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Uptake, distribution and biofortification of selenium in *Acmella Oleracea* (L.) R. K. Jansen

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Abstract *—Nutritional biofortification of foods is a promising alternative to reduce Selenium deficiency in the diet of populations. This study evaluated the biofortification capacity of jambu with Se. The experiment was completely randomized with six treatments and five repetitions, in hydroponics. Five doses of Se in the form of sodium selenate (1, 2, 3, 4 and 5 $mg.L^{-1}$) and the control dose were used. Biometric, macro and micronutrient analyzes were performed, as well as the Se content in the plant parts. The indices of translocation (TrI), tolerance (tI) and Nutrient Use Efficiency (Nue) were estimated. The results were submitted to ANOVA and principal components analysis for the construction of multivariate indicators, in order to specify the regression models. The plant obtained higher agronomic performance when submitted to a dose of 3$mg.L^{-1}$, with Se translocation above 70%. The tI and Nue indices indicated that jambu reached optimal growth with the dose of 3 $mg.L^{-1}$ of Se. The results obtained from the regression equation of the multivariate indicators of growth, mass and nutrition indicated that the ideal concentrations of Se varied between 2.77 and 3.36 $mg.L^{-1}$. The general indicator that captured the entire plant behavior showed that the optimal concentration for biofortification is 2.98 $mg.L^{-1}$ of Se. The daily consumption of 100 g of biofortified jambu at the indicated dose provides a daily content of 50.13µg of Se for the population, a sufficient amount of Se for a balanced diet.*

I. INTRODUCTION

The supply and consumption of sustenance is the main theme in reaching the objectives of sustainable development in the schedule 2030. Objectives such as "zero hunger", "good health and well-being" and "life upon earth" are related to the form we produce, access and consume food [1].

The problem of bad nutrition continues to be a challenge to be beaten in the world, increased with social-economic inequality among the majority population of the world, mainly in underdeveloped regions. According to FAO [2], although Africa is the region where the levels of food insecurity are elevated, it can be observed that in Latin America and Caribbean this insecurity has been increasing since 2014, putting nearly one-third of the region's inhabitants unable to obtain food or reduce its nutritional quantity and quality [3];[1].

The problem of food insecurity and desnutrition tends to be greater with the increase of world's population and the Covid-19 pandemic. This brings consequences in the world's desnutrition, making the objectives related to production and consumption of food up to 2030 established by ONU couldn't be reached [1].

As a nutritional alternative, the unconventional vegetables bring the possibility of biofortification with many chemical elements essential to life such as iron, zinc and selenium [4]. These are the elements that respond to nutritional deficiency of the population in developing countries [5];[2] and the ones that have low soil concentration.

According to Kabata-Pendias [6], the medium iron content in soil varies by 3 to 5%; the zinc content of 60 to 89 mg/kg and the Se content 0,44 mg/kg. Notably, among the elements that possess low soil disponibility, the Se is the principal, even being a essential element to humans, because its make selenoproteins that physiological processes such as the reduction in the activity of reactive oxygen species, modulation of the immune system, reduction of trace elements in the human body as well as reducing the risk of cancer [7];[8].

Nascimento [9] points that a diet with deficiency of this mineral results in higher susceptibility to many diseases, and according to Agência Nacional de Vigilância Sanitária (ANVISA), of the Health Ministerium [10], the daily recommendation of Se to adult population is of 8.25 at the very least and 319.75 $\mu g.day^{-1}$ at most.

The Se is incorporated into human nutrition via agricultural products, however it can be found many types of food poor in this micronutrient due to its low content in soil, especially in Brazil [11];[12];[13]. It can be highlighted the agronomic biofortification as a technique relatively inexpensive and efficient to the increase of nutrition quality in food with minerals through differentiated cultural treatments [14];[15].

Bouis and Saltzman [16] evidences that the biofortification is possible without putting the crop productivity at risk, just as Puccinelli et al. [17] shows that the biofortification of edible crops with Se can represent a alternative system to provide the element in human's diet via mineral nutrition of plants, keeping in mind that around one billion of people presents this mineral deficiency [18].

In this perspective, jambu (*Acmella oleracea* (L.) R. K. Jansen), an unconventional vegetable, arises as a promising alternative to biofortification strategies in Brazil, such as in countries such as Peru, India, Nephal, China, Mexico and other regions of Africa [19]. Because it's present chemical and nutritional characteristics adequated with presence of proteins, carbohydrates, fibers, aminoacids and other bioactive composts [20].

Specifically in Amazon, jambu is consumed along the year, being part of the traditional cuisine of the region. The vegetable is part of typical dishes in Amazon's gastronomy, mainly Paraense, as pato-no-tucupi and tacacá [21]. According to Gusmão and Gusmão [22] the average of consume in a traditional dish as tacacá is 30g of jambu, and in other traditional dishes known as "arroz paraense", the consumption of the vegetable can reach 600g. Therefore, the biofortification of this vegetable could help to minimize the desnutritional and hunger impacts, mainly in the teenager population of Amazon region, which presents higher levels of food insecurity [23].

Thus, this research aims to show the adequate agronomic conditions to jambu biofortification with Se, mainly in relation of dose/response of the plant to the element, it's potential of translocation and adequate daily consumption of biofortification jambu.

II. MATERIAL AND METHODS

The experimental phase was made with the vegetable *Acmella oleracea* (L.) R. K. Jansen, known as jambu, yellow-flower variety, between february and march of 2020. The experiment was installed in greenhouse at Federal Rural University of the Amazon (UFRA), soil departament, city of Belém-Brazil, predominant weather is equatorial hot humid, according to Af category of Koopen [24].

