Determination of phenological stage and nitrogen application dose in linseed crops

João Pedro Dalla Roza[1], Ivan Ricardo Carvalho[1], Leonardo Cesar Pradebon[1], Murilo Vieira Loro[2], Jaqueline Piesanti Sangiovo[1], José Antonio Gonzalez da Silva[1] and Caroline Huth[3]

Abstract – This study aimed to evaluate the effects of nitrogen doses on different phenological stages of linseed development during three agricultural years of cultivation. A randomized blocks experimental design was employed, organized in a three-factor design (3x5x3), consisting of three nitrogen applications (beginning of development of basal branching, pre-flowering, and grain filling), five doses of nitrogen (0; 30; 60; 90, and 120kg ha^{-1}), and three agricultural harvests (2020, 2021, and 2022). Each treatment had three replications, totaling 135 experimental units. At full physiological maturity, the crop cycle was evaluated, then 10 plants were randomly collected per experimental unit, the following characters were evaluated and measured: plant height, stem diameter, number of basal branches, height of insertion of the first capsule, grain weight per plant, number of grains per plant, number of branches per plant, and number of capsules. The analysis of variance was conducted at a 5% probability of error using the F test. The variables that showed a significant interaction were broken down to simple effects via polynomial regression with adjustment of the highest degree of the polynomial. The maximum technical nitrogen efficiency for linseed cultivation is 69.8 kg per hectare. Nitrogen fertilization at the basal branching stage enhances grain weight, number of grains per plant, and grain yield. The application of nitrogen when filling grains enhances grain productivity (> 1.6 tons of grains per hectare) regardless of the agricultural year.

Index terms: *Linum usitatissimum*; Phenology; Grain productivity; Regression; Nitrogen management.

Determinação do estádio fenológico e dose de aplicação de nitrogênio na cultura da linhaça

Resumo – Este trabalho teve como objetivo avaliar os efeitos de doses de nitrogênio em diferentes estádios fenológicos de desenvolvimento da linhaça, durante três anos agrícolas de cultivo. Utilizou-se delineamento experimental em blocos casualizados, organizado em esquema fatorial trifatorial (3x5x3), sendo: três momentos de aplicação de nitrogênio (início do desenvolvimento da ramificação basal, pré-florescimento e enchimento de grãos), cinco doses de nitrogênio (0; 30; 60; 90 e 120kg ha^{-1}) e três safras agrícolas (2020, 2021, 2022). Cada tratamento teve três repetições, totalizando 135 unidades experimentais. Em plena maturidade fisiológica foi avaliado o ciclo da cultura, em seguida foram coletadas aleatoriamente dez plantas por unidade experimental, avaliados e medidos os seguintes caracteres: altura da planta, diâmetro do caule, número de ramos basais, altura de inserção da primeira cápsula, peso do grão por planta, número de grãos por planta, número de ramos por planta e número de cápsulas. A análise de variância foi realizada com 5% de probabilidade de erro por meio do teste F. As variáveis que apresentaram interação significativa foram decompostas em efeitos simples por meio de regressão polinomial com ajuste do maior grau do polinômio. A máxima eficiência técnica de nitrogênio para o cultivo de linhaça é de 69,8 kg por hectare. A fertilização com nitrogênio no estágio de ramificação basal aumenta o peso dos grãos, o número de grãos por planta e o rendimento de grãos. A aplicação de nitrogênio no enchimento de grãos potencializa a produtividade de grãos (> 1,6 toneladas de grãos por hectare) independente do ano agrícola.

Termos de indexação: *Linum usitatissimum*; Fenologia; Produtividade de grãos; Regressão; Manejo nitrogenado.

Introduction

The linseed crop (*Linum usitatissimum* L.) belongs to the Linaceae family and originates from Asia. It stands out for its multiple purposes and oleaginous, fibrous, probiotic, and nutraceutical properties (Antonelli *et al.*, 2013). Used as an alternative between crops, linseed presents itself as an option for diversifying cultivation systems (Antonelli *et al.*, 2013). Linseed contains 33.5% dietary fiber, 32.3% lipids, and 14.1% proteins, considered a functional food, in addition to holding antimicrobial and bioactive compounds (Loro *et al.*, 2022).

