

## Development and production of crambe (*Crambe abyssinica*) under different nitrogen and phosphate fertilizers

Lúcia Helena Garófalo Chaves<sup>15\*</sup>, Eduardo Obadowski Ledur<sup>2</sup>, Josely Dantas Fernandes<sup>3</sup>, Iêde de Brito Chaves<sup>4</sup>

<sup>1</sup>Federal University Campina Grande, Campina Grande, PB, Brazil

<sup>2</sup>Federal Technological University of Paraná, Medianeira, PR, Brazil

<sup>3</sup>State University of Paraíba, Campina Grande, PB, Brazil

<sup>4</sup>Federal University of Paraíba, Areia, PB, Brazil

<sup>5</sup>Fellow of CNPq Research Productivity, level 1C, Brazil

\*Corresponding author: lhgafalo@hotmail.com

### Abstract

The aim of this study was to evaluate the effect of combinations of nitrogen (0; 30; 60; 90; 120 kg ha<sup>-1</sup>) and phosphorus (0; 50; 75; 100; 125 kg ha<sup>-1</sup>) rates on the plant height, shoot biomass production, grain, lipid, nitrogen and protein yields, and leaf N and P content in crambe cultivated in Typic Haplorthox soil (Dusky Red Latosol). A completely randomized design with three replications was used totaling 75 experimental units. The results were submitted to a variance analysis (F-test) and regression study with 5% probability (p≤0.05). The nitrogen increased biomass production, grain yield and leaf nitrogen and potassium content. Phosphorus promoted an increase in the phosphorus and potassium accumulation in plant leaves. The interaction of nitrogen with phosphorus only increased the plant height. The grain lipid, nitrogen and protein content were not significantly affected as a function of phosphorus and nitrogen application in soil. Apparently, the growth and production characteristics of crambe plants did not respond to phosphorus fertilization.

**Keywords:** Yield components; Oil components; *Crambe abyssinica* H.; Leaf N and K content.

**Abbreviations:** N\_nitrogen; P\_phosphorus; K\_potassium; OM\_organic matter; PH\_plant height; NB\_number of branches; SDM\_shoot dry matter; NS\_number of siliques; MGW\_1,000 grain weight; PROD\_productivity; LIPID\_lipid; NITRO\_nitrogen; PROT\_protein; ATP\_adenosine triphosphate; DF\_degree of freedom; CV\_Coefficient of variation.

### Introduction

Crambe (*Crambe abyssinica* H.) is native of the Mediterranean region from Ethiopia to Tanzania; however, it is cultivated in tropical and subtropical regions. In Brazil, research on the culture of crambe began in 1995 at the Foundation Mato Grosso do Sul to evaluate their behavior in the formation of ground cover (Pitol et al., 2010). However, with the advent of this oilseed, biodiesel production has become quite interesting option due to advantages such as earliness, rusticity and good adaptability to drought and frost. Furthermore, crambe does not compete with food crops for biofuel production (Jasper et al., 2010).

Crambe's cultivation is fully mechanized employing the same equipment being used for the traditional cultures for the production of grain. This plant has a height between 70 and 90 cm, with flowering from 35 days after seeding and has 35 to 60% oil (Pitol et al., 2010), low production cost, productivity between 1000 and 1500 kg ha<sup>-1</sup>, as well as higher yield compared to crops such as sunflower, radish, canola, jatropha, among others. The oil extracted from crambe seeds can be used as an industrial lubricant, corrosion inhibitor and also in the synthetic rubber manufacture because of high content of erucic acid in oil (50-60%). It can also be used in the plastics, nylon, adhesives manufacturing and electrical insulation.

The crambe, like other plants, is nutrient-demanding, especially in terms of K and N (Heinz et al., 2011), indicating the need to supply these nutrients by fertilization. Nitrogen is a constituent of various components of the plant cell, being part of the chlorophyll molecule and participating in protein synthesis reactions. The inclusion of phosphorus in fertilization stimulates root development, accelerates physiological maturity, encourages flowering and seed formation, and increases resistance to cold and grain yield (Cihacek et al., 1993; Silva et al., 2011).

When one of these elements is not available, the plant has difficulty expressing their potential and completes their life cycle. In this regard, the application of fertilizers efficiently is critical to efficiency in productivity.