The seeds of jambu were collected from the UFRA germplasm bank, placed in 128-cell polystyrene trays filled with coconut fiber. After germination, the seedlings were treated with Hoagland and Arnon nutrient solution [25], with 25% ionic strength, composed of 1 $mL.L^{-1}$ of NH_4NO_3, 4 $mL.L^{-1}$ of KNO_3, 5 $mL.L^{-1}$ of $Ca(NO_3)_2$, 2 $mL.L^{-1}$ of $MgSO_4$, 1 $mL.L^{-1}$ of Fe-EDDHA and 1 $mL.L^{-1}$ of Micronutrient solution.

To obtain better seedlings growth, it were thinning after 7 days of germination, keeping one seedling per cell. After 21 days, the four leaf seedlings were transplanted to vases with 2 L with milled silica, nutritive solution with 50% of ionic force. After 7 days of acclimatization, the seedlings were submitted to Se doses.

The experimental design was casualized, with six treatments and five repetitions, 30 plants in total. It used five doses of selenium (1, 2, 3, 4 and 5 $mg.L^{-1}$), applied in sodium selenate form (Na_2SeO_4) and control [17].

To keep the oxygenation of nutritive solution in the vases, it was drained in the afternoon end and replaced in the morning beginning. This procedure was daily until the experiment ended. Aiming to preserve the nutritive solution concentration, it was monitored the pH in each vase with phmeter GroLine model - HI98118, HANNA mark. Weekly, the solutions were renewed and the water loss by evaporation daily renewed. 50 days after the germination, it was performed the harvest of all plants.

Biometric Analysis

After the harvest, were measured the following variables: i) plant height (PH) - using pachymeter, measuring from the collector to the apex; ii) stem diameter (SD) - using pachymeter, measuring the stem 0.5 cm from the substrate and iii) inflorescence number (IN) - counting of total inflorescence emitted by the plant.

To determine the variables leaf dry mass (LDM), stem dry mass (SDM), inflorescence dry mass (IDM) and root dry mass (RDM), the plants were cleaned with deionized water and droughts in open air during 24 hours. Posteriorly, the plant parts were separated, placed in paper bags and taken to the greenhouse at 65° for 48 hours, until the plants reached constant weights. After 48 hours, the plant parts were removed and weighed on a precision scale [26].

Chemical analysis

The samples of leaf, stem, inflorescence and root of the jambu, after the drought, were milled in a porcelain bowl. Subsequently, a part of 0,25 g was removed of each part of the plant and placed in a teflon tube and added 4,0 ml of concentrated HNO_3, 2 ml of H_2O_2 (30% v/v) and 2 ml of ultrapure water. The solution stay in rest for one hour [26].

After the rest, the samples were placed to complete digestion in a microwave CEM model. The digestion occurs in three steps: temperature elevation from 0 to 180° C in 10 minutes in 800w, 180° temperature constant for 30 minutes and ventilation for 55 minutes. After the digestion the samples were filtered and swollen to 50 ml with deionized water [26].

The determination of macro and micro nutrients of jambu was performed through absorption atomic spectrometry of flame (Agilent Technology, model AA-200) equipped with deuterium lamps (to correction of background radiation) and lamps with hollow cathode to determination of K (766.5 nm), Mg (285.2 nm), Ca (422.7 nm), Fe (248.3 nm), Zn (213.9 nm), Mn (279.5 nm) and Cu (324.8 nm). It was used air-acetylene flame with output of 10 L/h of air and 2.0 L/h of acetylene [27]. The quantification was made through calibration curves with five points (external padronization). The curves of calibration showed R^2 to 0.99 and vary from 5 to 40 $mg.L^{-1}$ for K, 0.5 to 3 $mg.L^{-1}$ for Mg, 0.5 to 6 $mg.L^{-1}$ for Ca, 0.25 to 3 $mg.L^{-1}$ for Fe, 0.5 to 6 $mg.L^{-1}$ for Zn and 0.1 to 2 $mg.L^{-1}$ for Mn.

The selenium content in the plant was determined by spectrophotometry of atomic absorption with Varian AA 240 Z graphite furnace with tube atomizer graphite GTA 120 and backgroud broker Zeeman [28]. was used a hollow cathode lamp for the determination of Se at a wavelength of 196 nm, with palladium solution as modifier matrix. The carrier gas was argon 5.0 analytical, with pyrolysis temperature of 120°C, atomization of 1000°C and cleanup of 2600°C. All analyzes were performed in the Water Quality Laboratory of Amazon at the State University of the Pará.

Translocation index, tolerance and efficient use of nutrients

To quantify the ability of growth of the *Acmella oleracea* in Se presence, were determined the Translocation Index (*TrI)*, Tolerance (*tI*) and Nutrient Use Efficient (*Nue*), according methodologies proposed by Siddiqui and Glass [29] and Swiader et al. [30], with the following equations:

$$TrI = Aci/AcI * 100 \rightarrow (1)$$

Where Aci is the accumulation of Se in the plant parts (leaf, stem, inflorescence and root) and AcI is the accumulation of Se in root [31].

$$tI = Mi/cM \rightarrow (2)$$

Where Mi is the dry mass in the interest dose and cM is the control mass [32].

$$Nue = (TDM)^2/TCSe \rightarrow (3)$$

Where TDM is the total dry mass produced and TCSe is the total content of selenium in the plant.

Multivariate indicators

With objective of a better comprehension in jambu behavior due to Se presence, it were created multivariate indicators according with the methodology proposed by Santana and Santana [33], Oliveira [34] and Oliveira et al. [26], which utilize the technique of Principal Component Analysis to extract the components according with it’s descriptive importance to the data variance.

