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Anatomical Features of Sugarcane (*Saccharum officinarum* L.) Treated with Thiamine

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Authors' contributions

This work was carried out in collaboration between all authors. Author LAML designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SBR, VGV, VAA, WJMS, GGS, LSBA, IJPS and ACM managed the analyses of the study. Author PAMF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This worked aimed to know the anatomical features of sugarcane (*Saccharum officinarum* L.) treated with thiamine. A completely randomized experiment was used and designed in a double factorial scheme at 3x5 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 mgL^{-1}; 100 mgL^{-1}; 200 mgL^{-1}; 400 mgL^{-1} and 800 mgL^{-1}; fifteen treatments were made with five replications, 75 plots in total. Tissues from the leaves and roots were influenced by the exogenous action of

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thiamine as used at planting, displaying a positive response, doses above 400 mg L^{-1} can be a limiting factor to the development of these tissues. Factor regarding the sugarcane variety did not influence the anatomy of leaves and roots. Concentrations till 400 mg L^{-1} of thiamine, at the exogenous administration, promoted a better development on morph-anatomic features of leaves and roots in the planting of sugarcane seedlings.

Keywords: Saccharum sp.; vitamin; phloem; xylem; B1.

1. INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) belongs to family Poaceae and has a fasciculate root system that does not deeply reach its substrate, which may harm the development as it suffers water stress, once it does not have the capacity of water absorption in deeper layers of soil [1]. The use of synthetic or natural molecules at the exogenous administration in sugarcane seedlings can promote better roots at the initial stage, leading to a greater development of the stems, among these molecules, vitamin B1 highlights [2].

The active form of Vitamin B1, thiamine pyrophosphate (TPP), works as a cofactor to the reaction of enzymes that acts on carbohydrate synthesis as well as some amino acids [2]. Its synthesis occurs on the formation of independent compounds, mainly pyrimidine and thiazole. In prokaryotes the way of vitamin B1 synthesis has been explained, however, regarding eukaryotes, there is a lack of studies [3]. In *Arabidopsis thaliana*, Thi1 protein is likely responsible by thiazole syntesis, once a compuond related to TPP has been found in its structure [4].

These modifications in organs of plants as submitted to use of vitamins are barely studied, once leaves present high plasticity of adaptations as exposed to the environmental stimulus and even to these biomolecules [5]. That way, thiamine function on vegetables' metabolism can be related to process of formation of Acetil-Coa in the Krebs cycle, favoring the development of roots, which presents a higher cellular respiration [6], and, consequently, promotes a greater exploration of deeper layers of soil, guarantying a higher absorption of nutrients and water, which will be direct to aerial part of the plant, to the photosynthetic process [7].

Use of thiamine can cause alterations on the anatomy of plants, in its structures and arrangement of fundamental and vessels tissues [8], being necessary a deep knowledge of this features regarding the response to the use of this biomolecules. The transformations caused by the changes in the ambient where the vegetable was inserted [9] makes the symptomatology an important tool in understanding the damage caused by the mechanisms that cause morphological changes [10].

In this way, this worked aimed to know the anatomical features of sugarcane treated with thiamine.

2. MATERIALS AND METHODS

The experiment was carried out at College of Agricultural and Technological Sciences – São Paulo State University, in Dracena, São Paulo State, Brazil, geographical coordinates: 21° 29' 10.24" S and 51° 31' 41.29" W, with a 411m average above the sea level, on April 2016.

The local weather, according to Köppen classification, is the Cwa type: hot weather in summer and dry terms on winter, with the biggest rain rates between November and March. The annual average of temperature varies between 30.4°C and 19.2°C, average precipitation of 1311.6 mm and air humidity of 78%.

The soil was collected in the depth of 30-50 cm and classified as Argissolo red yellow [11], with the following chemical parameters, as shown in Table 1 [12]:

The experimental design was completely randomized with three varieties of sugarcane: RB86-7515; RB96-6928 and CTC-4, the second factor refers to doses of thiamine, in different five 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, in total 75 plots.

The plots were planted in plastic pots with a capacity of 9.0 dm^3 of sieved soil and corrected according to the nutritional requirements of the crop, the urea, super simple and potassium chloride fertilisers were used [12]. The experiment was conducted in an unprotected environment and irrigated according to the soil moisture.

Table 1. Soil chemical parameters

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%: Saturation Al.

Forty-five days after planting the total leaf area (TLA cm^2) was determined using the Easy leaf area image program [13]. At the same time, ultra structural characteristics of sugarcanes leaf were also evaluated, from fragment five with five-centimetre was taken from the median region of leaves from the central middle third of the stem and a five-centimetre fragment from the median root region. After 24 hours, the fragments were washed and stored in 70% ethanol until the date of analyses. All fragments of plant tissues received the pertinent procedures for dehydration, diaphanization, inclusion and embedding. By using a microtome that contains steel razors, eight- µm transversal sections were removed in each embedded fragment [14].

