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Research Article

Internal Efficiency of Macronutrients in Barley Depending on Nitrogen Nutrition

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Abstract
Bulgarian barley varieties Emon, Obzor, Krami, Kristi and Kaskadior were studied at three levels of nitrogen nutrition 0, 200 400 mg N.kg^{-1} soil in a pot experiment. The nitrogen levels were created by adding NH_4NO_3 dissolved in water. Each experimental treatment was studied in four replicates. Ten plants of each variety were grown in plastic pots filled with 5 kg alluvial-meadow soil under greenhouse conditions. The Internal efficiency (IE) of the absorbed nitrogen (N), phosphorus (P_2O_5), potassium (K_2O), calcium (CaO) and magnesium (MgO) was studied in relation to the grain (IEg) and biomass (IEb) formation. The internal efficiency was calculated as the ratio of the grain yield or biomass yield to the total absorbed nutrient at maturity (g.g^{-1}). It was established that the internal efficiency of macronutrients proven decreased in parallel with increased nitrogen input into the soil. Higher reduction of the internal efficiency of nitrogen by 25.5% for grain and by 29.4% for biomass was found at N_{400} level, compare to N_0. The studied barley varieties did not show proven differences in the internal efficiency of nitrogen, phosphorus and potassium for grain and biomass formation. A trend was found indicating the Kristi variety as more efficiently using the accumulated nitrogen for both grain (NIEg=43.9 g.g^{-1} N) and biomass formation (NIEb=106.7 g.g^{-1} N), regardless of the soil nitrogen. The Kaskadior variety could be referred to as less efficient. A strong negative relationship was found between the soil nitrogen level and the internal efficiency of nitrogen, phosphorus and potassium for grain and biomass formation, as well as a significant negative relationship (r=-0.536*) with the internal efficiency of magnesium for grain. Index of NIEg was in a strong positive correlation with the internal efficiency of phosphorus, potassium and magnesium for grain and biomass formation.

Keywords: Internal efficiency, macronutrients, nitrogen nutrition, barley.

Introduction

Globally, there is growing interest among scientific researchers and farmers in more efficient cereal genotypes [1; 2] and creating cultivation strategies for higher efficiency of nutrient use [3]. Nitrogen use efficiency has two main components – absorption efficiency and internal efficiency or using the absorbed nitrogen for grain/biomass/protein formation [4, 5]. The internal efficiency characterized the productivity per unit of nitrogen or other nutrient absorbed by plants. This index provides an integral assessment of the factors related to genotype on the use of absorbed nutrients and crop productivity [6]. The efficiency of nutrient uptake is defined as the ability of plants to absorb a particular macronutrient at a low level of soil availability or nutrient medium [7; 8]. Usually, however, crop selection is carried out under soil conditions that do not limit their growth and productivity [9]. It is known that barley yield is mainly related to the assimilation of nitrogen and phosphorus, which are the main cause of donor limitation at grain filling [10; 11; 12]. Nitrogen affects the development and maintenance of leaf area and photosynthetic efficiency and this nutrient manages the allocation of dry mass to the reproductive organs [13]. Plants take up

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potassium exclusively from the soil solution, which is in dynamic equilibrium with the exchangeable and, to a lesser extent, the non-exchangeable potassium stock in the soil [14]. The effectiveness of mineral nutrition depends on two integrated groups of plant factors, namely absorption efficiency and utilization efficiency [15]. Absorption efficiency is defined as the relative uptake of a given nutrient at a given provision or supply. Utilization efficiency represents the total or primary yield relative to the nutrient uptake. The genotypic specificity of mineral nutrition in cereals, including barley, has been proven and in a number of countries work is being done to adapt plant diagnostics according to the genotype [16]. There is a lack of sufficient research on Bulgarian barley varieties. The objective of this study was to determine the effect of the nitrogen nutrition level on the internal efficiency of absorbed macronutrients in barley varieties.

