

## CHARACTERISTICS OF NITROGEN USE EFFICIENCY AND THEIR MUTUAL RELATIONSHIP IN WINTER WHEAT CULTIVARS

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Field experiments with 12 winter wheat cultivars, which differed in N grain concentration, were established with three variants of N fertilisation (N0 = 0 kg N/ha, N1 = 100 kg N/ha, N2 = 200 kg N/ha) in the vegetative years 2004/05 and 2005/06 in Piestany (Borovce). The aims of experiment were to find out the effect of N applied (Nf) and available N (Nfs) on differences in N uptake and N utilisation. From the primary data of traits, grain yield (GY kg/ha), biomass production (BP kg/ha), and N concentration in grain (Ng) and in straw (Ns), other characteristics (NUP, NUE, NUTE) for available and applied N (Nfs, Nf, respectively) were calculated. In available N, significant differences were seen among cultivars in all characteristics but in applied N differences

in NUP and NUTE among cultivars were not significant. Genetic correlations among traits and characteristics were modified by N environment. Grain N concentration (Ng) was, at the low N rate, in a negative correlation with NUE ( $r = -0.683^+$ ) and in a strong negative correlation with NUTE ( $r = -0.956^{++}$ ). At a high N rate, these correlations had the same character, but were weaker and not significant ( $r = -0.560$  and  $r = 0.570$ ). Grain N concentration (Ng) was more determined by efficiency of N translocation than by quantity of N uptake. Selection on the higher NUPE and NUE will be limited by negative correlation between BP and N biomass concentration and by low genetic variability of all characteristics of N uptake and utilisation.

Key words: winter wheat, cultivar, nitrogen use efficiency, nitrogen uptake efficiency, nitrogen utilisation efficiency, available nitrogen

Productive dwarf cultivars and higher doses of nutrients, particularly nitrogen, participated in the increase of cereals yields in the second half of the last century (Austin *et al.* 1980; Uzik & Zofajova 2007). In the following decades, it is necessary to increase crop productivity to provide food for a growing population in conditions of decreasing arable land and use of biomass for the production of biofuels. Increasing the actual low efficiency of application of N fertilisers is an important goal of the research.

Plants are able to accumulate in the grain only 30%–40% of applied nitrogen from fertilisers (Raun & Johnson 1999) as a large proportion of N penetrates into groundwater and air. Nitrogen use effi-

ciency (NUE) is generally defined as grain yield obtained per the unit of applied or available nitrogen in soil. Moll *et al.* (1982) defined NUE as the product of nitrogen uptake efficiency (NUPE) and nitrogen utilisation efficiency (NUTE), where NUPE [%] is defined as all N in the above biomass at maturity divided by sum of the fertiliser N and soil N (N available) and NUTE defined as ratio of grain yield (in kg) to total N uptake in biomass (NUP in kg).

Increasing NUE can be achieved by improving NUPE or NUTE or by increasing the efficiency of both the components. During the past decade, numerous studies have been undertaken for insights into whether NUPE or NUTE plays a more important role in NUE under different N environments,

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but data in the literature are not unambiguous. Abeledo *et al.* (2008) report that both N conversion and N capture have played a role in the improvement of NUE. According to other authors (LeGouis *et al.* 2002; Wang *et al.* 2011), N uptake in biomass was the most important factor in NUE determination regardless of N level. Contrary results were obtained by Gaju *et al.* (2011) who found that the NUPE effect explained only a small amount of phenotypic variance in NUE amongst cultivars, but NUTE affected it up to 61% and 77% under high nitrogen (HN) and low nitrogen (LN) conditions, respectively.

In our previous research (Uzik & Zofajova 2009), we analysed the grain yield structure of 12 winter wheat cultivars, which represent the use in the wider Central European region. In the next work with the same set of cultivars (Uzik & Zofajova 2012b), we analysed the dynamics of N during grain filling. The aim of the present work was to detect, in the same cultivar set, the effect of genotype and N fertilisation on the differences in total N uptake and its utilisation for grain yield and biomass and for N accumulation in grain. Other objectives were to separate the effects of available N from applied N on the efficiency of N uptake and its utilisation and to find out the effect of N environment and genotype on the relationships among traits of biomass production and N accumulation and characteristics of the efficiency of N uptake and N utilisation.

## MATERIAL AND METHODS

Detailed description of methods for the establishment and management of the experiment was published in the previous papers (Uzik & Zofajova 2009, 2012b). Field experiments with 12 winter wheat cultivars were established in three variants of N fertilisation (N0 = 0 kg N/ha, N1 = 100 kg N/ha, N2 = 200 kg N/ha) and in three replications in the vegetation 2004/05 and 2005/06 in Borovce. Pea was the preceding crop.

Nutrition content in soil was the same in both years (N 0.098%, humus content 2.084%, pH 7.42, P 59.68 mg/kg, K 183.7 mg/kg, Mg 236.6 mg/kg, Ca 4910 mg/kg, NO<sub>3</sub>-N 14.2 mg/kg and NH<sub>4</sub>-N 1.942 mg/kg). P and K fertilisers in the form of superphosphate (26% P<sub>2</sub>O<sub>5</sub>) and potassium nitrate (60% K<sub>2</sub>O) at the

rates of 50 and 200 kg/ha, respectively, were applied before sowing. N fertilisers were applied in the divided rates, at N1 in the ratio 40 : 30 : 30 kg N/ha and at N2 in the ratio 100 : 70 : 30 kg N/ha, on three dates. The first date of regenerative N fertilisation was in the middle of March, the second date in the middle of April and the third date at the end of the first decade of May. N was applied in the form of ammonium calcite nitrate (27.5% N) in the first and in the second dates and in the form of calcite nitrate (15.5%) in the third date in both years.

The evaluated set consisted of seven foreign cultivars (1 – Evropa 90, released in 1990; 2 – Nevesinjka, 1990; 3 – Pobeda, 1990; 4 – Zlatka, 1996; 5 – Sonata from Serbia, 2000; 6 – Renan from France, 1989; 7 – Tamaro from Switzerland, 1992) and of five local cultivars (8 – Ilona, 1989; 9 – Malyska, 2001; 10 – Vanda, 2001; 11 – Petrana, 2002; 12 – Axis, 2003). Grain yield (GY, kg/ha) was assessed from each parcel and from the row 0.5 m of stand, a plant sample was collected for determination of harvest index (HI). N concentration in grain (Ng, %) and in straw (Ns, %) were determined by Dumas method using CNS–2000 analyser.

