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Initial development of maize plants grown with different combinations of nitrate and ammonium

Gildeon Santos Brito[1], Girlene Santos de Souza[2], Alfredo Teles de Jesus Neto[3], Cristian Martins de Souza[4], Anacleto Ranulfo dos Santos[5]

[1] Universidade Federal do Recôncavo da Bahia - Center for Agricultural, Environmental, and Biological Sciences, undergraduate in Agronomy, PIBIC FAPESB Scholarship Holder. E-mail: gildeon.9772@outlook.com

[2] Universidade Federal do Recôncavo da Bahia - Center for Agricultural, Environmental, and Biological Sciences. Associate Professor 4. Email: girlene@ufrb.edu.br. Author for correspondence

[3] Universidade Federal do Recôncavo da Bahia - Center for Agricultural, Environmental, and Biological Sciences, undergraduate in Agronomy, PIBIC CNPq Scholarship Holder. E-mail: alfredotelles008@outlook.com

[4] Universidade Federal do Recôncavo da Bahia - Center for Agricultural, Environmental, and Biological Sciences, undergraduate in Agronomy, PIBIC FAPESP Scholarship Holder. E-mail: cr-tiam@hotmail.com

[5] Universidade Federal do Recôncavo da Bahia - Center for Agricultural, Environmental, and Biological Sciences, Full Professor. E-mail: anacleto@ufrb.edu.br

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Abstract

Corn is one of the main commodities of Brazilian agribusiness, due to its wide use, whether for human or animal consumption or in the chemical and biofuel industries. Thus, this work was carried out to evaluate the influence of different proportions of nitrate and ammonium in the initial development of corn plants. The experiment took place in a greenhouse, by direct sowing, where the seeds were placed at a depth of 1 cm in plastic pots with a capacity of 6 dm^3 containing a mixture of previously sieved and washed with vermiculite, in a 2:1 ratio. Five proportions of nitrate and ammonium ions (NO_3^- :NH_4^+) were used: T1=100:0, T2=75:25, T3=50:50, T4=25:75 and T5=0:100, through nutrient solutions. After 35 days of cultivation, the following were evaluated: plant height, number of leaves, culm diameter, chlorophyll *a*, *b*, total and *a/b* ratio, root length, leaf fresh matter mass, dry matter mass of leaves, culm, roots, total dry mass, and root dry mass/shoot dry mass ratio, leaf area, specific leaf area, leaf area ratio, and leaf mass ratio. Nitrate as the only source of nitrogen provided a smaller increase in the chlorophyll *a* index of maize plants, which is the most important chlorophyll in the capture of light in the antenna complex. It is not recommended to cultivate corn plants with only nitrate or ammonium; however, it was observed that high doses of nitrate combined with low doses of ammonium in the culture medium provided satisfactory performance for most variables.

Keywords: Ionic interaction. Growth. *Zea mays L.*

Introduction

Corn (*Zea mays* L.) is considered the second most important cereal in the world, behind wheat and ahead of rice (LISBOA et al., 1999). Due to its great diversity of applications, both in human food and animal feed, corn crop has social and economic relevance (ROMANO, 2005).

Pinheiro et al*.* (2021) stated that corn is among the main crops worldwide. Its production chain is rich in strands, with great production capacity from the countless investments in technologies for genetic improvement and cultivation techniques. Thus, it is a culture that will always have market value, visibility, and profitability; corn is the target of many scientific studies and investments to better respond to fertilization, resist pests and diseases, obtain high productivity, high-quality grains, and other factors associated with production optimization.

Corn crops' necessity of nitrogen fertilization is one of the factors that most influence the number of studies regarding this fertilizer. According to Felisberto (2015), nitrogen fertilization has an important function in corn cropd since nitrogen (N) is the most absorbed nutrient by this species. Thus, in many production systems, nitrogen availability is almost always a limiting factor, which influences plant growth more than any other nutrient (BREDEMEIER; MUNDSTOCK, 1999).

This element is the main constituent of nucleotides, amino acids, and proteins; therefore, nitrogen is essential to life (BRAUN et al., 2013). In addition, Silva et al. (2010) also highlight that nitrogen is considered the most important factor, after water deficiency, to limit biomass production in natural ecosystems.