The first transversal sections without damage caused by the cut of plants tissues were chosen for preparation of the histological slides. These sections were fixed with patches (albumin), were tinted with safranin with a 1% ratio, and were set in microscope and glass slides wih Entellan®. All slides were observed with an Olympus optical microscope model BX 43, with an attached camera to the photographs of the cuts. Pictures were used to measure anatomic parameters through the software cellSens Standart that was calibrated with a microscopic ruler in the same gains.

By using transversal sections, the following morph-anatomic characteristics were measured: adaxial epidermal thickness (ADET); abaxial epidermal thickness (ABET); adaxial cuticle thickness (ADCT); abaxial cuticle thickness (ABCT); diameter of the beam buliform cells (DBBC); root phloem diameter (RPD); root xylem diameter (RXD) and thickness of the endoderm (TE). Five measurements were done for all characteristics in each microscope slide. Plots were represented by average value obtained on each characteristic. Values in micrometre (µm).

All variables were submitted to the F test ($p<0.05$) and the regression analysis was applied to the Thiamine doses, in which their models were tested: linear, quadratic and cubic [15]. For the sugarcane varieties, the Tukey test was applied at a 5% probability. Assistat 7.7 static software was used [16].

3. RESULTS

Variety RB96-6928 highlights among the studied varieties by presenting greater averages to the variable total leaf area (TLA), as Table 2 shows.

To others variables, a significant difference was not observed among the sugarcane varieties (Table1). However, as thiamine doses are considered, leaf area was not changed, which displays only the effect of sugarcane varieties, as Table 3 shows.

Only variety RB-96-6928 presented a linear response to the use of thiamine doses in the variable adaxial epidermis thickness (ADET) (Table 2), as Fig. 1 shows.

To the variable abaxial epidermis thickness (ABET) varieties of sugarcane RB86-7515 and RB96-6928 presented a quadratic growth curve as submitted to thiamine, in which they reach their peak at 441.17 and 410.44 mg L^{-1} doses. Doses above 400 mg L^{-1} presented a harmful effect, as Fig. 2 shows.

Table 2. Mean values of total leaf area (TLA); adaxial epidermal thickness (ADET); abaxial epidermal thickness (ABET); adaxial cuticle thickness (ADCT); abaxial cuticle thickness (ABCT); diameter of the beam buliform cells (DBBC) of sugarcane cultivated with thiamine

	TLA	ADET	ABET	ADCT	ABCT	DBBC
	cm²	---------------------------------- µm ----------------------------------				
RB86-7515	221.93b	9.89a	9.64a	4.93a	5.13a	4.93a
RB96-6928	453.16a	9.81a	9.57a	4.83a	4.75a	4.83a
CTC-4	260.81b	9.46a	8.56a	4.73a	4.32a	4.73a
MSD	121.64	1.19	1.38	0.71	0.91	0.71
CV%	57.25	18.03	21.96	21.71	28.25	21.71
MG	311.96	9.72	9.26	4.83	4.73	4.83
f	12.01**	0.43ns	2.19ns	0.24ns	2.30ns	0.24ns

*S: Stomata. MSD: Minimum significant difference. CV: Coefficient of variation. MG: Overall mean. f: value of F calculated in the analysis of variance; ** significant at the 1% probability level (p<0.01); * significant at the 5% probability level (0.01=<p<0.05); ns–not significant (p>=0.05). The averages in the column followed by the same letter do not differ statistically from each other. The Tukey test was applied at a 5% probability level. Source: August 2018.*

Table 3. The analysis of variance of the regressions of the thiamine doses applied, where the models were tested: linear, quadratic and cubic of variety sugarcane

System	Middle square							
	FV	GL	TLA	ADET	ABET	ADCT	ABCT	DBBC
	Concentration	4	16073.88	0.84	42.15	0.35	0.79	0.35
RB86-7515	Residue	21	27519.00	4.76	3.86	0.70	0.52	0.70
	Regression	1	Ns	Ns	Q**	Ns	Ns	Ns
	Concentration	4	43391.33	18.36	18.31	2.63	2.79	2.63
RB96-6928	Residue	21	57441.27	4.08	3.39	1.75	0.82	1.75
	Regression	1	Ns	L*	Q*	Ns	Ns	Ns
	Concentration	4	3804.79	1.52	11.85	0.21	0.55	0.21
CTC-4	Residue	21	20092.79	0.98	5.60	1.07	3.85	1.07
	Regression	1	Ns	Ns	Ns	Ns	Ns	Ns

*Ns–p>=0.05; *0.01=<p<0.05; **p<0.01. L: polynomial of 1st degree. Q: polynomial of 2nd degree. NL–number leaf; PH–plant height; DMAP– Dry mass of the air part. Source: August 2018.*

