DOI: http://dx.doi.org/10.1590/1678-4499.385

SOIL AND PLANT NUTRITION - Article

Nitrogen use efficiency in modern wheat cultivars

Matheus Henrique Todeschini, Anderson Simionato Milioli, Diego Maciel Trevizan, Elesandro Bornhofen, Taciane Finatto, Lindolfo Storck, Giovani Benin*

Universidade Tecnológica Federal do Paraná - Pato Branco (PR), Brazil.

ABSTRACT: The nitrogen use efficiency (NUE) is defined as the capacity of a given genotype in take advantage of the applied nitrogen (N) and transform it in biomass and grains. The objective of this study was to evaluate 12 wheat cultivars as to the NUE and its components. The experiment was conducted in a controlled environment, in a randomized block design with three replications. Twelve wheat cultivars were submitted to four N supply levels (0, 80, 160 and 240 kg of N·ha^{-1}). The data were submitted to analysis of variance, means multiple comparison, polynomial regression, and path analysis. The nitrogen remobilization efficiency (NRE) was the main NUE component of the evaluated cultivars, in both low and high conditions of nitrogen fertilization. In the cultivars average, the nitrogen utilization efficiency (NUtE) presented reduction tendency as the N supply was increased, tending to stabilization at the dose of 231 kg of N·ha^{-1}. The wheat cultivars Mirante, TBIO Itaipu, BRS Parrudo, and TBIO Iguaçu were the most efficient on the N use, and the first two were also efficient in remobilizing the N from the phytomass to the grains.

Key words: *Triticum aestivum*, nitrogen nutrition, path analysis, chlorophyll, yield.

*Corresponding author: benin@utfpr.edu.br

Received: Aug. 17, 2015 – Accepted: Nov. 21, 2015

INTRODUCTION

Nitrogen (N) is the most limiting nutrient for the production of wheat (Pan et al. 2006). Due to this fact and the possible environmental problems related to its use, the Nitrogen use efficiency (NUE) plays a fundamental role in sustainable grain production (Asplund et al. 2014). NUE is given by the ratio between grain yield (GY) and the amount of nutrient provided by the fertilizer (Moll et al. 1982; Cormier et al. 2013; Dai et al. 2013).

The mineral N fertilizer represents a significant cost in wheat production and may cause negative impacts on the environment through leaching and N_2O emissions (Cui et al. 2014). Management practices to help farmers increase productivity and reduce production costs should be studied to ensure agricultural sustainability (Kaneko et al. 2010). In this sense, studies indicate that the development and use of wheat cultivars with higher NUE can contribute to reduce the applied N amounts without decreasing GY (Barraclough et al. 2014; Gaju et al. 2014).

Cultivars that use N more efficiently is one of the main objectives of wheat breeding programs (Sadras and Lemaire 2014). The main components of NUE are N uptake efficiency (NUpE), N utilization efficiency (NUtE), and N remobilization efficiency (NRE) (Le Gouis et al. 2000). The NUpE is the ability of plants to absorb the N available in soil. The NUtE is the relationship between crop yield and total N absorbed by the plant (N in grain + N in phytomass), indicating the GY obtained from each unit of N absorbed by the plant. The NRE is the ability of plants to translocate the N after anthesis from the shoot to the grains. Cultivars with higher NRE tend to accelerate the senescence process and increase N levels in grains (Gaju et al. 2014).

In wheat, the NUE is smaller than 60% (Haile et al. 2012; Hawkesford 2012; Duan et al. 2014). Rahman et al. (2011) indicated values between 28.8 and 40.0 kg_{grains} per $kg_{N_{applied}}$ that depended on the genotype and N levels effect, which ranged from 80 to 120 kg $N{\cdot}ha^{-1}$. The variability of modern cultivars response to NUE has been attributed to NUpE (Sadras and Lemaire 2014), NUtE (Barraclough et al. 2010), and NRE (Kichey et al. 2007; Pask et al. 2012; Guo et al. 2014). The accumulation of phytomass (Giambalvo et al. 2010) and leaf chlorophyll content (Wani et al. 2011; Silva et al. 2014) are traits that have been linked to NUE and can, therefore, be applied for indirect selection of cultivars that use this nutrient more efficiently.

The first research stations investigating wheat crops date back to 1919 (Caierão et al. 2014) and were responsible for the development of pioneering cultivars, important for the Brazilian wheat. Beche et al. (2014) evaluated several Brazilian cultivars developed from 1940 to 2010 and observed that modern cultivars use N more efficiently and are more tolerant to low N availability compared to pioneer cultivars. In this study, we evaluated modern wheat cultivars regarding NUE and their components to establish the existence of genetic variability, useful for leveraging greater genetic progress in future breeding cycles.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse from May to October 2013.The 12 wheat cultivars used in this study were obtained from different breeding programs and were sown over an extensive area in the 2012 and 2013 harvest years in southern Brazil: BRS Gaivota (Embrapa Trigo – 2012), BRS Gralha Azul (Embrapa Trigo – 2012), BRS Parrudo (Embrapa Trigo – 2013), BRS Tangará (Embrapa Trigo – 2007), CD 150 (Coodetec – 2009), Fcep Cristalino (CCGL Fcep – 2006), Fcep Raízes (CCGL Fcep – 2007), Mirante (OR/Biotrigo Genética – 2009), TBIO Iguaçu (Biotrigo Genética – 2011), TBIO Itaipu (Biotrigo Genética – 2010), TBIO Mestre (Biotrigo Genética – 2013), and Topázio (OR Sementes – 2012).

Four N levels were evaluated: 0 (control), 3.7 (medium supply), 7.5 and 11.3 (higher supply) g N per pot. These levels represent, respectively, 0, 80, 160 and 240 kg $N{\cdot}ha^{-1}$. The experiment was performed in a factorial (12 cultivars × 3 N levels + control) completely randomized block design with three replications. Each experimental unit consisted of two pots of 20 L (35 × 30 cm) with 30 homogeneous plants each. Table 1 shows soil physicochemical characteristics. The soil pH was corrected with dolomitic limestone (TNRP 85%) to obtain a base saturation value close to 70%. Similarly, the soil was corrected with 60 $kg{\cdot}ha^{-1}$ of potassium and phosphorus.

