

# Aluminum stress in *Crambe abyssinica* Hochst

## *Estrés por aluminio en Crambe abyssinica Hochst*

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### ABSTRACT

Crambe is a sensitive specie to exchangeable aluminum, it require a well-fertilized and corrected soil. The objective of this study was to understand the impact of aluminum presence, in different concentrations, in the substrate on the germination and development of crambe. The experiment was carried out as a randomized complete block design with a 5x2 factorial arrangement in four replicates. The five concentrations of Al<sup>3+</sup> were 0, 15, 40, 80 and 120 mg dm<sup>-3</sup> Al<sup>3+</sup> and two temperatures (20 and 30 °C), with four replications. The relative growth rate, plant height, leaf number, total leaf area, specific leaf area, dry matter in each plant compartment (root, stem and leaf), leaf area ratio, stem mass ratio and leaf mass ratio were evaluate. Germination was favored by 20 °C. The limitation on plant growth was greater as the concentration of aluminum increased, presenting losses in growth, as well of production of dry matter and leaf development, also causing changes in the patterns of biomass allocation between the plant compartments.

**Keywords:** crambe, toxicity, germination, growth.

### RESUMEN

*Crambe es una especie sensible al aluminio intercambiable que requiere suelos bien fertilizados y corregidos. El objetivo de este trabajo fue evaluar distintas concentraciones de aluminio en la germinación y desarrollo de crambe. El experimento para germinación se realizó con un diseño de bloques completos al azar con arreglo factorial de 5x2, con cuatro repeticiones. Las cinco concentraciones de Al<sup>3+</sup> fueron 0, 15, 40, 80 y 120 mg dm<sup>-3</sup> y las temperaturas de 20 y 30° C. El experimento para desarrollo de la planta se realizó con un diseño de bloques completos al azar con cinco concentraciones de Al<sup>3+</sup> y cuatro repeticiones. Los parámetros evaluados fueron porcentaje de germinación, crecimiento de las plántulas y biomasa. Se analizó la tasa relativa de crecimiento, altura de planta, número de hojas, área foliar total, área foliar específica, materia seca en cada compartimento de la planta (raíz, tallo y hojas), razón de área foliar, razón de masa de raíz, tallo y hojas. El porcentaje de germinación aumentó a los 2 ° C. La mayor concentración de aluminio afectó el crecimiento, producción de materia seca y desarrollo de hojas. Estos resultados sugieren que el crambe es sensible al aluminio intercambiable.*

**Palabras clave:** crambe, toxicidad, germinación, crecimiento.

### Introduction

The crambe (*Crambe abyssinica* Hochst) is a plant species of the family Brassicaceae, native to the Mediterranean region, well adapted to different climatic conditions in Brazil (Souza *et al.*, 2009). Specific knowledge about its cultivation in Brazil is still scarce, making it necessary to develop studies on different phytotechnical aspects, in particular studies that allow development of new improved

cultivars and adequacy of management practices for its cultivation in Brazil (Colodetti *et al.*, 2012).

Crambe is mainly cultivated to be a source of feedstock for biodiesel production. The average crop yield has reached 1,507 kg of seeds per hectare; its seeds have around 34% oil content, which have desirable characteristics to be used for many purposes (Jasper *et al.*, 2010). However, it is severely affected by the presence of exchangeable aluminum in the soil (Broch & Roscoe, 2010).

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Therefore, it requires soils with proper correction and fertilization to ensure the full development of its root system.

There are various negative effects caused by aluminum; the main effect is related to its absorption, which inhibits the growth of roots, blocking the mechanisms of absorption and transport of water and nutrients. Consequently, the plant develops a limited root system, less capable of exploring the soil, resulting in susceptibility to drought and lodging, and to absorb and utilize nutrients, preventing the plant from expressing its agronomic potential (Castro & Oliveira, 2005; Rossiello & Netto, 2006). These negative effects on the development and functionality of the root system are originated from disturbances caused by aluminum in the cell division of the root meristem, and consequently in the interruption of mitosis of those cells (Nichol & Oliveira, 1995).

