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THIAMINE AS A PROMOTER ON INITIAL DEVELOPMENT IN VARIETIES OF SUGARCANE

Tiamina como promotora del crecimiento inicial en variedades de caña de azúcar

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ABSTRACT. This work aims to know thiamine response as a growth promoter in sugarcane varieties. A completely randomized experimental was installed and designed in a double factorial scheme at 3x4 levels, in which the first factor consists of a variety of sugarcane: RB86-7515; RB96-6928 and CTC-4; the second factor was thiamine doses in five levels: zero mg L^{-1}; 100 mg L^{-1}; 200 mg L^{-1}; 400 mg L^{-1} and 800 mgL^{-1}; fifteen treatments were made with five replications, 75 plots in total. Forty five days after planting the following variables were determined: ESI – emergence speed index; ALA – average leaf area; APL – aerial part length; DMAP – dry mass of aerial part ; DMR – dry mass of root; XD – leaf xylem vessel diameter and PD – leaf phloem vessel diameter. It is recommended doses of 400 mg L^{-1} Thiamine use as a promoter of growth in varieties RB86-7515; RB96-9628 and CTC-4 of sugarcane. Emergence speed index and diameter of xylem vases of leafs in CTC-4 varieties showed a negative response to Thiamine use. However, since the work was conducted only once, more studies are needed to prove the results.

Key words: *Saccharum* sp., vitamin, dry mass, xylematyc vasess, phloematic vasess

RESUMEN. El objetivo de este trabajo es conocer la respuesta de la tiamina como promotora de crecimiento en variedades de caña de azúcar. Se instaló un experimento mediante un diseño completamente aleatorizado en esquema factorial doble en los niveles 3x4, en el cual el primer factor consiste en variedades de caña de azúcar, siendo ellas: RB86-7515; RB96-6928 y CTC-4; el segundo factor fueron dosis de tiamina variando en cinco niveles, o sea: cero mg L^{-1}; 100 mg L^{-1}; 200 mg L^{-1}; 400 mg L^{-1} y 800 mg L^{-1}; que representan 15 tratamientos con cinco repeticiones, totalizando 75 parcelas. Cuarenta y cinco días después de la siembra se determinaron las siguientes variables: IVE - índice de velocidad de emergencia; AFM – área foliar media; CPA – longitud de la parte aérea; MSPA – masa seca de la parte aérea; MSR – masa seca de raíz; DX – diámetro de los vasos xilemáticos de la hoja y DF – diámetro de los vasos floemáticos de la hoja. Se recomienda el uso de 400 mg L^{-1} de Tiamina como promotora de crecimiento en las variedades RB86-7515; RB96-9628 y CTC-4. El índice de velocidad de emergencia y el diámetro de los vasos xilemáticos de las hojas de la variedad CTC-4 presentó una respuesta negativa al uso de la Tiamina. Sin embargo, como el trabajo se llevó a cabo sólo una vez es necesario realizar más estudios para la comprobación de los resultados.

Palabras clave: *Saccharum* sp., vitamina, masa seca; vasos xilemáticos, vasos floemáticos

INTRODUCTION

It is perceptible that the roots of a plant are as large as its ability to explore available soil, nutrients and water. It has been shown that most of the root system in sugarcane is found in the first 0.30 cm of soil (1).

The root system of the cane represents an important field of study, since it has an essential role in the regeneration of the ration after the harvest, in addition, directly influences the efficiency of the absorption of nutrients in the plants, in the resistance to the dry terms and in the tolerance to the attack of plagues, that affect the development of all the plant. However, the root system of sugarcane has been relegated to research, due to the difficulty of access and visualization, mainly in outdoor evaluations (2).