The studies on crambe fertilization for the conditions in Brazil are similar to small grains (Knights, 2002), such as canola (*Brassica napus* L. and *Brassica rapa* L.) and mustard (*Brassica juncea* L.) and are still very limited and controversial. So, there is no specific recommendation for its culture and more researches on this subject are needed. In this context, this study aims to evaluate the effect of application of N and P on growth and yield of crambe.

## Results and Discussion

### *Plant height, number of branches and shoot dry matter*

Nitrogen (N) is considered an essential element for plants, because it is present in the composition of the most important biomolecules, chlorophyll, many proteins and enzymes (Mifflin and Lea, 1976; Harper, 1994). In many production systems, the availability of nitrogen is often a limiting factor influencing plant growth more than any other nutrient. Phosphorus (P), when applied in adequate amounts, stimulates root development, accelerates physiological maturity, encourages flowering, seed formation and increases resistance to cold and productivity (Malavolta et al., 1997). However, the nitrogen fertilizer, like phosphorus, had no significant effect on plant height (Table 1), corroborating with Oliveira et al. (2013) who found no response to nitrogen for plant height when this nutrient was applied to cover. These results disagree with Vechiatto and Fernandes (2011) since they noted that nitrogen fertilization influenced positive development in plants crambe.

The plant height crambe obtained significant results at the 5% level by the F-test using the interaction of nitrogen with phosphorus (Table 1), despite presenting results different between treatments (Fig 1 and 2).

The number of branches and shoot dry matter production were significantly influenced by nitrogen application (Table 1) corroborating with Vechiatto and Fernandes (2011) who reported a significant difference between applied nitrogen (N) fertilization (0, 80 and 120 kg ha<sup>-1</sup>) in crambe. According to the authors, this demonstrates the importance of N application on dry matter production, providing increased soil organic matter. Fig 3 and 4 show the behaviors of the number of branches and shoot dry matter as a function of doses of applied nitrogen at crambe planting, respectively, in which the number of branches and shoot dry matter increased with increasing doses of nitrogen. Although the results of shoot dry matter observed by Soratto et al. (2013) (2.7 to 4.2 g/plant) were lower than those found in this study (from 11.03 to 18.97 g/planta without N e with 90 kg N ha<sup>-1</sup>, respectively), their behavior was similar, in which fertilizing with NPK increased the dry matter of plants crambe. The number of branches and shoot dry matter production was not affected by phosphorus application, contrary to what was observed by Rogério et al. (2012). These authors found a significant increase in dry matter as a function of the doses of phosphorus.

### *Number of siliques and weight of 1000 grains*

The number of siliques per plant in crambe was significantly influenced only by nitrogen fertilizer (Table 2). Its behavior has been depicted in Fig 5. The weight of 1,000 crambe grains was decreased significantly by N fertilizer application in the sowing furrow, with values ranging from 7.16 to 6.68 (Fig 6).

Phosphorus fertilizer application did not significantly influence on weight of 1,000 crambe grains (Table 2) corroborating with Oliveira et al. (2013). Contrarily, Silva et al. (2011) and Soratto et al. (2013) reported a weight increase in 1,000 crambe grains by P application in the sowing furrow. The values of 8.6 to 9.3 g obtained by Soratto et al. (2013) were slightly higher than those observed in this study (6.84 to

6.94 g), but within the range reported by Falasca et al. (2010) and Silva et al. (2011), which are between 6 and 10 g and between 6.3 and 7.7 g, respectively.

The grain yield was significantly influenced only by nitrogen fertilizing (Table 2), which mainly depends to fertilizer's rates (Fig 7). Pitol et al. (2010) observed that yield response of crambe is associated with nitrogen doses, in which applying 35 kg ha<sup>-1</sup> of nitrogen produced 1,361 kg ha<sup>-1</sup> yield.

Increased productivity without increasing 1,000 grains weight can be explained by the fact that the application of doses of nitrogen contributed to higher number of siliques.

Although the phosphorus is essential for increasing the plant productivity, as participating of the structure ATP (adenosine triphosphate) energy source in plant (Malavolta et al., 1997), increasing doses of phosphorus did not increase the grain yield (variation 1,736 to 1,801 kg ha<sup>-1</sup>) corroborating with Broch et al. (2010) and Pitol et al. (2010). However, Silva et al. (2011) and, Soratto et al. (2013) observed an increase in crambe grain yield, with the application of 40 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, of 0 to 90 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and application of 0, 150 and 300 kg ha<sup>-1</sup> of NPK, respectively. One possible explanation for not having response to fertilizing phosphorus in this study is related to chemical characteristics of the studied soil, which had good initial level of fertility, ie, eutrophic soil, with the initial content of phosphorus (18.8 mg dm<sup>-3</sup>) above the critical level for high levels of P in clay soils.

