

RESEARCH ARTICLE

# Wheat nitrogen utilization efficiency and yield as affected by nitrogen management and environmental conditions

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## ABSTRACT

Nitrogen (N) is the main nutrient for plant nutrition, however, its fertilization management is still very complex. To evaluate wheat N utilization efficiency (NUE) and yield in response to N fertilization management considering the influence of environmental conditions, an experiment was carried out in three field conditions in Southern Brazil: Londrina in rainfed and irrigation conditions, and Ponta Grossa in rainfed. A complete (2 × 2 × 2) + 1 factorial arrangement evaluated two N rates (40 and 80 kg ha<sup>-1</sup>), two N sources (ammonium nitrate and urea), two N timings of fertilization (at sowing or at the beginning of plant tillering), and additional treatment without N fertilization. Agronomic characteristics related to wheat productivity and N plant nutrition were evaluated. Irrigation increased the density of fertile spikes and the N accumulated in the shoot dry matter at anthesis, which partially explained the better grain yield in the irrigated condition. The higher N accumulation in the shoot dry matter was essential for grain yield increase in the environments with lower water deficit, based on their higher NUE for grain yield. Nitrogen fertilization reduced NUE for grain yield in Londrina (in rainfed and irrigation conditions), and increased NUE for shoot dry matter production in all environments. In a colder condition (i.e. Ponta Grossa), the use of a higher N rate at sowing provided greater grain yield, thousand-grain weight, and density of fertile spikes. Nitrogen rate can be reduced in warmer and wetter environmental conditions that favor the mineralization of soil organic matter and the decomposition of soybean straw. The choice for urea or ammonium nitrate can be based on economic criteria in environments with low water deficit and low potential for NH<sub>3</sub> volatilization. Nitrogen fertilization carried out exclusively at wheat sowing may be suitable to supply spring wheat N requirements.

**Keywords:** Ammonium nitrate; Nitrogen source of fertilizer; Nitrogen timing of fertilization; *Triticum aestivum*; Urea

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is worldwide used in human and animal nutrition. More than two million hectares are cultivated with wheat in Brazil, mainly in the states of Paraná and Rio Grande do Sul, which correspond to 85% of the national production (Conab, 2020a). However, Brazil has not been self-sufficient in wheat production, which leads it to import more than half of its demand to supply the domestic demand (Conab, 2020b). Improvements in grain yield through crop management can advance the competitiveness of the Brazilian wheat crop and eventually decrease its reliance on the external supply.

Fertilizer management has been associated to improvements in crop yield in historical time scales (Sinclair and Rufty, 2012). Among the essential mineral nutrients for plant development, nitrogen (N) stands out because it is present in several plant structures, such as cell walls, proteins, nucleic acids, enzymes, coenzymes, and chlorophyll molecules (Taiz et al., 2017). The N uptake by the roots is transported through the xylem via the respiratory chain, mainly in the forms of nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>). In addition, it is translocated via phloem from the leaves to other parts of the plant (such as grains and roots) in the forms of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, amino acids, and other assimilated products (Marschner, 2012).

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Nitrogen utilization efficiency (NUE) is usually calculated as the quotient of the grain dry mass and the N accumulated in the plant at maturity (Swiader et al., 1994). In wheat, yield is curvilinearly related to N uptake at maturity and opportunities to improve yield through increases in NUE are greater in high yielding environments (Oliveira Silva et al., 2020). Siddiqi and Glass (1981) proposed a specific efficiency index as a function of the nutrient concentration in the dry mass. They reported that plant growth is also related to the nutrient concentration in plant tissues and not only to the accumulated quantity since the growth only occurs from a minimum nutrient concentration in the plant tissue. According to Baligar et al. (2001), as this index takes into account both biometric and physiological parameters, it is an effective tool for assessing the nutritional status of the plants.

The complex dynamic of N in the soil and its interaction with weather conditions and plant residues released on the soil surface challenge the management of N fertilization (Tei et al., 2020). For example, when wheat is sown in heavy residue situations with a high carbon/N ratio, it is recommended to anticipate—at crop sowing—part or all N that would be provided as top dressing to reduce the effects of N immobilization by microbial activity (Wiethölter 2011; Marzi et al., 2020). However, high N rates at sowing can be ineffective to increase grain yield due to the lack of synchrony between the periods of N supply and higher plant demand (Chen et al., 2017; Clunes and Pinochet, 2020), decreasing the N use efficiency (Lollato et al., 2021). On the other hand, wheat plants that receive high N rates as top dressing are more prone to lodging (Khan et al., 2019).

The main N fertilizer sources used for wheat crop are ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) and urea [ $\text{CO}(\text{NH}_2)_2$ ], which have different agronomic efficiencies due to the influence of environmental conditions (mainly soil pH, humidity, and temperature) and the potential for ammonia ( $\text{NH}_3$ ) volatilization (Wiethölter, 2011; Santos et al., 2020). Ammonium nitrate contains 31% to 34% N, half in ammoniacal form and a half in nitric form, both readily available to plants. It is produced from the reaction of  $\text{NH}_3$  with nitric acid, presenting in the 2020 Brazilian crop season a cost per unit of N about 36% higher than urea (Conab, 2020c). Meanwhile, urea stands out as an N source due to its production from  $\text{NH}_3$  and carbon dioxide ( $\text{CO}_2$ ), which makes it less costly, associated with its high concentration of N (45%), which reduces its transport and storage cost. However, urea fertilization can result in greater N losses due to the activity of the urease enzyme (naturally present in the soil) that catalyzes the hydrolysis of urea into  $\text{NH}_4^+$ , which is susceptible to be converted into  $\text{NH}_3$  and lost by volatilization (Tasca et al., 2011). In addition, urea has restrictions on its simultaneous use with seed sowing, since

the direct contact of the seeds with the fertilizer can cause failures in the crop plant stand (Garcia et al., 2019).

Several studies evaluated the efficiency of N sources for some *Poaceae* crops, such as maize (Viero et al., 2014; Kaur et al., 2017), wheat (Viero et al., 2014; Galindo et al., 2019; Silva et al., 2019; Kaneko et al., 2020), rice (Liu et al., 2018; Amanullah et al., 2020), and sugar cane (Boschiero et al., 2018; Degaspari et al., 2020). However, the outcomes are inconclusive and with few exceptions (e.g. Hochman and Waldner, 2020) consider the influence of environmental and/or meteorological variables throughout the growing season. Thus, we hypothesize that the environmental conditions are key determinants of N release from N sources to plant uptake, influencing NUE and, consequently, wheat yield. Thus, the inclusion of environmental characteristics in the crop fertilization establishment can improve N management. Therefore, the aim of this work was to evaluate NUE and grain yield of the wheat crop in response to different sources, rates, and timings of N fertilization, considering the influence of the environmental conditions experienced in-season.

## MATERIAL AND METHODS

### Environmental description of the experimental sites

The experiment was carried out during the 2016 growing season in two edaphoclimatic environments on experimental farms of the Brazilian Agricultural Research Corporation (Embrapa) in the state of Paraná, Southern Brazil, one located in the region of Londrina ( $23^{\circ}11'37''$  S,  $51^{\circ}11'03''$  W; and 628 m a.s.l.) and another in Ponta Grossa ( $25^{\circ}08'59''$  S,  $50^{\circ}04'39''$  W; and 876 m a.s.l.). Both experiments were conducted under no tillage practices and the wheat crop was sown after a soybean [*Glycine max* (L.) Merrill] crop. In Londrina, rainfed ( $L_{\text{rainfed}}$ ) and irrigation ( $L_{\text{irrig}}$ ) conditions were used, while in Ponta Grossa only rainfed ( $PG_{\text{rainfed}}$ ), resulting in three different environments. The  $L_{\text{irrig}}$  and  $L_{\text{rainfed}}$  trials were arranged side by side, separated by 10-m distance between edges.

