Nitrogen fertilization split in green corn under rainfed system

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ABSTRACT

The objective of this work was evaluating the nitrogen fertilization splitting on the performance of green corn, grown in rainfed system. The experiment was carried out in Forquilha, Ceará state. Two varieties of corn (Sweet and Gorutuba) were used in the experiment, grown in five types of nitrogen fertilization in the crop cycle (one; three; six; nine; and twelve applications). The variables evaluated were: plant height, stem diameter, number of leaves of the plant, plant fresh mass, plant dry mass, ear mass plant^{-1}, marketable ear mass plant^{-1}, marketable husked ear mass plant^{-1}, marketable unhusked ear mass plant^{-1}, number of ear plant^{-1}, number of marketable ear plant^{-1}, length of husked marketable ear, length of marketable unhusked ear, diameter of marketable husked ear, diameter of marketable unhooked ear, number of rows marketable ear^{-1} and number of grains row^{-1} of marketable ear. The data were subjected to analysis of variance by the F test ($P < 0.05$) and the treatment means were compared by the test of Tukey ($P < 0.05$) and subjected to regression analysis. It was observed that the split of nitrogen fertilization promoted the development of the two varieties of corn studied. It was found that the split, performed in six applications, provided greater vegetative and reproductive development of the corn varieties.

Keywords: fertilizer; nitrogen; semi-arid; *Zea mays*.

INTRODUCTION

Corn (*Zea mays* L.) is one of the main cereals grown in Brazil, with about 81.36 million tons produced in the 2017/2018 harvest in an area of 16.63 million hectares and productivity of 4.89 tons per hectare (Conab, 2019). As a consequence, such crop plays a significant role in the Brazilian grain production (Cruz *et al.*, 2008). In addition, it is the most cultivated cereal in the world, with an average productivity of 5.75 tons per hectare, in which Brazil is the third largest producer, behind only the United States of America and China (FAO, 2017). However, in the semi-arid region of Brazil, productivity is still small, especially in family farming. According to Conab (2019), in the 2017/2018 harvest, the crop yield was 2.55 tons per hectare, a result caused by the non-use of appropriate technologies such as irrigation and balanced fertilization and also due to an irregular rainfall in the region. In Ceará state, where the cereal is produced mostly in family farming, productivity is 0.778 tons per hectare (Conab, 2019), demonstrating that the state can increase the productivity of corn crops.

Due to its versatility, corn adapts to different production systems, presenting a great economic importance, being used in several ways, since animal feeding to the high-tech industry (Embrapa, 2015; Portela *et al.*, 2016). It is one of the most cultivated and consumed cereals in the world, both for its productive potential, as well as for its chemical composition and nutritional value (Barrios & Basso, 2018; Fernandes *et al.*, 2007).

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Although corn is an input for a variety of products, the pork and poultry production chains consume approximately 70 to 80% of the corn produced in Brazil (Embrapa, 2015). However, the destination of this grain for human consumption is also of great importance, especially in the Northeast region, where the cultivation of corn and the commercialization of ear of green corn enables the addition of value to the product, allowing the producer to acquire a higher price than that of the corn destined for grains (Rocha *et al.*, 2011; Portela *et al.*, 2016). Thus, this product can be consumed both in its fresh form (cooked or roasted), as well as canned and processed in the form of tamale, cake, ice cream and several other traditional products, during all periods of the year (Pereira Filho *et al.*, 2003; Mamede *et al.*, 2009).

Similar to most crops, corn is a nutrient-demanding plant. A balanced mineral nutrition associated with adequate fertilization practices is vital for obtaining not only a high productivity but also an economically viable one (Portela *et al.*, 2016). Nitrogen is one of the most important nutrients for this crop as it removes large amounts of the mineral in the soil to the point of considering the nitrogen dose to be applied in the crop the most important decision in the management of fertilizers (Embrapa, 2015), with the nutrient being absorbed in greater amount by corn (Kappes *et al.*, 2014). According to Alonso *et al.* (2016), nitrogen has been a determining factor in increasing agricultural production over the past 50 years. Associated with favorable climatic and biological conditions, the correct management of soil fertility in terms of nitrogen fertilization is the main responsible for increasing crop productivity (Ribeiro *et al.*, 2018).

According to Amado *et al.* (2003), Cruz *et al.* (2008) and Silva *et al.* (2005), besides being the nutrient required in greater amount and the one that most influences corn productivity, nitrogen is also the one that most raises production costs, being the critical element for obtaining the maximum yield in production (Subedi & Ma, 2005). As reported by Ernani *et al.* (2005), the insufficient supply of nitrogen during the process of floral differentiation may reduce the differentiation of the number of eggs at the beginning of the ear, thus, negatively affecting the grain yield.

According to Araújo *et al.* (2004), the correct use of nitrogen fertilization in the corn crop increases the productivity indexes and the financial return to the producer, also contributing to the reduction of pollution risks to the agricultural system and the environment.