The predominant forms of mineral nitrogen available to plants are ammonium ions (NH_4^+) and nitrate (NO_3^-); therefore, it is extremely important to know the most appropriate proportions of these substances for each crop. Ammoniacal and nitric forms have different effects on plant growth and vigor, as well as on biomass production and reproduction (LANE; BASSIRIRAD, 2002), we also highlight that these effects vary from species to species.

Lasa et al. (2001; 2002) stated that excess nitrate is tolerable by most plants, mainly because their ions are accumulated in vacuoles and, thus, do not cause symptoms of toxicity to plants and may even perform osmotic functions. Although plants are not much affected by excess nitrate, their accumulation in vegetables and consequent consumption induces possible problems in humans and animals (SANTAMARIA, 2006).

On the other hand, most vascular plants develop severe toxic symptoms when grown only with ammonium (CAMPOS, 2013). Silva et al. (2010) stated that its root absorption depends on a single intake and is mediated by a carrier; however, when taken to the cell cytoplasm, it causes an electrostatic imbalance between the intra- and extracellular medium, and this process causes the cell to promote the contrary flow of positive charges to achieve neutrality.

Given its importance, this study was carried out to evaluate the influence of different proportions of nitrate and ammonium on the initial development of corn plants.

Material and methods

The experiment was carried out between March and April 2022, in a greenhouse of the Center for Agricultural, Environmental, and Biological Sciences (CCAAB) of the Universidade Federal do Recôncavo da Bahia (UFRB), in the city of Cruz das Almas, located at 200 m altitude above sea level, latitude of 12°40' S, and longitude of 39°06' W of Greenwich, located in the south of the region known as Recôncavo da Bahia. According to the Köppen classification, it has Aw to Am climate, a hot and humid tropical climate, with an average annual rainfall of 1224 mm, with higher occurrences of rain in the period from March to June.

The corn cultivar AG-1051 acquired in the local trade of the city of Cruz das Almas (BA) was used. The seeds were arranged at 1 cm depth in plastic pots of 6 dm^3, with a mixture of sand previously sifted (in a 2 mm sieve) and washed (in running water, until all organic matter residue was removed) with vermiculite, in the ratio of 2:1, respectively, with three seeds distributed per pot.

After seedling emergence, thinning was performed on the eighth day after sowing, leaving only the most vigorous and standardized seedling in terms of height and number of leaves.

The experiment was carried out with a completely randomized design and five replications. The treatments consisted of five proportions of nitrate and ammonium ions (NO_3^- :NH_4^+), in which: T1=100:0, T2=75:25, T3=50:50, T4=25:75, and T5=0:100. The respective treatments were adjusted according to the standard solution established by Hoagland and Arnon (1950) so that, during their cycle, each plant received the macronutrients in the concentration in mg L^{-1}: N=210, P=31, K=234, Ca=200, Mg=48, and S=64 (Table 1). The application of the treatments occurred eight days after sowing and was performed twice a week, totaling seven applications of 200 mL.

Irrigation was performed manually by applying 200 mL of water in each pot in the first week, and, as the water requirement increased,

Table 1. Table of stock solutions with volume (mL) to form 1L of modified nutrient solution, appropriate to the respective treatments.

Stock Solution (1M)	Proportions of (NO_3^-:NH_4^+)				
	0:100	25:75	50:50	75:25	100:0
	(mL L-1)				
KH_2PO_4	1.0	1.0	1.0	1.0	1.0
NH_4Cl	15.0	11.25	7.50	3.75	-
KCl	5.0	1.25	5.0	3.75	-
$CaCl_2$	5.0	5.0	1.25	-	-
$MgSO_4$	2.0	2.0	2.0	2.0	2.0
KNO_3	-	3.75	-	1.25	5.0
$Ca(NO_3)_2$	-	-	3.75	5.0	5.0
Micronutrients **	1.0	1.0	1.0	1.0	1.0
Iron – EDTA *	1.0	1.0	1.0	1.0	1.0

**Micronutrient solutions (g/l): H_3B0_3 = 2.86; $MnCl_2$ $4H_20$ = 1.81; $ZnCl_2$ = 0.10; $CuCl_2$ = 0.04; H_2Mo0_4 H_20 = 0.02. *Iron-EDTA Solution: A total of 26.1 g of disodium EDTA was dissolved in 286 ml of NaOH 1N + 24.9g $FeS0_4.7H_20$ and aerated for one night.

the amount increased to 300 mL. The water used for irrigation was obtained by Empresa Baiana de Águas e Saneamento S.A. (EMBASA), which sample was sent for analysis of chemical composition in the laboratory (Table 2).

