemun (44°84' N, 20°40' E; 88 m a.s.l.) Two cultivars of winter wheat, 'Pobeda' (medium late) and 'Renesansa' (medium early), were grown during the seasons 2010-2011 and 2011-2012 at three N fertilization levels (0, 75, and 150 kg N ha⁻¹). Preceding crop was maize (Zea mays L.) in both seasons. Wheat planting was done on in the optimal time (from 12-15 October). The sowing density was 500 seeds m⁻². Plot size was 5 m². A standard cultivation practice was applied. Nitrogen fertilizer KAN (27% N) was applied in March (early spring) at the tillering stage. Nitrogen fertilizer calcium ammonium nitrate -KAN (produced in "HIP-Azotara", Pancevo, Serbia) was applied in March (early spring) at the tillering stage at a rate of 0, 278, and 556 kg ha⁻¹, respectively. Fertilizer KAN contains roughly 8% Ca and 27% N (13.5% nitrate and 13.5% ammonium form).

Agrochemical soil characteristics and climatic conditions during the experiment

The soil type was a Chernozem (IUSS Working Group WRB, 2014) with pH in H_2O 6.2 (neutral reaction), 6.87% organic matter, 4.33% humus, 0.33% total N, 17.97 mg $NH_4{}^+\text{-}N\,kg^{-1}$, and 5.37 mg $NO_3{}^-\text{-}N\,kg^{-1}$. The P_2O_5 and K_2O were 5.4 and 18.4 mg 100 g^{-1} soil, respectively.

Climate diagram based on Walter and Lieth (1967) showed that in 2010-2011 season (total season rainfall 577.2 mm) was not drought period; in 2011-2012 season (total season rainfall 490 mm) period of drought were in November (beginning tillering stage), March (intensive process of tillering, rooting, and growth of foliage and beginning stem elongation stage), June and July (grain filling phase) (Figure 1).

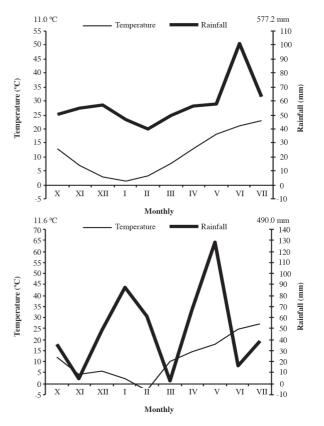


Figure 1. Climate diagram according to Walter in the 2010-2011 and 2011-2012 seasons for Zemun, Serbia.

Data collection

Grain yield (GY, kg ha⁻¹) was harvested at the ripening stage and expressed at 14% moisture. The number of spikes m⁻² (NS m⁻²) was recorded before harvest. At harvest, plant height (PH, cm), spike length (SL, cm), number of spikelets per spike (NSS), number of grains per spike (NGS), weight of grains per spike (WGS, g), and 1000-grain weight (TGW, g) were measured from 30 random spikes from each sub plot. Rain-use efficiency (RUE) calculated according to Gwenzi et al. (2008) (grain yield/total seasonal rainfall, kg ha⁻¹ mm⁻¹), N agronomic efficiency (NAE) according to Delogu et al. (1998) ((GY at N treatment - GY at zero N)/applied N at N treatment, kg grain increase kg⁻¹ N applied), and NUE according to Moll et al. (1982) (GY/N applied, kg grain kg⁻¹ N applied).

Statistical analysis

Design of the experiments was randomized complete block design with four replicates. Data components were analyzed using ANOVA with STATISTICA (version 10; StatSoft, Tulsa, Oklahoma, USA). The significance level was set at $P \leq 0.05$ and $P \leq 0.01$. Differences between parameters means were assessed using Duncan's Multiple Range Test at $P \leq 0.05$ level. Direct relationships between studied traits were analyzed with simple Pearson correlation coefficients.

