Revista ARACÊ
ISSN: 2358-2472

BIOFORTIFICATION OF RICE CULTIVARS IRRIGATED WITH LITHIUM DOSES VIA FOLIAR SPRAYING

https://doi.org/10.56238/arev7n2-103

Submitted on: 10/01/2025 **Publication date:** 10/02/2025

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Revista ARACÊ
ISSN: 2358-2472

SUMMARY
Although it is an essential trace element for human health, lithium (Li) intake is insufficient in several regions of the world. The biofortification of rice with this element is a technique that can contribute to minimize its deficiency in the population. Thus, the objective of this study was to determine the influence of Li doses applied via foliar on biofortification, physiological properties and production of irrigated rice cultivars. The experimental design was in randomized blocks in a 5x3 factorial arrangement, with four replications. The factors consisted of five Li doses (0, 50, 100, 150 and 200 g ha-1) and three irrigated rice cultivars (BRS Catiana, BRS Tropical and BRS Pampeira). The foliar application of LiOH· H2O provides an increase in the Li content in the grains of any of the rice cultivars under study. The supply of Li to rice plants does not influence their development, yield and physiological properties. There is genetic variability among rice cultivars regarding the accumulation of Li in the grains.

Keywords: Oryza Sativa L. Lithium Hydroxide. Foliar fertilization. Food Composition. Nutritional Quality.

Revista ARACÊ
ISSN: 2358-2472

INTRODUCTION

Lithium (Li) is an essential trace element in the human diet due to its importance for the proper functioning of the body (Kalinowska et al., 2013; Marshall, 2015). This trace element has been used in the prevention of cognitive decline and suicide, in the treatment of bipolar disorder and behavioral changes such as psychosis, neurosis, and schizophrenia (Brown et al., 2018; Schrauzer, 2002).

Although the doses of Li used in treatments are high, very low doses, in concentrations found naturally in the environment, they can promote beneficial effects on health (Szklarska and Rzymski, 2018). In this sense, there is evidence that insufficient intake of Li can affect the endocrine, cardiovascular, neuromuscular, renal, and dermatological systems (Kabata-Pendias and Mukherjee, 2007).

Although it is not considered an essential element for plant development and production, Li is easily absorbed by most plants (Schrauzer, 2002). The level of absorption and concentration of Li in their tissues is quite variable and depends on the species, the plant genotype, and its concentration in the soil (Jiang et al., 2014; Shahzad et al., 2016). This element can act on plants in two ways: to effect important metabolic functions and to stimulate development and production, when used in low concentrations; or promote physiological and biochemical changes with toxic effects that result in lower yield, when in high concentrations (Santos et al., 2019; Silva et al., 2019; Shahzad et al., 2017).

Cereal grains and vegetables are the main sources of Li for human consumption, which can supply up to 90% more than 90% of the daily consumption of the element (Schrauzer, 2002). Therefore, it is expected that a diet rich in these foods provides a reasonable amount of Li for the population. However, because Li has a very heterogeneous distribution in the earth's crust, even with diets based on cereals and vegetables, the intake of the element by populations in different regions of the world is extremely variable (Schrauzer, 2002). Given this, in several places on the planet, Li levels in the soil and therefore in water and food are low, which results in daily intake of the element below the suggested recommendation (Szklarska and Rzymski, 2018).

In this context, it is necessary to adopt measures that raise Li intake to satisfactory levels, among which agronomic biofortification of agricultural products emerges as a sustainable, efficient, and low-cost strategy (De Valença et al., 2017). This technique provides an increase in the concentration of minerals in plant tissues without interfering with their basic characteristics or plant yield (Andrade et al., 2018). Among the agronomic biofortification methods, foliar spraying of minerals, as it does not depend on the translocation of the root to the shoot and because it avoids losses due to leaching or immobilization in the soil, has proven to be highly efficient in the biofortification of different crops (Chan et al., 2021; Puccinelli et al., 2017; Ramos et al., 2019; Santos et al., 2019).

The use of agricultural products widely consumed by the population is essential for the success of biofortification programs. That said, rice (*Oryza sativa L.)*It occupies a prominent place for being the second most cultivated cereal in the world and the main irrigated crop on the planet. It is also a staple food for more than half of the world's population (Rao et al., 2017; Reis et al., 2018). Due to its global importance, this crop has wide aptitude to be used in such programs, in which it can serve as a vehicle for the delivery of micronutrients to the human body and supply specific nutritional deficiencies.

Due to the importance of research involving the biofortification of crops with Li, associated with the lack of information on the subject, especially about irrigated rice crops, in tropical climate regions, the objective of this study was to determine the influence of Li doses applied via foliar on biofortification, physiological properties and production of irrigated rice cultivars.

MATERIAL AND METHODS

EXPERIMENTAL CONDITIONS

The study was conducted using plastic pots, in an experimental field area without environmental protection, between April and September 2018, in the municipality of Lagoa da Confusion, Tocantins State, Brazil (10º46'51.0" S, 49º37'01.5" W, 188 m altitude). The climate of the region is tropical with summer rainfall (Aw) or tropical savannah (Alvares et al., 2013). The average annual temperature is 27.2°C and the average annual rainfall is 1882 mm. Data related to precipitation and temperature during the experimental period, collected at meteorological stations in the municipalities of Lagoa da Confusion and Pium, State of Tocantins, Brazil, are presented in Fig. 1 (INMET 2018).

