UNDER PEER REVIEW

Review Paper

ANATOMICAL FEATURES OF SUGARCANE TREATED WITH THIAMINE

.

ABSTRACT. This worked aimed to know the anatomical features of sugarcane treated with thiamine. A completely randomized experiment was installed 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 exogenous action of 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 influenced on the anatomy of leaves and roots. Concentrations till 400 mg L^{-1} of thiamine, at exogenous administration, promoted a better development on morph-anatomic features of leaves and roots in planting of sugarcane seedlings.

Keywords: *Saccharum* sp., vitamin, phloem, xylem, B1

1. INTRODUCTION

Sugarcane belongs to family Poaceae and has a fasciculate root system that do 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 exogenous administration in sugarcane seedlings can promote better roots at 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 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

UNDER PEER REVIEW

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 environmental stimulus and even to theses 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 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. MATERIAL 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]:

UNDER PEER REVIEW

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.

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} e 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 fertilizers were used [12]. The experiment was installed and conducted in an unprotected environment and irrigated according to the soil moisture factor.

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-centimeter was taken from the median region of leaves from the central middle third of the stem and a five-centimeter fragment from the median root region. After 24 hours, the fragments were washed and stored in 70% ethanol until the date of analyzes. 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 done in each embedded fragment.

The first transversal sections without damage caused by 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 in order to perform 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 [14].

UNDER PEER REVIEW

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.

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.

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
	cm^2	---------- 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.

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

UNDER PEER REVIEW

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.

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

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 Figure 1 shows.

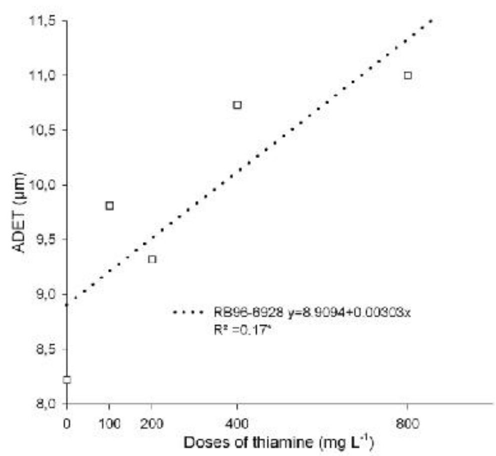

Fig. 1: Variable adaxial epidermis thickness (ADET) of varieity RB96-6928 thirty days after use of thiamine following the planting.

To the variable abaxial epidermis thickness (ABET) varieties of sugarcane RB86-7515 and RB96-6928 presented a quadratic growth curve as submitted to

UNDER PEER REVIEW

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 Figure 2 shows.

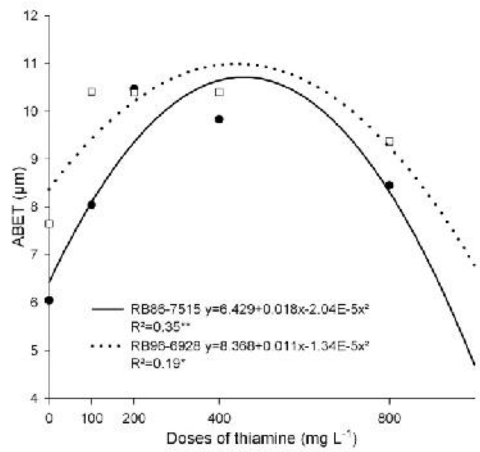

Fig. 2: Variable abaxial epidermis thickness (ABET) of varieties RB86-7515 and RB96-6928 thirty days after use of thiamine following the planting.

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.

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.

UNDER PEER REVIEW

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.

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.

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

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 Figure 3 shows.

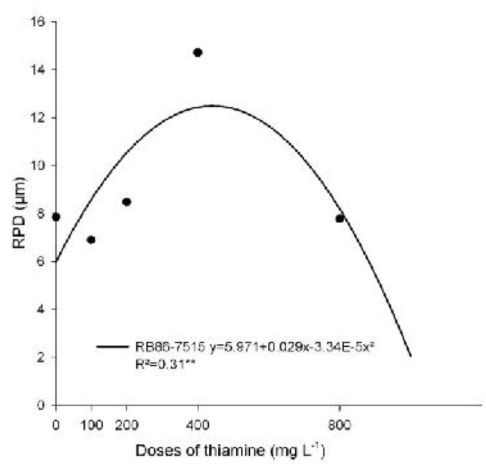

Fig. 3: Root Phloem Diameter (RPD) of the variety RB86-7515 thirty days after use of thiamine at the planting.