Material and methods

Bulgarian winter two-row barley varieties Emon, Obzor, Krami, Kristi and Kaskadior were studied at three levels of nitrogen nutrition 0, 200 400 mg $N.kg^{-1}$ soil in a pot experiment. The nitrogen levels were created by adding NH_4NO_3 dissolved in water. Each experimental treatment was studied in four replicates. Ten plants of each variety were grown in plastic pots filled with 5 kg alluvial-meadow *Mollic Fluvisols* [17] under the conditions of a polyethylene greenhouse. The main soil characteristics were as follows: $pH_{(H2O)}$ 7.3; humus content 3.2%; mineral nitrogen 43.8 mg $N.kg^{-1}$; available phosphorus 209 mg $P_2O_5.kg^{-1}$ (Egner-Ream method); and exchangeable potassium 450 mg $K_2O.kg^{-1}$ (extracted by 2N HCL). The plants were harvested at physiological maturity and separated into grain and aboveground biomass (straw+glume+stalks). The barley grain and biomass were dried for 48 hours at a temperature of 60°C to constant weight. The concentrations of nitrogen (N), phosphorus (P_2O_5), potassium (K_2O), calcium (CaO) and magnesium (MgO) were analyzed in the barley grain and biomass after wet digestion by H_2SO_4 and H_2O_2 as a catalyst [18]. Colorimetric methods were used for determination of nitrogen and phosphorus concentrations in barley plants. The concentration of potassium was analyzed by the flame photometer model PFP-7. The calcium and magnesium in the grain and biomass were determined in concentrated HCl and HNO_3 (ratio 3:1) on an AAS model Perkin-Elmer 200 Analyst after microwave mineralization in a Milstone 2000 mega furnace. The amount of each absorbed nutrient in the grain and in the biomass was calculated as the multiplication of its concentration by the dry weight of the barley grain and biomass, respectively. The Internal efficiency (IE) of each of the absorbed macronutrients was studied in relation to the grain (IEg) and biomass (IEb) formation. The internal efficiency was calculated in $g.g^{-1}$ as the ratio of the grain yield or biomass yield to the total absorbed nutrient at maturity [19; 20].

An overall analysis of variance (ANOVA) was performed to evaluate the effect of the experimental treatments on the referred variables. In order to establish the difference among the means Duncan's multiple range test at level of significance $p \leq 0.05$ was used. Correlation test with significance level reported ($p < 0.05$ or $p< 0.01$) was based on Pearson's correlation coefficient.

Results and discussion

The level of nitrogen nutrition significantly affected the use of nitrogen, phosphorus, potassium and magnesium absorbed at maturity by barley for the grain yield (Table 1). The internal efficiency of macronutrients proven decreased in parallel with increasing nitrogen input into the soil. This finding did not apply only to calcium from the studied nutrients, where there was no mathematically proven difference. The growing of barley varieties in higher nitrogen fertilization (400 mg $N.kg^{-1}$ soil) resulted in a lower internal efficiency by 25.5% for NIEg, by 18.5% for PIEg, by 20% for KIEg, by 13.1% for CaIEg and by 15.8% for MgIEg, compared to their values from the control level without nitrogen input N_0. The internal efficiency of magnesium use for grain formation showed the highest values, which on average for the studied barley varieties ranged from 458.5 $g.g^{-1}$ MgO (in N_0) to 386.1 $g.g^{-1}$ MgO (in N_{400}). Barley plants formed the least amount of grain per unit of absorbed potassium. In the present study with nitrogen levels of 0 – 400 mg $N.kg^{-1}$ soil, the average values of KIEg obtained were from 39.0 $g.g^{-1}$ K_2O (N_0 level) to 31.2 $g.g^{-1}$ K_2O (N_{400} level).

Table 1. Internal efficiency of nitrogen, phosphorus, potassium, calcium and magnesium for grain formation in barley

Parameter	N level	Min	Max	Average	% to N_0
NIEg	N_0	42.3	52.6	46.1 a*± 3.9	100
	N_{200}	35.7	42.4	38.2 b ± 2.7	78.8
	N_{400}	28.4	36.8	31.0 c ± 3.3	74.5
PIEg	N_0	69.8	79.3	76.3 a ± 4.1	100
	N_{200}	62.7	70.5	68.1 b ± 3.2	89.3
	N_{400}	58.1	64.5	62.2 c ± 2.5	81.5
KIEg	N_0	37.2	41.4	39.0 a ± 1.9	100
	N_{200}	32.7	37.4	34.5 b ± 1.8	88.5
	N_{400}	30.0	34.0	31.2 c ± 1.5	80.0
CaIEg	N_0	53.0	74.7	62.4 ns ± 8.0	100
	N_{200}	52.9	70.2	58.4 ± 6.9	93.6
	N_{400}	49.8	63.9	54.2 ± 5.6	86.9
MgIEg	N_0	396.6	527.6	458.5 a ± 57.6	100
	N_{200}	353.9	452.3	400.1 b ± 48.9	87.3
	N_{400}	328.8	443.0	386.1 c ± 44.3	84.2

*Values with identical letters within each nutrient are not significantly different at p<0.05 according to Duncan's multiple range test.