The N was applied in three phenological stages: I – 1.4 g N per pot at the base for all treatments except control; II – 65% of the remaining N at the start of tillering (Z 22; Zadoks et al. 1974); and III — 35% at the end of tillering (Z 39; Zadoks et al. 1974). Urea (45% N), diluted with water, was the N source. The control of pests and diseases followed the recommendations for wheat crop.

Measurements of a*, b* and a + b* chlorophyll contents were held in Z 6.5 (Zadoks et al. 1974) of each cultivar, using the handheld ClorofiLOG CFL 1030-Falker. The readings were performed on the center of the flag leaf in 20 plants per experimental unit.

At the time of anthesis (Z 60), three plants per experimental unit were randomly collected to determine the N accumulated in the straw. At physiological maturity (Z 90), the other plants were harvested. The grain fractions and phytomass (shoots) were manually separated. The grain mass was measured, and the humidity, standardized to 13% to determine GY. The fractions of phytomass were dried at 40 °C for 48 h and grounded in a Wiley mill. Subsequently, samples of phytomass and kernels were subjected to chemical analysis in order to determine the N concentration following the Kjeldahl method (Tedesco et al. 1995).

Total N in the grains, in phytomass at anthesis and in physiological maturity was measured by multiplying the N concentration (%) of the fraction by the phytomass production. The NUE measurements were calculated according to Moll et al. (1982), Guarda et al. (2004) and Foulkes et al. (2009): NUE ($g{\cdot}g^{-1}$) = GY/NS, where GY is the grain yield (g) and NS is the N supplied by the fertilizer (g); NUpE ($g{\cdot}g^{-1}$) = (NG + NPM)/NS, where NG is the amount of N in grains (g) and NPM is the amount of N in phytomass at physiological maturity (g); NUtE ($g{\cdot}g^{-1}$) = GY/(NG + NPM); and NRE (%) = NPM – (NPM – NG)/$N_{anthesis}$, where $N_{anthesis}$ is the total NitrogenN at anthesis (g).

The data were tested for normal distribution (Kolmogorov-Smirnov test, $p \leq 0.05$; Sprent and Smeeton 2007). Subsequently, the data were submitted to analysis of variance, considering the effects of cultivars, N levels and interaction as fixed. As a measure of experimental precision, selective accuracy was estimated (SA = $\sqrt{1 - 1/Fc}$), following Resende and Duarte (2007), for the main effects of N and cultivars. The effect of N levels was measured by polynomial regression analysis tested up to cubic degree. Since no significant interaction was observed, the regression analysis was performed for the means of the cultivars, which were compared by Scott-Knott hierarchical clustering algorithm (p = 0.05) in general and within each N level (low and high N supply). Additionally, after checking the multicollinearity between the explanatory variables (Montgomery and Peck 1981), path analysis was carried out (Wright 1921) to identify the direct and indirect effects of the measured traits on GY and NUE. These analyses were performed by the Genes software (Cruz 2013).

RESULTS AND DISCUSSION

All traits were significant ($p < 0.01$) for the cultivar effect and N levels (Table 2). The NUpE, NUtE, NRE, $N_{anthesis}$, NPM and N_{grains} values were also affected by the cultivar versus N level interaction, indicating differences in the responses of the cultivars to the different N levels. The experimental precision related to the effects of N and cultivar, assessed by the magnitude of SA, is very high (SA > 0.90) according to the criterion established by Resende and Duarte (2007) for all traits. This precision favors the discrimination among cultivars.

To compare the means of the cultivars within the N levels (Table 3), these were grouped into two classes: low (between 0 and 80 kg $N{\cdot}ha^{-1}$) and high (between 160 and 240 kg $N{\cdot}ha^{-1}$) supply. Still, in this table, for the traits that showed no interaction, the means of cultivars were compared by the average of the four N levels.

Table 1. Chemical analysis results of the soil used in the experiment.

pH	H + Al	Al^{+3}	Ca^{+2}	Mg^{+2}	K^{+}	P	OM	V	M
($CaCl_2$)	(cmolc·dm⁻³)					(mg·dm⁻³)	(g·dm⁻³)	(%)	
4.90	5.31	0.77	5.12	2.89	0.61	7.96	65.65	58.75	8.66

pH = Hydrogen potential; H + Al = Soil acidity potential; Al^{+3} = Aluminum; Ca^{+2} = Calcium; Mg^{+2} = Magnesium; K^{+} = Potassium; P = Phosphorus; OM = Organic matter; V = Base saturation; M = Aluminum saturation.

Table 2. Analysis of variance including mean square values of the effects of Nitrogen, cultivars, interaction and experimental error, with respective degrees of freedom, mean and selective accuracy for 11 plant traits of 12 modern wheat cultivars, under different N levels.

Traits	Sources of variation				Mean	Selective accuracy	
	N levels (DoF = 3)	Cultivars (DoF = 11)	Interaction (DoF = 33)	Error (DoF = 94)		N	Cultivar
GY	1,588.73*	603.62*	21.52	19.567	64.99	0.99	0.98
Chl_a	44.41*	17.85*	0.76	1.023	35.33	0.98	0.97
Chl_b	62.77*	22.08*	0.34	0.727	14.46	0.99	0.98
Chl_{a+b}	212.45*	79.08*	1.62	3.089	49.79	0.99	0.98
NUE	2,924.37*	395.02*	21.68	23.946	48.34	0.99	0.96
NUpE	0.45*	0.10*	0.02*	0.006	1.82	0.99	0.97
NUtE	1,494.05*	132.*	47.79*	5.994	28.32	0.99	0.97
NRE	1,223.19*	107.2*	17.06*	6.510	26.89	0.99	0.96
$N_{anthesis}$	15.35*	0.17*	0.13*	0.033	2.06	0.99	0.90
NPM	0.77*	0.04*	0.04*	0.002	0.51	0.99	0.97
N_{grains}	10.31*	0.42*	0.18*	0.024	1.93	0.99	0.97

*Significant by F-test ($p \leq 0.01$). DoF = Degrees of freedom; GY = Grain yield (g grains per plot); Chl_a = Chlorophyll a (Falker Index); Chl_b = Chlorophyll b (Falker Index); Chl_{a+b} = Total chlorophyll; NUE = N use efficiency – g grains per g N supplied ($g \cdot g^{-1}$; NUpE = N uptake efficiency – g N in the straw and grains per g N supplied ($g \cdot g^{-1}$); NUtE = N utilization efficiency – g grains per g N in the straw and grains ($g \cdot g^{-1}$); NRE = N remobilization efficiency (%); Nanthesis = Total N at anthesis (%); NPM = total N at physiological maturity (%); Ngrains = Total N remobilized to the grains (%).