RESULTS

Quantitative traits and grain yield

ANOVA showed that cultivar had highly significant effect on NS m⁻² and very significant on GY (Table 1). 'Pobeda' had significant higher NS m⁻² (533.7) and GY (4437 kg ha⁻¹) than 'Renesansa' (500.9 and 4265 kg ha⁻¹, respectively). The season had highly significant effect on WGS, and very significant on PH, NSS, TGW, NS m⁻², and GY. Values of these traits were significantly higher in the 2010-2011 season (favorable weather conditions) than in the 2011-2012. The N fertilization levels (75 and 150 kg N ha⁻¹) have highly significant effect on NSS, WGS, TGW, NS m⁻², and GY, and very significant on PH and SL. However, results showed that was not significant differences in values traits between these N levels, although the highest values of traits were recorded at 150 kg N ha-1. The interaction of season and N fertilization level had highly significant effect on PH. The interaction of cultivar and season had highly significant effect on $NS m^{-2}$.

Rain-use efficiency, N agronomic efficiency, and N use efficiency

ANOVA showed that cultivar can differ on RUE (Table 2). 'Pobeda' had significant higher RUE (8.32 kg ha⁻¹ mm⁻¹) than 'Renesansa' (8 kg ha⁻¹ mm⁻¹). The NUE was significantly higher in 2010-2011 season (31.46 kg grain kg⁻¹ N applied) than 2011-2012 (27.59 kg grain kg⁻¹ N applied). The N fertilization level (75 and 150 kg ha⁻¹) had highly significant effect on RUE (8.24 and 8.45 kg ha⁻¹ mm⁻¹), NAE (3.11 and 2.21 kg grain increase kg⁻¹ N applied) and NUE (58.62 and 29.96 kg grain kg⁻¹ N applied) compared to control (7.80 kg ha⁻¹ mm⁻¹, 0 kg

Table 1. Cultivar, season and nitrogen fertilization level effects on wheat quantitative traits.

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Factor		PH	SL	NSS	NGS	WGS	TGW	NS m ⁻²	GY
Cultivar (A)	Pobeda Renesansa	77.4 76.5	7.36 7.49	16.02 16.35		0.99 0.97	35.07 34.93	533.7a 500.9b	4437a 4265b
Season (B)	2010-2011 2011-2012	86.0a 67.9b		17.27a 15.09b		1.05a 0.90b		570.6s 464.0b	
N level, kg ha ⁻¹ (C)	0 75 150	78.6a	7.59a	16.51a	27.6	1.01a	35.17a	503.8b 521.1a 527.0a	4396a
F test Mean	A B	ns **	ns ns	ns **	ns ns	ns *	ns **	**	*
	C A×B	ns	ns	ns	ns ns	ns	ns	*	ns
	$A \times C$ $B \times C$	ns *	ns ns	ns ns	ns ns	ns ns	ns ns	ns ns	ns ns
	A×B×C	ns 77.0	ns 7.43	ns 16.18	ns 28.0	ns 0.98	ns 35.0	ns 517.3	ns 4351

PH: plant height (cm); SL: spike length (cm); NSS: number of spikelets per spike; NGS: number of grain per spike; WGS: weight of grain per spike (cm); TGW: 1000 grain weight (g); NS m⁻²: number of spike m⁻²; GY: grain yield (kg ha⁻¹).

Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range test (p \leq 0.05).

*, **Significant at the 0.05 and 0.01 probability levels, respectively; ns: nonsignificant.

Table 2. Cultivar, season, and N fertilization level effects on rain-use efficiency (RUE), N agronomic efficiency (NAE), and N use efficiency (NUE).

