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VARIABILITY AND HERITABILITY OF NITROGEN NUTRITION EFFICIENCY INDICATORS IN WINTER WHEAT

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ABSTRACT

Considering the very important role of nitrogen in plant life cycle, parameters of plant nitrogen nutrition efficiency represent a group of physiological traits suitable to contemporary wheat breeding aims. Therefore, the objectives of the study were to estimate variability of nitrogen nutrition efficiency indicators and to estimate their heritability in wheat. The experiment included 30 wheat cultivars and experimental lines, originating from Serbia: Small Grains Research Center and Institute of Field and Vegetable Crops, Novi Sad. The variability and broad - sense heritability of total nitrogen accumulation (ANt) at maturity, grain harvest index (GHI) and physiological efficiency of nitrogen (PEN) were analysed. The years when the researches were carried out had a highly significant effect on all tested indicators, as well as genotype and interaction year x genotype. Total nitrogen accumulation in mature plant was the most variable indicator (Cv 13.91%), while lower variability was registered for nitrogen harvest index and physiological efficiency of nitrogen (5.81% and 5.59% respectively). NHI heritability was lower (0.52) compared to the ANt and PEN (0.66 and 0.69, respectively). On the other hand, heritability of ANt and PEN were almost the same, but PEN was more repeatable than the ANt.

Key words: heritability, nitrogen nutrition efficiency, variability, wheat.

INTRODUCTION

Selection of advanced breeding materials for grain yield is a labour-intensive procedure and sometimes produces inaccurate results, due to the complex genetic behaviour of yield. Grain yield is influenced directly and indirectly by a number of factors, such as morphology, physiology, and especially environmental conditions. Grain yield in wheat (*Triticum aestivum* L.) has low heritability and shows high genotype x 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 potentially can make the selection process more efficient. So far, the use of this strategy is not well established in a large-scale breeding program, due to the lack of knowledge about the indirect traits.

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

The problem of including plant physiology in selection and wheat breeding programs has been present in investigations for a long time. Obstacles are results of sensitivity of physiological traits towards environmental conditions, complexity of physiological interactions and non existence of adequate methods (Asseng and Milroy 2006; Váňová et al., 2006; Estrada-Campuzano et al., 2008). According to Abeledo et al. (2003), some physiological traits can be used as breeding criteria, if their genetic variability and control, relationship

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with desirable traits (grain yield, mainly) and measurability can be defined and formulated. The importance of some physiological traits in wheat breeding under various environmental conditions was analysed by van Ginkel et al. (2001), Baker et al. (2004), Flowers et al. (2004), Săulescu et al., 2005, Pathak et al. (2008), Kandic et al. (2009).

Considering the very important role of nitrogen in plant life cycle, parameters of plant nitrogen nutrition efficiency represent a group of physiological traits suitable to contemporary wheat breeding aims.

Many authors (Andersson et al., 2004; Gallais and Coque, 2005) established that some of these parameters affect grain yield positively.

There are several reasons for which a progress in such breeding programs on winter wheat may be limited (Gomy et al., 2006). Firstly, numerous endo- and exogenous characteristics and mechanisms are involved in the complex plant response to water and nutrient shortages, but the most appropriate selection criteria are not always clearly defined. Secondly, there is a lack of 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). So, breeders need information about parameters, such as heritability, genetic and environmental variances under various conditions. They also need to know the dynamics of these parameters 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 and Aly, 2008). Although nitrogen use traits are strongly influenced by environment, modern molecular technologies would be helpful to modify them by breeding (Vinod, 2007).

Therefore, the objectives of the study were to estimate variability of nitrogen nutrition efficiency indicators and to estimate their heritability in wheat.

MATERIAL AND METHODS

The study was carried out at the Small Grains Research Center in Kragujevac (186 m.a.s.l.), Serbia, during three consecutive seasons (2001/02, 2002/03 and 2003/04). The soil type was smonitza in degradation (Vertisol). It is characterized by heavy mechanical composition, unstable, rough structure and low porosity (Jelic 1996).

