Development of Flaxseed Submitted to Nitrogen Doses[1]

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ABSTRACT

The flaxseed is little explored under Brazilian cultivation conditions, generating a gap in information about the appropriate management of the crop, in the country. Hence, the objective was to find the appropriate dose of nitrogen for the development of the variables: technical length, yield length, main stem diameter, number of grains per pod, plant grain yield, and plant dry mass of two flaxseed varieties, brown and golden, in places with subtropical and tropical climate in Brazil. The treatments consisted of a two-factor 2 x 6 experiment, consisting of two flax varieties (brown and golden) and six nitrogen doses (0; 30; 60; 90; 120 and 150 kg ha^{-1}), in randomized blocks with four repetitions and three different environments: Santa Maria and Frederico Westphalen, in the state of Rio Grande do Sul, and Viçosa, in the state of Minas Gerais. The brown variety obtained greater technical and yield length compared to the golden variety, on the other hand, the golden variety showed a higher number of grains per capsule and higher plant grain yield compared to the brown variety, in the three environments. The plant dry mass increased linearly and proportionally to the increase in nitrogen doses in Santa Maria and Frederico Westphalen environments. Therefore, flaxseed responds differently to nitrogen supplementation according to the cultivation environment, showing wide variability. The different doses of nitrogen influenced the variables analyzed and the varieties of flaxseed differently, depending on the environment.

Keywords: Linum usitatissimum L.; fertilizer; multi-environment; nitrogen.

INTRODUCTION

The grains of flaxseed (*Linum usitatissimum* L.) as they are also known, have been reaching prominence in the world due to the α-linolenic acid (ω-3) content shown in their oil (Madhusudhan, 2009; Lee *et al.,* 2021). In humans, ω-3 can prevent certain types of cancer, strokes, autoimmune diseases such as systemic lupus erythematosus, cardiovascular diseases (Morris & Vaisey-Genser, 2003), decrease bad cholesterol, diabetes (Ricklefs-Johnson *et al.*, 2017; Zhu *et al.,* 2020) and provide an increase in docosahexaenoic acid (DHA) that is directly related to the formation, development, and functioning of the brain and retina (Martin *et al.*, 2006).

The agronomic characteristics an ω-3 contents in the oil depend on the flax variety or cultivar (Dmitriev *et al.,* 2020), the cultivation conditions (Morris & Vaisey-Genser, 2003) and especially the fertilization used. Nitrogen fertilizers contribute to a greater extent to the variation in oil content in flax grains and, in addition, to high crop yields. In Brazil, the average grain yield is around 1,000 kg ha^{-1} (IBGE, 2016), while countries such as Sweden, France,

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and Canada showed averages of 3,918, 1,931, and 1,543 kg ha^{-1} respectively, evidencing the yield potential of the crop (Popovic, 2016). Thus, as in other crops, the flaxseed tends to vary its yield and its phenotypic expression according to the environment in which it is positioned and according to the availability of nutrients in the system, among these nutrients, nitrogen. Considered essential and an important fertility factor, nitrogen increases grain production promoting canopy formation that is necessary for photosynthesis, and consequently increasing crop yields by about 40% (Zörb *et al.*, 2018).

The flaxseed is little explored in research in Brazil, resulting in low use and technical knowledge about the crop, generating a lack of information about proper management, responses to fertilization, and, likewise, the appropriate supply of nitrogen. Studies on nitrogen manuring are carried out in countries such as Egypt (El-Gedwy *et al.*, 2018; Emam, 2019; Abdel-Kader & Mousa 2019; El-Gedwy, 2018), Ethiopia (Taddese & Tenaye, 2018), Kenya (Kariuki *et al.*, 2014), Iran (Khajani *et al.*, 2012), Canada (Ibrahim, 2009), China (Shi & Zhao, 2018; Zhang *et al.*, 2020), India (Chopra & Badiyala, 2016; Patel *et al.*, 2017), Russia (Prakhova & Turina, 2021), Iran (Khajani *et al.,* 2012), Japan (Hatanaka *et al.*, 2021) and Poland (Andruszczak *et al.,* 2015).