From 35 days after sowing, we evaluated: plant height (PH), number of leaves (NL), culm diameter (CD), chlorophyll *a* (CLA), chlorophyll *b* (CLB), chlorophyll total (CLT) and *a*/*b* ratio, root length (RL), leaf fresh matter mass (FLM), leaf dry matter mass (LDM), culm dry matter mass (CDM), root dry matter mass (RDM), total dry matter mass (TM), and ratio of root dry mass/ aerial part dry mass (DRM/APDM), leaf area (LA), specific leaf area (SLA), leaf area ratio (LAR), and leaf mass ratio (LMR).

The PH was measured with a graduated measuring tape in mm from the plant neck to the end of the last leaf; the CD measured approximately 3 cm from the substrate, with the aid of a digital caliper with an accuracy of 0.01 mm; the RL was measured with a graduated ruler from the upper base up to the volume corresponding to approximately 80% of the effective root system; chlorophyll indexes were evaluated between 8:00 and 10:00 a.m., using the Falker electronic meter model CFL1030, with readings performed on three leaves of the middle third of each plant, and the number of leaves obtained by direct manual counting.

Regarding the determination of dry phytomass, the components were partitioned into leaves, culms,

Table 2. Chemical characteristics of the water used for irrigation. Water supply by EMBASA.

Parameters	pH.	Ca^{2+}	Mg^{2+}	Na^+	K^+
		mmolc L-1			mg L-1
Results	4.5	5.5	2	1	3.9
Method	Embrapa Florestas, 2011.				

pH = ionic hydrogen potential. Ca^{2+} = calcium concentration. Mg^{2+} = magnesium concentration. Na^+ = sodium concentration. K^+ = potassium concentration.

and roots; the material was individually packed in paper bags and subjected to drying in a greenhouse with forced air circulation at 65ºC ± 2ºC, for a period of 72 hours, until reaching constant mass. The dry mass values were obtained on an analytical scale of precision of 0.001 g.

The determination of LA per plant was made by the ratio of LDM and dry mass matter of ten leaf discs, which were obtained with the aid of a known area driller (12 mm), always avoiding the central rib. The SLA, LAR, and LMR were determined from the values of LA, LDM, and TM, expressed in grams, using mathematical formulas described by Peixoto et al. (2011).

The data were submitted to variance analysis with the aid of the computational statistical program "R" (R Development Core Team, 2018), and according to the significance level, the Tukey test was applied at 5% probability for comparison of means.

Results and discussion

The different proportions of ions NO_3^- and NH_4^+ significantly influenced ($P<0.05$) chlorophyll indices, plant height, leaf fresh matter mass, leaf dry matter masses, culms, roots, total dry matter mass of the plant, and root dry matter mass/aerial part dry matter mass ratio.

Table 3 shows that plants grown exclusively with NO_3^- or NH_4^+ had the chlorophyll *a* increment negatively affected, with more severity associated with excess nitrate. Silva et al. (2010) obtained a similar result in sunflower plant cultivation, in which they observed that nitrogen in only one of these forms is unfavorable to the increase in leaf chlorophyll indices.

Chlorophylls *b* and total similar trends (Table 3), in which the supply of N, exclusively in the form of NO_3^-, impaired these variables, whereas the other treatments did not differ from each other. An important fact is that chlorophylls perform different purposes in plant metabolism. Chlorophyll *b*, for example, absorbs energy at different wavelengths than chlorophyll *a* and transfers it to the reaction center, optimizing the energy capture that effectively acts in photochemical reactions (TAIZ et al., 2017). However, N absorption in nitric form requires reduction processes for further incorporation into organic compounds, so nitrate is reduced to nitrite by nitrate reductase, and nitrite to ammonia by nitrite reductase. This process consumes a large amount of energy, carbon, and protons (BUCHANAN et al., 2000); this complex metabolic route prevents the rapid formation of organic molecules, including chlorophyll.

Via the relationship between chlorophylls *a*/*b*, we perceived a higher average for treatment only with NO_3^- (Table 3), which is a variable of great importance since it indicates photooxidative losses caused by high intensity irradiation (HENDRY; PRICE, 1993), in addition to indicating

Table 3. indices of *a* (CLA), *b* (CLB) ,and total (CLT) chlorophyll of corn plants grown under different nitrate and ammonium proportions. Cruz das Almas, BA, 2022.