The chemical analyses were carried out in the Agrochemical Laboratory of the Center, and indicated a moderate level of soil fertility and acidity (Table 1).

Table 1. Chemical characterization of soil (0-20 cm) before establishing experiments (summer 2001)

Chemical characteristics	Summer 2001
Water pH	6.23
KCl pH	5.15
Total N	0.25%
Available K (K_2O)	28.8 mg $100g^{-1}$
Available P (P_2O_5)	13.8 mg $100g^{-1}$
Organic matter	2.65%

The average temperatures and monthly rainfall during the wheat vegetation period (October - June) for the three seasons and the 30 years mean (1970-2000) are shown in Table 2. In all three years, the mean temperature was higher than the 30 yr average. There was considerable variability in rainfall amounts and distribution from year to year. The amount of rainfall was most suitable for plant growth in the third season. Rainfall (74.5 mm), received during the germination period (October - November) in the first season was less than in other two (97.00 mm and 111.8 mm) and long-term average (94.73 mm). Rainfall distribution during the rest of the vegetative period in the first season was improved but the total amounts of rainfall were less than long-term means.

Table 2. Weather conditions during the three test growing seasons and long-term (30-yr) mean (LTM) for winter wheat

Month	Average monthly temperatures (°C)				Monthly amounts of rainfall (l)			
	2001/02	2002/03	2003/04	LTM	2001/02	2002/03	2003/04	LTM
X	13.8	12.2	10.6	11.40	10.4	65.5	83.2	47.53
XI	4.6	9.7	8.9	5.90	64.1	31.5	28.6	47.20
XII	- 2.4	1.1	2.2	2.13	27.6	39.4	37.2	44.33
I	- 0.1	0.7	- 0.9	0.73	17.2	59.0	86.4	36.70
II	7.0	- 2.4	3.0	2.42	20.1	19.7	59.5	35.77
III	8.9	5.8	7.1	6.43	26.0	2.8	21.3	41.57
IV	10.8	10.8	12.8	11.22	63.7	37.2	52.3	50.77
V	18.4	19.9	14.5	16.24	38.6	42.3	50.3	65.43
VI	21.6	23.3	19.8	19.40	57.2	47.7	61.4	81.27
Season average					Total			
	9.18	9.01	8.67	8.43	324.9	345.1	483.2	624.43

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, 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 was done using standard procedures. The randomised complete block experimental design was used with five replicates in rows 1.5 m long, with spacing between rows of 0.20m. Sowing (200 grains per row) was done by hand (one genotype per row), during the optimal planting period for central Serbian conditions, for winter wheat (29.10. 2001, 15.11.2002 and 06.11.2003). NPK fertilizer, formulated 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). The samples were air – dried and the grain yield (GY, g m^{-2}) and biological yield (BY, g m^{-2}) were measured. All dry vegetative samples and grain were first ground and then plant N concentration was determined by the standard macro-Kjeldahl procedure. Nitrogen accumulation at maturity (Ant – g N m^{-2}) was calculated by multiplying the N concentration by BY. Moreover, the following parameters, related to N nutrition efficiency were calculated according to Arduini et al. (2006), as follows:

1. Nitrogen harvest index
   (NHI) = ANgrain / ANt x 100 (%)
2. Physiological efficiency of nitrogen
   (PEN) = GY / AN_t, (g_{grain} gN^{-1}).

The data were analysed by two factorial analysis of variance. Difference between mean values was tested by LSD test at P level 0.05 and 0.01 (Hadzivukovic, 1991).

The heritability in a broad sense, as a ratio of genetic/phenotypic variance, was calculated according to the formula:

$$h^2 = \sigma^2 g / \sigma^2 f \times 100$$ (Falconer, 1989).

RESULTS AND DISCUSSION

The years when the researches were carried out had a highly significant effect on all tested indicators, as well as genotype and interaction year x genotype (Table 3). These results suggest that environmental factors in this study played major role in the expression of all tested properties.