UNDER PEER REVIEW

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 Figure 4 shows.

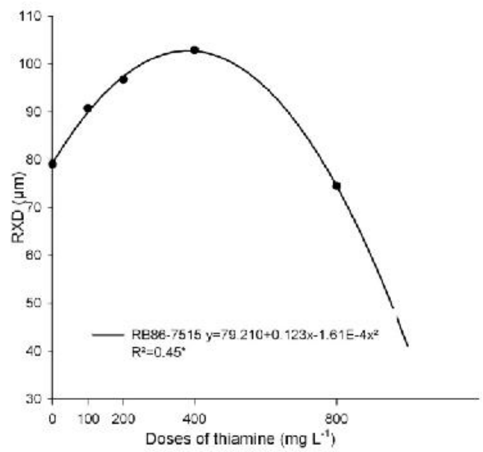

Fig. 4: Root Xylem Diameter (RXD) of variety RB86-7515 thirty days after use of thiamine at the planting.

To 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 Figure 5 shows.

UNDER PEER REVIEW

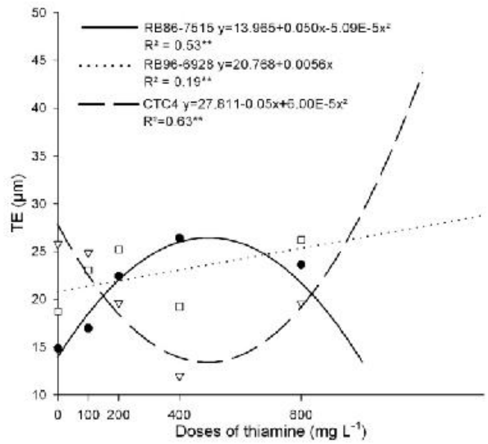

Fig. 5: Thickness of endoderm of varieties RB86-7515; RB96-6928 and CTC-04 thirty days after use of thiamine at the planting.

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 is 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 entails 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

UNDER PEER REVIEW

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 influenced on the anatomy of leaves and roots.

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

REFERENCES

1. Clemente PRA, Bezerra BKL, Silva VSG, Santos JCM, Endres L. 2017. Root growth and yield of sugarcane as a function of increasing gypsum doses. *Pesquisa Agropecuária Tropical*, 47(1): 110-117. http://dx.doi.org/10.1590/1983-40632016v4742563
2. Kamarudin AN, Seman IA, Yusof ZNB. 2017. Thiamine biosynthesis gene expression analysis in *Elaeis guineensis* during interactions with *Hendersonia toruloidea*. *Journal of Oil Palm Research*, 29(2): 218-226. http://dx.doi.org/10.21894/jopr.2017.2902.06
3. Park J, Dorrestein PC, Zhai H, Kinsland C, McLafferty FW, Begley TP. 2003. Biosynthesis of the thiazole moiety of thiamin pyrophosphate (Vitamin B1). *Biochemistry*, 42: 12430-12438. http://dx.doi.org/10.1021/bi034902z
4. Garcia AF, Dyszy F, Munte CE, Demarco R, Beltramini LM, Oliva G, Costa-Filho AJ, Araujo APU. 2014. THI1, a protein involved in the biosynthesis of thiamin in *Arabidopsis thaliana*: Structural analysis of THI1(A140V) mutant.

UNDER PEER REVIEW

Biochimica et Biophysica Acta, 1844: 1094–1103. http://dx.doi.org/10.1016/j.bbapap.2014.03.005

