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Agronomic Biofortification in Caupi Beans with Lithium

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Abstract

Biofortification of basic crops is ideal because of the broader consumption of staple foods by the majority of the population. Beans are one of the main constituents of many Brazilian dishes, for urban and rural populations. Li is essential to the mental and behavioral health of human beings. The objective of this work was to study the assimilation of Lithium (Li) by cowpea beans (*Vigna unguiculata*), with the application of lithium hydroxide (LiOH). The study was carried out in a greenhouse at the Federal University of Tocantins, Campus de Gurupi. The treatments were arranged in a completely randomized design in a 5 x 3 arrangement, with five doses of Li (0. 10. 20. 30 and 40 mg kg^{-1}); and three cultivars of Caupi beans (BRS Cauamé, BRS Itaim and BRS Nova Era) with four replicates. The application was performed via foliar, and the doses were divided into two applications of equal proportions, spaced in 15 days. Each experimental plot was represented by a vessel containing 10 kg of soil. Stem diameter, number of pods per plant, pod length, number of grains per pod, insertion of the first pod, plant height, mass of 100 grains, grain yield, lithium content, total nitrogen, crude protein and lipid content. The agronomic biofortification with Li occurred positively with the application of up to 26 kg Li ha^{-1} for the three cultivars, with up to 159.38% increase over the treatment without Li application. The results indicate that it is possible to increase the Li doses in Caupi with the use of lithium hydroxide (LiOH) in foliar fertilization.

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Keywords: mineral; Li concentration; fertilization; diet; biofortification.

1. Introduction

Caupi beans [*Vigna unguiculata* (*L.*) Walp] is one of the most important crops in the North and Northeast regions, its cultivation in Brazil reaches approximately one million hectares, with these two regions accounting for about 90% of the area total cultivated, in addition to being one of the main sources of protein for families in these regions [1].

Lithium is a chemical element, its symbol is Li, its atomic number is 3, its atomic mass is 7 and its structure contains three protons, and three electrons is an essential mineral for the mental and behavioral health of human beings, however, malnutrition in people caused by the lower availability of lithium (Li) it has not received due attention [2]. It is found in very small amounts in soil and water (around 0.004%). The largest natural deposits of Li are found in the Li triangle of America, in the brines of Bolivia (Solar de Uyuni), Chile (Solar de Atacama) and Argentina (Solar Del hombre muerto) [3]. Afghanistan has reserves of this mineral that may be the largest in the world [4]. The largest Brazilian reserves are in the states of Minas Gerais and Ceará [5].

Since 1949, Lithium has been used as an effective medicine in the treatment of Bipolar Disorder. Research shows that around the world, approximately 32 million (1.1% of the population) people over eighteen years of age, live daily with bipolar disorder, with emphasis on China, Brazil, India, USA, Australia, Canada and Great Britain [6]. However, a mitigating measure to control the manifestation of behavioral diseases and the increase in the percentage of suicide among young people in Europe has been the use of the “Lithium diet”, through water enriched with Li and food supplements (yeasts) [7].

The importance of Lithium for health has motivated the study of this mineral, not only by the pharmaceutical industry but also in vegetables that are part of the diet of humans and animals. There are few studies on the absorption and assimilation of Li by vegetables, due to some limitations. Li is transported to the plant cell via passive diffusion and its transport occurs actively using a Na^+/K^+ pump (ATPase), replacing the sodium ions (Na^+), although this transport is around twice as slow for Li from than Na+ [8].

In an attempt to revert to the lack of nutrients found in agricultural soils in Brazil, researchers are betting on agronomic food biofortification [9]. The purpose is that they are absorbed and accumulated in crops, increasing the content of these nutrients. Biofortification is characterized by an increase in the content of nutrients in food, through conventional genetic improvement or genetic engineering [10]. The choice of widely consumed culture is fundamental to the success of biofortification programs since the level of Li in a population is linked to the content of Li in these foods, with Caupi being one of the cultures capable of absorbing and assimilating lithium, even in small quantities.

Brazil is represented in the cultivation of beans by the 26 (twenty-six) units of the federation and the Federal District, which demonstrates the economic, cultural and food importance of the product. From the north to the south, some type of beans is available on the Brazilian table that satisfies the hunger of the population regardless of family income. Therefore, it is possible to observe the importance of agronomic biofortification of this grain with trace elements, such as Li.

Thus, the biofortification of Caupi beans (*Vigna unguiculata* (*L*) can play a vital role in providing food diversity, food and nutritional security and generating employment and income for Brazilians, demonstrating the agronomic biofortification strategy to increase the intake of Caupi beans.

Therefore, the aim of this study was to evaluate the accumulation and development of Caupi plants submitted to foliar application of different doses of lithium.

2. Material and Methods

2.1 Conditions Experimental

The work was conducted using plastic pots in a protected environment from February to May 2018 in the experimental area of the Federal University of Tocantins (UFT), Gurupi University Campus, located in the southern region of the State of Tocantins, Brazil, at the location of 11°43'45” latitude, 49°04'07” longitude and 280 m altitude. The climatic classification according to reference [11] is of the humid B1wA'a 'type with moderate water deficiency. The average annual temperature is 29.5°C, with an average annual rainfall of 1430 mm, with rain concentrations in summer and dry winter.

The soil used for growing the plants came from the 0-20 cm deep layer of a medium textured dystrophic Red-yellow Latosol [11]. The mixture was in the proportion 3: 1, being 3 of soil and 1 of sand, then placed in plastic pots with 8 dm^3 of volume.