### *Nitrogen, phosphorus and potassium in the protein and oil content in grains*

In oilseeds plants, nitrogen influences the synthesis of compounds of reserves of seeds, determining the protein levels in grains and oil production (Castro et al., 1999). However, in this study, the grain protein, lipid and nitrogen content were not influenced by fertilization (Table 2). Likewise, Rogério et al. (2012) did not observe significance influence on oil content when P<sub>2</sub>O<sub>5</sub> was applied at seeding rates. In this study we observed the grain lipid contents range of 35.22-33.60 % and of 33.91-34.51 % based on the nitrogen and phosphorus treatment levels, respectively. These values are similar to those observed by Adamsen and Coffelt (2005) and Soratto et al. (2013) that observed crambe seed oil contents varying between 33.8-39.5 % and 35.2-39.5 % in a study in Arizona, USA and São Paulo State, Brazil, respectively. The values obtained in present study were below those observed by Souza et al. (2009) that found average grain oil contents of 44.1 % in samples of unhusked grain of the crambe cultivar FMS Brilhante in the states of Mato Grosso and Mato Grosso do Sul.

### *Nitrogen, phosphorus and potassium in the foliar concentration*

The application of increasing doses of nitrogen at planting crambe influenced significantly the leaf nitrogen and potassium concentration. The phosphorus concentrations in the crambe leaves were not influenced by nitrogen fertilizer; however, the phosphorus concentrations were significantly influenced by phosphorus fertilizer. The phosphorus fertilizer also influenced the levels of potassium foliar (Table 3).

**Table 1.** Analysis of variance for plant height (PH), number of branches (NB) and shoot dry matter (SDM) under different levels of nitrogen and phosphorus fertilizer for plants crambe.

Source of variation	DF	Square Means		
		PH	NB	SDM
Block	2	0.029 <sup>**</sup>	13.49 <sup>**</sup>	26.96 <sup>ns</sup>
Nitrogen (N)	4	0.003 <sup>ns</sup>	8.09 <sup>**</sup>	129.72 <sup>**</sup>
Linear effect	1	-	28.69 <sup>**</sup>	358.18 <sup>ns</sup>
Quadratic effect	1	-	-	128.43 <sup>**</sup>
Phosphorus (P)	4	0.011 <sup>ns</sup>	0.26 <sup>ns</sup>	7.54 <sup>ns</sup>
N x P	16	0.004 <sup>*</sup>	0.66 <sup>ns</sup>	13.68 <sup>ns</sup>
N in P 1				
Linear effect	1	0.019 <sup>**</sup>	-	-
N in P 5				
Linear effect	1	0.0145 <sup>**</sup>	-	-
Quadratic effect	1	0.0117 <sup>*</sup>	-	-
P in N 1				
Quadratic effect	1	0.022 <sup>**</sup>	-	-
Residue	48	0.002	0.55	17.25
CV (%)		3.67	7.96	26.81
General mean		1.17	9.37	15.49

Significant at 0.05 (\*) and at 0.01 (\*\*) of probability; (ns) not significant by F-test; - DF: degree of freedom; CV: Coefficient of variation.

**Table 2.** Analysis of variance for number of siliques (NS), 1,000 grain weight (MGW), productivity (PROD), grain lipid content (LIPID), grain nitrogen content (NITRO) and grain protein content (PROT) under different levels of nitrogen and phosphorus fertilizer for plants crambe.

