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Biochemical differences in the initial growth of sugarcane varieties cultivated under different potassium doses and water conditions

Diferenças bioquímicas no crescimento inicial de variedades de cana-de-açúcar cultivadas sob doses de potássio e condições hídricas

Igor T. M. da Rocha[1]*, Anibia V. da Silva[2], Fernando J. Freire[1], Emídio C. A. de Oliveira[1], Edivan R. de Souza[1], Laurício Endres[3]

[1]Department of Agronomy, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil. [2]Department of Agronomy, Instituto Federal de Pernambuco, Vitória do Santo Antão, PE, Brazil. [3]Department of Agronomy, Universidade Federal de Alagoas, Maceió, AL, Brazil.

ABSTRACT - Sugarcane varieties have distinct biochemical and productive capacities, which are accentuated under water and nutrient stress conditions. Water stress promotes biochemical changes in plants, such as the overproduction of reactive oxygen species (ROS) that, depending on the concentration, degrade photosynthetic pigments. Current research indicates that K^+ plays an important role in increasing yield and tolerance to water stress. This study evaluated the effect of different K^+ levels on the initial growth of two sugarcane varieties under two water conditions. The experiment was conducted in a greenhouse using randomized blocks, with four replications, five K^+ doses (0; 0.5; 1; 2 and 6 mmol L^{-1}), two sugarcane varieties (RB92579 and RB992506), and two water conditions (hydrated plants and water stress). Data were analyzed in a 5x2x2 factorial scheme. Leaf carbohydrates, photosynthetic pigments, antioxidant enzyme activity (catalase, superoxide dismutase and ascorbate peroxidase) and dry matter production were assessed. K^+ doses promoted an increase in plant dry mass. The RB992506 variety had lower APX activity and higher levels of photosynthetic pigments, carbohydrates and dry matter than its RB92579 counterpart. Water conditions and interactions between factors had no effect on the variables studied. RB992506 is a promising variety and more tolerant to water stress due to its superior biochemical and productive traits when compared to RB92579.

Keywords: Saccharum spp. ROS. Leaf pigments. Water stress. Ascorbate peroxidase.

RESUMO - As variedades de cana-de-açúcar possuem capacidades bioquímicas e produtivas distintas, especialmente acentuadas em situação de estresse hídrico e nutricional. O déficit hídrico promove alterações bioquímicas nas plantas, as quais envolvem a superprodução de Espécies Reativas de Oxigênio (EROs) que, dependendo da concentração, proporcionam a degradação de pigmentos fotossintéticos. Pesquisas atuais indicam que o K^+ possui importante papel no aumento da produtividade e na tolerância ao déficit hídrico. Este estudo avaliou o efeito de doses de K^+ no crescimento inicial de duas variedades de cana submetidas a duas condições hídricas. O experimento foi montado em casa de vegetação em blocos ao acaso, com quatro repetições, sendo cinco doses de K^+ (0; 0,5; 1; 2 e 6 mmol L^{-1}), duas variedades de cana (RB92579 e RB992506), e avaliado em duas condições hídricas (plantas hidratadas e estresse hídrico). Os dados foram analisados estatisticamente em esquema fatorial 5x2x2. Foram avaliados os teores foliares de carboidratos, pigmentos fotossintéticos, atividade das enzimas antioxidativas (catalase, superóxido dismutase e ascorbato peroxidase) e a produção de matéria seca. As doses de K^+ promoveram aumento da massa seca das plantas. A variedade RB992506 apresentou menor atividade da enzima APX e maiores teores de pigmentos fotossintéticos, de carboidratos e de massa seca do que a variedade RB92579. As condições hídricas, bem como as interações entre os fatores, não promoveram efeito sobre as variáveis estudadas. A variedade RB992506 consiste em uma variedade promissora e mais tolerante ao estresse hídrico devido apresentar características bioquímicas e produtivas superiores as da variedade RB92579.

Palavras-chave: Saccharum spp.. EROs. Pigmentos foliares. Estresse hídrico. Ascorbato peroxidase.