Physical and chemical analyzes indicated that this soil had 72. 5 dag kg^{-1} of sand, 1. 5 dag kg^{-1} of silt, 22. 5 dag kg^{-1} of clay, pH in $CaCl_2$ of 4, 7, 1. 7 dag kg^{-1} of MO, P (Mehlich 1) 6.3mg dm^{-3}, 77.0 mg dm^{-3} of K, 1. 6 cmolc dm^{-3} of Ca, 0. 7 cmolc dm^{-3} of Mg, 0.0 cmolc dm^{-3} AL, 2.2 cmolc dm^{-3} H + Al, 29 g kg^{-1} of Fe and 53 % base saturation.

Based on the chemical analysis of the soil, liming was not necessary, however, 0.54 t ha^{-1} of agricultural gypsum was applied (26% CaO and 26% SO_4). After 15 days, basic sowing fertilization was carried out with 30 kg ha^{-1} of N, 30 kg ha^{-1} of P_20_5 and 20 kg ha^{-1} of K, using the sources: urea, monoammonium phosphate (MAP) and potassium chloride - KCl. Basic fertilization with micronutrients (Mo 1%, B 1.8%, Cu 0.8%, Mn 2%, Zn 7%) was supplied in the form of FTE BR12 (Fritted Trace Elements), with 42 kg ha^{-1}. In addition to the sowing fertilization, covering fertilization, 40 kg ha^{-1} of N, urea was used as source.

Each experimental unit consisted of a pot with two plants spaced 1 m between blocks and 0.35 m inside the blocks. Six Caupi seeds were sown per pot. At 15 days after plant emergence, thinning was carried out, leaving two seedlings per pot. At 20 days after sowing, the invaders were manually cleaned and nitrogen cover was fertilized. During the conduction of the experiment, the soil was kept moist through drip irrigation, using drip tape with variable irrigation shift, according to the development and necessity of the crop.

The application of doses of Li hydroxide (LiOH) was carried out via leaf in the morning (between 8:00 and 9:00 am) at 30 days after sowing, at the beginning of flowering and at 55 days after sowing, when the plants were in the stage of filling the grains and ripening the first pod, using standard LiOH solution (0.25 mg mL^{-1}). For example, 3.58 ml of the solution was used per pot with two plants for the treatment of 10 g ha^{-1} of Li. These doses of the standard solution were made up to 16.6 mL of water and applied uniformly to plants in each experimental unit. Doses were converted to hectare considering the population of plants generally employed for the culture beans Caupi (166. 66, 00 plants per hectare), adjusting doses per pot to the hectare. This application was carried out when the plants were in this stage due to the period of greatest metabolism of plant tissue that the accumulation of Li (grains) was desired.

For the application of lithium, a manual compression sprayer (Tramontina) was used with a maximum pressure of 2.5 bar (36.26 psi), a flow rate of 300 mL per minute, and an adjustable copper tip. The choice of this form of application was due to the effectiveness, safety and low cost of foliar spray with syrups promoting the biofortification of vegetables [12;13].

2.2 Treatments and Experimental Design

The treatments were arranged in a completely randomized design in a 5 x 3 factorial arrangement, with four replications. The first factor consisting of five doses of Li (0.0. 10. 20. 30 and 40 mg kg^{-1}), and the second consisting of three cultivars of Caupi beans (BRS Cauamé, BRS Itaim and BRS Nova Era) developed by Brazilian Agricultural Research Corporation [14].

The three bean cultivars Caupi were selected to obtain information on the general response of different genotypes in relation to the application of Li, for presenting good agronomic characteristics such as: precocity, uniformity, size that allows mechanized cultivation, and for being among the bean cultivars Caupi most cultivated in Brazil [15].

2.3 Characteristics Evaluated at 75 days after sowing

1. Height of Plant (AP, cm): made with a ruler graduated in millimeter, measuring the distance between the neck and the apex of the plant.

2. Height of insertion of the first pod (IPV, cm): it was determined with the aid of a ruler, corresponding to the distance between the base of the plant and the apex of the first pod, with maturation point (stage R9).

3. Length of pod (CV, cm): performed with a ruler graduated in millimeter, measuring the distance of the total length of the pod.

4. Plant diameter (mm): the stem diameter was determined using a digital caliper with the reading being taken 2 cm above the soil surface, with the reading being made after 55 days after emergence.

5. Mass of 100 grains (g), grain yield (g vase^{-1}): to quantify the mass of 100 grains (g) and grain yield (g vase^{-1}), the pods were threshed and the grains were placed in paper bags measuring 10 x 5 x 16 cm (length x width x height). The paper bags were placed in an oven with forced air circulation (Solab, model SL-102) at 70ºC. The grains remained in the greenhouse until they reached constant weight. The grain mass was evaluated on a precision scale of 0.01 g (Gehaka BK4000).

6. Number of Pods per plant (NVP): Number of pods in a sample of 10 random plants within the row.

7. Number of grains per pod (NGV): Obtained from the pods of 10 plants removed at random from the line.

2.4 Characteristics Evaluated at 75 days after sowing

Li concentration in the grains (mg kg^{-1}): The determination of the total Li content in the grains was carried out after the grains were ground in a Willey knife mill with a 1.27 mm sieve, then the material was subjected to nitro-perchloric digestion. The reading was performed in a Quimis flame photometer, for this the digest was diluted in the proportion of one part to five of deionized water (5 + 25 mL) and transferred to a 50 ml pool before making the reading.

For the calibration of the instrument, standards prepared by diluting Li standard solution (Li 1000 ppm, Specsol brand) were used. Initially, a calibration curve was estimated by diluting this standard.

2.5 Characteristics Evaluated at 75 days after sowing

One of the most versatile and effective extraction procedures that overcomes the difficulties faced with other methods is the methodology of [16], a simplified version of the classic procedure using chloroform-methanol proposed by [17].

