

## Productivity, Fatty Acid Profiles and Nitrogen Metabolism of Crambe Under Varied Nitrate Levels

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**Abstract:** The purpose of this research was to evaluate the effects of different nitrate ( $\text{NO}_3^-$ -N) doses (1.0, 2.5 and 7.5 mM) under nitrogen metabolism on Nitrogen (N), Phosphorus (P) and Potassium (K) concentrations, grain and oil yields and the oil composition of crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries), considering its utilization in biodiesel production or as a source of erucic acid. The experiment was conducted in a hydroponic system located in a greenhouse and at the end of the vegetative stage, the fresh weight and the  $\text{NO}_3^-$ -N levels of each plant part were evaluated. At the end of seed maturation,  $\text{NO}_3^-$ -N, amino-N, ammonium soluble sugars, total N P and K levels, nitrate reductase activity, grain and oil yields and the oil profile were determined. Under 1 mM  $\text{NO}_3^-$ -N plants displayed intense N reduction and assimilation activities, a high oil yield and high erucic acid levels. Moreover with 2.5 mM  $\text{NO}_3^-$ -N, the plants displayed an increase in oil quality for use as biodiesel because of high levels of myristic and palmitic acids and low levels of erucic and tetracosanoic acids. Therefore, the use of low  $\text{NO}_3^-$ -N levels for crambe cultivation can be a viable method for oil production both for erucic acid extraction and biodiesel purposes, reducing production costs and environmental problems.

**Key words:** *Crambe abyssinica* Hochst. ex. R.e. fries, brassicaceae, hydroponic system, biodiesel, seed composition, nitrate reductase

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### INTRODUCTION

Aiming to attend to the energy demand around the world and with the economic instability of the oil market and the growth perspectives of the population, there has been a great search for alternative sources of energy. In this context, biofuels have been spotlighted predominantly because of the large availability of biomass and the environmental benefits related to the use of this source (Hill *et al.*, 2006; Jefferson, 2006).

However, rising food prices have hindered the growth of biofuel production, likely due to the rerouting of a significant portion of agricultural production (Mitchell, 2008). This effect occurs because of important crops in food industry, such as soybean, corn, sugar cane, sunflower and rapeseed oil are currently the most important crops used for the production of biofuels.

Through, research performed by the IFPRI (International Food Policy Research Institute), it was concluded that the growth in demand for biofuels from

2000-2007 can be related to an increase of 30% on average for the price of the main grains used in the food industry with corn suffering a major impact, estimated at 39% (Rosegrant, 2008). For this reason, it is important to continue to search for biomass sources for the production of biofuels that are not commonly used by the food industry (Pinzi *et al.*, 2009).

In 2008, a major divulgation occurred, that is crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries), a plant with a short life cycle belonging to the Brassicaceae family was identified as a promising source for biodiesel production for several advantageous reasons. Among them are its precocity, characterized by a cycle of approximately 90 days, its high productivity (1000-1500 kg ha<sup>-1</sup>), low production cost and total oil percentage in grains (between 26 and 38%), a major value when compared with soybean (Pitol, 2008). Moreover, due to its toxicity, crambe cannot be consumed by humans and thus does not compete directly with food production. However, it must be highlighted that its cake represents an important

protein source for ruminants (30-32% crude protein), which can also be used as a fertilizer and that the oil is an important source of erucic acid for certain industries. Because of its characteristics, this Winter Brassicaceae has been presented as a promising alternative for biodiesel production in Brazil. However, even with the high potential of crambe for use in the biodiesel or erucic acid industry, very few research studies have been performed with the aim of determining ways to cultivate this species adequately. Among the required nutrients for vegetable metabolism, N is one of the most important because it is an essential element typically required in major quantities. Thus, NPK fertilizers have been widely used in agriculture, however these types of fertilizers are not commonly well applied, significantly influencing the cost of culture production. Moreover, the inadequate use of these fertilizers is widely related to river pollution, even to eutrophication and to soil and atmosphere contamination (Abrol *et al.*, 2007; Choudhury *et al.*, 2007). In addition, it is important to highlight that environmental factors can influence vegetable metabolism, significantly altering the profile of fatty acids produced and thus, the biodiesel quality (Francois and Kleiman, 1990; Pinzi *et al.*, 2009).