Factor		RUE	NAE	NUE	
		kg DM ha ⁻¹ mm ⁻¹	kg grain increase kg-1 N applied	kg grain kg ⁻¹ N applied	
Cultivar	Pobeda	8.32a	1.34	30.04	
(A)	Renesansa	8.00b	2.20	29.01	
Season	2010-2011	8.06	1.26	31.46a	
(B)	2011-2012	8.26	2.28	27.59b	
N level,	0	7.80b	0.0b	0.0c	
kg ha ⁻¹	75	8.24a	3.11a	58.62a	
(C)	150	8.45a	2.21a	29.96b	
Ftest	Cultivar (A) Season (B) N level (C)	ns **	ns ns **	ns **	
	$A \times B$	ns	ns	ns	
	$A \times C$	ns	ns	ns	
	$B \times C$	ns	ns	**	
Mean	$A \times B \times C$	ns 8.16	ns 1.77	ns 29.53	

Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range test (p \leq 0.05).

grain increase kg⁻¹ N applied, and 0 kg grain kg⁻¹ N applied, respectively). These levels of N did not differ for RUE and NAE; NUE was significantly higher at 75 kg N ha⁻¹ than 150 kg N ha⁻¹. The interaction of season and N fertilization level had highly significant effect on NUE.

Correlation between studied properties

Results show that the GY have very strong positive correlation with TGW (Table 3). PH was strong positively correlated with NSS, TGW, NS m-2 and GY, SL with NGS, NSS with WGS, NGS with WGS, and GY with NS m⁻². Moderate positive correlations were found for PH with NGS and WGS, SL with NSS and WGS, NSS with NGS, TGW, NS m⁻² and GY, WGS with TGW, GY and RUE, TGW with NS m⁻² and NAE, and NAE with NUE. Weak, low positive correlations were found for PH with SL (nonsignificant) and NUE, SL with RUE, NAE and NUE, NSS with RUE (nonsignificant) and NUE, WGS with NS m⁻², TGW with RUE (nonsignificant) and NUE (nonsignificant), NS m-2 with NUE (nonsignificant). Very weak to negligible correlations were found for PH with RUE and NAE, SL with TGW and NS m⁻², NSS with NAE, NGS with TGW, NS m⁻², NAE and NUE, WGS with NAE and NUE, NS m-2 with RUE and NAE, RUE with NAE and NUE.

DISCUSSION

Among wheat cultivars, significant differences were recorded for NS m⁻² and GY; 'Pobeda' had significant higher NS m⁻² and GY than 'Renesansa'. These traits are highly correlated, can be said that NS m⁻² is the important effective component in GY.

Plant height, NSS, WGS, TGW, NS m⁻², and GY were affected by season. Values for these traits were significantly

^{*, **}Significant at the 0.05 and 0.01 probability levels, respectively; ns: nonsignificant.

Table 3. Pearson correlation coefficient (r) between quantitative traits of two wheat cultivars ('Pobeda' and 'Renesansa'), three N fertilization levels (0, 75, and 150 kg N ha⁻¹), and two growing seasons (2010-2011 and 2011-2012).

Factor	PH	SL	NSS	NGS	WGS	TGW	NS m ⁻²	GY	RUE	NAE
SL	0.35ns	1.00								
NSS	0.80^{**}	0.62**	1.00							
NGS	0.44**	0.73**	0.58**	1.00						
WGS	0.69**	0.67**	0.70^{**}	0.86^{**}	1.00					
TGW	0.71**	0.12^{ns}	0.51**	0.19^{ns}	0.50**	1.00				
NS m ⁻²	0.82^{**}	$0.02^{\rm ns}$	0.57**	0.12^{ns}	0.37^{*}	0.67^{**}	1.00			
GY	0.74**	0.12^{ns}	0.53**	0.19^{ns}	0.51**	0.99^{**}	0.70^{**}	1.00		
RUE	0.11^{ns}	0.31*	0.25^{ns}	0.29^{*}	0.62**	$0.24^{\rm ns}$	0.08^{ns}	0.30^{*}	1.00	
NAE	0.01 ^{ns}	0.40^{**}	0.12^{ns}	0.15^{ns}	0.19^{ns}	0.42^{**}	0.16 ^{ns}	0.38**	0.18 ^{ns}	1.00
NUE	0.32^{*}	0.38**	0.33*	0.13^{ns}	0.16^{ns}	0.27^{ns}	0.20^{ns}	0.33*	0.04^{ns}	0.53**

^{*, **}Significant at the 0.05 and 0.01 probability levels, respectively; ns: nonsignificant.