The nitrogen absorption in corn plants is low in the first thirty days, increasing between the days forty and fifty after the emergence of the plants, a fact that motivates the splitting of fertilization for this crop (Melo *et al.*, 1999). According to Vitória *et al.* (2012), nitrogen fertilization is complex and should be carried out carefully because, on the one hand, the lack of this nutrient can seriously limit production, on the other hand, its excess can drastically reduce it.

The period of application of nitrogen fertilization has a great influence on the use of this nutrient by plants (Kappes *et al.*, 2014). Due to the complexity in the management of nitrogen fertilization, it is recommended that it is carried out in installments. Thus, it is essential to carry out studies that evaluate the best way to split nitrogen fertilization in order to achieve a better use of this resource (Melo *et al.*, 1999).

According to Ceretta *et al.* (2007), splitting nitrogen fertilization reduces the losses caused by leaching and allows the application of the nutrient in the phase of the greatest need of the crop. According to Perfeito *et al.* (2017), nitrogen split promotes the early harvest and allows greater mass yield. In view of these considerations, this study was conducted with the objective of evaluating the effect of nitrogen fertilizer splitting on the performance of green corn, cultivated in rainfed systems, in the Northern region of the state of Ceará, Brazil.

MATERIAL AND METHODS

The experiment was carried out in Sector II of the Forquilha Irrigated Perimeter (NH2), located in the municipality of Forquilha in the state of Ceará within the geographical coordinates 3°46’S latitude and 40°17’W longitude. According to the classification of Koppen, the climate of the region is of the hot Bsh-Semi-Arid type. During the conduction of the experiment, the precipitation was monitored, with collections performed daily, over the experiment (Figure 1), with the aid of a rain gauge. The accumulated rainfall during the survey period corresponded to 509 mm.

Two corn varieties were used in the experiment (BC 2158F1 Sweet Corn hybrid and BRS Gorutuba Corn), grown in five types of nitrogen fertilization splitting (P1- fertilization was performed only once, 10 days after planting (DAP) ; P3 – fertilization was split into three periods at 10, 40 and 70 DAP; P6 – fertilization was split into six periods, at 10, 22, 34, 46, 58 and 70 DAP;

P9 – fertilization was split into nine periods at 10, 17, 24, 31, 38, 45, 52, 59, 66 DAP; and P12 – fertilization was split into twelve periods over the cycle at 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65 DAP. The source of nitrogen (N) used in the study was urea, which contains 48% of N. The amount applied for each of the splitting (2.4 m^2) was 40 grams of urea (166.66 kg ha^{-1}), however, fractionated in each application, with the following amount of fertilizer in each repetition: P1 – 40 g; P3 – 13.35 g; P6 – 6.67 g; P9 – 4.45 g and P12 – 3.34 g. Nitrogen fertilization followed the fertilization recommended by Embrapa Milho e Sorgo (Coelho, 2006).

In the fertilization splitting, N applications were performed manually, with the aid of a hoe, used to build the furrows with a depth of about 2 cm, and spaced at 20 cm from the neck of the plant. The application of the fertilizer was carried out in linearly (next to the planting line). After distribution, the fertilizer was buried to avoid losses due to volatilization, simulating applications by small farmers in the region.

Both corn varieties were planted in 12 m long lines with five treatments (split) in each line. Each repetition was represented by a 2.4 m plot, with six holes in each plot. The spacing used was 0.4 meters between plants and 1 m between lines, with two plants per hole, totaling 50,000 plants per hectare. The design used for the experiment was of randomized blocks, in a 2 x 5 factorial scheme (two varieties of corn x five types of nitrogen fertilization split), with six replications.

Soil tillage and removal of weeds were performed using a harrowing before the experiment setting. Both corn varieties were sown on March 15, 2018, under no-tillage system, at a depth of 4 cm, with three seeds per hole. Seedling emergence occurred five days after sowing. Ten days after emergence, thinning was performed manually, focusing on seedlings with better development. The cultivation was carried out in a rainfed system and, for the control of spontaneous plants, manual weeding was adopted. There was no need to apply pesticides to control pests and diseases.

Growth was evaluated at 74 DAP. The growth variables evaluated in the study were the following: plant height (PH), measured by direct measurement from the plant base to the last fully expanded leaf; stem diameter (SD), directly determined with the aid of a digital caliper at five centimeters from the level of the soil surface; number of leaves of the plant (NL), by counting definitive leaves; plant fresh matter (PFM) performed by cutting the plants, followed by washing, drying at room temperature and weighing on a precision scale; dry mass of the plant (PDM), after being packed in appropriate bags and taken to the oven where they remained for 48 h at a temperature of 105 ºC. After this period, they were removed and weighed on a precision scale.