The soil at the experimental field in Londrina is a Rhodic Eutrudox, according to USDA Soil Taxonomy (Soil Survey Staff, 2014), or *Latossolo Vermelho entroférico* according to Brazilian Soil Classification System (Santos et al., 2018), with clay texture ( $732 \text{ g kg}^{-1}$  clay, and  $107 \text{ g kg}^{-1}$  sand). The landscape in the study site is smooth-rolling with mild slopes ( $\sim 15\%$ ). The soil had the following chemical characteristics in the surface layer (0–20 cm): pH in  $\text{CaCl}_2$  ( $0.01 \text{ mol L}^{-1}$ ) of 5.4,  $4.59 \text{ cmol}_c \text{ dm}^{-3}$  of potential acidity (H+Al),  $15.5 \text{ g dm}^{-3}$  of soil organic carbon,  $37.1 \text{ mg dm}^{-3}$  of available P (Mehlich-1),  $207 \text{ mg dm}^{-3}$  of exchangeable K,  $4.30 \text{ cmol}_c \text{ dm}^{-3}$  of exchangeable  $\text{Ca}^{2+}$ ,

2.24 cmol<sub>c</sub> dm<sup>-3</sup> of exchangeable Mg<sup>2+</sup>, base saturation of 60.5%, 0.01 cmol<sub>c</sub> dm<sup>-3</sup> of exchangeable Al<sup>3+</sup>, and cation exchange capacity (CEC) of 11.65 cmol<sub>c</sub> dm<sup>-3</sup>. The regional climate, according to the Köppen climate classification (Köppen, 1931), is humid subtropical (Cfa), with warmer and rainy summer, sparse frosts, no defined dry season, and with mean annual temperature and precipitation of 21.2 °C and 1,438 mm (Sibaldelli and Farias, 2019).

The landscape of Ponta Grossa is also smooth-rolling with mild slopes (~8%), and the soil at the experimental field is a Rhodic Hapludox (*Latosolo Vermelho distroférrico*), with clay texture (526 g kg<sup>-1</sup> clay, and 397 g kg<sup>-1</sup> sand), showing the following chemical characteristics in the surface layer (0–20 cm): pH of 4.7, 5.65 cmol<sub>c</sub> dm<sup>-3</sup> of potential acidity, 23.4 g dm<sup>-3</sup> of soil organic carbon, 7.9 mg dm<sup>-3</sup> of available P, 127 mg dm<sup>-3</sup> of exchangeable K, 2.94 cmol<sub>c</sub> dm<sup>-3</sup> of exchangeable Ca<sup>2+</sup>, 1.06 cmol<sub>c</sub> dm<sup>-3</sup> of exchangeable Mg<sup>2+</sup>, base saturation of 43.5%, 0.11 cmol<sub>c</sub> dm<sup>-3</sup> of exchangeable Al<sup>3+</sup>, and CEC of 9.97 cmol<sub>c</sub> dm<sup>-3</sup>. The regional climate is mesothermal humid subtropical (Cfb), with well-distributed rainfall, frequent frosts, and mean annual temperature and precipitation of 17.5 °C and 1,500 mm (Nitsche et al., 2019).

Meteorological variables were recorded during wheat growing season in meteorological stations located less than 800 m from the experimental areas. From this information the ten-day water balance, based on the method of Thornthwaite and Mather (1955), was calculated using spreadsheets in Excel<sup>TM</sup> developed by Rolim et al. (1998). This meteorological information was also used in the irrigated environment of Londrina for monitoring the need for water supplementation, which was confirmed in the field by means of tensiometers installed in the experimental area. A self-propelled sprinkler irrigation system was used for this purpose.

Prior to wheat sowing, an estimate of the amount of soybean straw in the experimental areas was performed by sampling four random sub-areas of 0.5 × 0.5 m (0.25 m<sup>2</sup>) within each plot. The straw samples were placed in a heater with forced air circulation at 65 °C during 72 h. After weighing the dry matter and dividing it by the sampled area, 4,550 and 5,870 kg ha<sup>-1</sup> of soybean straw dry matter were obtained for Londrina and Ponta Grossa, respectively.

### Experimental design and treatments

A randomized complete block design was used considering a complete (2 × 2 × 2) + 1 factorial arrangement plus a control with three replications. The factorial treatments were composed of two N rates (40 and 80 kg ha<sup>-1</sup>), two N sources (ammonium nitrate and urea), and two N timings of fertilization [at sowing or at the beginning of plant

tillering (growth stage GS21); Zadoks et al., 1974], and an additional treatment without N fertilization (N control). Nitrogen rates were based on technical indications for wheat crop in the state of Paraná (Foloni et al., 2016).

The experimental unit was 6 m long by 1.6 m wide (9.6 m<sup>2</sup>), consisting of nine 0.18 m spaced rows. The wheat was sown on April 29, 2016 in Londrina and on June 30, 2016 in Ponta Grossa, with a sowing-fertilizer drill developed for experimental plots and no tillage system. The seed density of 350 viable seeds m<sup>-2</sup> was established according to breeder's recommendation (Basso and Foloni, 2015), and seed depth was approximately 4 cm.

The wheat cultivar sown in the experiment was BRS Gralha-Azul (Basso and Foloni, 2015). It has been widely cultivated in the study region showing high grain yield, but it is prone to lodging, especially when high N rates are used in conditions of high water availability. It is classified as medium maturing with respect to phenology, with average crop cycle from seedling emergence until grain maturity of 110 days, and average plant height of 90 cm.

Nitrogen fertilization treatments applied at the day of wheat sowing were performed in furrow (depth of approximately 4 cm) before sowing using the same equipment used for sowing the crop later. Base fertilization was performed together with wheat sowing into the furrow of the previous N fertilization with 300 kg ha<sup>-1</sup> of 00–20–20 (N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O) formulated fertilizer, which was calculated according to soil chemical analysis and expected grain yield (CBPTI, 2016). Nitrogen fertilization treatments applied at GS21 were broadcast as top dressing.

Weeds, insects, and fungi diseases were controlled as needed using commercial pesticides according to technical recommendations for wheat production (CBPTI, 2016).

### Shoot dry mass, nitrogen utilization efficiency, grain yield and agronomic characteristics

Wheat shoot biomass was sampled when the plants were at anthesis (Zadoks growth stage GS69) by harvesting two rows 0.5 m long (0.18 m<sup>2</sup>). The shoot dry matter (SDM; kg ha<sup>-1</sup>) was determined after drying the samples, using a heater with forced air circulation at 65 °C during 72 h. Anthesis was chosen for this evaluation because it is the phenological stage with the highest N accumulation in the plant, thus presenting a greater contribution to wheat grain formation and yield (Wiethölter, 2011; Lollato et al., 2021).

The dried plant tissue samples were ground in a stainless steel Wiley mill, passed through a 0.1 mm sieve, for subsequent determination of N concentration in the SDM (N<sub>C<sub>SDM</sub></sub>; g kg<sup>-1</sup>) by the Kjeldahl method (Silva, 2009).

Nitrogen accumulated in the SDM ( $NA_{SDM}$ ;  $kg\ ha^{-1}$ ) i.e. an indirect measurement of N uptake was estimated by Equation 1:

$$N\ accumulated\ in\ the\ SDM\ (kg\ ha^{-1}) = \frac{SDM \times N\ concentration\ in\ the\ SDM}{1,000} \quad (Eq.1)$$

Nitrogen utilization efficiency for SDM ( $NUtE_{SDM}$ ;  $kg^2\ g^{-1}\ ha^{-1}$ ) production was based on the utilization index proposed by Siddiqi and Glass (1981), considering an area of one hectare, according to Equation 2:

$$NUtE_{SDM}\ (kg^2\ g^{-1}\ ha^{-1}) = \frac{(SDM)^2}{N\ accumulated\ in\ the\ SDM} \quad (Eq. 2)$$

Wheat grain yield ( $kg\ ha^{-1}$ ) was determined by harvesting plants at ripening (Zadoks growth stage GS92) using a self-propelled combine developed for small-plot of cereals. Harvested area covered seven rows wide by 6 m long, in each experimental plot. Grain moisture content was evaluated, recorded and adjusted to 13% for yield calculation. In addition, the hectoliter weight and the thousand-grain weight (IGW) were evaluated in the harvested grains.