One of the advantages of the method developed by [16] is the formation of a two-phase system based on the proportions of solvents added during the extraction process. The formation of this two-phase system is based on the theory of liquid-liquid balance of three components (chloroform/methanol/water). The determination of the solubilities of each component can be evaluated using a ternary diagram of the solubility of two liquids partially miscible with each other (chloroform and water) with a third (methanol), completely miscible in the other two.

For the extraction of lipids according to the method of [16], 2.5 g of each sample of dried and ground Caupi beans were weighed. In a 125 ml conical flask, 20 ml of methanol, 10 ml of chloroform and 0.8 ml of water were added. Stir for 30 minutes on the Nova Técnica shaking table (model NT 145) at 120 rppm, after complete homogenization, a single-phase was obtained and an additional 10 ml of chloroform and 10 ml of anhydrous sodium sulfate were added 1.5%, followed by stirring for 2 min. Remove the conical flask from the shaker, let it rest for 30 min until phase separation occurs (decantation-precipitation). Remove 15 ml of the precipitate and filter with filter paper in a separating funnel, measure 5 ml of the filtrate, pour into a previously tared beaker (50 ml), place to dry in an oven at 105ºc for 30 minutes, remove the beakers from the oven by placing in desiccator to cool, then weigh on an analytical balance.

P1 = weight of b ether after stove - weight of b ether empty

P2 = sample weight

p = weight of lipids (g) contained in 5 ml

g = sample weight (g)

% total lipids = (p x 4 x 100)/g

2.6 Determination of Nitrogen and Crude Protein in Grains

2.6.1 Nitrogen concentration in grains:

The determination of total N in plant tissue is a way to assess the nutritional status of the crop [18]. This process can also be carried out on grains, mainly on legumes such as beans, because from the amount of nitrogen, the amount of crude protein present in the sample can be determined. According to [19], the methodology described by [20] in 1883, is still widely used because it is reliable and has practically no changes over the years. [21, 22] describe that through this technique it is possible to indirectly determine the percentage of nitrogen and crude protein in samples.

To determine the total N of plant tissue and crude protein of Caupi beans in this experiment, [20] the micro-method was adopted [18]. The method consisted of heating a mixture with a small sample of ground beans 0.5 g, dissolved in a concentrated solution of sulfuric acid (H_2SO_4), in the presence of copper sulfate that acts as a catalyst accelerating the process. The mixture was heated to 370ºC, then the nitrogen present was determined by distillation followed by titration in the presence of sulfuric acid or hydrochloric acid.

2.6.2 Protein concentration in grains:

From the percentage of nitrogen present in the sample, it was possible to determine the crude protein according to the equation:

$$\mathbf{PB = \%\ N\ X\ 6.25}$$

Where: 6.25 correspond to the constant related to the protein content present in the vegetables, considering that the vegetable has 16% protein.

2.7 Statistical Analysis

The data obtained were subjected to analysis using the Tukey test, adopting 1 and 5% probability. Then they were submitted to regression analysis, evaluating determination coefficients using the *Sisvar* version 5.6 program [23]. The regression graphs were plotted using the statistical program *SigmaPlot* version 10®.

3. Results and Discussion

The summary of the analysis of variance (Table 1) shows that there was a significant effect at ($p \leq 0.05$) for cultivars in all characteristics, showing genetic variability between them. Regarding the doses, without showing a significant difference ($p \leq 0.05$) for diameter, number of pods and insertion of the first pod. For the interaction of cultivar versus doses, significant effects were observed for the characteristics of pod length, first pod insertion, plant height, mass of one hundred grains, grain yield, lithium content in the grain, total nitrogen in the grain, crude protein in the grain and content of lipids in the grain, being, therefore, the splitting of one factor inside the other.

Table 1: Summary of the analysis of variance regarding the evaluations: stem diameter (DIAM, cm), number of pods per plant (NV), plant height (ALT, cm), pod length (CV, cm), insertion of first pod (IPV, cm), number of grains per pod (NGV), mass of 100 grains (M100, kg ha^{-1}), grain yield (RED, g), lithium content in the grain (mg kg^{-1}) Total nitrogen (kg ha^{-1}), crude protein (%) and lipid content (%) in cultivars bean Caupi, Cauamé BRS BRS and BRS Vila Nova was a function of foliar fertilization of lithium hydroxide (0.10. 20. 30 and 40 mg ha^{-1} of Li) in a greenhouse, Gurupi-TO, 2020.

SOURCE OF VARIATION							
		Medium square					
FV	GL	DIAM	NV	ALT	CV	BTI	NGV
Block	3	0.35 ns	1.26 ns	4.94 ns	4.57 ns	0.11 ns	1.038 ns
Grow Crops	2	2.49 **	3.51 **	203.12 **	17.13 **	33.16 **	51.31 **
Dose	4	0.1 ns	1.37 ns	8.72 *	5.16 **	2.38 ns	1.516 **
C x D	8	0.29 ns	0.97 ns	14.53 **	10.74 **	10.20 **	0.316 ns
Residue	42	0.2	0.81	2.51	1.17	1.57	0.53
CV (%)		7.37	17.47	9.76	11.1	9.39	5.86
Average		6.08	5.16	16.23	9.78	13.34	12.51

		Medium square					
FV	GL	M100	RED	LI	NIT	PROT	LIP
Block	3	4.68 ns	2.53 ns	0.002 ns	0.229 ns	8.96 ns	0.0002 ns
Grow Crops	2	269.54 **	2187.46 **	0.005 *	1.64 **	67.33 **	1.7546 **
Dose	4	36.14 **	110.81 **	0.01 **	1.51 **	60.39 **	0.4505 **
C x D	8	22.69 **	94.34 **	0.005 **	0.69 **	27.17 **	0.2727 **
Residue	42	2.87	5.24	0.001	0.13	5.17	0.001
CV (%)		9.0	6.27	9.61	8.26	8.17	1.13
Average		18.85	36.53	0.441	4.45	27.84	2.88

FV: variation factor; ** significance at the level of 1 % probability ($p \leq 0.01$); * significant at the level of 5% probability ($0.01 \leq p \leq 0.05$); ns not significant ($p \leq 0.05$) by the F test.