NO_3^-:NH_4^+	CLA	CLB	CLT	CLA/CLB
0:100	37.7 b	16.21 a	53.91 a	2.34 b
25:75	38.91 ab	17.97 a	56.88 a	2.19 b
50:50	40.77 a	16.56 a	57.33 a	2.46 b
75:25	40.31 a	17.29 a	57.6 a	2.34 b
100:0	32.34 c	10.46 b	42.8 b	3.12 a
CV (%)	**3.34**	**9.79**	**4.77**	**7.87**

*Same letters do not differ from each other in columns, by Tukey test at 5% probability.

the additional quantity of chlorophyll *a* in comparison to chlorophyll *b*, of which chlorophyll *a* acts more effectively in the capture of light during the photosynthetic process. On the other hand, both are responsible for capturing light in the antenna complex, besides being primordial components of reaction centers in photosystems (TAIZ et al., 2017) and, for this reason, are very important molecules since they act directly in the production of photoassimilates that will promote the growth and development of the plant.

We verified that the proportion of 75% of NO_3^- promoted an increase of approximately 19.7% in the height of corn plants compared to the treatment with the absence of this ion (Table 4). This combination of the high nitrate concentration in the substrate with low ammonium concentration favored the increase in plant height by reducing ammonium toxicity. According to Hachiya et al. (2012), ammonium toxicity in plants is often accompanied by the depletion of organic acids and inorganic cations and the accumulation of ammonium, which consider all these factors as possible causes of NH_4^+ toxicity, which is a determining factor for plant growth suppression.

Regarding the variable number of leaves, no influence of the treatments used was observed to detect a statistical difference (Table 4). This is because ammonium is a source of rapid absorption and assimilation, whereas the preferred sites of NO_3^- reduction are leaves, aiding plants to take advantage of both forms of nitrogen.

In a study that evaluated proportions of NO_3^- and NH_4^+ in eucalyptus culture, Guimarães et al. (2014) obtained similar results for this variable, which was not statistically distinguished from the treatments used. Similarly, it was observed in the variable culm fresh matter mass that the difference in ion concentrations was not sufficient to promote statistical differences, which also revealed the efficient use of nitrate and ammonium.

In the fresh matter mass of the leaves (Table 4), we noted the plants developed differently according to the treatments. Plants grown with the proportion of 75% nitric N had an increase of approximately 33.15% of leaf fresh matter mass, compared to plants grown only with ammonium. Thus, it is evident that the supply with high concentrations of ammonium was not favorable to the increase of this variable, since the excess of ammonium causes a reduction in the tissue concentrations of Ca_2^+ and Mg_2^+ (ROOSTA; SCHJOERRING, 2008), chemical elements that directly participate in the metabolism of the vegetable and influence its growth and development.

The dry matter masses of the leaves and roots of corn plants were variables with similar

Table 4. Height (HGT), number of leaves (NL), leaves fresh matter mass (LFM) and culm fresh matter mass (SFM), leaf matter dry mass (LMDM), culm dry mass (CDM), and root dry mass (RDM) of corn plants grown under different nitrate and ammonium proportions. Cruz das Almas, BA, 2022.

NO_3^-:NH_4^+	HGT	NL	LFM	SFM	LMDM	CDM	RDM
	(cm)	(un)	- - - - - - - - - -	- - - - - - - -	(g)	- - - - - - - -	- - - - - - - - - -
0:100	78.42 b	8.4 a	17.69 C	24.74 a	3.18 b	2.26 b	3.35 b
25:75	79.92 b	8.6 a	20.29 bc	31.06 a	3.56 b	2.88 ab	3.21 b
50:50	87.06 ab	8.8 a	24.32 ab	27.99 a	4.32 ab	2.71 ab	3.63 ab
75:25	93.86 a	8.4 a	26.46 a	31.47 a	4.90 a	3.40 a	4.57 a
100:0	86.4 ab	8.4 a	21.45 abc	24.22 a	3.93 ab	2.43 b	3.78 ab
CV (%)	**8.3**	**9.68**	**12.59**	**16.88**	**15.15**	**16.88**	**16.26**

*Same letters do not differ from each other in columns, by Tukey test at 5% probability.

responses to the treatments used (Table 4). The best performance was observed with high concentrations of NO_3^- in the substrate; however, when it became an exclusive source of nitrogen, this performance was compromised, revealing that nitrate may be harmful due to the characteristics of need for a reduction since the absorption rate of NO_3^- usually exceeds the capacity of its reduction, causing this ion to accumulate and perform toxic functions (SOLOMONSON; BARBER, 1990).