The world's largest linseed producer is Canada, with approximately 950 thousand tons of grains in 2015, equivalent to 40% of all production and a sown area of 380 thousand hectares (Loro *et al.*, 2022). Although the worldwide cultivation of this crop has been growing, its production still needs to expand to meet market

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[1] Universidade Regional do Noroeste do Estado do Rio Grande do Sul, RS, Brasil. E-mail: dallaroza@gmail.com, carvalhoirc@gmail.com, leonardopradebon@gmail.com, sangiovojaque@gmail.com, jose.gonzales@unijui.edu.br

[2] Universidade Federal de Santa Maria, RS, Brasil. E-mail: muriloloro@gmail.com, ORCID: https://orcid.org/0000-0003-0241-4226

[3] Cisbra, Rua Ragnar Thorstemberg 288 - Lambari, Ijuí - RS, CEP 98700-000. E-mail: carol_huth@gmail.com

demand, especially in Brazil. This implies the need to develop genotypes adapted to our cultivation regions (Carvalho *et al.*, 2019; Carvalho *et al.*, 2023a; Carvalho *et al.*, 2023b) and management practices that maximize linseed productivity, such as fungicide management (Bruisma *et al.*, 2023), sowing densities (Loro *et al.*, 2022; Sangiovo *et al.*, 2022; Scarton *et al.*, 2023), and efficient management of the time (Pradebon *et al.*, 2023) and dose of nitrogen application (Hutra *et al.*, 2022). Nitrogen fertilization is a key agronomic practice to optimize grain yield, to which linseed responds positively, resulting in high grain yields and better quality (Antonelli, 2015).

Nitrogen is an important constituent of proteins, being the main nutrient for maintaining the productivity of crops. When applied, it is assimilated by the plant and associates with the carbon chains, promoting an increase in cellular constituents and, consequently, an increase in the vigor of regrowth and the production of dry weight of plants under favorable climatic conditions (Loro *et al.*, 2022).

According to Bilalis *et al.* (2010), among the studies conducted with linseed and nitrogen, nitrogen (N) is the main constituent, leading to variations in the oil content of seeds of this crop. As some research shows, the application of N in top dressing influences grain productivity and plant productivity components. Urea is an important nitrogen fertilizer, being the most used worldwide due to its lower cost and higher N content (Cunha *et al.*, 2011).

The Southern region of Brazil presents productive potential for the cultivation of linseed due to its ideal soil and climate conditions for this crop, which requires low temperatures. However, scientific studies are still incipient when it comes to characterizing the development of linseed and its relationship with productivity, especially on Brazilian soil (Bassegio *et al.*, 2012). Therefore, the importance of elucidating the relationships between crop development and yield and nitrogen fertilization is evident, especially considering the expansion of cultivation in the Southern region of Brazil. Determining the appropriate development stage of linseed for nitrogen application allows crop productivity to be enhanced and production costs to be minimized. Therefore, this work aimed to evaluate the effects of nitrogen doses on different phenological stages of linseed development during three cultivation seasons.

Material and methods

The experiment was conducted in the experimental area of the Regional University of Northwestern Rio Grande do Sul, in Augusto Pestana municipality - RS. Sowing was performed on May 13th and the harvest on November 11th. These dates (day and month) were the same over the three years of study. Soybeans were the predecessor crop at all times. Sowing fertilization was conducted with the application of 300kg ha^{-1} of N-P-K formula 05-25-25. The experiment site is located at 28° 26’ 20’’ S and 54° 00’ 23’’ W, 301 meters above sea level and its soil is classified as a typical dystroferric red latosol. The soil analysis of the experimental area showed the following characteristics: Clay = 66%; pH = 5.3; SMP index = 5.6; P = 20mg dm^3; K = 165mg dm^3; MO = 2.2%; Al = 0.5cmolc/dm^3; Ca = 3.41cmolc/dm^3; Mg = 1.25cmolc/dm^3; Cu = 6.93mg dm^3; Zn = 1.52mg dm^3; Mn = 23.3mg dm^3; and S = 8.7mg dm^3. The climate is classified, according to Köppen, as *Cfa* (humid subtropical).