Therefore, studies to determine the correct doses of N required by crambe metabolism and focusing on its utilization in biodiesel or erucic acid production are necessary. With these studies, it will be possible to avoid waste or deficiency situations and, consequently, a negative impact on culture productivity, the environment and also on the cost of its own production.

Therefore, the aim of this research was to evaluate the influence of different  $\text{NO}_3^-$ -N doses (1.0, 2.5 e 7.5 mM) on the growth, N metabolism aspects, oil production and composition of the crambe plant mainly with regard to its application in the biodiesel or erucic acid industry.

## MATERIALS AND METHODS

**General aspects and seed preparation:** The experiment was conducted using a hydroponic system in a greenhouse (Chemistry Department, Universidade Federal Rural do Rio de Janeiro) from October, 2011 to January, 2012.

The commercial crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) seeds used were previously treated under stirring with distilled water (15 min), 70% ethanol (1 min) and 2% sodium hypochlorite (15 min). After washing the seeds with distilled water, the sowing was performed using a sterilized substrate mixture composed of soil (40%), humus (30%) and clay (30%) in a growth chamber.

**Seedling acclimation and treatments:** When the seedlings reached 10 cm, they were moved to the greenhouse for 5 days of acclimation with a 50% reduction in the light intensity. After this stage, the light reduction was abolished and the seedlings were transferred to 1.7 L pots in the hydroponic system. The hydroponic system was composed of pots connected to an air pumping machine and in each pot, 1 seedling was placed. The plants were cultivated for 1 week in a Hogland and Arnon (1950) nutrient solution modified with 2 mM  $\text{NO}_3^-$ -N at 1/2 strength. Then, the treatments (1.0, 2.5 and 7.5 mM  $\text{NO}_3^-$ -N) were applied and a 1/2 strength Hogland and Arnon (1950) nutrient solution was used as a source of other nutrients. The nutrient solution was replaced weekly and every 2 days, the pH was measured and adjusted to 6.3. The solution volume was maintained by distilled water addition.

**Harvests and root, stem and leaf analyses:** At 68 Days After Germination (DAG), the first harvest was performed and each part of the plant had its fresh weight and the  $\text{NO}_3^-$ -N levels (Cataldo *et al.*, 1975) analyzed. At the end of the reproductive stage (103 DAG), the second harvest was performed and the fresh weight of the roots and stems was evaluated. At this stage for each plant part, the nitrate reductase activity was evaluated (Jaworski, 1971) and 0.5 g samples of fresh tissue were maintained in 20 mL of 80% ethanol and then used for amino-N (Yemm and Cocking, 1955),  $\text{NO}_3^-$ -N (Cataldo *et al.*, 1975), ammonium (Felker, 1977) and soluble sugar (Yemm and Willis, 1945) analyses. The residual materials were dried for dry weight determinations and 0.2 g samples were submitted to digestion for total N, P and K analyses.

**Quantitative and qualitative oil analyses:** The harvested grains were dried and weighed and the oil was extracted with a Soxhlet apparatus for 4 h with hexane. After solvent evaporation using a rotavapor, the oil quantity was then determined. Crambe oil samples (0.15 g) were esterified (Metcalf *et al.*, 1966) and then submitted to Gas Chromatography in an apparatus equipped with a Flame Ionization Detector (GC-FID) and Mass Spectrometry (CG-MS). Thus, it was possible to determine the composition and the relative percentage of fatty acids of each sample. GC-FID used for gas chromatography (Agilent, model: HP-5890, Santa Rosa, CA, USA) was equipped with a flame ionization detector at 290°C and a flow splitter injector at 240°C (1:30 ratio and an injection volume of 1  $\mu\text{L}$ ). Factor 4 (VF-5 m sec, 30 m  $\times$  0.25 mm i.d., DF = 0.25  $\mu\text{m}$ , Varian Inc., USA) was the capillary column used and helium was the carrier gas (Flow: 1 mL  $\text{min}^{-1}$ ). The column temperature was

programmed as follows: 200°C held for 1 min followed by heating at 5°C min<sup>-1</sup> to 290°C and holding constant for 10 min. Fatty acid identification was performed by CG-MS from the methyl ester derivatives under the same conditions used for the GC-FID. Each component's percentage was obtained by digital integration.