From the primary data of traits, additional characteristics were derived:

- Ns [kg/ha] – amount of soil N was defined as N amount taken by parcel with maximal N uptake ( $i j k l_{\max}$ ) on non-fertilised variant N0 (k) in each year (i) and replication (j) (twice it was cultivar (l) Tamaro and once Vanda),
- Nfs [kg/ha] – available N was expressed as sum of N quantity in fertilisers (Nf in kg/ha) and soil (Ns),
- BP [kg/ha] – biomass production was calculated from grain yield and harvest index (GY/HI),
- Nb [%] – concentration N in biomass at maturity (weighed average from Nv a Ng),
- NUP [kg/ha] – N uptake in biomass,
- NUPE [%] – efficiency of N uptake (NUP / Nfs) × 100,
- NAg [kg/ha] – N accumulation in grain,
- NAgE [%] – efficiency of N accumulation in grain (NAg / Nfs) × 100,
- NAs [kg/ha] – accumulation N in straw,

- NAsE [%] – efficiency of N accumulation in straw (NAs / Nfs) × 100,
- NUEg [kg/kg] – N (available) use efficiency (Nfs) on grain yield (GY / Nfs),
- NUEb [kg/kg] – N (available) use efficiency (Nfs) on biomass production (BP / Nfs),
- NUTE [kg/kg] – N utilisation efficiency (GY / NUP).

Characteristics for estimation of the effect of applied nitrogen (Nf) were determined as difference between the value of trait in non-fertilised variant and in fertilised one, which was calculated for each year (i), replication (j), N fertilisation (k) and cultivar (l). This manner of estimation has taken into account differences of mineralisation in the soil microenvironment and specific differences in N uptake among cultivars:

$$xf_{ijkl} = xfs_{ijkl} (k = N1 \text{ or } N2) - x_{ijkl} (k = N0),$$

where x can be GY, BP or NUP.

- NUPf = NUPfs – NUPs – N uptake of applied N fertiliser,
- NUPfE [%] = (NUPf / Nf) × 100 – efficiency of N uptake of applied N fertiliser,
- NAgf [kg/ha] = (NAgfs – NAgNs) – accumulation of applied Nf in grain,
- NAgfE [%] = (NAgf / Nf) × 100 – efficiency of accumulation of applied Nf in grain,
- NAsf [kg/ha] = (NAsfs – NAsNs) – accumulation of applied Nf in grain,
- NAsfE [%] = (NAsf / Nf) × 100 – efficiency of accumulation of applied Nf in grain,
- NUEgf [kg/kg] = (GYf / Nf) – use efficiency of applied N on grain yield, where GYf = GYfs – GYs,
- NUEbf = (BPf / Nf) – use efficiency of applied N on biomass production, where BPf = BPfs – BPs,
- NUTEgf [kg/kg] = (GYf / NUPf) – utilisation efficiency of applied N for grain yield.

Statistical analyses were performed by the software package Statgrafics plus for Windows. The combined ANOVA (year, N fertilisation, genotype and replication) of the data set and characteristics from all experiments were performed (not shown). Genotypic (cultivar) correlations were calculated

from average values for cultivars over years, replications and N0 variant of fertilisation (rGLN, low N rate); from average of N1 and N2 variants of fertilisation (rGHN, high N rate); and from average characteristics N0, N1 and N2 for rGF. By combinations of factors – year, N fertilisation, replication (2 × 3 × 2 = 12) – 12 environments differentiated by N level were formed, termed as N environments. N environments correlations (rE) were calculated from average data of 12 cultivars (hypothetic average cultivar) from each environment.

## RESULTS

*Nitrogen uptake (NUP kg/ha) and nitrogen uptake efficiency (NUPE %) of available nitrogen (Nfs) (Table 1)*

From the total average amount of available N, 320 kg N/ha, 224.4 kg N/ha (NUP) was taken up, i.e. 72.5% efficiency of N uptake (NUPE), by the crop. Characteristics of N uptake had low experimental error (*Ve*) and low genetic variability (*Vg*).

Significant differences were among cultivars in NUP and NUPE. From the other cultivars, only two were different by low NUP and NUPE – Nevesinjka (209.1 kg N/ha and 66.5%) and Zlatka (199.6 kg N/ha and 63.3%). From the total amount of available N (Nfs), only 169.8 kg/ha (NAG), i.e. 55.2% (NAGf), was distributed in the grain. Cultivars Pobeda (58.9%), Renan (59.2%) and Axis (58.4%) exhibited a high portion of N accumulation in the grain. Cultivar Zlatka accumulated a lower N portion in grain (50.2%) than cultivars Tamaro, Malyska and Vanda, which accumulated more than 20% in the straw (NAsE).

*Nitrogen uptake (NUP kg/ha) and nitrogen uptake efficiency (NUPE %) of applied nitrogen (Nf) (Table 2)*

Characteristics of efficiency of applied N rates estimated as difference between N fertilised and non-fertilised variant had a high coefficient of experimental error (*Ve*), which was not caused by high absolute value of MSe, but low difference among Nx – N0 variant, in which *Ve* was expressed. Differences among cultivars in the total uptake of applied N (NUPf) were not significant, but effects of year, N fertilisation (not shown) and cultivar were

T a b l e 1

N uptake, N uptake efficiency, N accumulation efficiency in grain and in straw of winter wheat cultivars at available N