Table 3. Mean squares of total nitrogen accumulation, nitrogen harvest index and physiological efficiency of nitrogen

Source of variance	df	Mean square of NNE indicators		
		Ant	NHI	PEN
Years	2	525.43**	190.38**	495.95**
Genotypes	29	93.06**	86.38**	62.02**
Interaction G*Y	58	38.14**	79.31**	42.63**
Error	356	4.35	10.79	4.18

Variation of total nitrogen accumulation (ANt) values was very large, from 7.13 g m^{-2} (KG 200/31) to 18.31 g m^{-2} (KG 10), with average value 10.93 g m^{-2} (Table 4). Cv varied from 4.70% (KG 253/4-1) to 19.83% (KG 100). Average nitrogen harvest index (NHI) of all analysed varieties was 75%, ranging from 67% (KG 10) to 79% (KG 165/2), with extreme Cv values of 1.08% in cultivar Studenica and 28.21%, in line KG 10. Table 4 also shows that genotype KG 165/2 revealed the highest average value of physiological efficiency of nitrogen (43.40 g gN^{-1}), while the lowest was registered in genotype Pesma (35.81 g gN^{-1}). Average value of this indicator, for all tested genotypes, was 39.87 g gN^{-1}. Cv ranged from 1.31% (KG100) to 17.04% (KG100/97).

Table 4. Average values and variability of ANt, NHI and PEN

Indicator / Genotype	ANt (g m^{-2})	S_X	Cv (%)	NHI (g m^{-2})	S_X	Cv (%)	PEN (g gN^{-1})	S_X	Cv (%)
Morava	16.02	1.58	17.17	74	1.66	5.16	37.29	0.63	4.93
Lepenica	8.44	0.85	13.91	75	1.83	5.32	39.34	0.75	5.18
Studenica	10.96	1.23	15.31	76	0.40	1.08	41.61	0.23	1.36
Takovcanka	10.59	1.06	15.55	72	1.75	5.17	41.64	0.82	4.89
Toplica	10.12	1.30	19.80	75	0.60	1.65	41.67	0.34	2.09
Srbijanka	10.32	1.47	18.90	71	1.91	5.75	40.58	0.80	5.67
KG - 100	13.12	1.73	19.83	72	0.37	1.08	38.61	0.20	1.31
Lazarica	8.75	1.08	18.93	73	0.77	2.34	43.30	0.47	2.43
Bujna	9.66	1.07	15.95	77	1.44	3.93	42.92	0.65	3.70
Matica	12.23	0.98	12.79	77	1.46	4.10	39.38	0.57	3.96
Vizija	11.89	0.75	12.34	72	0.55	1.55	38.09	0.21	1.39
Pobeda	9.72	0.94	14.45	78	2.69	7.64	41.98	1.08	7.69
Rana 5	10.27	0.59	8.78	76	1.85	5.40	41.79	0.95	5.45
Evropa 90	15.22	0.87	8.68	77	0.71	1.90	36.63	0.27	1.92
Renesansa	9.21	0.99	16.99	77	1.62	4.37	39.74	0.66	4.47
Tiha	10.64	1.14	15.30	74	2.57	7.56	37.94	1.05	7.65
Mina	11.79	0.63	9.44	71	2.34	7.42	39.81	0.94	7.45
Prima	7.28	0.68	11.31	77	2.50	7.24	41.52	1.10	7.45
Kremna	10.93	0.64	9.24	75	1.07	2.85	38.58	0.39	2.88
Rusija	10.65	0.62	8.17	74	1.16	3.20	40.27	0.50	3.45
Pesma	7.88	0.95	15.65	76	1.57	4.25	35.81	0.57	4.36
KG 200/31	7.13	0.98	17.82	74	1.96	5.07	37.71	0.74	5.12
KG 253/4-1	13.38	0.40	4.70	76	1.63	4.32	38.50	0.63	4.33
KG 115/4	10.80	1.06	17.27	77	4.24	12.11	40.66	1.78	12.05
KG 165/2	10.63	0.75	12.89	79	1.20	3.19	43.40	0.51	3.03
KG 56/1	11.27	1.49	16.54	77	2.06	5.59	41.08	1.38	8.68
KG 100/97	10.68	0.64	9.94	76	5.64	17.00	38.11	2.34	17.04
Perla	12.05	0.90	11.88	76	2.73	7.50	41.33	1.42	10.02
KG 224/98	7.81	0.46	9.77	74	0.77	2.25	37.18	0.29	2.16
KG 10	18.31	2.29	17.86	67	7.57	28.21	39.77	2.14	15.57
$\bar{X}$	10.93	1.00	13.91	75	1.95	5.81	39.87	0.81	5.59
	A**	B**	A x B**	A**	B**	A x B**	A**	B**	A x B**
$LSD_{0.05}$	0.47	1.50	2.59	0.75	2.36	4.09	0.46	1.47	2.54
$LSD_{0.01}$	0.62	1.97	3.42	0.98	3.11	5.38	0.61	1.93	3.35