In the cultivation of the plants, a mixture of soil, substrate (Bioflora®) and medium sand (0.5 mm) was used in the proportion of 6:1:1. This mixture was placed in plastic pots with 8 dm3 volume where the plants were grown. This soil came from the 0 to 20 cm deep layer of a dystrophic Red-Yellow Latosol (Embrapa, 2018). Physical and chemical analyses indicated that this soil had: clayey texture (52.5 dag kg1 of sand, 5.0 dag kg1 of silt, 42.5 dag kg1 of clay); pH in CaCl2 of 5.0; organic matter 3.9 dag kg1; phosphorus (Mehlich1)

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ISSN: 2358-2472

75.9 mg dm3; potassium 206.0 mg dm3; calcium 8.2 cmolc dm3; magnesium 1.0 cmolc dm3; aluminum 0.0 cmolc dm3; hydrogen + aluminum 2.0 cmolc dm3; iron 51.0 mg dm3; lithium 4 mg dm3 and base saturation 83%.

The sowing fertilization was carried out according to the physicochemical analyses of the soil, and consisted of 321.5 mg dm-3 of the fertilizer NPK 04-14-08 that provided the plants with 13.0, 45.0, 25.5, 35.6, 34.4 and 0.64 mg dm-3 of nitrogen (N), phosphorus (P2O5), potassium (K2O), calcium (Ca), sulfur (S) and zinc (Zn), respectively. In addition, micronutrient fertilization was carried out with the application of 12.5 mg dm-3 of FTE-BR12 fertilizer (Peninsula) that provided the plants with 1.12, 0.22, 0.10, 0.25, 0.43 and 0.01 mg dm-3 of zinc (Zn), boron (B), copper (Cu), manganese (Mn), iron Fe) and molybdenum (Mo), respectively.

EXPERIMENTAL INSTALLATION

Six rice seeds were sown per pot. At 15 days after plant emergence, thinning was carried out, leaving two seedlings per pot, sufficient quantity to obtain the total grain needed for the analyses and not compromise the development of the plants by competition. As top dressing, 55 mg of nitrogen and 22.5 mg K2O per dm-3 of soil were applied, divided into four applications between the beginning and the end of tillering of the crop. For this, the following mineral fertilizers were used: ueria, ammonium sulfate and potassium chloride. During the experiment, the soil was kept moist through individual daily irrigations in each experimental unit.

TREATMENTS AND EXPERIMENTAL DESIGN

The experimental design was in randomized blocks in a 5x3 factorial arrangement, with four replications. The first factor consisted of five doses of Li (0, 50, 100, 150 and 200 g $^{ha-1}$) using lithium hydroxide monohydrate (LiOH·H2O P.A). The second factor under study consisted of three irrigated rice cultivars (BRS Catiana, BRS Tropical and BRS Pampeira). Three rice cultivars were chosen to obtain information on the response of different genotypes about Li application. These cultivars were selected because they are in the group of the most cultivated in the region. Each experimental unit consisted of a pot with two plants spaced 1 m between blocks and 0.35 m inside the blocks. Each block was composed of 15 vessels (all treatments).

The application of Li doses was carried out via foliar, in the morning (between 8:00 and 9:00 am), when the plants were in the grain filling stage (R6). For this purpose, a standard LiOH solution was used·H2O (0.5 mg mL-1). For example, 1.21 mL of the solution per two-plant pot was used for the treatment of 50 g $^{ha-1}$ of Li. These doses of the standard solution were completed in 50 mL of water and applied uniformly on the plants of each experimental unit. The doses were converted to hectare considering the plant population normally used for irrigated rice cultivation (1,000,000.00 plants per hectare), extrapolating the doses per pot to hectare. For the application, a manual pre-compression sprayer (STIHL SG 11) was used with a maximum pressure of 2.5 bar (36.26 psi), a flow rate of 300 mL per minute, and an adjustable conical tip.

EVALUATIONS AND SAMPLING

To quantify the chlorophyll and gas exchange indexes, samples were collected seven days after Li application. To determine the components of yield, yield and Li concentration in rice grains, samples were collected at the end of the crop cycle.

Chlorophyll Index

Seven days after Li application, indirect quantifications of chlorophyll were performed with readings at ten different points of the flag leaves of the plants of each experimental unit, measuring: chlorophyll index *a* (chl *a* - ICF); chlorophyll index *b* (chl *b* - ICF); and total chlorophyll index (total chl - ICF), using a portable chlorophyll meter (ClorofiLOG; model CFL1030), which provides results in dimensionless units (ICF-Falker Chlorophyll Index).

Gas exchange

Gas exchange was evaluated seven days after Li application. Such analyses were carried out using twelve readings on the flag leaves of the plants of each plot, measuring: internal concentration of CO2 (*Ci* - µmol CO2 m2 $^{s-1}$); photosynthetic rate (*A* - µmol CO2 $^{m-2}$ s-1$^{); stomatal conductance (}$gs - *µmol H* $_{2O}$ $^{m-2}$ $^{s-1}$); transpiration (*E* - µmol H$_{2O}$ $^{m-2}$ $^{s-1}$) and water use efficiency (*US* - µmol CO $_2$ $^{mol-1}$ H2O). For this, an InfraRed Gas Analyzer (IRGA; LCi-SD model; ADC BioScientific Ltd.). The readings were carried out between 8:00 am and 11:00 am, with clear skies, to maintain homogeneous environmental conditions during the evaluations.