Cultivar	NUP [kg/ha]	NUPE [%]	NAG [kg/ha]	NAGe [%]	NAs [kg/ha]	NAsE [%]
Evropa 90	222.4 <sup>bcd</sup>	71.6 <sup>bc</sup>	171.3 <sup>bede</sup>	55.7 <sup>bede</sup>	51.0 <sup>ab</sup>	15.8 <sup>ab</sup>
Nevesinjka	209.1 <sup>ab</sup>	66.5 <sup>ab</sup>	163.3 <sup>ab</sup>	52.2 <sup>ab</sup>	45.8 <sup>ab</sup>	14.3 <sup>ab</sup>
Pobeda	238.4 <sup>d</sup>	75.9 <sup>c</sup>	184.0 <sup>e</sup>	58.9 <sup>e</sup>	54.4 <sup>bc</sup>	17.1 <sup>bc</sup>
Zlatka	199.6 <sup>a</sup>	63.3 <sup>a</sup>	157.5 <sup>a</sup>	50.2 <sup>a</sup>	42.2 <sup>a</sup>	13.1 <sup>a</sup>
Sonata	217.2 <sup>abc</sup>	70.7 <sup>bc</sup>	166.6 <sup>bede</sup>	54.8 <sup>abede</sup>	50.6 <sup>ab</sup>	15.9 <sup>ab</sup>
Renan	232.6 <sup>cd</sup>	76.1 <sup>c</sup>	179.4 <sup>e</sup>	59.2 <sup>e</sup>	53.1 <sup>bc</sup>	16.9 <sup>bc</sup>
Tamaro	232.8 <sup>cd</sup>	76.7 <sup>c</sup>	170.9 <sup>bede</sup>	56.5 <sup>bede</sup>	61.9 <sup>cde</sup>	20.1 <sup>cde</sup>
Hona	224.0 <sup>bcd</sup>	72.0 <sup>bc</sup>	177.1 <sup>cde</sup>	56.9 <sup>cde</sup>	46.9 <sup>ab</sup>	15.1 <sup>ab</sup>
Malyska	232.8 <sup>cd</sup>	76.1 <sup>c</sup>	159.6 <sup>abc</sup>	53.1 <sup>abc</sup>	73.2 <sup>e</sup>	23.0 <sup>e</sup>
Vanda	229.2 <sup>cd</sup>	75.0 <sup>c</sup>	163.3 <sup>abcd</sup>	53.8 <sup>abcd</sup>	65.9 <sup>de</sup>	21.2 <sup>de</sup>
Petrana	226.0 <sup>bcd</sup>	72.3 <sup>bc</sup>	164.1 <sup>abc</sup>	52.8 <sup>abc</sup>	61.9 <sup>cd</sup>	19.5 <sup>cd</sup>
Axis	228.3 <sup>bcd</sup>	73.6 <sup>c</sup>	180.1 <sup>de</sup>	58.4 <sup>de</sup>	48.2 <sup>ab</sup>	15.3 <sup>ab</sup>
$\bar{x}$	224.4	72.5	169.8	55.2	54.6	17.3
$LSD_{0.05}$	20.0	6.7	12.2	4.5	11.3	3.3
$V_g$ [%]	4.9	5.7	5.1	5.2	16.9	17.5
$V_e$ [%]	12.3	12.0	8.5	8.8	17.8	15.7

Means within columns with different letters are significantly different at  $\alpha = 0.05$

NUP – N uptake in biomass, NUPE – efficiency of N uptake, NAG – N accumulation in grain, NAGe – efficiency of N accumulation in grain, NAs – accumulation N in straw, NAsE – efficiency of N accumulation in straw

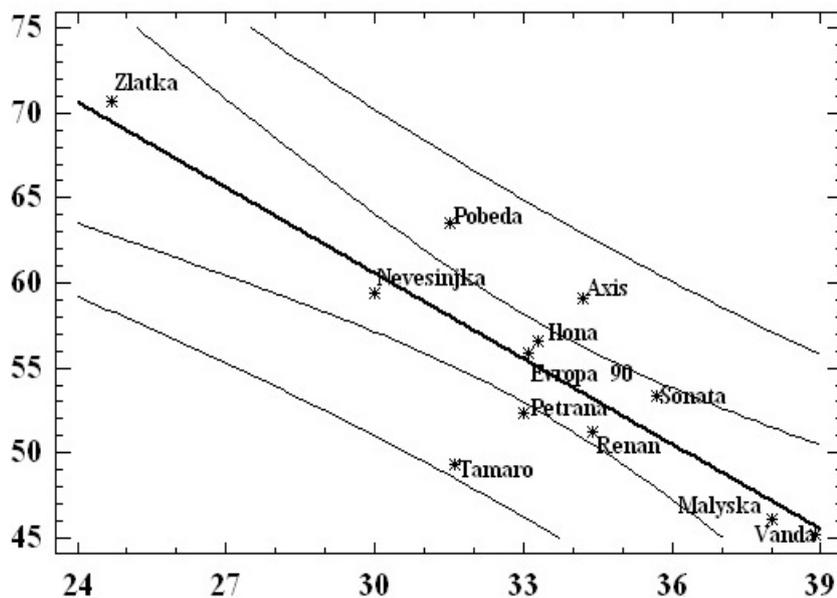


Figure 1. Relationship between  $NUE_g$  at variant N0 (x axis) and  $NUE_g$  [%] at variant N2 (y axis) (100% =  $NUE_g$  at variant N0)

significant for distribution of applied Nf in the grain (N<sub>Agf</sub>) and in the straw (N<sub>Asf</sub>). Interaction N fertilisation × cultivar was not significant (not shown). From the average rate of applied N (N<sub>f</sub> = 150 kg/ha) by the cultivar only 65.1 kg N/ha, i.e. 46.9% (NUPfE), was taken up. Differences among cultivars fluctuated up to the double value of the minimal difference (from 46.2 kg/ha Renan to 92.3 kg/ha Pobeda). Significant differences were among cultivars in N distribution in grain, namely in the amount (N<sub>Agf</sub>) and in efficiency (N<sub>AgfE</sub>), although experimental error was also high. On average, only 31.9 kg/ha or 24.0% from 150 kg of applied N was accumulated in grain.

High N grain accumulation showed cultivars Pobeda (40.6%), Ilona (36.0%) or Axis (34.9%) and low one cultivar Malyska (7.5%), in which high N portion accumulated in straw (35.1%). Portion of N accumulated in grain and in straw from applied N

was nearly the same (24.0% and 22.9%), while from available N (N<sub>f</sub>s) three times more was distributed in grain than in the straw (55.2% vs. 17.3%) (Table 1).

*Nitrogen use (NUE kg/kg) and nitrogen utilisation efficiency (NUTE kg/kg) of available nitrogen (N<sub>f</sub>s) (Table 3)*

On average, 25.4 kg of grain devolved on 1 kg of available N. High N<sub>UEg</sub> values had cultivars Vanda (27.8), Axis (26.7), Malyska (27.4) (all low grain quality) and low one Zlatka (21.1) (high grain quality) and Tamaro (23.3) (low HI). Higher available N (N<sub>f</sub>s) amounts significantly made lower N<sub>UEg</sub> in N<sub>2</sub> variant to 54.6% (N<sub>0</sub> = 100%).

