Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga
ISSN 1983-2125 (online)

http://dx.doi.org/10.1590/1983-21252024v3711728rc

Scientific Article

Nitrogen and potassium interaction in oxisol soils under BRS 394 wheat cultivation

Interação nitrogênio e potássio em latossolo sob cultivo de trigo BRS 394

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ABSTRACT - Potassium and nitrogen are essential nutrients and their uptake and distribution within plants must be coordinated for optimum growth and development. We evaluated the interaction between nitrogen and potassium fertilization on the development of BRS 394 wheat, and the nitrate and ammonium levels in Oxisol soils. A 5^2 factorial design was used in a greenhouse experiment, with four replicates in randomized blocks, corresponding to five doses of nitrogen (0, 70, 140, 210, and 280 mg dm^{-3}) and five doses of potassium (0, 50, 100, 150, and 200 mg dm^{-3}). The variables analyzed were shoot dry mass; chlorophyll index; nitrate reductase; nitrogen, potassium, and protein concentrations in the leaves; and nitrate and ammonium levels in the soil. There was an interaction between nitrogen and potassium doses on the chlorophyll index, with nitrogen doses between 152.67 and 163.53 mg dm^{-3} and potassium of 191.50 mg dm^{-3} providing the highest chlorophyll index. The other variables were influenced separately by the nitrogen dose. The highest nitrate reductase enzyme activity was 1.30 μmol NO h^{-1} g^{-1} Fresh Matter, which was reached at a nitrogen dose of 95 mg dm^{-3}. Shoot dry mass, concentrations of nitrogen and total protein in the leaves, and concentrations of nitrate and ammonium in the soil were adjusted to the linear regression model as a function of nitrogen fertilization. The dose of nitrogen that promoted the best development of BRS 394 wheat and the highest concentrations of nitrate and ammonium in Oxysol soils was 280 mg dm^{-3}.

Keywords: *Triticum aestivum*. Nitrate reductase. Nitrogen fertilization. Potassium fertilization.

RESUMO - O potássio e nitrogênio são nutrientes essenciais, e sua absorção e distribuição dentro da planta devem ser coordenadas para um crescimento e desenvolvimento ideais. Objetivou-se avaliar a interação entre adubação com nitrogênio e potássio no desenvolvimento do trigo BRS 394 e teores de nitrato e amônio em Latossolo Vermelho. Utilizando-se delineamento fatorial 52, com quatro repetições, em blocos casualizados, correspondendo a cinco doses de nitrogênio (0, 70, 140, 210 e 280 mg dm^{-3}) e potássio (0, 50, 100, 150 e 200 mg dm^{-3}). As variáveis analisadas foram massa seca da parte aérea, índice de clorofila, nitrato redutase, concentração de nitrogênio, potássio e teor de proteína nas folhas, teor de nitrato e amônio no solo. Houve interação entre as doses de nitrogênio e potássio para a variável índice de clorofila, sendo as doses de nitrogênio entre 152,67 e 163,53 mg dm^{-3} de nitrogênio e a dose 191,50 mg dm^{-3} de potássio que proporcionaram maiores índices de clorofila. As demais variáveis foram influenciadas isoladamente pelas doses de nitrogênio. A maior atividade da enzima nitrato redutase foi de 1,30 μmol NO h^{-1} g^{-1} Massa Fresca na dose de nitrogênio de 95 mg dm^{-3}. A massa seca da parte aérea, concentração de nitrogênio e proteína total nas folhas, e concentração de nitrato e amônio no solo apresentaram ajuste ao modelo de regressão linear em função da adubação nitrogenada. A dose de nitrogênio que promoveu melhor desenvolvimento do trigo BRS 394 e maiores concentrações de nitrato e amônio no solo foi 280 mg dm^{-3}.

Palavras-chave: *Triticum aestivum*. Nitrato redutase. Adubação nitrogenada. Adubação potássica.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: February 7, 2023.
Accepted in: September 8, 2023.

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INTRODUCTION

Wheat (*Triticum aestivum*) is widely consumed by humans and is one of the most essential cereal crops worldwide (FREITAS et al., 2018). In addition to its human consumption, wheat is widely used as silage, and the quality and yield of this cereal largely depend on the phenological stage and management of nutrients, such as nitrogen (WROBEL et al., 2018). The BRS 394 wheat cultivar has an early growth cycle and was specifically developed by EMBRAPA for cultivation in the Cerrado (Savannah) region, showing high potential for grain productivity and quality (ALBRECHT et al., 2020). However, fertilizer management is essential to achieve the envisioned results regarding wheat quality and productivity, because Savannah soils, such as Oxisols, are generally acidic and have low natural fertility.

Potassium and nitrogen are essential nutrients and their uptake and distribution within plants must be coordinated to achieve optimum growth and development. Potassium is involved in the charge balance between inorganic and organic anions and macromolecules, the control of membrane electrical potential, pH homeostasis, and the regulation of cellular osmotic pressure, whereas nitrogen is an essential component of amino acids, proteins, and nucleic acids. Nitrate (NO^{3-}) is usually the main source of nitrogen, although it also serves as a signaling molecule for plants (RADDATZ et al., 2020).

After being taken up by the plant, nitrate is assimilated into amino acids in a series of reactions facilitated by a known set of enzymes. However, when nitrate is available in excess, it may be temporarily stored in vacuoles before assimilation. Nitrogenous compounds formed during assimilation are stored in the source tissues (leaves) during vegetative growth, and are then remobilized to sinks (grains) during the reproductive phase in a process known as nitrogen remobilization (KANT, 2018).