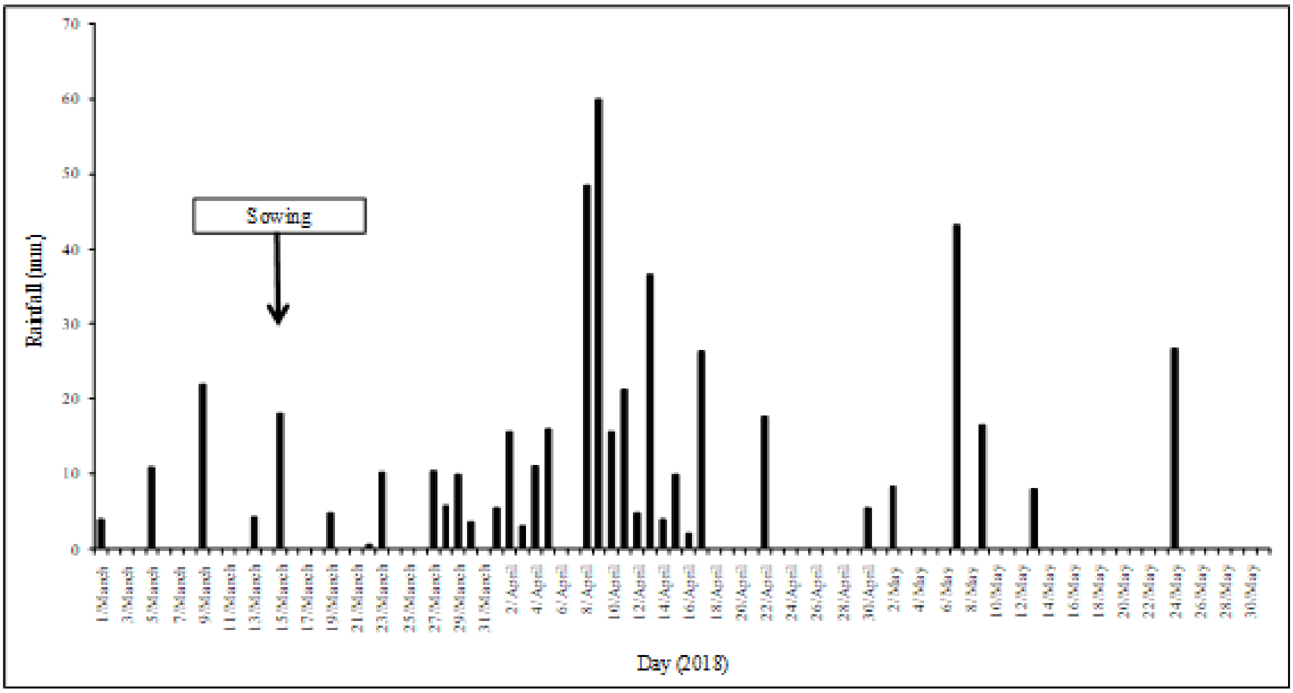

Figure 1: Rainfall distribution in Sector II in the Forquilha Irrigated Perimeter from March to June 2018.

At 75 DAP, Gurutuba corn was harvested and Sweet Corn was harvested at 82 DAP. The harvested material was taken to Plant Tissue Analysis Laboratory at the Ceará Federal Instituted/*Campus* of the Sobral city, where the production variables were evaluated: ear mass plant^{-1} (EM P^{-1}); marketable ear mass plant^{-1} (MEM P^{-1}); marketable husked ear mass plant^{-1} (MHEM P^{-1}), procedure performed after removal and separation of the ears in the plant, followed by washing, drying at room temperature and weighing on a precision scale; marketable unhusked ear mass plant^{-1} (MUEM P^{-1}), proceeded by removing the straws from the ears per plant and weighing them on a precision scale; number of ears plant^{-1} (NE P^{-1}), number of marketable ears plant^{-1} (NME P^{-1}), measured by the direct count of ears in the plant; length of the marketable husked ear (LMHE) and length of the unhusked marketable ear (LUME), obtained by measuring the ears with the aid of a ruler graduated in centimeters; diameter of the husked marketable ear (DHME) and the diameter of the unhusked marketable ear (DUME), determined directly with the aid of a digital caliper; number of rows marketable ear^{-1} (NR ME^{-1}); and grain number marketable ear row^{-1} (GN MER^{-1}), measured from direct count per ear.

The data obtained in the experiment were submitted to analysis of variance by the F test ($P < 0.05$) and when a significant effect was observed, the means of the treatments represented by qualitative variables were compared by the test of Tukey ($P < 0.05$), while the means of the quantitative variables were submitted to regression analysis, using the model that presented the best biological adjustment.

RESULTS AND DISCUSSION

A significant effect was found for the corn variety factor and for the number of nitrogen fertilization split factor ($P < 0.01$) in all growth variables analyzed in the crop (plant height, stem diameter, number of leaves, fresh weight of the plant and dry mass of the plant), and also a significant interaction was observed between the factors (corn variety x nitrogenous fertilization split) for most of these variables, with quadratic regression as the best fit for the nitrogen fertilization split factor in all analyzed growth variables (Table 1).