Interaction cultivar × N fertiliser can be expressed by relation between absolute value N<sub>UEg</sub> on N<sub>0</sub> variant (x) to relative value N<sub>UEg</sub> on N<sub>2</sub> variant in percentage (100% = N<sub>UEg</sub> on N<sub>0</sub> variant)

T a b l e 2

N uptake and distribution of N applied at winter wheat cultivars

Cultivar	NUPf [kg/ha]	NUPfE [%]	N <sub>Agf</sub> [kg/ha]	N <sub>AgfE</sub> [%]	N <sub>Asf</sub> [kg/ha]	N <sub>AsfE</sub> [%]
Evropa 90	54.8	38.3	23.5 <sup>ab</sup>	16.0 <sup>ab</sup>	31.3 <sup>a</sup>	22.3 <sup>a</sup>
Nevesinjka	78.1	59.1	44.9 <sup>cd</sup>	33.8 <sup>cd</sup>	33.2 <sup>a</sup>	25.3 <sup>ab</sup>
Pobeda	92.3	65.7	56.3 <sup>d</sup>	40.6 <sup>d</sup>	36.0 <sup>ab</sup>	25.0 <sup>ab</sup>
Zlatka	75.2	50.3	47.2 <sup>cd</sup>	32.7 <sup>cd</sup>	28.0 <sup>ab</sup>	17.6 <sup>abc</sup>
Sonata	55.6	40.7	23.7 <sup>ab</sup>	18.5 <sup>abc</sup>	31.9 <sup>abc</sup>	22.1 <sup>abc</sup>
Renan	46.2	33.0	16.8 <sup>a</sup>	12.6 <sup>a</sup>	29.3 <sup>abc</sup>	20.4 <sup>abc</sup>
Tamaro	48.3	36.7	14.9 <sup>a</sup>	12.2 <sup>a</sup>	33.3 <sup>abc</sup>	24.5 <sup>abc</sup>
Ilona	67.0	48.3	46.6 <sup>cd</sup>	36.0 <sup>d</sup>	20.3 <sup>abc</sup>	12.3 <sup>abc</sup>
Malyska	58.3	42.6	7.9 <sup>a</sup>	7.5 <sup>a</sup>	50.2 <sup>abc</sup>	35.1 <sup>abc</sup>
Vanda	49.5	35.6	14.8 <sup>a</sup>	12.7 <sup>a</sup>	34.6 <sup>abc</sup>	22.9 <sup>abc</sup>
Petrana	83.4	62.0	38.5 <sup>bc</sup>	30.6 <sup>bcd</sup>	44.9 <sup>bc</sup>	31.4 <sup>bc</sup>
Axis	72.7	50.8	47.8 <sup>cd</sup>	34.9 <sup>d</sup>	24.7 <sup>b</sup>	16.0 <sup>b</sup>
$\bar{x}$	65.1	46.9	31.9	24.0	33.2	22.9
<i>LSD</i> <sub>0.05</sub>	NS	NS	78.0	15.5	19.9	15.0
<i>V<sub>g</sub></i> [%]	23.3	23.2	51.9	48.9	16.0	13.8
<i>V<sub>e</sub></i> [%]	63.4	67.3	78.0	58.1	24.3	25.9

Means within columns with different letters are significantly different at  $\alpha = 0.05$

NUPf – N uptake of applied N fertiliser, NUPfE – efficiency of N uptake of applied N fertiliser, N<sub>Agf</sub> – accumulation of applied N<sub>f</sub> in grain, N<sub>AgfE</sub> – efficiency of accumulation of applied N<sub>f</sub> in grain, N<sub>Asf</sub> – accumulation of applied N<sub>f</sub> in straw, N<sub>AsfE</sub> – efficiency of accumulation of applied N<sub>f</sub> in straw

(y). Assuming that interaction was not found, relative values should be equal for each cultivar and among parameters  $x, y, r = 0$ . However, between NUEg absolute value in N0 variant and the relative on N2 there was a strong negative correlation ( $r = -0.851^{**}$ ) (Figure 1) while between absolute values in N0 and N2 variant no correlation was found ( $r = 0.110$ ). The cultivars with low NUEg in N0 variant reduced lesser NUEg in N2 variant than cultivars with high NUEg in N0 (compare Zlatka versus Vanda).

Among cultivars, statistically significant differences were for NUE for biomass (NUEb); also, interaction cultivar  $\times$  N fertilisation was significant (not shown). Coefficient NUEb was more than two times higher (57.9 kg/kg) than NUEg. NUEg and NUEb in cultivars were combined in different ways: Vanda (high NUEg and NUEb), Zlatka (low NUEg and NUEb) and Nevesinjka (high NUEg and

low NUEb). Differences in N (available) utilisation efficiency for grain (NUTE) among cultivars were highly significant. On average, 34.7 kg grain devolved on 1 kg of uptake N with the range from 29.9 in Tamaro to 37.8 kg grain per 1 kg N in Sonata.

*Nitrogen use (NUE kg/kg) and nitrogen utilisation efficiency (NUTE kg/kg) of applied N (Nf) (Table 4)*

Cultivars were significantly different in the reaction on applied N in biomass production increment (BPf) and in grain yield increment (GYf). Cultivars Nevesinjka, Pobeda, Zlatka, Malyska and Petrana positively responded on applied N by high BPf and cultivars Nevesinjka, Pobeda and Zlatka had the highest GYf. Cultivar Tamaro had only average biomass production increment and unambiguously the lowest increment of grain.