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The active form of vitamin B, thiamine pyrophosphate (TPP), is an indispensable cofactor for specific enzymes that act on the metabolism of amino acids and carbohydrates. Its biosynthesis occurs due to the independent formation of its compounds, such as pyridine and thiazole. The synthesis route of vitamin B in prokaryotes has been clarified; however, studies are lacking regarding eukaryotes. In Arabidopsis thaliana, the Thi1 protein is probably responsible for thiazole synthesis, once a compound for TPP was found in its structure (3). The vegetative propagation of potatoes in liquid cultures, as different concentrations of pantothenic acid, vitamins and thiamine were used, led to a positive response on the multiplication rate of the plant material (4).

The use of thiamine can cause changes in the anatomy of the plant, visible to the naked eye that, in fact, are derived from changes in the structures of the epidermis, fundamental or vascular tissues of plants, so it is necessary to have a deep knowledge of these transformations caused by changes in the environment (5). The symptomatology is widely used to evaluate the damage caused by biotic or abiotic factors. In this case, the structural aspects help to understand the mechanisms that cause the injuries (6).

The objective of this work is to know the different responses, using thiamine as a promoter of growth in sugarcane varieties.

MATERIALS AND METHODS

The experiment was carried out in the Faculty of Agricultural and Technological Sciences of the State University of São Paulo, in Dracena, State of São Paulo, Brazil, with geographic coordinates: 21 ° 29'10.24 "S and 51° 31' 41.29"Or, with an average of 411 m above sea level, in April 2016.

The local climate, according to the Köppen classification, is of the Cwa type: warm climate in summer and dry in winter, with the highest rainfall rates between November and March. The average annual temperature varies between 30.4 °C and 19.2 °C, the average precipitation of 1 311.6 mm and the humidity of the air of 78 %.

The soil was collected in the depth of 30-50 cm and classified as yellow red Argissolo (7), with the chemical parameters shown in the Table 1 (8).

The experimental design was completely randomized with three varieties of sugarcane: RB86-7515; RB96-6928 and CTC-4; the second factor refers to thiamine doses, in five different levels: null L^{-1}; 100 mg L^{-1}; 200 mg L^{-1}; 400 mg L^{-1} and 800 mg L^{-1}; within 15 treatments and 5 repetitions, 75 plots in total.

The plots were planted in plastic pots, with a capacity of 9 dm^3 of sifted soil and were corrected according to the nutritional requirements of the crop, urea fertilizers, super simple and potassium chloride were used. The experiment was installed and conducted in an unprotected and watered environment according to the soil moisture factor.

During the first 30 days, the following was evaluated or calculated: ESI- emergency speed index, according to the calculation formula ESI= PN / NDTE, in which PN=number of emergent grower and NDTE= number of emergency days of the cane.45 days after sowing, the following variables were evaluated or calculated: ALA-average leaf area (9) and NL=number of leaves; APL-aerial part length , established through a graduated centimeter rule; DMAP-dry mass of the aerial part and DMR - dry mass of the root, in which all the developed green mass was dried in a laboratory oven with circulation and air renewal at a temperature of 65 ° C, until reaching a constant weight . For the treatment 10 plants were used. Forty-five days after sowing, the ultrastructural characteristics of the sugarcane leaf, the five-centimeter fragment of the mid-leaf region of the central mid-third of the stem, and a five-centimeter fragment of the leaf were evaluated. Middle root region. The fragments were transported to the Laboratory of Plant Morphology and Forage in the Faculty of Agricultural and Technological Sciences - State University of São Paulo. All the collected material was immersed in F.A.A. 70 (37% formaldehyde, acetic acid and 70% ethanol in the ratio of 1.0: 1.0: 18.0 -V/V).

After 24 hours, the fragments were washed and stored in 70% ethanol until the analyze date (10). All fragments of plant tissues received the relevant procedures for dehydration, diaphanization, inclusion and embedding, by using a Leica microtome containing steel blades, cross sections of 8 μm were made in each embedded fragment.