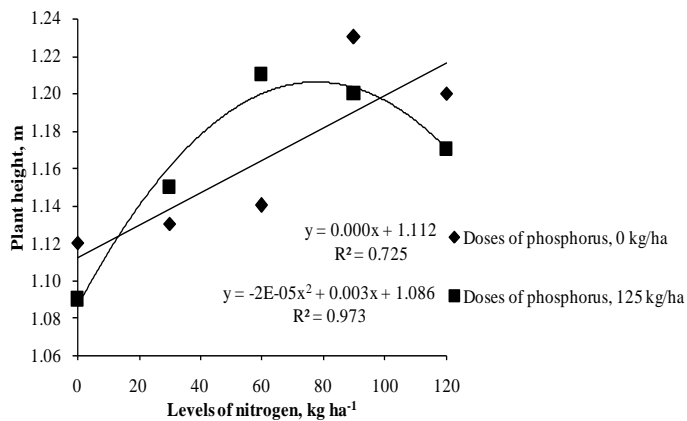
Source of variation	DF	Square Means					
		NS	MGW	PROD	LIPÍD.	NITRO	PROT
Block	2	202974.24 <sup>**</sup>	0.161 <sup>*</sup>	21904.69 <sup>ns</sup>	53.91 <sup>ns</sup>	0.11 <sup>ns</sup>	5.75 <sup>ns</sup>
Nitrogen (N)	4	320485.12 <sup>**</sup>	0.522 <sup>**</sup>	547899.49 <sup>**</sup>	7.96 <sup>ns</sup>	0.09 <sup>ns</sup>	4.50 <sup>ns</sup>
Linear effect	1	1098932.81 <sup>**</sup>	1.96 <sup>**</sup>	1217750.15 <sup>**</sup>	-	-	-
Quadratic effect	1	-	-	918244.15 <sup>**</sup>	-	-	-
Phosphorus (P)	4	28593.91 <sup>ns</sup>	0.023 <sup>ns</sup>	10586.48 <sup>ns</sup>	0.90 <sup>ns</sup>	0.13 <sup>ns</sup>	4.41 <sup>ns</sup>
N x P	16	43079.38 <sup>ns</sup>	0.019 <sup>ns</sup>	21672.11 <sup>ns</sup>	22.06 <sup>ns</sup>	0.08 <sup>ns</sup>	2.94 <sup>ns</sup>
Residue	48	23916.13	0.036	17020.83	22.94	0.05	1.94
CV (%)		14.67	2.75	7.38	13.97	6.91	7.20
General mean		1054.19	6.89	1767.85	34.28	3.10	19.36

Significant at 0.05 (\*) and at 0.01 (\*\*) of probability; (ns) not significant by F-test; - DF: degree of freedom; CV: Coefficient of variation.

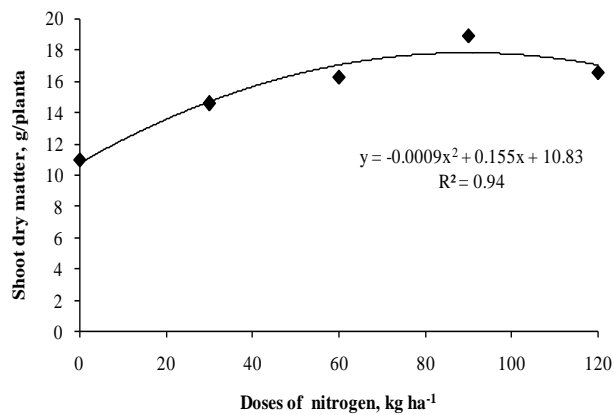
**Table 3.** Analysis of variance for leaf nitrogen, leaf phosphorus and leaf potassium under different levels of nitrogen and phosphorus fertilizer for plants crambe.

Source of variation	DF	Square Means		
		Leaf N	Leaf P	Leaf K
Block	2	179.60 <sup>*</sup>	12.38 <sup>**</sup>	254.27 <sup>**</sup>
Nitrogen (N)	4	411.71 <sup>**</sup>	1.21 <sup>ns</sup>	75.38 <sup>*</sup>
Linear effect	1	1586.71 <sup>**</sup>	-	-
Quadratic effect	1	-	-	161.57 <sup>*</sup>
Phosphorus (P)	4	95.41 <sup>ns</sup>	10.54 <sup>**</sup>	109.66 <sup>**</sup>
Linear effect	1	-	40.68 <sup>**</sup>	-
Quadratic effect	1	-	-	414.52 <sup>**</sup>
N x P	16	46.39 <sup>ns</sup>	1.29 <sup>ns</sup>	27.00 <sup>ns</sup>
Residue	48	47.98	0.84	26.92
CV (%)		17.69	17.62	16.65
General mean		39.16	5.19	31.16