Exchangeable aluminum sensitivity varies among species and may interfere with the development of the plants or the germination processes, causing reduction in the germination rate of seeds of some species (Custódio *et al.*, 2002). According to the information presented above, this study was conducted aiming to evaluate the influence of aluminum in different concentrations in the substrate on the germination and seedling growth of *C. abyssinica* Hochst.

## Material and Methods

### Acquisition of seeds

Seeds of *C. abyssinica* Hochst., cultivar FMS Brilhante, from the 2012 harvest, were selected to form a homogeneous sample. Selected seeds were stored in a cold room (3 °C) and kept at 10% humidity until the tests began.

### Germination of *C. abyssinica* Hochst in different concentrations of aluminum

The experiment was conducted in the laboratory of mineral nutrition (Laboratório de Nutrição Mineral de Plantas) of the Centro de Ciências Agrárias, Universidade Federal do Espírito Santo (CCA/UFES), located in Alegre-ES, during June, 2013. The effects of temperature and concentration of aluminum on germination of crambe were studied in a 5x2 factorial scheme. Five concentrations of

aluminum were selected based on preliminary tests: 0, 15, 40, 80 and 120 mg dm<sup>-3</sup>. Two temperatures were adopted, due to lack of specific standards for *C. abyssinica* Hochst, using temperatures commonly employed for other species of the family Brassicaceae (Brazil, 2009): 20 and 30 °C. The treatments were arranged in a completely randomized design, with four replications. The experimental plots consisted of dishes containing 25 seeds each.

The seeds were treated with a solution of sodium hypochlorite, washed with distilled water and transferred to Petri dishes (11 cm diameter). The dishes were prepared and covered with two sheets of filter paper (3 µ porosity, 80 g m<sup>-1</sup> specific weight), moistened with solutions of aluminum sulfate (Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>.16H<sub>2</sub>O) at different concentrations.

Each dish received 25 seeds and was transferred to a germination chamber (Biochemical Oxygen Demand) for incubation at 20 or 30 °C, according to the treatment. The seeds were kept for a period of seven days under fluorescent lamp (24-hour photoperiod under cool white light). After this period, the germination and growth of the seedlings were evaluated. Germination was determined from a direct count of the number of germinated seeds in relation to the total number of tested seeds, with results expressed as percentages. Seedling growth was determined by measuring (digital caliper, 0.01 mm precision) the length of radicle and hypocotyl of each germinated seedling.

The germinated seedlings were dried in a laboratory oven, with forced air circulation at 60 °C, until constant weight. After drying, the dry matter of the seedlings was determined in an electronic scale (0.001 g precision).

### Development of *C. abyssinica* Hochst in different concentrations of aluminum

The experiment was conducted in a greenhouse, installed in an experimental area of the Centro de Ciências Agrárias, Universidade Federal do Espírito Santo (CCA/UFES), located in Alegre-ES, during the months of July and August, 2013. Black plastic pots were filled with 8 kg of sand previously washed with deionized water, and received 10 seeds of crambe. After germination, emerged plants were thinned to form a uniform group, regarding size and vigor, keeping three plants per pot. The experiment followed a completely randomized design, with five treatments and four replications. The experimental

plot was studied based on the mean values of the three plants per pot.

The treatments consisted of applying modified nutrient solutions based on standard Hoagland solutions (Hoagland & Arnold, 1950), with addition of five levels of aluminum concentration, similar to the germination test (0, 15, 40, 80 and 120 mg dm<sup>-3</sup>). Nutrient solutions were prepared with deionized water using the following salts (Pro Analyze) as sources of nutrients: KNO<sub>3</sub>, MgSO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, K<sub>2</sub>SO<sub>4</sub>, CaSO<sub>4</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, CuSO<sub>4</sub>, H<sub>3</sub>BO<sub>3</sub>, MoO<sub>3</sub>, MnCl<sub>2</sub>, ZnSO<sub>4</sub>, FeSO<sub>4</sub>/EDTA and Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>.16H<sub>2</sub>O. The solutions were prepared and applied three times daily, to maintain the moisture in the substrate approximately constant. The solutions were applied uniformly across the surface, percolated through the sand and drained at the bottom of the pots.