Table 1. Chemical parameters of the soil

pH	MO	P	K	Ca	Mg	H + Al	Al	SB	CTC	V%	m%	S	B	Cu	Fe	Mn	Zn
$CaCl_2$	g dm^{-3}	mg dm^{-3}				$mmol_c$ dm^{-3}								mg dm^{-3}			
4,7	9,0	2,0	1,2	12	5,0	16,0	2,0	20,2	36,2	56,0	9,0	6,0	0,22	0,6	4,0	13,6	0,2

SB: sum of bases; V%: base saturation; m%: Al saturation

The first transverse sections without damage caused by cutting the tissues of the plants were chosen for the preparation of the histological slides. They were fixed with patches (albumin), stained with safranin in a proportion of 1% and placed in a microscope and glass slides with Entellan® patch (10).

All the slides were observed with an optical microscope Olympus model BX 43, with an attached camera to make the photographs of the cuts. The images were used to measure anatomical parameters through the cellSens Standart software, which was calibrated with a microscopic rule with the same gains (11).

By using cross sections, the following anatomical features of morphology were measured: diameter of the xylematic vessels of the leaves (XD) and diameter of the phloem vessels of the leaves (PD). Five measurements were made for all the features on each microscope slide. The plots were represented by the average value obtained in each characteristic. All the variables were subjected to the F test ($p < 0.05$) and the regression analysis was applied to the thiamin doses, in which their models were tested: linear, quadratic and cubic (12). The static software Assistat 7.7 (13) was used.

RESULTADOS Y DISCUSIÓN

As shown in Figure 1, a significant quadratic effect was observed in the doses of thiamin in the sugarcane types RB86-7515 and RB96-9628 in the variable emergency rate index, however, a linear effect was observed decreasing to the CTC-4 variety.

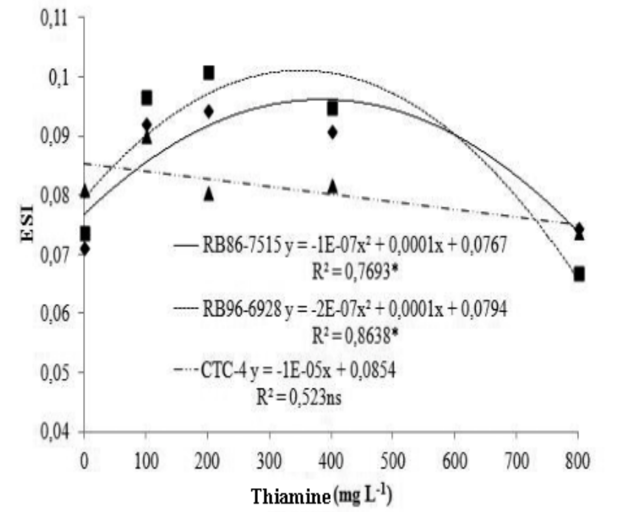

Figure 1. Index of emergency speed in sugar cane subjected to thiamine dose

Peak doses of 400 and 350 mg L^{-1} of thiamine were observed in the varieties RB86-7515 and RB96-9628, which show a positive response in the emergence speed in sugarcane. However, the use of thiamine in CTC-4 was not significant because of its application. Due to the stimulation triggered by the use of thiamine in the physiology of the sugarcane gems, its appearance was high; however, when there was an increase in the doses of this compound, there was a decrease in this index where doses higher than 400 mg L^{-1} of thiamine, in solution, were harmful for the cultivation of sugarcane. The opposite results were observed when studying the spread of Brazilian oak (14), since there was no positive response to the use of thiamine in the participation of oak, which did not release new outbreaks.

It is known that thiamin is a precursor of the NAD molecule (15), which is important in the energy balance in the electron transport chain, which can play a key role in the availability of energy, in the growth of cane shoots of sugar, in the early stages of development; In addition, it acts on the biosynthesis of the thiazole molecule, as a precursor of the decarboxylase enzymes that favor the vegetable.

A significant quadratic effect was observed on the use of thiamine in varieties of sugarcane in the average leaf area, as shown in Figure 2.