The NUpE, NUtE, NRE, $N_{anthesis}$, NPM and N_{grains} values differed among the cultivars for low and high N supply (Table 3), indicating the presence of several mechanisms responsible for the increase in NUE. Five cultivars (Topázio, BRS Parrudo, TBIO Iguaçu, TBIO Mestre, and TBIO Itaipu) were observed in the group with higher NUpE and low N supply. However, under high N supply, only one of these five cultivars (BRS Parrudo) remained in the group with the highest NUpE, showing efficient N uptake in both supply levels. NUpE had low amplitude variation among cultivars (1.54 to 1.89 $g \cdot g^{-1}$ for low N supply and 1.76 to 2.09 $g \cdot g^{-1}$ for high N supply), corroborating Haile et al. (2012). NUpE depends on the cultivar ability to recover the N applied. This possibly happened because the investigated cultivars are elite genotypes, with efficient N uptake.

Regarding total N in phytomass at physiological maturity (NPM), only the Fcep Raízes cultivar remained in the best cultivar group for both N supply levels. Under high N supply, the BRS Gaivota and CD 150 are among the best genotypes and under low N supply; they represent intermediate cultivars for the NPM. The behavioral differences of cultivars in low and high N supply are also observed for NUtE, $N_{anthesis}$ and N_{grains}. Overall, the cultivar BRS Parrudo had the highest NUpE, NUtE and $N_{anthesis}$ for high N supply and greater NUpE in low N supply.

NUE was higher for the cultivar Mirante (58.55 $g \cdot g^{-1}$), followed by TBIO Itaipu (55.25 $g \cdot g^{-1}$), BRS Parrudo (52.73 $g \cdot g^{-1}$), and TBIO Iguaçu (52.24 $g \cdot g^{-1}$), and lower for the BRS Gaivota (37.59 $g \cdot g^{-1}$) (Table 3). The variation range of chlorophyll a* (Chl_a: 33.6 to 37.5), b* (Chl_b: 12.5 to 16.9) and total a + b* (Chl_{a+b}: 46.1 to 54.4) indicated genetic variability for these traits. The highest chlorophyll levels were observed in BRS Parrudo (Chl_a: 37.5; Chl_b: 16.9; and Chl_{a+b}: 54.4). The cultivar Mirante had high levels of chlorophyll (Chl_a: 36.6; Chl_b: 15.9; and Chl_{a+b}: 52.6), the highest GY (74.3 g) and NUE (58.5 $g \cdot g^{-1}$).

The NUtE ranged between 25.64 and 38.91 $g \cdot g^{-1}$ for low N supply and between 18.86 and 28.70 $g \cdot g^{-1}$ for high N supply. The highest values were observed for the cultivars Mirante and TBIO Iguaçu (low N supply) and cultivars Mirante, TBIO Iguaçu, BRS Parrudo and TBIO Itaipu (high N supply); these cultivars also had high NUE (Table 3).

Table 4 shows the variation range of the results based on four N levels, per cultivar (traits with interaction) and overall. The data fitted polynomial regression models since the coefficients of determination (R^2) are high for each cultivar and low for overall. NUpE displayed increasing linear behavior for six cultivars and quadratic for four ones, with a maximum point within the limits studied. The critical point (CP, max) of the NUpE was observed at 222, 216, and 204 kg $N \cdot ha^{-1}$ for the cultivars BRS Parrudo, →

Table 3. Means for the traits of 12 modern wheat cultivars submitted to low (0 and 80 kg N·ha^{-1}) and high (160 and 240 kg N·ha^{-1}) N supply.