The Gurutuba corn variety showed a higher height than that of Sweet corn for all splitting conditions (Figure 2A). The Gurutuba corn variety had a higher height (196.83 cm) when the nitrogen fertilizer was split in 5.9 applications, while the Sweet corn variety showed higher PH (171.56 cm) with the nitrogen fertilization split in 5.4 times. According to Rodrigues Júnio *et al.* (2014), the presence of nitrogen fertilization increases leaf chlorophyll, promoting the development of the plant. In addition, if this nutrient is applied in installments, as shown by the results of Gross *et al.* (2006), a direct influence is observed on plant height besides providing a significant increase in corn productivity. The raise in plant height in response to the nitrogen supply in the corn crop was also evidenced by Lana *et al.* (2009) and Santos *et al.* (2010).

Nitrogen fertilization influenced the SD of both corn varieties analyzed in the experiment (Figure 2B). For both varieties, the stems of the plants had the highest diameter, 18.55 and 18.88 mm, for Sweet and Gurutuba, respectively, with fertilization split in 6.7 and 6.1 applications. Cruz *et al.* (2008) also found an increase in stem diameter in corn plants, 15 days after sowing, with the split application of nitrogen. In addition to the supporting structure of the plant, the stem also acts as a storage site for soluble solids to be later used in grain formation (Fancelli & Dourado-Neto, 2000).

The Gurutuba corn variety showed the highest NL in all nitrogen fertilization splitting conditions (Figure 2C), when compared to the Sweet corn variety. As the splitting with 6.6 applications is the best treatment, resulting in a NL of 12.52, which was 10.8% higher than the Sweet variety, which presented a NL of 11.17, with 6.2 applications.

Both corn varieties studied had the highest PFM when they received nitrogen fertilization split into 6.3 and 5.1 applications, providing a PFM of 220.13 and 260.04 g plant^{-1} of Sweet corn and Gurutuba, respectively. As for the variable PDM, it was observed that the Gurutuba variety had a mass of 93.03 g plant^{-1} for 5.6 applications, while the mass in Sweet variety was 95.07 g plant^{-1} for a number of nitrogen fertilizer applications corresponding to 6.6. In this split fertilization, with a greater number of applications, the Sweet variety has a 3% greater mass, differing significantly from the Gurutuba variety (Figures 2D and 2E). It is observed that the fertilization installments between 5.1 and 6.6 applications are the best interval between treatments for the vegetative development of Sweet and Gurutuba corn varieties, contributing significantly to a greater production of phytomass in the crop. These results are similar to those obtained by Cardoso *et al.* (2011), who found that the top-dressing nitrogen application increased the plant height.

A significant effect was observed for the corn variety factor, for the number of nitrogen fertilization splitting factor and for the interaction between the factors (corn variety and nitrogen fertilization split) ($P < 0.01$), for all variables (mass of ears plant^{-1}, number of ears plant^{-1}, mass of marketable ears plant^{-1}, number of marketable ears plant^{-1}, mass of unhusked marketable ears plant^{-1} and mass husked marketable ears plant^{-1}) related to the reproductive development of plants, in which the quadratic regression model was placed as the best fit, presenting a highly significant effect for all variables related to the reproductive development of corn plants (Table 2).

Table 1: Summary of analysis of variance and regression for the variables plant height (PH), stem diameter (SD), number of leaves (NL), plant fresh mass (PFM) and plant dry mass (PDM) of two varieties of green corn (Sweet and Gurutuba), grown in rainfed system with nitrogen fertilization split. Sobral-CE, 2018

SV	DF	Mean Square				
		PH (cm)	SD (mm)	NL	PFM (g)	PDM (g)
Variety (V)	1	6869.40**	19.51**	13.06**	924.73**	153.92**
Installment (I)	4	1488.64**	37.43**	6.73**	15377.71**	7032.67**
V * I	4	148.27*	7.03**	0.31^{ns}	7101.03**	776.59**
Residue	50	55.42	1.03	0.57	95.65	16.47
CV (%)		4.26	6.28	6.90	4.75	5.71
Regression (nitrogen fertilization split)						
Linear	1	845.59**	9.44**	4.14*	17430.68**	1268.15**
Quadratic	1	3194.63**	131.57**	18.87**	38272.07**	18530.44**

*, **Significant at 5 and 1%, respectively by the F test; ^{ns}Not-significant by the F test; SV = Source of variation; CV = Coefficient of variation.

Table 2: Summary of the analysis of variance for the variables ear mass plant^{-1} (EM P^{-1}), number of ear plant^{-1} (NE P^{-1}), mass of marketable ear plant^{-1} (MME P^{-1}), number of marketable ear plant^{-1} (NME P^{-1}), marketable unhusked ear mass plant^{-1} (MUEM P^{-1}) of both varieties of green corn and mass of marketable ear straw plant^{-1} (MPEC P^{-1}) (Sweet and Gurutuba), grown in a rainfed system with nitrogen fertilization split Sobral-CE, 2018