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***Corresponding author:**
<igor.rocha@ufrpe.br>

INTRODUCTION

Water stress is one of the factors that most affects crop yield and is related to the lack of rain for dryland crops (CAMPOS; SANTOS; NACARATH, 2021).

Sugarcane varieties exhibit contrasting genotypes with potential tolerance to water stress, as observed in their biochemical, physiological and productive traits (SANTOS et al., 2014; SIMÕES et al., 2019). Identifying these traits is important in recommending the varieties most tolerant to water stress.

The morphological traits of sugarcane (yield, plant height, stem diameter) are the most widely used for variety selection. However, under similar planting conditions and environments, the productive potential of current varieties is not significantly different (SILVA et al., 2018). Given that the morphological traits of sugarcane do not change during short periods of water stress, (MAIA JÚNIOR et al., 2018), identifying biochemical characteristics is an alternative in selecting varieties tolerant to abiotic stress.

Photosynthetic pigments (chlorophyll *a*, b, total chlorophyll and carotenoids), carbohydrate content and antioxidative enzyme activity (catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX)) are biochemical traits sensitive to environmental changes related to water and salt stress and are used to characterize varieties tolerant to these types of stress (SIMÕES et al., 2019; MORAIS et al., 2020).

In addition to genotypic traits, plant tolerance to abiotic stress can also be increased by nutrient supplementation. For example, K^+ supplied in above-recommended doses for maximum crop yield increases abiotic stress tolerance (RAO; REDDY; KUNDO, 2014; SILVA et al., 2021).

K^+ is biochemically important to plants (ANSCHÜTZ; BECKER; CHABALA, 2014). Particularly for sugarcane, large amounts of K^+ are required, and it absorbs more than other nutrients, including N (SALVIANO et al., 2017). In addition, raising yield by applying increasing amounts depends on the variety (UCHÔA et al., 2009).

K^+ has many functions in plants, such as activating different enzymatic complexes responsible for metabolizing carbohydrates and proteins and detoxifying reactive oxygen species (ROS) (EROs) (WANG et al., 2013). Plant K^+ deficiency increases ROS generation, which compromises plant development due to their toxic effect, primarily in cell membranes (AHMAD; MAATHUIS, 2014). In addition, an increase in ROS caused by K^+ deficiency may result in oxidative damage in leaf pigments, decreasing their content (CAVALCANTE et al., 2015) and contributing to reducing photosynthetic capacity and crop yield.

Thus, there is a need to know the intrinsic genotypic traits of new sugarcane varieties and compare them with those previously established in the sugarcane ethanol industry, in order to confirm their viability, mainly under drought conditions when plants are subjected to water stress for a few months every year. Thus, the aim of the present study was to assess the biochemical and productive responses of two sugarcane varieties under different K^+ concentrations in the presence and absence of water stress.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Department of Agronomy of the Federal Rural University of Pernambuco, located at 8°54'28'' S, 34º52'53'' W. Study onset was on July 20, 2016, plants emerged on August 8 and were harvested on November 11.

The soil used in the test was chemically characterized as follows: [pH_{water}=5.13; P: 15.81 mg dm^{-3}; Na^+: 0.02 $cmol_c$ dm^{-3}; K^+: 0.05 $cmol_c$ dm^{-3}; (Ca^{2+}+Mg^{2+}): 2.3 $cmol_c$ dm^{-3}; Al^{3+}: 0.0 $cmol_c$ dm^{-3}; (H+Al): 1.5 $cmol_c$ dm^{-3}], and total organic carbon (TOC) content was 2.32 g kg^{-1} (DONAGEMA et al., 2011). The results were used to calculate potential ($CEC_{potential}$: 3.87 $cmol_c$ dm^{-3}) and effective cation exchange capacity ($CEC_{effective}$: 2.37 $cmol_c$ dm^{-3}), base saturation (V: 61%) and aluminum saturation (m: 0%). Soil K^+ content was classified as low (<0.08 cmolc dm^{-3}), according to the Pernambuco fertilizer recommendation manual (CAVALCANTE et al., 2008).