Low N supply		High N supply		Low N supply		High N supply		Traits with no interaction		
Cult	NUpE	Cult	NUpE	Cult	NUtE	Cult	NUtE	Cult	Chl_a	Chl_b
1	1.73 b*	1	1.76 c	1	33.12 c	1	20.78 b	1	35.01 c	14.37 c
2	1.81 a	2	1.94 b	2	30.27 d	2	22.15 b	2	36.70 b	15.73 b
3	1.83 a	3	2.09 a	3	32.63 c	3	28.70 a	3	37.47 a	16.94 a
4	1.78 b	4	1.99 b	4	38.84 a	4	27.52 a	4	36.62 b	15.95 b
5	1.54 d	5	1.80 c	5	36.01 b	5	23.05 b	5	35.11 c	14.36 c
6	1.59 d	6	1.84 c	6	27.59 e	6	18.86 b	6	34.85 c	13.44 d
7	1.89 a	7	1.96 b	7	28.04 e	7	28.03 a	7	34.19 d	13.05 d
8	1.72 b	8	1.81 c	8	25.64 e	8	21.23 b	8	36.37 b	15.70 b
9	1.81 a	9	1.98 b	9	38.91 a	9	25.71 a	9	34.75 c	13.78 c
10	1.69 c	10	1.82 c	10	30.09 d	10	20.43 b	10	35.49 c	14.48 c
11	1.80 a	11	1.99 b	11	34.55 c	11	27.11 a	11	33.58 d	12.53 d
12	1.66 c	12	1.87 c	12	32.71 c	12	27.63 a	12	33.89 d	13.23 d
	NRE		**NRE**		**$N_{anthesis}$**		**$N_{anthesis}$**		**Chl_{a+b}**	**GY**
1	32.69 b	1	23.79 b	1	1.61 b	1	2.57 c	1	49.37 c	66.42 c
2	27.26 c	2	21.34 c	2	1.49 b	2	2.31 c	2	52.42 b	57.79 e
3	32.43 b	3	22.41 c	3	1.63 b	3	2.92 a	3	54.41 a	71.59 b
4	37.30 a	4	26.41 a	4	1.45 b	4	2.58 c	4	52.56 b	74.32 a
5	33.07 b	5	21.72 c	5	1.49 b	5	2.50 c	5	49.47 c	62.36 d
6	26.64 c	6	16.11 d	6	1.45 b	6	2.70 b	6	48.29 c	50.95 f
7	26.31 c	7	20.63 c	7	1.88 a	7	2.67 b	7	47.23 d	61.97 d
8	29.16 c	8	20.67 c	8	1.50 b	8	2.49 c	8	52.08 b	60.18 d
9	32.75 b	9	23.65 b	9	1.60 b	9	2.68 b	9	48.52 c	70.62 b
10	32.80 b	10	21.68 c	10	1.49 b	10	2.44 c	10	49.96 c	63.45 d
11	35.73 a	11	26.21 a	11	1.58 b	11	2.50 c	11	46.13 d	74.91 a
12	31.84 b	12	22.86 b	12	1.47 b	12	2.41 c	12	47.11 d	65.34 c
NPM		**NPM**		**N_{grains}**		**N_{grains}**		**Cultivars**		**NUE**
1	0.33 d	1	0.52 d	1	1.73 a	1	2.82 a	1 BRS Tangará		49.39 c
2	0.50 a	2	0.59 c	2	1.37 c	2	2.21 d	2 TBIO Mestre		42.83 d
3	0.44 b	3	0.67 b	3	1.57 b	3	2.15 d	3 BRS Parrudo		52.73 b
4	0.41 c	4	0.47 d	4	1.55 b	4	2.45 c	4 Mirante		58.55 a
5	0.29 d	5	0.64 b	5	1.51 b	5	2.31 c	5 BRS Gralha Azul		45.86 d
6	0.36 c	6	0.74 a	6	1.35 c	6	2.17 d	6 BRS Gaivota		37.59 e
7	0.39 c	7	0.52 d	7	1.82 a	7	1.81 e	7 Topázio		45.96 d
8	0.37 c	8	0.72 a	8	1.79 a	8	2.40 c	8 CD 150		44.42 d
9	0.39 c	9	0.65 b	9	1.35 c	9	2.35 c	9 TBIO Iguaçu		52.24 b
10	0.36 c	10	0.66 b	10	1.65 a	10	2.62 b	10 Fcep Cristalino		47.17 c
11	0.36 c	11	0.61 b	11	1.78 a	11	2.36 c	11 TBIO Itaipu		55.25 b
12	0.55 a	12	0.72 a	12	1.38 c	12	1.85 e	12 Fcep Raízes		48.06 c

*Means with different letters differ by the Scott-Knott test ($p = 0.05$). Cult = Cultivars; NUpE = N uptake efficiency – g N in straw and grains per g N supplied (g·g^{-1}); NUtE = N utilization efficiency – g grains per g N in the straw and grains (g·g^{-1}); Chl_a = Chlorophyll a (Falker Index); Chl_b = Chlorophyll b (Falker Index); NRE = N remobilization efficiency (%); $N_{anthesis}$ = Total N at anthesis (%); Chl_{a+b} = Total chlorophyll; GY = Grain yied (g grains per plot); NPM = Total N at physiological maturity (%); N_{grains} = Total N remobilized to the grains (%); NUE = N use efficiency – g grains per g N supplied (g·g^{-1}).

Table 4. Polynomial regression analysis for 11 plant traits of 12 modern wheat cultivars submitted to four Nitrogen levels (x, between 0 and 240 kg N·ha^{-1}), per cultivar and overall.