Plants were collected 10 and 30 days after emergence and used to determine the evolution of dry matter and to calculate the relative growth rate (RGR) in each aluminum concentration. After 30 days of cultivation, the growth of the plants was evaluated. Plant height was measured with a graduated ruler (0.1 cm precision). Leaves were counted and collected to determine the total leaf area of each plant (Area meter LICOR LI-3100, 0.01 cm<sup>2</sup> precision). The specific leaf area (SLA) was obtained from the ratio between the surface and the dry weight of leaf discs of known area (cut with a hole puncher).

Roots were washed and separated from the substrate. Plants were collected and plant compartments were separated and placed in paper bags, which were dried to determine the dry matter accumulation in each (roots, stems and leaves), following the same drying methodology described in the germination test. The leaf area ratio (LAR) was calculated as the ratio between leaf area and

total dry matter. The leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) were calculated by the ratio between dry matter of leaves, stems and roots and the total dry matter, respectively.

### Statistical Analysis

The data were subjected to analysis of variance (p≤0.05) and, according to the significance of the factors, the Tukey test (p≤0.05) were used for the qualitative factors and regression analysis for the quantitative factors. Regression models were chosen based on the significance of the model and coefficients, using Student's t-test (p≤0.05); also considering the coefficient of determination (R<sup>2</sup>). Data analysis was performed using the statistical software "GENES" (Cruz, 2013).

### Results and Discussion

#### Germination of *C. abyssinica* Hochst in different concentrations of aluminum

Both the aluminum concentration and the temperature at which the seeds were subjected, as well as the interaction between those two factors, influenced the development of the seedlings. This fact is corroborated by the analysis of variance, which showed significance for the sources of variation and their interaction in relation to germination and lengths of radicle and hypocotyl. Dry matter did not show interaction, but was affected by the effect of temperature and aluminum concentration singly.

In Table 1, it is presented the mean comparison between the two temperatures (20 and 30 °C) for each variable (germination, radicle length and hypocotyl length), within each level of aluminum concentration.

Table 1. Means of germination, radicle length and hypocotyl lengths of seedlings of *Crambe abyssinica* Hochst exposed to increasing concentrations of aluminum in tests with 20 and 30 °C (after 7 days).

Aluminum concentration (mg dm <sup>-3</sup> )	Germination (%)		Radicle length (mm)		Hypocotyl length (mm)	
	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
0	85.33 A	81.33 A	57.41 A	49.12 B	24.84 B	33.27 A
15	92.00 A	72.00 B	45.34 A	15.08 B	23.29 B	28.05 A
40	86.67 A	65.33 B	36.56 A	12.70 B	24.99 A	26.84 A
80	86.67 A	45.33 B	16.93 A	6.33 B	18.55 A	15.18 B
120	92.00 A	45.33 B	10.75 A	3.45 B	19.41 A	9.24 B

Means followed by the same letter in the row do not differ significantly by the Tukey test at 5% probability.

Temperature affected germination, radicle length and hypocotyl length of seedlings of crambe in different magnitudes for each concentration of aluminum (Table 1). Overall, these results suggest that the harmful effect of higher concentrations of aluminum can be intensified by higher temperatures.

In the absence of aluminum, there was no difference in the percentage of germination of seeds when subjected to temperatures of 20 or 30 °C. However, with the addition of aluminum, the temperature of 20 °C led to significantly greater means for germination than 30 °C. It is assumed that the temperature of 30 °C promoted the deleterious effects of increasing levels of  $Al^{3+}$  on germination, which was not observed at 20 °C. The higher temperature may have promoted transpiration, and consequently increased the contact of the toxic element with the roots and its absorption.