A randomized blocks experimental design was employed, organized in a three-factor design (3x5x3), consisting of three nitrogen (urea - N 46%) applications (beginning of development of basal branching, pre-flowerings and grain filling), five doses of nitrogen (0; 30; 60; 90 and 120 kg ha^{-1}), and three agricultural harvests (2020, 2021, and 2022).

The experimental units consisted of 17 sowing rows, spaced 0.18m apart, six meters long, totaling an area of approximately 18m^2. Sowing was conducted using a seeder, with a sowing density of 60 viable seeds m^{-1}. A usable area of 10 m^2 was considered. Phytosanitary management was performed to minimize abiotic effects (pests and diseases) on plant development.

At full physiological maturity, the crop cycle (CYCLE, days) was evaluated, then 10 plants were randomly collected per experimental unit. The following characters were evaluated and measured: plant height (PH, cm), stem diameter (SD, mm), number of basal branches (NBB, units), height of insertion of the first capsule (HIFC, cm), grain weight per plant (GWP, grams), number of grains per plant (NGP, units), number of branches per plant (NBP, units), and number of capsules (NC, units).

Meteorological information on mean air temperature (Tmed, °C), minimum air temperature (Tmin, °C), maximum air temperature (Tmax, °C), and precipitation (Prec, mm) were expressed to better understand the results (NASA POWER, 2023). The data obtained were subjected to the assumptions of the statistical model, normality of errors, and homogeneity of residual variances. Subsequently, descriptive analysis was conducted using dispersion diagrams and central tendency of the minimum, mean and maximum air temperature variables.

Analysis of variance was conducted at a 5% probability of error using the F test. The interaction between moments of nitrogen application × nitrogen doses × agricultural harvests was tested. The variables that showed a significant interaction were broken down to simple effects via polynomial regression with adjustment of the highest degree of the polynomial with significance based on the Student’s t test at 5% significance. Significant quadratic phenomena were subjected to estimation of maximum technical efficiency via the ratio between linear and quadratic coefficients, by the formula, $x = -b \pm \frac{\sqrt{\Delta}}{2*a}$, and cubic, Met= $\frac{2x\beta \pm \sqrt{4x\beta^{2\,2} - 12x\beta\, j x\beta^{3\,3}}}{6x\beta\, 3}$.

The qualitative effects of nitrogen application times and agricultural harvests, when in the presence of a significant interaction, were broken down to simple effects by the mean comparison test using the Tukey test at 5% significance. All statistical analyses were performed by the *R* software (R CORE TEAM, 2023), using the *ggplot2*, *metan*, *agricolae*, and *Exp. Des.Pt*. packages.

Results and discussion

During the study period, different meteorological conditions were recorded (Figure 1). The occurrence of cold was a determining factor for cultivation. High

