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Effects of Preplantation Phosphate on the Inner Morphology of Sugarcane Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. Author LAML designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RH and PAMF managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Phosphorus is considered an essential element for sugarcane, assuming great importance in rooting, tillering and final stem yield. In order to evaluate the effects of pre-planting phosphating on the internal morphology of sugarcane leaves, an experiment with the RB867515 variety was carried out in a randomized block design with 4 replications, in a factorial scheme 2x4, being two sources of phosphorus (decanted phosphate and monoammonium phosphate) and four doses of phosphorus (0; 80; 120; 160 kg ha^{-1} P_2O_5). At 120, 240 and 362 days after planting the following characteristics were evaluated: Abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness, phloem vessel diameter and phloem vessel diameter. Phosphorus doses influenced the development of sugarcane leaf-bearing vessels at harvest. Concentrations above 160 kg ha^{-1} the P_2O_5 presented lower mean phloem diameter values in sugarcane leaves. Concentrations with 80 kg ha^{-1} the P_2O_5 presented greater xylem diameter in sugarcane leaves.

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1. INTRODUCTION

Leaves are organs responsible for 90% of the accumulated dry mass in sugarcane, resulting from photosynthetic activity [1]. As a result, the macro and micromorphological modifications of each cultivar, as well as the effects caused by them, should be increasingly studied to improve the understanding and direction of relevant research [2].

The symptomatology is widely used to assess the damage caused by biotic or abiotic factors. In this case, structural aspects help in understanding the mechanisms that cause injuries [3]. However, it is important to point out that the changes visible to the naked eye are derived from modifications of the dermal, fundamental or vascular tissue structures of the plants, making it necessary to have a thorough knowledge of these transformations motivated by environmental variations [4,2].

Plant morphophysiology depends not only on the presence of light, but also on attenuation and light quality, as well as the availability of soil nutrients that influence the process of vegetative and reproductive development [5,6]. Study also demonstrates the importance of plant morphological and functional knowledge. Medeiros et al. [7] found significant increases in stomatal polar diameter upon nitrogen application, which provides gas exchange and more efficient sweating control.

The knowledge of leaf morphology, the functions of plant tissues and their possible modifications caused by the absence of nutrients, is an important tool in decision-making process regarding the appropriate management to be employed, as well as predicting the losses estimated by not controlling nutritional deficiency. Knowledge of crop nutritional status through leaf diagnosis is an efficient tool, as the plant is the soil nutrient extractor itself, enabling a direct and accurate nutritional diagnosis [8].

The objective of this work was to evaluate the effects of pre-planting phosphating on the internal morphology of sugarcane leaves.

2. MATERIALS AND METHODS

The experiment was carried out from July 2012 to October 2013, at the Santa Mercedes Plant, in the municipality of Tupi Paulista, State of São Paulo, in Longitude west 51º 36' 53.83", Latitude south de 21º 24' 59.85" and level of 396 m.

The climate, according to the Koppen classification is of the type Aw, It is characterized by warm summer and dry winter seasons, with the highest rainfall from November to March. The annual temperature averages are 30.4ºC of maximum, 19.2ºC minimum and average relative humidity of 78% and accumulated precipitation of 1311.6 mm.

The soil of the area was classified as Argisol red yellow [9] with good drainage. At the time of the installation of the experiment in July 2012, soil sampling was performed at the depths of 0 – 20 cm and 20 – 40 cm and their chemical attributes are described in Table 1.

