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Nitrogen Metabolism in Coffee Plants Subjected to Water Deficit and Nitrate Doses

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HIGHLIGHTS

- In N-sufficient plants, water deficit reduces leaf NR activity, but increases leaf GS activity.
- In N-deficient plants, water deficit do not affect leaf NR activity, but increases leaf GS activity.
- Under water deficit young coffee-plants increase the content of proline in the roots.
- Good N nutrition increases amino acids and proteins in leaves of coffee under water deficit.

Abstract: Nitrogen uptake is essential for coffee growth and development, resulting in important effects on the biomass and final crop yield. Thus, like most nutrients, nitrogen is absorbed by the roots using water as a mean of transport, so that water stress and nitrogen can directly and indirectly affect various physiological processes. The aim of this paper was to evaluate the nitrogen metabolism in young plants of four varieties of coffee trees (*Coffea arabica* L.) submitted to water deficit (WD) and nitrogen supply. We have done a triple factorial (2 x 4 x 4) experiment entirely randomized. The plots received combinations of high or low N doses (7mmol/L and 2.8 mmol/L NO_3^-), four water potentials (0; -0.4; -0.8; and -1.6 MPa), and four varieties (Mundo Novo IAC379-19, Acauã F6 of IBC – PR 82010, Catuaí Vermelho IAC 44, and Catuaí Amarelo IAC 62). One hundred and forty days after the begining of the experiment (140 days after the beginning of N stress and 82 days after the beginning of WD stress) the activity of the enzymes nitrate reductase (NR) and glutamine synthetase (GS), concentration of nitrate, free proline, amino acids (TAA), and total proteins were determined in samples of leaf and root tissues. There were differences between varieties independently of WD and N dose for leaf NR, being 'Acauã' the cultivar that presented the highest and 'Catuaí Vermelho' the lowest value to this trait. The WD promoted an increase on the proline concentration in the roots. With low N dose, the activity of GS presented linear increases in response to WD. It was concluded that in young coffee

plants under WD, proline can be involved in the osmotic adjustment, having its synthesis in the roots increased. Under WD, plants with good nitrogen nutrition presented larger leaf concentration of soluble amino acids and total soluble proteins. The varieties studied do not present differentiated responses to WD.

Keywords: *Coffea arabica* L.; glutamine synthetas; nitrate reductase; polyethylene glycol; proline.

INTRODUCTION

Water, the natural resource indispensable to the survival of all living beings, is one of the environmental conditions that has the greatest influence on the growth and productivity of cultures. Among other functions, it is essential for the absorption of soil nutrients by the plants. However, this resource is becoming scarce mainly due to the anthropic action in the hydrographic basins.

In consequence of recent climate changes, periods of accentuated and continuous water deficits have also had negative consequences for coffee culture. Thus, studies on the coffee tree tolerance to water deficit are fundamental to improve plant growth and production under these conditions [1, 2].

In the world context, coffee is one of the agricultural products of great relevance, being the second largest generator of foreign exchange, behind only oil. The nitrogen (N), a nutrient that has its absorption strongly influenced by water availability, is the most required by coffee trees, especially in the fruiting phase, when it is strongly drained from the leaves to the fruits. To meet this demand, high doses of fertilizers are used to increase N absorption and assimilation inside the plant. Nitrogen also favors the growth and formation of roots and leaves, production and translocation of photoassimilates, and the development of flower and fruit buds [3], thus, being necessary in all stages of the coffee-plant development [4].

Nitrate (NO_3^-) is the main form of nitrogen absorbed by cultivated plants; however, in order to be incorporated into numerous organic compounds from which it is a part of, its reduction to ammonium (NH_4^+) is necessary. This nitrate reduction process requires the activity of two enzymes: (1) nitrate reductase (NR), and (2) nitrite reductase (NRI) [5, 6].

NR catalyzes the first stage of the nitrogen assimilation process, reducing nitrate to nitrite (NO_2^-) in the cytosol. Then, NO_2^- is transported from the cytosol to the chloroplasts in the leaves and to the plastids in the roots, where it is reduced to NH_4^+ by NRI activity. Subsequently, NH_4^+ is rapidly assimilated into amino acids by the sequential actions of the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT), in the called the GS/GOGAT route [6, 7].

N assimilation is essential to the growth and development of the coffee tree, resulting in important effects on the phytomass and the culture final productivity. Thus, as most nutrients, N is absorbed by roots using water as a means of transportation, hence water and N stresses can direct or indirectly affect several physiological processes, such as the efficient use of radiation, net carbon assimilation, due to limitations stomatic and non stomatic, and even the translocation of the N itself in the plant [8].

When subjected to several types of abiotic stresses, mainly the hydric, plants can present alterations regarding physiological and metabolic responses. The increase of compatible solutes, like proline, is an example of these responses. Such behavior has been related to the plant tolerance, among others, to water stress, for it is able to maintain the plant turgor, through the reduction of the root water potential which favors water absorption [9]

By the other hand, under N stress influence, amino acid metabolism is greatly modified, not only by the reduction of protein synthesis, but also by the induction of rapid degradation of the already formed proteins, which leads to an increase in free amino acids and amines. As a result, there is an increase in proline synthesis caused by the increase of polyamines, ammonia, arginine, ornithine, glutamine and glutamate derived from catabolic routes [10].

The increase in proline amounts can stimulate different cellular functions such as osmotic adjustment, carbon and nitrogen reserve, which is used in growth for post stress recovery, detoxification of excess ammonia, proteins and membranes stabilization, and elimination of free radicals [9, 11].

In view of the explained above we can hypothesize that differences in nitrogen metabolism among coffee varieties may confer adaptive advantage to some of them when under water stress.

The objective of this paper was to evaluate the nitrogen metabolism in four varieties of coffee trees (*Coffea arabica* L.) submitted to water deficit and nitrate doses in the vegetative phase.

MATERIAL AND METHODS

Plant Material and growth conditions

The experiment was carried out in a greenhouse at the Agronomy Department of the Federal University of Viçosa, Minas Gerais, Brazil (20 ° 45 'S, 42 ° 15' W and 650 meters of altitude). During the conduction of the experiment, the maximum, average, and minimum temperatures ranged between 23.8 and 29.2 ºC, 15.8 and 22.0 ºC, and 10.8 and 17.7 ºC respectively. The relative humidity of the air remained between 76.4 and 82.04 %. Six-month-old plants of *Coffea arabica* L. produced in hydroponic system were used: Mundo Novo IAC379-19, Acauã, F6 of IBC – PR 82010, Catuaí Vermelho IAC 44, and Catuaí Amarelo IAC 62. During the initial 58 days, the plants received two doses of N-nitrate as a mean of obtain plants with differentiated N status. Each experimental unit received 4.5 liters of hydroponic solution. The hydroponic solution used for the plant growth contained: 2.8 mmol L^{-1} NO_3^- as a low level of nitrate (LN), and 7 mmol L^{-1} NO_3^- as a high level of nitrate (HN), 1 mmol L^{-1} P, 4.8 mmol L^{-1} K, 1 mmol L^{-1} Mg, 1 mmol L^{-1} S, 2.1 mmol L^{-1} Ca, 40 µmol Fe-EDTA, 23 µmol L^{-1} B, 0.8 µmol L^{-1} Cu, 12 µmol L^{-1} Mn , 0.3 µmol L^{-1} Mo, and 1 µmol L^{-1} Zn. The containers received constant aeration throughout the experiment and pH corrections to maintain the value around 6 ± 0.2, using HCl or NaOH. Replacement of nutrients were carried out, based on the reduction of electrical conductivity (EC), admitting up to 30% depletion, except for nitrogen. The criterion of 30% reduction in the nitrate concentration was adopted using the LAQUA Twin Nitrate NO_3 Meter® sensor. As the volume of the solution decreased with evapotranspiration, it was replaced with deionized water until the total volume of the vessel was completed.