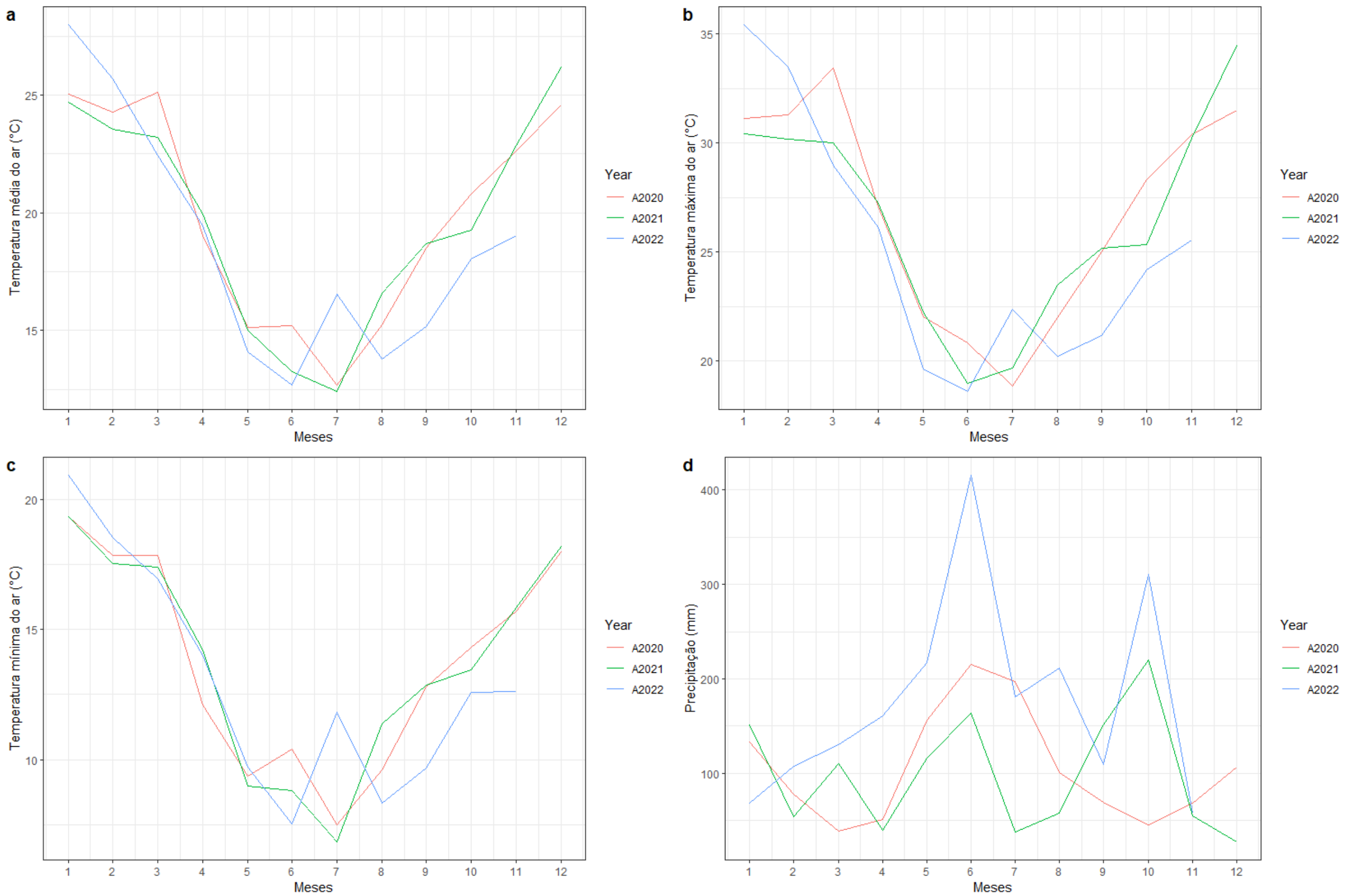

Figure 1. Meteorological data, referring to maximum air temperature (°C), mean air temperature (°C), minimum air temperature (°C), and precipitation (mm) for the 2020, 2021, and 2022 harvests, in the municipality of Augusto Pestana, RS
Figura 1. Dados meteorológicos, referentes à temperatura máxima do ar (°C), temperatura média do ar (°C), temperatura mínima do ar (°C) e precipitação (mm) para as safras 2020, 2021 e 2022, no município de Augusto Pestana, RS

temperatures close to 32°C harm the crop during periods of flowering and grain filling and may cause reductions in the differentiation processes of some components (Stanck *et al.*, 2018; Furlan *et al.*, 2022); however, the crop is sensitive to the occurrence of frost, especially in its seedling phase and flowering period. Variations in the mean air temperature were also recorded during the study period. The highest mean air temperatures were observed in the months of June and July, including the periods comprising the beginning of basal branching and of flowering for the year 2022. However, the highest mean temperatures were observed from August to September in 2021, a period of full flowering, whereas higher mean temperatures during capsule formation and filling were evident in 2020.

In 2021, the highest maximum temperatures were recorded from September to November, surpassing those of both 2020 and 2022, with temperatures above 25°C (Figure 1b). This period is understood as decisive for linseed productivity, as it comprises the reproductive period of the crop from flowering to grain filling. According to Stanck (2017), high temperatures during the flowering and grain filling season can directly affect the quality of the grain and also reduce the oil content, as well as flower abscission.