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Evaluation of plant growth and yield

At the end of the crop cycle, the plants were harvested and plant height (cm), number of panicles per pot, number of grains per panicle, spikelet sterility (%), weight of 100 grains (g) and grain yield (g $^{pot-1}$) were evaluated. To quantify the mass of 100 grains and grain yield, the samples were dried in an incubator with forced air circulation (Solab, model SL-102) at 60ºC. The grains remained in the incubator until they reached constant weight. Next, the grain mass was evaluated on a 0.01 g precision scale (Gehaka BK4000).

Li concentration in grains and estimation of daily Li intake

The determination of Li concentrations in grains (mg $^{kg-1}$) was carried out at the Laboratory of Research in Environmental and Biofuel Chemistry (LAPEQ) of the Federal University of Tocantins, Brazil. In summary, after drying in an oven with forced air circulation (Solab, model SL-102) at 60ºC, the grains were hulled and the brown rice samples were ground and homogenized. Then, an aliquot (approximately 200 mg) was digested in a nitro-perchloric solution (HNO 3-$HClO_4$) (2:1) and a catalytic mixture composed of anhydrous sodium sulfate (Na2SO4) and copper sulfate pentahydrate (CuSO4.5H2O). The volume of digested extract was completed to 50 mL with ultrapure water.

The Li contents in the acquired extracts were measured by microwave-induced plasma atomic emission spectroscopy (MP-AES) (Agilent Technologies, model 4210). For the calibration of the instrument, six standards (0, 2, 4, 6, 8 and 10 ppm) were used, prepared by dilution of a standard solution of Li 100 ppm (LiOH· H2O, Vetec brand). Soon after, a calibration curve with these standards was estimated (coefficient of determination - R2 = 0.999, limit of detection - LD = 0.000374 and limit of quantification - LQ = 0.001247). After obtaining the Li concentration in the digested extract, the Li concentrations (mg $^{kg-1}$) were calculated in the mass of the samples used.

The estimate of daily Li intake by consumption of biofortified rice grains was calculated using Equation (2), as described by Ramos et al. (2023) (adapted):

$$IdLi = CLig*Cda \qquad (2)$$

Where *IdLi* = estimated daily intake of Li by an adult (mg $^{day-1}$), *CLig* = Li concentration in grains (mg $^{kg-1}$) and *Cda* = average daily consumption of rice per person in Brazil (0.146 kg $^{day-1}$) (CONAB 2019).

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STATISTICAL ANALYSIS

For the cultivar factor, the data were submitted to analysis of variance (ANOVA) by applying the F test ($\alpha = 0.05$). If there were significant differences, the means of the treatments were compared using Tukey's test ($\alpha = 0.05$). For the dose factor, the data were submitted to regression analysis. The criteria used in the selection of the regression curves were the significance of the equation and its coefficients ($\alpha = 0.05$), the highest regression coefficient (R^2) and the simplicity of the equation. For these analyses, the statistical program Sisvar was used (Ferreira, 2011). The figures and regression plots were created using Sigma Plot version 12.5 (Systat Software, Chicago, IL, USA).

RESULTS

There was significance ($p < 0.05$) of the interaction between rice cultivars and Li doses, exclusively, for the concentration of Li in the grains. A significant effect ($p < 0.05$) was found among the rice cultivars for all the traits evaluated, except for the number of panicles per pot, internal CO2 concentration (*Ci*) and water use efficiency (*EUA).* The effect of Li doses influenced ($p < 0.05$) only the Li concentration in the grains (Table 1).

Tabela 1. Resumo da análise de variância com os quadrados médios para as características: altura de plantas (AP), número de panículas por vaso (NPV), número de grãos por panícula (NGP), esterilidade de espiguetas (EE), massa de 100 grãos (M100), rendimento de grãos (RG), índice de clorofila a (Chl *a*), índice de clorofila b (Chl *b*), índice de clorofila total (Chl total), concentração interna de CO_2 (*Ci*), assimilação líquida de CO_2 (*A*), condutância estomática (*gs*), transpiração (*E*), eficiência do uso da água (*EUA*) e concentração de Li nos grãos (CLig) de três cultivares de arroz irrigado submetidas à aplicação foliar de doses (0, 50, 100, 150 e 200 g/ha) de Li ($LiOH{\cdot}H_2O$).