In the literature Shi & Zhao (2018) and Cui *et al.* (2019) report that excessive nitrogen application reduces the rate of plant dry biomass accumulation. At the advanced growth stage, it destroys the carbon and nitrogen balance and consequently affects the synthesis of photosynthetic products and their translocation to grain (Yan *et al.*, 2018), reflecting lower grain weight, often non-viable and low vigor. The content of non-structural carbohydrates shown in various flaxseed plant organs has a negative correlation with nitrogen concentration (Yan *et al*, 2018), thus reducing the rate of contribution of these non-structural carbohydrates to yield before flowering, which in turn decreased flax grain yield. In addition, the high application of nitrogen can favor lodging, which, in addition to reducing grain yield, makes mechanical harvesting difficult and also increases the cost to the farmer.

This study is based on the lack of studies related to nitrogen fertilizers in the flaxseed crop, as well as the lack of knowledge about appropriate supply under cultivation conditions in Brazil. Hence, the objective was to estimate the appropriate nitrogen dose for the development of the variables technical length, yield length, main stem diameter, number of grains per pod, plant grain yield, and plant dry mass of two flax varieties, brown and golden, in places with subtropical and tropical climate in Brazil.

MATERIAL AND METHODS

The experiment was carried out in a test network in three different environments: Santa Maria (29º43’23”S and 53º43’15”W, at an altitude of 95m) and Frederico Westphalen (27º39’56”S and 53º42’94”W, at 493 m altitude), in the state of Rio Grande do Sul (RS); and Viçosa (20º 45’ 14” S and 42º 52’ 55” W, at an altitude of 648 m), in the state of Minas Gerais (MG). The climate, according to the Koppen classification, is classified as Cfa for the municipalities of the state of RS and Cwa for the municipality of MG. (Alvares *et al.,* 2013). The meteorological characterizations of each location were obtained from automatic meteorological stations, located close to the experiments and are represented in Figure 1.

The treatments consisted of a two-factor 2 x 6 experiment, consisting of two flax varieties (brown and golden), grown by producers in the region of Panambi-RS-Brazil with seeds provided by CISBRA company, and six nitrogen doses (0; 30; 60; 90; 120 and 150 kg ha^{-1}) in randomized blocks with four repetitions. The randomization of the treatments followed the structure of split plots, where the flax varieties were randomized in the main plots and the nitrogen doses in the subplots. The experimental units used were plots of 2.5 m in length by 2.0 m in width, equivalent to an area of 5 m² and seeding was carried out manually in June with density be 80.0 kg of seeds viable per hectare. Each plot consisted of ten lines with a spacing of 20 cm between lines, considering a useful area of eight central lines per plot.

The study lasted 162 days, from June to November 2020. The soils of the different flax cultivation regions are classified as: Arenic Dystrophic Red-Yellow Argisol for the municipality of Santa Maria, Alumino-Ferric Red Latossol for the municipality of Frederico Westphalen-RS, and Red Yellow Argisol for the municipality of Viçosa-MG (Embrapa, 2013). The base manuring was carried out according to the results of the soil analysis of each place and with the application of the NPK 5-20-20 formula, appropriate to the expectation of production of 2 t ha^{-1}, according to the manuring and liming, ROLAS (2004) for the crop of flaxseed. The nitrogen application hauling to the crop was performed in a single dose from the beginning of branching and was provided in the form of urea.

Figure 1: Daily averages of maximum, minimum and average temperatures and precipitation in Santa Maria (A) and Frederico Westphalen (B), in the state of Rio Grande do Sul, and Viçosa (C), in the state of Minas Gerais, in the period 06/01 to 11/09 of 2020.

Upon reaching the point of harvest maturation, 20 plants were harvested per plot, which was cut close to the ground in a sequence of five plants, at four random points in the useful area of the plot. Subsequently, the collected plants were taken to the laboratory for analysis of the variables: technical length (cm) obtained by measuring the base of the plant to the region of insertion of the first stems with capsules; yield length (cm) estimated from the calculation of the plant height measurement minus technical length; main stem diameter (mm) measured at 10 cm above the base of the plant; the number of grain per pod; plant grain yield (g) and plant dry mass (g).