Table 1. Chemical attributes of the soil at the time of the experiment installation in July 2012

	Depth	
	0 – 20 cm	**20 – 40 cm**
pH $CaCl_2$	5.6	5.2
Organic matter g dm^{-3}	13	9.0
P mg dm^{-3} (resin)	2.0	3.0
K $mmol_c$ dm^{-3} (resin)	2.7	2.5
Ca $mmol_c$ dm^{-3} (resin)	13	12
Mg $mmol_c$ dm^{-3} (resin)	7.0	7.0
H + Al $mmol_c$ dm^{-3}	18	20
Al $mmol_c$ dm^{-3}	0	0
Sum of bases $mmol_c$ dm^{-3}	23	22
CTC $mmol_c$ dm^{-3}	41	42
Base saturation (V%)	56	52
Saturation Al (m%)	0	0
S (SO_4^{-2}) mg dm^{-3}	8.0	11

Phosphate sources were analyzed in the laboratory to determine the following concentrations of P_2O_5: total, water-soluble, citric acid soluble (CAS, 2%) and soluble in neutral ammonium citrate [10]. The results of the nutrient content of decanted phosphate and mono ammonium phosphate are presented in Table 2.

The experimental design was randomized blocks with 7 treatments and 4 replications, totalling 28 experimental units, in factorial scheme 2 x 4, being two sources of phosphorus (DP – Decanted Phosphate; MAP – Mono Ammonium Phosphate) and four doses of phosphorus (0; 80; 120; 160 kg ha^{-1} P_2O_5). Only a control treatment was considered to have the same concentration of P_2O_5, as shown in Table 3.

In August 2012, the preparation of the area for installation of the experiment was started, liming was performed by applying 2.34 t ha^{-1} dolomitic limestone with the (70%) to raise the V% from the ground to 60% and the plaster was in total area, with 1,2 t ha^{-1} of agricultural plaster with 16% sulfur. Phosphate estimation was performed on the day (04 October 2012) by following the treatments in Table 3, later the sugarcane variety was planted RB867515, with 500 kg ha^{-1} of compost 05-25-25 second [11]. It was applied 0.25 kg ha^{-1} doses of Fipronil diluted in a soil conditioner composed of organic liquid matter in the form of humic and fulvic acids in the dosage of 250 L ha^{-1}, to prevent pest attacks on sugar cane tails and to provide organic matter and humic and fulvic acids.

The experimental units were composed of 10.0 m x 9.0 m, in total of 90.0 m². Each experimental unit contained six sugarcane rows with 1.50 m between lines. No large number of invasive plants was found that could hinder the development of sugarcane in the experimental units during the experiment.

At 120, 240 and 362 days after planting, four leaves +1 were collected in each experimental unit. On each leaf, a fragment approximately 5 cm long was taken from the middle region of each leaf. All plant tissue fragments were submitted to procedures related to dehydration, diaphanization, inclusion and blocking and with the aid of a microtome, 8.0 µm cross sections were performed in each tissue fragment [12].

The slides were observed under an optical microscope with a camera attached to perform measurements of histological variables using an image program, calibrated with a microscopic

Table 2. Chemical analysis of the fertilizers used in the experiment

Source	Nutrient	Solubility	Content %
		P (total)	12.67
		P (Water)	4.81
DP	Phosphor	P (CAS + Water)	10.43
		P Citric acid	10.58
	Nitrogen	N (total)	9.15
		P (total)	50.10
MAP		P (Water)	44.19
	Phosphor	P (CAS + Water)	49.42
		P Citric acid	47.46

DP – Decanted Phosphate; MAP – Mono Ammonium Phosphate

Table 3. Description of treatments

Treatments	P_2O_5 (kg ha^{-1})	DP (kg ha^{-1})	MAP (kg ha^{-1})
1	0	---	---
2	80	631.41	---
3	120	947.12	---
4	160	1262.82	---
5	80	---	159.68
6	120	---	239.52
7	160	---	31936

Obs.: Doses calculated from the total content of P_2O_5. DP – Decanted Phosphate; MAP – Mono Ammonium Phosphate

ruler at the same magnification, where the following tissues were measured: ABET – abaxial epidermis thicknesse; ADET – adaxial epidermis thicknesse; MT – mesophyll thickness; PD – phloem diameter and XD – xylem diameter. For each slide, ten measurements were performed to obtain an average for each anatomical parameter. Then, these averages represented the value of each plot.