Methyl esters were identified by retention time using reference data obtained by the injection of standard methyl esters (Sigma-Aldrich®). In addition, for the identification of fatty acids was used a comparison between the fragmentation pattern of the samples and the NIST library data.

**Statistical and experimental design:** Each treatment was composed of 5 replicates (plants) arranged in a completely randomized experimental design. An ANOVA was performed and the treatment means obtained were compared using Tukey's test at the p = 0.05 level with Sigma Stat 3.2 software (Inc, Chicago, IL, USA).

## RESULTS

**Biomass and N metabolism:** At the end of the vegetative stage, 7.5 mM NO<sub>3</sub><sup>-</sup>-N significantly increased the fresh weight of the leaves when compared to 1.0 mM NO<sub>3</sub><sup>-</sup>-N (Fig. 1a). For the stems, the different NO<sub>3</sub><sup>-</sup>-N doses did not have a significant influence on the fresh weight production at this stage. However at the end of the reproductive stage, 7.5 mM NO<sub>3</sub><sup>-</sup>-N allowed for a high fresh weight of the stem (Fig. 1b). A similar response trend to the NO<sub>3</sub><sup>-</sup>-N doses applied was observed for the dry weight (Fig. 1).

The effects of the NO<sub>3</sub><sup>-</sup>-N levels on the different plant parts reveal the level of influence of the treatments and the preferential site for NO<sub>3</sub><sup>-</sup>-N storage in crambe (Table 1). At the end of the vegetative stage, the highest NO<sub>3</sub><sup>-</sup>-N levels in every part of the plants submitted to the largest dose were observed. Moreover at the end of the reproductive stage, a high NO<sub>3</sub><sup>-</sup>-N level was observed in the stems. These results indicate that the NO<sub>3</sub><sup>-</sup>-N levels in the plant stems cultivated with 7.5 mM NO<sub>3</sub><sup>-</sup>-N were maintained at a high level during the cycle. Another aspect, shown in Table 1, is the high NO<sub>3</sub><sup>-</sup>-N content in the plant stems cultivated with this dose during both stages.

Plants cultivated with 7.5 mM NO<sub>3</sub><sup>-</sup>-N displayed major nitrate reductase activities in their stems. In contrast, this behavior was different in the roots because a high enzymatic activity was observed in the plants cultivated with 1.0 mM of this compound (Fig. 2a). Figure 2b shows that in the roots, the highest levels of soluble sugars were detected in the plants cultivated with

Table 1: NO<sub>3</sub><sup>-</sup>-N levels and contents in the roots, stems and leaves of crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) plants cultivated with different NO<sub>3</sub><sup>-</sup>-N doses in nutrient solutions (1.0, 2.5 and 7.5 mM) at the end of the vegetative and reproductive stages (68 and 103 DAG, respectively)

NO <sub>3</sub> <sup>-</sup> -N dose (mM)	NO <sub>3</sub> <sup>-</sup> -N level (moles g <sup>-1</sup> FW)					
	End of the vegetative stage (68 DAG)			End of the reproductive stage (103 DAG)		
	Root	Stem	Leaf	Root	Stem	Leaf <sup>b</sup>
1.0	0.00 <sup>b</sup>	0.00 <sup>f</sup>	0.00 <sup>f</sup>	0.00 <sup>a</sup>	0.02 <sup>b</sup>	-
2.5	2.74 <sup>b</sup>	14.19 <sup>b</sup>	1.43 <sup>b</sup>	0.02 <sup>a</sup>	2.05 <sup>b</sup>	-
7	10.93 <sup>a</sup>	48.78 <sup>a</sup>	24.56 <sup>a</sup>	1.57 <sup>a</sup>	25.79 <sup>a</sup>	-
	NO <sub>3</sub> <sup>-</sup> -N content (mg plant <sup>-1</sup> )					
1.0	0.00 <sup>b</sup>	0.00 <sup>f</sup>	0.00 <sup>f</sup>	0.17 <sup>a</sup>	0.14 <sup>b</sup>	-
2.5	15.16 <sup>a</sup>	77.90 <sup>b</sup>	26.54 <sup>b</sup>	0.29 <sup>a</sup>	28.56 <sup>b</sup>	-
7.5	67.11 <sup>a</sup>	356.72 <sup>a</sup>	493.43 <sup>a</sup>	9.30 <sup>a</sup>	255.96 <sup>a</sup>	-