There was a clear interference of temperature on radicle length. The means for this variable were higher at 20 °C, regardless of the concentration of  $Al^{3+}$  in the solution (Table 1). The effect of the lower temperature on the growth of the seedlings is in accordance with that observed by Martins *et al.* (2012).

A different behavior was found for the hypocotyl length of crambe seedlings. The development of shoots increased at the temperature of 30 °C for  $Al^{3+}$  concentrations between 0 and 15  $mg\ dm^{-3}$ . However, increasing aluminum concentration to 40  $mg\ dm^{-3}$ , both temperatures began promote equivalent development. Concentrations of aluminum in solution above 80  $mg\ dm^{-3}$  caused the behavior to be reversed (Table 1). It is possible that in the absence or low concentration of  $Al^{3+}$  in solution, the higher temperature favored the initial growth of the aerial part instead of the root. This same stimulus was not detected when the seedling was subject to stress caused by higher concentrations of toxic aluminum, which ends up limiting the development of the seedling as a whole at concentrations over 40  $mg\ dm^{-3}$ .

The dry matter accumulated by the seedlings did not show interaction between temperature and  $Al^{3+}$  concentration, but was influenced by each of the factors individually. The dry matter in the test at 20 °C was 23.68% greater than 30 °C, with means of 4.23 g and 3.42 g per plant, respectively, confirming better development of crambe seedlings at the lower temperature.

Regression analyses for each variable as a function of the aluminum concentration are presented

in Figure 1. Overall, it is noted that the increase in the aluminum concentration caused deleterious effects on the development of crambe seedlings associated with each temperature.

The germination in the test at 20 °C did not suffer interference from increasing levels of  $Al^{3+}$  in solution, while in the test at 30 °C it presented a linear decrease in germination percentage (Figure 1A). Although the temperature of 20 °C promoted germination, it did not limit the destructive effects of toxic aluminum in seedlings of crambe. Even the seedlings that sprouted still presented damage in early development, with impairment and slowing of radicle development and hypocotyl expansion. There is a trend in other oleaginous species with potential for biodiesel production that increasing concentrations of aluminum in solution up to 20  $mmol\ L^{-1}$  cause reduction in seedling emergence, harming not only the germination potential but also the emergence rate (Gordin *et al.*, 2013).

Radicle length showed an exponential decrease as a function of increasing concentrations of  $Al^{3+}$  in solution at 30 °C and a linear reduction up to 120  $mg\ dm^{-3}$  at 20 °C. The aggressiveness of the deleterious effects of aluminum on root development of crambe was more pronounced with the temperature of 30 °C, with a severe reduction in the length of radicles already at a concentration of 15  $mg\ dm^{-3}$  of  $Al^{3+}$  in solution.

Inhibition of the root growth is the most easily recognized symptom of toxicity of aluminum. This limitation causes the plant to explore a smaller volume of soil, generating great difficulty in absorbing water and nutrients in the quantity demanded, resulting in impairment of the formation of the other organs (mainly leaves) and causing drastic reduction in growth and productivity (Lima *et al.*, 2007; Macedo *et al.*, 2008; Marin *et al.*, 2004). Compared with physic nut, another oilseed species, Macedo *et al.* (2011) and Lana *et al.* (2009) reported that aluminum toxicity induced reduction of shoot and root lengths, and caused darkening of the meristematic tissue of the root tips.

The effect of aluminum concentrations on the length of hypocotyls in crambe seedlings showed a linear decrease in length in both temperatures (Figure 1C). However, this reduction was less severe at 20 °C. Possibly, the temperature of 20 °C enhanced germination, allowing the seedling to develop better and resist longer against the effects of the toxic element present in the solution.