Nitrogen utilization efficiency for grain yield ( $NUtE_{GY}$ ;  $kg\ kg^{-1}$ ) was estimated according to Crusciol et al. (2003) by means of the Equation 3 (grain yield at GS92, and N accumulated in the SDM at GS69; both in  $kg\ ha^{-1}$ ):

$$NUtE_{GY}\ (kg\ kg^{-1}) = \frac{Grain\ yield}{N\ accumulated\ in\ the\ SDM} \quad (Eq. 3)$$

Additionally, plant height and the density of fertile spikes (spikes  $m^{-2}$ ) were evaluated at GS69, and plant lodging (by the visual method adapted from Embrapa, 2009) at GS92.

### Statistical analysis

Statistical analyzes for each environment were performed using the R statistical software (R Core Team, 2020), through the 'ExpDes.pt' package (Ferreira et al., 2011). We used Shapiro-Wilk's test to evaluate the model's assumptions regarding normality of residuals, and Bartlett's test regarding variance homogeneity. According to these tests, no data transformation was needed. Since all assumptions required for a valid analysis of variance (ANOVA) were met, F-test ( $P \leq 0.05$ ) was performed. When the subject factors (i.e. the main effects) were significant but the interaction was not,

the comparisons between the two treatment means were performed within each factor by the F-test from ANOVA. On the other hand, when the interaction between factors was significant, the comparisons between the treatment means for one factor were performed individually within each level of the other factor by the Student-Newman-Keuls' test ( $P < 0.05$ ) (Wei et al., 2012).

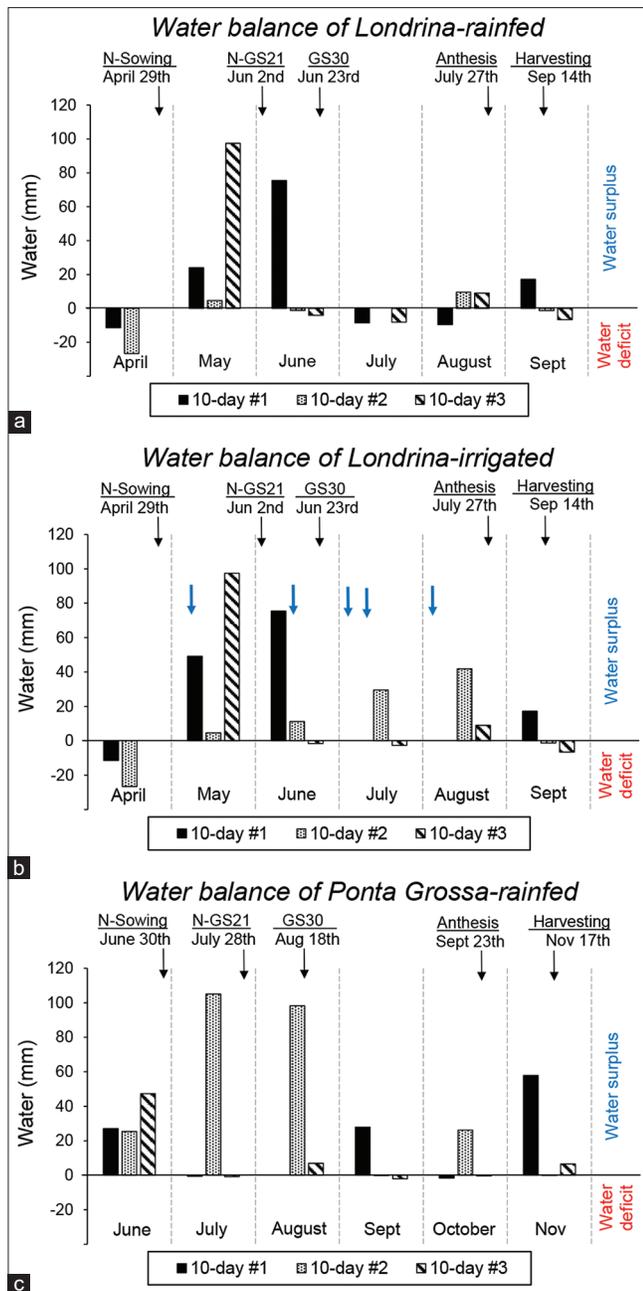
## RESULTS

### Water balance during wheat growing season

The three water balances at the field experiments are shown in Fig. 1. During the entire crop season in  $L_{rainfed}$ , there were an average temperature of  $17.6\ ^\circ C$  and total accumulated precipitation of 465.3 mm (data not shown). However, the rainfall distribution was irregular, resulting in an accumulated water deficit of 31.4 mm, concentrated in the phenological stages from stem elongation to the beginning of grain formation (Fig. 1a). On the other hand, in  $L_{irrig}$  the five 25-mm irrigation events carried out in drought periods increased the accumulated water supply to 590.3 mm and reduced the water deficit to an accumulated of only 4.3 mm (Fig. 1b). In  $PG_{rainfed}$  the average temperature was  $16.4\ ^\circ C$  and there was a suitable volume (526.4 mm) and distribution of rainfall during the wheat growing cycle, resulting in a low water deficit of 5.6 mm (Fig. 3c).

From wheat sowing to GS21 the soil moisture conditions were favorable for seed germination and initial establishment of the plant stand in all sites (Fig. 1). Then, there were some periods of water deficit in  $L_{rainfed}$ , distributed between the last third of tillering until the beginning of grain formation, with the culm elongation stage being the most affected by water stress (Fig. 1a). In turn, anthesis occurred in periods with low water availability in the three environments (Fig. 1), especially in  $L_{rainfed}$ , where the rainfall was reestablished two weeks after anthesis.

The fertilization at wheat sowing was carried out into moist soil; however, it was followed by 8, 5, and 12 days without significant water supply (i.e. accumulated rainfall  $\leq 4.3$  mm) in  $L_{rainfed}$ ,  $L_{irrig}$ , and  $PG_{rainfed}$ , respectively (data not shown). The top-dressing N fertilization at GS21 was carried out amid suitable water availability in  $L_{rainfed}$  and  $L_{irrig}$ , and drought conditions in  $PG_{rainfed}$  (Fig. 1a, 1b, and 1c). In  $L_{rainfed}$  and  $L_{irrig}$  there was a total of 78.3 mm of rainfall in the four days following fertilization, which was followed by a 39-day drought period, during which three 25-mm irrigations were carried out in  $L_{irrig}$  (Fig. 1b). Meanwhile, in  $PG_{rainfed}$  there was only 2.2 mm of rainfall in the 10 days following the top-dressing fertilization (data not shown).



**Fig 1.** Ten-day water balance in the wheat growing season of 2016 in Londrina [(April 29<sup>th</sup> to September 14<sup>th</sup> = 139 days; rainfed (a) and irrigation (b) conditions] and Ponta Grossa [(June 30<sup>th</sup> to November 17<sup>th</sup> = 141 days; rainfed (c) conditions]. Blue arrows (b) indicate irrigations of 25 mm carried out on May 4<sup>th</sup>, Jun 17<sup>th</sup>, July 1<sup>st</sup>, July 13<sup>th</sup>, and August 2<sup>nd</sup>. GS21 = Zadoks growth stage at the beginning of tillering; GS30 = Zadoks growth stage at the beginning of stem elongation

### Grain yield components and agronomic characteristics in wheat crop

Wheat grain yield averaged 3,117, 4,291, and 5,965 kg ha<sup>-1</sup> in  $L_{rainfed}$ ,  $L_{irrig}$ , and  $PG_{rainfed}$ , respectively (Table 1). Nitrogen fertilization increased grain yield by 25.2%, 19%, and 13.8% in  $L_{rainfed}$ ,  $L_{irrig}$ , and  $PG_{rainfed}$  as compared to the treatment without N fertilization (N control). Analyzing only the

factorial with N treatments, there was no effect of N rate or N source or N timing on grain yield in  $L_{rainfed}$  and  $L_{irrig}$  (Table 1). However, in  $PG_{rainfed}$  there was an interaction between N timing and N rate. The addition of 80 kg ha<sup>-1</sup> N at wheat sowing yielded 16% greater than 80 kg ha<sup>-1</sup> N at GS21, and 7.8% greater than 40 kg ha<sup>-1</sup> N at sowing (Table 2).