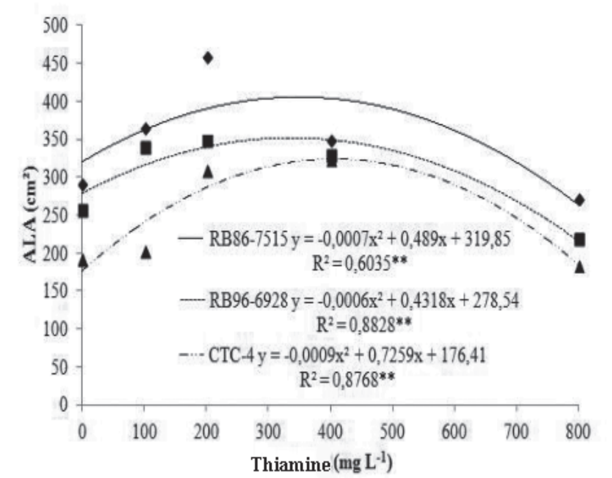

Figure 2. Average leaf area of sugar cane subjected to thiamine dose

Maximum points of 349.28; 359.83 and 403.27 mg L^{-1} were observed respectively in RB86-7515, RB96-9628 and CTC-4, which demonstrates the effectiveness of the use of this compound in sugar cane cultivation, since it provided an increase in its leaf area up to the dose of 400 mg L^{-1}. In the event that a plant has a larger leaf area, its photosynthetic rate is enhanced and, consequently, there is an increase in its growth (16).

The action of thiamine on the synthesis of thiazole should occur in chloroplasts (17), while the final step of pyrophosphorylation of thiamine to diphosphate (DPT) apparently occurs exclusively in the cytosol, which then serves as a cofactor in enzymatic reactions that occur in the mitochondria, which can favor the supply of energy to the cells. Unless alternative routes of thiamine are discovered in the mitochondria and chloroplasts, the important organelles promote a higher leaf area index in these plants, which influences the growth of their aerial part (3,18), as shown in Figure 3.

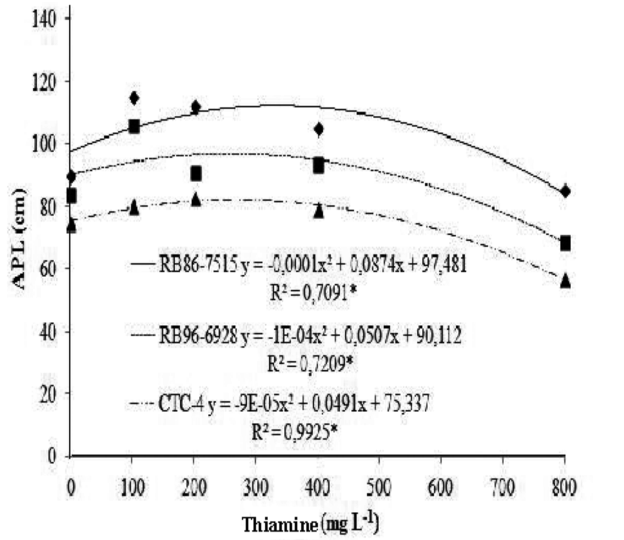

Figure 3. Length of aerial part of sugarcane submitted to thiamine dose

A quadratic effect was observed in the variable of length of the aerial part in the varieties RB86-7515, RB96-9628 and CTC-4 when subjected to the use of thiamine, in which they reached their peaks of growth in doses of 437.00; 253.5 and 272.77 mg L^{-1}, respectively.

The development of the aerial part of the plant is directly related to the rate of carbon assimilation of photosynthesis in the leaves (19), since, due to its elevation of the aerial part of the leaf, it can contribute to its development, demanding a greater amount of energy for its metabolism of structural protein (3,15) and promoting a greater delay of atmospheric carbon in its dry mass aerial part, according to Figure 4.

The varieties RB86-7515, RB96-9628 and CTC-4 showed a significant quadratic effect in the variable dry mass of the aerial part. Again, the doses of thiamine around 400 mg L^{-1} had positive results, due to the great metabolic process that occurs in the aerial part of the plants, mainly in the leaves. Thiamine may have provided greater efficiency in these processes, since it provides a greater amount of energy to your metabolism (15,20), in which thiamine may undergo phosphorylation in its hydroxyl group, in order to produce monophosphate esters, diphosphate and triphosphate. Then DPT acts as a cofactor of mitochondrial pyruvate dehydrogenase during glucose and favors energy availability.