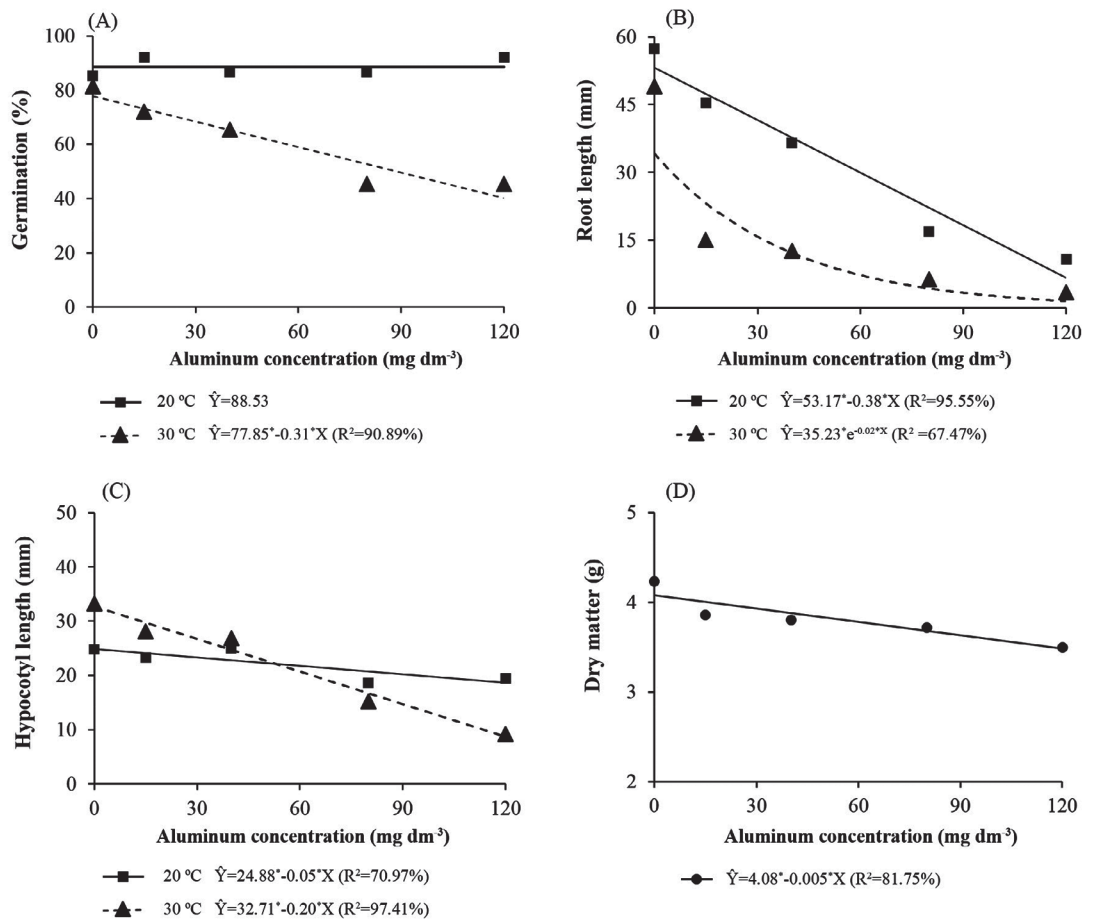


Figure 1. Regression analysis for germination (A), radicle length (B), hypocotyl length (C) and dry matter (D) of crambe seedlings subjected to increasing concentrations of aluminum, at temperatures of 20 and 30 °C (after 7 days).

As previously stated, there was no interaction between the effect of temperature and aluminum concentration on the accumulation of dry matter; however, aluminum concentration caused a significant effect. It was observed linear decrease of dry matter production of crambe seedlings was a function of increasing aluminum concentration, directly undermining the accumulation of biomass (Figure 1D). As result of the limited growth of the radicles, water absorption and nutrient uptake, transport and utilization are severely impaired. Thereby, several plant species decreased production of biomass when exposed to aluminum toxicity (Tabaldi *et al.*, 2007).

The influence of increasing aluminum concentration on the development of crambe seedlings in the tests at 20 and 30 °C can be

observed (Figure 2). Temperature may have acted as a conditioning agent to the severity of the effect of aluminum on crambe seedlings. The temperature of 30 °C promoted more deleterious effects of Al<sup>3+</sup> on these plants compared to 20 °C.