The highest averages for the stem diameter are observed for the cultivars BRS Cauamé and BRS Itaim, probably due to the genetic characteristics of each cultivar which, according to Embrapa [14], are larger and, consequently, larger in diameter than the others (Table 2). Reference [22], applying Li by foliar fertilization in doses ranging from 28 to 45 g Li ha^{-1} had a stimulating effect on agronomic characteristics.

Table 2: Averages diameter stem and m three-bean cultivars Caupi (BRS Cauamé BRS BRS Vila and Nova Era), grown in five doses of lithium (0. 10. 20. 30 and 40 mg ha^{-1} Li) Gurupi-TO, 2020.

	Li doses (mg ha^{-1})					
GROW CROPS	0	10	20	30	40	AVERAGE
BRS CAUAM IS	5.95	6.18	5.98	6.25	6.18	6.66 a
BRS ITAIM	5.90	6.55	6.73	6.35	6.58	6.42 a
BRS NOVA ERA	5.98	5.57	5.45	5.87	5.75	5.71 b
GENERAL AVERAGE	5.94	6.1	6.05	6.15	6.17	-

Different letter averages in the column differ by the Tukey test at the 5% probability level.

Regarding cultivars, a higher average is observed for BRS Nova Era, probably due to the genetic characteristic of the cultivar, which according to [11] has undetermined growth (Table 3).

For the characteristic number of pods to be cultivated, BRS Nova Era presented the highest average. The cultivar Itaim showed the best performance at the dose of 20 mg ha^{-1} lithium with 6.25 units. As for the cultivar BRS Cauamé up to the dose of 30 mg ha^{-1}, there was an increase in the number of pods, however, compared to zero dosage there was no explanatory difference.

Table 3: Average number of pods in three cultivars of Caupi beans, BRS Cauamé, BRS Itaim and BRS Nova Era, cultivated in five doses of lithium (0. 10. 20. 30 and 40 mg ha^{-1} of Li) Gurupi-TO, 2020.

	Li doses (mg ha^{-1})					
GROW CROPS	0	10	20	30	40	AVERAGE
BRS CAUAM IS	5.50	4.65	4.75	5.00	4.75	4.95 b
BRS ITAIM	4.25	5.25	6.25	5.50	4.25	4.90 b
BRS NOVA ERA	6.00	6. 25	5.00	6.00	5.00	5.65 a
GENERAL AVERAGE	5.25	5.38	5.33	5.50	4.67	-

Different letter averages in the column differ by the Tukey test at the 5% probability level.

As for plant height (Figure 1 A), it was found that the quadratic model was the one that best fit for all cultivars. For the cultivars BRS Cauamé and BRS Itaim the doses that promoted the highest heights were 20.87 and 15 kg ha^{-1}, with heights of 19.99 and 22.27 cm respectively, with a gain of 28.80% and 18.77% in relation to plants that did not receive doses of lithium. This better performance is probably attributable to the genetic characteristics of the cultivars, the cultivar BRS Cauamé obtained greater gain, with 28.80% in relation to the control and has an undetermined growth. It is also observed that BRS Itaim always presented higher averages of height, regardless of Li doses, however, it has determined growth. The doses of Li had a negative influence on the height of the cultivar BRS Nova Era, with a loss of 31.51% in the dose of 24.76 grams in relation to the control. BRS Itaim and BRS Cauamé They are larger than Cultivar BRS Nova Era. [14].

For the characteristic pod length to be cultivated, BRS Itaim showed maximum response at a dose of 19.65 mg ha^{-1} of 11.95cm lithium, about 29.60% greater than the treatment without Li application (Figure 1 B). The genetic characteristics of this cultivar may have favored, as it has determined growth and consequently, in addition to a longer pod length, it obtained a greater number of grains per pod. For the cultivars BRS Cauamé and BRS Nova Era there was a loss of 13.13% and 34.8% respectively at doses 40 and 22.23 mg ha^{-1} of lithium, with a shorter pod length of 11.32 cm and 6.52 cm compared to treatment without lithium plication. The cultivar

BRS Itaim showed higher averages in the presence of Li up to the dose of 30g ha^{-1}, probably because it is a more rustic cultivar and of determining growth.