	Fonte de variação					
	Graus de liberdade					
	Cultivares (S)	Doses (D)	C x D	Residuais		
Características	2	4	5	42	Média	CV (%)
AP	904.96**	2.78^{ns}	11.17^{ns}	12.45	79.67	4.43
NPV	0.35^{ns}	19.05^{ns}	30.62^{ns}	21.58	41.60	11.17
NGP	1693.02*	758.82^{ns}	325.16^{ns}	332.59	101.28	18.01
EE	3106.23**	164.00^{ns}	81.33^{ns}	81.03	37.64	23.91
M100	0.03**	0.01^{ns}	0.00^{ns}	0.00	2.13	3.38
RG	4491.16**	134.94^{ns}	130.90^{ns}	140.45	51.95	22.81
Chl *a*	27.31**	3.14^{ns}	3.41^{ns}	3.76	29.07	6.68
Chl *b*	8.14**	1.81^{ns}	1.20^{ns}	1.10	10.08	10.40
Chl *t*	65.32**	9.64^{ns}	7.32^{ns}	8.49	39.15	7.44
Ci	578.52^{ns}	1111.24^{ns}	132.28^{ns}	767.98	276.91	10.01
A	29.92**	2.12^{ns}	0.59^{ns}	1.61	5.95	21.33
gs	0.01**	0.00^{ns}	0.00^{ns}	0.00	0.11	21.77
E	20.05**	0.57^{ns}	1.92^{ns}	2.58	4.92	32.65
EUA	0.15^{ns}	0.13^{ns}	0.20^{ns}	0.16	1.34	30.19
CLig	8.41**	2.62**	0.81**	0.04	3.80	5.86

**Significativo a 1% de probabilidade ($p < 0.01$); *significativo a 5% de probabilidade ($p < 0.05$); ^{ns}não significativo ($p > 0.05$) pelo teste F; CV: Coeficiente de Variação.

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PLANT GROWTH AND YIELD

For the plant height trait (Figure 2A), the cultivar BRS Tropical showed the highest average (87 cm), followed by the cultivar BRS Pampeira (77 cm). The cultivar BRS Catiana showed the lowest average for this trait (74 cm). There was no significant difference between rice cultivars for number of panicles per pot (Figure 2B). Regarding the response of rice cultivars to the number of grains per panicle (Figure 2C), BRS Pampeira presented a higher average (109 grains per panicle), although it did not differ from BRS Catiana, while BRS Tropical presented a lower average (91 grains per panicle). Regarding the sterility of the spikelets (Figure 2D), the highest percentage of sterile spikelets was found for the cultivar BRS Tropical (52%), followed by BRS Catiana (31%) and BRS Pampeira (30%), although the last two did not differ statistically from each other.

For a weight of 100 grains, BRS Catiana had a higher average (2.17 grams), although it did not differ from BRS Tropical, with an average of 2.13 grams, while BRS Pampeira had a lower average for this trait (2.09 grams) (Figure 2E). Regarding grain yield (Figure 2F), not differing significantly from each other, the cultivars BRS Pampeira and BRS Catiana stood out with higher averages (61 and 60 grams per pot, respectively). With an average of 35 grams per pot, BRS Tropical was lower.

Figure 2. Plant height (A), number of panicles per pot (B), number of grains per panicle (C), sterility of spikes (D), weight of 100 grains (E) and grain yield (F) of irrigated rice cultivars, regardless of Li doses. Means followed by the same lowercase letter do not differ from each other (Tukey, $p < 0.05$).

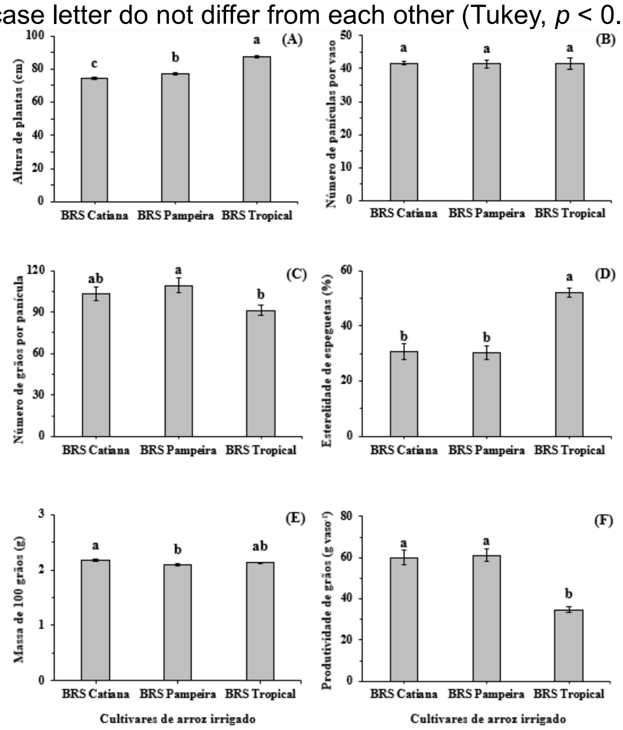

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Figure 3. Chlorophyll *a* (A), chlorophyll *b* (B) and total chlorophyll (C) of irrigated rice cultivars, regardless of Li doses. Means followed by the same lowercase letter do not differ from each other (Tukey, $p < 0.05$).