Other factors can influence the development of the aerial part and the accumulation of the dry mass, such as: luminosity (11), nutrients and even the presence of heavy metals that release oxidative stress in the plant, which initiates those metals in their tissues (17, 21), in which the action of thiamin favors antioxidant stress through the enzyme decarboxylase in their physiology (18,20,22).

The development of the aerial part can be enhanced with the greater absorption of nutrients and water by the root system, as indicated in Figure 4B. It has been reported that patchouli propagules that remain with the leaves, have a higher percentage of rooting (23).

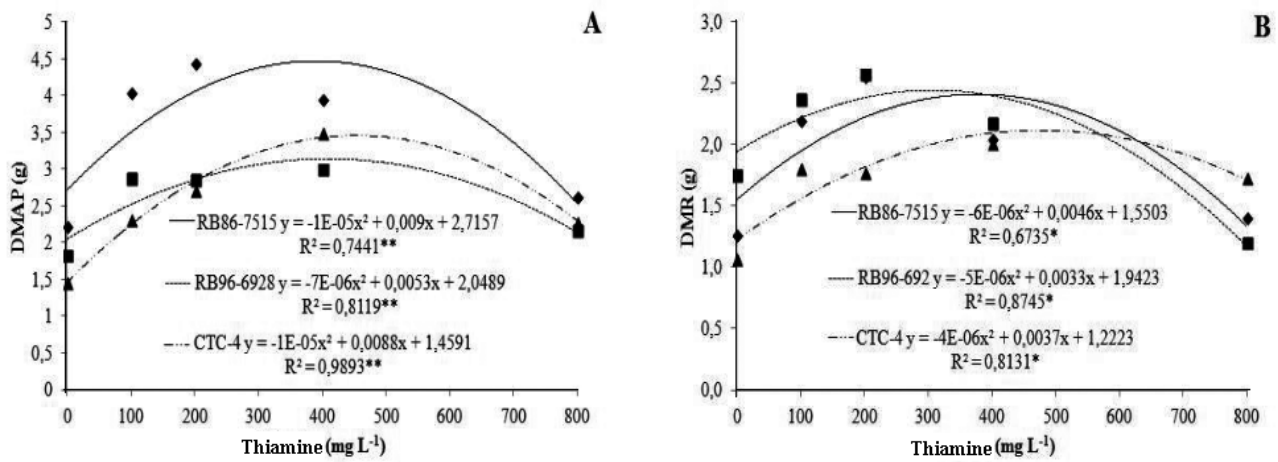

Figure 4. Dry mass of aerial parts and root of sugarcane subjected to thiamine dose

A significant quadratic effect was observed in RB86-7515, RB96-9628 and CTC-4 subjected to doses of thiamine, which showed an increasing development of the root to a dose close to 400 mg L^{-1}. The plants need thiamine for the development of the root (17); however, some species, such as clover, do not have thiamin as requirements for that function. Due to a greater provision of thiamine to the stem and root apical cells, growth is favored, which shows that sugarcane is susceptible to the use of thiamine in the phases of establishment of the cultivar in its substrate, mainly in its root development.

This increase in the development of plant organs, such as leaf, stem and root, is related to the biosynthesis of organic molecules and carbon accumulation through photosynthesis. The metabolism of vegetables can be improved or maximized since their tissues show a greater development and perform their functions in their organs, as shown in Figure 5.

The xylem shows specific functions to the vegetal organism, in that way it stands out among the main tissues of the plant, since it has the capacity to transport sap, rich in salts coming from the soil.

As described (24), these tissues transport thiamin of exogenous origin, regardless of the energy conditions available for plant metabolism. In this way, a significant linear effect was observed on the variable diameter of the xylem vessels of the sugar cane leaves in the varieties RB86-7515 and CTC-4, which did not occur in the variety RB96-9628, as shown in Figure 6.