SV	DF	Mean Square					
		EM P^{-1} (g)	NE P^{-1}	MME P^{-1} (g)	NME P^{-1}	MUEM P^{-1} (g)	MPEC P^{-1} (g)
Variety (V)	1	18500.16**	0.77**	114216.35**	0.15**	73365.66**	4382.04**
Installment (I)	4	18295.78**	0.63**	19039.29**	0.26**	10719.42**	1855.23**
V * I	4	9505.38**	0.15**	12199.39**	0.05**	8433.37**	456.35**
Residue	50	48.49	0.03	80.91	0.01	90.60	121.13
CV (%)		4.88	17.65	5.46	15.91	7.73	26.33
Regression (nitrogen fertilization split)							
Linear	1	44303.63*	0.00^{ns}	0.39^{ns}	0.10**	19.75^{ns}	6.93^{ns}
Quadratic	1	5259.15**	2.23**	60171.67**	0.53**	37520.94**	2556.48**

**Significant at 1% by the F test; ^{ns}Not-significant by the F test; SC = Source of variation; CV = Coefficient of variation.

Both corn varieties showed variation for the total mass of ears/plant, according to the number of installments of nitrogen fertilization applied (Figure 3A). The Sweet corn variety had the highest value for total mass of ears in installments of one and three applications. However, based on the nine-applications splitting, the Gurutuba corn variety was superior, with the highest value for total mass of ears plant^{-1}. For this variable, the Sweet corn variety performed better with fertilization split in one application. On the other hand, the Gurutuba variety had a higher total mass of ears plant^{-1} in the treatment with six applications of nitrogen fertilization.

No difference was found between the two corn varieties with respect to NE P^{-1} when nitrogen fertilization was performed in up to six applications. However, Sweet corn variety was superior for this variable when the fertilization was split into nine and twelve applications (Figure 3B). With nine applications, the Gurutuba variety produced 21% less ears than the Sweet variety, thus showing greater productive potential when fertilized

