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The possibilities of using physiological efficiency of nitrogen in wheat breeding in terms of ecological agriculture

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Summary: Inadequate amounts of fertilizers, especially nitrogen, have negative effects on ecosystems and food safety. On the other side, fertilizing, with an improvement in cropping systems, mainly in developed countries, has provided a food supply sufficient for both animal and human consumption. Therefore, with the increasing world population, the main task for the following decades is to develop highly productive agriculture, whilst at the same time preserving the quality of the environment. A multidisciplinary approach to winter wheat breeding and the inclusion of physiological indicators of nitrogen nutrition efficiency in this process could be helpful in reaching this goal. This study deals with the physiological efficiency of nitrogen as one of the physiological indicators, its relation with grain yield, heritability and variance and evaluation of Serbian winter wheat genotypes according to this indicator. The highest values of the physiological efficiency of nitrogen were registered at KG 165/2 and Lazarica. These genotypes, selected as superior in terms of this indicator, could be considered as desirable in wheat breeding theory, for improvement of production efficiency, environmental protection and development of ecological agriculture. However, further investigations of other physiological parameters, their correlations and correlations with yield and grain quality of wheat are necessary to define breeding programs and to obtain reliable results in this area.
Key words: breeding, efficiency, grains, nitrogen, physiological efficiency of nitrogen, wheats

Introduction

Grain yield of wheat (*Triticum aestivum* L.) is influenced directly and indirectly by many factors, such as morphological, physiological, and especially environmental conditions. Grain yield in wheat has low heritability and shows high genotype × environment interaction, and hence, selection becomes more difficult in a given environment (Prasad et al., 2007). A good understanding of the factors responsible for growth and development is necessary to identify indirect selection tools for improving grain yield in wheat (Araus et al., 2001). Reynolds et al. (2001) emphasized the potential of using different morpho-physiological selection criteria to complement empirical selection for grain yield, which could potentially make the selection process more efficient. So far, the use of this strategy has not been well established in a large-scale breeding program, due to the lack of knowledge of the indirect traits.

A better understanding of relatively simple crop-physiological characteristics that determine yield in a wide range of conditions may be an instrument for assisting future breeding. Physiological traits may be selected either directly or by using molecular-biology tools.

The question of including plant physiology in wheat breeding programs has been analyzed by researchers for a long time. Obstacles come from the sensitivity of physiological traits towards environmental conditions, the complexity of physiological interactions and nonexistence of adequate methods (Asseng & Milroy 2006; Váňová et al., 2006). According to Abeledo et al. (2003), some physiological parameters can be involved as breeding criteria if their genetic variability and control, relationship with desirable traits (grain yield, mainly) and measurability can be defined and formulated. The role of physiological parameters of nitrogen accumulation and use efficiency in wheat breeding under different environmental conditions was analyzed by van Ginkel et al. (2001), Baker et al. (2004), Flowers et al. (2004) and Pathak et al. (2008).

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Considering the importance of nitrogen in the plant life cycle, indicators of plant nitrogen nutrition efficiency represent a group of physiological traits suitable to contemporary wheat breeding aims. Many authors (Andersson et al., 2004; Gallais & Coque, 2005) noticed that some of these parameters positively affect grain yield. Górny et al. (2006) reported some of the most important reasons limiting involving of parameters of plant nitrogen status in wheat breeding programs as criterias and development of such programs. First of all, large number of endo- and exogenous traits and mechanisms are parts in the complex plant response to water and nutrient shortages, but the most appropriate selection criteria are not always clearly defined. Further, there is not enough detailed information either on the genetic nature or variation ranges in the complex utilization efficiency and adaptation to reduced soil resources among the native wheat germplasm.