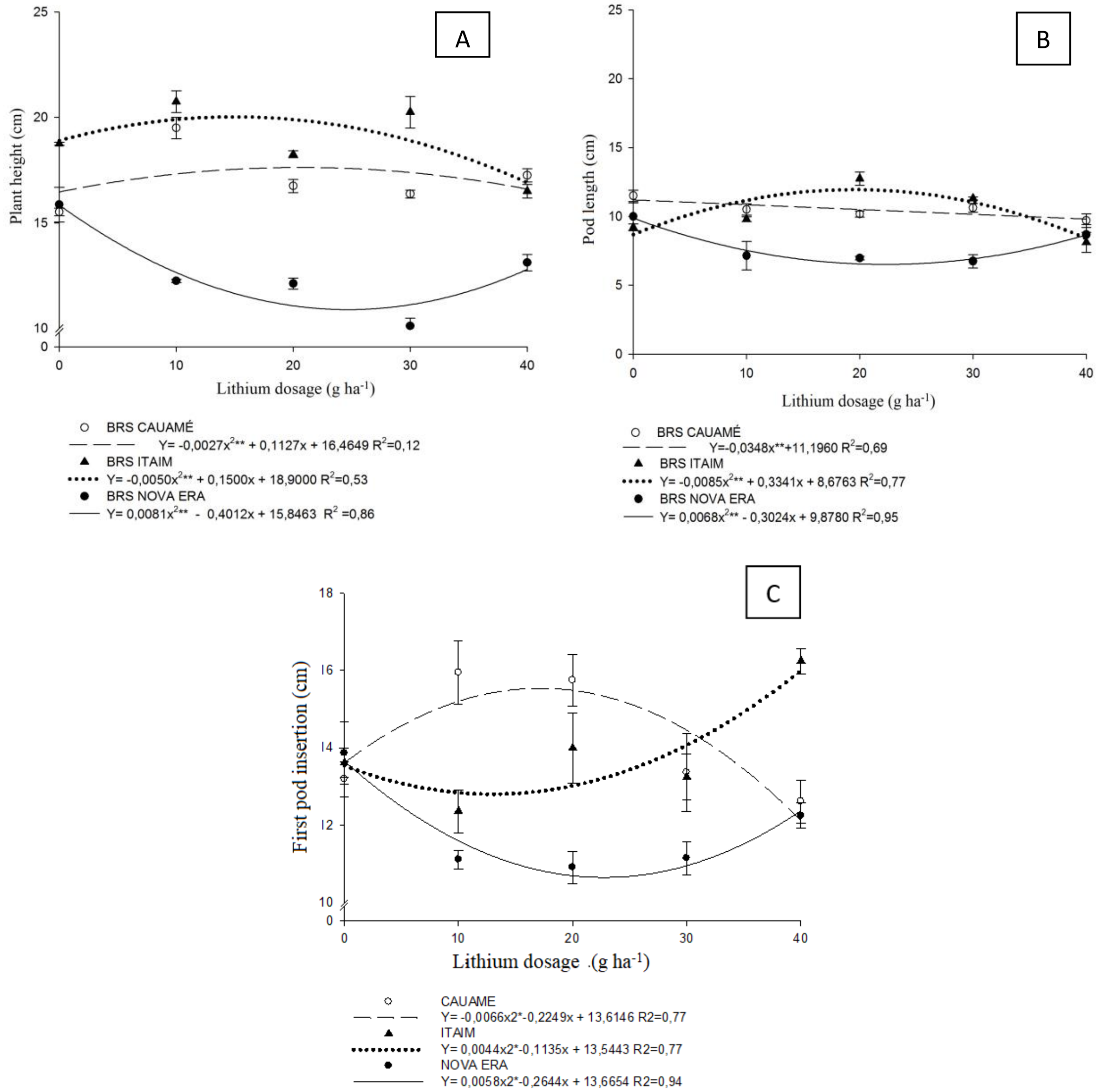


Figure 1: Response pattern found for studies involving the application of Li in Caupi beans (BRS Cauamé, BRS Itaim and BRS Nova Era) and its effect on plant height (A), pod length (B), first pod insertion (C) depending on the leaf application of lithium hydroxide (0. 10. 20. 30 and 40 g Li ha^{-1}). The bars indicate standard deviation from the mean, Gurupi-TO, 2020.

The insertion of the first pod of the cultivar BRS Cauamé was stimulated up to the dose 17.03 mg ha^{-1} of Li, with insertion of 11.69 cm, about 11.44% higher than the treatment without application of Li (Figure 1 C). For the cultivars BRS Itaim and BRS Nova Era there was a loss of 23.22% and 5.95%, respectively, in doses 12.89 and 22.79, with insertion of the first pod of 12.81 cm and 10.65 cm in relation to treatment without application of lithium. The cultivar BRS Cauamé showed greater insertion in the presence of Li up to the dose of 30 g ha^{-1}, this cultivar has undetermined growth, in addition to greater insertion it obtained a larger diameter and consequently better grain yield.

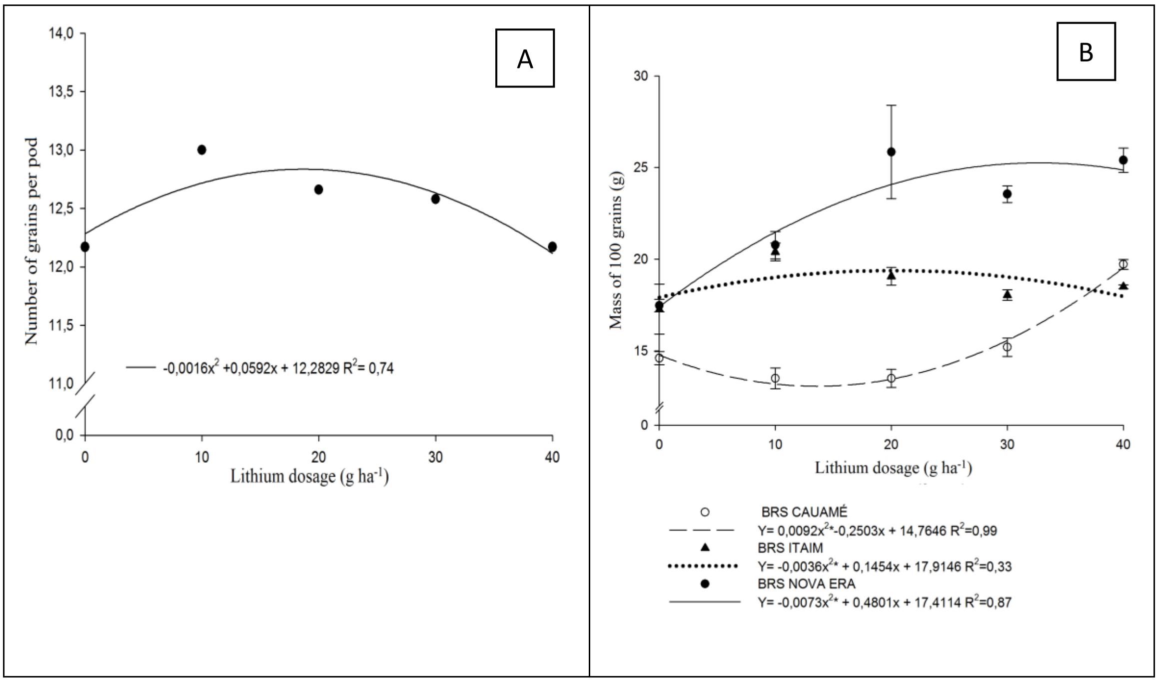
Regarding the number of grains per pod, the highest averages were for cultivars BRS Itaim (14.30) and BRS Cauamé (12.05), probably due to the genetic characteristics of each cultivar, which according to [11] the BRS Itaim and BRS Cauamé cultivars are larger than Cultivar BRS Nova Era (Table 4).