Different letters for the same plant part during each stage indicate significant differences (Tukey's test, p ≤ 0.05); leaves were absent at the end of the reproductive stage

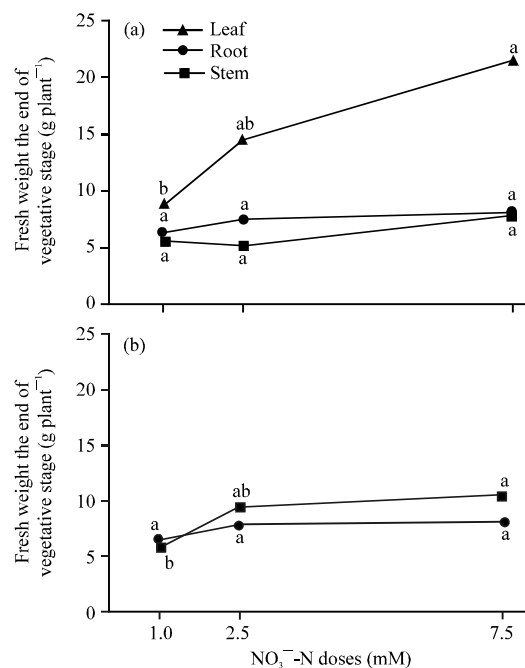


Fig. 1: a) Fresh weight (g plant<sup>-1</sup>) of each part of the crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) plants cultivated with different NO<sub>3</sub><sup>-</sup>-N doses in nutrient solutions (1.0, 2.5 and 7.5 mM) at the end of the vegetative; b) Reproductive stages. Different letters for the same plant part indicate significant differences (Tukey's test, p ≤ 0.05)

1.0 mM NO<sub>3</sub><sup>-</sup>-N. The soluble sugar content in the roots of the plants cultivated under this dose was also high (Table 2). Ammonium was also found in high levels in the roots of the plants cultivated with 1.0 mM NO<sub>3</sub><sup>-</sup>-N at the end of their life cycle (Fig. 2c). At this stage, the highest amino-N levels were observed in the roots of these plants (Fig. 2d).