The efficiency of an indirect selection criterion compared to direct selection depends on the heritability of the indirect trait, along with the genetic correlation between the direct and indirect traits (Prasad et al., 2007). Therefore, breeders need facts about parameters, like heritability, genetic and environmental variances under various conditions. They also need information about their dynamics under increased N availability. In addition, adequate genetic variability for the traits that determine N efficiency is compulsory (Manal, 2007). Genotypic variation was reported for N uptake and/or N utilization efficiency in bread wheat (Le Gouis et al., 2002) and maize (Manal & Aly, 2008). Although parameters of N use are strongly affected by the environment, modern molecular technologies could be helpful in changing them by breeding (Vinod, 2007).

The challenge in contemporary wheat selection and breeding is to integrate genetic and phenotypic information to develop a new model of cultivars adapted to specific environments and cultivation practices. Keeping in mind the increasing demand for food, the requirement for a more environmentally-friendly agriculture and future risks arising from climate change and role of nitrogen in plant life cycle, the issue of nitrogen use efficiency and its indicators is of key importance to overcome environmental concerns in conventional systems and production limitations in organic systems. Therefore, the objectives of this study were to estimate the variability of physiological efficiency of nitrogen, its heritability and variance and its correlation with grain yield in winter wheat. The obtained results could be useful as guidelines in ecological agricultural practice and for further wheat breeding programe for improved nitrogen nutrition efficiency and realization of its contemporary goals.

Material and Methods

The study was carried out at the Small Grains Research Center in Kragujevac (186 m.a.s.l.), Serbia in three consecutive seasons (2001/02, 2002/03 and 2003/04) on vertisol soil. According to both World Reference Base for Soil Resources (WRB) and the USDA soil taxonomy (Kurtzman et al., 2016), vertisols are soils with a high content of clay minerals that shrink and swell as they change water content.

The meteorological data were collected from Meteorological Station, situated on the grounds of Small Grains Center in Kragujevac.

The experiment included 30 wheat cultivars and experimental lines, originating from Serbia: Small Grains Research Center, Kragujevac (Morava, Lepenica, Studenica, Takovcanka, Toplica, Srbijanka, KG 100,

Table 1. Average monthly temperatures during the three test growing seasons and long-term (30-yr) mean (LTM) for winter wheat

Month	Average monthly temperatures (°C)			
	2001/02	2002/03	2003/04	LTM
X	13.8	12.2	10.6	11.40
XI	4.6	9.7	8.9	5.90
XII	- 2.4	1.1	2.2	2.13
I	- 0.1	0.7	- 0.9	0.73
II	7.0	- 2.4	3.0	2.42
III	8.9	5.8	7.1	6.43
IV	10.8	10.8	12.8	11.22
V	18.4	19.9	14.5	16.24
VI	21.6	23.3	19.8	19.40
$\overline{X}$	Season average			
	9.18	9.01	8.67	8.43

Table 2. Monthly amounts of rainfall during the three test growing seasons and long-term (30 years) mean (LTM) for winter wheat

Month	Monthly amounts of rainfall (l)			
	2001/02	2002/03	2003/04	LTM
X	10.4	65.5	83.2	47.53
XI	64.1	31.5	28.6	47.20
XII	27.6	39.4	37.2	44.33
I	17.2	59.0	86.4	36.70
II	20.1	19.7	59.5	35.77
III	26.0	2.8	21.3	41.57
IV	63.7	37.2	52.3	50.77
V	38.6	42.3	50.3	65.43
VI	57.2	47.7	61.4	81.27
	Total			
	324.9	345.1	483.2	450.57

Table 3. Mean squares of physiological efficiency of nitrogen

Source of variance	Df	Mean square of physiological efficiency of nitrogen
Years	2	495.95**
Genotypes	29	62.02**
Interaction	58	42.63**
Error	356	4.18

Lazarica, Bujna, Matica, Vizija, KG – 200/31, KG – 253/4 – 1, KG – 115/4, KG – 165/2, KG – 56/1, KG – 100/97, Perla, KG - 224/98 and KG – 10) and Institute of Field and Vegetable Crops, Novi Sad (Pobeda, Rana 5, Evropa 90, Renesansa, Tiha, Mina, Prima, Kremna, Rusija, Pesma).