Soil N availability is an important limiting factor in wheat production. Nitrogen deficiency negatively affects grain yield and quality in cereal crops by affecting nutrient uptake, photosynthesis rate, respiratory efficiency, and enzyme activities (LIU et al., 2020). In contrast, K is involved in the initiation of nitrogen metabolic processes, which activate nitrate reductase and starch synthase, thereby creating a balance between protein and carbohydrate production, respectively. Therefore, a shortage of K leads to a breakdown of these processes, and plants suffer even with the availability of other nutrients. Consequently, Ca^{2+}, Mg^{2+}, NO^{3-}, and PO_4^{3-}, as well as plant hormones and enzymes, cannot be translocated, and the source-drain relationship is disrupted (COSKUN; BRITTO; KRONZUCKER, 2017).

This study hypothesized that the correct combination of N and K increases the production and improves the nutritional value of BRS 394 wheat, thus increasing the efficiency of fertilization and reducing production costs. In the Savannah region, where wheat growth is in the expansion phase, the activity of the nitrate reductase enzyme can serve as a complementary tool in the diagnosis of a plant's nutritional status, guiding the recommended application of K and N fertilizers. In this context, the objective of this study was to evaluate the interaction between N and K fertilization on the development of BRS 394 wheat and nitrate and ammonium levels in Oxisol.

MATERIALS AND METHODS

The experiment was conducted between May and August 2020 in a greenhouse at the Graduate Program in Agricultural Engineering at the Federal University of Rondonópolis in the city of Rondonópolis-MT (16º28'15"S, 50º38'08"W, 284 m a.s.l.). The climate in the region according to Köppen is tropical, with an average temperature of 24.8 °C, average annual precipitation between 1200 and 1800 mm, and the rainy season is concentrated in the spring and summer months (October to March).

The experimental design was in randomized blocks, employing a response surface study based on a modified central composite experimental design of a 5^2 factorial, fractional (LITTELL; MOTT, 1975), five nitrogen doses (0, 70, 140, 210, and 280 mg dm^{-3}), and five potassium doses (0, 50, 100, 150, and 200 mg dm^{-3}), totaling 13 nitrogen x potassium combinations (mg dm^{-3}), being:0-0, 0-100, 0-200, 70-50, 70-150, 140-0, 140-100, 140-200, 210-50,210-150, 280 -0, 280-100, 280-200 and four repetitions, for a total of 52 experimental units.

In the presentation of results, the graphs of response surfaces are provided with coded equations (Table 1) to insert any values of factor levels (nitrogen and potassium doses) in the models of response surfaces, and the conversion of the original variables to the coded variables, represented by X1 and X2, was performed using Equations 1 and 2, following the criteria of Cecon and Silva (2011).

$$X1 = \frac{N\dfrac{(0+280)}{2}}{\dfrac{(280-0)}{2}} = \frac{N-140}{140} \quad (1)$$

$$X2 = \frac{K\dfrac{(0+200)}{2}}{\dfrac{(200-0)}{2}} = \frac{K-100}{100} \quad (2)$$

X1: coded variable nitrogen
X2: coded variable potassium
N: nitrogen dose
K: potassium dose

Table 1. Levels of the nitrogen and potassium factors in the original and coded scale considered in the response surface in a modified central composite design.

Experiment	Coded variables		Original variables	
	X1(N)	X2(K)	N	K
			mg dm^{-3}	
N-K Wheat cultivar BRS 394	-1.0	-1.0	0	0
	-0.5	-0.5	70	50
	0	0	140	100
	0.5	0.5	210	150
	1.0	1.0	280	200

The soil used came from an experimental area cultivated with wheat in nitrogen and potassium fertilization experiments for six consecutive years, and after each crop, the field remained fallow. Each experimental unit (pot) was collected in the plot for its respective treatment in the 0–0.2 m depth layer. The soil was first sieved through a 4 mm mesh to fill the 5 dm^{-3} pots, and then through a 2 mm mesh for chemical and particle-size characterization, according to Teixeira et al. (2017) (Table 2). The soil was classified as an Oxisol (SOIL SURVEY STAFF, 2022).

Ten wheat seeds were sown per pot and thinned 15 days after emergence (DAE), leaving three plants per pot. Phosphate fertilization (P_2O_5) at 150 mg dm^{-3} was applied at seeding, using a single superphosphate as the phosphorus source. Micronutrients were applied in the form of FTE BR 12 (9% Zn, 1.8% B, 0.8% Cu, 2% Mn, 3.5% Fe, and 0.1% Mo) at 50 mg dm^{-3}.

Table 2. Chemical and particle-size characterization of each plot collected from the 0–0.20 m layer.