After this period, to induce the water deficit, the solutions were renewed and calculated amounts of PEG 6000 (Polyethylene glycol) were added to generate water potentials of -0.4; -0.8; and -1.6 MPa [12]. The quantities used per liter of solution for the defined water potentials were 172.6 g, 240.2 g, and 335.8 g, respectively. In order to gradually impose the deficit, these quantities were divided into 6 parts and applied daily for six days. The plants remained under stress for a period of 76 days.

At 140 days, approximately 1.0 g of fine roots and 7 leaf discs with an approximate weight of 0.45 g were removed from fully expanded leaves, present in the middle third of coffee plants. These samples were washed in deionized water, stored in liquid nitrogen and kept at - 80 °C for further analysis. Then the plants were harvested, the leaves and roots were separated, washed with deionized water, dried in an oven with forced air circulation at 70 °C until constant weight, and ground in a mill type Wiley and 20 mesh.

Determination of total nitrogen in the dry matter of leaves and roots, and nitrate in the dry matter of the roots

To determine total N, the samples were submitted to sulfuric digestion and the determining of total N by the micro Kieldahl method.

To determine NO_3^- in the roots, extraction was carried out using the method described by Cataldo and coauthors, [13]. Nitrate concentrations were determined by the colorimetric method described by Doane and Horwath [14].

Determination of enzymatic activity

Preparing extracts for analysis of enzymatic activities (NR and GS)

The extraction was executed according to the protocol proposed by Radin [15], adapted by Cambraia *et.al.,* [16], with some adjustments for the coffee plant.

Determination of nitrate reductase (NR) activity

In order to determine the activity of nitrate reductase, the in vitro assay was used following the methodology of Radin [24], adapted by Cambraia and coauthors, [16].

Determination of glutamine synthetase (GS) activity

The activity of glutamine synthetase was determined through the method proposed by Elliott [17], which is based on the formation of λ-glutamylhydroxamate.

Determination of free amino acids and soluble proteins

Methanolic extraction for analysis of free amino acids and soluble proteins

The extraction was carried out following protocol proposed by Lisec and coauthors, [18]. The precipitate was washed twice in 70% ethanol, and later used to quantify protein [19].

Determination of free amino acids

The concentration of free amino acids was determined as described by Gibon and coauthors, [19].

Determination of total soluble protein

The concentration of total soluble protein was determined according to Gibon and coauthors, [19] and Bradford reagent solution [20].

Determination of free proline

The concentration of free proline was determined in accordance to the methodology described by Shabnam [21].

Experimental design and statistical analysis

The experimental units received combinations of three factors, that is, two doses of nitrogen (high-HN and low-LN), four water potentials: 0; -0.4; -0.8; and -1.6 MPa, and four varieties: Mundo Novo, Acauã, Catuaí Vermelho, and Catuaí Amarelo. Those composed of a triple factorial scheme (2 x 4 x 4) in a randomized block design with three true repetitions(each repetition was a plant) for the variables NR, GS, nitrate, amino acids and proteins, and with two repetitions for proline and NR in roots.

The data were submitted to the Shapiro Wilk normality test, an assumption for analysis of variance. The protein variable to meet the assumption was transformed into a Neperian logarithm (Ln). No data transformation was necessary for the other variables.

After meeting the assumption, the obtained data were subjected to analysis of variance. Qualitative data (N doses and varieties) were compared using the Tukey test at 5% probability. Quantitative data (water potential) were subjected to polynomial regression analysis. The models were chosen based on the biological behavior and significance of the regression coefficients, using the t test at 5% probability, and on the determination coefficient. Statistical analyzes were performed with the aid of the SISVAR 5.6 program [22], at the level of 5% probability, and the adjustments to the regression models were made using the statistical graphic program Sigma Plot 10.0® (Systat Software Inc.).

RESULTS

Concentrations of total nitrogen in the dry matter of leaves and roots and of nitrate in the dry matter of roots

Shoot dry matter (Table 1) do not showed significant differences ($P<0.05$) to the interaction between N and WD stresses. The mean values of shoot dry matter production were 39.9 g under HN supply and 38.4 under LN supply. Under no water stress the plants produced a mean of 45.0 g of shoot dry matter, while under -1.6 MPa of water potential they produced a mean of 34.9 g of dry matter.

Regarding root dry matter production (Table 1) there were significant differences ($P<0.05$) for the interaction between N and WD stresses. As a general pattern Acauã stood out with a mean of 15.6 g, being followed by Catuaí Amarelo (12.1 g), Catuaí Vermelho (9.8 g), and Mundo Novo (9.1 g). Root dry matter increased from 9.12 g to 14.2 g in response to N stress. The difference was most sharp with some degree of WD, except for Mundo Novo.

There was no significant interaction between the three factors studied, for any of the variables analyzed ($P <0.05$). In HN, the concentration of total nitrogen (g kg^{-1}) in both leaves and roots was higher in relation to the observed in LN dose in all studied water potentials (Table 2).

The concentration of nitrate (NO_3^-) in the roots of the four varieties did not vary in response to the water deficit or to the doses of N. However, there was an interaction between the nitrogen doses and the different water potentials. When the water deficit was applied to plants under low nitrogen availability, there was no significant difference in the root nitrate content, with an average value of 2.77 mg g^{-1} of dry matter (Figure 1A). Yet, at a high nitrogen dose, there was a decrease in the nitrate content as the water deficit increased.

This decrease followed a quadratic function and was accentuated when it went from the stress-free condition to -0.4 MPa of stress. It was observed that in HN, the concentration of nitrate reached the minimum point in the water potential -1.03 MPa (Figure 1A). The root nitrate content was higher in the HN dose only when the plants did not suffer water restriction (0.0 MPa). Under any degree of water restriction, this concentration was equal to that of plants grown with LN dose (Table 2).