Cultivar	NUpE = a + bx + cx^2	R^2	CP	NUtE = a + bx + cx^2	R2	CP
1 BRS Tangará	1.76 – 0.0018x + 0.0000095x^2	0.49	95	40.35 – 0.221x + 0.000583x^2	0.91	189
2 TBIO Mestre	1.77 + 0.00083x	0.46	-	36.03 – 0.161x + 0.00042x^2	0.89	192
3 BRS Parrudo	1.67 + 0.0044x – 0.00001x^2	0.84	222	32.32 + 0.0537x – 0.00036x^2	0.77	75
4 Mirante	1.73 + 0.00127x	0.78	-	47.41 – 0.218x + 0.00053x^2	0.83	205
5 BRS Gralha Azul	1.40 + 0.0039 – 0.000009x^2	0.89	216	46.44 – 0.3059x + 0.00088x^2	0.89	174
6 BRS Gaivota	1.46 + 0.00408x – 0.00001x^2	0.96	204	28.81 – 0.0465x	0.82	-
7 Topázio	1.87 + 0.00042x	0.45	-	32.33 – 0.1082x + 0.00038x^2	0.48	142
8 CD 150	1.72	-	-	28.63 – 0.0919x + 0.00026x^2	0.84	176
9 TBIO Iguaçu	1.89	-	-	45.54 – 0.1971x + 0.00046x^2	0.96	214
10 Fcep Cristalino	1.66 + 0.00079x	0.71	-	31.23 – 0.0497x	0.77	-
11 TBIO Itaipu	1.74 + 0.0013x	0.90	-	40.19 – 0.158x + 0.00043x^2	0.77	184
12 Fcep Raízes	1.57 + 0.00156x	0.63	-	37.01 – 0.128x + 0.00038x^2	0.59	168
	NRE = a + bx + cx^2			**$N_{anthesis}$ = a + bx + cx^2**		
1 BRS Tangará	33.02 + 0.031x – 0.00038x^2	0.89	41	1.51 – 0.0037x + 0.000046x^2	0.91	40
2 TBIO Mestre	28.47 – 0.0348x	0.71	-	1.38 + 0.0043x	0.83	-
3 BRS Parrudo	35.59 – 0.06819x	0.86	-	1.27 + 0.0083x	0.96	-
4 Mirante	37.36 + 0.0063x – 0.00028x^2	0.85	12	1.27 + 0.0062x	0.85	-
5 BRS Gralha Azul	38.20 – 0.164x + 0.00039x^2	0.88	210	1.27 + 0.00604x	0.91	-
6 BRS Gaivota	30.34 – 0.112x + 0.0002x^2	0.94	280	1.12 + 0.0079x	0.98	-
7 Topázio	28.85 – 0.067x + 0.000119x^2	0.91	281	1.72 + 0.0017x + 0.000015x^2	0.94	56
8 CD 150	30.77 – 0.0488x	0.82	-	1.30 + 0.00576x	0.95	-
9 TBIO Iguaçu	34.65 – 0.0538x	0.73	-	1.36 + 0.00647x	0.78	-
10 Fcep Cristalino	34.62 – 0.06149x	0.85	-	1.26 + 0.0058x	0.93	-
11 TBIO Itaipu	36.06 + 0.0018x – 0.00024x^2	0.93	4	1.33 + 0.00592x	0.98	-
12 Fcep Raízes	34.79 – 0.0621x	0.60	-	1.19 + 0.00622x	0.76	-
	NPM = a + bx + cx^2			**N_{grains} = a + bx + cx^2**		
1 BRS Tangará	0.317 + 0.00091x	0.68	-	1.13 + 0.0176x – 0.000044x^2	0.97	200
2 TBIO Mestre	0.36 + 0.0037x – 0.000012x^2	0.78	154	1.07 + 0.0084x – 0.000013x^2	0.98	323
3 BRS Parrudo	0.39 – 0.00068x + 0.000011x^2	0.81	31	1.38 + 0.00403x	0.74	-
4 Mirante	0.42 – 0.0024x + 0.000014x^2	0.60	85	1.013 + 0.0144x – 0.00003x^2	0.94	240
5 BRS Gralha Azul	0.18 + 0.0035x – 0.000006x^2	0.93	291	0.994 + 0.0138x – 0.00003x^2	0.86	230
6 BRS Gaivota	0.31 + 0.0019x	0.81	-	1.19 + 0.0047x	0.88	-
7 Topázio	0.27 + 0.0035x – 0.000011x^2	0.77	159	1.81	-	-
8 CD 150	0.35 + 0.00164x	0.64	-	1.62 + 0.00389x	0.67	-
9 TBIO Iguaçu	0.41 – 0.0017x + 0.000014x^2	0.90	61	0.92 + 0.0144x – 0.000035x^2	0.92	205
10 Fcep Cristalino	0.35 + 0.00128x	0.47	-	1.45 + 0.00568x	0.95	-
11 TBIO Itaipu	0.30 + 0.00153x	0.87	-	1.23 + 0.0157x – 0.000046x^2	0.88	170
12 Fcep Raízes	0.46 + 0.001379x	0.64	-	1.01 + 0.0113x – 0.000034x^2	0.85	166
Traits with no interaction N × cultivar						
	Chl_a = 34.11 + 0.01018x	0.34	-	Chlb = 12.98 + 0.0123x	0.40	-
	Chl_{a+b} = 47.09 + 0.0225x	0.37	-	GY = 57.59 + 0.0617x	0.36	-
	EUN = 58.36 – 0.08x	0.89	-			

NUpE = N uptake efficiency – g N in straw and grains per g N supplied (g·g^{-1}); CP = Critical point (maximum or minimum); NUtE = N utilization efficiency – g grains per g N in the straw and grains (g·g^{-1}); NRE = N remobilization efficiency (%); $N_{anthesis}$ = Total N at anthesis (%); NPM = Total N at physiological maturity (%); N_{grains} = Total N remobilized to the grains (%); Chl_a = Chlorophyll a (Falker Index); Chl_b = Chlorophyll b (Falker Index); Chl_{a+b} = Total chlorophyll; GY = Grain yield (g grains per plot); NUE = N use efficiency – g grains per g N supplied (g·g^{-1}).

BRS Gralha Azul and BRS Gaivota, respectively. The NUpE decreased with increasing N supply for the cultivar BRS Tangará, while CD 150 and TBIO Iguaçu did not change. Guarda et al. (2004) and Asplund et al. (2014) also reported increasing NUpE for increasing N supply levels.

The NUtE decreased linearly for the cultivars BRS Gaivota and Fcep Cristalino. Also, it showed quadratic response with minimum point (at the levels indicated in the table) for BRS Tangará, TBIO Mestre, Mirante, BRS Gralha Azul, Topázio, CD 150, TBIO Iguaçu, TBIO Itaipu and Fcep Raízes and maximum point (N = 75 kg N·ha^{-1}) for BRS Parrudo. The means of the cultivars showed a tendency to reduce the NUtE as the N supply level increased, stabilizing at 231 kg N·ha^{-1} (minimum CP). The differential behavior of cultivars with different N supply levels, regarding the NUtE, is important when defining the management and choosing the cultivar.

The NRE displayed a decreasing linear behavior for six cultivars and quadratic responses for the others. Maximum CPs were obtained for lower N supply for the cultivars BRS Tangará (41 kg N·ha^{-1}), Mirante (12 kg N·ha^{-1}) and TBIO Itaipu (4 kg N·ha^{-1}). Furthermore, maximum CP was observed close to the highest N supply for the cultivars BRS Gralha Azul, BRS Gaivota and Topázio. The high N levels caused the $N_{anthesis}$ to increase, and the cultivars were unable to remobilize the N at the same rate when N supply was high compared to low supply. Barbottin et al. (2005) and Kichey et al. (2007) also reported that the NRE depends on the N level, and the highest remobilization rates occurred for lower N supply.

The $N_{anthesis}$ is the main source of N to the grains (Gaju et al. 2014). The highest protein concentrations in the grain are related to the higher remobilization of post-anthesis N (Barraclough et al. 2014; Bogard et al. 2010). According to Kichey et al. (2007), much of the N found in grains comes from the remobilization of N stored in the shoots and roots of the plant before anthesis. It is noteworthy that, as the N supply increases, most cultivars respond positively, demonstrating an average $N_{anthesis}$ increase of 0.0063% per kg of N applied. NPM and N_{grains} also responded positively to an increasing N supply. For N_{grains}, the means of the cultivars showed a quadratic response, with CP of 224 kg N·ha^{-1} (data not shown).