The basic processing and pre-sowing preparation of the soil were performed using standard procedures. The randomized complete block experimental design was used with five replicates in rows 1.5 m long, with spacing between rows of 0.20 m.

Sowing (200 grains per row) was manual (one genotype per row), during the optimal planting period for central Serbian conditions, for winter wheat (29 October 2001, 15 November 2002 and 6 November 2003). NPK fertilizer (8:24:16) was applied at the rate of 300 kg ha^{-1} before sowing in each season. Eight grams of nitrogen (260 kg KAN ha^{-1}) per row were added at the tillering stage of development in each season.

Plant samples of each genotype were taken at maturity (five plants per replication). The samples were air-dried and the grain yield (GY, g m^{-2}) and biological yield (BY, g m^{-2}) were measured per plant and recalculated using number of plants per unit area. All dry vegetative samples and grain were first ground and then plant N concentration was determined by the standard macro-Kjeldahl procedure (Musinger and Mckinney, 1982). Nitrogen accumulation at maturity (ANm - gN m^{-2}) was calculated by multiplying the N concentration by BY. Physiological efficiency of nitrogen (PEN) was calculated as the ratio between GY and ANm (g grain gN^{-1}) (Arduini et al., 2006).

Table 4. Average values ($\overline{X}$), standard error (SE_X) and coefficient of variation (Cv) of PEN (g gN^{-1}).

Genotype	2001/02			2002/03			2003/04		
	$\overline{X}$	SE_X	Cv (%)	$\overline{X}$	SE_X	Cv (%)	$\overline{X}$	SE_X	Cv (%)
Morava	39.90	0.65	3.64	28.57	0.63	4.93	43.38	2.26	11.65
Lepenica	41.44	1.00	5.37	32.17	0.75	5.18	44.43	0.33	1.68
Studenica	44.36	0.36	1.84	38.54	0.23	1.36	41.94	0.92	4.93
Takovcanka	44.73	0.28	1.40	37.37	0.82	4.89	42.81	1.28	6.71
Toplica	45.23	0.58	2.89	35.90	0.34	2.09	43.87	1.27	6.47
Srbijanka	45.69	0.67	3.28	31.63	0.80	5.67	44.43	1.21	6.10
KG – 100	43.30	0.59	3.02	33.85	0.20	1.31	38.67	1.26	7.27
Lazarica	43.01	0.59	3.06	43.19	0.47	2.43	43.69	0.64	3.27
Bujna	48.95	0.24	1.09	39.28	0.65	3.70	40.53	0.17	0.93
Matica	40.73	0.19	1.07	32.14	0.57	3.96	45.27	0.39	1.91
Vizija	39.22	0.61	3.48	33.31	0.21	1.39	41.73	1.27	6.80
Pobeda	44.44	0.41	2.08	31.41	1.08	7.69	50.10	1.03	4.61
Rana 5	39.52	0.60	3.42	39.06	0.95	5.45	46.80	0.63	3.03
Evropa 90	40.32	3.43	19.00	31.91	0.27	1.92	37.68	0.22	1.29
Renesansa	39.17	1.51	8.63	32.99	0.66	4.47	47.05	0.70	3.35
Tiha	38.54	0.47	2.71	30.63	1.05	7.65	44.65	1.37	6.87
Mina	42.91	0.49	2.53	28.31	0.94	7.45	48.21	1.03	4.79
Prima	45.66	0.98	4.79	33.10	1.10	7.45	45.82	0.60	2.91
Kremna	42.51	0.60	3.17	30.48	0.39	2.88	42.76	0.33	1.70
Rusija	44.47	0.63	3.19	32.41	0.50	3.45	43.92	0.43	2.17
Pesma	38.14	1.09	6.40	29.18	0.57	4.36	40.11	0.65	3.65
KG 200/31	37.91	0.51	3.00	32.16	0.74	5.12	43.07	0.70	3.63
KG 253/4-1	41.86	0.46	2.45	32.67	0.63	4.33	40.98	0.71	3.86
KG 115/4	44.54	0.40	2.02	33.06	1.78	12.05	44.39	0.62	3.14
KG 165/2	44.16	0.40	2.05	37.62	0.51	3.03	48.42	0.27	1.26
KG 56/1	42.14	0.30	1.62	35.62	1.38	8.68	45.46	0.72	3.56
KG 100/97	38.41	0.19	1.12	30.67	2.34	17.04	45.25	0.59	2.94
Perla	47.17	0.59	2.80	31.76	1.42	10.02	45.08	0.45	2.23
KG 224/98	39.20	0.30	1.73	29.82	0.29	2.16	42.51	1.34	7.04
KG 10	40.17	0.91	5.07	30.74	2.14	15.57	48.39	0.66	3.06
	A**	B**	A × B**						
$LSD_{0.05}$	0.46	1.47	2.54						
$LSD_{0.01}$	0.61	1.93	3.35						