The density of fertile spikes averaged 387, 456, and 535 spikes m<sup>-2</sup> in  $L_{rainfed}$ ,  $L_{irrig}$ , and  $PG_{rainfed}$  (Table 3). Nitrogen fertilization increased the density of fertile spikes by an average of 18.7% in  $L_{rainfed}$ , as compared to the N control. In addition, in  $PG_{rainfed}$  the application of 80 kg ha<sup>-1</sup> N increased the density of fertile spikes by 14.4% compared to 40 kg ha<sup>-1</sup> N.

The grain features showed different responses to the environments and N treatments. The hectoliter weight was similar in the three sites and was not influenced by N fertilization or by the studied factors (i.e. N rate, source, and timing), showing high phenotypic stability (Table 3). In turn, the TGW increased on average 4.9% in  $L_{irrig}$  due to N fertilization, as compared to the N control (Table 1). In  $PG_{rainfed}$ , N fertilization at sowing increased the TGW by 4.2% compared to GS21 N timing. Regarding the N source, urea increased TGW by 5.7% in relation to ammonium nitrate, considering only the N rate of 80 kg ha<sup>-1</sup> in  $L_{rainfed}$  (Table 4). However, in  $L_{irrig}$  there was a 4.5% TGW decrease due to the use of urea instead of ammonium nitrate in the fertilization carried out at sowing. In addition, ammonium nitrate provided higher TGW when supplied at sowing compared to GS21 (Table 4).

In  $L_{rainfed}$ , N fertilization increased plant height by an average of 7.5% (Table 1). Plants fertilized at sowing showed an average height of 4.1% higher than those fertilized at GS21 in  $L_{rainfed}$ . On the other hand, increasing the N rate from 40 to 80 kg ha<sup>-1</sup> resulted in plants 4.7% shorter in  $L_{rainfed}$ .

The wheat crop grown in Londrina had severe lodging in all treatments (including the N control), with averages of 91% in  $L_{rainfed}$  and 97% in  $L_{irrig}$  (Table 3). In  $L_{irrig}$  the fertilization with urea increased lodging compared to ammonium nitrate.

The SDM averaged 6,615, 7,559, and 8,172 kg ha<sup>-1</sup> in  $L_{rainfed}$ ,  $L_{irrig}$ , and  $PG_{rainfed}$  (Table 1). Nitrogen fertilization increased SDM by 79%, 37.4% and 17.6% in  $L_{rainfed}$ ,  $L_{irrig}$  and  $PG_{rainfed}$  in comparison to the N control. In  $L_{irrig}$ , SDM increased by 7.3% with urea compared to ammonium nitrate; however, SDM decreased by 7.1% when N rate increased from 40 to 80 kg ha<sup>-1</sup>. On the other hand, in  $L_{rainfed}$  there was an interaction between N source and N timing so that the addition of ammonium nitrate at sowing increased the SDM by 24.6% compared to its application at GS21, and by 20.3% due to its replacement by urea (both applied at

**Table 1: Significance of the analysis of variance of the main effects (N rate, N source, and N timing of fertilization) and their interactions; and averages of grain yield, thousand-grain weight, plant height, and shoot dry matter of the wheat cultivar BRS Gralha-Azul in three environments: Londrina in rainfed ( $L_{rainfed}$ ) and irrigated ( $L_{irrig}$ ) conditions, and Ponta Grossa in rainfed ( $PG_{rainfed}$ )**

Source of variation	Df <sup>(c)</sup>	Grain yield			Thousand-grain weight			Plant height			Shoot dry matter		
		$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$
Block	2	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
N rate (R)	1	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*	*
N source (S)	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*
N timing (T)	1	ns	ns	***	ns	*	**	*	ns	ns	*	ns	ns
R x S	1	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
R x T	1	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
S x T	1	ns	ns	ns	ns	*	ns	ns	ns	ns	*	ns	ns
R x S x T	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Addit. vs Factorial <sup>(a)</sup>	1	**	*	***	ns	*	ns	*	ns	ns	***	***	*
CV (%) <sup>(b)</sup>		11.0	10.7	5.0	3.3	3.4	2.8	4.4	2.8	4.6	12.7	8.2	9.7
Factor	Treatment	Grain yield (kg ha <sup>-1</sup> )			Thousand-grain weight (g)			Plant height (cm)			Shoot dry matter (kg ha <sup>-1</sup> )		
		$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$
N rate	40 kg ha <sup>-1</sup>	3,084	4,289	5,986	35.8	31.9	36.3	80.6 a	87.4	87.4	6,827	8,081 a	7,947
	80 kg ha <sup>-1</sup>	3,292	4,448	6,107	35.8	32.2	35.7	76.8 b	86.9	88.9	7,086	7,509 b	8,673
N source	AN <sup>(d)</sup>	3,282	4,342	6,066	35.4	32.2	36.0	78.2	86.9	88.6	7,239	7,519 b	7,935
	Urea	3,095	4,395	6,027	36.4	31.9	36.0	79.3	87.3	87.7	6,674	8,071 a	8,684
N timing	Sowing	3,238	4,448	6,324	36.0	32.6	36.8 a	80.6 a	87.2	89.7	7,354	7,831	8,638
	GS21 <sup>(e)</sup>	3,138	4,289	5,769	35.8	31.5	35.3 b	76.9 b	87.0	86.5	6,559	7,759	7,982
Addit. vs Factorial	Additional	2,547 b	3,670 b	5,313 b	36.2	30.5 b	35.9	73.2 b	88.2	87.5	3,887 b	5,674 b	7,069b
	Factorial	3,188 a	4,368 a	6,047 a	35.9	32.0 a	36.0	78.7 a	87.1	88.1	6,956 a	7,795 a	8,310a

<sup>(a)</sup>Additional treatment = control without N fertilization; <sup>(b)</sup>CV = coefficient of variation. <sup>(c)</sup>Df = degrees of freedom (note: df from error=16) <sup>(d)</sup>AN = ammonium nitrate. <sup>(e)</sup>GS21 = Zadoks growth stage at the beginning of tillering. ns, \*, \*\* and \*\*\* = not significant, and significant at the  $P \leq 0.05$ ,  $\leq 0.01$  and  $\leq 0.001$  by the F-test. Means followed by different lowercase letters in the column, individually for each factor, differ from each other by the F-test. Comparisons with significant interaction between main effects have further interpretations in the Tables 2 (grain yield/ $PG_{rainfed}$ ), 4 (thousand-grain weight/ $L_{rainfed}$  and  $L_{irrig}$ ), 5 (shoot dry matter/ $L_{rainfed}$ ), 6 (shoot dry matter/ $PG_{rainfed}$ )

**Table 2: Unfolding of the significant interaction from ANOVA: 'N rate x N timing' for grain yield of the wheat cultivar BRS Gralha-Azul grown in Ponta Grossa in rainfed ( $PG_{rainfed}$ ) conditions**

N timing	Grain yield in $PG_{rainfed}$ (kg ha <sup>-1</sup> )	
	N rate	
	40 kg ha <sup>-1</sup>	80 kg ha <sup>-1</sup>
Sowing	6,086 aB	6,562 aA
GS21 <sup>(1)</sup>	5,886 aA	5,653 bA

Means followed by different lowercase letters in the column and uppercase letters in line differ from each other by the Student-Newman-Keuls' test ( $P \geq 0.05$ ). <sup>(1)</sup>GS21 = Zadoks growth stage at the beginning of tillering

sowing) (Table 5). Finally, in  $PG_{rainfed}$ , SDM was influenced by the interaction among some studied factors (Table 1). Considering the N rate of 40 kg ha<sup>-1</sup>, urea applied at sowing resulted in an average SDM increase of 26.7% in relation to its addition at GS21 or the use of ammonium nitrate (also at sowing) (Table 6).

### Nitrogen uptake and utilization efficiency by wheat plants

Nitrogen concentration in the SDM was not influenced by N fertilization as compared to the N control (Table 7). It increased by 5.8% in  $L_{irrig}$  and 8.9% in  $PG_{rainfed}$  due to N rate increase from 40 to 80 kg ha<sup>-1</sup>. Nitrogen fertilization at GS21

increased N concentration in the SDM by 19%, 12%, and 16.4% in  $L_{irrig}$  (with ammonium nitrate; Table 8),  $L_{rainfed}$  and  $PG_{rainfed}$  (Table 7), as compared to N fertilization at sowing. In addition, in  $L_{irrig}$ , the fertilization with urea increased the N concentration in the SDM by 9.8% compared to ammonium nitrate, both applied at sowing (Table 8).