The effect of increasing K^+ levels in nutrient solution on the biochemical traits of two sugarcane crops was assessed: one with good recovery after short periods of water stress and extensively cultivated in Brazil (RB92579), and the other recently launched onto the sugarcane ethanol market (RB992506).

The experiment used a randomized block design with four repetitions, five K^+ concentrations$^+$ (0, 0.5, 1, 2 and 6 mmol L^{-1}) and two sugarcane varieties (RB992506 and RB92579), totaling 40 plots (pots). All assessments were carried out at two different times, corresponding to two water conditions (hydration: leaf samples collected in the morning after irrigation, and water stress: samples collected two days after irrigation).

Sugarcane stems (20 cm long) and two buds were planted in 20 L plastic pots. A 150 mL Hoagland and Arnon (1950) nutrient solution containing K^+ concentrations of 0. 0.5, 1, 2 and 6 mmol L-1 was applied weekly, maintaining the concentrations of the remaining nutrients. The pots were irrigated with 500 mL of distilled water every two days up to 30 days after planting, and thereafter, with daily 1L applications.

At 90 days after emergence (DAE), the plants were irrigated at the end of the afternoon and the first fully expanded leaves (position +1) were collected in the morning of the following day. Following each collection, the plants were not irrigated for two days (water stress). After this period, a new collection of +1 leaves was conducted, involving the middle thirds, without the midrib. The samples were packed in aluminum foil and submersed in liquid N for 10 to 20 seconds and then stored in a Styrofoam box with ice for subsequent determination of the biochemical variables.

Carbohydrates

The soluble sugars were determined after lyophilization of 20 mg of leaf tissue, ground in a mortar and added with liquid N in order to obtain fine powder. The samples were then ground with 2 mL of distilled water and added with a further 2 mL of water. Next, they were centrifuged at 3,000 rpm for 15 minutes in ambient temperature. The supernatant was collected and stored in 2 mL microtubes and once again centrifuged for 10 minutes at 6,000 rpm, then used to determine sugar content. 50 µL of supernatant + 450 µL of distilled water was added with 500 µL of phenol (5%) and 2.5 mL of concentrated H_2SO_4. The mixture was submitted to vortex agitation for 10-20 seconds. The tubes were cooled in a tray with ice and spectrophotometer readings were conducted at 490 nm (DUBOIS et al., 1956).

Antioxidant complex enzymes

A total of 100 mg of plant tissue were ground with liquid N to obtain the fine powder. The powder was ground with 2 mL of reaction medium (TFK buffer + pH 7.5 + sodium ascorbate + EDTA+ Triton X), added with 0.3 g of PVPP and ground again until a homogeneous extract was obtained. The extract was transferred to 2 mL microtubes and centrifuged for 14 minutes at 10,000 rpm at 4 ºC. The supernatant was collected and stored for analysis. Ascorbate peroxidase (APX) activity was determined in a spectrophotometer at 290 nm, using a quartz cuvette with readings every 5 seconds, for 1 minute. In order to set up the reaction, 334 µL of TFK buffer, 1566 µL of water, 20 µL of sodium ascorbate, 20 µL of hydrogen peroxide (10 mM) and 60 µL of extract (NAKANO; ASADA, 1981) were added. For catalase (CAT), the readings occurred every five seconds for 1 minute, at 240 nm in a quartz cuvette. The reaction extract was set up with 334 µL of TFK buffer, 1356 µL of water, 250 µL of hydrogen peroxide (100 mM) and 60 µL of extract (HAVIR; MCHALE, 1987).

Superoxide dismutase (SOD) activity was determined in the extract obtained by grounding 300 mg of +1 leaves with 2 mL of TFK extractor, pH 7.8, in a spectrophotometer at 560 nm. After the samples were ground, they were centrifuged for 15 minutes at 15,000 rpm at 4 ºC. Fifty µL aliquots were added with 2.95 mL of reaction medium (TFK buffer, pH 7.8 + 100 mM EDTA + 75 µM NBT + 13 mM methionine + 2 µM riboflavin). After the mixture was formed, the samples were packed in a box with fluorescent light for 4 minutes (GIANNOPOLITIS; RIES, 1977).

