Physiological characterization of sugarcane varieties under oxidative stress caused by the herbicide paraquat

Caracterização fisiológica de variedades de cana-de-açúcar submetidas ao estresse oxidativo causado pelo herbicida paraquat

Alan Arthuso PAVANI[1; 2]; Ana Carolina Ribeiro GUIMARÃES[3]; Caio Augusto de Castro Grossi BRUNHARO[4]; Pedro Jacob CHRISTOFFOLETI[5]

[1] Parte da dissertação de mestrado apresentada pelo primeiro autor
[2] Engenheiro Agrônomo, Universidade de São Paulo, alanpavani@gmail.com
[3] Engenheiro Agrônomo, Universidade de São Paulo,acguimaraes@gmail.com
[4] Autor para correspondência (Correspondig author): Engenheiro Agrônomo, Universidade de São Paulo. Departamento de Produção Vegetal, ESALQ/USP, caio.brunharo@yahoo.com.br
[5] Engenheiro Agrônomo, Professor, Universidade de São Paulo, pjchrist@usp.br

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Abstract

Drought is the main factor limiting crop yields. A plant's response to drought is the production of free radicals and drought-tolerant varieties are those characterized by a reduced production of free radicals under water stress. The mechanism of action of photosystem I inhibitor herbicides also results in the formation of free radicals that promote lipid peroxidation, hence destroying cell membranes. This phenomenon is known as oxidative stress and is similar to the response of plants to drought. The objective of this study is to physiologically characterize the sugarcane varieties SP83-2847, SP83-5073, SP80-3280 and CTC2 under levels of oxidative stress caused by the application of paraquat in different doses and to correlate the paraquat tolerance of these varieties with drought tolerance. The relative chlorophyll content (CC), the potential quantum efficiency of photosystem II (Fv/Fm), the visual rating of phytotoxicity (VR) and the fresh biomass (FB) were used as physiological parameters. Oxidative stress was imposed by the application of paraquat at 0 (D0), 25 (D1), 50 (D2), 100 (D3) and 200 (D4) g ha^{-1}. The evaluations were performed at 24, 48 and 960 hours (40 days) after treatment. There was a negative correlation of the paraquat doses and chlorophyll content (CC) estimated by SPAD, quantum efficiency of photosystem II (Fv/Fm), visual phytotoxicity ratings and the fresh biomass. The response patterns of the varieties known as tolerant to drought to the oxidative stress caused by paraquat are not clear, suggesting different mechanisms from oxidative stress caused by drought, questioning the hypothesis that tolerant varieties to oxidative stress caused by herbicides would also be tolerant to drought.

Additional keywords: drought; phytotoxicity; reactive oxygen species.

Resumo

O déficit hídrico é um dos principais fatores limitantes da produtividade agrícola, tendo como consequência na planta a produção de radicais livres, sendo que variedades mais tolerantes à seca apresentam comportamento de menor produção de radicais livres em situação de déficit hídrico. Da mesma forma, a aplicação de herbicidas inibidores do fotossistema I tem como consequência de seu mecanismo de ação, a formação de radicais livres, que promovem a peroxidação dos lipídeos, que por sua vez destroem as membranas celulares, através do fenômeno conhecido como estresse oxidativo, semelhante ao provocado pela reação da planta submetida ao déficit hídrico. Sendo assim, o objetivo deste trabalho foi caracterizar fisiologicamente as variedades SP83-2847, SP83-5073, SP80-3280 e CTC2, submetidas a níveis de estresse oxidativo, ocasionados pela aplicação de doses do herbicida paraquat, e assim correlacionar a tolerância destas variedades ao herbicida com a tolerância à seca. O conteúdo relativo de clorofila (CC), a eficiência quântica potencial do fotossistema II (Fv/Fm), a nota visual de fitotoxicidade (NF) e a fitomassa fresca (MF) foram utilizados como parâmetros fisiológicos de avaliação. O estresse oxidativo foi imposto pela aplicação do herbicida paraquat nas doses 0 (D0), 25 (D1), 50 (D2), 100 (D3) e 200 (D4) g ha^{-1} de paraquat. As avaliações foram realizadas nos períodos de 24, 48 e 960 horas após a aplicação dos tratamentos. O conteúdo relativo de clorofila (CC) estimado através do índice SPAD, a eficiência quântica potencial do fotossistema II (Fv/Fm), a nota visual de fitotoxicidade (NF) e a massa fresca (MF) foram influenciados negativamente pelas doses do paraquat. Os padrões de resposta ao estresse oxidativo ocasionado pela aplicação do paraquat, nas variedades conhecidas como tolerantes ao déficit hídrico, não indicam claramente que são diferentes dos padrões de resposta ao estresse oxidativo ocasionado pelo déficit hídrico, fato que não corrobora claramente a hipótese de que variedades tolerantes ao estresse oxidativo ocasionado por herbicidas também seriam tolerantes ao déficit hídrico.

Palavras-chave adicionais: espécies reativas de oxigênio; fitotoxicidade; seca.

Introduction

Sugarcane is a renewable source with potential for energy production and since the 1970's, due to the emerging energy crisis, sugar--cane yields have become a target for investment in Brazil. The importance of sugarcane can be attributed to its multiple uses - forage for feeding cattle or raw material for the production of brown sugar, molasses, rum, sugar and ethanol. Its importance derives not only from the production yields and from the jobs generation, but also represents a relevant component in the population diet.