Sample	pH	P	K	Ca	Mg	Al	H	SB	CEC	V	OM
N–K	$CaCl_2$	mg dm^{-3}	------------------	------------------	------------------	$cmol_c$ dm^{-3}	------------------	------------------	------------------	%	g dm^{-3}
0–0	5.50	19.00	77.80	3.25	1.23	0.00	3.48	4.68	8.15	57.42	31.20
0–100	5.40	10.80	66.70	2.85	1.08	0.00	3.13	4.10	7.22	56.79	26.30
0–200	5.50	6.10	61.60	2.70	1.04	0.00	2.85	3.90	6.75	57.78	24.10
70–50	5.20	7.60	72.70	2.60	1.00	0.00	3.83	3.79	7.62	49.74	28.70
70–150	5.30	13.40	80.80	2.75	1.04	0.00	3.30	4.00	7.30	54.79	26.30
140–0	5.50	13.00	47.50	3.25	1.25	0.00	3.73	4.62	8.35	55.33	32.10
140–100	5.40	11.70	42.40	2.85	1.08	0.00	3.05	4.04	7.09	56.98	25.60
140–200	5.30	6.90	38.40	2.60	1.00	0.00	3.48	3.70	7.17	51.60	26.30
210–50	5.40	15.90	66.70	3.00	1.12	0.00	3.48	4.29	7.76	55.28	29.50
210–150	5.50	13.00	63.60	2.80	1.08	0.00	3.17	4.05	7.22	56.09	25.60
280–0	5.30	13.40	53.50	3.10	1.16	0.00	3.95	4.40	8.35	52.69	31.20
280–100	5.50	11.70	50.50	2.95	1.12	0.00	3.05	4.20	7.25	57.93	26.30
280–200	5.40	10.80	48.50	2.65	1.08	0.00	3.23	3.86	7.08	54.52	25.60
			Sand			Silt			Clay		
			------------------	------------------	------------------	g Kg^{-1}	------------------	------------------	------------------		
			295			125			580		

OM: organic matter; CEC: cation exchange capacity; SB: sum of bases; V: base saturation.

The N and K fertilizers were applied according to the treatment. Potassium fertilizer was supplied in the form of potassium chloride (KCl) at the time of sowing, and nitrogen fertilizer was applied in installments in the form of urea (45% N), with 30% of the recommended quantity applied at planting and the remainder 15 days after plant emergence.

During cultivation, the soil moisture was maintained at 60% of the water-holding capacity by irrigation on the surface of the pots and controlled by daily weighing of the closed pots, compensating for losses by leaching.

The monitoring of temperature and relative humidity inside the greenhouse was performed using a thermo-hygrometer (Incoterm, model: 7666.02.0.00) placed in the center of the experiment, programmed to perform daily readings throughout the experiment. From these readings of maximum and minimum temperatures and relative humidity, an average temperature of 29.48 ºC and average relative humidity of 47.14% over the course of the study were calculated (Figure 1).

The chlorophyll index (SPAD) was measured in the middle third of the leaves (+1 and +2) with a developed leaf tongue through the average reading of five random leaves. Evaluations were performed at 15, 30, and 45 DAE using a Minolta SPAD-502 chlorophyll meter.

The activity of the reductase enzyme was determined according to the methodology described by Majerowicz et al. (2003). Thirty days after emergence, the diagnostic leaves (+1 and +2) were randomly collected in the experimental units to reach the required mass of 0.5 g. The material was collected, packed in plastic bags, and stored in a thermal box with ice until transfer to the laboratory. Collections for nitrate reductase enzyme activity were performed at 9:00 am, 11:00 am, and 1:30 pm, when the plants had received at least three hours of sunlight and nitrate reductase had already reached its maximum activity (HAGEMAN; REED, 1980). The leaves were cut into smaller pieces using scissors and placed in 10 mL syringes. For each 0.5 g of leaf, 5 mL of incubation solution (K_2HPO_4 0.05 M pH 7.5, KNO_3 0.05 M + propanol 1%) was added.

After placing the plunger into the syringe, it was reversed to expel air and pull the reaction medium. The syringe output was closed and the plunger was pulled three times to create a vacuum with the plant material submerged in the medium. The syringes were covered with aluminum foil, stored in the dark, and incubated for 45 min at room temperature. Thereafter, 1.0 mL aliquots of the reaction medium were removed and transferred to test tubes, and 1.0 mL sulfanilamide and 1.0 mL n-naphthyl ethylenediamine were added. The tubes were shaken and allowed to stand for 15 min for color development. The optical density was measured using a spectrophotometer (Quimis, model: Q798U2M) at 540 nm. The enzyme activity was expressed as µmol NO_2^- g Fresh $Matter^{-1}$ h^{-1}.

At 80 d after sowing (Est 11.4), at the harvest maturity stage, according to Large (1954), the plants were collected and packed in paper bags, identified, and transferred to an oven with forced air circulation at a constant temperature of 65 ºC until a constant mass was obtained. To analyze the total nitrogen concentration in the leaves, samples from each treatment were separated after drying in a closed circulation stove. Leaves were ground with a crusher and sent to the laboratory for analysis. The materials were subjected to the Kjeldahl method, as described by Malavolta, Vitti and Oliveira (1997).