Meanwhile, in 2021, the lowest minimum temperatures were recorded in July (Figure 1c), with temperatures from 1°C to 2°C. As mentioned by Stanck (2017), the linseed crop requires cold for development to occur suitable, with temperatures below −4°C being harmful. Regarding precipitation, the year of 2022 revealed the greatest volumes from June to October, with records from 300 to 400mm. According to a study by Bosco *et al.* (2020), the crop requires from 400 to 750mm to complete its cycle.

In the analysis of variance (Table 1), the interaction "year × moments of nitrogen application × doses" showed a significant effect on stem diameter, thousand grain weight, number of basal branches, and grain yield. The interaction "moments of nitrogen application × dose" revealed a significant effect for the number of grains per plant. Regarding "year × dose", the interaction revealed a significant effect for the height of insertion of the capsule and number of grains per plant. For the "year × moments of nitrogen application" interaction, significant effects were observed for the height of insertion of the capsule. Moreover, the variable dose influenced plant height, and the variable year revealed a significant effect for cycle, grain weight per plant, and number of basal branches.

In the years 2020 and 2022, the highest plant heights were observed,

Table 1. Summary of the analysis of variance for the three sowing years regarding times of nitrogen application, in the municipality of Augusto Pestana of the Northwest of the State of Rio Grande do Sul, Brazil

Tabela 1. Resumo da análise de variância para três anos de semeadura, épocas de aplicação de N no município de Augusto Pestana do Noroeste do estado do Rio Grande do Sul, Brasil

FV[1]	MS[2]										
	DF[3]	HIFC[4]	PH[5]	SD[6]	CYCLE[7]	GWP[8]	TGW[9]	NGP[10]	NBB[11]	NBP[12]	GY[13]
BLOCK	2	7.99	113.84	0.71*	71.14*	0.00005	0.013	131.81	0.63125	1.11	39698.67
YEAR	2	2235.42*	954.00*	3.91*	12332.14*	0.88*	2.30*	15580.71*	161.51*	27.74*	3904665.41*
PERIOD	2	110.54*	64.41	0.96*	0.36	0.0030	0.021	55.30	1.74*	3.63	75818.14
DOSE	4	108.54*	97.85*	0.35*	0.36	0.016	0.0073	339.05	0.9723	0.28	33441.74
Y*P	4	98.06*	68.60	0.56*	0.36	0.0095	0.023	842.34	0.88716	2.22	209167.93*
Y*D	8	35.89*	46.43	0.25*	0.36	0.0043	0.066	653.11*	1.08778	2.25	49884.40
P*D	8	34.24	49.90	0.70*	0.36	0.0042	0.32	210.73*	0.9388	2.29	107670.13*
Y*P*D	16	25.00	35.64	0.22*	0.36	0.0044	0.58*	135.32	1.61289*	1.12	124314.25*
RESIDUE	88	28.42	37.83	0.12	3.58	0.0064	0.030	290.19	0.71196	1.22	33564.67
CV (%)		8.5	7.98	15.01	1.13	28.97	5.31	25.67	2.95	26.16	15.21

[1]FV: Factor of Variation; [2]MS: Mean square; [3]DF: Degrees of freedom; [4]HIFC: Height of Insertion of the first capsule; [5]PH: Plant height; [6]SD: Stem diameter; [7]CYCLE: Cycle; [8]GWP: Grain weight per plant; [9]TGW: Thousand grain weight; [10]NGP: Number of grains per plant; [11]NBB: Number of basal branches; [12]NBP: Number of branches per plant; [13]GY: Grain yield.

[1]FV: Fator de Variação; [2]MS: Média quadrada; [3]DF: Graus de liberdade; [4]HIFC: Altura de Inserção da primeira cápsula; [5]PH: Altura da planta; [6]SD: Diâmetro da haste; [7]CICLO: Ciclo; [8]GWP: Peso de grãos por planta; [9]TGW: Peso de mil grãos; [10]NGP: Número de grãos por planta; [11]NBB: Número de ramos basais; [12]NBP: Número de filiais por planta; [13]GY: Produtividade de grãos.