### Development of *C. abyssinica* Hochst in different concentrations of aluminum

During the period of the experiment, the mean temperature was 20.64 °C, ranging from 19.98 °C mean minimum to 21.37 °C mean maximum, being favorable to the initial development of crambe based on the results obtained in the previous experiment. The presence of aluminum significantly influenced the parameters of plant development, generating restrictions to growth and accumulation of biomass



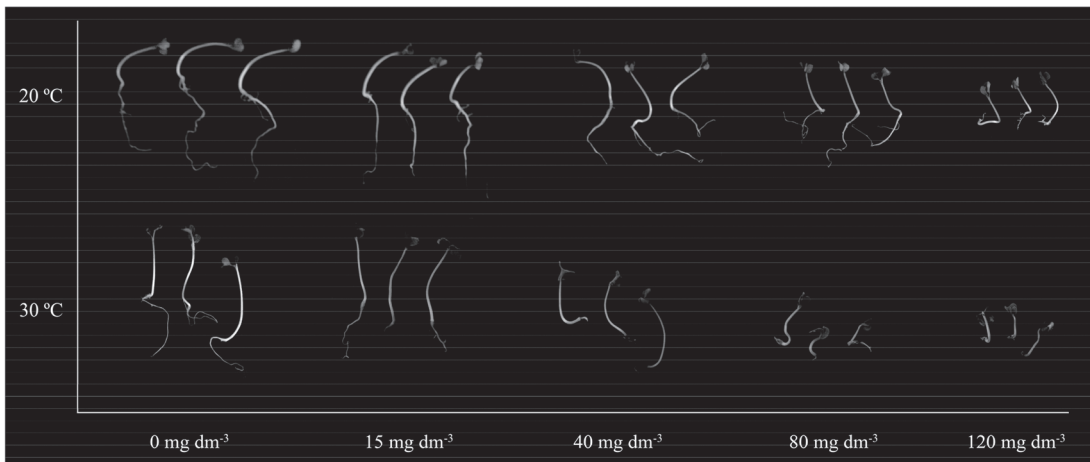


Figure 2. Seedlings of *Crambe abyssinica* Hochst subjected to increasing concentrations of aluminum at temperatures of 20 and 30 °C (after 7 days).