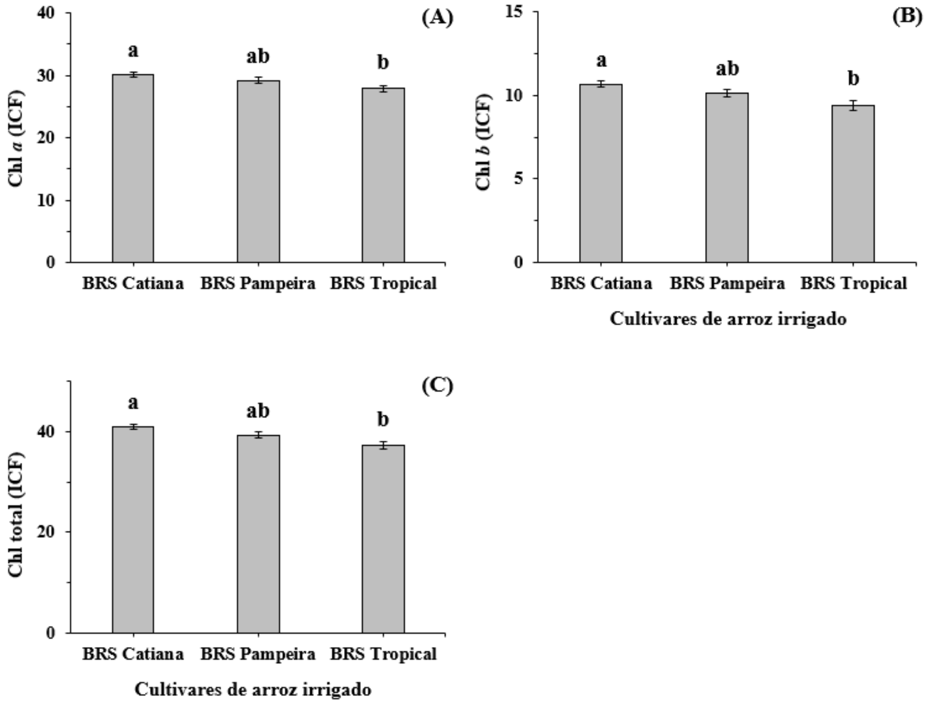
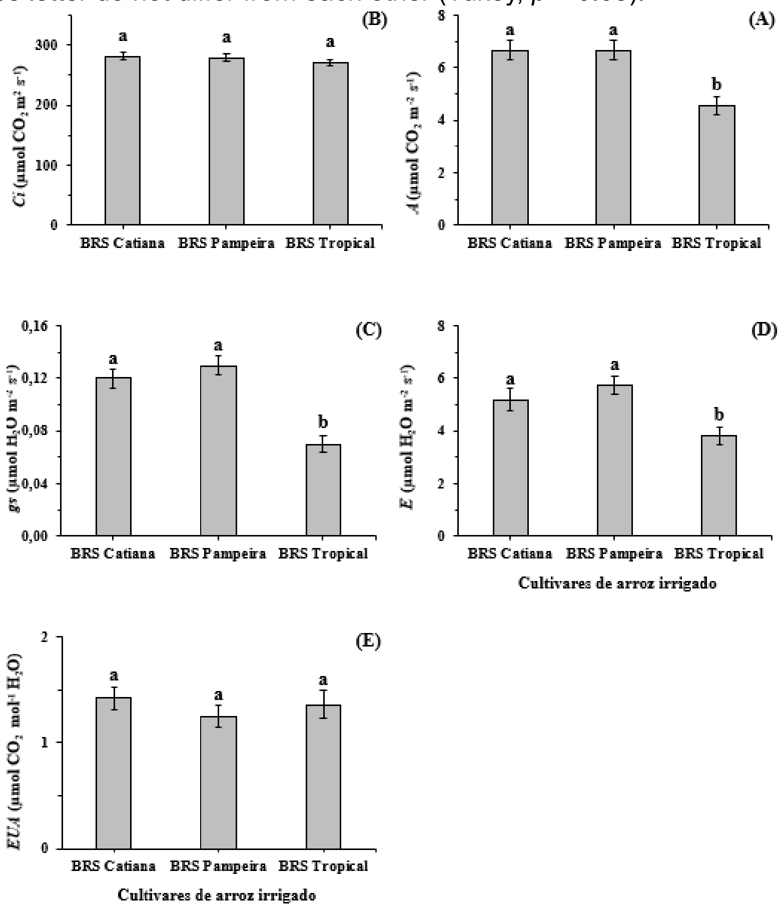


Figure 4. Net assimilation of CO2 (A), internal concentration of CO2 (B), stomatal conductance (C), transpiration (D) and water use efficiency (E) of irrigated rice cultivars, regardless of Li doses. Means followed by the same lowercase letter do not differ from each other (Tukey, $p < 0.05$).



PHOTOSYNTHETIC PIGMENTS AND GAS EXCHANGE

The photosynthetic pigments chl *a*, chl *b* and total chl showed similar behavior. The cultivar BRS Catiana exhibited higher indexes of these pigments, while BRS Tropical exhibited lower indexes. With intermediate means, BRS Pampeira did not differ statistically from the other cultivars (Figures 3A, 3B and 3C). Regarding the physiological indicators photosynthetic rate, stomatal conductance and transpiration, the cultivars BRS Pampeira and BRS Catiana showed higher averages, in which they differed significantly from BRS Tropical, which exhibited a lower average for these characteristics (Figures 4A, 4C, 4D).

LI CONCENTRATION IN GRAINS

Differently from what occurred in grain yield, photosynthetic pigments and gas exchange, the cultivar BRS Tropical showed the highest concentration of Li in the grains, followed by BRS Catiana and BRS Pampeira, respectively. The foliar application of Li provided efficient accumulation of the element in the grains in all rice cultivars. For BRS Catiana and BRS Pampeira, there was a linear increase in the concentration of Li in rice grains as a function of the increase in the doses applied to the plants. For BRS Tropical, a quadratic response was verified, with an increase in the concentration of Li in rice grains up to a dose of 102 g $^{ha-1}$. From then on, a decrease in the concentration of the element in the grains was observed with the increase in the applied dose (Figure 5).