Table 1. Shoot dry matter mass (SDM) and root dry matter mass (RDM) of four coffee cultivars submitted to high (HN) or low (LN) doses of nitrogen and different water potentials (MPa).

SDM (g)								
	0 MPa		-0,4 MPa		-0,8 MPa		-1,6 MPa	
Variety	HN	LN	HN	LN	HN	LN	HN	LN
C.Vermelho	35,81Aa	40,59Aa	35,29Aa	34,62 Aa	34,92Aa	33,57Aa	27,22Aa	31,79Aa
Acauã	53,30Aa	38,97Aa	35,94Aa	44,01 Aa	45,95Aa	32,41Aa	36,25Aa	36,14Aa
C. Amarelo	53,24Aa	37,33Aa	41,31Aa	40,27 Aa	30,4 Aa	31,74Aa	32,47Aa	38,82Aa
Mundo Novo	56,49Aa	44,32Aa	46,36Aa	52,2 Aa	37,46Aa	35,78Aa	35,59Aa	41,16Aa
Average	49,71	40,30	39,73	42,78	37,18	33,38	32,88	36,98
CV	26,2							
RDM (g)								
	0 MPa		-0,4 MPa		-0,8 MPa		-1,6 MPa	
Variety	HN	LN	HN	LN	HN	LN	HN	LN
C.Vermelho	8,43 Bb	16,85Aa	6,28Aa	12,48 Ba	6,20 Aa	10,48ABa	5,31 Ab	12,14Aa
Acauã	16,78Aa	19,35Aa	9,72Ab	23,06 Aa	12,53Aa	17,4 Aa	11,58Aa	14,13Aa
C. Amarelo	14,27Ba	19,24Aa	8,24Ab	15,48ABa	8,22 Aa	11,64ABa	8,13 Aa	11,95Aa
Mundo Novo	13,20Ba	13,22Aa	7,19Aa	12,21 Ba	4,95 Aa	7,72 Ba	4,48 Aa	10,17Aa
Average	13,17	17,17	7,86	15,81	7,98	11,81	7,38	12,10
CV (%)	31,88							

The averages followed by at least one capital letter in the column and lower case in the row for each water deficit did not differ significantly at the 5% probability level by Tukey's test.

The concentration of nitrate (NO_3^-) in the roots of the four varieties did not vary in response to the water deficit or to the doses of N. However, there was an interaction between the nitrogen doses and the different water potentials. When the water deficit was applied to plants under low nitrogen availability, there was no significant difference in the root nitrate content, with an average value of 2.77 mg g^{-1} of dry matter (Figure 1A). Yet, at a high nitrogen dose, there was a decrease in the nitrate content as the water deficit increased. This decrease followed a quadratic function and was accentuated when it went from the stress-free condition to -0.4 MPa of stress. It was observed that in HN, the concentration of nitrate reached the minimum point in the water potential -1.03 MPa (Figure 1A). The root nitrate content was higher in the HN dose only when the plants did not suffer water restriction (0.0 MPa). Under any degree of water restriction, this concentration was equal to that of plants grown with LN dose (Table 2).

In the water potential 0.0 MPa, the activity of nitrate reductase in HN was approximately four times greater than the activity in LN. However, as the water potential decreased, the difference in NR activity between doses also decreased, that is, in water potentials -0.4 and -1.6 MPa, the enzymatic activity in HN was approximately three and two times superior in relation to those observed in LN , respectively (Table 2).

Concerning to the activity of glutamine synthetase (GS) there was an interaction between nitrogen doses and water potentials ($P < 0.05$). At both doses of nitrogen, the activity of GS increased as the water potential decreased (Figure 1C). In the average of the four varieties cultivated with HN dose, the GS activity increased with the increase of the water deficit, according to the quadratic function. In this case, the maximum point was reached with -0.96 MPa. When in low N availability, GS activity increased linearly with water deficit. There was also a difference in activity among the doses of N in water potential -1.6 MPa, whereas in LN, the activity of GS was 22% higher than in HN (Table 2).

Table 2. Concentration of total nitrogen in leaves and roots, nitrate in roots, activity of nitrate reductase (NR) and glutamine synthetase (GS) in coffee leaves submitted to high (HN) or low (LN) doses of nitrogen and different water potentials (MPa).

	Ψ (MPa)			
Dose de N	**0.0**	**-0.4**	**-0.8**	**-1.6**
	Total N in leaves (g kg^{-1} of DM)			
HN	30.99 a	23.41 a	24.20 a	25.67 a
LN	21.08 b	20.58 b	21.34 b	22.45 b
CV (%)	8.43			
	Total N in roots (g kg^{-1} of DM)			
HN	29.09 a	20.95 a	21.82 a	22.75 a
LN	17.91 b	16.07 b	17.07 b	17.20 b
CV (%)	13.03			
	Nitrate in roots (mg g^{-1} of DM)			
HN	13.93 a	1.85 a	2.92 a	3.47 a
LN	2.17 b	1.99 a	3.13 a	3.78 a
CV (%)	65.77			
	NR (NO_2^- h^{-1} g^{-1} of FM)			
HN	0.38 a	0.21 a	0.19 a	0.19 a
LN	0.09 b	0.07 b	0.13 a	0.09 b
CV (%)	48.02			
	GS (λ-GH h^{-1} mg^{-1} of FM)			
HN	0.15 a	0.17 a	0.23 a	0.18 a
LN	0.19 a	0.19 a	0.19 a	0.23 b
CV (%)	29.58			

Averages followed by the same letter do not differ by the Tukey test at the 5% probability level.

Regardless of water deficit and availability of N, there was a difference in NR activity among varieties (Figure 2A). For this variable, the highest value was obtained in the Acauã variety and the lowest value in Catuaí Vermelho. The Acauã variety showed NR activity 31% higher than the NR activity of Catuaí Vermelho.

Concerning the NR activity in roots, the HN dose differed from the LN dose, whereas the enzymatic activity with the HN dose was 43% superior in relation to the LN dose ($P < 0.05$) (Figure 2B).

Concentration of proline, amino acids, and proteins

With reference to the N doses, it was found that HN promoted a higher concentration of proline in roots (Figure 3A), and concentration of total amino acids (TAA) in leaves ($P < 0.05$) (Figure 3B), regardless of the variety or water deficit. It was observed that the levels of free proline in HN were approximately 49% higher than in LN. The concentration of TAA in leaves in HN was 1.7 times superior to its concentration in LN.

There was a linear increase in proline content with increased water deficit, ($P < 0.05$) (Figure 4). The concentration of proline in water potential -1.6 MPa (15.90 μmol g^{-1} FM) was almost 10 times higher than in the control (1.62 μmol g^{-1} FM).