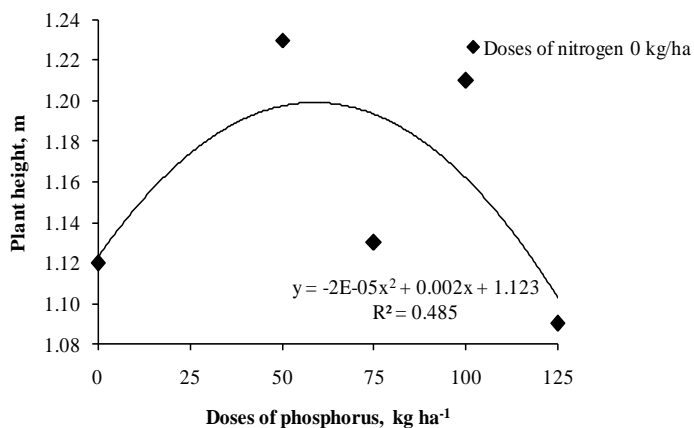
Significant at 0.05 (\*) and at 0.01 (\*\*) of probability; (ns) not significant by F-test; - DF: degree of freedom; CV: Coefficient of variation.



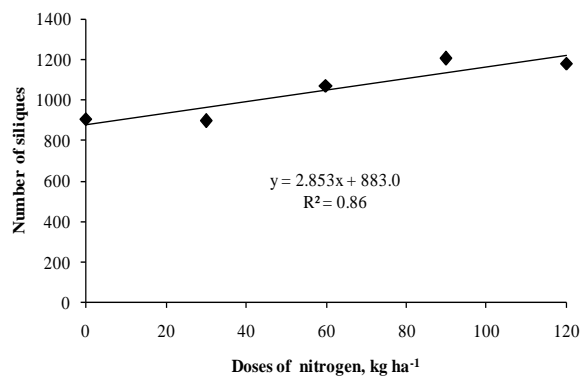
**Fig 1.** Crambe plant height as a function of the interaction of nitrogen with 1 dose (0 kg ha<sup>-1</sup>) and 5 (125 kg ha<sup>-1</sup>) phosphorus.



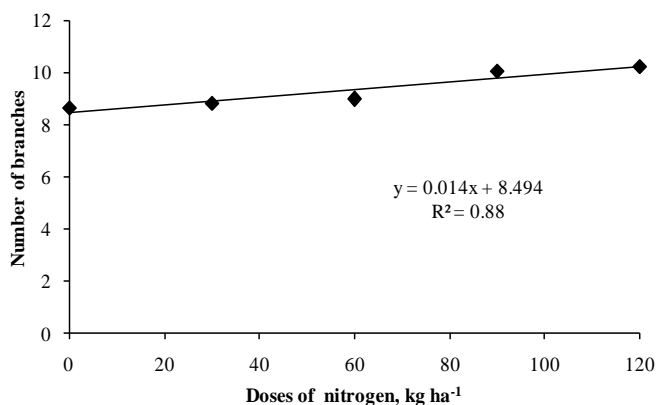
**Fig 4.** Shoot dry matter as a function of doses of nitrogen.



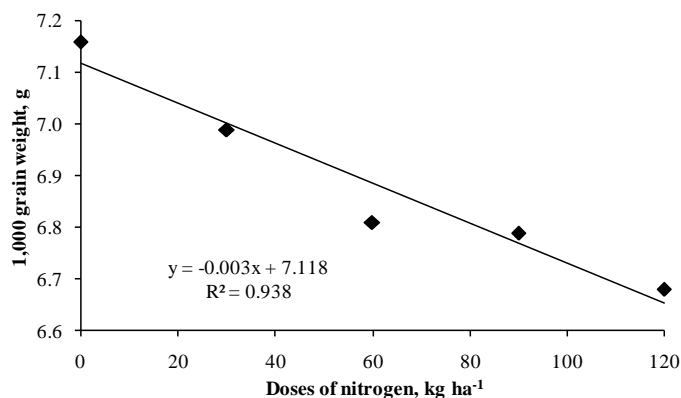
**Fig 2.** Crambe plant height as a function of the interaction of phosphorus with 1 doses (0 kg ha<sup>-1</sup>) of nitrogen.