Table 2. Comparison test of means for the variables plant height (PH), cycle (CYCLE), grain weight per plant (GWP), number of branches per plant (NBP), and number of capsule (NC) in the municipality of Augusto Pestana of the Northwest of the State of Rio Grande do Sul, Brazil in the years of 2020, 2021, and 2022

Tabela 2. Teste de comparação de médias das variáveis altura de planta (AP), ciclo (CICLO), peso de grãos por planta (GWP), número de ramos por planta (NBP) e número de cápsula (NC) no município de Augusto Pestana do Noroeste do estado do Rio Grande do Sul, Brasil entre os anos de 2020, 2021 e 2022

YEAR	PH	CYCLE	GWP	NBP	NC
2020	79.80 a	168.82 b	0.19 b	6.10 a	8.72 b
2021	71.72 b	183 a	0.19 b	5.77 a	13.21 a
2022	79.59 a	150 c	0.43 a	4.61 a	12.68 a

It presents an average test, where the variables are year (YEAR), plant height (PH), cycle (CYCLE), grain weight per plant (GWP), number of branches per plant (NBP), and number of capsules (NC).

Apresenta um teste de média, onde as variáveis são ano (ANO), altura de planta (PH), ciclo (CICLO), peso de grãos por planta (GWP), número de ramos por planta (NBP) e número de cápsulas (NC).

recording 79.80 and 79.59 centimeters, respectively (Table 2). The longest duration of the crop cycle was recorded in 2021, with 183 days, whereas 2020 and 2022 recorded cycles of 168.82 and 150 days. The lowest air temperature during the vegetative stage of the crop promoted a longer cycle duration (Figure 1). The linseed plant needs milder temperatures for appropriate development. The plant can delay its flowering when it is not cold, increasing its cycle, despite its adequate photoperiod.

The highest grain weight per plant was observed in 2022, which is associated with the highest accumulation of precipitation. The number of branches on the main stem did not differ significantly between years. Although weather conditions were different, the environment did not influence the number of main branches. This suggests that this character may be determined by a greater proportion of genetic effects. The highest means for the number of capsules were observed in 2021 and 2022 (13.21 and 12.68, respectively).

In the analysis of maximum technical efficiency (MTE), interactions between year and moments of nitrogen application were observed and estimated. Based on the quadratic equation of the variable number of basal branches, the prediction of all variables was conducted considering 69.8kg of N ha^{-1}. This dose promoted the greatest increase in linseed grain productivity, being the main variable desired by plant breeders and farmers. Thus, the other characters were predicted based on the dose of 69.8kg of N ha^{-1}. The mean height of insertion of the first capsule was estimated as 63.03 centimeters in

Table 3. Maximum technical efficiency, the seven variables are height of insertion of the first capsule (HIFC), plant height (PH), stem diameter (SD), thousand grain weight (TGW), number of grains per plant (NGP), number of basal branches (NBB), grain yield (GY). General in YEAR represents the three years of cultivation. For MANAGEMENT, General is for any time, which can also be during basal branching (BB), grain filling (GF), full flowering (FF) in the municipality of Augusto Pestana of the Northwest of the State of Rio Grande do Sul, Brazil
Tabela 3. Máxima eficiência técnica, onde havia sete variáveis, sendo: altura de inserção da primeira cápsula (HIFC), altura de planta (PH), diâmetro do caule (SD), peso de mil grãos (TGW), número de grãos por planta (NGP), número de ramos basais (NBB) e produtividade de grãos (RG). Foram obtidos os anos agrícolas onde (Geral) é para os três anos de cultivo, sob manejo de nitrogênio (Geral) é para qualquer época de aplicação (ramificação basal (BB), enchimento de grãos (GF) e floração plena (FF)) no município de Augusto Pestana do Noroeste do estado do Rio Grande do Sul, Brasil