For total soluble proteins in leaves, the concentrations were only lower in LN for the varieties Mundo Novo and Catuaí Amarelo ($P < 0.05$) (Figure 5). There was an interaction between N doses and varieties when analyzed for total protein concentration. The varieties Catuaí Amarelo and Mundo Novo differed in relation to nitrogen doses. In HN dose, the protein concentration was 40% and 38% higher than the LN dose, in C. Amarelo and Mundo Novo, respectively. However, there was no significant difference in the quantity of proteins from C. Vermelho and Acauã varieties, when compared among N doses (Figure 4).

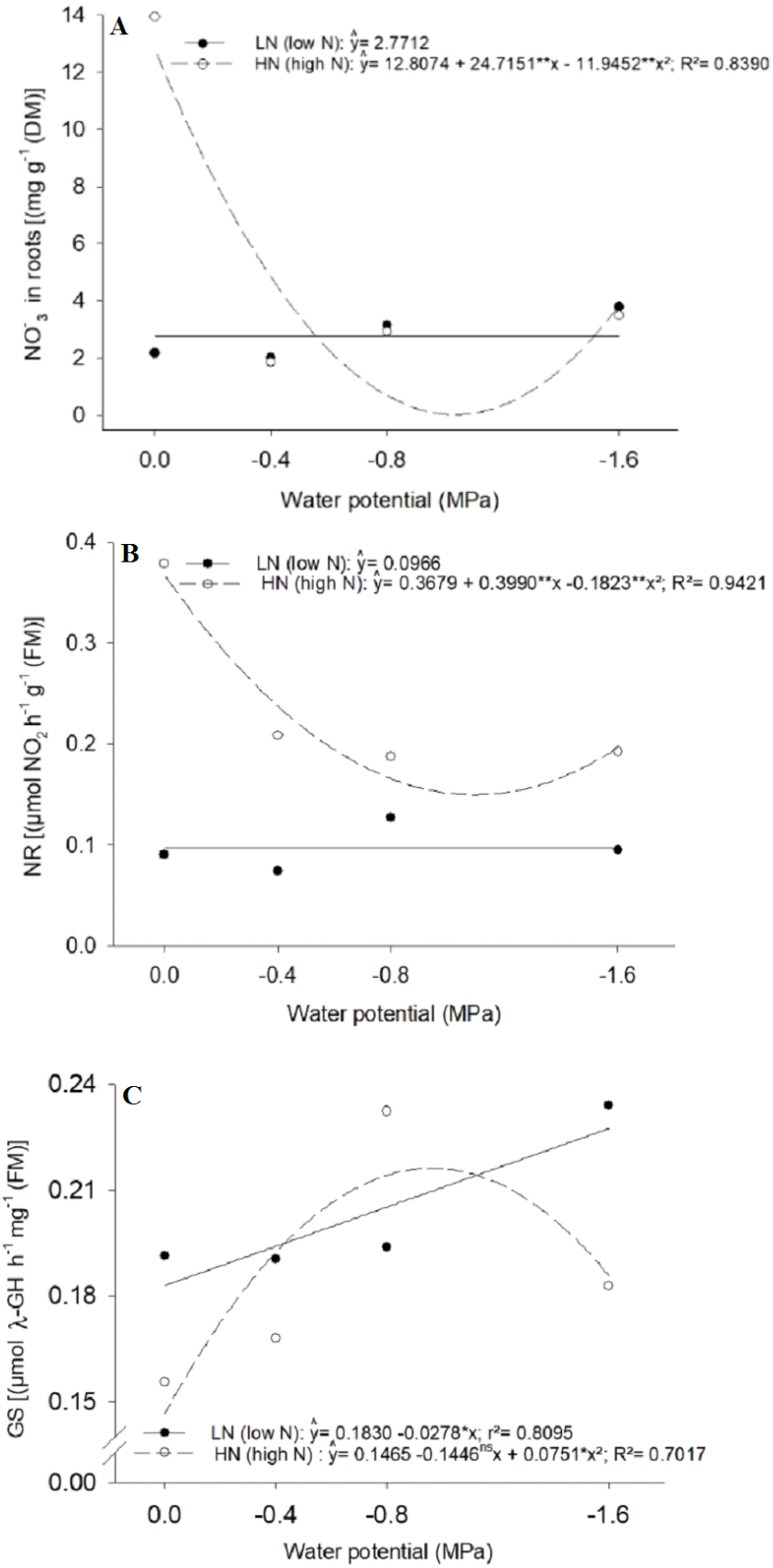

Figure 1. Concentration of nitrate in roots (A), activity of nitrate reductase (B) and activity of glutamine synthetase (C) in coffee leaves submitted to high (HN) or low (LN) doses of nitrogen and different water potentials (MPa).