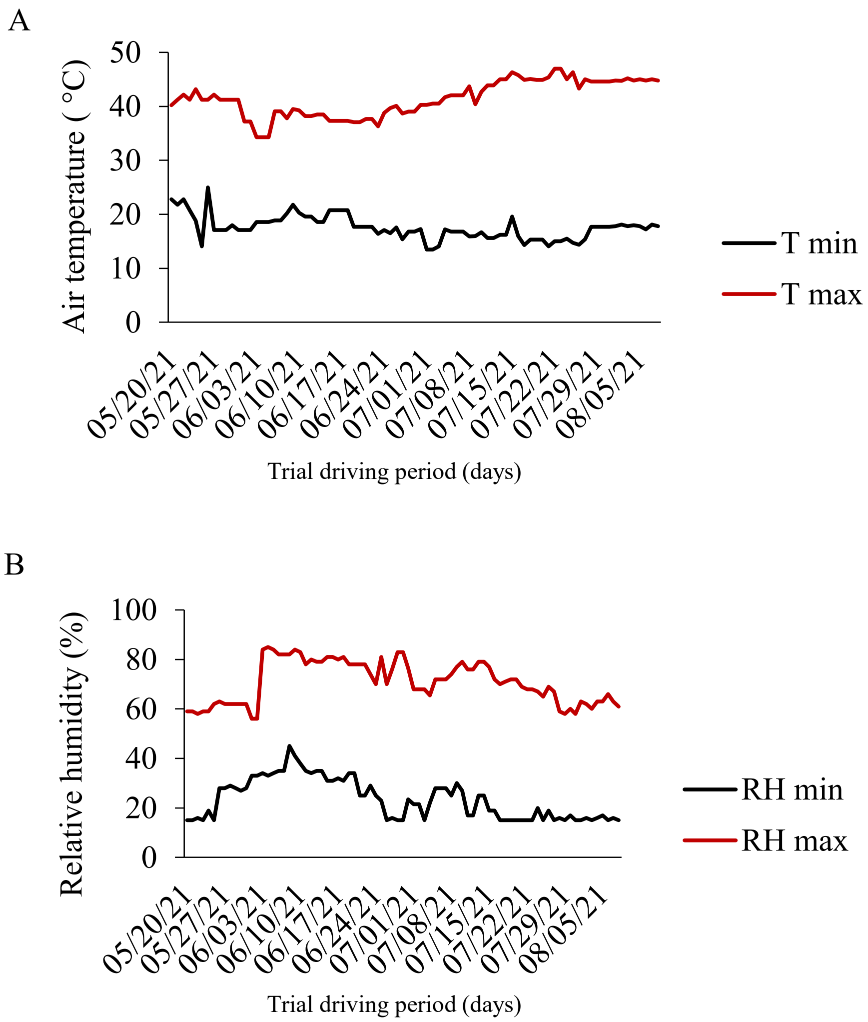

Figure 1. Fluctuations in daily air temperature (A) and relative humidity (B) in the greenhouse.

For the analysis of K^+ concentration in leaves and grains, parts of the leaf and grain samples were used (for nitrogen analysis), and analyses were performed according to the methods described by Malavolta, Vitti and Oliveira (1997). The protein content of the wheat leaves and grains was obtained by multiplying the N-total content by 6.25.

The determination of inorganic nitrogen (ammonium and nitrate) was performed using soil collected from the pot profile, which was packed in plastic bags and kept in a thermal box with ice. The soil was stored in a freezer at a temperature of <15 ºC.

The ammonium and nitrate concentrations were determined using the steam distillation method described by Raij et al. (2001) and Teixeira et al. (2017). A 20 mL aliquot of the solution extracted with KCl was used for N-mineral determination. In the first distillation, MgO was added for $N\text{-}NH^{4+}$ determination, and in the second distillation of the same sample, Devarda's alloy was added for $N\text{-}NO^{3-}$ determination. To quantify the ammonium and nitrate contents, a soil density of 1 g cm^{-3} was considered as standard.

The results were subjected to analysis of variance and, when significant, analyzed using polynomial regression for the interaction between nitrogen and potassium; in cases where the interactions were not significant, first- and second-degree regression studies were performed. A significance level of up to 5% was used for all statistical analyses. The results were subjected to statistical analysis using the software R® v. 3.4.2 (R CORE TEAM, 2018) and the MINITAB.

RESULTS AND DISCUSSION

Nitrate and ammonium content in soil

For the nitrate and ammonium contents in the soil, no significant interaction was detected between nitrogen and potassium doses. However, significant effects on nitrate and ammonium contents in the soil were observed only for nitrogen doses, with adjustment of the results to the linear regression model (Figures 2A and 2B).