5. Li C, Wang M, Wu X, Chen D, Lv H, Shen J, Qiao Z, Zhang W. 2016. THI1, a thiamine thiazole synthase, interacts with Ca^{2+}-Dependent protein kinase CPK33 and modulates the S-Type anion channels and stomatal closure in Arabidopsis. *Plant Physiology*, 170: 1090-1104. https://doi.org/10.1104/pp.15.01649
6. Idris ZHC, Abidin AAZ, Subki A, Norhana Z, Yusof B. 2018. The effect of oxidative stress towards the expression of thiamine biosynthesis genes (THIC and THI1/THI4) in oil palm (*Elaeis guineensis*). *Tropical Life Sciences Research*, 29(1): 71–85. http://dx.doi.org/10.21315/tlsr2018.29.1.5
7. Pourcel L, Moulin M, Fitzpatrick TB. 2013. Examining strategies to facilitate vitamin B1 biofortification of plants by genetic engineering. *Frontiers in Plant Science*, 4: 1-8. http://dx.doi.org/10.3389/fpls.2013.00160
8. Woodward JB, Abeydeera ND, Paul D, Phillips K, Rapala-Kozik M, Freeling M, Begley TP, Ealick SE, Mcsteen P, Scanlon MJ. 2010. A maize thiamine auxotroph is defective in shoot meristem maintenance. *The Plant Cell*, 22: 3305–3317. http://dx.doi.org/10.1105/tpc.110.077776
9. Raven PH, Eichhorn SE, Evert RF. 2014. *Biologia Vegetal*. 8.ed. Guanabara Koogan. 850p.
10. Reis AR, Barcelos JPQ, Osório CRWS, Santos EF, Lisboa LAML, Santini JMK, Santos MJD, Junior EF, Figueiredo PAM, Lavres J, Gratão PL. 2017. A glimpse into the physiological, biochemical and nutritional status of soybean plants under Ni-stress conditions. *Environmental and Experimental Botany*, 144: 76–87. http://dx.doi.org/10.1016/j.envexpbot.2017.10.006
11. Empresa Brasileira de Pesquisa Agropecuária - Embrapa. 2013. *Sistema brasileiro de classificação de solos*. 3.ed. Brasília, 353p.
12. Raij B, Cantarella H, Quaggio JÁ, Furlani AMC. 1996. *Recomendações de adubação e calagem para o Estado de São Paulo*. 2.ed. Campinas: IAC. 285p.
13. Easlon HM, Bloom AJ. 2014. Easy leaf área: Automated digital image analysis for rapid and accurate measurement of leaf area. *Applications in Plant Sciences*, 2(7): 1-4. https://doi.org/10.3732/apps.1400033

UNDER PEER REVIEW

14. Lisboa LAM, Lapaz AM, Bottan AP, Yoshida CHP, Santos LFM, Figueiredo PAM, Viana, RS. 2018. Ethephon Action on Germination and Leaf Ultrastruture of *Urochloa brizantha* cv. Marandu. *Australian Journal of Basic and Applied Sciences*, 12(4): 22-28. http://dx.doi.org/10.22587/ajbas.2018.12.4.5
15. Banzatto D, Kronka, SN. 2013. *Experimentação Agrícola*. 4.ed. Funep, 237p.
16. Silva FAZ, Azevedo CAV. 2016. The Assistat Software Version 7.7 and its use in the analysis of experimental data. *African Journal Agriculture Resarch*, 11(39): 3733-3740. http://dx.doi.org/10.5897/AJAR2016.11522
17. Rebeille F, Douce R. 2011. *Biosynthesis of Vitamins in Plants Part A, Volume 58: Vitamins A, B1, B2, B3, B5 (Advances in Botanical Research)*. Academic Press; 1 ed.. 322p.
18. Begley TP, Ealick SE, Mclafferty FW. 2012. Thiamin Biosynthesis - still yielding fascinating biological chemistry. *Biochemical Society Transactions*, 40(3): 555–560. http://dx.doi.org/10.1042/BST20120084
19. Minhas AP, Tuli R, Puri S. 2018. Pathway Editing Targets for Thiamine Biofortification in Rice Grains. *Frontiers in Plant Science*, 9(975): 1-8. https://doi.org/10.3389/fpls.2018.00975
20. Friedman R, Levin N, Altman A. 1986. Presence and Identification of Polyamines in Xylem and Phloem Exudates of Plants. *Plant Physiology*, 82(4): 1154-1157. https://doi.org/10.1104/pp.82.4.1154
21. Martinis J, Gas-Pascual E, Szydlowski N, Crèvecoeur M, Gisler A, Bürkle L Fitzpatrick, T. B. 2016. Long distance transport of thiamine (vitamin B1) is concomitant with that of polyamines in Arabidopsis. *Plant Physiology Preview*, 171(1): 542-553. https://doi.org/10.1104/pp.16.00009
22. Zimmermann MH, Milburn JA. 1975. Transport in plants I: Phloem transport. New Series, 1.ed. 525p. http://dx.doi.org/10.1007/978-3-642-66161-7