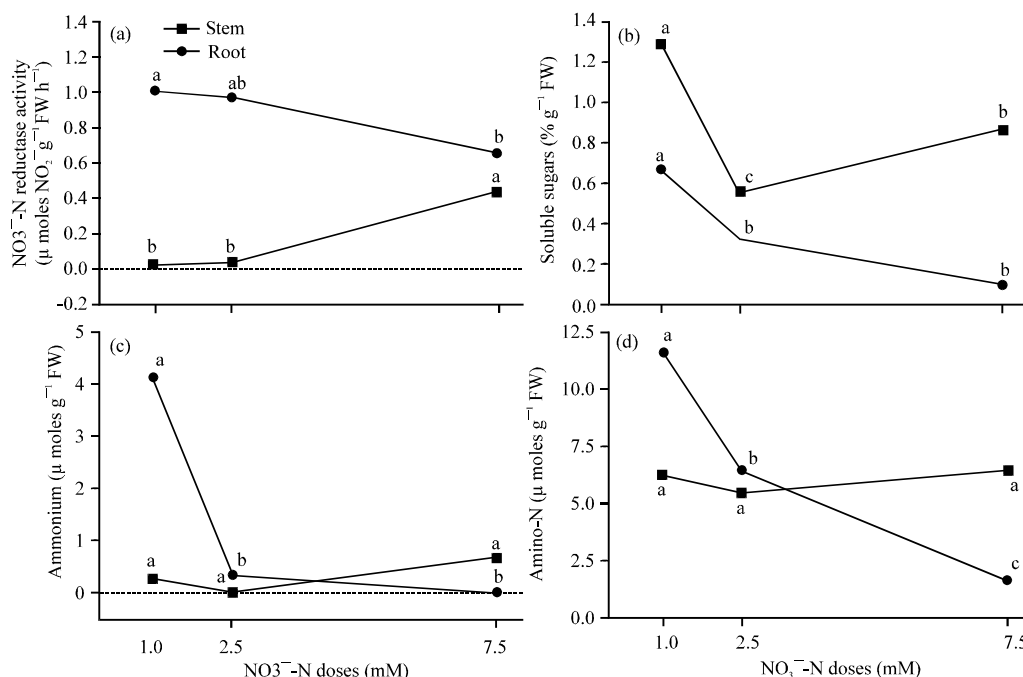


Fig. 2: a)  $\text{NO}_3^-$ -N reductase activity; b) Soluble sugar; c) Ammonium; d) Amino-N levels in the roots and stems of crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) plants cultivated with different  $\text{NO}_3^-$ -N doses in nutrient solutions (1.0, 2.5 and 7.5 mM) and harvested at the end of the reproductive stage (103 DAG). Different letters for the same plant part indicate significant differences (Tukey's test,  $p \leq 0.05$ )

Table 2: Soluble sugar contents ( $\text{g plant}^{-1}$ ) in the roots and stems of crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) plants cultivated with different  $\text{NO}_3^-$ -N doses in nutrient solutions (1.0, 2.5 and 7.5 mM) and harvested at the end of the reproductive stage (103 days after germination).

$\text{NO}_3^-$ -N dose (mM)	Soluble sugar content ( $\text{mg plant}^{-1}$ )		
	Root	Stem	Leaf <sup>b</sup>
1.0	43.50 <sup>a</sup>	77.80 <sup>a</sup>	-
2.5	29.60 <sup>a</sup>	61.90 <sup>a</sup>	-
7.5	8.20 <sup>b</sup>	84.60 <sup>a</sup>	-

Different letters for the same plant part during each stage indicate significant differences (Tukey's test,  $p \leq 0.05$ ); leaves were absent at the end of the reproductive stage

Table 3: Total N, P and K levels ( $\text{mg g}^{-1}$  DW) and contents ( $\text{mg plant}^{-1}$ ) in the roots and stems of crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) plants cultivated with different  $\text{NO}_3^-$ -N doses in nutrient solutions (1.0, 2.5 and 7.5 mM) and harvested at the end of the reproductive stage (103 days after germination)

$\text{NO}_3^-$ -N dose (mM)	Level ( $\text{mg g}^{-1}$ DW)					
	Total N		P		K	
	Root	Stem	Root	Stem	Root	Stem
1.0	16.12 <sup>b</sup>	4.16 <sup>b</sup>	1.09 <sup>a</sup>	0.25 <sup>b</sup>	0.18 <sup>a</sup>	0.47 <sup>c</sup>
2.5	15.43 <sup>b</sup>	5.71 <sup>b</sup>	0.71 <sup>b</sup>	0.31 <sup>b</sup>	0.10 <sup>ab</sup>	0.72 <sup>b</sup>
7.5	23.91 <sup>a</sup>	14.22 <sup>a</sup>	0.76 <sup>b</sup>	0.64 <sup>a</sup>	0.07 <sup>b</sup>	0.84 <sup>a</sup>
Content ( $\text{mg plant}^{-1}$ )						
1.0	8.58 <sup>a</sup>	5.14 <sup>b</sup>	0.62 <sup>a</sup>	0.28 <sup>b</sup>	0.13 <sup>a</sup>	0.61 <sup>b</sup>
2.5	10.01 <sup>a</sup>	16.50 <sup>b</sup>	0.45 <sup>a</sup>	0.88 <sup>b</sup>	0.11 <sup>a</sup>	2.04 <sup>ab</sup>
7.5	15.44 <sup>a</sup>	52.49 <sup>a</sup>	0.49 <sup>a</sup>	2.29 <sup>a</sup>	0.02 <sup>a</sup>	3.06 <sup>a</sup>

Different letters in the same plant part in each division (level or content) indicate significant differences (Tukey's test,  $p \leq 0.05$ )

The results in Table 3 show that a high level of total N was detected in the roots and stems of plants cultivated with 7.5 mM  $\text{NO}_3^-$ -N. The P levels displayed a different behavior between the roots and stems of the plants cultivated with various  $\text{NO}_3^-$ -N supplemental levels (Table 3). The highest levels of P were observed in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N, whereas the lowest levels were observed in the stems of the plants cultivated with 7.5 mM  $\text{NO}_3^-$ -N. K levels increased significantly in the stems as the  $\text{NO}_3^-$ -N supply increased, however in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N, a high K level was detected (Table 3). The pH value in the nutrient solution showed a tendency toward acidification during the 1.0 mM  $\text{NO}_3^-$ -N treatment as the experiment progressed (Fig. 3).