Biological efficiency of applied N on grain yield (Nf), so the difference between grain yield in fertilised variants minus non-fertilised variant to applied

T a b l e 3

N use (NUE) and utilisation (NUTE) efficiency for cultivars at available N (Nfs)

Cultivar	NUEg [kg/kg]	NUE [kg/kg]		NUEb [kg/kg]	NUTE [kg/kg]
		N0	N2 [%] (N0 = 100%)		
Evropa 90	24.9 <sup>bcd</sup>	33.1 <sup>bcd</sup>	55.9 <sup>abc</sup>	55.3 <sup>b</sup>	34.5 <sup>bcd</sup>
Nevesinjka	24.5 <sup>bc</sup>	30.0 <sup>b</sup>	59.4 <sup>bcd</sup>	49.8 <sup>a</sup>	36.8 <sup>ef</sup>
Pobeda	25.7 <sup>cdef</sup>	31.5 <sup>bc</sup>	63.5 <sup>cd</sup>	58.1 <sup>bc</sup>	33.7 <sup>bc</sup>
Zlatka	21.1 <sup>a</sup>	24.7 <sup>a</sup>	70.7 <sup>d</sup>	45.4 <sup>a</sup>	33.4 <sup>bc</sup>
Sonata	26.9 <sup>efg</sup>	35.7 <sup>def</sup>	53.4 <sup>abc</sup>	56.5 <sup>b</sup>	37.8 <sup>f</sup>
Renan	25.5 <sup>cdef</sup>	34.4 <sup>cde</sup>	51.2 <sup>ab</sup>	58.7 <sup>bcd</sup>	33.1 <sup>b</sup>
Tamaro	23.3 <sup>b</sup>	31.6 <sup>bcd</sup>	49.3 <sup>ab</sup>	62.6 <sup>cd</sup>	29.9 <sup>a</sup>
Ilona	26.0 <sup>cdefg</sup>	33.3 <sup>bcd</sup>	56.6 <sup>abc</sup>	59.5 <sup>bcd</sup>	35.8 <sup>de</sup>
Malyska	27.4 <sup>fg</sup>	38.0 <sup>ef</sup>	46.1 <sup>a</sup>	62.4 <sup>cd</sup>	35.3 <sup>cde</sup>
Vanda	27.8 <sup>g</sup>	38.9 <sup>f</sup>	45.2 <sup>a</sup>	64.1 <sup>d</sup>	36.4 <sup>def</sup>
Petrana	25.3 <sup>cde</sup>	33.0 <sup>bcd</sup>	52.3 <sup>abc</sup>	60.0 <sup>bcd</sup>	34.6 <sup>bcd</sup>
Axis	26.7 <sup>defg</sup>	34.2 <sup>cde</sup>	59.1 <sup>bcd</sup>	62.2 <sup>cd</sup>	35.9 <sup>de</sup>
$\bar{x}$	25.4	33.2	54.6	57.9	34.7
<i>LSD</i> <sub>0.05</sub>	1.9	4.1	12.5	5.4	1.9
<i>Vg</i> [%]	7.35	11.2	13.4	9.6	6.1
<i>Ve</i> [%]	9.21	8.7	15.9	9.2	7.5

Means within columns with different letters are significantly different at  $\alpha = 0.05$   
 NUEg – N (available) use efficiency (Nfs) on grain yield, NUE – N use efficiency, NUEb – N (available) use efficiency (Nfs) on biomass production, NUTE – N utilisation efficiency

N ( $NUE_{gf} = GYf/Nf$ ) was only 4.7 kg grain per 1 kg Nf, so five times lower than in available N (Table 3). Significant differences were present among cultivars; some of them produced approximately 10 kg grains per 1 kg of applied fertilisers as Nevesinjka (9.9), Pobeda (10.5) and Zlatka (11.0), while cultivars Malyska and Vanda utilised applied N only for vegetative biomass production and on the contrary their grain yield decreased at higher N rates (Table 4).

Biological efficiency of applied N on biomass production was five times lower (13.0 kg;  $BPf/1kg\ Nf$ ) than available N and differences among cultivars were statistically significant. Cultivars were significantly different in the ratio  $NUE_{gf} : NUE_{bf}$ . Higher biomass production per 1 kg of applied N than grain production was seen in cultivars Evropa 90, Nevesinjka, Pobeda, Tamaro and Petrana and in other cultivars biomass production equalled to the

grain production, and so biomass increase was only in grain (Zlatka, Sonata, Renan, eventually Axis).

Utilisation efficiency of applied N for grain ( $NUTE_{f}$ ) e.g. ratio of the difference of grain yield between fertilised and non-fertilised variant to the difference of amount of N uptake between fertilised and non-fertilised variants was on average low; only 2.05 kg of grain and differences among cultivars were not statistically significant due to the high experimental error.

Cultivars can be categorised into several groups according to utilisation of applied N on grain production. Into the first group belonged cultivars which produced on 1 kg N above average grain yield: Zlatka 28.4, Pobeda 16.8 and Renan 15.2 kg/kg; into the second group belonged cultivars which accumulated N (Nf) in vegetative biomass and made lower the grain yield: Malyska – 40.3, Vanda – 28.6 and Tamaro – 4.7 kg/kg. Nearly all cultivars utilised

T a b l e 4

N use (NUE) and N utilisation (NUTE) efficiency for cultivars at N applied

Cultivar	GYf [kg]	BPf [kg]	$NUE_{gf}$ [kg/kg]	$NUE_{bf}$ [kg/kg]	$NUTE_{f}$ [kg/kg]
Evropa 90	340 <sup>cd</sup>	1950 <sup>cdef</sup>	4.8 <sup>cde</sup>	12.9 <sup>abcd</sup>	5.6
Nevesinjka	1210 <sup>ef</sup>	3220 <sup>ef</sup>	9.9 <sup>fg</sup>	25.7 <sup>d</sup>	8.7
Pobeda	1450 <sup>ef</sup>	3560 <sup>f</sup>	10.5 <sup>g</sup>	26.2 <sup>d</sup>	16.8
Zlatka	1560 <sup>f</sup>	2800 <sup>def</sup>	11.0 <sup>g</sup>	18.7 <sup>cd</sup>	28.4
Sonata	350 <sup>cd</sup>	1530 <sup>cde</sup>	3.0 <sup>bcd</sup>	12.1 <sup>abcd</sup>	5.9
Renan	120 <sup>bc</sup>	590 <sup>abc</sup>	1.4 <sup>abc</sup>	4.2 <sup>abc</sup>	15.2
Tamaro	50 <sup>abc</sup>	1590 <sup>cde</sup>	1.1 <sup>abc</sup>	13.8 <sup>bcd</sup>	-4.7
Ilona	900 <sup>def</sup>	-260 <sup>ab</sup>	7.3 <sup>efg</sup>	-1.5 <sup>ab</sup>	8.2
Malyska	-460 <sup>ab</sup>	2530 <sup>def</sup>	-2.1 <sup>a</sup>	20.9 <sup>cd</sup>	-40.3
Vanda	-570 <sup>a</sup>	-720 <sup>a</sup>	-1.1 <sup>ab</sup>	-4.8 <sup>cd</sup>	-28.6
Petrana	380 <sup>cd</sup>	2540 <sup>def</sup>	3.9 <sup>cde</sup>	19.3 <sup>cd</sup>	2.4
Axis	870 <sup>de</sup>	1330 <sup>bcd</sup>	6.2 <sup>def</sup>	8.9 <sup>abcd</sup>	7.0
$\bar{x}$	520	1720	4.7	13.0	2.05
$LSD_{0.05}$	660	1770	4.1	17.8	NS
$V_g$ [%]	134.7	77.4	95.1	76.4	930
$V_e$ [%]	22.5	155	47.0	92.9	34.0