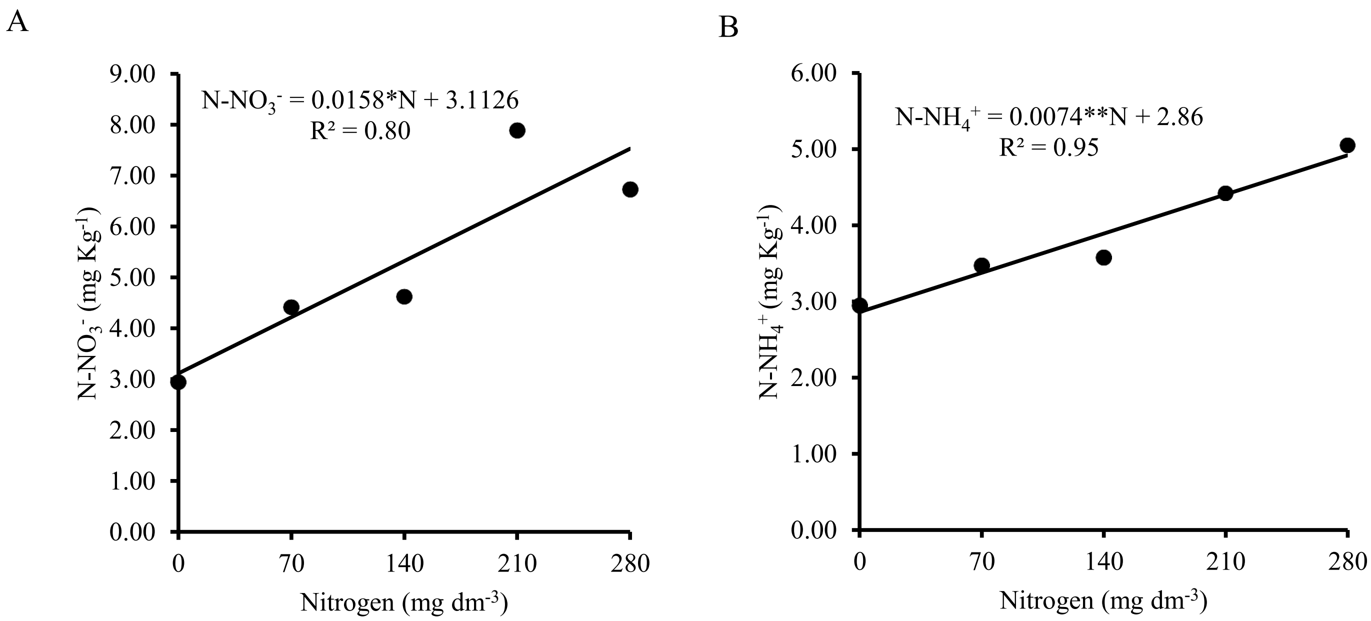

Figure 2. Content of nitrate (A) and ammonium (B) of soil samples cultivated with the wheat cultivar BRS 394 as a function of nitrogen doses. ** and * = significant at 1% and 5% probability, respectively.

The nitrate content in the soil ranged from 3.11 to 7.53 mg kg^{-1} under the no-application and high-nitrogen supply conditions, respectively. Thus, the addition of increasing doses of nitrogen increased the nitrate availability in the studied soil samples. The ammonium content of the soil increased linearly with nitrogen supplementation, with a 72.37% variation between the lowest and highest nitrogen doses applied.

The soil nitrate content was higher than the ammonium content in the proportion of the sampled soil. When studying 18 areas cultivated with wheat under dryland conditions with an average annual rainfall of 601.6 mm, Miao, Wang and Li (2015) found a maximum and minimum soil nitrate content value of 45.7 mg kg^{-1} and 2.2 mg kg^{-1}, respectively. For ammonium content in the same areas, the maximum value they found was only 3.6 mg kg^{-1}.

A concern related to the nitrogen cycle is denitrification, a process mediated by microorganisms, in which NO_3^- is sequentially reduced to produce nitrogen gas (N_2). This is one of the main causes of nitrogen loss from agricultural soils, and under conditions of low O_2 availability, the process has the potential to produce nitrous oxide (N_2O), a potent greenhouse gas (WAFULA et al., 2015).

Mendes-Santos, Kandasamy and Cid-Rigobelo (2017) showed that plant growth is not related to the availability of nitrogen during the first days of development, because the levels of nitrate and ammonium in soil of a corn crop inoculated with *Azospirillum brasilense* 20 days after sowing showed no difference between the treatments and the control.

Chlorophyll index (SPAD)

At 15 days after emergence, the variable chlorophyll index (SPAD) showed an isolated effect only for the doses of K, fitting the quadratic regression model, with the best results (43.28 SPAD) at a dose of 47.0 mg dm^{-3} (Figure 3A). Multiple regression analysis showed a significant difference in the SPAD at 30 and 45 days after emergence, fitting the quadratic regression model (Figures 3B and 3C).

The chlorophyll index at 30 days after emergence showed the maximum point (50.63 SPAD) in the combination of nitrogen doses of 152.67 mg dm^{-3} and potassium doses of 191.50 mg dm^{-3}. However, at 45 days the highest index (46.97 SPAD) was observed when wheat was fertilized with 163.53 mg dm^{-3} nitrogen and 191.50 mg dm^{-3} potassium.

Chlorophyll is the most important pigment in leaves and is responsible for the green color. The chlorophyll content of leaves can be used as an indicator of nitrogen in plants, because it is an essential element in the synthesis of photosynthetic proteins (TAIZ et al., 2017).

Schlichting et al. (2015) studied indirect and direct chlorophyll index methods in wheat crops and found a high correlation between the chlorophyll index and nitrogen concentration in leaves. The chlorophyll index can thus be used to help assess the nitrogen nutritional status of plants. Freitas et al. (2018), when studying the influence of combinations of nitrogen and potassium on field-grown wheat crops in the Savannah region, demonstrated the isolated effects of fertilization, with the highest SPAD values of 47 at the 196 kg ha^{-1} nitrogen dose and 42.7 for the 148 kg ha^{-1} potassium dose.