The N accumulated in the SDM was strongly influenced by the environment, with averages of 92, 119, and 135 kg ha<sup>-1</sup> in  $L_{rainfed}$ ,  $L_{irrig}$ , and  $PG_{rainfed}$  (Table 7). In addition, N fertilization increased the N accumulated in the SDM by an average of 98% in  $L_{rainfed}$  and 41.4% in  $L_{irrig}$ . Increasing N rates from 40 to 80 kg ha<sup>-1</sup> increased the N accumulated in the SDM by 20.1% in  $PG_{rainfed}$ , without changes in the other environments. In  $L_{irrig}$ , urea fertilization at sowing increased by 24.8% the N accumulated in the SDM compared to ammonium nitrate (Table 8). In addition, N timing of fertilization influenced the effect of ammonium nitrate in  $L_{irrig}$ , so that its application at GS21 increased by 25% the N accumulated in the SDM, in relation to its use at sowing (Table 8).

The environmental effect on  $NUtE_{SDM}$  was small (Table 7). However,  $NUtE_{SDM}$  increased by 63%, 34%, and 27% in  $L_{rainfed}$ ,  $L_{irrig}$ , and  $PG_{rainfed}$  as a result of N fertilization, in

**Table 3: Significance of the analysis of variance of the main effects (N rate, N source, and N timing of fertilization) and their interactions; and averages of density of fertile spikes, hectoliter weight, and plant lodging of the wheat cultivar BRS Gralha-Azul in three environments: Londrina in rainfed ( $L_{rainfed}$ ) and irrigated ( $L_{irrig}$ ) conditions, and Ponta Grossa in rainfed ( $PG_{rainfed}$ )**

Source of variation	Df <sup>(c)</sup>	Density of fertile spikes			Hectoliter weight			Plant lodging		
		$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$
Block	2	ns	ns	ns	ns	ns	ns	ns	ns	-
N rate (R)	1	ns	ns	*	ns	ns	ns	ns	ns	-
N source (S)	1	ns	ns	ns	ns	ns	ns	ns	*	-
N timing (T)	1	ns	ns	ns	ns	ns	ns	ns	ns	-
R x S	1	ns	ns	ns	ns	ns	ns	ns	ns	-
R x T	1	ns	ns	ns	ns	ns	ns	ns	ns	-
S x T	1	ns	ns	ns	ns	ns	ns	ns	ns	-
R x S x T	1	ns	ns	ns	ns	ns	ns	ns	ns	-
Addit. vs Factorial <sup>(a)</sup>	1	*	ns	ns	ns	ns	ns	ns	ns	-
CV (%) <sup>(b)</sup>		10.1	11.1	12.5	4.0	1.2	6.0	3.3	2.4	-

Factor	Treatment	Density of fertile spikes (spikes m <sup>-2</sup> )			Hectoliter weight (kg hL <sup>-1</sup> )			Plant lodging (%)		
		$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$
N rate	40 kg ha <sup>-1</sup>	408	467	508 b	79.3	79.2	78.1	91.7	97.5	0
	80 kg ha <sup>-1</sup>	379	435	581 a	78.1	79.1	78.1	91.7	98.7	0
N source	AN <sup>(d)</sup>	397	453	556	78.1	79.4	77.9	92.1	97.0 b	0
	Urea	390	450	533	79.3	78.8	78.4	91.2	99.2 a	0
N timing	Sowing	408	449	570	78.7	79.5	78.2	92.1	97.9	0
	GS21 <sup>(e)</sup>	379	454	519	78.7	78.7	78.0	91.2	98.3	0
Addit. vs Factorial	Additional	331 b	491	463	80.2	79.3	76.8	91.7	96.8	0
	Factorial	393 a	451	544	78.7	79.1	78.1	91.7	98.1	0

<sup>(a)</sup>Additional treatment = control without N fertilization; <sup>(b)</sup>CV = coefficient of variation. <sup>(c)</sup>Df = degrees of freedom (note: df from error = 16) <sup>(d)</sup>AN = ammonium nitrate. <sup>(e)</sup>GS21 = Zadoks growth stage at the beginning of tillering. ns, \*, \*\* and \*\*\* = not significant, and significant at the  $P \leq 0.05$ ,  $\leq 0.01$  and  $\leq 0.001$  by the F-test. Means followed by different lowercase letters in the column, individually for each factor, differ from each other by the F-test

**Table 4: Unfolding of the significant interactions from ANOVA: 'N source x N rate' and 'N source x N timing' for thousand-grain weight (TGW) of the wheat cultivar BRS Gralha-Azul grown in Londrina in rainfed ( $L_{rainfed}$ ) and irrigated ( $L_{irrig}$ ) conditions**

N source	TGW in $L_{rainfed}$ (g)		TGW in $L_{irrig}$ (g)	
	N rate		N timing	
	40 kg ha <sup>-1</sup>	80 kg ha <sup>-1</sup>	Sowing	GS21 <sup>(1)</sup>
Ammonium nitrate	36.1 aA	34.8 bA	33.3 aA	31.1 aB
Urea	35.9 aA	36.8 aA	31.8 bA	31.9 aA

Means followed by different lowercase letters in the column and uppercase letters in line differ from each other by the Student-Newman-Keuls' test ( $P \geq 0.05$ ). <sup>(1)</sup>GS21 = Zadoks growth stage at the beginning of tillering

**Table 5: Unfolding of the significant interaction from ANOVA: 'N source x N timing' for shoot dry matter (SDM) production at anthesis of the wheat cultivar BRS Gralha-Azul grown in Londrina in rainfed ( $L_{rainfed}$ ) conditions**

N timing	SDM in $L_{rainfed}$ (kg ha <sup>-1</sup> )	
	N source	
	Ammonium nitrate	Urea
Sowing	8,031 aA	6,676 aB
GS21	6,447 bA	6,671 aA

Means followed by different lowercase letters in the column and uppercase letters in line differ from each other by the Student-Newman-Keuls' test ( $P \geq 0.05$ ). GS21 = Zadoks growth stage at the beginning of tillering

comparison to the N control (Table 7). Nitrogen fertilization at sowing (compared to GS21) increased  $NUtE_{SDM}$  by 44% in  $L_{rainfed}$  (only for ammonium nitrate; Table 8), 11.3% in  $L_{irrig}$

(Table 7), and 38.3% in  $PG_{rainfed}$  (in most cases; Table 9). Increasing N rates from 40 to 80 kg ha<sup>-1</sup> in  $L_{irrig}$  (Table 7) and  $PG_{rainfed}$  (only for urea at sowing; Table 9) reduced  $NUtE_{SDM}$  by 12.3% and 20.6%, respectively. In addition, in  $L_{rainfed}$ , the application of ammonium nitrate at sowing increased  $NUtE_{SDM}$  by 25.4% compared to urea (Table 8).

The  $NUtE_{GY}$  was lower in Londrina compared to Ponta Grossa, with averages of 35.4, 36.7, and 45.4 kg kg<sup>-1</sup> in  $L_{rainfed}$ ,  $L_{irrig}$ , and  $PG_{rainfed}$  (Table 7). Nitrogen fertilization reduced  $NUtE_{GY}$  only in Londrina, with an average decrease of 38% in  $L_{rainfed}$  and 15% in  $L_{irrig}$ , as compared to the N control (Table 7). The N rates, sources, and timings did not influence  $NUtE_{GY}$  in  $L_{rainfed}$ . However, in  $PG_{rainfed}$ , the fertilization at sowing increased  $NUtE_{GY}$  by 17.1% compared to GS21; while increasing N rate from 40 to 80 kg ha<sup>-1</sup> decreased the  $NUtE_{GY}$  by 15.7% (Table 7). Finally, in  $L_{irrig}$ , the ammonium nitrate applied at sowing provided the highest  $NUtE_{GY}$ , which was on average 26.7% greater than the other combinations between N rate and N timing (Table 8).