After data collection, the residual normality test proposed by Shapiro-Wilk and the variance homogeneity test proposed by Oneil and Matthews were performed. An analysis of variance was carried out for the response variables, and the factors were analyzed by the Tukey test at 0.05 of error probability, except for the nitrogen doses, which were adjusted to polynomial models, to find the ideal nitrogen dose for obtaining the best result, and the expression used $MET = \frac{-\beta 1}{2 \times \beta 2}$ when the regression model was quadratic and the expression $MET = \frac{-2 \times \beta 2 \pm \sqrt{4 \times \beta_2^{\,2} - 12 \times \beta_1 \times \beta_3}}{6 \times \beta_3}$ for cubic regression models. All statistical analyzes were performed adopting a 5% error and with the *ExpDes* and *ggplot2* packages of the R software (R Development Core Team, 2020).

RESULTS AND DISCUSSIONS

The assumptions of the analysis of variance, homogeneity, and normality of the experimental residues were 100% met and the coefficients of variation (CV%) ranged from 3.20 to 23.40% (Table 1), giving reliability to the results.

Table 1: Comparison of averages for the cultivation of brown and golden flaxseed concerning the plant technical length (PTL, cm), plant yield length (PYL, cm), main stem diameter (MSD, mm), number of grains per capsule (NGC), plant dry mass (PDM, g) and plant grain yield (PGY, g), in the state of Rio Grande do Sul (Santa Maria and Frederico Westphalen) and in the state of Minas Gerais (Viçosa), Santa Maria – RS, 2022

Variety	Santa Maria						Frederico Westphalen					
	PTL	**PYL**	**MSD**	**NGC**	**PDM**	**PGY**	**CT**	**PTL**	**PYL**	**MSD**	**NGC**	**PDM**
brown	-	18.17^{a}	1.88^{b}	6.34^{b}	4.66^{b}	-	58.42^{a}	10.50^{a}	-	7.98^{b}	-	-
golden	-	11.82^{b}	2.08^{a}	6.96^{a}	5.95^{a}	-	54.12^{b}	8.79^{b}	-	8.850^{a}	-	-
C.V. (%)	7.23	14.75	8.88	10.50	17.72	23.40	3.20	17.92	8.88	9.01	20.08	14.50
Means	64.67	14.99	1.98	6.65	5.30	1.00	56.27	9.65	1.79	8.41	2.81	1.06
				Viçosa								
brown	82.76^{a}	12.46^{a}	-	8.87^{b}	-	2.58^{a}						
golden	73.48^{b}	10.15^{b}	-	9.183^{a}	-	1.93^{b}						
C.V. (%)	12.54	23.11	18.29	5.90	9.20	5.27						
Means	78.12	11.31	2.32	9.02	0.75	2.25						

Different letters in the column differ by 5% error by Tukey's test.

In the analysis of variance, the environment of Santa Maria - RS showed significance in the interaction "variety *x* nitrogen dose" only in the variable plant grain yield. The variables yield length and main stem diameter were significant for the "variety" factor and the "nitrogen dose" factor. In the variables technical length and plant dry mass, the significance occurred only for the factor "nitrogen dose", while for the variable number of grains per capsule there was significance only in the "variety" factor.

For the environment of Frederico Westphalen - RS, there was a significant interaction between the factors "variety x nitrogen dose" for the variable technical length. The variables main stem diameter, plant dry mass, and plant grain yield showed significance for the factor "nitrogen dose". The variables yield length and number of grains per capsule showed a significant effect only for the "variety" factor.

In Viçosa - MG, there were no significant interactions between the factors "variety x nitrogen dose" for any of the variables studied and, also, it was not significant for the factor "nitrogen dose", showing exclusively the effect of the variety for the variables technical length, yield length, number of grains per capsule and plant dry mass.