The productivity of nitrogen, phosphorus, potassium, calcium and magnesium for biomass formation in barley decreased in parallel with increasing nitrogen nutrition levels (Table 2). This corresponded to the results obtained for the internal efficiency of macronutrients for the grain formation. The influence of soil nitrogen was not statistically proven only in relation to CaIEb, where it was established as a trend. Barley varieties used each of the studied macronutrients most efficiently for biomass formation at the N_0 level, where the values were 112.0 for NIEb, 184.4 for PIEb, 94.1 for KIEb, 150.7 for CaIEb, and 1104.5 for MgIEb. The higher nitrogen level (N_{400}) reduced the internal efficiency of macronutrients for barley biomass formation, compared to that of unfertilized plants (N_0). The largest reduction of 29.4% was found in relation to the internal efficiency of nitrogen. The values for the other macronutrients decreased by 13.9% for PIEb, by 15.2% for KIEb, by 8.2% for CaIEb and by 10.8% for MgIEb. The productivity of absorbed magnesium at maturity for barley biomass formation was the highest and it was varied depending on the nitrogen level from 984.8 $g.g^{-1}$ MgO (N_{400}) to 1104.5 $g.g^{-1}$ MgO (N_0). The internal efficiency of potassium for biomass formation of barley changed in dependence of the soil nitrogen level from 94.1 $g.g^{-1}$ K_2O (in N_0) up to 79.8 $g.g^{-1}$ K_2O (in N_{400}).

Table 2. Internal efficiency of nitrogen, phosphorus, potassium, calcium and magnesium for biomass formation in barley

Parameter	N level	Min	Max	Average	% to N_0
NIEb	N_0	101.6	124.4	112.0 a* ± 8.2	100
	N_{200}	88.5	103.6	94.9 b ± 10.6	84.7
	N_{400}	70.8	92.0	79.1 c ± 8.1	70.6
PIEb	N_0	162.4	199.6	184.4 a ± 13.8	100
	N_{200}	153.5	183.4	169.2 b ± 10.6	91.8
	N_{400}	145.9	173.5	158.7 c ± 9.8	86.1
KIEb	N_0	88.0	100.8	94.1 a ± 20.3	100
	N_{200}	80.3	92.7	85.8 b ± 5.1	91.2
	N_{400}	74.8	87.0	79.8 b ± 4.7	84.8
CaIEb	N_0	127.5	181.7	150.7 ns** ± 8.0	100
	N_{200}	129.2	174.0	145.3 ± 18.2	96.4
	N_{400}	124.7	163.5	138.4 ± 15.7	91.8
MgIEb	N_0	953.5	1227.6	1104.5 a ± 118.6	100
	N_{200}	874.4	1105.1	993.8 b ± 114.7	90.0
	N_{400}	819.3	1112.3	984.8 c ± 108.6	89.2

*Values with identical letters within each nutrient are not significantly different at p<0.05 according to Duncan's multiple range test.
**Not significant different

Table 3. Average internal efficiency of nitrogen, phosphorus, potassium, calcium and magnesium for grain and for biomass formation in barley varieties

Variety	NIEg	PIEg	KIEg	CaIEg	MgIEg
Obzor	37.9 ns**	68.5 ns	37.6 ns	69.6 a*	382.8 b
Emon	36.5	71.2	34.4	57.6 b	397.9 ab
Krami	38.4	63.5	35.6	58.4 b	474.0 a
Kristi	43.9	70.3	33.7	53.8 b	459.8 a
Kaskadior	35.6	70.8	33.4	52.4 b	360.5 b
Average	*38.5*	*68.9*	*34.9*	*58.4*	*415.0*
	NIEb	PIEb	KIEb	CaIEb	MgIEb
Obzor	94.0 ns	170.3 ab	93.5 ns	173.1 a	952.9 bc
Emon	95.0	185.5 a	89.5	150.0 b	1036.7 ab
Krami	92.8	153.9 b	86.2	141.5 bc	1148.3 a
Kristi	106.7	171.0 ab	82.1	131.0 c	1118.4 a
Kaskadior	86.9	173.2 ab	81.7	128.5 c	882.4 c
Average	*95.1*	*170.8*	*86.6*	*144.8*	*1027.7*