## DISCUSSION

### Weather and field conditions influences on wheat crop

The assessment of the three environments allowed for a wide range in yielding conditions. The  $PG_{rainfed}$  was the most

favorable environment to wheat growth and yield, which was 91% greater than  $L_{rainfed}$ . While all environments had precipitation amounts that could be considered non-limiting

**Table 6: Unfolding of the significant triple interaction from ANOVA: 'N rate x N source x N timing' for shoot dry matter (SDM) at anthesis of the wheat cultivar BRS Graha-Azul grown in Ponta Grossa in rainfed ( $PG_{rainfed}$ ) conditions**

N timing	SDM in $PG_{rainfed}$ ( $kg\ ha^{-1}$ )			
	N rate			
	40 $kg\ ha^{-1}$		80 $kg\ ha^{-1}$	
	Ammonium nitrate	Urea	Ammonium nitrate	Urea
Sowing	7,584 aB	9,551 aA	8,989 aA	8,427 aA
GS21	7,163 aA	7,491 bA	8,006 aA	9,270 aA
N source	N rate			
	40 $kg\ ha^{-1}$		80 $kg\ ha^{-1}$	
	Ammonium nitrate	Urea	Ammonium nitrate	Urea
	40 $kg\ ha^{-1}$	80 $kg\ ha^{-1}$	40 $kg\ ha^{-1}$	80 $kg\ ha^{-1}$
Sowing	7,584 aB	8,989 aA	9,551 aA	8,427 aA
GS21	7,163 aA	8,006 aA	7,491 bB	9,270 aA

Means followed by different lowercase letters in the column [individually for each unfolding (N source/N rate; or N rate/N source)] and uppercase letters in line (comparing ammonium nitrate with urea individually for each N rate; or comparing 40 with 80  $kg\ ha^{-1}$  N individually for each N source) differ from each other by the Student-Newman-Keuls' test ( $P \geq 0.05$ ). GS21 = Zadoks growth stage at the beginning of tillering

to wheat production (Passioura and Angus, 2010; Patrignani et al., 2014), the lower water deficit and particularly better moisture distribution during the season, as well as the more suitable temperature for the winter crop in  $PG_{rainfed}$ , could justify the greater yields in this environment. In fact, the later sowing (on June 30<sup>th</sup>) in  $PG_{rainfed}$  allowed its tillering stage to occur at lower temperatures, which for spring wheat favor tiller development and the production of fertile spikes, which is one of the main components of wheat yield (Slafer et al., 2014; Lorenzo et al., 2015). In addition, the better water availability in  $PG_{rainfed}$  favored the uptake of nutrients such as N, which is essential for increasing the plant tillering (Yousaf et al., 2014; Zhang et al., 2020) and grain development.

The additional water supply in  $L_{irrig}$  was decisive for increasing the average grain yield (38% higher than in  $L_{rainfed}$ ). Irrigation increased the density of fertile spikes and the N accumulated in the SDM, which partially explains the better grain wheat in  $L_{irrig}$ . Considering that the TGW was reduced by irrigation, other yield components (such as the number of spikelets/spike and grains/spikelet), besides the number of spikes  $m^{-2}$ , were likely responsible for the higher yield in  $L_{irrig}$ . Additionally, the lower TGW

**Table 7: Significance of the analysis of variance of the main effects (N rate, N source, and N timing of fertilization) and their interactions; and averages of N concentration in the shoot dry matter (SDM), N accumulated in the SDM, and N utilization efficiency (NUE) for grain yield (GY) and for SDM production at anthesis of the wheat cultivar BRS Graha-Azul in three environments: Londrina in rainfed ( $L_{rainfed}$ ) and irrigated ( $L_{irrig}$ ) conditions, and Ponta Grossa in rainfed ( $PG_{rainfed}$ )**

Source of variation	Df <sup>(c)</sup>	N concentration in the SDM			N accumulated in the SDM			NUE for SDM			NUE for GY		
		$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$
Block	2	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N rate (R)	1	ns	*	**	ns	ns	**	ns	**	ns	ns	ns	**
N source (S)	1	ns	ns	ns	ns	**	ns	ns	ns	*	ns	ns	ns
N timing (T)	1	**	***	***	ns	**	ns	**	*	***	ns	*	**
R x S	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R x T	1	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
S x T	1	ns	**	ns	ns	***	ns	*	ns	ns	ns	**	ns
R x S x T	1	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
Addit. vs Factorial <sup>(a)</sup>	1	ns	ns	ns	***	***	ns	***	***	**	***	*	ns
CV (%) <sup>(b)</sup>		9.9	5.6	6.0	14.7	7.7	12.9	15.6	11.5	10.7	15.4	13.1	13.9
Factor	Treatment	N concentration in the SDM ( $g\ kg^{-1}$ )			N accumulated in the SDM ( $kg\ ha^{-1}$ )			NUE for SDM ( $kg^2\ g^{-1}\ ha^{-1}$ )			NUE for GY ( $kg\ kg^{-1}$ )		
		$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$	$L_{rainfed}$	$L_{irrig}$	$PG_{rainfed}$
N rate	40 $kg\ ha^{-1}$	13.7	15.4 b	15.7 b	93	124	124 b	510	529 a	515	33.4	35.1	49.4 a
	80 $kg\ ha^{-1}$	14.4	16.3 a	17.1 a	102	122	149 a	498	464 b	511	32.9	36.9	41.9 b
N source	AN <sup>(d)</sup>	14.2	15.7	16.7	101	118	132	525	485	484	32.7	37.5	47.2
	Urea	13.9	16.0	16.2	93	129	140	482	509	542	33.6	34.4	44.2
N timing	Sowing	13.3 b	15.0	15.2 b	97	118	131	558	523 a	573	33.7	38.4	49.3 a
	GS21 <sup>(e)</sup>	14.9 a	16.6	17.7 a	97	129	141	449	470 b	453	32.6	33.6	42.1 b
Addit. vs Factorial	Additional	12.5	15.3	17.5	49 b	87 b	124	310 b	372 b	404 b	53.1 a	42.3 a	43.2
	Factorial	14.1	15.8	16.4	97 a	123 a	136	504 a	497 a	513 a	33.1 b	36.0 b	45.7

<sup>(a)</sup>Additional treatment = control without N fertilization; <sup>(b)</sup>CV = coefficient of variation. <sup>(c)</sup>Df = degrees of freedom (note: df from error=16) <sup>(d)</sup>AN = ammonium nitrate. <sup>(e)</sup>GS21 = Zadoks growth stage at the beginning of tillering. ns, \*, \*\* and \*\*\* = not significant, and significant at the  $P \leq 0.05$ ,  $\leq 0.01$  and  $\leq 0.001$  by the F-test. Means followed by different lowercase letters in the column, individually for each factor, differ from each other by the F-test. Comparisons with significant interaction between main effects have further interpretations in the Tables 8 (N conc. SDM/ $L_{irrig}$ , N accum. SDM/ $L_{irrig}$ , NUE for SDM/ $L_{rainfed}$ , NUE for GY/ $L_{irrig}$ ), and 9 (NUE for SDM/ $PG_{rainfed}$ )

**Table 8: Unfolding of the significant interactions from ANOVA: 'N source × N timing' for N concentration in the shoot dry matter (SDM), N accumulated in the SDM, and N utilization efficiency (NUE) for grain yield (GY) and for SDM production at anthesis of the wheat cultivar BRS Gralha-Azul grown in Londrina in rainfed ( $L_{rainfed}$ ) and irrigated ( $L_{irrig}$ ) conditions**

N timing	N source			
	Ammonium nitrate	Urea	Ammonium nitrate	Urea
	N concentration in the SDM in $L_{irrig}$		N accumulated in the SDM in $L_{irrig}$	
	(g ka <sup>-1</sup> )		(kg ha <sup>-1</sup> )	
Sowing	14.3 bB	15.7 aA	105 bB	131 aA
GS21	17.0 aA	16.2 aA	131 aA	126 aA
	NUE for SDM in $L_{rainfed}$		NUE for GY in $L_{irrig}$	
	(kg <sup>2</sup> g <sup>-1</sup> ha <sup>-1</sup> )		(kg kg <sup>-1</sup> )	
Sowing	620.3 aA	496.4 aB	42.7 aA	34.1 aB
GS21	430.5 bA	467.7 aA	32.4 bA	34.7 aA

Means followed by different lowercase letters in the column and uppercase letters in line differ from each other by the Student-Newman-Keuls' test ( $P \geq 0.05$ ). GS21 = Zadoks growth stage at the beginning of tillering