Comparing the varieties of flaxseeds, brown and golden in the environment of Santa Maria – RS, for the variables main stem diameter, the number of grains per pod, and plant grain yield, it appears that the golden variety obtained values of 2.08 cm, 6.96 and 5.95 g significantly higher compared to the values of the brown variety 1.88 cm, 6.34 and 4.66 g, respectively. As for the yield length variable, the brown variety showed the highest average for this variable (18.17 cm), compared to the golden variety (11.82 cm) (Table 1).

Similarly, in Frederico Westphalen – RS, the brown flaxseed variety showed a significantly higher mean value of yield length (10.50 cm), when compared to the golden variety (8.79 cm). It also showed a significantly higher value of the technical length of 58.42 cm compared to the golden variety with 54.12 cm. However, concerning the variable number of grains per pod, the golden variety showed a higher mean value of 8.85, compared to the brown with 7.98, in Frederico Westphalen - RS (Table 1).

In Viçosa – MG, the brown variety showed significantly the highest averages of technical length (82.76 cm), yield length (12.46 cm), and plant dry mass (2.58 g). While for the variable number of grains per pod, the result was the opposite, as the golden variety had the highest average of 9.18 grains per pod, compared to the brown variety with 8.87 grains. This superiority in the number of grains per capsule was also verified in the environments of Santa Maria and Frederico Westphalen.

The prominence shown by the brown variety concerning technical length and yield length, in the three cultivation environments, may be linked to the genetic variability of flax, which is quite wide (Velho & Lúcio, 2021). Another point to be taken into consideration is the association of technical and yield length with the total height of the plant since both are fractions of the total height. Fila *et al.*, (2018), reported that plant height was negatively associated with grain yield. Similarly, with this result found by the authors, in the present study, the variables number of grains per capsule and plant grain yield obtained the lowest averages in the brown variety, compared to the golden variety, in the three environments. Birhanu *et al.*, (2020), evaluating 12 flax genotypes in Southern Tigray upland and water stress, observed a significant positive correlation between the number of grains per capsule and grain yield. From this correlation, it was possible to infer that the golden variety, with significantly higher averages in the number of grains per capsule, was more productive compared to the brown variety, regardless of the environment in which it was cultivated.

In the regression analysis of the interaction "variety x nitrogen doses" in the environment of Santa Maria – RS under the variable plant grain yield, there was a significant quadratic polynomial adjustment for the golden variety, with a point of maximum technical efficiency (MTE) at the dose of 125.14 kg ha^{-1} of nitrogen (Figure 2). The brown variety, on the other hand, did not show significant polynomial model adjustments. In a study with flaxseed in Dingxi, Gansu province in China, Zhang *et al.* (2020) found that grain yield increased with intermediate doses of nitrogen. Compared to the conventional recommendation for flax crop in China (150 kg ha^{-1} of nitrogen), grain yield under the lower nitrogen doses of 120, 90, 60, and 30 kg ha^{-1} increased by 15.63, 18.21, and 16.39% respectively, the yield of flax grains.

For the regression analysis of the factor "nitrogen dose", under the average of the brown and golden varieties, in the Santa Maria – RS environment, the variables yield length (Figure 3a), and plant dry mass (Figure 3d) were adjusted to linear polynomial models, with a respective increment of 78 and 182% in the highest nitrogen dose (150 kg ha^{-1}), concerning the control treatment (0 kg ha^{-1}). The yield length represents the place where the capsules are inserted, which, in turn, contain the seeds. Thus, it can be a relevant variable for obtaining higher yields. Mohammadi *et al.* (2022) reported positive and significant associations between grain yield and yield length. El-Yamanee (2011) reported that increasing nitrogen doses induced a significant increase in yield length. Kulpa & Danert (1962), classified plants with a yield length equivalent to 1/2 of the total plant, as appropriate for the production of large seeds. In addition, the linear increase in plant dry mass is also related to the increase in nitrogen doses and can be attributed to the increases in technical length, yield length, and main stem diameter, which occurred in nitrogen-supplemented flaxseed plants. This growing increase in plant dry mass is also reported by Shi & Zhao (2018) and Cui *et al.* (2019), where excessive nitrogen application reduced the rate of flax plants dry biomass accumulation.