The indicators were constructed to represent the dimensions which compose the plant development, grouping the variables of each dimension: growth indicator – ***gI*** (group the variables plant height, stem diameter – and inflorescence number), plant mass accumulation – ***mI*** (leaf dry mass, stem dry mass, inflorescence dry mass and root dry mass), Nutrient Content – ***nI*** (content of K, Ca, Mg, Fe, Zn and Mn) and general (***gnI***) of the research, grouping the total set of explanatory variables, all the components were considered to be reach 100% of total mass variation of the data [33], [26].

The indicators creation followed the sequence proposed by Oliveira et al. [26]: *i)* it was estimated the vector with relative participation of the values $\lambda\left(\lambda_{kj}/\sum\lambda_k\right)$; *ii)* it was estimated the coefficients of the matrix of absolute values $\left(\alpha_{kj}\right)$ of eigenvectors of each transformed component; *iii)* it was estimated the relative coefficients of the eigenvectors matrix $\left(\alpha_{kj}/\sum\alpha_k\right)$, and; *iv)* the linear combination of the descriptor variables and matrix multiplication was performed to estimate the weights as in equations 4 and 5.

$$\theta_{j(kx1)} = \left(a_{kj}/\sum a_k\right)_{(kxk)} \cdot \left(\lambda_{kj}/\sum\lambda_k\right)_{(kx1)} \rightarrow (4)$$

Com

$$\theta_1 + \theta_2 + \ldots \theta_p = 1\left(j = gI, mI, nI, gnI\right) \rightarrow (5)$$

Therefore, it was formed the mathematical model used to estimate the weights associated with the descriptive variables of plant behavior, as reference the relationed dimensions: growth, mass accumulation, nutrient content and general behavior.

Lastly, each indicator was obtained by the weights vector multiplication θ by the values of explanatory variables related to the content aspects, growth, mass accumulation and general ***gI, mI, nI*** and ***gnI***. Equations (6 – 9): [26].

$$gI = \theta_{PH} \cdot PH + \theta_{SD} \cdot SD + \theta_{IN} \cdot IN \rightarrow (6)$$

$$mI = \theta_{LDM} \cdot LDM + \theta_{SDM} \cdot SDM + \theta_{IDM} \cdot IDM + \theta_{RDM} \cdot RDM \rightarrow (7)$$

$$nI = \theta_k \cdot K + \theta_{Mg} \cdot Mg + \theta_{Ca} \cdot Ca + \theta_{Fe} \cdot Fe + \theta_{Zn} \cdot Zn + \theta_{Mn} \cdot Mn \rightarrow (8)$$

$$\begin{aligned} gnI &= \theta_{PH} \cdot PH + \theta_{SD} \cdot SD + \theta_{IN} \cdot IN + \theta_{LDM} \cdot LDM + \theta_{SDM} \cdot SDM + \theta_{IDM} \cdot IDM \\ &+ \theta_{RDM} \cdot RDM + \theta_k \cdot K + \theta_{Mg} \cdot Mg + \theta_{Ca} \cdot Ca + \theta_{Fe} \cdot Fe + \theta_{Zn} \cdot Zn + \theta_{Mn} \cdot Mn \rightarrow (9) \end{aligned}$$

Regression analysis

The specification of the multiple regression system used to represent the phenomenon studied, contemplating the effects of selenium doses on growth, mass accumulation, nutrient content in jambu plants, and general behavior of the vegetable, was defined by equations (10 – 13):

$$\begin{aligned} gI &= b_{10} + b_{11}D_s + b_{12}D_s^2 + \upsilon_1 \rightarrow (10) \\ mI &= b_{20} + b_{21}D_s + b_{22}D_s^2 + \upsilon_2 \rightarrow (11) \\ nI &= b_{30} + b_{31}D_s + b_{32}D_s^2 + \upsilon_3 \rightarrow (12) \\ gnI &= b_{40} + b_{41}D_s + b_{42}D_s^2 + \upsilon_4 \rightarrow (13) \end{aligned}$$

Which: ***gI*** is the growth indicator of the plant; ***mI*** is the plant mass indicator; ***nI*** is the plant nutrient content and ***gnI*** is the plant general indicator; D represents the selenium doses with linear response; D² the selenium doses with quadratic response (control, 1mg.L^{-1}, 2mg.L^{-1}, 3 mg.L^{-1}, 4 mg.L^{-1} and 5 mg.L^{-1}); *b* are the equations interceptors (*i*= 1, 2, 3, 4); *b* are the parameters associated with the equations variables; υ are the random error terms of the equations.

The data of Se content (leaf, stem, inflorescence, root and aerial part), translocation, tolerance, nutrient use efficient, growth, mass and nutrient content (leaf, stem, inflorescence and root), were submitted to variance analysis (ANOVA) and the averages were compared by the Skott-Knott (SK) test, $p<0.05$. The data of the indicators were submitted to regression analysis to the estimation of maximum response of Se in the plant. The analysis were performed through the R software, version 3.5.2 [35].

III. RESULTS

Se Content

The results showed that the concentration increase of Se in nutritive solution results in the bigger uptake of the element in all plant parts. The higher values of the element were found in leaves when submitted to 5 mg.L^{-1} concentration of Se. The inflorescence also presented high content of the element in dose 5 mg.L^{-1}. Stem and root differ statistically of leaf and inflorescence, absorbing minor content of Se in all doses (Table 1).

The Se content in the aerial part of the plant is the basic element to the calculations of element quantity that will be consumed by population, considering that leaf, stem and inflorescence are the edible parts of jambu. The Se content in the aerial part grows with the doses increase (Table 1). However, doesn't have significant difference between the doses of 3 and 4 mg.L^{-1}. The 5 mg.L^{-1} dose of Se, in solution, allowed the higher uptake of the element in the aerial part in relation to the other doses and control.