The direct and indirect effects of the seven traits on the NUE (Table 5) and GY (Table 6) were examined by path analysis. It was observed a direct effect (DE) with sign and magnitude similar to the correlation coefficient (r) of the NRE over the NUE under low (DE = 0.94, r = 0.617) and high (DE = 0.65, r = 0.721) N supply, corroborating the studies of Barraclough et al. (2014), Guo et al. (2014) and Le Gouis et al. (2000). Although NUtE has been associated with the NUE (r = 0.487 and r = 0.516), this was mainly due to indirect effects (IE) of NRE (IE = 0.587) under low N supply. This result indicates that, for a group of modern cultivars, using the available N is linked to the ability of the cultivars to remobilize the nutrient toward the grains. In addition, under low N supply, the $N_{anthesis}$ affected directly the NUE (DE = 0.568) due to the DE of NREs with opposite sign canceling the correlation. This DE was not observed for high N supply (DE = 0.006). →

Table 5. Direct and indirect effects of chlorophyll a, chlorophyll b, Nitrogen uptake efficiency, Nitrogen utilization efficiency, Nitrogen remobilization efficiency, total Nitrogen at anthesis and total Nitrogen at physiological maturity traits on Nitrogen use efficiency for low (0 and 80 kg N·ha^{-1}) and high (160 and 240 kg N·ha^{-1}) Nitrogen supply, Pearson correlation coefficient (r) and model coefficient of determination (R^2).

Effects	Chl_a	Chl_b	NUpE	NUtE	NRE	$N_{anthesis}$	NPM
Low Nitrogen supply (NC = 45)							
Direct on NUE	0.091	−0.117	0.221	0.315	0.942	0.568	0.251
Indirect via Chl_a		0.084	0.021	−0.023	−0.008	0.024	0.022
Indirect via Chl_b	−0.108		−0.034	0.034	0.015	−0.036	−0.035
Indirect via NUpE	0.052	0.065		−0.050	−0.079	0.146	0.084
Indirect via NUtE	−0.078	−0.091	−0.071		0.196	−0.151	−0.130
Indirect via NRE	−0.084	−0.122	−0.337	0.587		−0.558	−0.423
Indirect via $N_{anthesis}$	0.150	0.178	0.376	−0.273	−0.337		0.207
Indirect via NPM	0.062	0.075	0.096	−0.104	−0.113	0.091	
Sum (r)	0.085	0.072	0.272*	0.487*	0.617*	0.084	−0.023
R^2	0.836						

...continue

Table 5. Continuation...

Effects	Chl_a	Chl_b	NUpE	NUtE	NRE	$N_{anthesis}$	NPM
			High Nitrogen supply (NC = 19)				
Direct on NUE	−0.183	0.205	0.027	0.155	0.654	0.006	0.023
Indirect via Chl_a		−0.159	−0.040	0.006	0.002	−0.019	−0.003
Indirect via Chl_b	0.178		0.040	−0.007	−0.003	0.031	0.016
Indirect via NUpE	0.006	0.005		0.010	−0.003	0.012	0.000
Indirect via NUtE	−0.005	−0.005	0.057		0.086	−0.011	−0.070
Indirect via NRE	−0.007	−0.008	−0.072	0.362		−0.411	−0.327
Indirect via $N_{anthesis}$	0.001	0.001	0.003	0.000	−0.004		0.001
Indirect via NPM	0.000	0.002	0.000	−0.010	−0.011	0.005	
Sum (r)	−0.011	0.041	0.015	0.516*	0.721*	−0.387*	−0.360*
R^2	0.551						

*Correlation (r) significant by t-test ($p < 0.05$); Chl_a = Chlorophyll a (Falker Index); Chl_b = Chlorophyll b (Falker Index); NUpE = N uptake efficiency; NUtE = N utilization efficiency; NRE = N remobilization efficiency (%); $N_{anthesis}$ = Total N at anthesis (%); NPM = Total N at physiological maturity (%); NUE = N use efficiency; NC = Number of condition (collinearity).

Table 6. Direct and indirect effects of chlorophyll a, chlorophyll b, Nitrogen uptake efficiency, Nitrogen utilization efficiency, Nitrogen remobilization efficiency, total Nitrogen at anthesis and total Nitrogen at physiological maturity traits on grain yield for low (0 and 80 kg N·ha^{-1}) and high (160 and 240 kg N·ha^{-1}) Nitrogen supply, Pearson correlation coefficient (r) and model coefficient of determination (R^2).

Effects	Chl_a	Chl_b	NUpE	NUtE	NRE	$N_{anthesis}$	NMP
			Low Nitrogen supply (NC = 45)				
Direct on GY	−0.052	0.084	0.051	−0.038	1.058	0.987	0.309
Indirect via Chl_a		−0.048	−0.012	0.013	0.005	−0.014	−0.013
Indirect via Chl_b	0.078		0.025	−0.024	−0.011	0.026	0.025
Indirect via NUpE	0.012	0.015		−0.012	−0.018	0.034	0.020
Indirect via NUtE	0.009	0.011	0.009		−0.024	0.018	0.016
Indirect via NRE	−0.095	−0.137	−0.378	0.659		−0.627	−0.475
Indirect via $N_{anthesis}$	0.260	0.308	0.653	−0.474	−0.585		0.360
Indirect via NPM	0.076	0.092	0.118	−0.128	−0.139	0.113	
Sum (r)	0.290*	0.326*	0.465*	−0.004	0.286*	0.538*	0.241*
R^2	0.945						
			High Nitrogen supply (NC = 19)				
Direct on GY	−0.001	−0.028	0.093	0.052	1.321	0.930	0.381
Indirect via Chl_a		−0.001	0.000	0.000	0.000	0.000	0.000
Indirect via Chl_b	−0.024		−0.006	0.001	0.000	−0.004	−0.002
Indirect via NUpE	0.020	0.018		0.034	−0.010	0.043	0.000
Indirect via NUtE	−0.002	−0.002	0.019		0.029	−0.004	−0.024
Indirect via NRE	−0.014	−0.017	−0.145	0.731		−0.831	−0.661
Indirect via $N_{anthesis}$	0.096	0.141	0.433	−0.065	−0.585		0.189
Indirect via NPM	0.007	0.030	−0.001	−0.172	−0.191	0.077	
Sum (r)	0.082	0.141	0.392*	0.581*	0.564*	0.211	−0.117
R^2	0.977						

*Correlation (r) significant by t-test ($p < 0.05$). NUE = N use efficiency; Chl_a = Chlorophyll a (Falker Index); Chl_b = Chlorophyll b (Falker Index); NUpE = N uptake efficiency; NUtE = N utilization efficiency; NRE = N remobilization efficiency (%); $N_{anthesis}$ = Total N at anthesis (%); NPM = Total N at physiological maturity (%); GY = Grain yield; NC = Number of condition (collinearity).