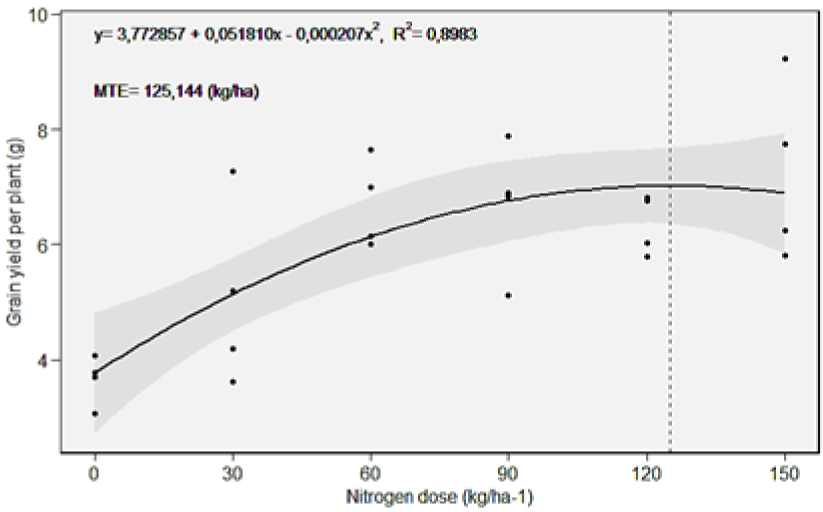

Figure 2: Relation between nitrogen doses (kg ha^{-1}) and flaxseed plant grain yield, golden variety, in Santa Maria - RS. Santa Maria - RS, 2022.