**Production of grain and oil and the fatty acid profiles:**

The grain yield did not display significant variation with the different  $\text{NO}_3^-$ -N doses applied, however the oil yield showed alterations (Fig. 4). Plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N had a high oil yield and did not exhibit a significant difference when compared with the plants cultivated with the highest  $\text{NO}_3^-$ -N dose. An evaluation of the fatty acid profiles of the crambe oil showed that 2.5 mM  $\text{NO}_3^-$ -N presented high percentages of myristic and palmitic acids (Table 4). Furthermore, plants cultivated with 2.5 mM  $\text{NO}_3^-$ -N also showed low

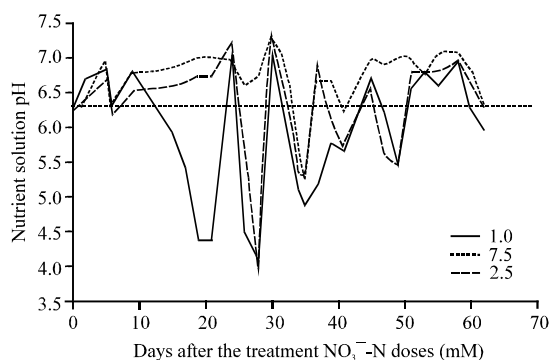


Fig. 3: The pH variation in the nutrient solutions used for the growth of crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) plants cultivated with different  $\text{NO}_3^-$ -N doses (1.0, 2.5 and 7.5 mM) after the treatments and until the end of the reproductive stage (103 days after germination)

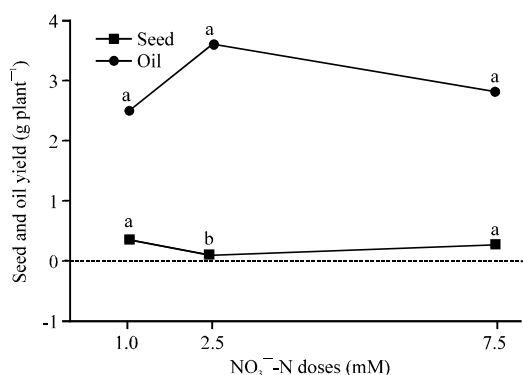


Fig. 4: Seed and oil yields ( $\text{g plant}^{-1}$ ) of crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) plants cultivated with different  $\text{NO}_3^-$ -N doses in nutrient solutions (1.0, 2.5 and 7.5 mM) and harvested at the end of the reproductive stage (103 DAG). Different letters in the same line indicate significant differences (Tukey's test,  $p \leq 0.05$ )

Table 4: Fatty acid levels (%) in oil from crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries) plants cultivated in nutrient solutions supplemented with different  $\text{NO}_3^-$ -N doses (1.0, 2.5 and 7.5 mM) compared with commercial seeds

Fatty acid	Oil composition (%)			
	Commercial seeds	$\text{NO}_3^-$ -N dose (mM)		
		1.0	2.5	7.5
Myristic (14:0)	0.1 <sup>ab</sup>	0.1 <sup>ab</sup>	0.8 <sup>a</sup>	0.0 <sup>b</sup>
Palmitic (16:0)	2.4 <sup>ab</sup>	1.7 <sup>b</sup>	3.6 <sup>a</sup>	20.0 <sup>ab</sup>
Stearic (18:0)	1.1 <sup>a</sup>	1.1 <sup>a</sup>	1.8 <sup>a</sup>	1.4 <sup>a</sup>
Oleic (18:1)	22.5 <sup>a</sup>	20.4 <sup>b</sup>	21.3 <sup>ab</sup>	22.2 <sup>ab</sup>
Linoleic (18:2)	8.3 <sup>a</sup>	5.4 <sup>b</sup>	7.0 <sup>ab</sup>	6.6 <sup>ab</sup>
Eicosanoic (20:0)	1.3 <sup>a</sup>	1.4 <sup>a</sup>	1.3 <sup>a</sup>	1.5 <sup>a</sup>
Erucic (22:1)	55.0 <sup>ab</sup>	58.5 <sup>a</sup>	41.2 <sup>b</sup>	54.6 <sup>ab</sup>
Tetracosanoic (24:1)	0.7 <sup>ab</sup>	0.9 <sup>ab</sup>	0.2 <sup>b</sup>	1.1 <sup>a</sup>