VARIABLES	YEAR	MANAGEMENT	Equation	R^2	Prediction (MTE: 69,8 kg of N ha^{-1})
HIFC	General	General	$y = 61.2893 + 0.0250x$	0.7	63.03 cm
PH	General	General	$y = 75.275 + 0.0294x$	0.5	77.32 cm
SD	2021	BB	$y = 2.2158 + 0.0370x - 0.0370x^2 + 0.00001x^3$	1	4.3 mm
SD	2022	GF	$y = 2.2333 + 0.0209x - 0.0002x^2$	0.8	3.49 mm
SD	2022	FF	$y = 3.2276 - 0.0203x + 0,0002x^2$	0.8	2.00 mm
SD	2022	BB	$y = 1.9556 + 0.0048x$	0.1	2.29 mm
TGW	2021	FF	$y = 3.2879 + 0.0028x$	0.7	3.48 g
TGW	2022	BB	$y = 3.0553 + 0.0152x + 0.0004x^2 - 0.000002x^3$	0.7	4.19 g
NGP	General	General	$y = 48.3941 + 0.1846x$	0.6	61.27 uni
NBB	2021	GF	$y = 3.0857 + 0.0698x - 0.0006x^2$	0.7	7.95 uni
NBB	2021	FF	$y = 2.5667 + 0.0178x$	0.6	3.80 uni
NBB	2021	BB	$y = 3.7333 - 0.0422x + 0.0004x^2$	0.5	6.70 uni
GY	2020	GF	$y = 1.366.2700 + 5.1363x$	0.7	1,694.78 kg
GY	2020	BB	$y = 1.366.5530 + 22.6934x - 0.5807x^2 + 0.0033x^3$	0.9	1,366.55 kg
GY	2022	FF	$y = 1.102.3880 - 19.2523x + 0.4788x^2 - 0.0024x^3$	1	875.48 kg

cases without differences for moments of nitrogen application, and the mean plant height was 77.32 centimeters in cases without differences for any factor.

Stem diameter showed a significant difference for moments of nitrogen application. In 2021, with the application conducted during basal branching, stem diameter reached 4.3 mm. However, in 2022, the stem diameter exhibited differences across the three moments of nitrogen application. During grain filling, the stem diameter measured 3.49mm, while in full flowering, it reduced to around 2.00 mm. Notably, with nitrogen applied during basal branching, a slight increase was noted, with plants reaching a diameter of 2.29mm.

Regarding the thousand grain weight, differences were obtained for moments of nitrogen application. In 2021, applying nitrogen during full flowering led to a thousand grain weight of 3.48 grams. Meanwhile, in 2022, with the application carried out during basal branching, thousand grain weight reached 4.19grams.

The variable number of grains per plant showed no difference between years, with plants yielding an average of 61.27 grains across application moments. Similarly, the number of basal branches per plant did not differ between years, but rather between moments of nitrogen application. When application was conducted during the grain filling period, the plants presented 7.95 branches; during full flowering, 3.80

branches; and during basal branching, 6.70 branches were obtained.

For the grain yield in 2020, differences were noted according to the moments of nitrogen application. When it was conducted during grain filling, the plants presented a grain yield of 1.694.78 kg ha^{-1}, whereas, when applied during basal branching, 1.366.55 kg ha^{-1} was obtained. For the year 2022, with moments of nitrogen application carried out in full differentiation of axillary buds, a yield of 875.48 kg ha^{-1} was observed. Studies in RS reported linseed grain productivity varying from 455.54 to 1,649.39 kg ha^{-1} in the agricultural years of 2020 and 2021 (Loro *et al.*, 2022) and from 477.93 to 1,649.39 kg ha^{-1} in the years of 2020, 2021, and 2022 (Scarton *et al.*, 2023). In cultivation on sowing dates, Pradebon *et al.* (2023) observed linseed grain productivity ranging from 791.97 to 2,675.47 kg ha^{-1}. Therefore, the results observed are consistent with the productive performance of linseed.

Conclusions

- The maximum technical nitrogen efficiency for linseed cultivation is 69.8kg per hectare;
- Nitrogen fertilization at the basal branching stage enhances grain weight, number of grains per plant, and grain yield;
- The application of nitrogen during grain filling enhances grain productivity (> 1.6 tons of grains per hectare) regardless of the agricultural year.

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