Table 1 - Medium Se content in jambu plants in hydroponics

Se doses in $mg.L^{-1}$	Se content in plant parts in $\mu g.kg^{-1}$				
	Leaf	Stem	Inflorescence	Root	Aerial part
1	166.40±15.63dA	29.22±5.75cB	34.66±7.86dB	15.79±2.11dC	230.28±17.40 d
2	258.05±28.13cA	35.03±2.51cC	63.97±14.00cB	23.78±1.84cC	350.05±23.76 c
3	338.35±22.10bA	41.67±4.14bC	124.40±18.45bB	35.48±4.17bC	504.73±35.90 b
4	329.29±48.75bA	54.56±7.66aC	127.68±11.80bB	55.32±6.95aC	511.55±51.45 b
5	400.74±35.50aA	63.05±10.85aC	177.94±5.84aB	61.41±7.21aC	641.74±24.02 a

The lowercase letters points the significant differences between the Se doses; the uppercase letters points the significant differences between the plant parts, according with Scott-Knott ($p<0.05$) test.

Translocation Index (*TrI*)

The Se translocation was higher in 1, 2 and 3 $mg.L^{-1}$ doses, with more than 75% of the element translocated to the leaf (Table 2). In doses with 4 and 5 $mg.L^{-1}$, the translocation was also high, with that more than 70% of Se moved from the root to the leaf. The stem was the jambu part, beyond the leaf, which more translocated Se, with 15.16% of translocation in 4 $mg.L^{-1}$ dose. The inflorescence showed higher capacity of translocation in 3, 4 and 5 $mg.L^{-1}$ doses. Doesn't have differences in the capacity of translocation in root (Table 2).

The results shows the potential of mobility of the element in jambu, and also stimulate the growth and accumulation of biomass by the plant.

Table 2. Translocation index in jambu plants supplemented with increasing Se doses

Se Dosesin $mg.L^{-1}$	Translocation index (%)			
	Leaf	Stem	Inflorescence	Root
1	75.84±2.49 aA	14.41±3.47 aB	4.83±1.86 bC	4.90±0.69 aC
2	77.09±3.72 aA	12.05±2.02 bB	6.26±2.09 bC	4.57±0.67 aC
3	75.03±1.30 aA	11.62±1.10 bB	7.86±1.10 aC	5.46±0.81 aC
4	70.75±3.80 bA	15.16±2.30 aB	7.82±1.00 aC	6.25±1.32 aC
5	73.29±3.90 bA	13.64±2.05 aB	7.52±1.13 aC	5.52±1.14 aC

The lowercase letters points the significant differences between the Se doses; the uppercase letters points the significant differences between the plant parts according to the Scott-Knott ($p<0.05$) test.

Tolerance index (*tI*) and Nutrient Use Efficiency (*Nue*)

The results showed a significant increase in the tolerance index of jambu to the Se until the 3 $mg.L^{-1}$ dose, demonstrating that the element promoted increase in the dry mass production in relation to the control. However, the plant begins to show a decrease in dry mass production in 4$mg.L^{-1}$ dose, with a significant difference to the 3 $mg.L^{-1}$ dose. In 5 $mg.L^{-1}$ dose, the ***tI*** had a significant decrease, however, higher than the control dose, indicating that in 5 $mg.L^{-1}$ concentration, the plant answered with more efficiency in mass production than the plants without the element (Figure 1A).

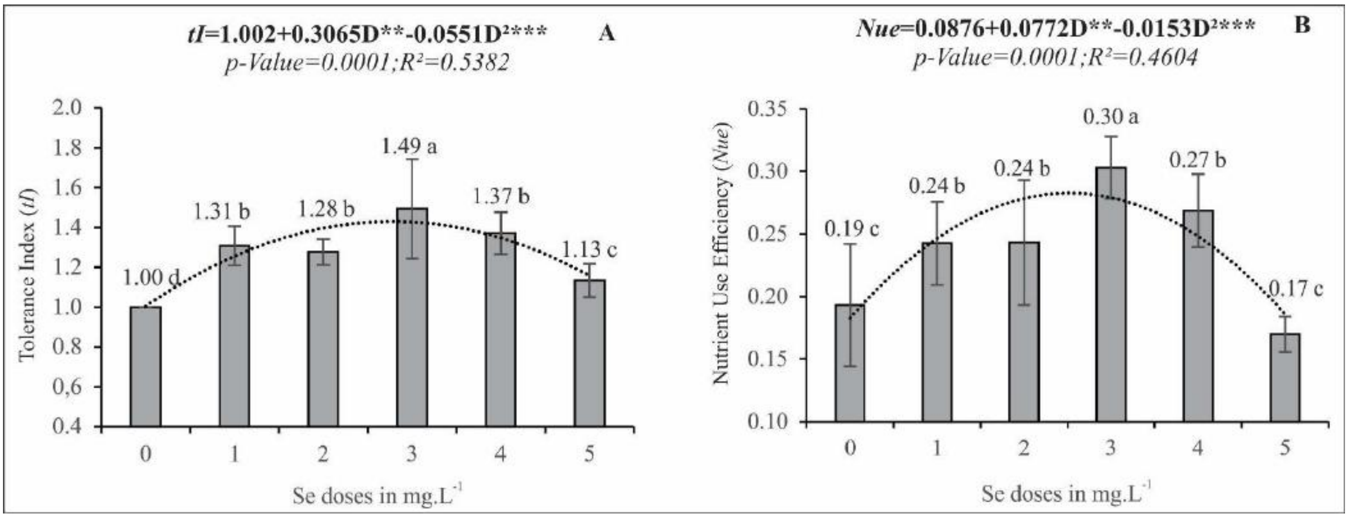

*Fig.1.Tolerance index (A) and Nutrient Use Efficiency in µg.planta^{-1} (B), in jambu plants submitted to increasing doses of Se. The bars are the standard deviation; the lowercase letters points the significant differences between the Se doses according to the Scott-Knott ($p < 0.05$) test. **significant to 1% of probability; *significant to 5% of probability (test F); D=dose; D²=square dose.*