PH: plant height; SL: spike length; NSS: number of spikelets per spike; NGS: number of grain per spike; WGS: weight of grain per spike; TGW: 1000 grain weight; NS m⁻²: number of spike m⁻²; GY: grain yield; RUE: rain-use efficiency; NAE: N agronomic efficiency and N use efficiency (NUE).

higher in favorable environmental conditions in 2010-2011 season. Drought stress during stem elongation stage (late March to early April in 2012) reduced the PH and NSS. Plants responded by reducing PH because stem growth was slow. Also, other researchers showed that drought stress during stem elongation stage reduced the PH (Shamsi and Kobraee, 2011; Gevrek and Atasoy, 2012) and NSS (Sener et al., 2000; Gevrek and Atasoy, 2012). In June and July 2012, water stress during grain filling stage shortened grain filling period and reduced WGS and TGW. Garcia del Moral et al. (2003) and Ilker et al. (2011) pointed that drought after flowering of wheat adversely affects DM translocation in the grain. Bauder (2001) reported that drought stress during maturity stage decreases yield about 10%. NS m-2 is a trait dependant on the intensity of tillering. The intensity of tillering is a varietal characteristic which is caused by environmental factors. Wheat sown in 2010 had a good set of plants as a result of favorable weather conditions during germination and tillering. On the other hand, the drought in the phase of germination 2011 (beginning November) and early tillering (second half of November) resulted in a sparse set of plants, and lower intensity of tillering, which resulted in lower NS m⁻². Grain yield was significant higher in 2010-2011. In June 2012, the wheat was exposed to high temperatures and lack of water leading to a shortening of grain filling process and accelerated ripening, reduction of WGS and TGW and hence yield. In addition, the NS m⁻² was higher in the 2010-2011 season, and this feature had a highly significant correlation with the GY. In both seasons there was no shortening of SL, because there was enough water at the time of head (inflorescence) emergence and flowering. The drought stress at flowering stage reduces the SL. Gevrek and Atasoy (2012) reported that SL was not significantly affected by drought stress after postanthesis stage. The NGS did not differ in seasons because there were unfavorable climatic conditions (heat stress, drought) during flowering and pollination (end of May).

Plant height, SL, NSS, WGS, TGW, NS m⁻², and GY were significantly increased with increasing N level. However, there was no significant difference between 75 and 150 kg N ha⁻¹. Also, many authors reported that

increasing N level increased PH (Ali et al., 2000; Sobh et al., 2000; Jan et al., 2002; Iqtidar et al., 2006; Ali et al., 2011), SL (Ali et al., 2000; Asif et al., 2009; El-Gizawy, 2009; Ali et al., 2011; Iqbal et al., 2012; Gheith et al., 2013), NSS (Modhej et al., 2008; Ali et al., 2011; Iqbal et al., 2012), WGS (Noureldin et al., 2013), TGW (Ali et al., 2000; Iqtidar et al., 2006; Ali et al., 2011; Abedi et al., 2011) and NS m⁻² (Ahmed Seham et al., 2009; Ansar et al., 2010; Njuguna et al., 2010; Abedi et al., 2011; Iqbal et al., 2012). Many studies have shown that the best yields achieved with 70 to 120 kg N ha⁻¹ (Teixeira Filho et al., 2007; 2010; Njuguna et al., 2010; Espindula et al., 2010; Abedi et al., 2011; Iqbal et al., 2012; Noureldin et al., 2013). High yields achieved in the control can be attributed to the high content of organic matter and humus in the soil in which they conducted experiments, since in the process of mineralization, which is more intensive in terms of sufficient amounts of rainfall and higher air temperatures in March and April, a significant amount of available N for plants is released (Cabrera et al., 2005). NGS was higher in both N fertilization levels compared to control, however differences were not significant. Gevrek and Atasoy (2012) found that N application had no significant effect on NGS. On the other hand, Iqtidar et al. (2006) and Abedi et al. (2011) reported that NGS significantly increased with increasing N fertilization levels.