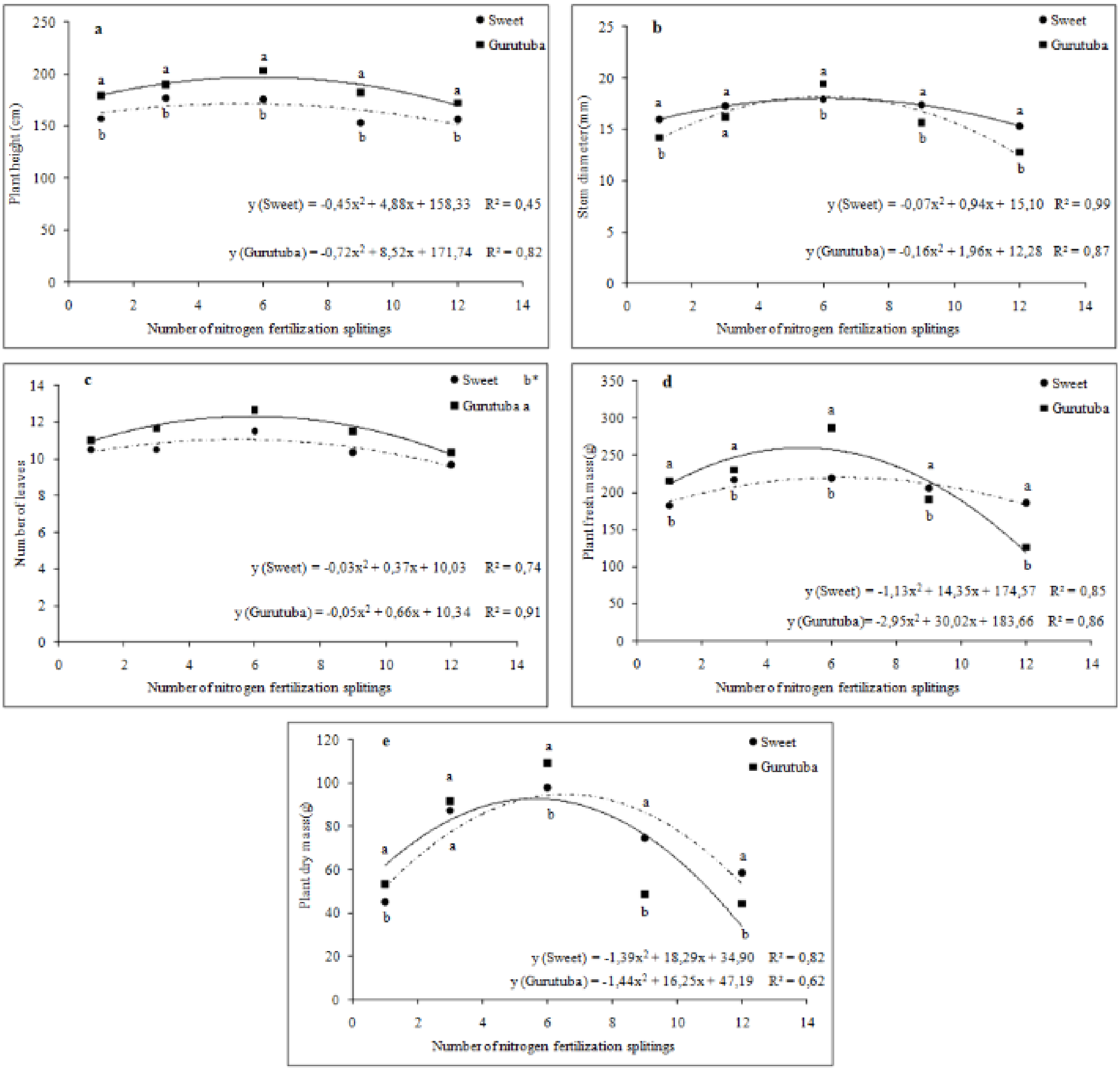

Figure 2: Relationship between the number of nitrogen fertilization splits and the growth variables (a - plant height; b - stem diameter; c - number of leaves; d - plant fresh mass; e - plant dry mass), related to vegetative development of the corn varieties Sweet (dotted line) and Gurutuba (full line).

at the correct time. Nitrogen fertilization applied in 10.5 times allows the largest number of ears per plant. Such result was the opposite to that obtained by Souza *et al.* (2016), who did not verify the effect of nitrogen fertilization on Sweet corn plant.

Regarding MEC P^{-1} variable, the value found for Sweet corn was 429.39 g, showing a value for mass greater than Gurutuba, which was 422.24 g, with 7.7 and 5.9 N installments, respectively In relation to NEC P^{-1}, the highest values were observed in both corn varieties when the plants received the treatment with nitrogen fertilization in 5.5 and 6 applications for Sweet and Gurutuba corn, respectively, showing a NEC P^{-1} of 0.78 and 0.82 Even with the lowest number of marketable ear plant^{-1} (Figure 3D), the Sweet corn variety showed the highest mass of husked marketable ear plant^{-1} (Figure 3C), the highest mass of unhusked marketable ear plant^{-1} (Figure 3E) and the highest mass of straw of marketable ear plant^{-1} (Figure 3F), for most treatments with nitrogen fertilization, particularly in the treatment with six applications of nitrogen fertilization, which presented the best results for most of these variables (Figure 3) Cardoso *et al* (2011) also observed that the