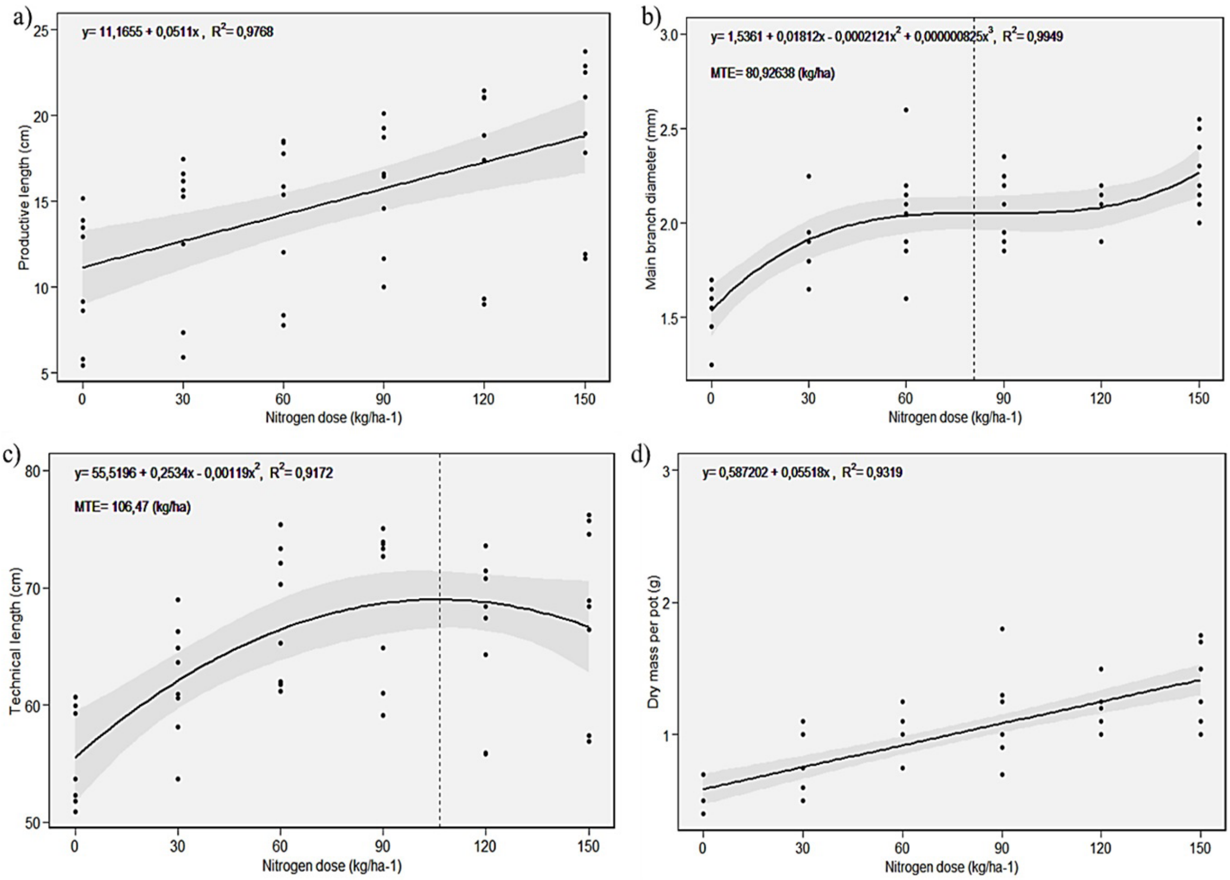

Figure 3: Relation between nitrogen doses (kg ha^{-1}) and yield length (a), main stem diameter (b), technical length (c), and plant dry mass (d) of flaxseed (average of brown and golden variety) in Santa Maria - RS. Santa Maria - RS, 2022.

For the variable technical length (Figure 3c), in the environment of Santa Maria – RS and the "nitrogen dose" factor, there was a quadratic polynomial adjustment, with a point of maximum technical efficiency at the dose of 106.47 kg ha^{-1} of nitrogen. On the other hand, the variable main stem diameter (Figure 3b) obtained a significant cubic polynomial adjustment, with a point of maximum technical efficiency estimated at the dose of 80.92 kg ha^{-1} of nitrogen, but it is observed in the figure that the highest dose of N (150 kg ha^{-1}) was the one that provided the highest average main stem diameter. The increase in these variables associated with the increase in nitrogen doses may be related to the action of this macronutrient in the increase of meristematic activity and cell division, causing an increase in the number and also the size of cells in the stem of flaxseed. El-Gedway (2018) reports that flax plants fertilized with 70 kg ha^{-1} of nitrogen obtained greater technical length and main stem diameter. The increase in the main stem diameter can be a very valid characteristic to be measured, flax plants with a larger diameter are more resistant to lodging caused by abiotic factors, such as wind and rain. Lodging is more frequent in flax than in wheat, for example (Vera *et al.*, 2012).

In the environment of Frederico Westphalen – RS, it was found that the increase in nitrogen doses provided a linear increase in the technical length of the golden variety of flaxseed plants, with an increase of 16.17% in the highest nitrogen dose (150 kg ha^{-1}), concerning non-supplementary plants (0 kg ha^{-1}) (Figure 4a). There was also a significant polynomial adjustment for the brown variety, however, the adjustment was quadratic with the point of maximum technical efficiency of the nitrogen doses under the technical length of the flax plants of the brown variety, at the dose of 121.90 kg ha^{-1} of nitrogen (Figure 4b). In studies with flaxseed crops El-Gedwy *et al.* (2018), Abdel-Kader & Mousa (2019) and Emam (2019) reached similar results where there was an increase in technical plant length with nitrogen supplementation.