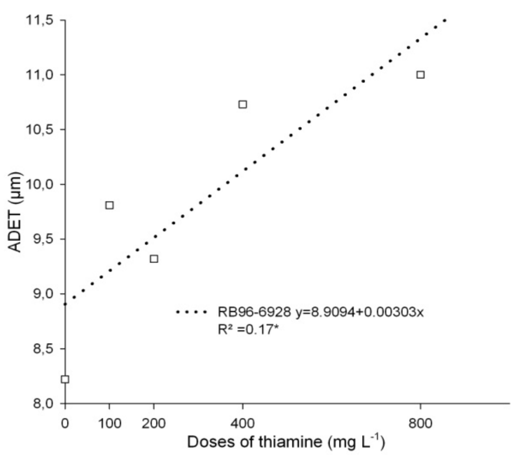

Fig. 1. Variable adaxial epidermis thickness (ADET) of varieity RB96-6928 thirty days after use of thiamine following the planting

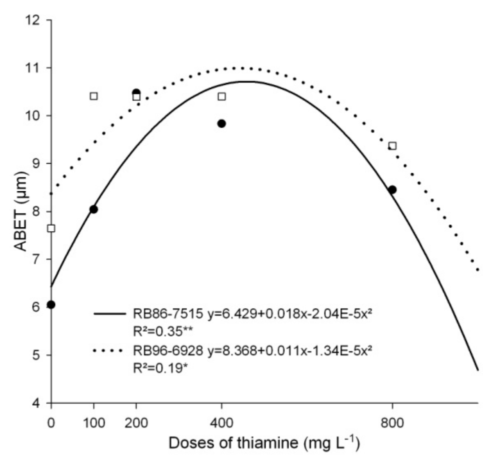

Fig. 2. Variable abaxial epidermis thickness (ABET) of varieties RB86-7515 and RB96-6928 thirty days after use of thiamine following the planting

No statistical difference was observed among the sugarcane varieties for the variables: root phloem diameter (RPD); root xylem diameter (RXD) and thickness of endoderm (TE) as Table 2a demonstrates.

When is considered the effect of the application of thiamine on sugarcane, variety RB86-7515 presented a quadratic response in the variable root phloem diameter, as Table 3a shows.

There was an increase in the phloem diameter of the variety RB86-7515 till, approximately, 434.13 mg L-1 thiamine doses, in which concentrations above 400 mg L^{-1} of thiamine may is a limiting factor for the development of sugarcane root phloem, as Fig. 3 shows.

It similarly occurs with Root Xylem Diameter (RXD), since only variety RB86-7515 presents a quadratic response to the use of thiamine at the sugarcane planting, reach its peak at 381.98 mg L^{-1} thiamine doses, as Fig. 4 shows.

Table 2a. Mean values of root phloem diameter (RPD); root xylem diameter (RXD) and thickness of the endoderm (TE) of sugarcane cultivated with thiamine

	RPD (µm)	RXD (µm)	TE (µm)
		-------------------------------- µm --------------------------------	
RB86-7515	9.99a	96.91a	22.47a
RB96-6928	9.14a	95.21a	20.83a
CTC-4	9.02a	88.73a	20.37a
MSD	2.15	9.31	2.13
CV%	33.70	14.60	14.73
MG	9.38	93.62	21.22
f	0.70ns	2.49ns	3.13ns

*S: Stomata. MSD: Minimum significant difference. CV: Coefficient of variation. MG: Overall mean. f: value of F calculated in the analysis of variance; **significant at the 1% probability level (p<0.01); *significant at the 5% probability level (0.01=<p<0.05); ns–not significant (p>=0.05). The averages in the column followed by the same letter do not differ statistically from each other. The Tukey test was applied at a 5% probability level. Source: August 2018.*

Table 3a. The analysis of variance of the regressions of the thiamine doses applied, where the models were tested: linear, quadratic and cubic of variety sugarcane

System	Middle Square				
	FV	GL	RPD	RXD	TE
	Concentration	4	112.61	2623.75	261.40
RB86-7515	Residue	21	8.87	157.85	16.13
	Regression	1	Q**	Q**	Q**
	Concentration	4	12.61	29.50	64.92
RB96-6928	Residue	21	10.05	364.07	5.17
	Regression	1	Ns	Ns	L**
	Concentration	4	26.81	24.78	363.47
CTC-4	Residue	21	10.46	125.37	11.34
	Regression	1	Ns	Ns	Q**

*Ns–p>=0.05; *0.01=<p<0.05; ** p<0.01. L: polynomial of 1st degree. Q: polynomial of 2nd degree. NL–number leaf; PH–plant height; DMAP–Dry mass of the air part. Source: August, 2018.*