Different letters in the same line indicate significant differences (Tukey's test,  $p \leq 0.05$ )

percentages of erucic and tetracosanoic acids. Plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N showed high percentages of erucic acid in oil.

## DISCUSSION

In rape (*Brassica campestris* L.) and Chinese cabbage (*Brassica chinensis* var. *Oleifera makino* et Nenoto) which are members of the Brassicaceae family (as is crambe), Chen *et al.* (2004) observed that the stem is the main site for  $\text{NO}_3^-$ -N storage. Thus, the results observed in Table 1 illustrate that crambe follows this same tendency of  $\text{NO}_3^-$ -N storage in the stem when compared with these species. It must also be considered that at the end of the vegetative stage, plants cultivated with 7.5 mM  $\text{NO}_3^-$ -N had approximately  $49 \mu\text{moles g}^{-1}$  of fresh weight of  $\text{NO}_3^-$ -N, however at the end of the cycle, this value decreased by 50% (Table 1). The higher remobilization and availability of N- $\text{NO}_3^-$  in the plants cultivated with 7.5 mM of this compound likely have a direct correlation with the high levels of fresh weight observed due to the possibility of this compound incrementally affecting the protein synthesis process.

The nitrate reductase activity observed in the stems suggests that in the plants subjected to the higher dose, the utilization of the available  $\text{NO}_3^-$ -N is occurring at a high rate likely also contributing to the high fresh weight production in this plant part at the end of the cycle (Fig. 2a). In the roots, the obtained results reveal that at this stage, this is the main organ involved in the  $\text{NO}_3^-$ -N reduction process. However in this organ, the observed behavior of the enzymatic activity was distinct when compared with the results detected in the stem once a high level was achieved by the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N which is a dose limit between the high and low affinity  $\text{NO}_3^-$ -N transport system. This result can be related to a higher rate of activation for the mechanisms involved in the induction of enzymatic activity.

The nitrate reductase activities in the roots and leaves can be stimulated by elevating sugar levels (Crawford, 1995; Lillo *et al.*, 2004). In the roots, the highest levels and also a high content of soluble sugars were detected in the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N (Fig. 2b and Table 2). This accumulation is possibly related to the lowest level of  $\text{NO}_3^-$ -N available because this would limit the N assimilation processes, thus lowering the utilization of carbon skeletons. Consequently, it is possible that the high level of soluble sugars in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N led to an increase in nitrate reductase activity due to the stimulatory effect of these compounds on the enzyme. It is also important to highlight that the behavior

of the nitrate reductase activity and the soluble sugar levels in the roots cultivated with the different doses of  $\text{NO}_3^-$ -N were similar which supports this hypothesis (Fig. 2a, b).

The high levels of ammonium at the end of the life cycle in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N are likely related to a high nitrate reductase activity a central enzyme for the pathway involved in ammonium formation even during late developmental stages which could have contributed to the low  $\text{NO}_3^-$ -N levels observed in the plants cultivated with the lowest dose of this ion (Table 1 and Fig. 2c). Another hypothesis is that the high ammonium levels observed in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N could be related to the senescence acceleration of these plants because the peaks in ammonium production originated by the degradation of the proteins in the leaves increase during this developmental stage (Lam *et al.*, 1996). However according to Souza and Fernandes, the activities of the enzymes involved in N metabolism typically decrease with senescence which was not observed with the nitrate reductase of the plants submitted to the lowest dose, weakening the earlier senescence hypothesis.