Table 4. An average number of grains per pod of three Caupi bean cultivars, BRS Cauamé, BRS Itaim and BRS Nova Era, grown in five doses of lithium (0; 10; 20; 30 and 40 mg ha^{-1} and Li) Gurupi-TO, 2020.

	Li doses (mg ha^{-1})					
GROW CROPS	0	10	20	30	40	AVERAGE
CAUAME	11.50	12.50	11.50	12.50	12.25	12.05 b
ITAIM	14.25	15.00	14.00	14.00	14.25	14.30 a
NOVA ERA	10.75	11.50	11.00	11.50	11.25	11.20 b
GENERAL AVERAGE	12.17	13.00	12.17	12.67	12.58	-

Different letter averages in the column differ by the Tukey test at the 5% probability level.

It is observed for number of grains per pod, the three cultivars had positive quadratic responses with the increase of Li doses, the range of doses of greater efficiency was between 10 and 30 g Li ha^{-1}. The differences between cultivars are attributed to genetic issues that according to [14] the number of grains per pod can vary from 9 to 12 among these three cultivars. Regarding the mass of 100 grains, it can be observed that the cultivar BRS Nova Era and BRS Itaim had 41.09 and 22.32 grams, in the maximum efficiency doses of 32.88 lithium and 20.19 g ha^{-1} with gain of 135.2% and 29.24%, respectively in relation to dose 0. Cultivar BRS Cauamé had 10.55% loss in dose 13.2 g ha^{-1} with a mass of 100 grains of 13.06 grams, however, when submitted to the highest dose 40 (g ha^{-1}), there was an increase of 66.22% for the cultivar BRS Cauamé (Figure 2 B). [24] stated that the concentration in solution ranging from 1 to 64 mg dm^{-3} Li had a stimulating effect on the biomass of corn (*Zea mays*) grown in nutrient solution.



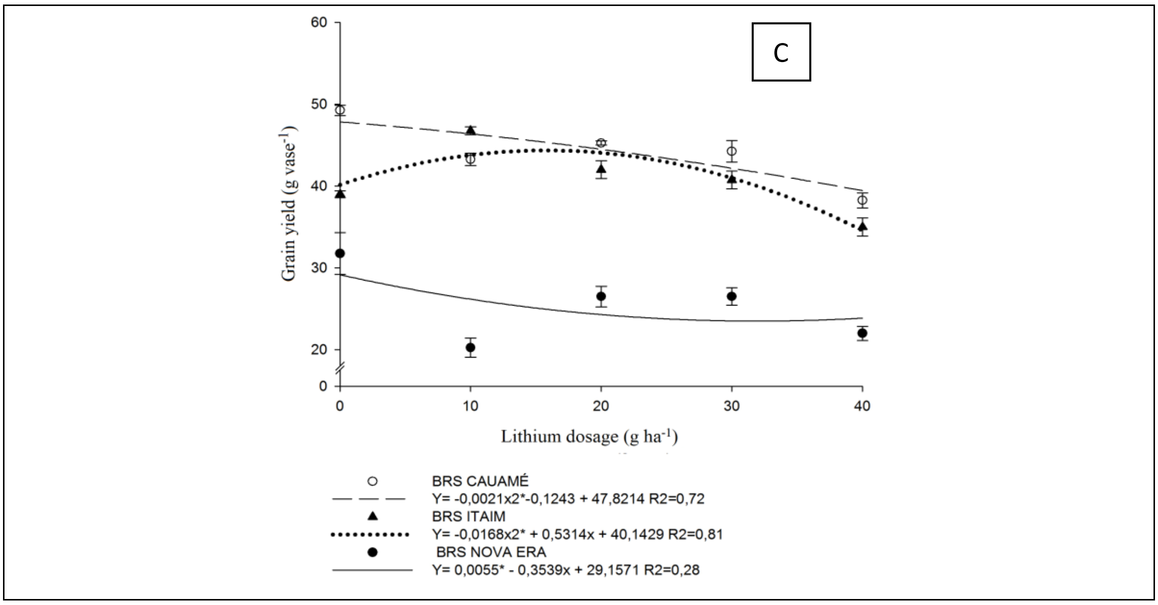


Figure 2: Response pattern found in studies involving the application of Li in Caupi culture and its effect on the number of grains per pod (A), mass of 100 grains (B), grain yield (C) as a function of the foliar application of lithium hydroxide (0. 10. 20. 30 and 40 g Li ha^{-1}). The bars indicate the standard deviation of the mean, Gurupi-TO, 2020.