Figure 5. Li concentration in the grains of three irrigated rice cultivars as a function of Li doses. The vertical line segments represent the mean ± SE of the data.

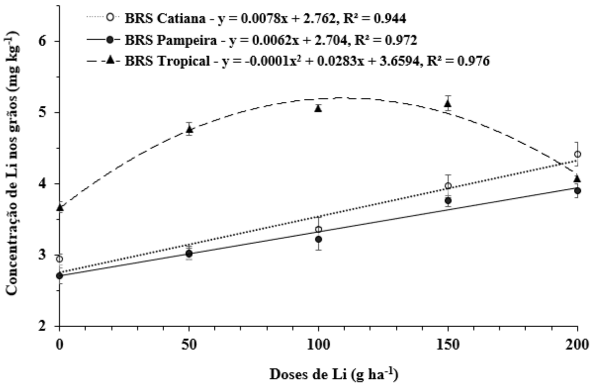


Figura 5. Concentração de Li nos grãos de três cultivares arroz irrigado em função da aplicação de doses de Li. Os segmentos de linha vertical representam a média ± SE dos dados.

With Li supplementation, the concentration of the element in the grains increased by an average of 1.41, 1.44 and 1.50 times for BRS Tropical, BRS Pampeira and BRS Catiana, respectively, when comparing the concentration of the element in the grains at the dose that promoted greater accumulation with the control treatment. The Li concentration in the grains reached an average of 3.90, 4.42 and 5.20 mg $^{kg-1}$ for the cultivars BRS Pampeira, BRS Catiana and BRS Tropical, respectively, at the maximum dose for BRS Catiana and BRS Pampeira and at the dose of 102 g $^{ha-1}$ for BRS Tropical. It was observed that the difference in Li accumulation in grains depends on the rice cultivars, which in turn depends on the Li doses applied.

DISCUSSION

PLANT GROWTH AND YIELD

The difference between the rice cultivars for most of the evaluated traits is probably due to intrinsic genetic attributes of each cultivar. For plant height, there was an amplitude of 13 cm between the cultivar that presented the largest (BRS Tropical) and the smallest size (BRS Catiana) (Figure 2A). The plant heights of the cultivars under study were slightly lower than those described by their breeders, and there was no risk of lodging in this case.

As for the number of grains per panicle, the cultivar BRS Pampeira presented a higher average, while BRS Tropical, lower. BRS Catiana was intermediate (Figure 2C). According to SOSBAI (2018), environmental factors and genetic particularities regulate this characteristic, which may explain the variation between the cultivars in this study. In rice cultivation, low tillering of plants results in a lower number of panicles per area, which in turn can reflect in lower grain yield. In this case, a higher number of grains per panicle is essential to compensate for the losses resulting from low tillering.

One of the factors that most affect grain yield in rice is the sterility of spikelets. Environmental factors, especially temperature extremes (below 17°C and above 35°C) in the flowering period, and genetic factors can strongly influence this characteristic (SOSBAI, 2018). Similarly to plant height, BRS Tropical exhibited a higher percentage of sterile spikelets. On the other hand, BRS Catiana and BRS Pampeira did not differ reciprocally (Figure 2D). As the sterility of spikelets has a high negative correlation with grain yield (Terra et al., 2015), the distinct behavior presented by the cultivars can be explored in genetic improvement programs for the selection of more productive rice plants.

The high rate of sterile spikelets (above 30%) for all cultivars is certainly due to the temperature extremes cited by SOSBAI (2018) observed during the flowering period of the crop (Figure 1).

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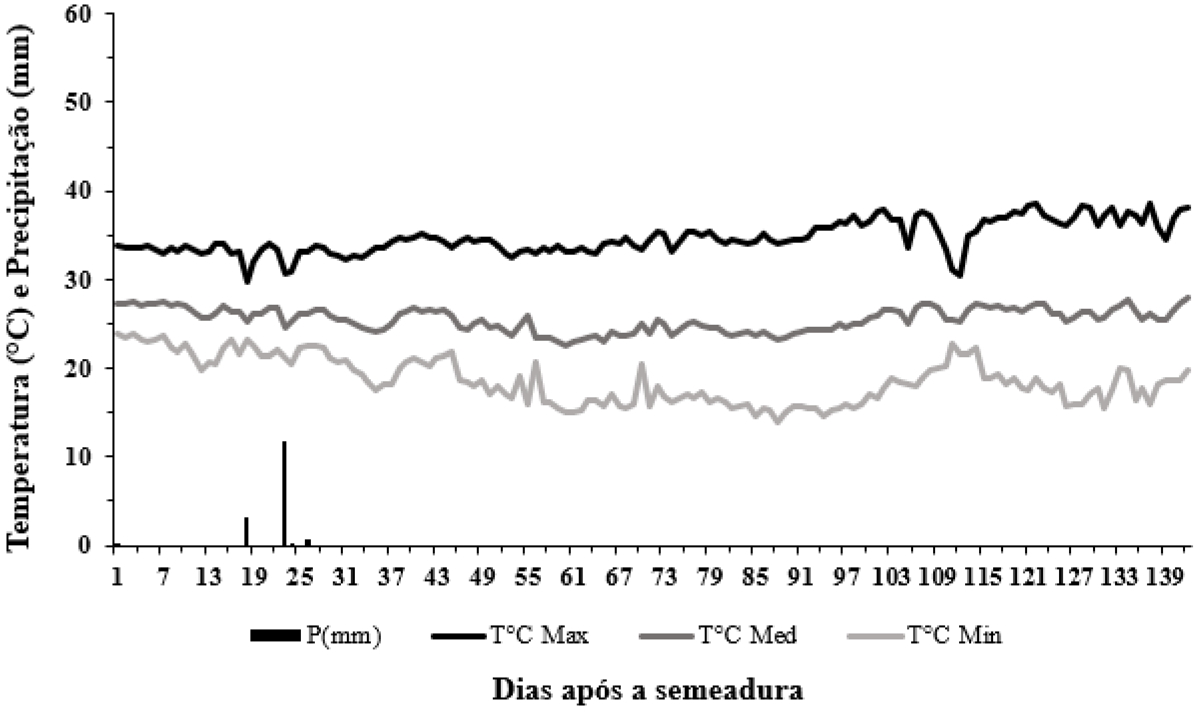
Figure 1. Rainfall and maximum, average and minimum temperatures during irrigated rice cultivation between April and September 2018, Lagoa da Confusion, Tocantins, Brazil.