All values were submitted to analysis of variance by the test F ($p<0.05$), and their averages were compared by Tukey test at 5% probability for phosphorus sources and doses. All statistical procedures followed the method proposed by [13]. The program used for the analysis was the Assistat 7.6 Beta [14].

3. RESULTS AND DISCUSSION

No significant differences were found in leaf tissue measurements when collected at 120 and 240 days after planting due to the application of treatments as shown in Table 4.

Studies by [15] and [16] obtained similar results by analyzing the anatomy of leaves of sugarcane varieties. Again at 240 days after sugarcane planting, there was no significant difference in leaf anatomical parameters for sources and doses of phosphorus as observed in Table 5.

Table 4. Mean leaf anatomical parameters of sugarcane cultivated with sources and doses of phosphorus, collected at 120 days after planting in 2013

	ABET	ADET	MT	PD	XD
Source of P_2O_5		---------- µm ----------			
DP	13.58	12.15	288.74	8.48	54.04
MAP	14.06	12.61	285.96	8.53	54.34
MSD	1.17ns	1.07ns	20.99ns	0.88ns	6.04ns
P_2O_5 (kg ha^{-1})					
0	14.51	12.62	297.33	8.76	54.50
80	13.43	12.29	285.20	8.36	51.04
120	13.11	11.89	301.51	8.86	56.86
160	14.23	12.74	265.35	8.05	54.35
SD	0.68	0.54	15.73	0.36	3.15
MSD	2.22ns	2.03ns	39.64ns	1.67ns	11.41ns
CV(%)	11.67	11.91	10.01	14.26	15.28

*** – significant at the 1% probability level (p<0.01); * – significant at 5% probability level (0.01=<p<0.05); ns – not significant (p>=0.05);SD – standard deviation; MSD – minimal significant sifference; CV – coefficient of variation; DP – decanted phosphate; MAP – mono ammonium phosphate; ABET – abaxial epidermis thicknesse; ADET – adaxial epidermis thicknesse; MT – mesophyll thickness; PD – phloem diameter and XD – xylem diameter*

Table 5. Mean leaf anatomical parameters of sugarcane cultivated with sources and doses of phosphorus, collected at 240 days after planting in 2013

	ABET	ADET	MT	PD	XD
Fonte de P_2O_5		---------- µm ----------			
DP	15.82	18.14	282.28	12.15	54.96
MAP	15.76	17.60	295.41	12.32	53.38
MSD	1.21ns	1.12ns	15.39ns	1.13ns	4.79ns
P_2O_5 (kg ha^{-1})					
0	16.01	18.69	283.41	12.58	57.32
80	15.41	17.22	295.23	11.23	52.62
120	15.42	17.73	290.51	12.15	53.79
160	16.33	17.84	286.22	12.98	52.97
SD	0.67	0.53	10.68	0.77	2.24
MSD	2.29ns	2.12ns	29.07ns	2.14ns	9.04ns
CV(%)	10.52	8.61	7.30	12.72	12.11

*** – significant at the 1% probability level (p<0.01); * – significant at 5% probability level (0.01=<p<0.05); ns – not significant (p>=0.05);SD – standard deviation; MSD – minimal significant difference; CV – coefficient of variation; DP – decanted phosphate; MAP – mono ammonium phosphate; ABET – abaxial epidermis thicknesse; ADET – adaxial epidermis thicknesse; MT – mesophyll thickness; PD – phloem diameter and XD – xylem diameter*

Morfoanatomic characteristics of leaves such as epidermis thickness and parenchyma thickness can directly influence the decrease in surface area, which contributes to the reduction of sweating and mainly photosynthetic factors [17]. The mesophyll thickness found in the study was higher than the results found by [2] by studying the effects on sugarcane leaf tissues after herbicide application in weed control.