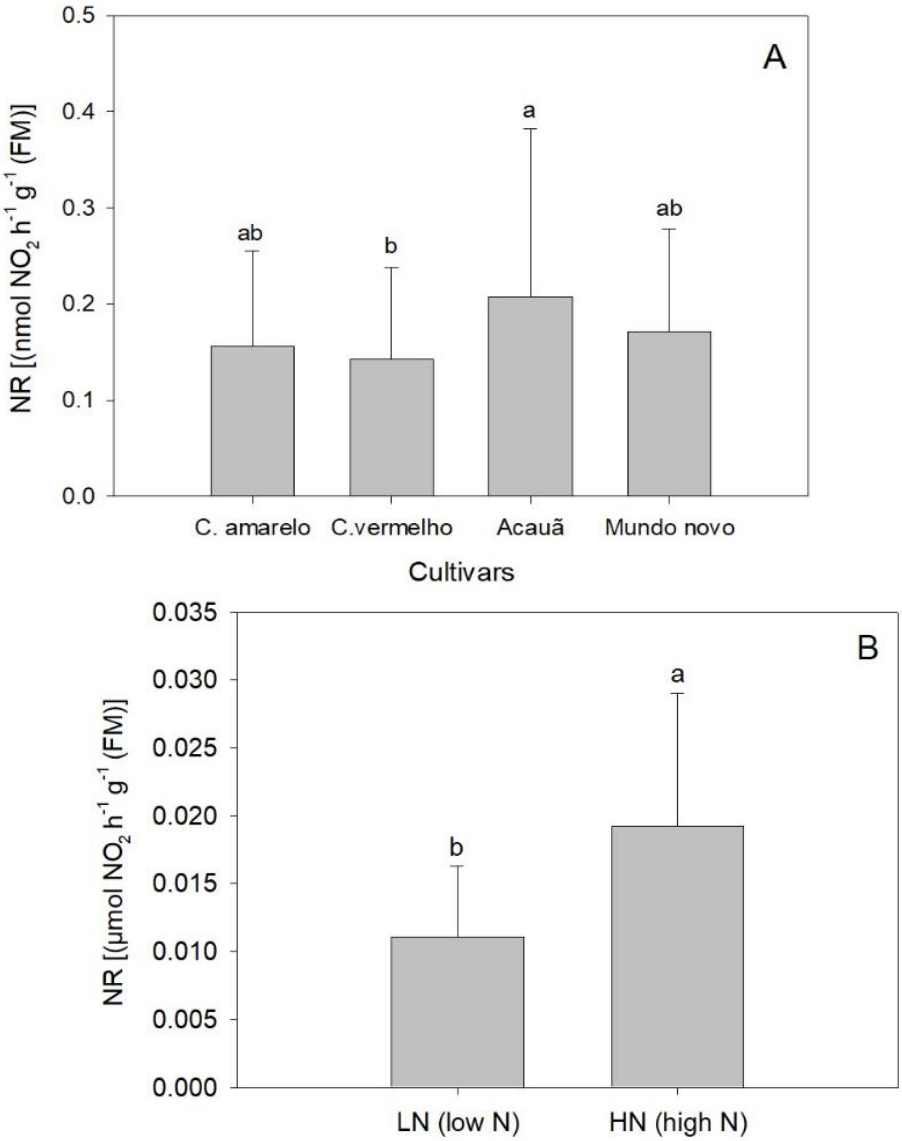

Figure 2. (A) Activity of nitrate reductase (NR) in leaves of young plants of four coffee varieties with high (HN) or low nitrogen (LN). (B) Average nitrate reductase (NR) activity in roots of young coffee plants grown with high (HN) or low nitrogen (LN). Means followed by the same letter do not differ by the Tukey test at the 5% probability level.

DISCUSSION

The plants were cultivated with adequate N supply and without water stress previously to the settlement of N and water stresses, what allowed them maintain the shoot dry matter production relatively stable when the stresses were imposed by reducing the N concentrations, especially of the root tissues (Table 1). Besides that, when the water stress was established (June) the low temperatures also limit the growth rates, preventing sharp differences.

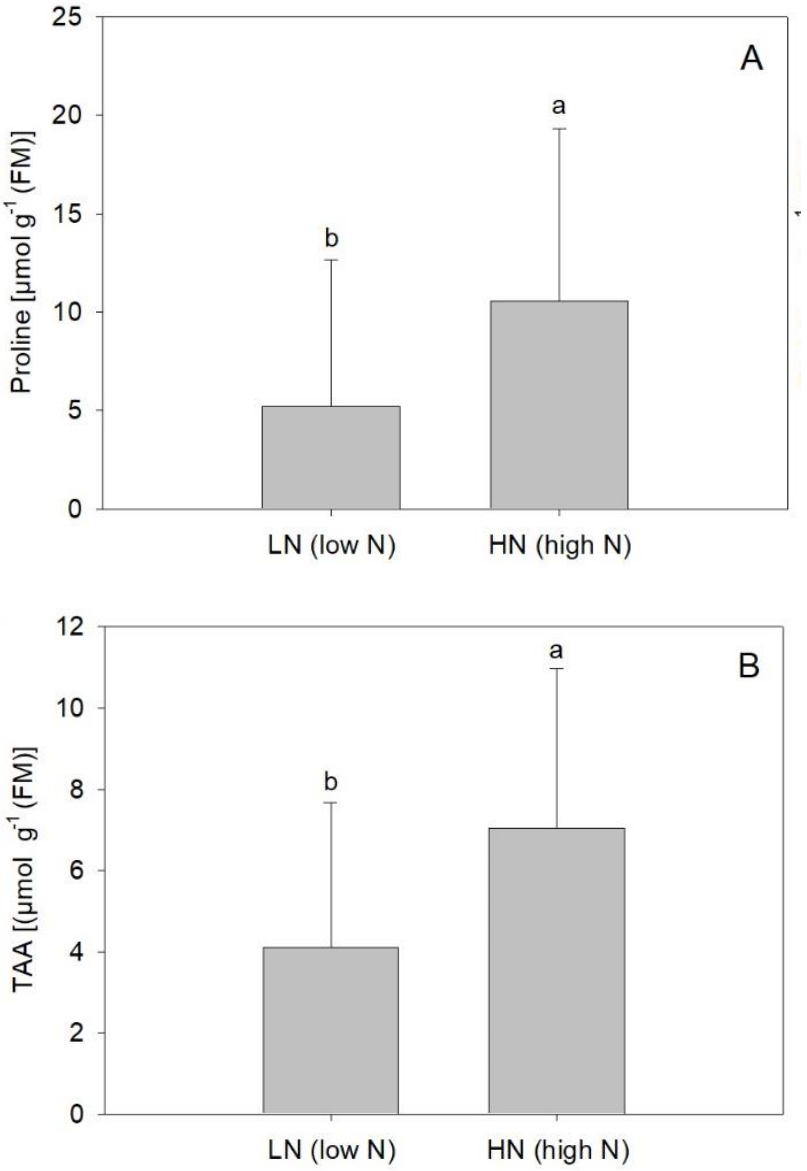

Figure 3. Concentration of free proline in roots (A) and total amino acids (B) in leaves of young coffee plants submitted to high or low nitrogen. Averages followed by the same letter do not differ by the Tukey test at the 5% probability level.

Another interesting response was the significant increase of root dry matter of Catuaí Vermelho, Catuaí Amarelo and Acauã when submitted to low nitrogen dose in some degree of water stress. This result is in accordance with Wu and coauthors [23], who studied the effect of water stress and N supply on seedlings of the tree species *Sophora davidii*. They stated that low concentrations of the nutrient in the soil stimulate root growth, while high concentrations promote greater biomass partition to the shoot.

The assimilation of nitrogen, fundamental for the growth, development, and productivity of plants, is influenced by the water deficit. Thus, the water shortage responses of the enzymes involved in the nitrate reduction into ammonium and its incorporation into organic compounds play an important role in the survival of plants [24].

According to this paper, the exposure of coffee varieties to a high dose of N and low values of water potential promoted a decrease in the content of nitrate in roots, and, consequently, a decrease in the activity of the NR enzyme (Figure 1A). It is believed that this lower concentration is the result of less absorption of nitrate by plants with an increase in water deficit. Besides controlling NR activity through stimulating its synthesis, the nitrate acts on transport proteins associated with nitrate absorption and storage in the vacuole, and on nitrite reductase, glutamine synthetase (GS), and glutamine-2-oxoglutarate aminotransferase enzymes (GOGAT).