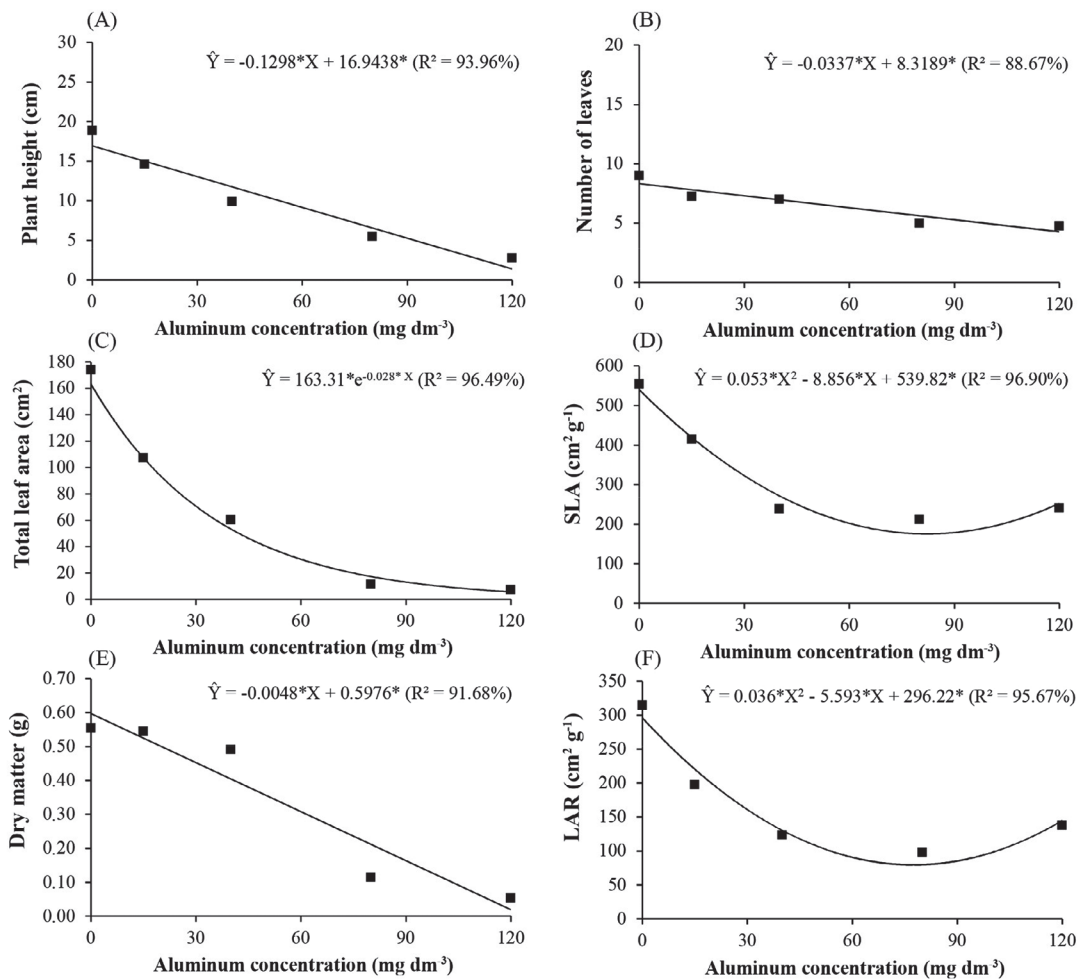


Figure 3. Regression analysis for plant height (A), number of leaves (B), total leaf area (C), specific leaf area (D), dry matter (E) and leaf area ratio (F) of plants of crambe subjected to increasing concentrations of aluminum (after 30 days).

as the concentration of this element increased in the substrate.

Plant height, number of leaves and total dry matter of crambe were greatly limited by the toxic effects of aluminum. The limitation of root growth prevented the development of the plant as a whole, causing a linear decrease in growth and leafiness of the plants as a function of the concentration of this toxic element in the substrate (Figure 3A, 3B and 3E).

While plants without the influence of aluminum had mean height of 19 cm and 0.56 g accumulated dry matter, the plants subjected to high aluminum concentration showed poor emergence and stagnated their growth under 3 cm height and 0.05 g accumulated biomass. Nutritional stress in crambe plants was studied by Colodetti *et al.* (2013), who described limitations in the accumulation of biomass by the plants when subjected to different kinds of nutritional stresses, highlighting the importance of adequate correction and fertilization of the soil for crambe crops.

Leaf area suffered drastic reduction in the presence of aluminum, even in the lowest concentration studied in the experiment, showing

exponential losses with the increase in concentration of  $\text{Al}^{3+}$  (Figure 3C). Similarly, specific leaf area began to show low values with the addition of aluminum, to a minimum in the concentration of  $83 \text{ mg dm}^{-3}$ , and increased again above that level. This fact can be explained by the development of thicker, irregularly shaped leaves in reaction to the effect of aluminum. At high concentrations, the formation of leaves is compromised and the few leaves that develop show extremely low values of dry matter, causing an increase in the values of specific leaf area (Figure 3D).

The leaf area ratio decreased with the increase of aluminum concentration in the substrate until the level of  $78 \text{ mg dm}^{-3}$ , showing a slight increase from that point (Figure 3F). Aluminum toxicity causes a discrepancy between the development of leaf area and production of dry matter in crambe plants, with only slightly higher values at higher aluminum concentrations due to severe impairment caused to the production of biomass, which becomes more severe than the impairment caused on leaf area.