The mean values, standard error, and variability of traits were analyzed by a two-factorial analysis of variance, but the significance of differences was tested by the LSD test at P level 0.05 and 0.01 (Hadzivukovic, 1977). The coefficients of genetic and phenotypic correlations and tests of their significance were determined according to Chaudhary et al. (1999). The heritability in a broad sense, as a ratio of genetic/ phenotypic variance, was calculated according to the formula h2 = σ2g / σ2f x 100 (Falconer, 1989).

Results and Discussion

The average temperatures and monthly rainfall during the wheat vegetation period (October-June) for the three seasons and the 30-year mean (1970-2000) are shown in Tables 1 and 2. In all three years, the mean temperature was higher than the 30-year average. There was considerable variability in rainfall amounts and distribution, during vegetative seasons, from year to year.

The meteorological variables such as temperature and precipitation, during the certain years, when the researches were carried out. had a highly significant effect on tested indicator, as well as genotype and interaction year × genotype (Table 3). These results suggest that environmental factors in this study played a major role in the expression of tested property.

The lowest PEN (28.31 g grain gN^{-1}) was registered in the second investigation year, in Mina cultivar while the highest value of this indicator was 50.10 g grain gN^{-1} (cultivar Pobeda, the third investigation year) (Table 4).

Mostly, the variation of PEN varied from 0.93% to 11.65%. The higher variability was noticed in cultivars Evropa 90, KG 100/97, KG 10 and KG 115/4 (19.00%, 2001/02, 17.04%, 2002/03, 15.57%, 2002/03 and 12.05%, 2002/03, respectively) (Table 4). The coefficient of variation is and indicator of sample representativity and shows percentage variation of observation units (Hadzivukovic, 1977; Dragovic, 2008). Generally, the main problem in biological and agricultural sciences is high variation due to so many influences that are difficult to control by experimental methods (Micic and Bosancic, 2012). In this context, the question of the level of variation allowed is very important. According to some authors, Cv in biological and agricultural investigations have to be between 5% and 30%. Values below and above these ones demand additional testing of studied samples. Coefficients of variation in this investigation did not exceed, in most cases, the upper allowed limit that eliminates systemic influence of unnoticed factors on statistical units of observation.

Genotype KG 165/2 revealed the highest average value of PEN (43.40 g grain gN^{-1}) while the lowest was calculated in genotype Pesma (35.81 g gN^{-1}). The average value of this indicator, for all tested genotypes, was 39.87g grain gN^{-1}.

Variability among genotypes and experimental years indicated the genetic distance between investigated varieties and differences of environmental factors during the growing period. Success in breeding depends on genetic and phenotypic variability that influence the expression of individual genotypes. Genotype and environment interactions are important sources of variation in crop breeding programs. Accordingly, varieties with low yield variability components are more stable and have higher potential for grain yield. The results of Aynehband et al. (2010) pointed out the high and positive interrelationship of many nitrogen status indicators, like nitrogen use and translocation efficiency and total nitrogen accumulation in mature plant and grain yield in wheat.