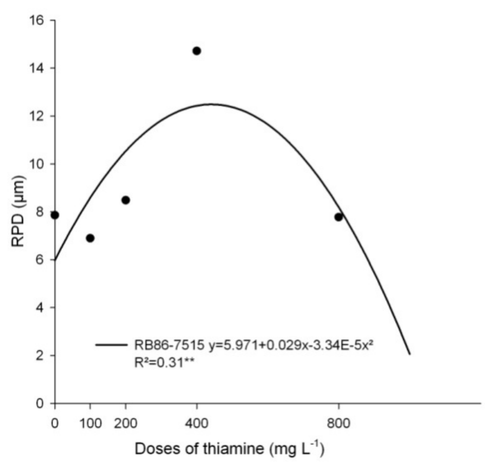

Fig. 3. Root Phloem Diameter (RPD) of the variety RB86-7515 thirty days after use of thiamine at the planting.

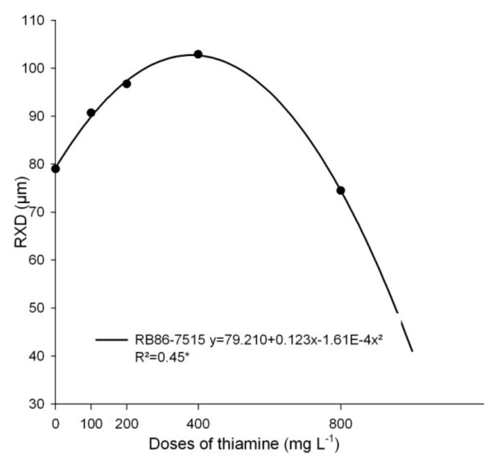

Fig. 4. Root Xylem Diameter (RXD) of variety RB86-7515 thirty days after use of thiamine at the planting

The variable thickness of endoderm (TE) all studied varieties significantly responded to the presence of thiamine at the planting. RB86-7515 presented a quadratic response till 491.15 mg L^{-1} thiamine doses, while CTC-4 displayed a negative quadratic response to the increasing of thiamine till 416.55 mg L^{-1} doses, however, RB96-6928 present a linear positive response, as Fig. 5 shows.

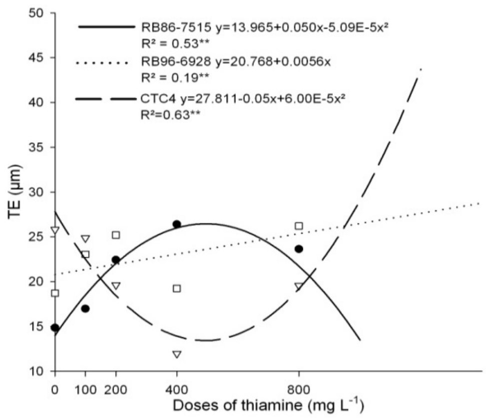

Fig. 5. Thickness of endoderm of varieties RB86-7515; RB96-6928 and CTC-04 thirty days after use of thiamine at the planting

4. DISCUSSION

Due to agronomic differences between varieties of sugar cane and the capacity of adaptation to different environmental conditions, these variations may reflect the productivity of culture, especially with the variation in leaf area, since, by reducing it, the photosynthetic rate impaired, harming the carbon fixation in the culture' dry mass [9].

Non-variation of leaves' and roots' anatomical features among the studied varieties affirms the importance of the exogenous use of thiamine, once the used doses of the vitamin entail a better development of leaf endoderm, due to the some carbohydrates' promoting action [3,17,2]. It was expected sharp changes in other areas of tissues, as in cuticle of the leaves, once it presents a high accumulation of oils and waxes, also, with the possible action of thiamine as cofactor in the synthesis of Acetyl-CoA, it could have entailed a bigger deposition of theses biomolecules [5,7].

Sheath cells of the vascular bundle could also have shown a greater development, since they present high biochemical reactions, mainly in the action of RuBPco molecule in the Calvin cycle [6,18,19] which could have potentialized in its reactions, leading to a greater development of the leaf area, and even enhancing the opening of the stomatal fissure, in which recent researches display that thiamine may act as an important factor in the opening and closure process, influencing the photosynthetic rate [5].

Due to the presence of thiamine, some polyamines may turn into synthesized and were carried by the vases of the vegetal organs, the presence of these biomolecules is an indicator of the reactions triggered by the presence of this vitamin [20,21,22], that way, conductors vases of the roots was influenced by the exogenous application at the planting, showing a positive response till 400 mg L^{-1}, doses above can be a limiting factor to the development of the tissues.

5. CONCLUSION

Factors regarding the sugarcane variety did not influence the anatomy of leaves and roots.

Concentrations till 400 mg L^{-1} of thiamine, at the exogenous administration, promoted a better development on morph-anatomic features of leaves and roots in the planting of sugarcane seedlings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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