Studies by [18] showed that the productive and anatomical characteristics of three forages in response to phosphorus and age found interaction between treatments and concluded that phosphorus and leaf age increased sclerenchyma and epidermis thickness. [19] does not confirm these results, studying guava, reported that as the thickness of the epidermis increases, the thickness of the mesophyll decreases.

Leaves with thicker tissues exhibit greater efficiency in water use and metabolism development [20], making the plant more tolerant to water stress, light, providing better development of plant material. For the measured thickness of the epidermis the values are similar to those found by [16], who studied five sugarcane cultivars. [4] report the importance of epidermal cell thickness because they play a protective role due to their position in the histology of the plant, lining its organs, protecting it from adverse environmental actions.

For the characteristic diameter phloem (PD) and diameter of the xylem vessels in the sugarcane leaves collected at 362 days after planting was significant at the tested doses. The dose with 160 kg ha^{-1} de P_2O_5 presented the lowest average values for the characteristic diameter of the phloem vessels, showing that the number of vessels may have influenced the diameter. For the average values of xylem vessel diameter, the treatment with 80 kg ha^{-1} de P_2O_5 presented the best results, as shown in Table 6.

This result can be explained due to the mobility of phosphorus inside the plant that occurs by phloem. This element is transported to younger tissues by root absorption or even by the migration of older organs to meristematic regions. Due to the synthesis of ATP and protein large amounts of phosphorus and sulfur are used in other regions and mainly in regions responsible for the storage of metabolized substances proven by [21]. The averages found in diameter of the phloem vessels are similar to the results found by [2] that were around 9.0 µm.

Studies by [22] with varieties of sugarcane, and found higher values of metaxylem still report that the xylem is formed by a remarkable circle containing approximately between 10 to 12 vessels, and this number increases with leaf thickening and ageing. The average values of xylem vessel diameter were higher than those found by [2] which presented means of 29 µm. The observed characteristics confirm the descriptions of positive changes in the diameters of the phloem vessels, which may directly influence the transport of nutrients and organic compounds and indirectly the photosynthesis, consequently the growth and development of plant organs [23].

Table 6. Mean leaf anatomical parameters of sugarcane grown with sources and doses of phosphorus, collected at 362 days after planting in 2013

	ABET	ADET	MT	PD	XD
Fonte de P_2O_5		---------- µm ----------			
DP	12.78	13.95	287.22	10.39	46.78
MAP	12.44	14.75	277.20	10.55	47.35
MSD	1.24ns	1.79ns	49.71ns	0.63ns	3.69ns
P_2O_5 (kg ha^{-1})					
0	11.54	14.66	315.26	11.26a	43.42b
80	13.80	14.01	268.82	10.81a	54.31a
120	12.60	13.80	308.57	10.81a	43.75b
160	12.51	14.92	236.18	9.01b	46.78b
SD	0.74	1.23	36.13	1.03	5.59
MSD	2.35ns	3.39ns	93.91ns	1.20**	6.97**
CV(%)	13.55	17.15	24.13	8.36	10.74

*** – significant at the 1% probability level (p<0.01); * – significant at 5% probability level (0.01=<p<0.05); ns – not significant (p>=0.05);SD – standard deviation; MSD – minimal significant difference; CV – coefficient of variation; DP – decanted phosphate; MAP – mono ammonium phosphate; ABET – abaxial epidermis thicknesse; ADET – adaxial epidermis thicknesse; MT – mesophyll thickness; PD – phloem diameter and XD – xylem diameter*

4. CONCLUSIONS

Phosphorus doses influenced the development of sugarcane leaf-bearing vessels at harvest.

Concentrations above 160 kg ha^{-1} the P_2O_5 presented lower mean phloem diameter values in sugarcane leaves.

Concentrations with 80 kg ha^{-1} the P_2O_5 presented greater xylem diameter in sugarcane leaves.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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