Regarding the grain yield was obtained responses between different cultivars, while for the BRS and BRS Cauamé Nova Era was reduced 6.64% and 26.11% at maximum doses 29.59 and 32.17 mg ha^{-1} to BRS Vila had a yield of 42.04 g/vase to the dose of 15.81 mg Li ha^{-1} with an increase of 7.79% compared to control, with subsequent reduction 93.36% in relation to the highest dose 40 mg ha^{-1} (Figure 2 C).

For lithium content, the cultivars BRS Itaim and BRS Cauamé showed maximum response in the dose of 24 and 26 mg ha^{-1} of lithium, presenting lithium content in the grains of 0.83 and 0.61 mg ha^{-1}, providing an increase of 159.38% and 56.41%, respectively in relation to the treatment without application of Li. As for the cultivar BRS Nova Era, the maximum lithium content in the grains was 0.43 kg ha^{-1} in the dose 0.0005 kg ha^{-1} com gain of 0.01%. The increase in the levels of Li in the grains occurred up to 26 mg kg^{-1} of Li and with this dose, the three cultivars of Caupi beans obtained up to 159.38% increase in relation to the treatment without application of Li (Figure 3 A). It was observed that in the Caupi culture there was a translocation of Li present in the plant to the produced grains, showing that it is possible to perform biofortification through foliar application, but the high amounts applied can compromise productivity. However, even resulting in lower productivity, biofortified food can be worth more and, consequently, compensate for production, mainly for meeting the needs of this element in human food, and beans are considered primary sources of nutrients for the population. Reference [25], noted that applying increasing doses of Li increased the levels of Li in the leaves of lettuce cultivars. [25] explain that unlike monocots, dicots have shown to be able to absorb large amounts of Li.

The analysis of nitrogen in the grains in the three cultivars showed that the concentration of this element is directly correlated with the increase in the doses of Li applied via leaf (Figure 3 B). For the cultivars BRS Cauamé, BRS Itaim and BRS Nova Era, the nitrogen levels increased until the application of 31.04; 16.05 and 22.5 mg kg^{-1} of Li, leading to a maximum value of 7.59; 5.18 and 4.67% nitrogen, respectively. Thus, the application of Li through foliar fertilization increased by approximately 137.19; 19.08 and 17.63% the contents of this element in the grains of the cultivars BRS Cauam is, BRS Itaim and BRS Nova Era, respectively, in comparison with the treatments without application of Li. According to [18], in samples of plant tissue, almost all nitrogen (N) is found in organic form, represented by amino acids and proteins.

When the cultivars of Caupi beans were subjected to 40 g Li ha^{-1}, the maximum applied dose, there was a significant reduction in most agronomic characteristics. However, no necrotic or chlorotic spots were observed on the leaves, regardless of their stage of maturation, even at the maximum applied dose.