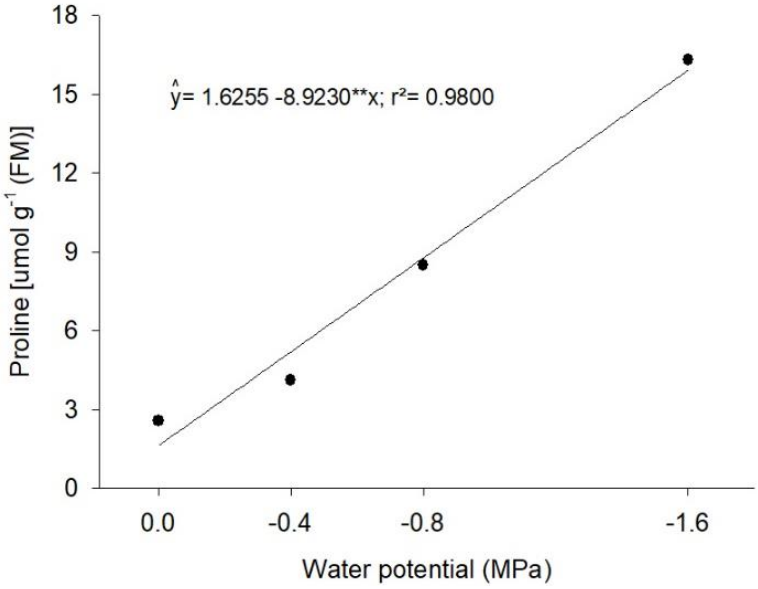

Figure 4. Concentration of free proline in roots (µmol g^{-1} FM) of young coffee plants submitted to different water potentials.

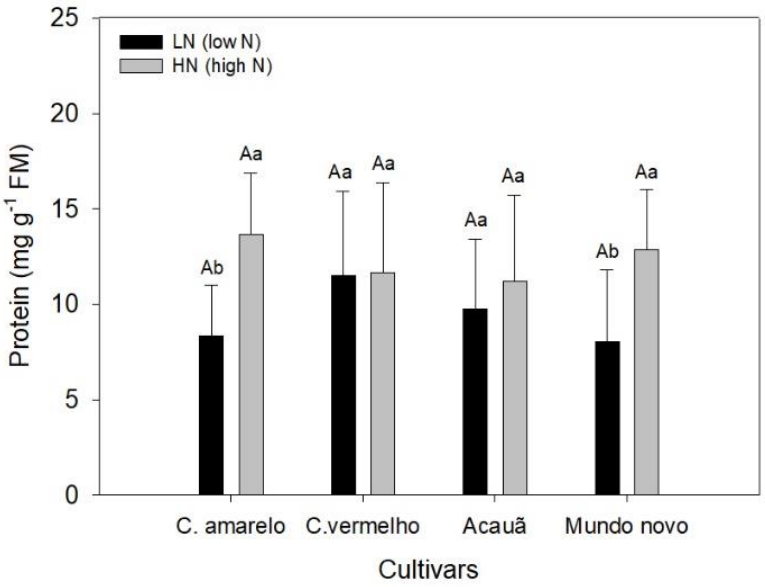

Figure 5. Concentration of total soluble proteins (mg g^{-1} FM) in leaves of young coffee plants submitted to high (HN) or low (LN) nitrogen. Means followed by the same capital letters do not differ of each other by the Tukey test at the level of 5% probability and compare cultivars. Averages followed by the same lower case letters do not fiffer of each other by Fischer's F at the level of 5% probability and compare low N and high N.

Environmental factors such as light, temperature, and N and water availability absolutely alter the activity of NR enzyme in leaves, as well as in roots, which explain the results obtained for the activity of NR enzyme [25]. N availability in the growth medium induces protein synthesis of nitrate reductase enzyme, for example. However, when the plant undergoes a water stress condition, the NR activity can be limited due to a reduction of nitrate absorption by the roots, because of this stress [26, 27].

Reis and coauthors, [28], evaluated the NR activity in coffee trees submitted to different doses and different systems of application of nitrogen, and observed that its activity responded to nitrogen fertilization, that is, directly interfering in productivity. According to Khouri [29], in his work, the decrease in the NR activity because of the water deficit effect occurred due to the reduction in the nitrate flow to the roots, as a result of insufficient water, which is an essential substrate in the formation and activity of this enzyme. The results of the literature presented above prove that even with high nitrogen availability, plants under water deficit tend to decrease NR activity, corroborating the results found in this study (Figure 1B) (Table 1).

Among the varieties, there was a greater activity of NR in Acauã (Figure 1), indicating that under water deficit this variety has some mechanism that makes it possible to maintain the enzymatic activity with less change. According to Pereira and Baião, [30] in the field the variety of Acauã is characterized by high vigor and certain tolerance to water deficit, and can be indicated for drier regions. Carvalho and coauthors [31], evaluated the behavior of seven new coffee cultivars aged 3.5 years to water stress through measurements of water potential in the pre-dawn. They concluded that varieties from Acauã, showed greater tolerance to stress water, unlike the varieties from Catuaí.

In addition to the greater activity in leaves, in HN the roots also showed greater activity of NR than in LN (Figure 2B).). The coffee plant shows a high potential to reduce and assimilate nitrate in leaves as well as in roots. This partition of NR activity between the organs is basically regulated by the efficiency of the root system in exporting nitrate to the aerial part, that is, with an increase in the availability of nitrate, the coffee tree roots demonstrate a high capacity to increase their export to the leaves [32, 33].

Carelli and Fahl [26] observed that NR activity occurred in both organs in adult coffee plants; however, the activity in roots was inferior than in leaves, which confirms the results found in this study. The authors also found, in the same study, a greater accumulation of nitrate in roots than in leaves, and almost no NR activity was detected in the period prior to the nitrate application. According to Melo and coauthors, [34], there was a decrease in the assimilation of nitrogen, and, consequently, a reduction in NR activity in roots of the cultivar Siriema under water deficit. This is in agreement with the data found in this work, where the activity of NR in roots was higher in HN dose, and in plants subjected to lower water stress (Figure 2B).

The ammonium produced by the nitrate reduction is incorporated into organic compounds, then forming the amino acids. These reactions are due to the activity of glutamine synthase (GS) and glutamate synthase (GOGAT) enzymes. GS plays a central role in N metabolism in plants, because it is responsible for the initial assimilation of ammonium [35, 36]. In this study, an increase in GS was observed as the water deficit increased, in both nitrogen doses (Figure 1C).

Two isoforms of glutamine synthetase (GS) are present in plants, one located in the cytoplasm (GS1) and the other in the chloroplast (GS2). GS1 has the function of ammonium assimilation into the cytoplasm in a dark condition, while GS2 found in the leaves is governed by light and ammonium ($NH4^+$), in addition to the use of $NH4^+$ from photorespiration. This $NH4^+$ formed during photorespiration can be reassimilated by plants in the chloroplast [37, 38]. The reassimilation of $NH4^+$ made available during photorespiration in C3 plants represents about 90% of the flow through the GS /GOGAT route in leaves. Thus, the lower nitrate reductase activity found in this work, as well as the nitrate accumulation, may have favored the increase in GS activity, that is, increased the assimilation of $NH4^+$ resulting from the photorespiratory process, alternatively to the normal pathway of the ammonium, formed by the NR activity [39].