**Fig 5.** Number of siliques per plant as a function of doses of nitrogen.



**Fig 3.** Number of branches as a function of doses of nitrogen.



**Fig 6.** Thousand grain weight of crambe as a function of doses of nitrogen.

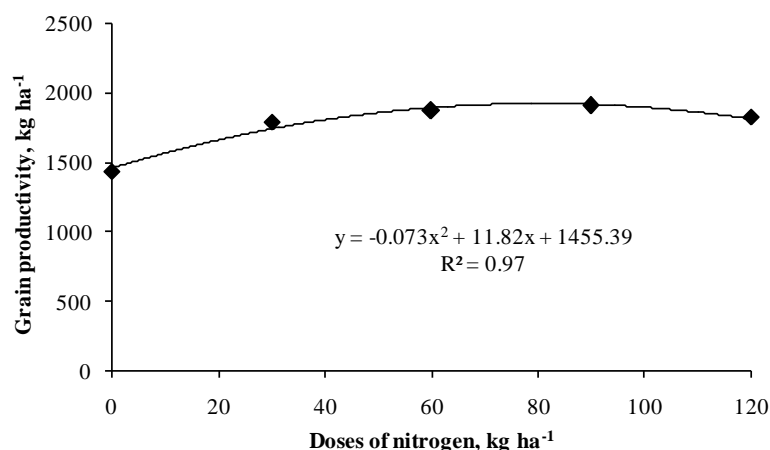


Fig 7. Grain productivity of crambe as a function of doses of nitrogen.

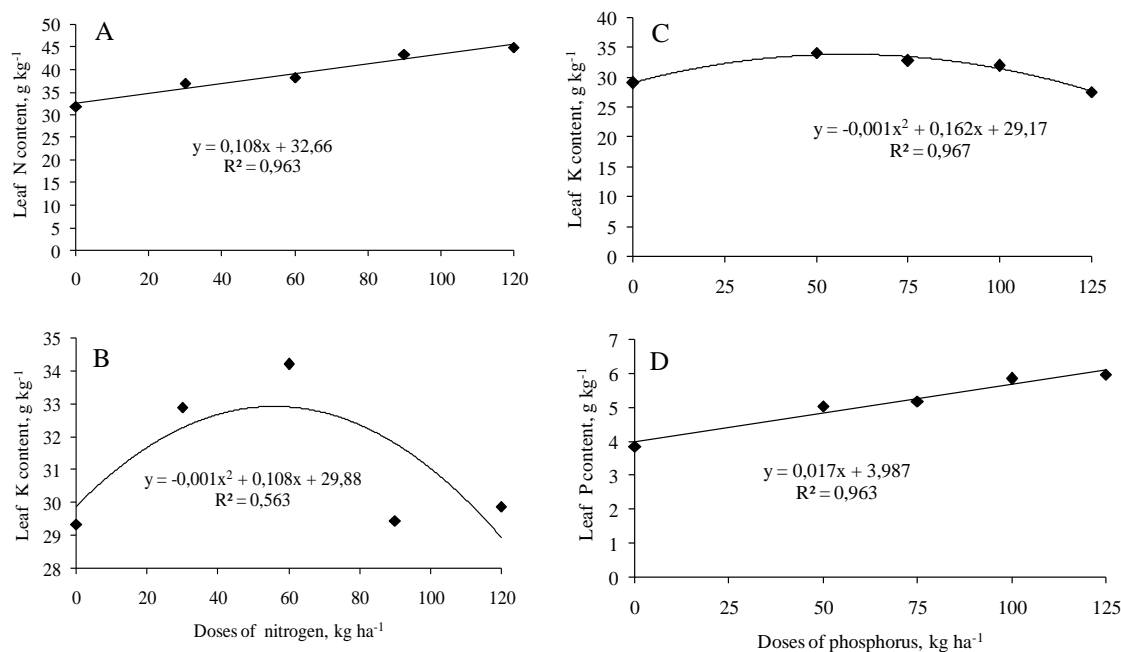


Fig 8. Nitrogen (A) and potassium (B) leaf as a function of doses of nitrogen and potassium (C) and phosphorus (D) leaf as a function of doses of phosphorus.

Leaf N and K content as a function of different doses of N are shown in Fig 8. The data were fitted to a linear and quadratic equation, respectively, which demonstrates the importance of N application at development of leaves. The same way, the contents of K and P as a function of different P doses were adjusted in a quadratic and linear equation, respectively (Fig 8).