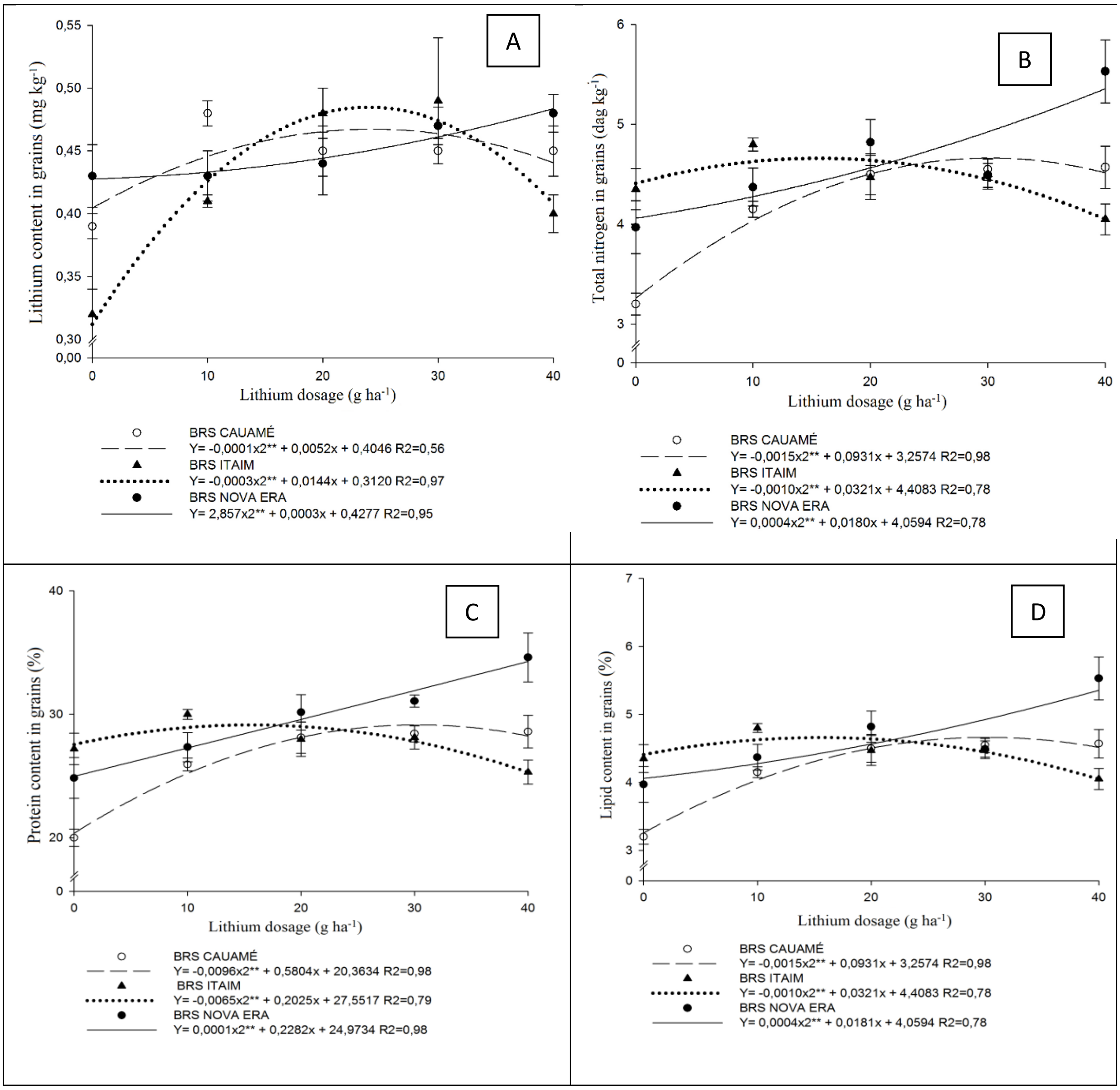


Figure 3: Response pattern found for studies involving the application of Li in Caupi crops and its effect on lithium content in grains (A), total nitrogen concentration in grains (B), protein content in grains (C), lipids in grains (D) as a function of foliar application of lithium hydroxide (0. 10. 20. 30 and 40 mg Li ha^{-1}) The bars indicate standard deviation of the mean, Gurupi-TO, 2020.

The same response behavior was observed for the characteristic protein content (%), (Figure 3 C). The increase in protein content occurred positively up to doses of 30.22; 15.57 and 1.14 mg ha^{-1} Li, increasing about 133.35; 18.72 and 1.57% with maximum protein values of 46.67, 32.28 and 25.23% for the cultivars BRS Cauamé, BRS Itaim and BRS Nova Era, respectively, in relation to the treatment without application of Li. Reference [26] suggests that protein values should be between 16 and 36%. The variation in protein content depends not only on the gene that controls the synthesis and accumulation of specific protein fractions but also on genes that control other factors, such as nutrient absorption, plant vigor, maturation, yield and size of grains.

The analysis of the lipid content in the grains revealed that the concentration of this element is directly correlated with the increase in Li doses applied via leaf (Figure 3 D). For the cultivars BRS Cauamé, BRS Itaim and BRS Nova Era, lipid contents increased up to the application of 31.03; 16.5 and 22.62 mg ha^{-1} Li, reaching a maximum value of 7.59; 5.18 and 4.67% lipids, respectively. Thus, the application of Li through foliar fertilization increased by about 261.43; 83.04 and 45.94% the contents of this element in the grains of the cultivars BRS Cauamé, BRS Itaim and BRS Nova Era, respectively, compared to treatments without Li application.

The differences in pod length, insertion of the first pod, plant height, mass of 100 grains, yield, lithium content, total nitrogen, crude protein and lipids in Caupi beans are also due to the genetic characteristics of these genotypes. In this context, the plants of the BRS Nova Era cultivar showed lower height (Figure 1A), pod length (Figure 1B), first pod insertion (Figure 1C), number of grains per pod (Figure 3A), grain yield (Figure 2C) and consequently less lithium content in the grains than the plants of the cultivars BRS Cauamé and BRS Itaim (Figure 3 A).

However, the application of Li, in addition to promoting an increase in the levels of this element in bean grains, also promotes an increase in grain yield in cultivars without compromising nutritional quality, thus configuring a quadratic behavior in the results.

According to the Brazilian Institute of Geography and Statistics - IBGE [27], the average food consumption of beans per person in Brazil is 41 g day^{-1} and, according to FAO [28] (Food and Agriculture Organization), the daily recommendation for lithium is (1-10 mg day^{-1}). Thus, relating to these values with the results obtained in this study, it is estimated that the biofortified beans at a dose of 10 g ha^{-1} will contribute to the increase of 5.5 % of the recommended daily intake (RDI). In this study, the best dose was 26 mg ha^{-1} which, the increase will be 14.3% of the RDI, but according to the Ministry of Health [29], the maximum tolerance limit (LMT) in food for Li is up to 10 mg day^{-1}, much lower than the concentration of 0.83 mg kg^{-1} obtained for this dose.

According to the quadratic equation presented in Figure 3 A, it is calculated that the maximum dose of Li that can be applied to the soil in order not to exceed the LTM concentration of 10 mg day^{-1} is 313 mg ha^{-1} as lithium hydroxide for medium-textured dystrophic Red-Yellow Latosol, however, it is not recommended, due to the loss of productivity in much lower doses, which can cause phytotoxicity in the plant. In view of this, excess lithium in bean grains at risk of food poisoning is unlikely.

In humans, the literature reports a case of mild intoxication manifested by severe nausea and vomiting as a result of an intentional overdose of a food supplement containing Li by ingesting 18 tablets with a total dose of 83 mg Li [30].

The daily consumption of beans per person in Brazil is 41 g day^{-1}, the highest lithium content among the three cultivars was 0.49 mg kg^{-1} of lithium in the cultivar BRS Itaim, contributing 0.2%.

4. Conclusion

The foliar application of Li hydroxide resulted in an increase in Li contents in the grains of the three Caupi cultivars, resulting in their biofortification. Biofortification occurred positively with the application of 24 and 26 g ha^{-1} of lithium for the cultivars BRS Itaim and BRS Cauamé.

The foliar application of Li promoted a positive response in the nutritional characteristics of the three Caupi cultivars, increasing the quality of the grains. The Li doses that promoted a positive increase were 16 and 31g Li ha^{-1} on average for the three cultivars.

The cultivar BRS Nova Era showed to be superior in the number of the characteristic of grains per pod, pod length and also promoted, through the lowest dose, the highest accumulation of Li in the grains.

The cultivar BRS Itaim, besides being superior in agronomic characteristics with greater height, greater number of grains per pod and longer pod length, also accumulated a higher lithium content in the grains.

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