Means within columns with different letters are significantly different at  $\alpha = 0.05$

GYf – grain yield increment, BPf – biomass production increment,  $NUE_{gf}$  – use efficiency of applied N on grain yield,  $NUE_{bf}$  – use efficiency of applied N on biomass production,  $NUTE_{f}$  – utilisation efficiency of applied N for grain

applied uptake N (Nf) for biomass production except cultivars Ilona and Vanda, which made lower biomass production.

*Genetic correlations among traits and characteristics on the low and high N rate (Table 5)*

NUEg on low N rate was in a stronger relationship with NUP ( $r = 0.735^{++}$ ,  $R^2 = 50\%$ ) than with NUTE ( $r = 0.548$ ,  $R^2 = 30\%$ ); on the contrary, on high N rate NUEg was in a stronger relationship with NUTE ( $r = 0.656^+$ ) than with NUP ( $r = 0.444$ ,  $R^2 = 20\%$ ).

The amount of N uptake (NUP) determined the differences among cultivars in NUEg, on the low N rate to 50%, but on the high N rate only to 20% ( $rGx$  (NUP  $\times$  NUEg) = 0.570). No correlation was between NUP and its utilisation for grain NUTEg.

Significant differences among cultivars were in NUTEg (29.9 to 37.83 kg grain per kg N) (Table 3) and were more causally determined by HI than by N uptake, as HI determined NUTEg on low N rate, circa 36% ( $r = 0.569$ ) and on the high N rate, up to 64% ( $r = 0.809^{++}$ ).

Positive correlations among some variables were expected as one variable is the component of the other one, for example, NUP  $\times$  NAg = (NAs + NAg)  $\times$  NAg. Relation NUP  $\times$  NAg was modified by N content rate in the environment. At the low N rate, NUP determined N in grain (NAg) was very strong ( $r = 0.964^{++}$ ) and weaker on the high N rate ( $r = 0.499$ ). At the low N rate cultivars were not differentiated in efficiency of N translocation in the grain, but at N abundance in the environment the amount of N in grain was more dependent on ef-

T a b l e 5

Genetic ( $rGLN$  low N rate,  $rGHN$  high N rate) and N environments ( $rE$ ) correlation among production biomass, grain yield, N concentration and characteristics of N use efficiency for grain yield and biomass production

Combination of traits	$rGLN$	$rGHN$	$rGx$	$rGf$	$rE$
NUEg $\times$ NUP	0.735 <sup>++</sup>	0.444	0.570	0.762 <sup>++</sup>	-0.747 <sup>++</sup>
NUEg $\times$ NUTE	0.548	0.656 <sup>+</sup>	0.603 <sup>+</sup>	0.807 <sup>++</sup>	0.924 <sup>++</sup>
NUP $\times$ NUTE	-0.157	-0.337	-0.281	0.430	-0.870 <sup>++</sup>
HI $\times$ NUTE	0.569	0.809 <sup>++</sup>	0.714 <sup>++</sup>	0.644 <sup>+</sup>	0.694 <sup>+</sup>
NUP $\times$ NAg	0.964 <sup>++</sup>	0.499	0.585 <sup>+</sup>	0.873 <sup>++</sup>	0.908 <sup>++</sup>
NAs $\times$ NAg	0.681 <sup>+</sup>	-0.531	-0.244	-0.411	0.192
Ng $\times$ GY	-0.720 <sup>++</sup>	-0.495	-0.752 <sup>++</sup>	0.082	0.864 <sup>++</sup>
Ng $\times$ NUEg	-0.683 <sup>+</sup>	-0.560	-0.758 <sup>++</sup>	0.126	-0.696 <sup>+</sup>
Ng $\times$ NUTEg	-0.956 <sup>++</sup>	-0.570	-0.796 <sup>++</sup>	-0.074	-0.754 <sup>++</sup>
Ng $\times$ NUP	-0.028	0.082	-0.039	0.498	0.961 <sup>++</sup>
Ng $\times$ Ns	-0.337	-0.687 <sup>+</sup>	-0.615 <sup>+</sup>	0.166	0.119
NUEb $\times$ NUP	0.829 <sup>++</sup>	0.808 <sup>++</sup>	0.866 <sup>++</sup>	0.616 <sup>+</sup>	-0.757 <sup>++</sup>
Nb $\times$ BP	-0.744 <sup>++</sup>	-0.859 <sup>++</sup>	-0.905 <sup>++</sup>	-0.365	0.647 <sup>+</sup>
Nb $\times$ NUP	-0.214	-0.424	-0.597 <sup>+</sup>	0.448	0.897 <sup>++</sup>
Nb $\times$ NUEb	-0.719 <sup>++</sup>	-0.867 <sup>++</sup>	-0.900 <sup>++</sup>	-0.406	-0.928 <sup>++</sup>
Nb $\times$ NUTEb	-0.998 <sup>++</sup>	-0.998 <sup>++</sup>	-0.991 <sup>++</sup>	-0.371	-0.996 <sup>++</sup>