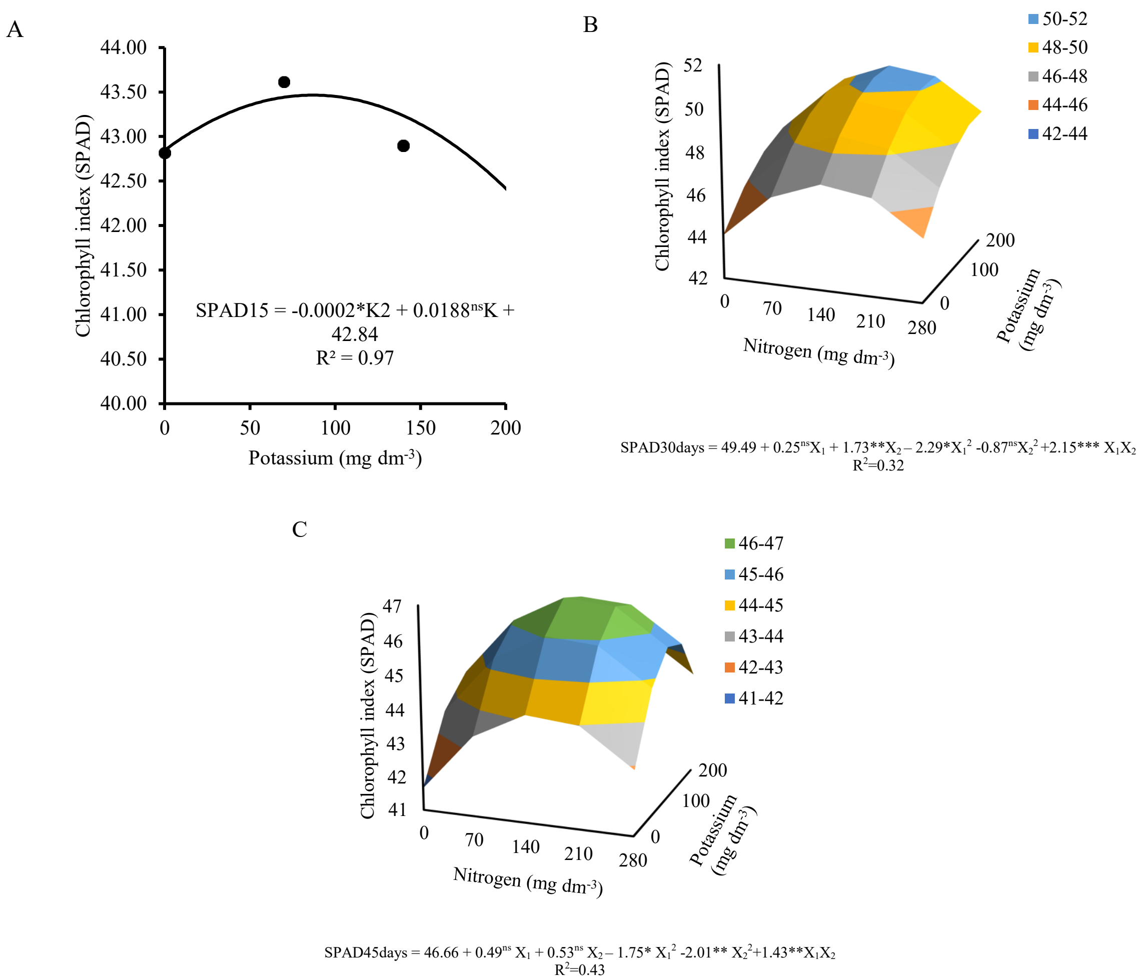

Figure 3. Chlorophyll index (SPAD) as a function of potassium doses at 15 days after plant emergence of the wheat cultivar BRS 394 (A), and at 30 (B) and 45 (C) days as a function of nitrogen and potassium doses. ^{ns},***, ** and * = not significant and significant at 0.1%, 1% and 5% probability, respectively. X1 and X2 = codings for nitrogen and potassium doses, respectively.

Shoot dry mass

For shoot dry mass, there was no significant interaction between the nitrogen and potassium doses. Shoot dry mass showed an isolated effect on the application of nitrogen, with an increase of 39.26% when comparing the nitrogen dose of 280 mg dm^{-3} to the absence of nitrogen application (Figure 4).

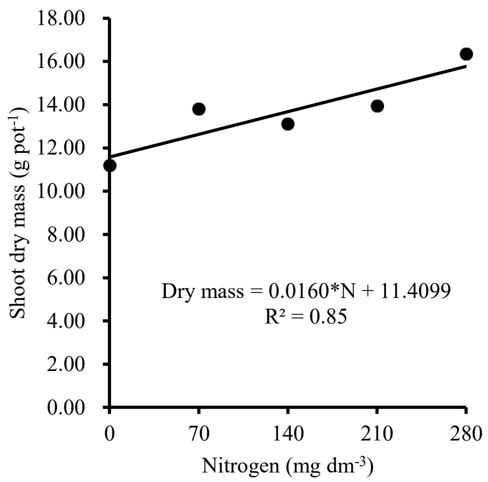

Figure 4. Shoot dry mass of the wheat cultivar BRS 394 as a function of nitrogen doses. * significant at 5% probability.

The increase in the production of the shoot dry mass of wheat plants occurred because nitrogen increased tillering and, consequently, the number of leaves, resulting in a high rate of photosynthesis, higher production of assimilates, and consequently higher dry mass. Morais et al. (2016), when evaluating the effect of nitrogen (0, 100, 200, 300,400 mg dm^{-3} of N) and potassium (0, 90, 180, 270, and 360 mg dm^{-3} of K_2O) levels on Piatã grass, observed significant effects on the shoot dry mass in three consecutive cuts with an interval of 30 days between them. In the first cut, the greatest shoot dry mass occurred for the nitrogen and potassium doses of 295 and 108 mg dm^{-3}, respectively. In the second and third cuts, the greatest production occurred for the nitrogen and potassium doses of 254 and 158 mg dm^{-3} in the second and 250 and 306 mg dm^{-3} in the third cuts, respectively.