Leaf pigment analysis

The +1 leaves of each treatment were collected and analyzed for chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids. 50 mg of leaf tissue was incubated for 24 hours in a refrigerator with 5 mL of 80% acetone, determined in a spectrophotometer at 480 nm (carotenoids), 645 nm (chlorophyll *b*) and 663 nm (chlorophyll *a*) (HENDRY; GRIME, 1993).

Dry matter

All the plant shoot material contained in the pots was collected, dried in a forced air oven until constant weight and weighed on a precision scale.

The experiment used a 5x2x2 factorial design, with five K^+ doses (0, 0.5, 1, 2, and 6 mmol L^{-1}), two sugarcane varieties (RB92579 and RB992506) and two water conditions (hydration and water stress). The data underwent analysis of variance and when the factors were significant, regression analyses were carried out for the K^+ doses or Tukey's test for the sugarcane varieties and hydration conditions ($p<0.05$ and <0.01). The regression parameter coefficients were tested using the t-test and the means with Tukey's test ($p<0.05$ and 0.01). Statistical analyses were performed with SISVAR software, version 5.8 (FERREIRA, 2011).

RESULTS AND DISCUSSION

Analysis of variance (Tables 1, 2 and 3) demonstrated a significant effect ($p<0.05$) of K^+ concentrations on dry matter production. The varieties also differed significantly in terms of carbohydrates, dry matter production, chlorophyll *a*, *b*, total chlorophyll, carotenoids and APX ($p<0.01$).

However, water condition (hydration and water stress) and the interaction between hydration x K^+ dose did not influence the variables assessed (Tables 1, 2 and 3). According to Simões et al. (2018), water stress in the sprouting and tillering phases does not compromise sugarcane yield, but rather increases it. This may explain the fact that suspending irrigation had no effect on the traits assessed in the present study.

In addition, the lack of a significant effect of the interaction between K^+ doses and hydration conditions on the variables assessed was likely due to the short water stress evaluation period (two days of water deprivation). Silva et al. (2021) assessed the physiological traits of sugarcane submitted to water stress and K^+ doses, and only observed a K^+ dose effect when irrigation was suspended from the 4th assessment day onwards.

The leaf pigments only differed statistically for the varieties ($p<0.01$) (Table 1).

The results showed that the RB92579 variety exhibited lower chlorophyll *a*, *b*, total chlorophyll and carotenoid values than those of RB992506 (Figures 1A, B, C and D). Differences in leaf pigment contents between sugarcane varieties were also observed by Santos et al. (2014), and, according to Silva et al. (2014), higher values in this trait denote greater water stress tolerance.

Although literature data indicate that K^+ deficiency reduces chlorophyll content due to ROS degradation (AHMAD et al., 2014), even without K^+ application in the nutrient solution, plants did not exhibit significant differences in the chlorophyll *a*, *b*, total chlorophyll and carotenoid content in the present study (Table 1). This is likely due to the K^+ reserves contained in the stalks used as seed in the experiment and the short assessment period (two days of suspended irrigation).

Table 1. Clorophyll *a*, *b*, total chlorophyll and carotenoids as a function of increasing potassium concentrations in the nutrient solution in different sugarcane varieties in irrigated plants and after water stress, analysis of variance and coefficient of variation of the data.