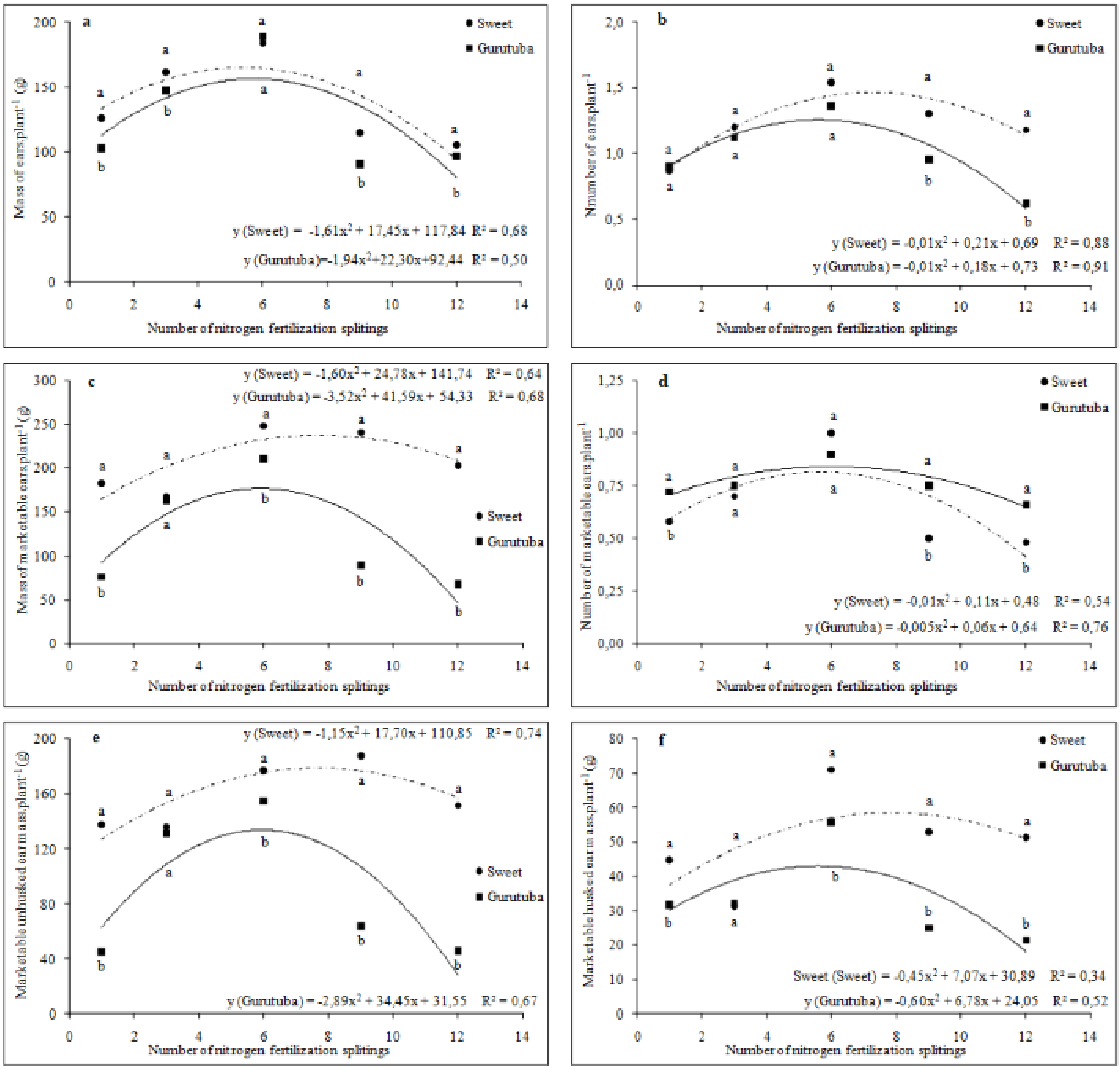

Figure 3: Relationship between the number of nitrogen fertilization installments and the variables (a - mass of ears.plant^{-1}; b - number of ears.plant^{-1}; c - mass of marketable ears.plant^{-1}; d - number of marketable ears.plant^{-1}; e - marketable unhusked ear mass.plant^{-1}; f - marketable husked ear mass plant^{-1}) related to the reproductive development of the sweet green varieties Sweet (dotted line) and Gurutuba (full line).

application of nitrogen in topdressing increased the height of the ear, the number of grains per ear and the grain yield of the corn crop grown under no-tillage system. Similar results were also obtained by Marković *et al.* (2017) when studying the development of corn plant plants under irrigation.

The results obtained in this study support the statement by Alonso *et al.* (2016), when reporting that adequate amount of nutrients is a fundamental aspect to improve agricultural production. Currently, the fertilization rates recommended for farmers are very general and, in some cases, they are not related to the amount needed for the crop and its availability in the soil, resulting in an unbalanced and inefficient use of fertilizers and high production costs.

A significant effect was observed for the factors corn variety and number of nitrogen fertilization splits ($P < 0.01$) for all variables related to reproductive performance analyzed in the crop (length of marketable husked ear, diameter of marketable husked ear, length of unhusked marketable ear, diameter of unhusked marketable ear, number of rows.marketable ear^{-1} and number of grains.row of marketable ear^{-1}). However, no significant interaction was found between the factors (corn variety and nitrogen fertilization split) for any of these variables (Table 3).

Table 3: Summary of analysis of variance for the variables: length of husked marketable ear (LHME), diameter of husked marketable ear (DHME), length of unhusked marketable ear (LUME), diameter of unhusked marketable ear (DUME), number of rows marketable ear^{-1} (NR ME^{-1}) and number of grains row of marketable ear^{-1} (NR ME^{-1}) of both varieties of green corn (Sweet and Gurutuba), grown in a rainfed system with nitrogen fertilization split. Sobral-CE, 2018

SV	DF	Mean Square					
		LHME (cm)	**DHMECP (mm)**	**LUME (cm)**	**DUME (mm)**	**NR ME^{-1}**	**NG RME^{-1}**
Variety (V)	1	68.03*	139.81**	27.97**	353.61**	13.06*	421.35**
Installment (I)	4	51.72**	78.58**	23.83**	114.51*	43.26**	228.05**
V * P	4	0.59^{ns}	33.19^{ns}	1.68^{ns}	30.64^{ns}	1.98^{ns}	22.30^{ns}
Residue	50	12.38	16.72	2.95	39.15	2.19	9.76
CV (%)		16.72	9.22	10.30	15.85	10.38	10.48
Regression (nitrogen fertilization split)							
Linear	1	1.75^{ns}	113.85*	16.61*	331.45**	90.29**	95.69**
Quadratic	1	145.46**	84.89*	36.99**	48.93^{ns}	13.60*	273.38**

**, *Significant at 1 and 5%, respectively by the F test; ^{ns}Not-significant by the F test; SC = Source of variation; CV = Coefficient of variation.