The variety RB86-757 had a positive response to the use of thiamine, since the CTC-4 had an opposite result, with a negative response. These results were not expected, since the other varieties responded positively, which makes further studies necessary.

With the increase in the diameter of the xylem vessels, the volume of non-metabolized sap may have occurred proportionally to this increase. In this way, a greater availability of salt, water and minerals to the leaves, leads to a greater delay of the dry mass in the aerial part (Figure 4A). A greater efficiency of the materials absorbed by the xylem could have provided a better development of the phloem of the leaves and the root.

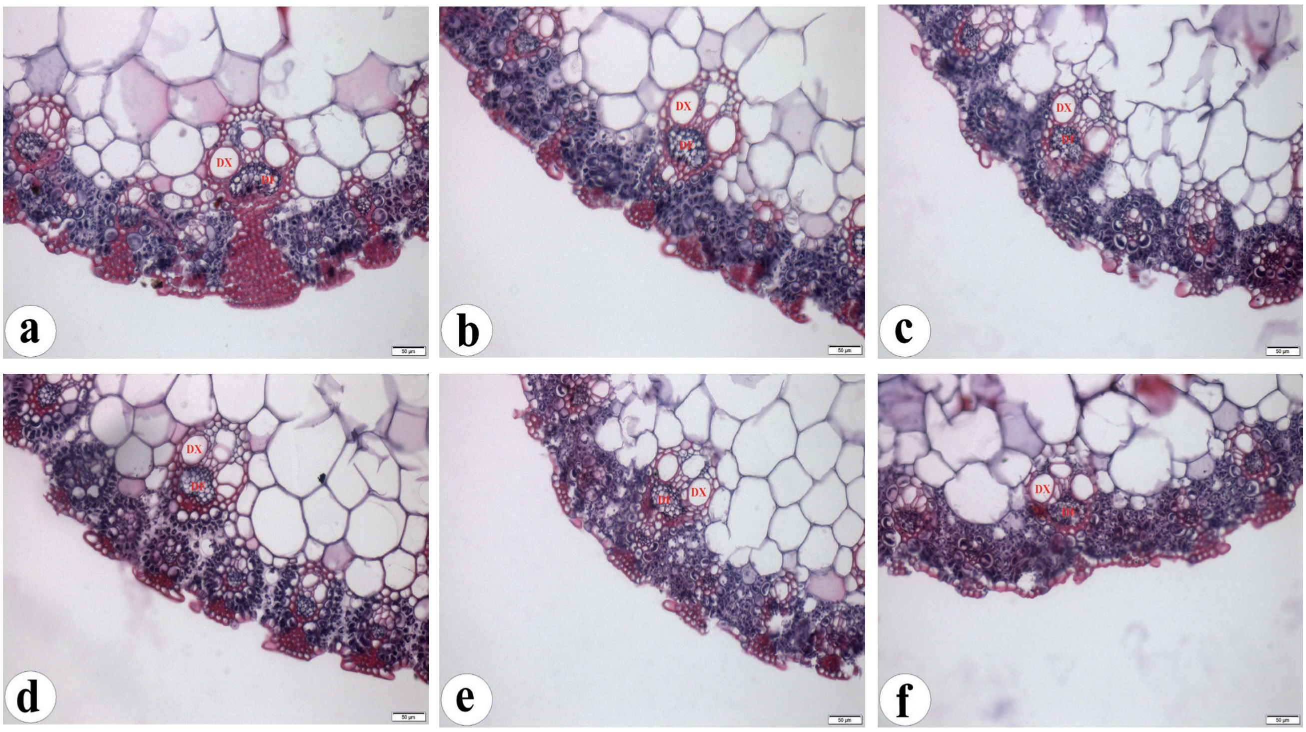

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Figure 5. XD- diameter of xylem vessels of sugarcane leaves and PD- diameter of the phloem vessel; a- leaf RB86-7575 with null mg L^{-1} of thiamine; b- leaf RB86-7575 with 400 mg L^{-1} of thiamin; c- RB96-9628 leaf with null mg L^{-1} of thiamin; d- RB96-9628 leaf with 400 mg L^{-1} of thiamin; e- CTC-4 leaf with null mg L^{-1} of thiamine and f- leaf CTC-4 with 400 mg L^{-1} of thiamin