In the analysis of nutrient use efficient (***Nue***), it is related the total dry mass produced by the plant with the total nutrient content. Thus, it can be observed in the figure 1B that occurs a higher uptake of nutrient by the plant in doses 1, 2, 3 and 4 mg.L^{-1}, however without presenting statistical differences between the doses to influence de ***Nue*.** On the other hand, in 5 mg.L^{-1} dose, the ***Nue*** value was minor than in other doses, indicating that in this concentration the plant begins to reduce its uptake of nutrients in a way to harm the biomass production.

Jambu mass and growth

In table 3, the values of the individual variables and mass are presented. The doses 2, 3 and 4 mg.L^{-1} of Se influence the jambu growth (PH). In 5 mg.L^{-1} dose, the PH begins to reduce its growth. In relation to SD, there is no difference between the element doses, presenting differences only in relation to control. The variable IN does not doesn't present Se influence.

Table 3. Effect of Se concentrations on growth variables and mass gain of jambu plants grown in nutrient solution.

Se Doses in mg.L^{-1}	Growth and mass variables						
	PH	SD	IN	LDM	SDM	IDM	RDM
Control	20.4±1.28b	5.24±0.34b	14.00±1.41a	1.60±0.19b	1.57±0.36c	0.39±0.05b	1.01±0.15c
1	22.34±2.14a	6.10±0.65a	16.80±4.26a	1.96±0.24a	2.09±0.17b	0.57±0.07a	1.32±0.10a
2	22.54±1.35a	6.10±0.34a	18.00±1.58a	1.88±0.22a	2.15±0.29b	0.59±0.06a	1.20±.10b
3	23.81±1.19a	6.654±0.51a	16.20±1.92a	2.084±0.16a	2.62±0.29a	0.59±0.07a	1.44±0.10a
4	23.86±1.75a	6.04±011a	16.80±3.27a	2.022±0.13a	2.60±0.25a	0.57±0.08a	1.05±0.14c
5	21.53±0.33b	5.87±0.43a	19.20±1.18a	1.77±0.11b	2.11±0.27b	0.40±0.04b	0.86±0.06d

Subtitle: PH - Plant Height (cm); SD - Stem Diameter (mm); IN - Inflorescence Number; LDM - Leaf Dry Mass (g.plan^{-1}); SDM - Stem Dry Mass (g.plant^{-1}); IDM - Inflorescence Dry Mass (g.plant^{-1}); RDM - Root Dry Mass (g.plant^{-1}). The lowercase letters points the significant differences between treatments, according to Scott-Knott ($p < 0.05$) test.

To the variables which determine the plant mass gain, it can be observed that with the doses 1, 2, 3 and 4 mg.L^{-1} promoted higher leaf biomass (LDM), with significant difference to the control plants and the plants with 5 mg.L^{1} (Table 3). In the stem, the doses of 3 and 4 mg.L^{-1} were the ones which promoted higher mass accumulation as indicated in SDM.

The inflorescence, through the IDM variable, presented similar behavior with the leaves, with more mass gain in 1, 2, 3 and 4 mg.L^{-1} doses, statistically differing of the 5 mg.L^{-1} and control dose.

The Se in root promoted more mass gain (RDM) with 3 mg.L^{-1} dose differing from other doses. It can be noticed that the 5 mg.L^{-1} dose affected in a negative way the biomass gain of the jambu root, indicating toxicity signs.

Nutrient Content

The Se had an influence on the content of macronutrients K, Mg and Ca in jambu. with the increase in the concentration of the element up to the dose of 3 mg.L^{-1}, there was greater uptake of macronutrients, with a significant difference for the control plants. The plant's ability to capture K, Mg and Ca begins to be harmed from a dose of 4 mg.L^{-1}, reaching the maximum dose reduction at 5 mg.L^{-1} (Table 4).

Table 4 – Total content of nutrients absorbed by jambu exposed to increasing doses of Se.

Se doses in mg.L^{-1}	Macronutrientes content in g.kg^{-1}			Micronutrient content in mg.kg^{-1}		
	K	Mg	Ca	Fe	Zn	Mn
Control	78.54±2.26 e	19.90±1.38 e	4.14±0.17 c	1.76±0.15 c	3.28±0.09 e	0.25±0.01 b
1	99.06±4.17 c	25.25±0.66 c	9.25±0.26 b	2.02±0.12 b	3.46±0.15 d	0.26±0.03 b
2	103.77±1.91 b	26.25±0.54 b	9.16±0.25 b	2.11±0.06 b	3.80±0.13 b	0.24±0.01 b
3	108.67±1.91 a	32.46±0.59 a	9.89±0.66 a	2.10±0.07 b	4.27±0.14 a	0.30±0.02 a
4	102.48±2.64 b	26.70±0.47 b	9.17±0.52 b	2.38±0.08 a	3.60±0.08 c	0.25±0.03 b
5	93.70±3.91 d	24.14±0.80 d	8.76±0.29 b	2.06±0.06 b	3.62±0.09 d	0.24±0.01 b

The lowercase letters points the significant differences between treatments according to the Sscott-Knott ($p < 0.05$) test

The micronutrient Fe, despite having the same absorption tendency of K, Mg and Ca, had its highest content in jambu with 4 mg.L^{-1} of Se in solution, with a reduction in Fe content with an increase in the dose of Se. Zn showed a higher absorption capacity up to the dose of 3 mg.L^{-1}, when it starts a significant reduction in subsequent doses.