We also found that excess ammonium was unfavorable. This is because nitrate reductase (NR) activity is known to decrease when the concentration of NO_3^- in the external environment is low and that of NH_4^+ is high (HELALI et al., 2010). Also, according to Hachiya et al. (2012), ammonium toxicity is related to physiological processes closely linked to nitrate signaling, absorption, and reduction.

The variable culm dry matter mass was responsive to the different proportions of the ions used (Table 4), revealing that the use of a greater amount of nitrate is favorable; however, by becoming the only source of N, the plants do not develop appropriately. This is due to the process of reducing nitrate to nitrite and ammonium since this process requires the transfer of approximately eight electrons and represents about 25% of the total energy consumed by roots and aerial parts (TAIZ et al., 2017)—energy that the plant ceases to invest in other biomass accumulation processes. We also observed that the supply with only NH_4^+ caused a reduction of approximately 33.53% compared to the treatment that promoted the best result, which may be tied to competition in the culture medium, in which ammonium since it has a load similar to ions such as Ca^{+2} and K^+, makes it impossible to absorb and assimilate them (HOLZSCHUH et al., 2009).

Ammonium in greater proportions than nitrate also provided decreases in total dry matter mass production (Table 5), even if it is regarded as the preferable source of N in terms of energy cost (HELALI et al., 2010). We clearly noted that the averages between treatments that have suffered with ammonium toxicity do not distance themselves from the others, since plant cells promote their rapid conversion into amino acids, to avoid the toxicity of ammonium (TAIZ et al., 2017).

The RDM/APDM ratio concerns the average, in grams, that the plant accumulates in root mass for each accumulated gram of aerial part mass (SILVA et al., 2010). The highest mean for this variable was observed in plants cultivated only with ammonium, with no statistical difference from 50% nitrate (Table 5). This may have occurred due to the lower energy expenditure required for the assimilation of ammonium, since,

Table 5. Total dry matter mass (TM), root/aerial part dry matter mass ratio (RDM/APDM), leaf mass ratio (LMR), leaf area (LA), specific leaf area (SLA), and leaf area ratio (LAR) of corn plants grown under different proportions of nitrate and ammonium. Cruz das Almas, BA, 2022.

$NO_3^-:NH_4^+$	TM	RDM/APDM	LMR	LA	SLA	LAR
	(g)	- - - - - - - - - g/g - - - - - - - -		(cm²)	- - - - - - - - cm².g⁻¹ - - - - - - -	
0:100	8.78 b	0.62 a	0.36 a	21.14 a	6.64 a	2.40 a
25:75	9.65 b	0.50 b	0.37 a	21.01 a	5.91 a	2.19 a
50:50	10.66 ab	0.52 ab	0.40 a	20.86 a	4.95 a	2.01 a
75:25	12.88 a	0.55 ab	0.38 a	29.41 a	6.02 a	2.29 a
100:0	10.14 b	0.59 ab	0.39 a	25.13 a	6.41 a	2.49 a
CV (%)	**13.25**	**11.02**	**7.86**	**23.98**	**19.35**	**22.33**

*Same letters do not differ from each other in columns, by Tukey test at 5% probability.

according to Britto & Kronzucker (2002) and Kronzucker et al. (2001), the energy expenditure resulting from the allocation of photoassimilates to NO_3^- is 12 ATP, whereas for NH_4^+ it is 2 ATP.

Regarding the leaf mass ratio (Table 5), we noted no significant difference for this variable, and the means were considered similar. Regarding leaf area, specific leaf area, and leaf area ratio, statistical analysis did not discriminate significance for the different proportions of ions used, since ammonium (NH_4^+) and nitrate (NO_3^-) ions were the main mineral N forms available to plants, which can assimilate both efficiently.

Conclusions

The cultivation of corn plants with the exclusive supply of nitrate impairs the increase in the chlorophyll *a* index of corn plants.

Parameters related to leaf area are not influenced by different proportions of nitrate and ammonium.

High doses of nitrate combined with low doses of ammonium provide satisfactory performances in the initial development of corn plants.

Therefore, we do not recommend growing corn plants only with nitrate or ammonium as a nitrogen source.

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