	Leaf content			
	Chl *a* (mg g^{-1} FM)[1]	Chl *b* (mg g^{-1} FM)	Total chl (mg g^{-1} FM)	Carotenoids (µmol mg^{-1} FM)
	F-test in analysis of variance			
K^+ (mmol L^{-1})	0.79^{ns}	0.54^{ns}	0.73^{ns}	1.16^{ns}
Variety	12.89**	6.56**	11.32**	14.80**
Water condition	0.43^{ns}	0.39^{ns}	0.42^{ns}	3.36^{ns}
K^+ x Variety	0.43^{ns}	0.46^{ns}	0.43^{ns}	0.55^{ns}
K^+ x W. cond.[2]	1.22^{ns}	1.15^{ns}	1.20^{ns}	1.77^{ns}
Var[3] x W. cond.	2.71^{ns}	2.16^{ns}	2.59^{ns}	2.26^{ns}
K^+ x Var x W. cond	1.87^{ns}	1.81^{ns}	1.86^{ns}	2.23^{ns}
CV%[4]	30.5	27.84	29.73	20.62

[1]Fresh matter; [2] Water condition; [3]Variety; [4]Coefficient of variation = (Standard deviation/Mean) x 100.**Significant at 1% probability. [ns] Not significant.

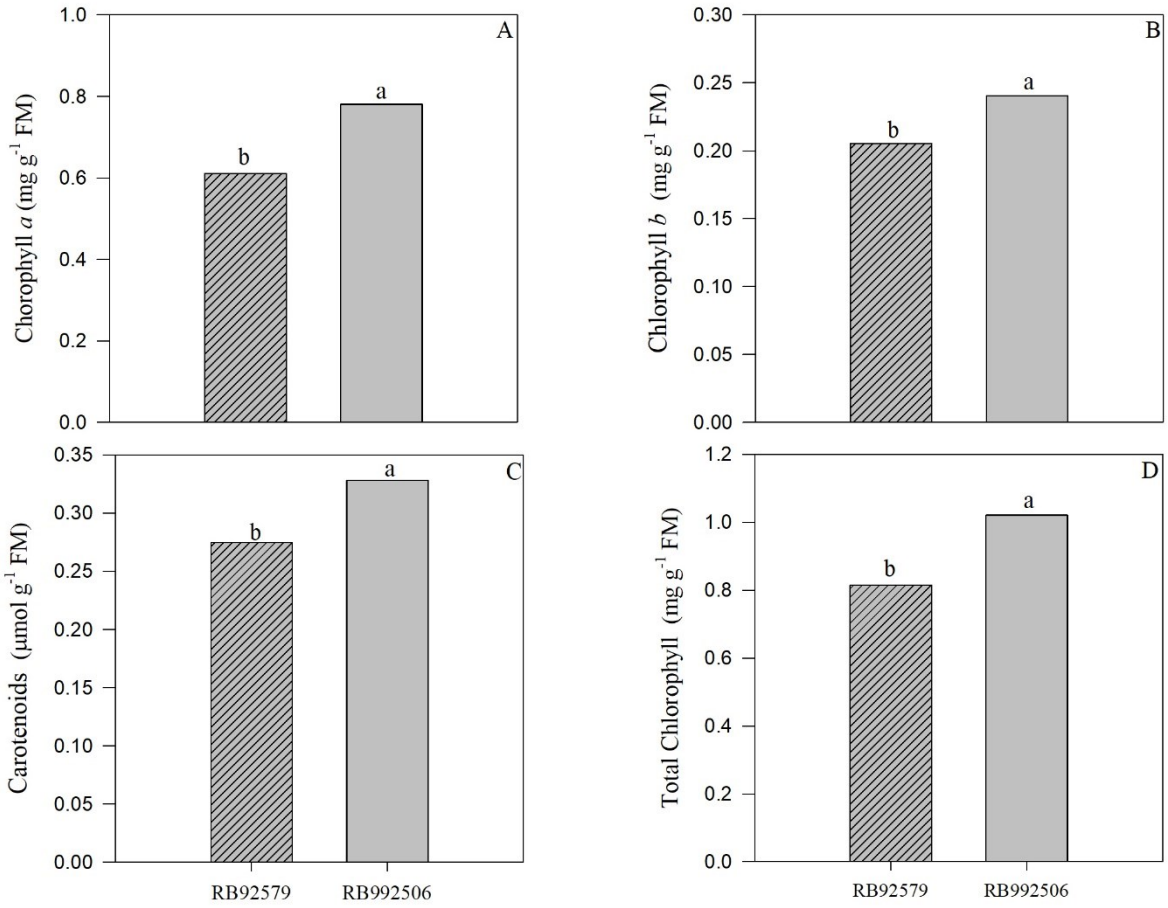

Figure 1. leaf content of chlorophyll *a* (A), chlorophyll *b* (B), carotenoids (C) and total chlorophyll (D) in different sugarcane varieties.