However, the contribution of nitrogen fertilization to the production of shoot dry mass was positive, because as the nitrogen doses increased, the shoot dry mass increased. Unlike the present study, Carvalho et al. (2016), when studying combinations of nitrogen and potassium in the wheat cultivar BRS 254 in a greenhouse, observed an interaction between fertilizers and, consequently, an increase in shoot dry mass.

Proper nitrogen management is necessary because excessive nitrogen application can have various environmental impacts, such as atmospheric pollution, soil degradation, and excessive input costs (ZHANG et al., 2015; PRAVALIE, 2021; SHI et al., 2022). Therefore, studying the combination of fertilizers is essential to achieve their rational and effective use.

Nitrogen concentration in the leaves

There was no significant interaction between nitrogen and potassium doses on nitrogen concentration in wheat leaves. However, an isolated effect of the applied nitrogen dose was observed, which fit the increasing linear regression model. Analyzing the concentration of nitrogen in wheat leaves, an increase of 66.18% was observed when comparing the highest nitrogen dose applied within the experimental interval with its absence (Figure 5).

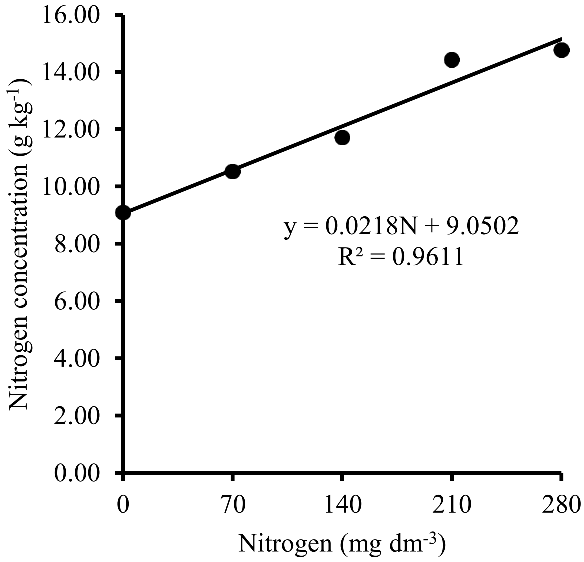

Figure 5. Nitrogen concentration in leaves of the wheat cultivar BRS 394 as a function of nitrogen doses. * = significant at 5% probability.

Malavolta, Vitti and Oliveira (1997) considered adequate nitrogen concentrations in the diagnostic leaves of wheat to be between 30 and 33 g kg^{-1}. However, in this study, the concentrations were below the range considered adequate for the crop (15.05 g kg^{-1} at a dose of 280 kg N ha^{-1}) because of the period when the analyses were performed, considering that nitrogen was transferred to the productive part of the plant during the reproductive phase.

Melero et al. (2013) studied the development and productivity of wheat in a no-till system and observed, as in this study, that the increase in nitrogen doses resulted in an increase in leaf nitrogen content. The use of fertilizers, especially nitrogen fertilizers, increases the production of dry matter in grasses, and the importance of nitrogen includes the production of different biomolecules of physiological importance and the physiological development of crops (MEHRABI; SEPASKHAH, 2019).

Nitrogen management is essential, because there are physiological consequences for wheat plants when this nutrient is in excess, such as less accumulation of many non-enzymatic antioxidants that are primarily involved in oxidative scavenging, less resistance to pathogen attack and heat stress, as well as consequences for productivity, such as lower grain yield (KONG et al., 2017). The results of the present study corroborate those of Fang et al. (2018), who studied nitrogen fertilization in common buckwheat (*Fagopyrum esculentum* M.) in China and observed an increase in the total nitrogen concentration in wheat leaves with increasing doses of nitrogen fertilization.

Nitrate reductase in leaves

Nitrate reductase activity in wheat leaves was only influenced by the nitrogen concentration, and the interaction between nitrogen and potassium was not significant, fitting the quadratic regression model. In the leaves, nitrate reductase at 30 DAE showed the highest value (1.30 µmol NO h^{-1} g^{-1} Fresh Matter) when plants were subjected to a treatment with a nitrogen dose of 95 mg dm^{-3} (Figure 6). Thus, the increase in nitrogen supply, starting at 95 mg dm^{-3}, promoted a decrease in enzyme activity.

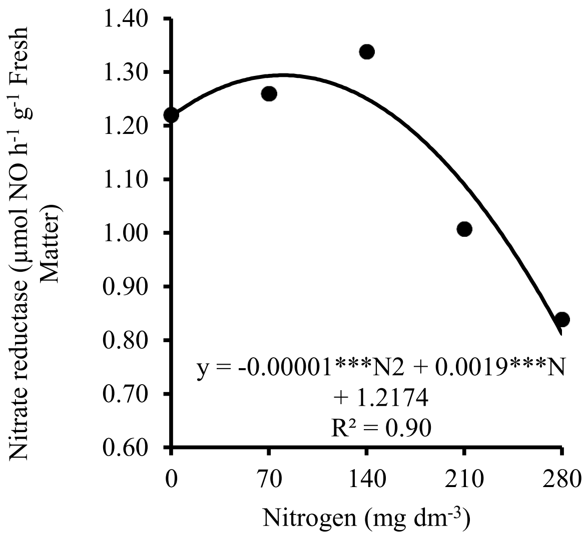

Figure 6. Nitrate reductase enzyme activity as a function of nitrogen doses in leaves of the wheat cultivar BRS 394. *** = significant at 0.1% probability.