This is explained by the linear increase of $N_{anthesis}$ in most cultivars, with increasing N levels (Table 4); thus, high N supply results in high amount of $N_{anthesis}$, and the RNE is presented as a measure of the highest association with NUE (DE = 0.654) (Table 5).

The association between $N_{anthesis}$ and GY results from the DE on the GY, under low (DE = 0.987) and high (DE = 0.930) N supply (Table 6). Accordingly, under low N supply, it is possible to select cultivars with greater NUE from the indirect selection on NRE or $N_{anthesis}$. Under high N supply, N saturation probably occurs, and the NUE is then explained by the cultivars' ability to remobilize N from the straw to the grains, resulting in a higher grain quality and higher GY. Under low N supply, Beche et al. (2014) observed high DE of the NRE on GY while, under high N supply, they observed higher effect of NUtE and NUpE. This divergence in results might be explained by the different set of cultivars used in that study, characterized by pioneering and modern cultivars, whereas, in this study, we used only modern cultivars.

The Chl_a, Chl_b, and Chl_{a+b} parameters are dependent on the cultivar (Table 3) and the N level applied (Table 4). However, the results in Tables 5 e 6 showed no important IE and DE on the NUE and GY. For low N supply, the correlation of GY with Chl_a and Chl_b is significant due to the IE of the $N_{anthesis}$. Thus, in this study, the evaluation of Chl_a, Chl_b and Chl_{a+b} was important to estimate the NUE.

CONCLUSION

The efficient use of Nitrogen by the evaluated cultivars resulted especially from the highly efficient way these cultivars were able to remobilize the absorbed Nitrogen to grain production. It is possible to select wheat cultivars with increased Nitrogen use efficiency from the indirect selection on Nitrogen remobilization efficiency or total Nitrogen at flowering. The Mirante, TBIO Itaipu, BRS Parrudo, and TBIO Iguaçu wheat cultivars are the most efficient in Nitrogen use, and the first two are the most efficient in Nitrogen remobilization.

REFERENCES

Asplund, L., Bergkvist, G. and Weih, M. (2014). Proof of concept: nitrogen use efficiency of contrasting spring wheat varieties grown in greenhouse and field. Plant Soil, 374, 829-842. http://dx.doi.org/10.1007/s11104-013-1895-6.

Barbottin, A., Lecomte, C., Bouchard, C. and Jeuffroy, M. H. (2005). Nitrogen remobilization during grain filling in wheat: genotypic and environmental effects. Crop Science Society of America, 45, 1141-1150. http://dx.doi.org/10.2135/cropsci2003.0361.

Barraclough, P. B., Howarth, J. R., Jones, J., Lopez-Bellido, R., Parmar, S., Shepherd, C. E. and Hawkesford, M. J. (2010). Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. European Journal of Agronomy, 33, 1-11. http://dx.doi.org/10.1016/j.eja.2010.01.005.

Barraclough, P. B., Lopez-Bellido, R. and Hawkesford, M. J. (2014). Genotypic variation in the uptake, partitioning and remobilization of nitrogen during grain-filling in wheat. Field Crops Research. 156, 242-248. http://dx.doi.org/10.1016/j.fcr.2013.10.004.

Beche, E., Benin, G., Bornhofen, E., Dalló, S. C., Sassi, L. H. and Oliveira, R. (2014). Eficiência de uso de Nitrogênio em cultivares de trigo pioneiras e modernas. Pesquisa Agropecuária Brasileira, 49, 948-957. http://dx.doi.org/10.1590/S0100-04X2014001200005.

Bogard, M., Allard, V., Brancourt-Hulmel, M., Heumez, E., Machet, J. M., Jeuffroy, M. H., Gate, P., Martre, P. and Le Gouis, J. (2010). Deviation from the grain protein concentration-grain yield negative relationship is highly correlated to postanthesis N uptake in winter wheat. Journal of Experimental Botany, 61, 4303-4312. http://dx.doi.org/10.1093/jxb/erq238.

Caierão, E., Scheeren, P. L., Silva, M. S. and Castro, R. L. (2014). History of wheat cultivars released by Embrapa in forty years of research. Crop Breeding and Applied Biotechnology, 14, 216-223. http://dx.doi.org/10.1590/1984-70332014v14n4a34.

Cormier, F., Faure, S., Dubreuil, P., Heumez, E., Beauchêne, K., Lafarge, S., Praud, A. and Le Gouis, J. (2013). A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, 126, 3035-3048. http://dx.doi.org/10.1007/s00122-013-2191-9.

Cruz, C. D. (2013). GENES — a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum. Agronomy, 35, 271-276. http://dx.doi.org/10.4025/actasciagron.v35i3.21251.

Cui, Z., Wang, G., Yue, S., Wu, L., Zhang, F. and Chen, X. (2014). Closing the N-use efficiency gap to achieve food and environmental security. Environmental Science and Technology, 48, 5780-5787. http://dx.doi.org/10.1021/es5007127.

Dai, X., Zhou, X., Jia, D., Xiao, L., Kong, H. and He, M. (2013). Managing the seeding rate to improve nitrogen-use efficiency of winter wheat. Field Crops Research, 154, 100-109. http://dx.doi.org/10.1016/j.fcr.2013.07.024.