Leaf carbohydrate content in the RB992506 variety was higher than that of RB92579 (Table 2 and Figure 2). The difference between varieties may be a function of their respective genotypic capacities. Despite the difference between varieties, the carbohydrate content of both was around 40 mg g^{-1} of dry matter, a value similar to that obtained by Cavalcante et al. (2015) (30-40 mg g^{-1} MS).

Table 2. Carbohydrate content and dry matter production as a function of increasing potassium (K^+) concentrations in a nutrient solution in different sugarcane varieties in irrigated plants and after water stress, analysis of variance and coefficient of variation of the data.

	Carbohydrates (mg g^{-1} DM)(1)	Dry matter (g/pot)
	F-test in analysis of variance	
K^+ (mmol L^{-1})	1.52^{ns}	4474.78*
Variety	5.63*	52818.97*
Water condition	0.38^{ns}	
K^+ x Variety	1.16^{ns}	940.10^{ns}
K^+ x W. cond.(2)	0.73^{ns}	
Var(3) x W. cond.	2.55^{ns}	
K^+ x Var x W. cond	0.45^{ns}	
CV%(4)	14.03	22.55

[1]Dry matter; [2] Water condition; [3]Variety; [4]Coefficient of variation = (Standard deviation/Mean) x 100.**Significant at 5% probability. [ns] Not significant.

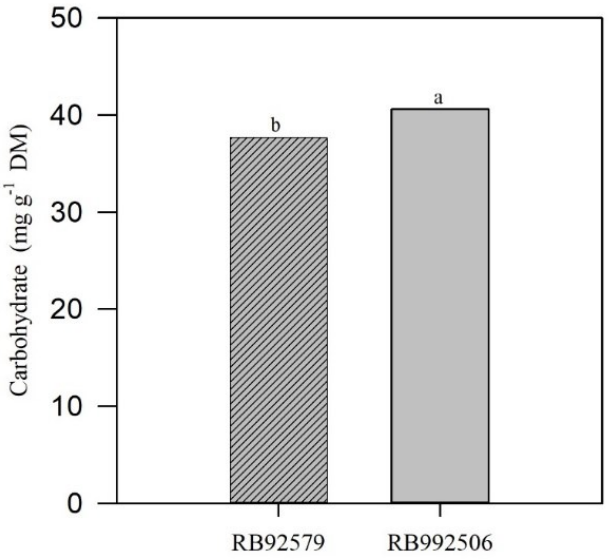

Figure 2. Leaf carbohydrate content in the sugarcane varieties.

The amount of K^+ applied in the nutrient solution and the sugarcane varieties had significant effects ($p<0.05$) on dry matter production (Table 2 and Figure 3). The K^+ applied caused a quadratic increase in sugarcane dry matter production, and 6 mmol L^{-1} de K^+ in the nutrient solution increased it by about 30% when compared to the control (Figure 3A). Among the varieties, RB992506 produced 35% more dry matter than RB92579 (Figure 3B). Several studies observed an increase in sugarcane yield due to the higher amount of K^+, in both the field and a greenhouse, as well as different yield increases in the varieties with the same amount of K^+ applied (UCHÔA et al., 2009; CAVALCANTE et al., 2015; JAISWAL et al., 2021).

In relation to dry matter production, according to Agathokleous, Feng and Peñuelasb (2020), leaf pigments are the molecules responsible for plant photosynthesis, which, in turn, is responsible for chemical energy production. This justifies the greater dry matter production by RB992506, since it displayed higher leaf pigment (Figures 1A, B, C and D) and carbohydrate (Figure 2) content.