**Table 9: Unfolding of the significant triple interaction from ANOVA: 'N rate × N source × N timing' for N utilization efficiency (NUE) for shoot dry matter (SDM) production at anthesis of the wheat cultivar BRS Gralha-Azul grown in Ponta Grossa in rainfed ( $PG_{rainfed}$ ) conditions**

N timing	NUE for SDM in $PG_{rainfed}$ (kg <sup>2</sup> g <sup>-1</sup> ha <sup>-1</sup> )			
	N rate			
	40 kg ha <sup>-1</sup>		80 kg ha <sup>-1</sup>	
	Ammonium nitrate	Urea	Ammonium nitrate	Urea
Sowing	538 aB	660 aA	569 aA	524 aA
GS21	410 bA	451 bA	417 bB	533 aA
	N source			
	40 kg ha <sup>-1</sup>		80 kg ha <sup>-1</sup>	
	Ammonium nitrate	Urea	Ammonium nitrate	Urea
Sowing	538 aA	569 aA	660 aA	524 aB
GS21	410 aA	417 bA	451 bA	533 aA

Means followed by different lowercase letters in the column [individually for each unfolding (N source/N rate; or N rate/N source) and uppercase letters in line (comparing ammonium nitrate with urea individually for each N rate; or comparing 40 with 80 kg ha<sup>-1</sup> N individually for each N source) differ from each other by the Student-Newman-Keuls' test ( $P \geq 0.05$ ). GS21 = Zadoks growth stage at the beginning of tillering

due to irrigation might have just been a compensating mechanism behind more grains m<sup>-2</sup> (Slafer et al., 2021), as the distal grains are naturally lighter than the central grains in a spikelet. Maqbool et al. (2015) also obtained an increase in these yield components with the use of irrigation in wheat crops, corroborating our results. Thus, wheat grain yield is highly dependent on the genotype × environment interaction (Du et al., 2020).

Plant height was reduced by the higher water deficit in  $L_{rainfed}$  compared to the other two environments. In fact, water turgor is an essential factor for cell expansion (Zubairova et al., 2016) and consequently for stem elongation. Shorter

culms are generally more structurally resistant (Piñera-Chaves et al., 2016), which helps to explain the lower lodging in  $L_{rainfed}$  compared to  $L_{irrig}$ .

Plant lodging was null in  $PG_{rainfed}$ , despite greater plant height, spike density per area, grain yield, N accumulated in the SDM, and water availability (as compared to  $L_{rainfed}$ ), all factors that predispose plants to lodging (Ma et al., 2016; Khan et al., 2020). Another factor that increases lodging is the wind speed (Ma et al., 2016). However, the wind blasts were smaller in the environment with higher lodging, i.e. the highest values were 15 m s<sup>-1</sup> in  $L_{rainfed}$  and  $L_{irrig}$  (at the 111<sup>th</sup> day of the growing cycle) and 42.5 m s<sup>-1</sup> in  $PG_{rainfed}$  (also at the 111<sup>th</sup> day of the cycle), both at an intermediate grain formation stage (data not shown). Therefore, we hypothesize that the plant lodging tolerance in  $PG_{rainfed}$  might have been associated with the greater culm resistance favored by the environmental conditions. In this context, Zhang et al. (2017) evaluated the culm structure and concluded that wheat plants that have culm with a thicker cell wall and denser basal internodes are less susceptible to lodging and that these features can be improved with the proper use of N. In addition, the optimization of N fertilization management can also reduce plant lodging—including for susceptible cultivars—by favoring the accumulation of lignin and cellulose, which can decrease the rate of culm breaking (Chen et al., 2018; Khan et al., 2020).

The better water availability in  $L_{irrig}$  and  $PG_{rainfed}$  (compared to  $L_{rainfed}$ ) increased grain yield proportionally more than SDM. Therefore, plants allocated a greater amount of biomass for grain formation while the water was not a limiting factor for vital processes, such as nutrient uptake, photosynthesis, and photoassimilate translocation for grain formation.

The N concentration in the SDM was higher in environments with lower water deficit ( $L_{irrig}$  and  $PG_{rainfed}$ ). Therefore, considering that the N concentration has a high positive correlation with cellular metabolic activity (Marschner, 2012), this fact helps to explain the higher grain yields in these environments compared to  $L_{rainfed}$ .

The N uptake by the plants can be indirectly evaluated by means of the N accumulated in the plant biomass. There was a higher N accumulated in the SDM in  $L_{irrig}$  and  $PG_{rainfed}$  compared to  $L_{rainfed}$ , i.e. the N uptake was compromised by the higher water deficit in the latter. The higher N accumulation in the SDM was important for grain yield increase, based on the higher  $NUE_{GY}$  values in the environments with lower water deficit whose average  $NUE_{GY}$  was 28.2% higher than in  $L_{rainfed}$ .

### Nitrogen fertilization effects on wheat crop

In general, N fertilization improved grain yield and SDM in the three environments, with greater percentage increases in the site with the higher water deficit ( $L_{rainfed}$ ). This can be attributed to the increase of  $NH_4^+$  and  $NO_3^-$  concentrations in the soil solution, which increased the amount of N transported in the soil and its uptake by the root system. Therefore, in these environments with limitations to provide N (either related to soil N concentration or due to N deficiency induced by water deficit), the response to N fertilization was proportionally greater while the accumulated water deficit increased. In addition,  $L_{rainfed}$  was the only environment with an increase in the density of fertile spikes in response to N fertilization, which justifies the higher response of the wheat crop by increasing grain yield and SDM production. Finally, the SDM increase in  $L_{rainfed}$  (in response to N fertilization) was proportionally greater than the grain yield increase, i.e. in conditions of water deficit, the plants prioritized the applied and uptake N for the production of vegetative structures at the expense of grain formation.

The increase of TGW due to N fertilization in  $L_{irrig}$  contributed to the increase of grain yield in this irrigated environment. In addition, as  $L_{rainfed}$  and  $PG_{rainfed}$  had higher average TGW (36 g) compared to  $L_{irrig}$  (31.9 g), it shows that there was still an opportunity to increase TGW in  $L_{irrig}$ . The decrease of TGW in irrigated environments was observed by Yan et al. (2019) when the fertilizer was provided in a smaller amount than necessary for wheat development, because the nutritional deficiency—mainly of N—results in the production of lighter grains, even if the wheat crop is favored by suitable water availability.

The plant height increased due to N fertilization, but only in  $L_{rainfed}$ . This is due to the increase of  $NH_4^+$  and  $NO_3^-$  concentrations in the soil solution, as previously discussed. The higher water availability in  $L_{irrig}$  and  $PG_{rainfed}$  likely favored the mineralization of the soil organic matter and the decomposition of the soybean straw resulting in a greater N supply for plant uptake to reach greater plant height.

Plant lodging was not influenced by N fertilization, as compared to the N control. This was not expected, because N usually increases lodging (Teixeira Filho et al., 2007; Teixeira Filho et al., 2010; Zhang et al., 2017; Wu et al., 2019). In  $L_{rainfed}$  and  $L_{irrig}$  all treatments had a high lodging rate, indicating that other factors (besides N) must be involved in this process. Considering the regional average of about 2,500 kg ha<sup>-1</sup> of wheat grain yield (CONAB, 2020a), there is evidence that the higher grain weight in  $L_{rainfed}$  (3,117 kg ha<sup>-1</sup>) and in  $L_{irrig}$  (4,291 kg ha<sup>-1</sup>) may have predisposed the plants to lodging (due to the increased grain load per plant).

Nitrogen fertilization did not change the N concentration in the SDM at anthesis. Therefore, based on Equation 1, the increments of N accumulated in the SDM were exclusively due to the increase of SDM production. Thus, there is an indication that the plant used a physiological buffer to maintain the N concentration in its tissues within a sufficiency range for this phenological stage.

The SDM increases were proportionally greater than the grain yield increases in response to N fertilization. As a result, there were increases in  $NUtE_{SDM}$  in the three environments and a reduction in  $NUtE_{GY}$  in  $L_{rainfed}$  and  $L_{irrig}$ . These changes in  $NUtE$  were of higher magnitude in  $L_{rainfed}$  because this was the environment proportionally more responsive to N fertilization in terms of grain yield, SDM production, and N accumulated in the SDM. Therefore,  $NUtE$  is strongly controlled by environmental conditions, especially by water availability which directly influences plant response to N fertilization.