The highest amino-N level in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N (Fig. 2d) can be related to the metabolic data previously observed in the roots of these plants which displayed high nitrate reductase activities and elevated carbon skeleton levels, possibly due to the higher availability of soluble sugars and the abundance of ammonium, an important substrate for free amino acid formation (Fig. 2a-c and Table 2).

The high level of total N detected in the roots and stems of the plants cultivated with 7.5 mM  $\text{NO}_3^-$ -N is indicated in Table 3. In this context, it is possible that a high  $\text{NO}_3^-$ -N availability allowed for an increase in the storage of that nutrient, predominantly in the stems (Table 1) which contributed to the high levels of N observed.

N reduction and assimilation appear to be more intensive in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N because of the high nitrate reductase activity and the elevated concentrations of amino-N, soluble sugars and ammonium that were detected (Fig. 2). The high-energy dependence of N reduction and assimilation likely increased the P demand in the roots. Furthermore, the high nitrate reductase activity in the stems of the plants cultivated with the highest dose generated a similar effect on the P requirement which could be the reason for the higher levels of P in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N and for the lowest levels in the stems of the plants cultivated with 7.5 mM  $\text{NO}_3^-$ -N, as shown in Table 3.

The high level of K in the roots of the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N can be related to the pH

variation in this nutrient solution because acidification can increase K uptake due to the presence of histidine residues in the pores of plant K channels which exhibit a response with pH reduction and can lead to the increase in K uptake (Hoth *et al.*, 2001) (Table 3 and Fig. 3). The acidification of the nutrient solution was likely caused by the significant activation of the  $\text{H}^+$  extrusion mechanism which aims to increase  $\text{NO}_3^-$ -N uptake because this process is performed via 2  $\text{H}^+$  symport. Thus, the activation of this mechanism can be generated at the same time an increase in K uptake in the roots of the plants cultivated with the lowest  $\text{NO}_3^-$ -N dose.

While the different  $\text{NO}_3^-$ -N doses applied did not display a significant influence on the grain yield, the plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N had a high oil yield and did not exhibit significant differences when compared with the plants cultivated with 7.5 mM  $\text{NO}_3^-$ -N. In this context, it is important to emphasize that Taylor *et al.* (1991) observed an increase in oil yield with a reduction in the N supply in canola (*Brassica napus* L.), a crucifer-like crambe. Moreover, other authors have reported stagnation or a reduction in the grain yield in canola when the N supply is increased (Gammellvind *et al.*, 1996; Sieling and Christen, 1997).

An evaluation of the fatty acid profile of the crambe oil showed that 2.5 mM  $\text{NO}_3^-$ -N provided good quality parameters for its use as biodiesel (Table 4), justified due to the high percentages of myristic and palmitic acids which contribute to a reduction in the iodine value and an increase in the cetane number and calorific value (Knothe, 2008; Pinzi *et al.*, 2009). Moreover, the plants cultivated with 2.5 mM  $\text{NO}_3^-$ -N presented low percentages of erucic and tetracosanoic acids, contributing to a decrease in the unsaturation level. Thus, the plants cultivated with 2.5 mM  $\text{NO}_3^-$ -N showed an increase in oil quality which is important for its application in the biodiesel industry. However, under 1.0 mM  $\text{NO}_3^-$ -N plants showed a high erucic acid level in oil, an important industrial component.

## CONCLUSION

Thus, plants cultivated with 1.0 mM  $\text{NO}_3^-$ -N, the limiting dose between the high and low affinity  $\text{NO}_3^-$ -N transport system, displayed high intensity N reduction and assimilation at the end of their life cycle compared with the other plants. Furthermore with this  $\text{NO}_3^-$ -N dose, it was possible to obtain a high oil yield and erucic acid level without significant losses in grain yield. However, it is important to highlight that considering the oil use for biodiesel production a superior fatty acid profile was obtained from the crambe plants cultivated with 2.5 mM  $\text{NO}_3^-$ -N. Therefore, the results indicate that is possible to obtain good yield parameters from crambe plants with the

aim being biodiesel production or erucic acid extraction with the use of low  $\text{NO}_3^-$ -N doses indicating the possibility for the production of this culture combined with reductions in cost and environmental impacts.

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