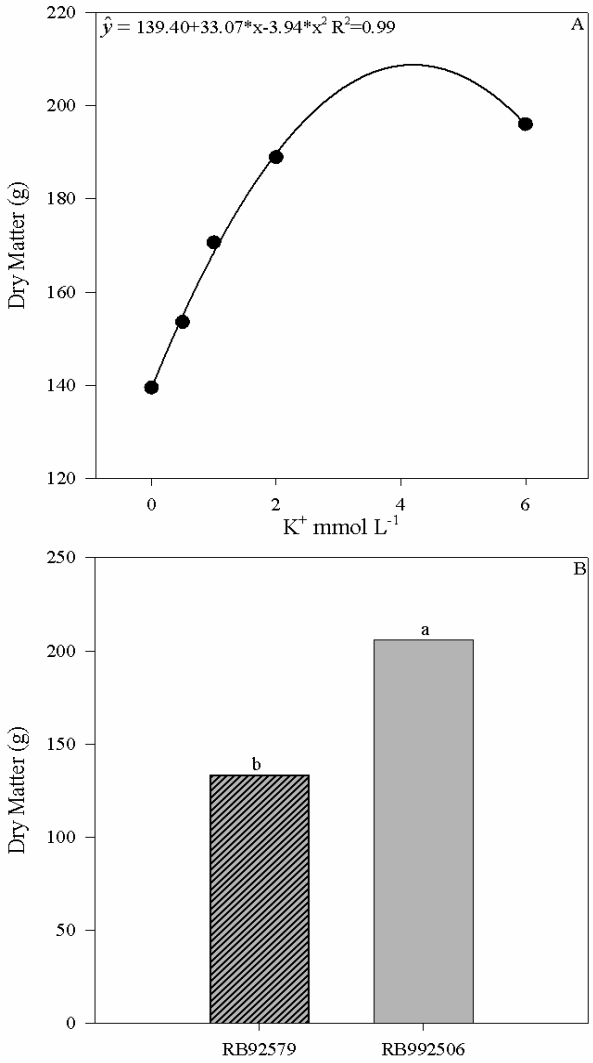

Figure 3. Dry matter production of sugarcane plants as a function of increasing K^+ added with nutrient solution (A) in different sugarcane varieties (B).

APX, an antioxidant complex enzyme, was influenced ($p<0.01$) by the variety, but SOD and CAT were not ($p>0.01$). Water condition and K^+ doses also had no influence ($p>0.01$) on the antioxidant complex enzymes (Table 3).

SOD, CAT and APX are known as antioxidant complex enzymes, since they cleanse molecules that cause oxidative damage in cells. The sharp increase in activity of these enzymes indicates that the plant is under stress, and it is essential that the excess ROS generated be metabolized and not compromise the cells (BARBOSA et al., 2014; NADARAJAH, 2020).

Given that the conditions imposed (water stress and K^+ concentrations) did not produce significant differences ($p>0.01$) for CAT, SOD and APX enzymes (Table 3), the cause of APX enzyme variation was solely as a function of the variety (Table 3 and Figure 4). These data corroborate the results obtained by Morais et al. (2020), who observed differences in CAT, SOD and APX activity in five sugarcane varieties submitted to water stress and saline.

Studies report that plant K^+ deficiency increases antioxidant complex enzyme activity (SOD, CAT and APX) due to the increase in ROS (WANG et al., 2012; AHMAD et al., 2014). In the present study, however, the data show that K^+ deficiency had no effect with an increase in antioxidant complex enzyme activity (Table 3). This may be due to the K^+ reserves in the stalks used in the experiment and the short assessment period.

It was also observed that the APX activity of the sugarcane varieties differed significantly ($p<0.01$). RB92579 obtained higher enzyme activity values than those of RB992506 (Figure 4). Differences in antioxidant complex enzyme activity are common and indicate the variety most tolerant of water stress (WANG et al., 2012; SANTOS et al., 2014).