The highest Mn content was notably induced by the dose with 3 mg.L^{-1} of Se, and the other doses of Se did not interfere with the absorption of the micronutrient.

Multivariate Indicators

The jambu plants when submitted to substances presence, being nutrients, beneficial elements or toxic, tend to answer according to the exposure level and assimilation form to these substances. Many variables can be used to point to the beneficial effect or harm effect of substances in plants, which can be pointed biometric variables, physiologic, biochemical, chemical and others. However, most studies isolate these variables to individualize the effects in each plan condition. In this work, in addition to this traditional analysis, the global behavior of jambu in response to selenium was evaluated. And, based on this new knowledge, the optimal dose of the element in nutrient solution was estimated, considering the indicators generated from the multivariate analysis.

Growth Indicator (*gI*) and Mass (*mI*)

This dimension includes the results of the growth indicator (***gI***), composed by the variables plant height (PH), stem diameter (SD) and inflorescence number (IN) and mass (***mI***) composed by the variables leaf dry mass (LDM), stem dry mass (SDM), inflorescence dry mass (IDM) and root dry mass (RDM), and the individual results of the variables.

Although some variables shows differences between the Se doses, the growth indicator, which allowed the analysis of the total plant behavior, does not reveal significant differences statistically between Se doses, however, it shows a positive difference of the control plants. From the regression specified for the ***gI***, the dose of 3.36 mg.L^{-1} of Se was estimated, which generated the maximum growth of jambu and that can provide more than 17% growth in relation to non-biofortified plants.

The mass gain indicator (***mI***) has information about the variables leaf dry mass, stem, inflorescence and root.

It can be observed that the dose with 3 mg.L^{-1} promoted higher biomass gain in jambu, differentiating itself from other doses and control.

The dose of 5 mg.L^{-1} showed lower mass gain in relation to the other doses, but still stood out in relation to the control plants. The behavior of the indicator shows that there was an increase in jambu mass gain with increasing Se doses up to 3 mg.L^{-1}. Subsequently, a decrease occurs as the concentration of Se in the nutrient solution increases.

The regression model for the ***mI*** allowed to estimate the concentration levels 2.77 mg.L^{-1} which results in maximum production of biomass, with an increase of 47% in relation to the plant non–supplemented.

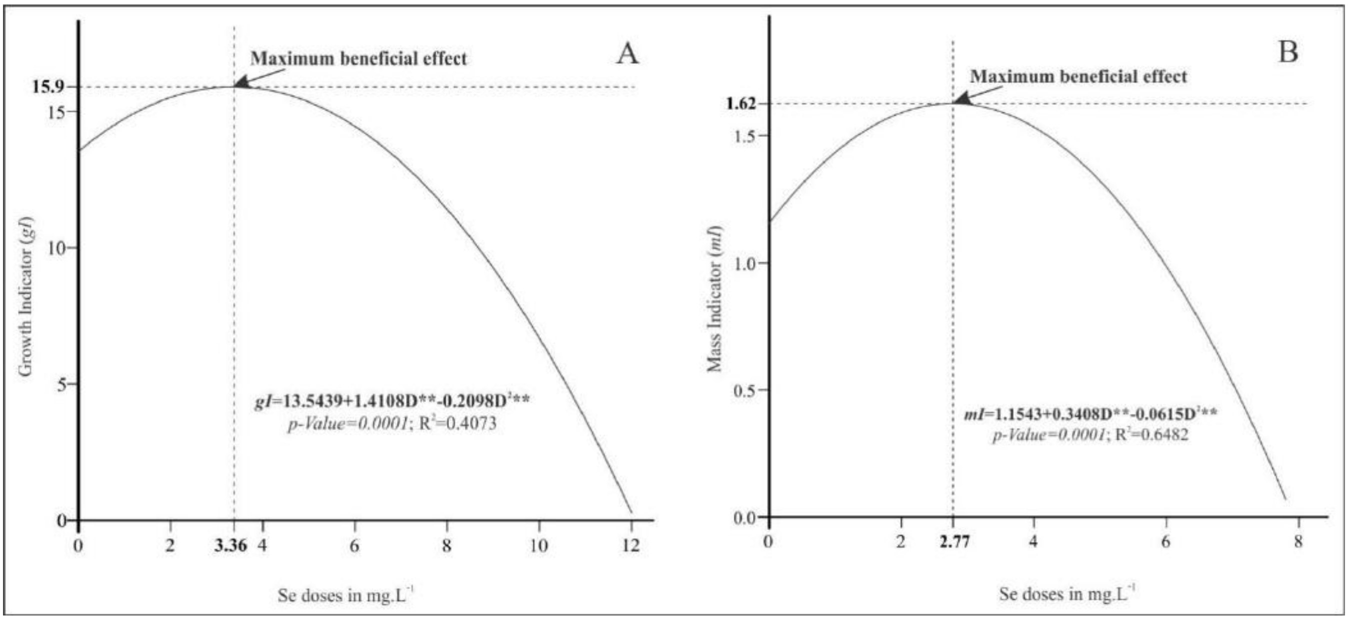

*Fig.2.Growth Indicator (A) and Mass Indicator (B) of jambu plants submitted to increasing doses of Se. The bars are the standard deviation; the lowercase letters points the significant differences between the Se doses according to the Scott-Knott ($p < 0.05$) test. **significant to 1% of probability; *significant to 5% of probability (test F); D=dose; D²=square dose.*