According to variability, the tested parameter of the nitrogen status of wheat plant could be important from the grain yield and wheat breeding point of view, but it is only one aspect of this matter. Data about correlations between PEN and grain yield could be an important contribution to the interpretation of this issue.

Table 5. Three-year average values of PEN (g gN^{-1})

Genotype	$\overline{X}$	SE_X	Cv (%)
Morava	37.29	0.63	4.93
Lepenica	39.34	0.75	5.18
Studenica	41.61	0.23	1.36
Takovcanka	41.64	0.82	4.89
Toplica	41.67	0.34	2.09
Srbijanka	40.58	0.80	5.67
KG – 100	38.61	0.20	1.31
Lazarica	43.30	0.47	2.43
Bujna	42.92	0.65	3.70
Matica	39.38	0.57	3.96
Vizija	38.09	0.21	1.39
Pobeda	41.98	1.08	7.69
Rana 5	41.79	0.95	5.45
Evropa 90	36.63	0.27	1.92
Renesansa	39.74	0.66	4.47
Tiha	37.94	1.05	7.65
Mina	39.81	0.94	7.45
Prima	41.52	1.10	7.45
Kremna	38.58	0.39	2.88
Rusija	40.27	0.50	3.45
Pesma	35.81	0.57	4.36
KG 200/31	37.71	0.74	5.12
KG 253/4-1	38.50	0.63	4.33
KG 115/4	40.66	1.78	12.05
KG 165/2	43.40	0.51	3.03
KG 56/1	41.08	1.38	8.68
KG 100/97	38.11	2.34	17.04
Perla	41.33	1.42	10.02
KG 224/98	37.18	0.29	2.16
KG 10	39.77	2.14	15.57
$\overline{X}$	39.87	0.81	5.59

Table 6. Genetic and phenotypic correlations between grain yield and physiological efficiency of nitrogen

Genetic correlations			Phenotypic correlations	
Year	Trait	PEN	Trait	PEN
1	Grain yield	0.01	Grain yield	0.16
2		0.38**		0.31**
3		0.27*		0.22*

Table 7. Broad-sense heritability of PEN

	Indicator		
			PEN
h^2_b	Year	1	0.67
		2	0.72
		3	0.69
	$\overline{X}$		0.69

Numerous previous investigations have indicated the impact of parameters of nitrogen status in wheat on yield and its components and significant correlation coefficients between them (Nikolic, 1999; Kichey et al., 2007; Zhou et al., 2018). The physiological efficiency of nitrogen is one of the indicators of nitrogen utilization which influences grain filling and formating. However, interdependence PEN and GY does not always have to be highly significant. Many factors influence the expression of that relationship, like the total supply of wheat plants with nitrogen. Their interdependence in this investigation varied from insignificant to statistically high significant (Table 6). Therefore, the role of this parameter in wheat breeding programs with the aim of better and more economical yield is not negligible.

Heritability is not a constant value, since decisions by the breeder can influence the magnitude of the value and the amount of genetic improvement obtained from selection. Its studies help the plant breeder to predict the interaction of genes in successive generations and are essential for effective breeding programs (Todorovic et al., 2011). Heritability estimates provide an indication of the expected response to selection in segregating populations, and in theory, both h^2b and h^2n can vary from 0 to 1. According to Johnson et al. (1955), heritability values are classified as low (0-30%), moderate (30-60%) and high (60% and above). Obtained results about heritability (h^2b) of PEN in this investigation varied from 0.67 to 0.72 (Table 7).

These results indicate that PEN can be transmitted in a high percentage from parents to their offsprings, and can therefore be used as a parameter in the creation of a breeding program on wheat. The importance of such a note is emphasized by the significant correlation relationships of this trait with grain yield. A high estimate indicates how well the evaluation of the parents will predict what the progenies will be like with a particular combination of breeding material and technique of evaluation. The h^2b overestimates the response to selection as it includes non-additive effects.