*Values with identical letters within each nutrient are not significantly different at p<0.05 according to Duncan's multiple range test.
**Not significant different

The studied barley varieties did not demonstrate proven genotypic differences in terms of the internal efficiency of using the absorbed nitrogen, phosphorus and potassium for grain and biomass formation (Table 3). A trend was found indicating the Kristi variety as more efficiently using the accumulated nitrogen for both grain (NIEg=43.9 $g.g^{-1}$ N) and biomass formation (NIEb=106.7 $g.g^{-1}$ N), regardless of the soil nitrogen level within the range of 0 – 400 mg $N.kg^{-1}$ soil. The Kaskadior variety could be referred to as less efficient. The internal efficiency of nitrogen of this variety was 35.6 g grain g^{-1} N and 86.9 g biomass g^{-1} N, which indicated a decrease of 9.0% for NIEg and 8.6% for NIEb. The average values of the internal efficiency of phosphorus and potassium for grain were 68.9 $g.g^{-1}$ P_2O_5 and 34.9 $g.g^{-1}$ K_2O, respectively, and those of PIEb and KIEb were 170.8 $g.g^{-1}$ P_2O_5 and 86.6 $g.g^{-1}$ K_2O, respectively. The two indicators changed within narrow limits for individual barley varieties.

The Obzor variety was distinguished by a higher internal efficiency of calcium for grain and for biomass formation. Our results demonstrated proven higher internal efficiency of magnesium for grain and biomass in the Krami and Kristi varieties. The Kaskadior variety was characterized as the least efficient in terms of the internal efficiency of calcium and magnesium for grain and biomass formation.

Table 4. Correlation of nitrogen level, internal efficiency of N, P, K, Ca and Mg for grain formation, and internal efficiency of N, P, K, Ca and Mg for biomass formation

	NIEg	PIEg	KIEg	CaIEg	MgIEg	NIEb	PIEb	KIEb	CaIEb	MgIEb
N level	-0.899**	-0.888**	-0.892**	-0.473	-0.536*	-0.892**	-0.710**	-0.788**	-0.294	-0.422
NIEg		0.791**	0.786**	0.383	0.726**	0.987**	0.570*	0.630*	0.179	0.618*
PIEg			0.701**	0.305	0.275	0.820**	0.925**	0.663**	0.196	0.194
KIEg				0.787**	0.488	0.777**	0.535*	0.941**	0.632*	0.385
CaIEg					0.138	0.395	0.253	0.842**	0.956**	0.093
MgIEg						0.690**	0.053	0.318	-0.041	0.968**
NIEb							0.651**	0.669**	0.227	0.614*
PIEb								0.623*	0.256	0.056
KIEb									0.781**	0.287
CaIEb										-0.022

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

A strong negative relationship was found between the level of nitrogen nutrition of barley and the internal efficiency of nitrogen, phosphorus and potassium for grain and biomass formation, as well as a significant negative relationship (r=-0.536*) with the internal efficiency of magnesium for grain (Table 4). A very strong positive relationship was established between the internal efficiency of nitrogen, phosphorus, potassium, calcium and magnesium for grain and for biomass. The NIEg index was strongly positively correlated with the internal efficiency of phosphorus, potassium and magnesium for grain formation and it was significantly positively

correlated with the indices of these elements for barley biomass formation.

Conclusions

The internal efficiency of nitrogen, phosphorus, potassium, calcium and magnesium in barley plants proven decreased in parallel with increased soil nitrogen input in range. Higher reduction of the internal efficiency of nitrogen by 25.5% for grain and by 29.4% for biomass was found at N_{400} level, compare to N_0. The studied barley varieties Emon, Obzor, Krami, Kristi and Kaskadior did not show proven differences in the internal efficiency of nitrogen, phosphorus and potassium for grain and biomass formation. A trend was found indicating the Kristi variety as more efficiently using the accumulated nitrogen for both grain (NIEg=43.9 $g.g^{-1}$ N) and biomass formation (NIEb=106.7 $g.g^{-1}$ N), regardless of the soil nitrogen. The Kaskadior variety could be referred to as less efficient. A strong negative relationship was found between the soil nitrogen level and the internal efficiency of nitrogen, phosphorus and potassium for grain and biomass formation, as well as a significant negative relationship (r=-0.536*) with the internal efficiency of magnesium for grain. Index of NIEg was in a strong positive correlation with the internal efficiency of phosphorus, potassium and magnesium for grain and biomass formation.

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