To the integrated analysis in nutrients uptake by jambu, it was constructed the nutritional indicator (***nI***) which make possible to observe the plant behavior when exposed to Se (Figure 3A). It can be noticed that the ***nI*** answer presented higher capacity to demonstrate how occurs the nutrients uptake, evidencing a absorption model in quadratic form, with the higher value in the dose 3 mg.L^{-1} and significant decrease up to 4 mg.L^{-1} dose. This response corroborates with the individual analysis of each nutrient (Table 4). According to the regression of indicator ***nI***, it was estimated that the dose promote the maximum nutrient absorption by jambu, 2.92 mg.L^{-1} of Se, in nutritive solution with increase of uptake capacity of nutrients in 45%. It is noteworthy that the value of the indicator in the dose with 5 mg.L^{-1} was significantly higher than the control, showing that although the plant reduces the concentration of nutrients in its tissues, this dose generates a greater value in the indicator and, therefore, , higher content of absorbed nutrients than plants not supplemented with Se (Figure 3A).

The general behavior of jambu when biofortified with Se, captured by the general indicator (***gnI***), showed the highest efficiency of the plant at the dose with 3mgL^{-1}, the ***gnI*** evidences the significant difference of the set of all explanatory variables stimulated by the element and makes the adequate model for the analysis of the biofortification of this vegetable (Figure 3B).

In all models, the data fit the 2° degree polynomial. In this aspect, the ***gnI*** translates the grouped behavior of all the explanatory variables analyzed of the biofortified jambu.

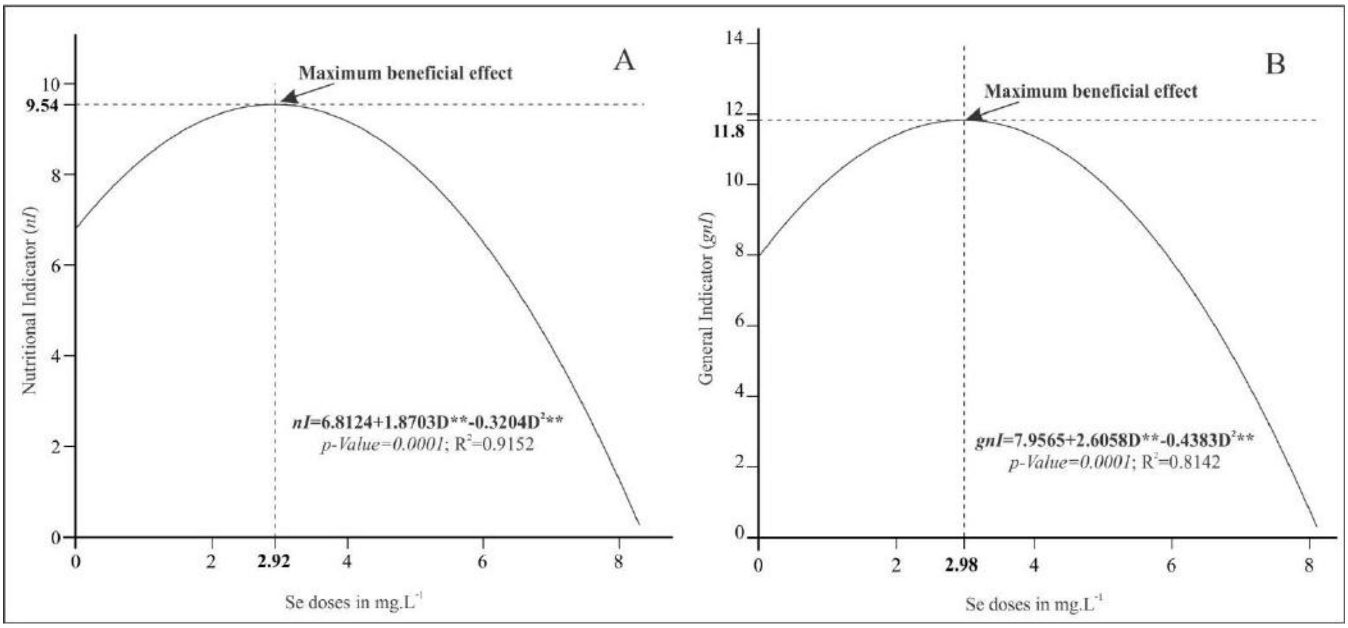

*Fig.3. Nutrient Indicator (A) and General Indicator (B) of jambu plants submitted to increasing doses of Se. The bars are the standard deviation; the lowercase letters points the significant differences between the Se doses according to the Scott-Knott ($p < 0.05$) test. **significant to 1% of probability; *significant to 5% of probability (test F); D=dose; D²=square dose.*

According to the mathematical model of ***gnI*** (Figure 3B), the plant has its development optimized in the concentration with 2.98 mg.L^{-1} and the possibility of development is 54% higher than jambu plants not biofortified with Se.

IV. DISCUSSION

The results showed that the Se content in the plant, especially in the leaf, inflorescence and stem (aerial part) was higher as the doses of the nutrient solution were increased, indicating similarity with the results obtained by Ramos, et al. [36] for lettuce cultivars subjected to Se in the form of selenate, by Puccinelli et al. [17] for basil plants, Sindelárova et al. [37] and Silva et al. [38] for broccoli and radish.