Table 3. Antioxidant complex enzymes in the leaf as a function of increasing potassium concentrations in the nutrient solution in different sugarcane varieties in irrigated plants and after water stress, analysis of variance and coefficient of variation of the data.

	Leaf content		
	CAT [(1)]	SOD [(2)]	APX [(3)]
	F-test in analysis of variance		
K^+ (mmol L^{-1})	2.93^{ns}	2.67^{ns}	1.36^{ns}
Variety	6.43^{ns}	0.77^{ns}	12.55**
Water condition	2.08^{ns}	6.03^{ns}	2.70^{ns}
K^+ x Variety	0.37^{ns}	3.12^{ns}	0.57^{ns}
K^+ x W. cond.[(4)]	0.37^{ns}	0.32^{ns}	1.24^{ns}
Var[(5)] x W. cond.	0.74^{ns}	1.86^{ns}	1.11^{ns}
K^+ x Var x W. cond.	2.16^{ns}	1.44^{ns}	0.28^{ns}
CV%[(6)]	26.81	81.35	32.36

[1]Catalase (Unit min^{-1} mg^{-1} prot.)[(1)]; [2]Superoxide dismutase (Unit min^{-1} mg^{-1} prot.)[(3)]; [3]Ascobate peroxidase (Unit mg^{-1} prot.)[(2)]; [4]Water condition; [5]Variety; [6]Coefficient of variation = (Standard deviation/Mean) x 100. **Significant at 1% probability. [ns]Not significant.

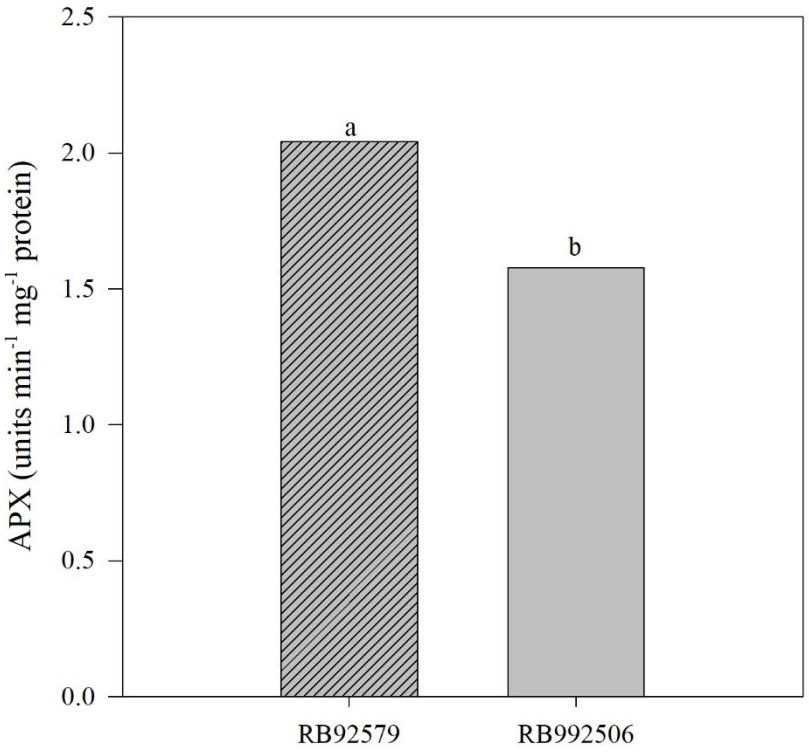

Figure 4. Ascorbate peroxidase enzyme (APX) in sugarcane varieties.

CONCLUSIONS

The new sugarcane variety (RB992506) was promising as a replacement for RB92579 due to its superior water stress tolerance (lower APX activity, higher leaf pigment and carbohydrate content) and higher yield. The increased yield observed with higher K^+ doses indicates that this nutrient is essential in guaranteeing adequate sugarcane yield.

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