### Nitrogen fertilizer management for wheat crop

The N fertilization management considered the combination of rates, sources, and timings of N application, focusing on the evaluation of the influence of environmental conditions on the outcomes.

Nitrogen fertilization management did not influence wheat grain yield in most cases, i.e. it was indifferent to the application of 40 or 80 kg ha<sup>-1</sup> N, in the form of ammonium nitrate or urea, applied at sowing or at GS21. This result was not expected, as the N rates were based on technical indications for wheat crop in the state of Paraná (Foloni et al., 2016), especially considering the high grain yields obtained in the three environments, as the optimal N rate is usually greater in higher yielding environments (Lollato et al., 2019). Thus, it can be inferred that a significant part of the N uptake by the plants might have derived from the native N present in the soil and in the soybean straw. In fact, the conditions of low water deficit and suitable temperatures (averages of 17.6 °C in Londrina and 16.4 °C in Ponta Grossa) likely favored microbial activity, allowing the mineralization of the soil organic matter and the decomposition of the straw, which were important to provide N to the plants. In this context, there was a large amount of soybean straw in Londrina (4,550 kg ha<sup>-1</sup>) and in Ponta Grossa (5,870 kg ha<sup>-1</sup>) at the time of sowing, representing an additional source of N.

Analyzing the other agronomic and nutritional features of the wheat crop, it turns out that they were influenced by at least one of the three ways of N fertilization management, except for the hectoliter weight that remained unchanged.

The increment of the density of fertile spikes due to the increase of N rate is due to the increase of plant tillering, as it was observed in other studies (Shirazi et al., 2014; Yang et al., 2019). On the other hand, reductions of plant height and SDM by increasing the N rate were not expected, and are likely associated with some nutritional imbalance. In this context, positive effects of N rate increasing on wheat height and SDM are frequently reported (Pradhan et al., 2018; Si et al., 2020), whereas negative effects have been verified only at high rates of N ( $\geq 180 \text{ kg ha}^{-1}$ ) (Lu et al., 2015; Si et al., 2020).

The increases of N concentration and N accumulated in the SDM due to the increase of N rate were expected in all sites, however, they happened only in environments with higher water availability (i.e.  $L_{\text{irrig}}$  and  $PG_{\text{rainfed}}$ ). These outcomes contributed to the decreases of  $NUtE_{\text{SDM}}$  in  $L_{\text{irrig}}$  and  $NUtE_{\text{GY}}$  in  $PG_{\text{rainfed}}$ , corroborating results obtained in field conditions by Zheng et al. (2017). This reinforces that water availability is a determining factor of the N fertilizer utilization efficiency by plants (Shirazi et al., 2014). The  $NUtE_{\text{GY}}$  is an agronomic characteristic mainly determined by the plant genetic features, thus, the selection of wheat cultivars considering this characteristic allows to maximize grain yield with the lower expenditure of N fertilizers (Cohan et al., 2019).

The N sources affected several wheat crop characteristics, being influenced by the environmental conditions. But, these effects were predominantly observed when unfolding the interactions with other N management practices. Therefore, the effects of N sources were variable and inconclusive. However, we found that the differences between the N sources were predominantly expressed when fertilization was carried out at wheat sowing. In addition, better effects of urea fertilizer happened more frequently in environments with lower water deficit throughout the crop cycle, i.e. in  $L_{\text{irrig}}$  and  $PG_{\text{rainfed}}$ .

The option for urea fertilizer is more suitable for environments with greater water availability immediately following its application since its faster solubilization and infiltration into the soil reduces the N losses due to  $\text{NH}_3$  volatilization (Lara Cabezas et al., 1997; Santos et al., 2020). In addition, the placement of urea into the soil together with seed sowing is another practice adopted to reduce  $\text{NH}_3$  volatilization and, consequently, increase urea agronomic efficiency (Wiethölter, 2011). In the present study, there were unfavorable conditions for  $\text{NH}_3$  volatilization after the top-dressing fertilization carried out at GS21 in  $L_{\text{rainfed}}$  and  $L_{\text{irrig}}$ , because there were 78.3 mm of rainfall in the four days following this fertilization, which incorporated urea into the soil. On the other hand, in  $PG_{\text{rainfed}}$  the topsoil and the plant residues on the soil surface remained dry in the

11 days following the top-dressing fertilization, and the soil pH (4.7) was very acidic, conditions that inhibit the activity of the enzyme urease (Perin et al., 2020). Therefore, these environmental conditions were unfavorable to  $\text{NH}_3$  volatilization, bringing agronomic efficiency closer between the two N fertilizer sources.

Considering the N fertilization timing, better results were predominantly obtained with N applied at sowing, as compared to top dressing at GS21. Analyzing this information, it is clear the higher frequency of 'N timing fertilization effects' with ammonium nitrate (compared to urea), indicating that the best timing for wheat fertilization may be influenced by the N source.

Nitrogen fertilization carried out exclusively at wheat sowing is generally not an efficient practice to maximize grain yield and SDM. Some studies reported higher wheat yield when N fertilization was performed only in top dressing (in a single or split application) in phenological stages from GS21 onwards (Shirazi et al., 2014; Si et al., 2020; Lollato et al., 2021). However, some of the reports showing advantages of a split N application evaluated winter wheat, which has a much longer crop cycle as compared to spring wheat. To the best of our knowledge, there are no studies showing advantages of the exclusive N fertilization at sowing related to higher increases of wheat grain yield compared to other N timing methods of fertilization. A potential cause of this discrepancy was the addition of N fertilizer in furrow prior to sowing in the current study as compared to broadcast immediately after sowing in many other studies. In this context, care must be taken to avoid that high N rates cause a saline effect that impairs seed germination and seedling development. In addition, in environments with sandy soils associated with a high amount of rainfall the N losses by leaching are intense, which means that N fertilization must be split out to synchronize the supply with the demand of N by the plant along its growing cycle.

On the other hand, the higher values of N concentration and N accumulated in the SDM obtained in treatments fertilized at GS21 can be an advantage when the objective is to increase the protein concentration in wheat grains, as reported by Lollato et al. (2021). This becomes more important for the farmer when there is a bonus/premium paid by the bakery industry for wheat with higher gluten content.

The decision on how to manage N fertilization depends on the expected environmental conditions throughout the crop cycle, especially on water availability, and must consider a strategy that allows for the best economic return, either by reducing costs or by increasing the yield and/or quality of the grains.

## CONCLUSIONS

The N fertilization management must consider the expected and/or the history of environmental conditions during the wheat growing cycle in each environment. The N rate can be reduced in warmer and wetter environmental conditions that favor the mineralization of soil organic matter and the decomposition of soybean straw. The choice for urea or ammonium nitrate can be based on economic criteria in environments with low water deficit and low potential for  $\text{NH}_3$  volatilization. Nitrogen fertilization carried out exclusively at wheat sowing may be suitable to supply spring wheat N requirements, reducing operating costs.

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## Authors' contributions

Letícia Ferreira: investigation, analysis and interpretation, visualization, writing – original draft. Sérgio Silva: conceptualization, study design, methodology, data acquisition, analysis and interpretation, supervision, validation, writing – original draft. Rômulo Pisa Lollato: visualization, writing – review & editing. Eric Batista Ferreira: statistical guidance, visualization. Oriol Kölln: visualization, writing – review & editing.

## Abbreviations

$L_{\text{irrig}}$	Londrina in irrigated conditions
$L_{\text{rainfed}}$	Londrina in rainfed conditions
NUtE	Nitrogen utilization efficiency
NUtE <sub>GY</sub>	Nitrogen utilization efficiency for grain yield
NUtE <sub>SDM</sub>	Nitrogen utilization efficiency for shoot dry matter production
PG <sub>rainfed</sub>	Ponta Grossa in rainfed conditions

GS21	Growth stage at the beginning of plant tillering
GS69	Growth stage at anthesis
GS92	Growth stage at ripe grain ready for harvest
SDM	Shoot dry matter
TGW	Thousand-grain weight

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