The translocation index (***TrI***) showed great efficiency to evaluate the uptake and partition of the Se element in jambu, with the leaves storing more than 70% in the first doses applied. At higher doses, ***TrI*** showed a reduction in the translocation potential. According to White [18], Se and sulfur (S) have great chemical similarity, competing for absorption routes. At low concentrations, Se acts positively on oxido-reductase enzymes with glutathione peroxidase, catalase, superoxide dismutase and ascorbate peroxidase, reducing the activities of reactive oxygen species [39].

The presence of Se at doses above the carrying capacity, especially in species that do not accumulate the metal, causes nutritional imbalance, affecting growth and production. This behavior was detected in this study through the tolerance index (***tI***), demonstrating that jambu tolerates up to 5 mg.L^{-1} of Se in the nutrient solution. From this dose, there is a reduction in the Nutrient Use Efficiency (***Nue***). Therefore, the results show that jambu achieves greater nutritional efficiency with ***Nue*** in the order of 0.29±0.03 g.plant^{-1} and an ***tI*** in the order of 1.49±0.24 g.plant^{-1} with 3 mg.L^{-1} of Se in solution.

This reduction of ***tI*** and ***Nue*** may occur due to competition for absorption routes between Se and S in the nutrient solution. The higher concentration of Se causes a lower absorption of S, replacing it in sulfur amino acids, glutathione and other compounds of the sulfhydryl group. This reduced dry biomass production and plant growth. This behavior was described by White et al. [39] in *Arabidopsis thaliana*, and shows the reduction in translocation capacity in some plants with increasing Se concentration in the medium.

The performance of jambu against Se is more evident in the multivariate indicators. The behavior of the growth indicator (***gI***) showed that the presence of Se induced an improvement of 17 % in the variables that compose it, showing a curve of maximum growth reached at the estimated dose of 3.36 mg.L^{-1} of Se in the nutritive solution. Therefore, the growth of jambu reaches the technical optimum with this dose.

The mass indicator (***mI***) shows a gain of 47%, with the maximum efficiency estimated at 2.77 $mg.L^{-1}$ of Se. This indicator also presents quadratic behavior, indicating that as the maximum efficiency dose is exceeded, there will be a reduction in mass gain.

As seen in the ***Nue*** index, doses close to 3 $mg.L^{-1}$ of Se produce greater absorption of nutrients, a fact corroborated by the nutritional indicator (***nI***) that shows a value of 2.90 $mg.L^{-1}$ as the ideal dose for the highest nutrient concentration with a 45% increase in the plant's ability to uptake nutrients. Also similar to the ***Nue*** and ***tI*** indices, as well as the growth and mass indicators, ***nI*** presents a polynomial behavior, with a reduction in the nutrient absorption capacity as the doses of Se are increased.

Regarding the evaluation of indicators, the general indicator (***gnI***) presents similar behavior to the other indicators and indices and shows that the ideal dose for the best development of jambu in response to selenium is 2.98 $mg.L^{-1}$. Values above this level can cause the plant to reduce its development potential.

Mostofa et al. [40], Reis et al. [41] and Silva et al. [42], show that concentrations of up to 100 $mg.L^{-1}$ of Se in nutrient solution caused a drastic reduction of chlorophyll, carotenoids and dry biomass in rice seedlings and a 90% reduction in the concentration of chlorophyll and carotenoids in beans caupi, a fact that may be related to the incorporation of Se into proteins and amino acids, resulting in selenoproteins in higher concentration than sulfur proteins and amino acids [43];[44]. In this work, the Translocation, Tolerance, Nutrients Use Efficiency, as well as the multivariate indicators showed that the optimal range of biofortification for jambu ranges from 2.77 $mg.L^{-1}$ to 3.36 $mg.L^{-1}$, with maximum value of Se supplementation in the order of 2.98 $mg.L^{-1}$ in nutrient solution.

Se is an essential element in human metabolism and the consumption of fortified foods can play a role in mitigating the effects of heavy metal intoxication, in addition to acting to prevent cancer and cardiovascular diseases [44];[45]. In addition, the biofortification of a plant such as jambu, which already brings with it antioxidant compounds, dietary fibers, proteins, carbohydrates, among other bioactive compounds, is an excellent alternative to reach populations in nutritional fragility around the world and especially in the Amazon due to its high consumption in the region.

What should be observed regarding biofortification with Se are the daily intake limits of the element. For the diet of the adult population, ANVISA recommends at least 8.25$\mu g.day^{-1}$ and up to 319.75$\mu g.day^{-1}$ can be ingested. Values below 8.25$\mu g.day^{-1}$ are considered deficient and above 319.75$\mu g.day^{-1}$ can cause toxicity [46];[47].

The daily consumption of 100 g of jambu biofortified with 2.98 $mg.L^{-1}$ will provide 50.13 $\mu g.day^{-1}$ of Se, above the minimum recommended by ANVISA, thus guaranteeing easy access for populations that are food insecure. to a product, which in addition to being biofortified, is a source of proteins, carbohydrates, among other compounds.

V. CONCLUSIONS

The results indicated that jambu can be biofortified with small doses of Se in nutrient solution by increasing dry biomass production, nutrient assimilation and plant growth. The construction of multivariate indicators proved to be adequate to understand the behavior of the plant in the face of exposure to Se. above all in terms of the general indicator, the ideal dose of Se in a nutrient solution is 2.98 $mg.L^{-1}$, which should be used as a beacon to produce a biofortified vegetable that is safe for human consumption.

The daily consumption of 100 g of jambu biofortified with 2.98 $mg.L^{-1}$ of Se provides a daily dose of 50.13 µg of Se, being an excellent nutritional alternative for several countries where it is grown, especially those that suffer from scourge of malnutrition, assisting in government policies to achieve the goals of “zero hunger”, good health and well-being”, of the sustainable development of the 2030 Agenda, especially after the COVID-19 pandemic.

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