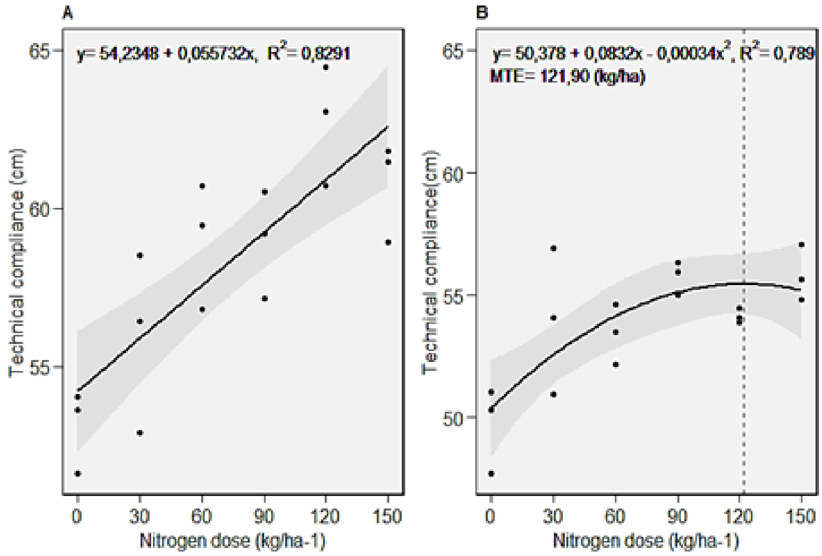

Figure 4: Relation between nitrogen doses (kg ha^{-1}) and the flaxseed plant technical length, golden variety (a) and brown variety (b), in Frederico Westphalen - RS. Santa Maria - RS, 2022