<sup>+</sup>  $P < 0.05$ ; <sup>++</sup>  $P < 0.01$

NUEg – N (available) use efficiency (Nfs) on grain yield, NUP – N uptake in biomass, NUTE – N utilisation efficiency, HI – harvest index, NAg – N accumulation in grain, NAs – N accumulation in straw, Ng – N concentration in grain, GY – grain yield, NUEg – N (available) use efficiency (Nfs) on grain yield, NUTEg – N utilisation efficiency for grain, Ns – N concentration in straw, NUEb – N (available) use efficiency (Nfs) on biomass production, Nb – concentration N in biomass at maturity, BP – biomass production was calculated from grain yield and harvest index, NUTEb – N utilisation efficiency for biomass

iciency of N translocation than on N in biomass. This also indicates strong correlation between NAg and NAs at the low N rate ( $r = 0.681^+$ ), but negative and not a significant one ( $r = -0.531$ ) at the high N rate. N grain concentration (Ng) on the low N rate was in negative correlation with the grain yield ( $r = -0.720^{++}$ ) with the use of N on grain (NUEg) ( $r = -0.683^+$ ) and in negative a very strong correlation with NUTE ( $r = -0.956^{++}$ ) (Figure 2a). At the high N rate correlations had the same character, but were weaker and non-significant in the same order ( $r = -0.495$ ,  $r = -0.560$  and  $r = -0.570$ ) (Figure 2b). N concentration in grain (Ng) was not at all determined by the amount of N uptake, but by efficiency of translocation N from straw, when between Ng and Ns there was a strong negative correlation ( $r = -0.687^+$ ) on the high N rate and weaker on the low rate ( $r = -0.337$ ). Similarly, like between N grain concentration and grain yield, negative correlations were also between N biomass concentration and biomass production, however, not modified by N level in the environment.

#### *N environments correlations among traits and characteristics (Table 5)*

Correlations among traits and characteristics conditioned by variability of N environment ( $rE$ ) were positive if expressed in absolute values, but if characteristics were expressed in N units, correlations were negative due to decreasing of efficiency of N unit with increasing of N amount in the envi-

ronment, e.g.  $rE(\text{Ng} \times \text{GY}) = +0.864^{++}$  but  $rE(\text{Ng} \times \text{NUTEg}) = -0.754^{++}$ .

## DISCUSSION

The difference between total uptake of 224.4 kg N/ha and applied 150 kg N/ha must come from mineralisation of soil N that was necessary to estimate for objective assessment of N efficiency available N (Nfs) and N from applied fertilisers (Nf). Ns amount determined by chemical analyses of soil can be lower than N uptake, so efficiency of N uptake is overestimated (Gaju *et al.* 2011) and *vice versa* estimation Ns according to non-fertilised variant (Guarda *et al.* 2004) can be underestimated, as it is limited by average genotypic capacity of N uptake (sink). The strong correlation between Nfs and N biomass concentration ( $R^2 = 93\%$ ) documented reliability of Ns estimation by the plot of non-fertilised variant with maximal N uptake in each replication and year (Figure 3). NUPE of available Nfs, circa 70% and eventually applied N circa 65% in biomass correspond at HI = 0.4 to circa 30% of apparent N recovery N in grain (ARf %), which is in accordance with the literature data (Ortiz-Monasterio *et al.* 1997; Raun and Johnson 1999; Guarda *et al.* 2004).

There are two ways how to improve N uptake, either better fertiliser management or better crop va-

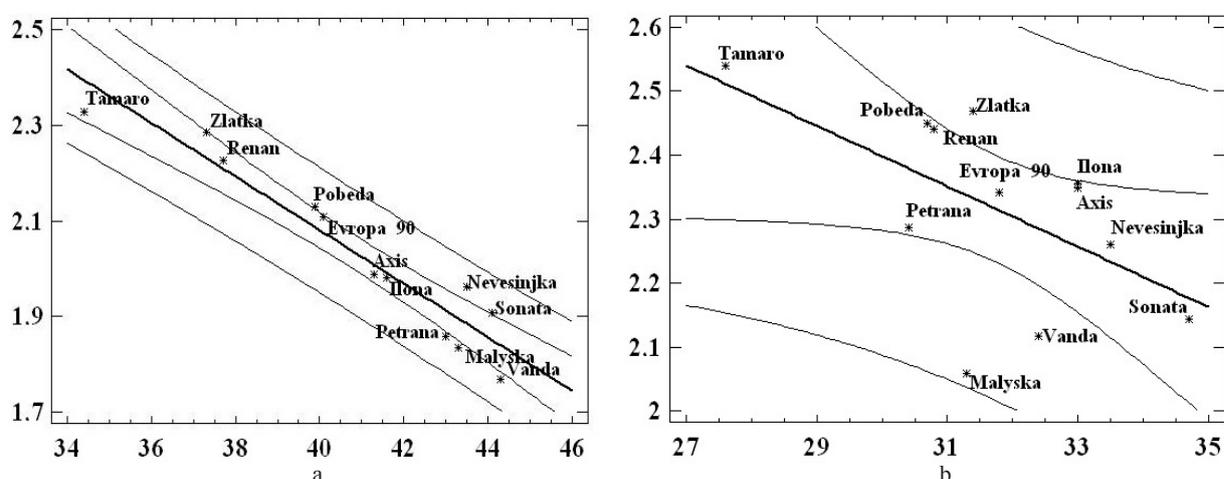


Figure 2. Relationship between NUTE [kg/kg] (x axis) and N concentration in grain (Ng) [%] (y axis) in low N environment (a) and high N environment (b)

rieties. The second way, breeding varieties capable of recovering more N from the soil and fertilisers, assumes the presence of genetic variability in sink (NUP). Genetic progress in biomass production in one of two basic sink components for NUP was low or none (Austin *et al.* 1980; Uzik & Zofajova 2007). In the evaluated cultivar set, low genetic differences were found in NUP in which variability of biomass production contributed positively ( $r = 0.877^{++}$ ), but N concentration had a reducing effect ( $r = -0.597^{+}$ ). Similarly, Barraclough *et al.* (2010) found differences in NUP whereby most of the variation was due to differences in biomass rather than to differences in N concentration.

For increasing efficiency of nitrate intake from the topsoil, the higher root density is generally recommended (Bengough *et al.* 2006). Wang *et al.* (2011) obtained genotype KN9204, producing biomass and root dry weight per plant significantly higher than standard on the high N rate, but had under average N grain concentration and N biomass concentration. This is in congruence with the trend of present genetic progress when grain yield was achieved by the redistribution of biomass and not by its raising (Austin *et al.* 1980; Uzik & Zofajova 2007). Also the translocation of dry matter of roots has been involved in the genetic progress in grain yield (Uzik & Zofajova 2007).