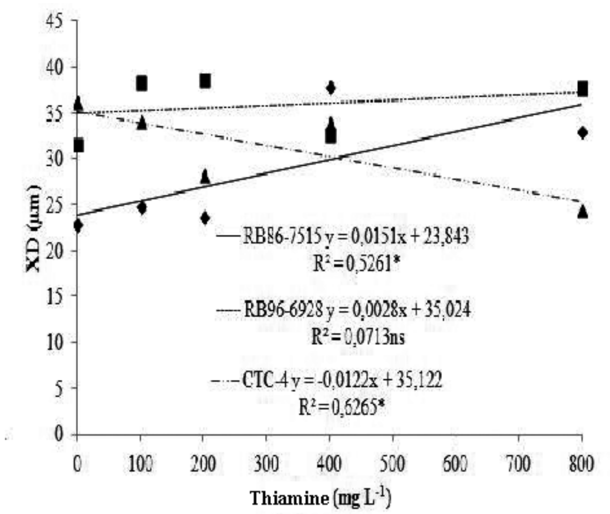

Figure 6. XD: Diameter of the vessel of the leaf xylem of sugarcane leaves subjected to doses of thiamin

It has been reported that xylem exudate shows a higher concentration of substances such as putrescine and spermidine, in the polyamine group, which shows that the substances are transported throughout the plant (25). This long-distance translocation also supports the hypothesis that polyamines have a regulatory role in the growth of the plant, as well as its response to stress, which shows the translocation of thiamine through this tissue (26,27). The presence of thiamine in the metabolism of the plant, caused by the distribution of this compound by the conductive vessels may have caused an increase in the diameter of the phloem vessels, as shown in Figure 7.

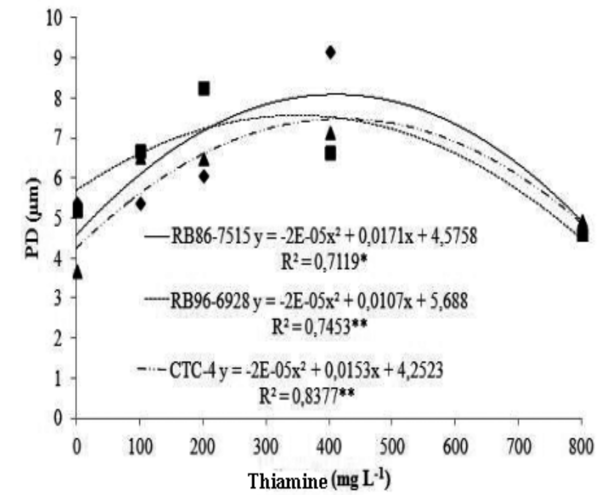

Figure 7. PD: diameter of the phloem vessel of sugar cane leaf subjected to thiamine dose

After the distribution of thiamine to other areas of the plant, through the xylem and the phloem, this compound fulfills its function as a precursor of thiamine pyrophosphate (TPP) (17), which begins to act as an indispensable co-factor for bound enzymes to the metabolism of carbohydrates and amino acids, its biosynthesis is produced by the independent formation of its compounds, mainly pyrimidine and thiazole (3,15,20,28), in this way the metabolism of sugarcane cells was benefited for the presence of thiamine.

CONCLUSIONS AND RECOMMENDATIONS

- The rate of appearance and the diameter of xylem vessels of leaves in CTC-4 varieties showed a negative response to the use of thiamine.
- Since the work was done only once, more studies are needed to test the results.
- Doses of 400 mg L^{-1} of thiamine use are recommended as a growth promoter in the varieties RB86-7515; RB96-9628 and CTC-4 of sugar cane.

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