The variability among cultivars for 100-grain mass (Figure 2E) should be linked to genetic attributes specific to each genotype. In rice plants, husk size, grain density, and karyopsis development, which is related to carbohydrate translocation, are varietal aspects that control this trait (Boldieri et al., 2010; Dalchiavon et al., 2012). Heavier grains associated with the other productive characteristics reflect in higher grain yield per unit area.

The fact that BRS Catiana and BRS Tropical have a higher mass of 100 grains is possibly due to the fact that the former has a smaller number of grains per panicle (Figure 2C) and the latter has, in addition to a smaller number of grains per panicle, a higher percentage of sterile spikelets (Figure 2D). In these cases, there may have been a distribution of photoassimilates to a smaller amount of grains, which resulted in greater mass.

Grain yield was significantly influenced by rice cultivars (Figure 2F). This disparity between the cultivars is certainly due to genetic factors and environmental conditions, which interfere with the agronomic properties of the crop. According to Freitas et al. (2007), grain yield in rice crops, as it is a quantitative characteristic, controlled by a large number of genes, is defined by the interaction of its components: number of panicles per unit area, number of grains per panicle, spikelet fertility index and grain weight. Therefore, in this study, the cultivars BRS Pampeira and BRS Catiana have a lower spikelet sterility index and a higher number of grains per panicle and a mass of 100 grains, respectively, which resulted in higher grain yields of these cultivars. On the other hand, the least productive cultivar (BRS Tropical) had a higher spikelet sterility index and a lower number of grains per panicle, a fact that compromised its grain yield.

PHOTOSYNTHETIC PIGMENTS AND GAS EXCHANGE

Photosynthetic pigments and gas exchange were also related to grain yield. Thus, the cultivars BRS Catiana and BRS Pampeira, which were more productive, obtained higher chlorophyll indices (Figures 3A, 3B and 3C) and, likewise, higher averages for the physiological indicators photosynthetic rate, stomatal conductance and transpiration (Figures 4A, 4C, 4D).

It is already well established that chlorophyll molecules are essential for the photosynthetic process, in which they are responsible for the collection and conversion of light energy into biochemical energy and for the transport of electrons within the reaction centers (Tanaka and Tanaka, 2006). According to Ramesh et al. (2002), the chlorophyll content in the leaf is the best indicator of photosynthetic activity in rice plants. These authors demonstrated in their research that grain yield in rice depends on the chlorophyll content in the leaves, a fact that confirms the results of the present study.

According to Ambavaram et al. (2014), the photosynthetic rate is the main component of plant yield, so that more than 90% of plant biomass comes from photosynthetic products. In summary, in this process, plants use energy from sunlight to produce biochemical energy, which serves to convert atmospheric carbon dioxide (CO2) into organic carbon (C6H12O6), which is the basis for plant development and production (Evans, 2013). In view of the above, it is possible to conclude that the higher chlorophyll and photosynthetic rate indices were the main responsible for the higher grain yield of the cultivars BRS Catiana and BRS Pampeira.

In this context, stomatal conductance and sweating are directly associated with the photosynthetic rate. According to Lawson and Vialet-Chabrand (2019), stomatal movement controls the uptake of CO2 used in photosynthesis and the loss of water through transpiration, so this activity plays an essential role in plant productivity, which validates the results presented in this study.

The fact that the application of Li did not cause an effect on the growth, yield, photosynthetic pigments and gas exchange of rice cultivars may be related to the fact that the doses used in this study were not sufficient to influence such characteristics and, allied to this, due to the differentiated capacity of plant species and genotypes to behave when cultivated in the presence of Li (Jiang et al., 2014; Shahzad et al., 2016).