Duan, Y., Shi, X., Li, S., Sun, X. and He, X. (2014). Nitrogen use efficiency as affected by phosphorus and potassium in long-term rice and wheat experiments. Journal of Integrative Agriculture, 13, 588-596. http://dx.doi.org/10.1016/S2095-3119(13)60716-9.

Foulkes, M. J., Hawkesford, M. J., Barraclough, P. B., Holdsworth, M. J., Kerr, S., Kightley, S. and Shewry, P. R. (2009). Identifying traits to improve the nitrogen economy of wheat: recent advances and future prospects. Field Crops Research, 114, 329-342. http://dx.doi.org/10.1016/j.fcr.2009.09.005.

Gaju, O., Allard, V., Martre, P., Le Gouis, J., Moreau, D., Bogard, M., Hubbart, S. and Foulkes, M. J. (2014). Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. Field Crops Research, 155, 213-223. http://dx.doi.org/10.1016/j.fcr.2013.09.003.

Giambalvo, D., Ruisi, P., Miceli, G. D., Frenda, A. S. and Amato, G. (2010). Nitrogen use efficiency and nitrogen fertilizer recovery of durum wheat genotypes as affected by interspecific competition. Agronomy Journal, 102, 707-715. http://dx.doi.org/10.2134/agronj2009.0380.

Guarda, G., Padovan, S. and Delogu, G. (2004). Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. European Journal of Agronomy, 21, 181-192. http://dx.doi.org/10.1016/j.eja.2003.08.001.

Guo, Z., Zhang, Y., Zhao, J., Shi, Y. and Yu, Z. (2014). Nitrogen use by winter wheat and changes in soil nitrate nitrogen levels with supplemental irrigation based on measurement of moisture content in various soil layers. Field Crops Research, 164, 117-125. http://dx.doi.org/10.1016/j.fcr.2014.05.016.

Haile, D., Nigussie, D. and Ayana, A. (2012). Nitrogen use efficiency of bread wheat: effects of nitrogen rate and time of application. Journal of Soil Science and Plant Nutrition, 12, 389-409. http://dx.doi.org/10.4067/S0718-95162012005000002.

Hawkesford, M. J. (2012). The diversity of nitrogen use efficiency for wheat varieties and the potential for crop improvement. Better Crops, 96, 10-12.

Kaneko, F. H., Arf, O., Gitti, D. D. C., Arf, M. V., Chioderoli, C. A. and Kappes, C. (2010). Manejo do solo e do Nitrogênio em milho cultivado em espaçamentos reduzido e tradicional. Bragantia, 69, 677-686. http://dx.doi.org/10.1590/S0006-87052010000300020.

Kichey, T., Hirel, B., Heumez, E., Dubois, F. and Le Gouis, J. (2007). In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilization to the grain correlates with agronomic traits and nitrogen physiological markers. Field Crops Research, 102, 22-32. http://dx.doi.org/10.1016/j.fcr.2007.01.002.

Le Gouis, J., Beghin, D., Heumez, E. and Pluchard, P. (2000). Genetic differences for nitrogen uptake and nitrogen utilization efficiencies in winter wheat. European Journal of Agronomy, 12, 163-173. http://dx.doi.org/10.1016/S1161-0301(00)00045-9.

Moll, R. H., Kamprath, E. J. and Jackson, W. A. (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy Journal, 74, 562-564. http://dx.doi.org/10.2134/agronj1982.00021962007400030037x.

Montgomery, D. C. and Peck, E. A. (1981). Introduction to linear regression analysis. New York: John Wiley.

Pan, J., Zhu, Y., Jiang, D., Dai, T., Li, Y. and Cao, W. (2006). Modeling plant nitrogen uptake and grain nitrogen accumulation in wheat. Field Crops Research, 97, 322-336. http://dx.doi.org/10.1016/j.fcr.2005.11.006.

Pask, A. J. D., Sylvester-Bradley, R., Jamieson, P. D. and Foulkes, M. J. (2012). Quantifying how winter wheat crops accumulate and use nitrogen reserves during growth. Field Crops Research, 126, 104-118. http://dx.doi.org/10.1016/j.fcr.2011.09.021.

Rahman, M. A., Sarker, A. Z., Amin, M. F., Jahan, H. S. and Akhter, M. M. (2011). Yield response and nitrogen use efficiency of wheat under different doses and split application of nitrogen fertilizer. Bangladesh Journal of Agricultural Research, 36, 231-240. http://dx.doi.org/10.3329/bjar.v36i2.9249.

Resende, M. D. V. and Duarte, J. B. (2007). Precisão e controle de qualidade em experimentos de avaliação de cultivares. Pesquisa Agropecuária Tropical, 37, 182-194.

Sadras, V. O. and Lemaire, G. (2014). Quantifying crop nitrogen status for comparisons of agronomic practices and genotypes. Field Crops Research, 164, 54-64. http://dx.doi.org/10.1016/j.fcr.2014.05.006.

Silva, C. L., Benin, G., Bornhofen, E., Beche, E., Todeschini, M. H. and Milioli, A. S. (2014). Nitrogen use efficiency is associated with chlorophyll content in Brazilian spring wheat. Australian Journal of Crop Science, 8, 957-964.

Sprent, P. and Smeeton, N. C. (2007). Applied nonparametric statistical methods. 4. ed. Boca Raton: Chapman & Hall.

Tedesco, M. J., Gianello, C., Bissani, C. A., Bohnen, H. and Volkweiss, S. J. (1995). Análise de solo, plantas e outros materiais, 2. ed. Porto Alegre: UFRGS.

Wani, B. A., Ram, M., Yasin, A. and Singh, E. (2011). Physiological traits in integration with yield and yield components in wheat (*Triticum aestivum* L.) Study of their genetic variability and correlation. Asian Journal of Agricultural Research, 5, 194-200. http://dx.doi.org/10.3923/ajar.2011.194.200.

Wright, S. (1921). Correlation and causation. Journal of Agricultural Research, 20, 557-585.

Zadoks, J. C., Chang, T. T. and Konzac, C. F. (1974). A decimal code for the growth stages of cereals. Weed Research, 14, 415-421. http://dx.doi.org/10.1111/j.1365-3180.1974.tb01084.x.