Willem (2007) found that the heritability values for the N uptake components, Ngrain, Ntotal and nitrogen harvest index (NHI) depend on the level of nitrogen nutrition. Their heritability, therefore, was high and increased at the HN (high nitrogen) treatment. With the exception of the NutEYld (nitrogen utilization efficiency for grain yield) component at the LN (low nitrogen) treatment, the broad-sense heritability values of all the components of nitrogen utilization efficiency, including PEN, were high and increased by the HN treatment.

Conclusion

Keeping in mind serious ecological risks and increasing food demand, the important goal of contemporary wheat selection and breeding is to create new model of cultivar, high yielding, but adapted to ecological and other sustainable agricultural systems that means, among the others, successful cultivation under conditions of limited nutrient use, i.e. ban on synthetic nitrogen use. Considering the role of N in the plant life cycle, one possible way to solve this problem and overcome such contradiction is to improve nitrogen use efficiency, through improving some important indicators of it, like the physiological efficiency of nitrogen. The PEN varied from 28.31 g grain gN^{-1} (Mina cv) to 50.10 g grain gN^{-1} (Pobeda cv). On average, genotype KG 165/2 revealed the highest value of PEN (43.40 g grain gN^{-1}) while the lowest was calculated in genotype Pesma (35.81 g gN^{-1}). The average value of this indicator, for all tested genotypes, was 39.87 g grain gN^{-1}. The analysed material (KG 165/2, KG 56/1, Studenica, Takovcanka, Lazarica, Toplica, Bujna, Pobeda, Rana 5, Prima, Mina and Perla) showed high efficiency of nitrogen use and could be interesting in further investigations and involved in wheat breeding program as a source of desirable traits, as well as for sustainable agricultural systems such as ecological agriculture. Heritability in broad-sense was 0.69. Correlation between PEN and grain yield, mostly were statistically significant. Thus, obtained results indicate the possibility to use the tested indicator in wheat breeding programs as criteria, but further investigations are necessary to provide more precise information on this issue.

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Mogućnosti korišćenja fiziološke efikasnosti azota u oplemenjivanju pšenice u smislu ekološke poljoprivrede

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Sažetak: Uprkos nepoželjnim efektima po životnu sredinu, upotreba đubriva u poljoprivredi, posebno azotnih, obavezan je preduslov za dobijanje dovoljnih količina hrane za ljudsku i stočnu ishranu. Stoga je izazov, u nastupajućim decenijama kada se očekuje povećanje ljudske populacije, razviti visoko-produktivne i efikasne poljoprivredne sisteme, a istovremeno doprineti očuvanju i zaštiti ekosistema. U ostvarenju tog cilja od značajne pomoći može da bude multidisciplinarni pristup oplemenjivanju pšenice, kao najvažnije hlebne vrste, kao i uključivanje fizioloških pokazatelja efikasnosti ishrane biljaka azotom u taj proces. Prema tome, u ovom radu se analizira fiziološka efikasnost azota (FEN), njegova heritabilnost i varijabilnost, povezanost sa prinosom zrna, a zatim se sa aspekta ovog indikatora ocenjuju genotipovi ozime pšenice selekcionisani u Srbiji. Najbolje vrednosti FEN zabeležene su kod genotipova KG 165/2 i Lazarica. Na osnovu ovih rezultata izdvojeni genotipovi se mogu smatrati nosiocima poželjnih osobina, sa aspekta teorije oplemenjivanja pšenice, unapređenja proizvodne efikasnosti, zaštite životne sredine i razvoja ekološke poljoprivrede. Za definisanje oplemenjivačkih programa i dobijanje pouzdanih rezultata neophodno je istraživanje ostalih fizioloških parametara, njihovih međusobnih korelacionih veza i korelacionih veza sa prinosom i kvalitetom zrna pšenice.

Ključne reči: azot, efikasnost, fiziološka efikasnost azota, oplemenjivanje, pšenica, zrno

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