Santos et al. (2019) and Silva et al. (2019) studying the effect of the application of lithium sulfate (Li_2SO_4) and lithium hydroxide (LiOH) on soybean and lettuce plants, respectively, with doses ranging from 100 to 1,200 times higher than the highest used in the present study, observed significant effects for most of the traits, which ranged from beneficial, in the smallest doses, to toxic, in the largest. There is also the hypothesis that Li is absorbed and accumulated more efficiently by eudicots than monocots. In this regard, Hawrylak-Nowak et al. (2012) found in their studies that, under the same conditions, sunflower plants accumulated the element in the leaves about five times more than corn plants. Dias et al. (2021) and Chan et al. (2021), evaluating the response of eudicot plants: cowpea and chia, respectively, to the application of Li, in the same region, observed significant effects of the doses (0, 10, 20, 30 and 40 g $^{ha-1}$) on several traits, even though these doses of Li are five times lower than those evaluated in the present trial.

In addition to the factors mentioned above, the moment of application of Li (grain filling) certainly contributed to the fact that the element did not influence the agronomic and physiological attributes of the rice cultivars under study, since at the time of application, the plants were already in the conclusion phase of their cycle, in which, supposedly, it was no longer possible for Li to act on such characteristics.

LI CONCENTRATION IN GRAINS AND ESTIMATION OF DAILY LI INTAKE

In figure 5, for Li concentration in grains, the opposite effect of what occurred in grain yield was observed in relation to the cultivar factor. The cause of the less productive cultivar (BRS Tropical) having a higher concentration of Li in the grains must be related to the fractionation of the same doses of the element applied to the plants in a lower mass of grains produced by this cultivar. On the other hand, the cultivars with higher yields diluted the applied Li doses in a higher grain mass, which resulted in a lower concentration of the element. This situation may also be related to the genetic particularities of each cultivar. Dias et al. (2021) studying the agronomic biofortification of cowpea with Li also found significant variation between three cultivars for the content of the element in the grains.

Revista ARACÊ
ISSN: 2358-2472

Also in figure 5, there was an increase in the concentration of Li in rice grains according to the increase in the doses of the element applied to the plants, except for the cultivar BRS Tropical, which had an increase in the concentration of Li in the grains up to the dose 102 g $^{ha-1}$ followed by a decrease. According to Qiao et al. (2018), the adaptation or tolerance of plants to this element gives them a specific ability to accumulate varying amounts of Li in different plant organs.

The finding of Li accumulation in rice grains even in the control treatment must be related to the fraction of Li existing in the soil (4 mg dm3). According to Robinson et al. (2018), the content of this element in the soil can vary from < 1 to 200 mg $^{kg-1}$. Although the concentration of Li in food is mainly related to the content of the element in the soil (Schrauzer, 2002), the selection of plant cultivars with greater capacity for absorption and accumulation of Li in edible parts is essential for the success of biofortification programs with the element.

The main result sought in biofortification programs is the increase in the concentration of microelements in the edible parts of plants without harming their productivity (Andrade et al., 2018). That said, the results obtained in this study demonstrate that in addition to promoting the increase in the concentration of the element in the grains, the foliar application of Li did not harm the growth, yield, photosynthetic pigments and gas exchange of the plants of all the cultivars evaluated, which demonstrates that the foliar application of Li is an efficient strategy with the possibility of use in biofortification programs with the element in rice crops. This event is positive, because it was possible to increase the Li content in rice grains without negative effects of the element on plants.

The intake of Li has been used with positive results in the treatment of mood instability and in the reduction of aggression rates. In addition, it has recently been proposed that several foods be enriched with the element to meet its nutritional deficiency in the population (Goldstein and Mascitelli, 2016). Thus, considering the results obtained in this work, combined with the importance of rice for the food security of the world population, the foliar application of Li can represent a valuable technique for biofortification of the grains of this crop, which can contribute to the increase of the intake of the element by people and to the prevention of diseases.

Considering that the recommendation for a minimum daily intake of Li for healthy adults, 70 kg, is 1 mg (Marshall, 2015) and that, in Brazil, the average per capita consumption of rice is 0.146 kg $^{day-1}$ (CONAB, 2019), based on Equation (2), it is found that the consumption of rice grains biofortified with Li, when using the doses that provided the greatest accumulation of the element in each cultivar, it can satisfy 57, 65 and 76% of the minimum recommended daily intake of the element for the cultivars BRS Pampeira, BRS Catiana and BRS Tropical, respectively.

The biofortification of food cultures with Li has been increasingly studied in several regions of the world, however, to date no food biofortified with the element has been commercialized (Rzymski et al., 2017). Therefore, the results presented in this study can contribute to the development of these enriched foods, which are capable of promoting the improvement of the health and well-being of the population. To this end, additional research covering combinations of sources, doses and forms of application of Li, in addition to the inclusion of a greater number of rice varieties, is essential.

CONCLUSION

The foliar application of $LiOH \cdot H2O$ promotes an increase in the Li content in the grains of the three rice cultivars under study. The supply of Li to rice plants does not influence their growth, yield and physiological properties. There is genetic variability among rice cultivars regarding the accumulation of Li in the grains. In addition, the consumption of rice grains biofortified with Li, when using the doses that provided the greatest accumulation of the element per cultivar, can supply up to 76% of the minimum recommended daily intake of the element. Even though it was carried out in only one period, but under homogeneous conditions and with strict phytosanitary control, this study provides subsidies for the strategic choice of Li doses and irrigated rice cultivars suitable to be used in possible biofortification programs with the element, which is relevant for research and can contribute to reduce its deficiency in regions naturally poor in Li.

THANKS

The authors would like to thank Simone Mota Fernandes Ramos for her support in this study.

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