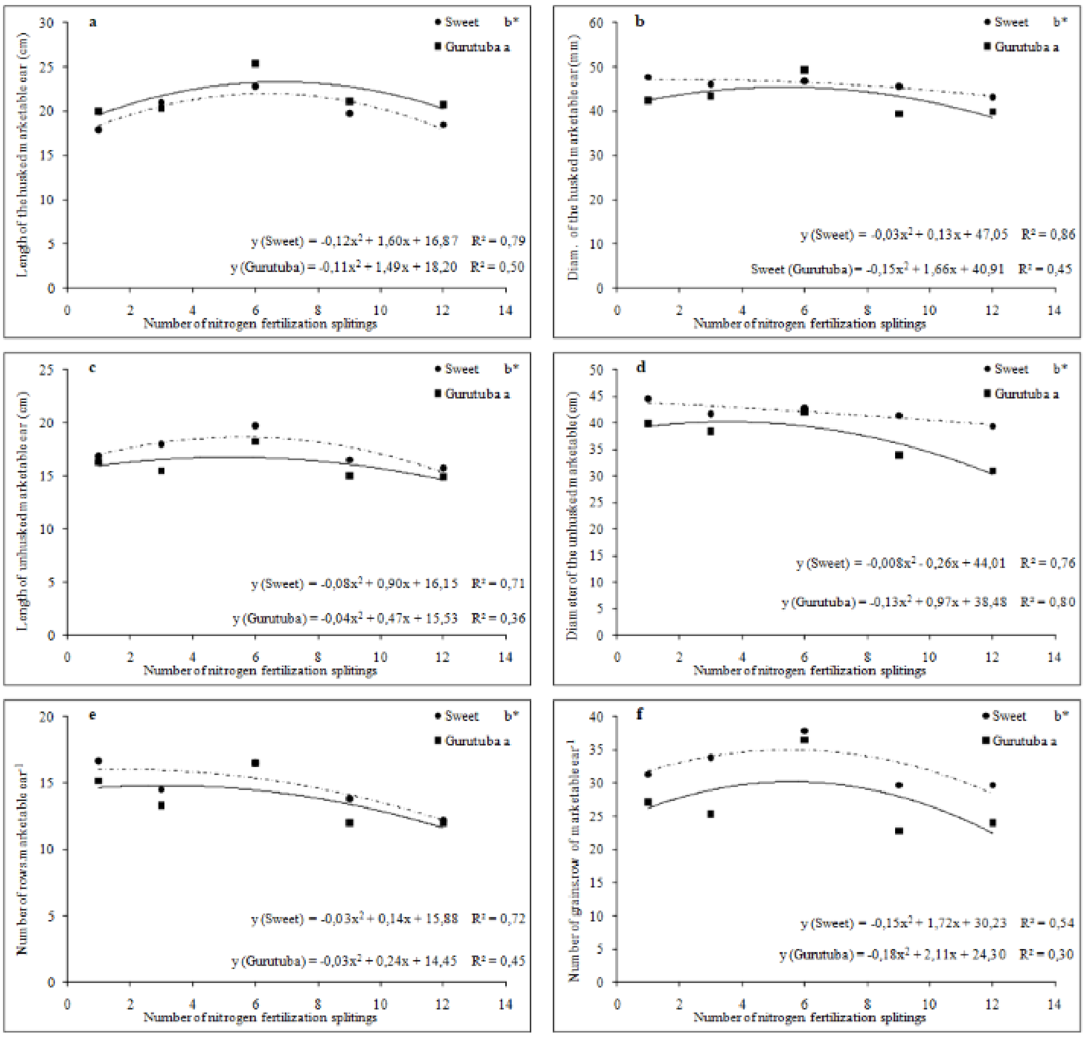

Figure 4: Relationship between the number of nitrogen fertilization splits and the variables (a - length of the husked marketable ear; b - diameter of the husked marketable ear; c - length of unhusked marketable ear; d - diameter of the unhusked marketable ear; e - number of rows.marketable ear^{-1}; f - number of grains.row of marketable ear^{-1}) related to the reproductive performance of the Sweet (dotted line) and Gurutuba (full line) green corn varieties.

Nitrogen fertilization split into six applications was also the treatment that showed the best results for the development of ears of Sweet and Guratuba corn varieties (Figure 4). Husked marketable ears of the Gurutuba variety showed greater length (Figure 4A). However, the Sweet corn variety had the largest diameter of the husked marketable ear (Figure 4B), the largest length of the unhusked marketable ear (Figure 4C), the largest diameter of the unhusked marketable ear (Figure 4D), the largest number of rows/marketable ear (Figure 4E) and the highest number of grains row of marketable ear^{-1} (Figure 4F). Such results refute those obtained by Casagrande & Fornasieri Filho (2002), where it was not observed any effect of application periods and nitrogen doses in the off-season corn in relation to productivity, the number of rows per ear, the number of grains and the mass of thousand grains.

Considering that the cost of the nitrogen fertilization split is relatively low (Rodrigues Júnior *et al.*, 2014), this strategy may be viable because it reduces the risks of nitrogen losses through applications in conditions that promotes the loss of the nutrient, either through leaching and volatilization and denitrification (Alonso *et al.*, 2016), causing negative effects on the environment. According to Cardoso *et al.* (2011), most nitrogen losses occur through denitrification and/or ammonia volatilization. These results corroborate the importance and the positive effect that the nitrogen fertilization split has on the optimal development of the corn crop.

CONCLUSIONS

Nitrogen fertilization split carried out in six applications promotes the vegetative and reproductive development of the varieties of Sweet and Gurutuba sweet corn varieties grown in rainfed system in the northern region of the state of Ceará, therefore, showing to be an alternative to increase the productivity of the semi-arid family farmer.

Sweet and Gurutuba sweet corn varieties show differentiated responses in the vegetative and reproductive development according to the type of splitting of the nitrogen fertilization applied.

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