Water resources in the Republic of Serbia are limited and about 1% of arable land is irrigated (Mandic et al., 2013). Wheat is not irrigated so the key to a stable production is rain-use efficiency (RUE). Genotypes with improved RUE are particularly beneficial under low rainfall conditions. 'Pobeda' had a better use of rainfall than 'Renesansa' since its RUE value was significantly higher. The NUE depends of the water availability. In our experimental fields, water was a limiting factor during the cropping season 2011-2012 with 490 mm and NUE had lower value. RUE, NAE, and NUE significantly increased with increase N fertilization level than in control. NAE and NUE were reduced in highest N level, especially NUE. This can be attributed to N loss in ecosystem. Russell (1967) found that average NAE of 7.2 kg grain increase

kg⁻¹ N applied at 25 kg N ha⁻¹ and 5.1 kg grain increase kg⁻¹ N applied at 50 kg N ha⁻¹. Serret et al. (2008) reported that NAE significantly reduced in the highest N fertilizer level. Hooper (2010) reported that average NAE for wheat about 20-25 kg grain increase kg⁻¹ N applied worldwide, 33 kg grain increase kg⁻¹ N applied in Australia and about 15-20 kg grain increase kg⁻¹ N applied in South Australia. Also, reported that N fertilization increased NUE, but the highest N level reduced NUE. Somarin et al. (2010) and Noureldin et al. (2013) reported that increased N level reduced NUE.

Correlation coefficients between GY and PH, TGW and NS m-2 were significant and high. Also, GY had a significantly moderate positive correlation with NSS, WGS, RUE, NUE, and NAE. These traits were important for selection and breeding criteria to GY improving. Many authors reported that in wheat grown in the different environments GY was significantly correlated with NSS and TGW (Lad et al., 2003; Kashif and Khaliq, 2004; Aycecik and Yildirim, 2006; Akram et al., 2008; Joshi et al., 2008; Wu et al., 2012). Contrary to our research, Shahid et al. (2002), Akram et al. (2008), and Joshi et al. (2008) reported that GY was negatively correlated with PH. In regard to traits for which no significant correlation was determined (PH with RUE and NAE, SL with TGW, NS m-2 and GY, NSS with RUE and NAE, NGS with TGW, NS m⁻², GY, NAE and NUE, WGS with NAE and NUE, TGW with NUE, NS m⁻² with RUE, NAE and NUE, and RUE with NAE and NUE), it is possible to conduct simultaneous selection/breeding for both traits. This relates particularly to correlation between RUE and NUE.

CONCLUSIONS

'Pobeda' had higher grain yield (GY) than 'Renesansa', because it had higher NSm⁻² and better used the rainfall during growing seasons. Drought in certain stages of development of plants in 2011-2012 led to a reduction in yield components (number of spikelets per spike, weight of grain per spike, 1000 grain weight, number of spikes per square meter) and GY. Also, N use efficiency (NUE) was lower in this season. Results showed that plant height, yield component, GY, rain-use efficiency, N agronomic efficiency, and NUE significantly increased with increasing N fertilization levels. However, application of 75 or 150 kg N ha⁻¹ did not differ significantly, except for NUE that significantly decreases at higher N level. Therefore, the application of 75 kg N ha-1 gives the minimum loss of N in the ecosystem as well as the lowest cost of production of wheat in Serbia.

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