Figure 4A illustrates the great damage caused by higher concentration of aluminum on the growth

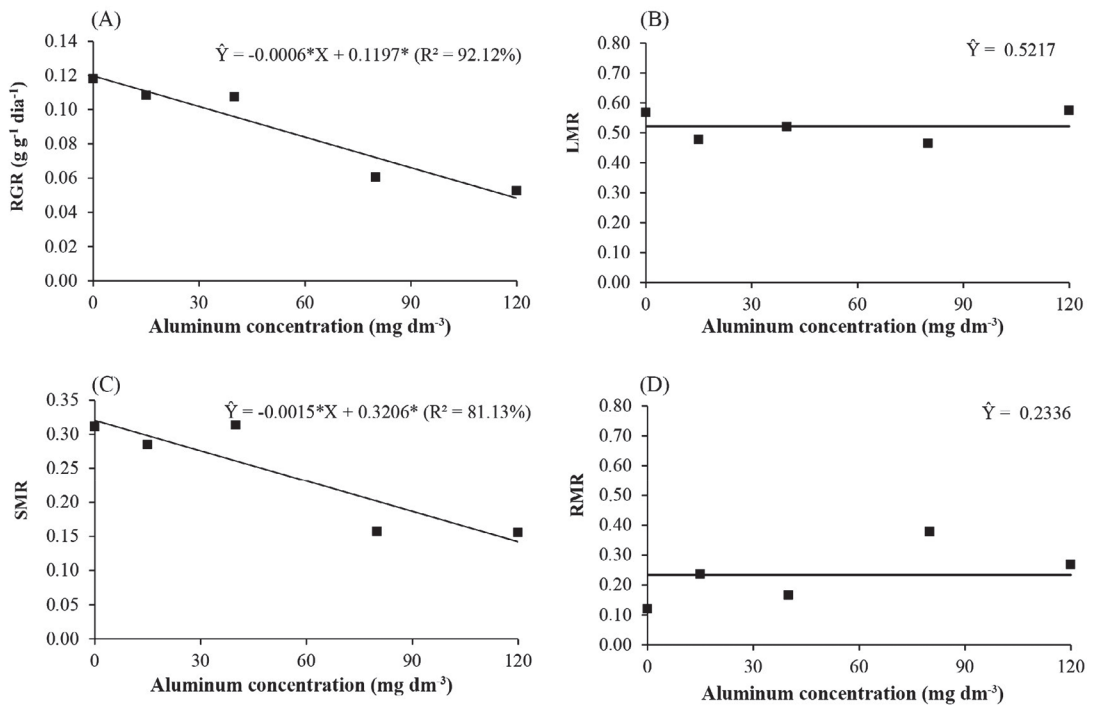


Figure 4. Regression analysis for relative growth rate (A), leaf mass ratio (B), stem mass ratio (C) and root mass ratio (D) of plants of crambe subjected to increasing concentrations of aluminum (after 30 days).

rate of crambe. The speed with which the plant is able to produce and accumulate biomass was linearly compromised by aluminum toxicity, a direct result of the loss of photosynthetic machinery efficiency of the plants, preventing normal growth and retarding the production of photosynthates.

The partition of biomass among plant compartments was only modulated by toxic aluminum in relation to dry matter accumulation in stems. However, plants allocated a smaller proportion of dry matter in support structures as the stress became more severe (Figure 4C). Although the total dry matter accumulation decreased with the addition of aluminum in the substrate, no change in the allocation pattern of dry matter to the leaves and roots was observed (Figure 4B and 4D).

## Conclusions

The initial development of *Crambe abyssinica* Hochst is greatly limited by the presence of aluminum in the substrate, with damage being caused to germination and growth of the plants.

Temperature can influence the intensity of deleterious effects of aluminum stress. Overall, with or without the presence of aluminum stress, the temperature of 20 °C provides better germination and initial development than 30 °C.

The limitation caused to plant development is more severe as the aluminum concentration in the substrate increases; we report loss in growth, biomass accumulation, leafiness and changes in the pattern of biomass allocation between the plant compartments.

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