Martinez and coauthors, [40] report that after 96 hours in deficit of -1.5 MPa, young plants of the Catuaí Amarelo and Mundo Novo coffee varieties grown in nutrient solution showed a marked increase in the expression of the NIA2 gene that codes for nitrate reductase, and also of the gene GLN1.3 that codes for glutamine synthetase. It is known that the water deficit induces reduction in the availability of nutrients such as nitrogen, since water allows their absorption by the root system. As a result, taking into account the lack of water, and an increase in the concentration of ammonium, it is likely that there could be an improvement in the efficiency of use of nitrogen by the plant, consequently preventing the $NH4^+$ ion from accumulating in toxic levels [41], thus explaining the results of the increase in GS activity (Figure 1C).

Regardless of the varieties and N doses, with increasing water deficit there was an increase in the concentration of proline in roots (Figure 5). When suffering from abiotic stresses, especially water stress, plants tend to accumulate compounds called compatible solutes or osmoprotectors, such as proline. The function of these solutes is to preserve cell turgidity, integrity of proteins, and cellular structures, and they also provide a decrease in the osmotic potential in lack of water situations [42, 43]

In a study characterizing morphological and physiological responses of different *Coffea* genotypes submitted to water stress, Almeida and coauthors [44], observed that the plants survived as a result of osmotic adjustment with increased proline content. Yet, Silva and coauthors, [45], found that during water stress, proline levels increased in a Conilon coffee clone sensitive to water deficit. Tounekti and coauthors, [46], studied the effect of water deficit in four coffee varieties grown for two years in pots containing substrate and irrigated with water and nutrient solution, noting that the varieties with greater drought tolerance, showed greater capacity of osmotic adjustment by proline accumulation.

Concerning the N doses, it was verified that the high dose promoted a higher concentration of proline in roots (Figure 3A) and TAA in leaves (Figure 3B), regardless of the variety or degree of water deficit. For total soluble proteins in leaves, the concentrations were only lower in HN for the Mundo Novo and Catuaí Amarelo varieties (Figure 5).

The total amino acid and protein concentration was higher in HN than in LN, that is, even with water deficiency, the highest dose of N favored a greater synthesis of amino acids (Figure 3B) and, consequently, of proteins (Figure 5), when compared to a lower dose of N. In addition to their particular functions, the amino acids formed under water deficit can be used as a source of nitrogen for protein synthesis, as well as they can be used in the energy supply for plants to recover more quickly from the water stress condition [47].

The amino acid increase in response to water deficits may be due to a decrease in protein synthesis, or a greater protein degradation. However, the free amino acid increase, mainly proline, helps to decrease the negative effects of water deficit on plants [48, 49].

CONCLUSION

In young coffee plants under high nitrogen availability, water deficit negatively affects nitrogen metabolism, by reducing the nitrate concentration in roots and NR activity in leaves.

In young coffee plants submitted to a low dose of N, the low nitrate concentration in roots and the low NR activity in leaves did not change with increasing water deficit, while the GS activity shows linear increments in response to increases in water deficit.

The water deficit has less impact on the N assimilation in coffee plants well supplied with this nutrient. At any deficit level, plants with high nitrogen nutrition show a higher leaf concentration of soluble amino acids and total soluble proteins.

In young coffee plants under water deficit, the proline synthesis in roots increases, suggesting their participation in osmotic adjustment. The effect is greater in plants well supplied with N.

The varieties Mundo Novo, Acauã, Catuaí Vermelho, and Catuaí Amarelo in general do not present differentiated responses to water deficit regarding N concentrations in tissues, NR and GS activities, and concentrations of proline in roots, total amino acids and proteins in leaves.

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REFERENCES

1. Da Matta FM, Grandis A, Arenque BC, Buckeridge MS. Impacts of climate changes on crop physiology and food quality. Int. Food Res. 2010; 43: 1814-23.
2. Menezes-Silva PE, Sanglard LM, Ávila RT, Morais LE, Martins SC, Nobres P, Da Matta FM. Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. J. Exp. Bot. 2017; 68: 4309-22.
3. Teixeira WF, Fagan EB, Silva JO, Silva PGD, Silva FH, Sousa, MC, Canedo SDC. [Nitrate Reductase Activity and Growth of Swietenia macrophylla King under Shading Effect]. Floresta Ambient. 2013; 20: 91-8.
4. Salamanca-Jimenez A, Doane TA, Horwath WR. Coffee response to nitrogen and soil water content during the early growth stage. J. Plant Nutr. Soil Sci. 2017; 180: 614-23.
5. Debouba M, Dguimi HM, Ghorbel M, Gouia H, Suzuki A. Expression pattern of genes encoding nitrate and ammonium assimilating enzymes in *Arabidopsis thaliana* exposed to short term NaCl stress. J. Plant Physiol. 2013; 170: 155-60.
6. Taiz L, Zeiger E, Møller IM, Murphy A. Fisiologia e desenvolvimento vegetal. Porto Alegre: Artmed Editora; 2017; 858p.
7. Cantú T, Vieira CE, Piffer RD, Luiz GC, de Souza SGH. Transcriptional modulation of genes encoding nitrate reductase in maize (*Zea mays*) grown under aluminum toxicity. Afr. J. Biotechnol. 2016; 15: 2465-73.
8. Reis AR, Favarin JL, Gallo LA, Malavolta E, Moraes MF, Lavres Junior J. Nitrate reductase and glutamine synthetase activity in coffee leaves during fruit development. Rev. Bras. Ciênc. Solo. 2009; 33: 315-24.
9. Kishor PBK, Sreenivasulu N. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue ? Plant, Cell and Environment. 2014; 37: 300–11.
10. Sodek L. Metabolismo do nitrogênio. Fisiologia Vegetal. 2004; Rio de Janeiro: Guanabara Koogan, 94-113 p.
11. Paulus D, Dourado Neto D, Frizzone JA, Soares TM. [Production and physiologic indicators of lettuce grown in hydroponics with saline water] Hortic. Bras. 2010; 28: 29-35.
12. Vilela F, Filho L, Sequeira E. [Table of Osmotic Potential as a Function of Polyethylene Glycol 6000 Concentration and Temperature.]. Pesq. Agropec. Bras. 1991; 26: 1957-68.
13. Cataldo DA, Maroon M, Schrader LE, Youngs VL. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. in Soil Sci. Plant Anal. 1975; 6:71-80.