Determination of N in biomass by N content in the environment on 93% indicates that N resorption by wheat root from the environment is rather passive than active; however, correlation coefficient between Nfs and Nb varied among cultivars from  $r = 0.66$  (Malyska, low grain quality) to  $r = 0.90$  (Zlatka and Nevesinjka, high grain quality) (not shown). Probably it is possible to select genotypes having the greatest capacity to accumulate an excess of N during periods of abundant N supply (Hirel & Lemaire 2006).

No relation was shown between NUP and NUTE in this experiment as well as in the historical set of cultivars (Barraclough *et al.* 2010; Uzik & Zofajova 2012a). The ratio of grain yield to NUP (NUTE) is not determined by NUP but by the HI, in which genetic progress was found (Austin *et al.* 1980; Uzik & Zofajova 2007) and which is latent in the formula ( $NUTE = (GY / BP) \times Nb = HI / Nb$ ). In the evaluated set, differences among cultivars in NUTE were determined by HI, on the low N rate (30%) and on the high N rate (up to 65%). Genetic variability of NUTE may be interpreted by HI variability and by N dilution in biomass also in the set of cultivars historically less differentiated.

We found out that in low N rate environment, NUE was more determined by NUPE (54%) than by NUTE (30%), but in high N rate environment NUTE was more significant than NUPE, which corresponds with the results of several authors (Ortiz-Monasterio *et al.* 1997; LeGouis *et al.* 2002) but is not in accord with other authors mentioned in the introduction. Barraclough *et al.* (2010) observed that NUTE was the key component determining genetic variation in GY under either low or high N conditions. On the contrary, Wang *et al.* (2011) found NUPE determines NUE more than NUTE regardless of N conditions. The differently reported results may be due to different variability of tested genotypes in HI and Nb and N level in environment which modifies the relationships.

Between grain yield (or  $NUE_g$ ) and N grain concentration, a negative correlation was found out, stronger on the low N rate than on the high N rate, which is analogous to other authors (Barraclough *et al.* 2010). But it is not consistent with the data of Wang *et al.* (2011) who found out only weak correlation on the low and high N rate. We have found out

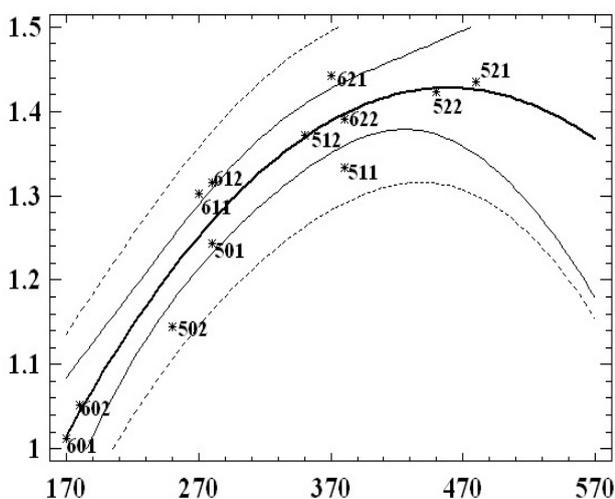


Figure 3. Relationship between amount of available N in environment (Nfs [kg/ha] (x axis) and N biomass concentration (Nb) (y axis) at environment ijk where i = 5, 6 year, j = 0, 1, 2 N variant, k = 1, 2

strong negative correlations between NUTE and Ng, which in the low N environment was so strong that it can be rightfully marked as functional (Barraclough *et al.* 2010) and was as strong as in the historical set of genotypes (Barraclough *et al.* 2010; Uzík & Zofajova 2012a). In the high N environment, correlation between NUTE and N grain concentration was also negative, but weak, which indicates that the power of linkage is dependent on environment and has no pleiotropic character.

Correlation between Ng and NUTE is more easily interpreted when characteristic NUTE is expressed in primary variables from which it was derived  $(GY / BP) \times (N_v + N_g) = HI / (N_s + N_g)$ . As it is seen, genetic correlation between Ng and grain yield per one unit of N uptake was modified by selection, namely, by redistribution of dry matter in favour of grain yield (HI) and by efficiency of N translocation, which altered the ratio straw Ns to grain Ng. Between the year of cultivar release and ratio Ns / Nba (N concentration in straw to N concentration in biomass at anthesis) marked as RNT (relative N translocation) and ratio Ng : Ns marked as RNA (relative N accumulation) was a very strong correlation (Uzík & Zofajova 2012b). The assumption that negative correlation between NUTE and Ng is possible to modify by selection was demonstrated on hypothetical populations (Uzík & Zofajova 2012a).

Characteristics of efficiency of uptake and utilisation of available N (Nfs) had a low genetic variability and interaction genotype  $\times$  N (Nfs) was not observed. Characteristics of applied N had a higher genetic variability, namely, in the uptake of applied N four times and in accumulation in grain (NAgf) ten times higher. The method could be utilised for the detection of genotypes with more effective utilisation of applied N as Guarda *et al.* (2004) similarly exploited it for estimation of genetic progress for apparent nitrogen recovery (ARf) and in agronomic efficiency of applied N (Ae%).

In accordance with the opinion of other authors (Barraclough *et al.* 2010), namely, for organic farming (Konvalina *et al.* 2009), it is necessary to extend genetic variability for characteristics of efficiency of uptake and utilisation of N. Simultaneous selection in the environments at low N and high N availability will allow easier detecting of favourable

recombinants and separate effects of available and applied N.

## CONCLUSIONS

Among cultivars there were low but significant differences in N uptake (NUP) and in efficiency of N uptake of available N (soil Ns and applied Nf), while cultivars were significantly differentiated in the distribution of N in straw and grain and in the utilisation for grain formation (NUTE).

NUE was about the same proportion determined by NUPE and by NUTE, while no relation was present between them, which means that breeding progress and differentiation among cultivars in grain yield were conditioned by HI and not by quantity of N uptake.

The controversial relationship between utilisation of N uptake on grain yield (NUTE) and N grain accumulation (Ng) was conditioned by the quantity of N available in environment. In the environment between NUTE and Ng there was a strong significant negative correlation ( $r = -0.956^{++}$ ) at low N rate, which was weaker and non-significant at high N rate ( $r = -0.570$ ).

It is necessary to extend genetic variability for traits of efficiency of uptake and utilisation of N. Simultaneous selection in the environments at low N and high N availability will allow easier detection of favourable recombinants and evaluation of the effects of available and applied N.

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