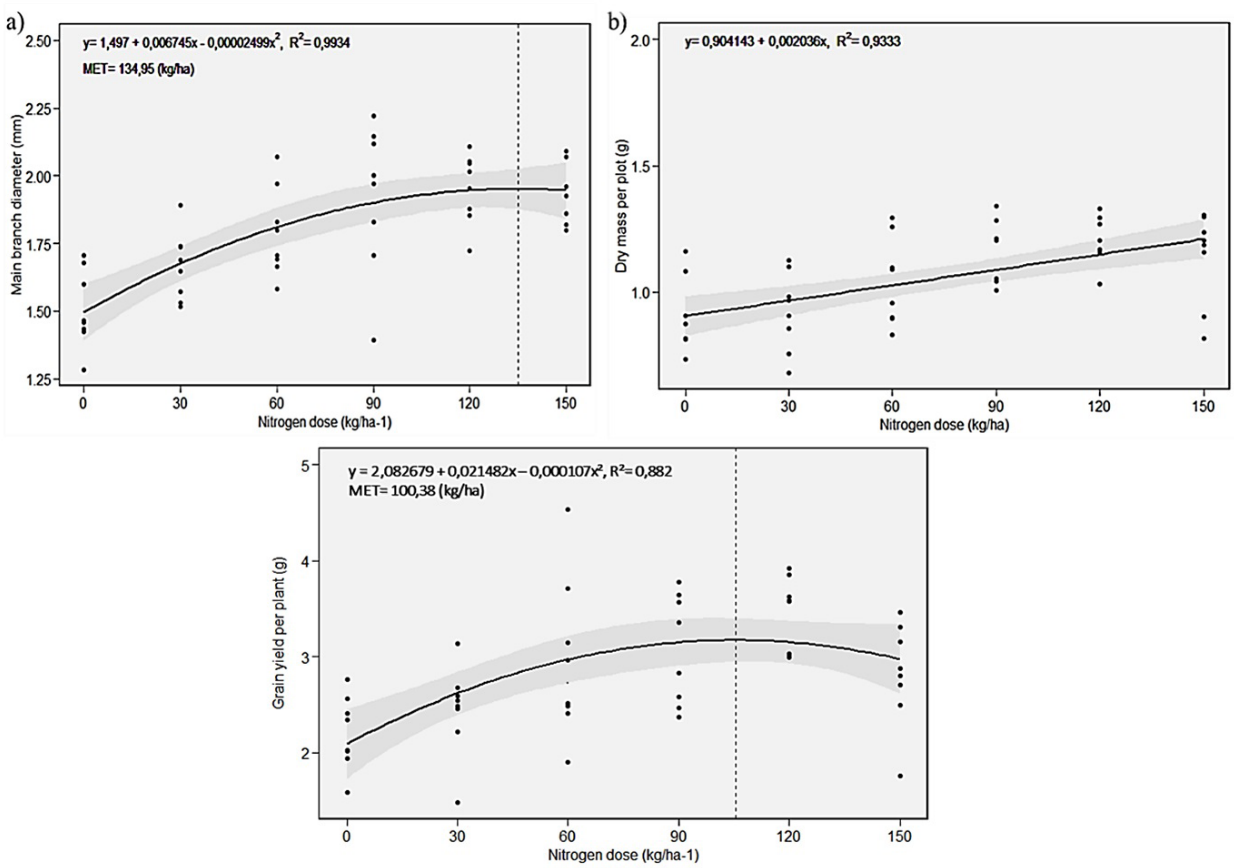

Figure 5: Relation between nitrogen doses (kg ha^{-1}) and the main stem diameter (a), plant dry mass (b), and flaxseed plant grain yield (c) (average of brown and golden variety) in Frederico Westphalen - RS. Santa Maria - RS, 2022.

Concerning the regression analysis of the "nitrogen doses", still in the environment of Frederico Westphalen – RS, the variables main stem diameter (Figure 5a) and plant grain yield (Figure 5c) showed a significant polynomial and quadratic adjustment, with maximum technical efficiency at the dose of 134.95 and 100.38 kg ha^{-1} of nitrogen, respectively. In contrast, Zhang *et al.* (2020) in a similar study with flaxseed reported that 90 kg ha^{-1} was the best nitrogen dose to improve flax grain yield, i.e., much lower doses than those found in the present study. However, similarly, the sharp increase in grain yield associated with the increase in nitrogen doses was also observed by Khajani *et al.* (2012), Kariuki *et al.* (2014), Andruszczak *et al.* (2015), Chopra & Badiyala (2016), Patel *et al.* (2017), Taddese & Tenaye (2018) and Yan *et al.* (2018). In addition, the increase in the main stem diameter with nitrogen doses was observed by El-Yamanee (2011). El-Gedway *et al.* (2018), reported that the dose of 70 kg ha^{-1} of nitrogen provided the greatest thickness in the main stem diameter, a value much lower than the dose of 134.95 kg ha^{-1} found in the present study.

In the variable plant dry mass (Figure 5b), in the environment of Frederico Westphalen – RS, the significant polynomial adjustment was linear and as the nitrogen doses increased, increased the flaxseed plants dry mass. It was also possible to observe that the highest dose of nitrogen (150 kg ha^{-1}) provided an increase of 34.44% in the plant dry mass, compared to the control treatment (0 kg ha^{-1}). This linear increase in plant dry mass also occurred in the environment of Santa Maria – RS. In contrast, Zhang *et al.* (2020) found that the dry mass increased by 36.13% with the application of 90 kg ha^{-1} of nitrogen, a dose much lower than the linear increase found in the present study with the highest doses. The authors also report that the application of doses above 90 kg ha^{-1} of nitrogen reduced the number of total flax plants' dry mass. Shi & Zhao (2018) and Cui *et al.* (2019) state that excessive nitrogen application reduced the rate of plant dry biomass accumulation.

The increase in plant dry mass due to the application of nitrogen fertilizers can be explained by the effect of nitrogen stimulation on growth attributes, maximizing the use of photosynthesis and, consequently, the number of metabolites, as well as the formation of new stem cells providing. Thus, an increase in technical length, yield length, and main stem diameter. This tendency of increasing dry mass with the application of nitrogen fertilizer was also observed by Khajani *et al.* (2012), Kariuki *et al.* (2014), Andruszczak *et al.* (2015), Chopra & Badiyala (2016) and Patel *et al.* (2017).

In general, it is possible to infer that flaxseed responds differently to nitrogen supplementation according to the cultivation environment, showing wide variability.

CONCLUSION

The brown variety obtained greater technical and yield length compared to the golden variety.

The golden variety showed a higher number of grains per capsule and higher plant grain yield compared to the brown variety, in the three environments (Santa Maria – RS, Frederico Westphalen – RS, and Viçosa – MG).

The plant dry mass increased linearly and proportionally to the increase in nitrogen doses in the environments of Santa Maria – RS and Frederico Westphalen – RS. The different doses of nitrogen applied influenced the variables analyzed and the varieties of flaxseed differently, depending on the environment.

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