14. Doane TA, Horwáth WR. Spectrophotometric determination of nitrate with a single reagent. Anal. Lett. 2003; 36: 2713-22.
15. Radin JW. Distribution and development of nitrate reductase activity in germinating cotton seedlings. Plant Physiol. 1974; 53:458-63.
16. Cambraia J, Pimenta JA, Estevão MM, Sant'Anna R. Aluminum effects on nitrate uptake and reduction in sorghum. J. Plant.Nutr. 1989; 12:1435-45.
17. Elliott WH. Isolation of glutamine synthetase and glutamotransferase from green peas. J. Biol.Chem. 1953; 201:661-72.
18. Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR. Gas chromatography mass spectrometry–based metabolite profiling in plants. Nat. Protoc. 2006. 1: 387.
19. Gibon Y, Blaesing OE, Hannemann J, Carillo P, Höhne M, Hendriks JH, Stitt M. A robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. Plant Cell. 2004; 16, 3304-25.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976; 72: 248-54.
21. Shabnam N, Tripathi I, Sharmila P, Pardha-Saradhi P. A rapid, ideal, and eco-friendlier protocol for quantifying proline. Protoplasma. 2016; 253: 1577-82.
22. Ferreira, D. F. Sisvar: a computer statistical analysis system. Ciênc. Agrotec. 2011; 35: 1039-42.
23. Wu F, Bao WK, Li FL, Wu N. Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. Environ. Exp. Botany.2008; 63:248–55.
24. Peloso AF, Tatagiba SD, Amaral JFT, Cavatte PC, Tomaz MA. Efeito da aplicação de piraclostrobina no crescimento inicial de café arábica em diferentes disponibilidades hídricas. Coffee Sci. 2017; 12: 498-507.
25. Yanagisawa S. Transcription factors involved in controlling the expression of nitrate reductase genes in higher plants. Plant Sci. 2014; 229: 167-71.
26. Carelli MLC, Fahl JI. Partitioning of nitrate reductase activity in *Coffea arabica* L. and its relation to carbon assimilation under different irradiance regimes. Braz J. Plant Physiol. 2006; 18: 397-406.
27. Lima JDN, Moraes WS, Silva SHGM, Ibrahim FN, Silva AC. Acúmulo de compostos nitrogenados e atividade da redutase do nitrato em alface produzida sob diferentes sistemas de cultivo. Pesq. Agropecu. Trop. 2008;38:180-7.
28. Reis AR, Junior EF, Haga KI. Atividade da redutase do nitrato em folhas de cafeeiro em função da adubação nitrogenada. Acta Sci. Agron. 2007; 29: 269-76.
29. Khouri CR. [Nitrate reductase activity, content of nitrogen and carbohydrates in coffee plant influenced by shading and phenology phase]. Masters dissertation. Viçosa: Universidade Federal de Viçosa, Brasil; 2007; 63 p.
30. Pereira AA, Baião AC. Cultivares. In: Sakiyama NS, Martinez HEP, Tomaz MA, Borém A. [Arabica coffee: from the sowing to the harvest]. Viçosa: Publisher: UFV; 2015; pp.24-45.
31. Carvalho CHS, Matiello JB, Jordão Filho M, Borato PB, Ferreira GL. [Evaluation of water potential in coffee trees of new cultivars]. In: Anais do 43º Congresso Brasileiro de Pesquisas Cafeeiras, Brasília: Embrapa, DF; 2017; CD Rom.
32. Smirnoff N, Todd P, Stewart GR. The occurrence of nitrate reduction in the leaves of woody plants. Ann. Bot. 1984; 54: 363-374.
33. Gojon A, Plassard C, Bussi C. Root/shoot distribution of NO_3^- assimilation in herbaceous and woody species. A whole plant perspective on carbon–nitrogen interactions. The Hague: SPB Academic Publishing; 1994;131-147.
34. Melo EF, Fernandes-Brum CN, Pereira FJ, Castro EMD, Chalfun-Júnior A. Anatomic and physiological modifications in seedlings of *Coffea arabica* cultivar Siriema under drought conditions. Cienc. Agrotec. 2014;38:25-33.
35. Gregerson RG, Miller SS, Twary SN, Gantt JS, Vance CP. Molecular characterization of NADH-dependent glutamate synthase from alfalfa nodules. 1993; Plant Cell. 5:215-26.
36. Donato VMTS, Andrade AGD, Souza ESD, França JGED, Maciel GA. [Enzymatic activity in sugar cane varieties cultivated in vitro under nitrogen levels]. Pesq. Agropec. Bras. 2004; 39: 1087-93.
37. Wallsgrove RM, Turner JC, Hall NP, Kendall AC, Bright SW. Barley mutants lacking chloroplast glutamine synthetase—biochemical and genetic analysis. Plant Physiol. 1987; 83: 155-8.
38. Edwards JW, Coruzzi GM. Photorespiration and light act in concert to regulate the expression of the nuclear gene for chloroplast glutamine synthetase. The Plant Cell. 1989; 1: 241-8.
39. Stitt M, Müller C, Matt P, Gibon Y, Carillo P, Morcuende R, Krapp A. 2002. Steps towards an integrated view of nitrogen metabolism. J. Exp. Bot. 53, 959-70.
40. Martinez HEP, Souza BP, Caixeta ET, Carvalho FP, Clemente JM. Water deficit changes nitrate uptake and expression of some nitrogen related genes in coffee-plants (*Coffea arabica* L.). Scientia Horticulturae, 2020; 267: 109254.
41. Hirel B, Lea PJ. Ammonia assimilation. In: Lea PJ, Morot-Gaudry JF. Plant nitrogen. Berlin:Springer; 2001; 79-99 pp.
42. Bartels D, Sunkar R. Drought and salt tolerance in plants. Crit. Rev. Plant Sci. 2005; 24: 23-58.

43. Giannakoula A, Moustakas M, Mylona P, Papadakis I, Yupsanis T. Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. J. Plant Physiol. 2008;165: 385-96.
44. Almeida JASD, Carvalho CR, Silvarolla MB, Arruda FB, Braghini MT, Lima VB, Fazuoli LC. [Characterization of morphologic and physiologic response In coffee genotype plants under water stress]. In: Anais do 5º Simpósio de Pesquisa dos Cafés do Brasil, Brasília: Embrapa; 2007; CD Rom.
45. Silva VA, Antunes WC, Guimarães BLS, Paiva RMC, Silva VDF, Ferrão MAG, Loureiro ME. [Physiological response of Conilon coffee clone sensitive to drought grafted onto tolerant rootstock]. Pesq. Agropec. Bras. 2010; 45: 457-64.
46. Tounekti T, Mahdhi M, Al-Turki TA, Khemira H. Water relations and photo-protection mechanisms during drought stress in four coffee (*Coffea arabica*) cultivars from southwestern Saudi Arabia. S. Afr. J. Bot. 2018; 117:17-25.
47. Suguiyama VF, da Silva EA, Meirelles ST, Centeno DDC, Braga MR. Leaf metabolite profile of the Brazilian resurrection plant *Barbacenia purpurea* Hook (Velloziaceae) shows two time-dependent responses during desiccation and recovering. Front. Plant Sci. 2014; 5: 96.
48. Catala R, Ouyang J, Abreu IA, Hu Y, Seo H, Zhang X, Chua NH. The Arabidopsis E3 SUMO ligase SIZ1 regulates plant growth and drought responses. Plant Cell. 2007; 19: 2952-66.
49. Kaur G, Asthir BJBP. Proline: a key player in plant abiotic stress tolerance. Biol. Plant. 2015; 59: 609-19.