## Materials and Methods

### Location of the experiment

A field experiment was carried out in Serranópolis do Iguaçú, Paraná State, Brazil (54° 02' W, 25° 24' S; 300 m asl) on a typical Haplorthox soil (dusky red latosol) with 730 g kg<sup>-1</sup> clay, 160 g kg<sup>-1</sup> silt and 110 g kg<sup>-1</sup> sand. The climate of the region

is subtropical humid mesothermal, in summer exceeding 22 °C and in winter below 18 °C. During the experimental period the data of rainfall ranged from 75 mm (May) to 160 mm (June), having no rainfall occurred in the month of August. In relation to temperature varied from 17 to 23 °C.

### Plant materials

The crambe (*Crambe abyssinica* Hochst. Former. RE Fries) cultivar FMS-Brilhante was used, which originates from the Foundation Mato Grosso do Sul, MT, Brazil (FMS).

### Soil analysis

Before sowing crambe, the chemical properties of the surface layer (0-0.20 m) were determined according to Embrapa

(2006). In 2012, the soil characteristics were as follows: organic matter (OM), 30.88 g dm<sup>-3</sup>; pH (H<sub>2</sub>O), 5.3; P, 18.8 mg dm<sup>-3</sup>; K, calcium, magnesium, and hydrogen + aluminum, 0.22; 5.87; 2.82 and 4.28 cmol<sub>c</sub> dm<sup>-3</sup>, respectively; and base saturation of 67.55 %; sulfate, copper, iron, manganese, and zinc, 6.67; 8.19; 32.0; 112.0 and 1.36 mg dm<sup>-3</sup>, respectively.

### Experimental design and treatments

The experiment was carried out in a randomized block design with a total of three blocks separated by two meters, in a 5 × 5 factorial experiment (five phosphorus levels and five nitrogen levels) totaling 75 experimental units. Each experimental plot consisted of 5 feet long and 4 feet wide (20 m<sup>2</sup>) with a spacing of one meter between plots.

The crambe cultivar FMS Brillhante was mechanically sown on 22/05/ 2012 at a spacing of 0.40 m between rows. The phosphorus (P) levels in kg ha<sup>-1</sup> were: 0; 50; 75; 100 and 125 added as simple super phosphate; the nitrogen (N) levels in kg ha<sup>-1</sup> were: 0; 30; 60; 90; 120 added as urea. Before sowing crambe, the soil was fertilized with 40 kg K<sub>2</sub>O ha<sup>-1</sup> (potassium chloride), with the P treatments and half dose of N treatments applied in the sowing furrow. The other half of the N was applied coverage for thirty days after emergence. Harvest was done manually from the 5 central rows.

### Collection of vegetal material

In the period of inflorescence, about 70 days after sowing, the third leaf from the apex to the base was collected approximately of 30 plants taken at random in each plot. These leaves were washed and dried in an oven with forced air circulation at 70 °C until constant weight. The dry matter was weighed (g plant<sup>-1</sup>) and triturated in mill type Willey to determine the analysis of vegetal tissues.

The shoot height of random 10 plants in each plot (2 × 3 m), harvested 107 days after sowing, was measured (cm plant<sup>-1</sup>) using a meter scale and in each plant the number of branches was counted. In the same time, the grains (siliques) were counted and harvested manually to determine the 1,000 grain weight and grain yield (productivity). The grain yield was calculated for a moisture content of 0.13 kg kg<sup>-1</sup>. Similarly, samples of grain from each plot were oven-dried at 70 °C for 24 h before grinding and analyzed.

### Determination of macronutrient in leaves and nitrogen, protein and oil content in grains

The leaves and the grain analyzes were conducted at the Laboratory of Irrigation and Salinity, in which the determinations of foliar levels of nitrogen, phosphorus and potassium and the concentrations of nitrogen, protein and oil in the grain were performed according to the methodology of Malavolta et al. (1997).

### Statistical analysis

The experimental data were analyzed by ANOVA using F-test. For the significant data, regression analysis was used with adjustment of the greatest determination coefficients ( $p \leq 0.05$ ). All analyses were performed using statistical software SISVAR (Ferreira, 2009).