Bakaeva (2020) explored soil nitrogen content and nitrate reductase activity in the leaves of winter wheat using nitrogen fertilizers and found that nitrate reductase enzyme activity in leaves can serve as a criterion for assessing plant supply. According to the author, in the future, the activity of this enzyme may be used as an indicator for the optimization of nitrogen nutrition in plants for metabolic processes. According to Viana and Kiehl (2010), nitrate reductase is an enzyme that participates in the regulation of nitrogen metabolism, which is why it may be indirectly associated with crop productivity. This is because plants with high nitrate reductase activity would have greater assimilation of the available nitrate, and a greater ability to respond to nitrogen fertilization.

According to Batista et al. (2020), the response of wheat cultivars to nitrogen supply is variable and cannot be generalized. However, nutrients must mainly be provided during the thinning and elongation stages of the stem, to enhance the growth rate, productivity, and grain quality.

In this study, the highest enzyme activity was found at a dose of 95 mg dm^{-3}, unlike shoot dry mass (Figure 4) and leaf nitrogen concentration (Figure 5), which showed higher values at the maximum dose (250 mg dm^{-3}). This may be due to the fact that the activity of the reductase enzyme, both in leaves and roots, is greatly altered by environmental factors such as light, temperature, and water availability.

Potassium concentration in the leaves

In the analysis of variance for potassium concentrations in wheat leaves, there was no significant interaction between nitrogen and potassium doses. The potassium concentration in the leaves was not significantly affected by the nitrogen and potassium doses, and did not fit the regression models.

Using the wheat cultivar Quartz, Bazzo et al. (2020) applied N to foliar and soil cover and observed no increase in K in the leaves. The non-interference of N applied at the concentration of K is explained by the fact that this nutrient is not a part of the carbohydrate molecules in cereal grains, but is a factor in coenzymes and the establishment of cell turgor (TAIZ et al., 2017).

Protein content of leaves

In the analysis of protein content in wheat leaves, there was no significant interaction between nitrogen and potassium doses. However, an isolated significant response was observed as a function of the nitrogen dose. The concentration of crude protein in the dry matter increased linearly with increasing doses of nitrogen, with an increase of 67.32% compared to the highest dose of nitrogen in the treatment without the application of this nutrient (Figure 7).

The results of the present study are similar to those observed by Wrobel et al. (2018) when studying the quality of wheat silage produced under nitrogen fertilizer doses of 88 and 148 kg ha^{-1}, in which they found a crude protein content of 6.88% and 7.05% at the floury grain phenological stage, respectively.

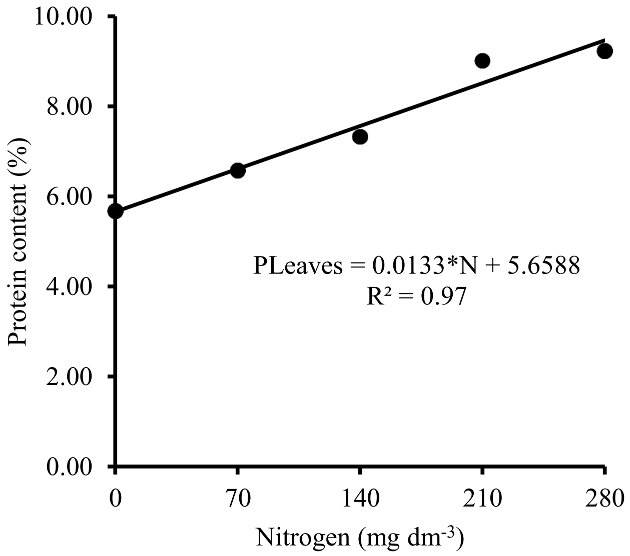

Figure 7. Protein in leaves of the wheat cultivar BRS 394 as a function of nitrogen doses. * = significant at 5% probability.

To analyze the influence of cutting regime on the production and nutritional value of wheat cv. BRS Umbu for forage, Carletto et al. (2020) observed crude protein content of 21.08% with one cut and 19.79% with two cuts 57 days after planting. Corroborating the results of the present study, there seems to be a trend toward a reduction in crude protein content with later harvests. It is possible that an increase in the protein components of the senescent leaves and stems contributes to this. According to Mancipe-Muñoz et al. (2021), crude protein contents ranging from 14.2% to 23.9% of the dry mass were observed when evaluating 25 wheat cultivars from different countries in the thickened pod or milky-pasty grain stages.

CONCLUSIONS

The dose of nitrogen that promoted the best development of BRS 394 wheat and the highest concentrations of nitrate and ammonium in the soil were 280 mg dm^{-3} and 560 kg ha^{-1}. The nitrate content in the soil was higher than that in the soil at all nitrogen doses. Enzyme activity responded significantly only to nitrogen fertilization. The dose of 95 mg dm^{-3} of nitrogen provided the best result; however, doses above this value caused a reduction in the activity of nitrate reductase.

ACKNOWLEDGEMENTS

The authors appreciate the contributions of the Soil and Water Practices Group (GPAS) of the Federal University of Rondonópolis.

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