Variability among genotypes and experimental years indicated genetic distance between investigated varieties and differences of environmental factors during growing period. Success in breeding depends on genetic and phenotypic variability that influence expression of individual genotypes. Genotype and environment interactions are important sources of variation in crop breeding programs (Mustățea et al., 2009). Accordingly, varieties with low variability of yield components are more stable and have higher potential for grain yield. The results of Nikolic (2009) and Aynehband et al. (2010) pointed out the high and positive interrelationship of many nitrogen status indicators and grain yield in wheat, especially of total nitrogen accumulation in mature plant. Correlation between grain yield and physiological efficiency of nitrogen was statistically significant, but moderate. Nitrogen harvest index showed various relationships with grain yield, ranging from moderate and negative to moderate and positive. In any case, tested parameters of nitrogen status of wheat plant are important from the grain yield point of view, and their investigation is interesting from the wheat breeding point of view, too.

When selecting the best method for characterizing yield stability in a breeding program, an important criterion is heritability. 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. Heritability estimates provide an indication of the expected response to selection in segregating populations, and in theory, both h^2_b and h^2_n can vary from 0 to 1. A high estimate indicate how well 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^2_b overestimates the response to selection as it includes non-additive effects (Morkel, 2007).

Nitrogen harvest index heritability (h^2_b) was lower compared to the ANt and PEN (Table 5), indicating that NHI is more environmentally influenced than these indicators. On the other hand, heritability of ANt and PEN were almost the same, although both traits showed a higher/lower heritability in some investigation years.

Generally, these indicators are more repeatable than the NHI. High heritability is suggestive of the scope for higher genetic gain in grain yield through selection (Todorovic et al., 2011).

Morkel (2007) found that the heritability values for the N uptake components, Ngrain, Ntotal and NHI were high, and were 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.

Table 5. Broad - sense heritability of ANt, NHI and PEN

Indicator			ANt	NHI	PEN
h^2_b	year	1	0.79	0.46	0.67
		2	0.52	0.43	0.72
		3	0.68	0.66	0.69
	X		0.66	0.52	0.69

CONCLUSION

The investigated material represents a desirable source of variability in wheat breeding programs, taking into account the indicators of nitrogen nutrition efficiency.

Variation of total nitrogen accumulation (ANt) values was very large, from 7.13 g m^{-2} (KG 200/31) to 18.31 g m^{-2} (KG 10), with average value 10.93 g m^{-2}. Cv varied from 4.70% (KG 253/4-1) to19.83% (KG 100). Average nitrogen harvest index (NHI) in all analysed varieties was 75%, ranging from 67% (KG 10) to 79% (KG 165/2), with extreme Cv values of 1.08%, in cultivar Studenica and 28.21%, in KG 10). Average value of PEN was 39.87 g gN^{-1} and genotype KG 165/2 had the highest average value of physiological efficiency of nitrogen (43.40 g gN^{-1}), while the lowest was registered in genotype Pesma (35.81 g gN^{-1}). Cv ranged from 1.31% (KG100) to 17.04% (KG100/97). Heritability in broad-sense was 0.52, 0.66 and 0.69 for NHI, ANt and PEN, respectively.

Obtained results indicate the possibility to use the tested indicators in wheat breeding programs as criteria, but further investigations are necessary to provide more precise information on this issue.

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