### Conclusions

The application of nitrogen and phosphorus fertilization influenced the productive and nutritional characteristics of crambe plants. The nitrogen increased biomass production, grain yield and leaf nitrogen and potassium content. Phosphorus promoted an increase in the phosphorus and potassium accumulation in plant leaves. The interaction of nitrogen with phosphorus increased the plant height only. The grain lipid, nitrogen and protein content were not significantly affected as a function of phosphorus and nitrogen application in the soil. Apparently, the growth and production characteristics of crambe plants did not respond to phosphorus fertilization contrary to nitrogen.

### Acknowledgments

The authors thanks the Coordination for the Improvement of Higher Education Personnel (CAPES) for project financing and Mato-Grosso do Sul Foundation for providing crambe seeds.

### References

- Adamsen FJ, Coffelt TA (2005) Planting date effects on flowering, seed yield, and oil content of rape and crambe cultivars. *Ind Crops Prod.* 21:293-307.
- Castro C, Balla A, Castiglioni VBR, Sfredo GJ (1999) Levels and methods of nitrogen supply for sunflower. *Sci Ag.* 56:827-833.
- Chhacek Lj, Carcoana R, Jacobson K (1993) Phosphorus and zinc fertilization effects on dry matter yield and phosphorus and zinc uptake by crambe. *J Plant Nutr.* 16:1829-1836.
- Empresa Brasileira de Pesquisa Agropecuária - Embrapa (2006) Manual methods of soil analysis. Embrapa Solos, Rio de Janeiro.
- Falasca SL, Flores N, Lamas MC, Carballo SM, Anschau A (2010) *Crambe abyssinica*: An almost unknown crop with a promissory future to produce biodiesel in Argentina. *Inter J Hydr Ener.* 35:5808-5812.
- Ferreira DF (2009) Estatística básica. 2nd ed UFLA. Lavras.
- Harper JE (1994) Nitrogen metabolism. In: Boote KJ, Bennett JM, Sinclair TR et al. (ed) *Physiology and determination of crop yield.* ASA/CSSA/SSSA, Madison.
- Heinz R, Garbiate MV, Viegas Neto AL, Mota LHS, Correia AMP, Vitorino ACT (2011) Decomposition and nutrient of crambe and fodder radish residues. *Ci Rural* 41:1549-1555.
- Jasper PA, Biaggioni MAM, Silva PRA, Seki AS, Bueno CO (2010) Analysis of energy in the crambe (*Crambe abyssinica* Hochst) produced in no tillage. *Eng Ag.* 30:395-403.
- Knights EG (2002) Crambe: A North Dakota case study. A report for the rural industries research and development corporation. RIRDC Publication No. W02/005, Kingston.
- Malavolta E, Vitti GC, Oliveira AS (1997) Avaliação do estado nutricional de plantas: princípios e aplicações. Potafos. Piracicaba.
- Mifflin BJ, Lea PJ (1976) The pathway of nitrogen assimilation in plants. *Phytochemistry* 15:873-885.
- Oliveira RC, Aguiar CG, Viecegli CA, Primieri C, Barth EF, Bleil Junior HG, Sanderson K, Andrade MAA, Viana OH, Santos R, Parizotto RR (2013) Cultura do crambe. Cascavel, ASSOESTE. 70p.

- Pitol C, Broch DL, Roscoe R (2010) Tecnologia e produção: crambe. In: Broch DL, Roscoe R. Fertilidade do solo, adubação e nutrição do crambe. Fundação MS, Maracaju.
- Rogério F, Santos JI, Silva TRB, Migliavacca RA, Gouveia B, Barbosa MC (2012) Effect of phosphorus doses in the development of crambe culture. *Bio J.* 28:251-255.
- Silva TRB, Lavagnolli RF, Nolla A (2011) Zinc and phosphorus fertilization of crambe (*Crambe abyssinica* Hochst). *Food Agri Envi.* 9:264-267.
- Soratto RP, Souza-Schlick GG, Fernandes AM, Souza EFC (2013) Effect of fertilization at sowing on nutrition and yield of crambe in second season. *R Bras Ci Solo* 37:658-666.
- Souza ADV, Fávoro SP, Ítavo LCV, Roscoe R (2009) Chemical characterization of seeds and presscakes of physic nut, radish and crambe. *Pesq Agr Bras.* 44:1328-1335.
- Vechiatto CD, Fernandes FCS (2011) Effects of nitrogen on doses in crambe culture coverage. *Cult o Saber* 4:18-24.