Currently, most research on plants is dedicated to seek and develop new varieties of plants resistant to environmental stresses, either biotic or abiotic. However, the ability to survive under stress conditions required some adjustments (HUMMEL et al., 2010).These evolutionary adaptations involve a complex set of variables, which have not been fully covered.

Different varieties of sugar cane respond differently to herbicides and thus, herbicide intoxication is frequent and may reduce crop yield (PROCÓPIO et al, 2004). The tolerance of a sugarcane genotype to an herbicide depends on the phenological development stage of the crop at the time of the herbicide application (FERREIRA et al., 2005).

The use of some specific types of herbicides, e.g. paraquat, results in electron accumulation and generation of toxic radicals in the plant chloroplast, similarly to oxidative stress caused by drought (LAW et al., 1983; COCHEME & MURPHY, 2009). In this work, we verify the hypothesis that the response pattern of sugarcane varieties to paraquat positively correlates to the patterns of response to water deficit, with the formation of reactive oxygen species (ROS). This has been hypothesized in the literature (MILLER et al., 2010). Based on this hypothesis, it would be possible to identify drought tolerance parameters without subjecting varieties directly to drought, but only through observation of the varieties reaction to paraquat application.

Thus, this study aimed to physiologically characterize four varieties of sugarcane under different levels of stress caused by the application of different doses of paraquat, an inducer of oxidative stress. Specifically, the objectives were to (i) identify patterns of response of sugarcane to paraquat-induced oxidative stress, (ii) identify physiological parameters related to tolerance/sensitivity off our varieties of sugarcane under study, and (iii) establish parameters for evaluation of oxidative stress tolerance in genotypes of sugarcane.

Material and methods

The treatments consisted of a combination of two factors, varieties of sugarcane and doses of paraquat, in four and five levels, respectively, with four replications, total of 20 treatments and 80 (20x4) plots. The varieties used were SP80-3280, SP83-2847, SP83-5073 and CTC2. The factor corresponding to dose of herbicide consisted of five doses of paraquat applied in post-emergence conditions of sugarcane (four leaves stage), at 0.0 (D0), 25 (D1), 50 (D2), 100 (D3) and 200 (D4) g ha^{-1}.

The experimental design was randomized complete blocks in a 4x5 factorial design, with four replications. Each plot consisted of three polyethylene pots, containing one sugarcane plant in each pot.

The parameters Fv/Fm (potential quantum efficiency of photo system) and CC (chlorophyll content) were evaluated 24 hours, 48 hours and 960 hours (40 days) after application of the treatments. The parameter FB(Fresh Biomass) was evaluated 40 days after treatment application. The parameter VR (Visual Rate) was measured at intervals of 1, 2 and 40 days after treatment application. Regarding the data analysis, the F-test was used in the analysis of variance and the averages compared by Scott-Knott test at 5 % probability (SCOTT & KNOTT, 1974).

The plants used in the trial were obtained from pieces of stem with buds meristem, that underwent a heat treatment process that ensures the health and quality of the material used. The seedlings were preselected among the three months-old buds grown individually and planted in pots containing 8 L of substrate Norplant (Norplant®,Brazil). The fertilization consisted of 2 g L^{-1}Basacote Plus 12M, 9-12-15 (N-P_2O_5--K_2O), incorporated into the substrate. The Basacote is a slow release fertilizer recommended for use with substrates.

Treatments were applied during post-emergence of sugarcane, 120 days after planting. This application was performed by a precision sprayer, pressurized with CO_2, operated at 40 psi and sprayed through a single nozzle TeeJet, 110.02VS, with width of 0.5 m and volume proportional to an application of 200 L ha^{-1}.

After application, the plants were cultivated under ideal conditions of growth. Agricultural operations were taken to control pests and diseases as required based on daily monitoring of the experiment.

The visual evaluations for herbicide injuries were carried out 1 (24 h), 2 (48 h) and 40 days (960 h) after the application, using a visual rating, ranging from 0 (no symptoms) to 10 (total death of the plants).The assessments of chlorophyll fluorescence were performed after 1 (24 h), 2 (48 h) and 40 days (960 h) of treatment with paraquat in a "Portable Chlorophyll Fluorometer" (WFP 2000 Hinz Walz, Germany) equipped with a Saturation Pulse Control Unit. The analyzes were performed following the methodology described by SCHREIBER et al. (1986) and GENTY et al. (1989) based on the evaluation of the emission of fluorescence by the upper surface of the leaves. The parameters were obtained in two intensities, medium and high, with average intensities of 100 and 325 µmol m^{-2} s^{-1}photons, respectively. The data collected in all measurements and continuous monitoring were analyzed in spreadsheets and charts in Excel® 2007, considering the average of three readings for each parameter measured.

Before the analyses, all plants were kept in the dark for a period of 30 minutes for complete oxidation of the electron transport system components; the following parameters were then determined: Fo, Fm, and Fv/Fm. Parameters Fo and Fm were obtained from direct reading and the ratio Fv/Fm was calculated according to the following formula (BILGER et al. 1995; WALZ, 1993):

$$Fv/Fm = (Fm - FO)/Fm$$

Where: FO: minimum fluorescence obtained with all PSII reaction centers opened while the thylakoid membrane is in the non-energized state, that is, adapted to the dark; Fm: maximal fluorescence obtained when all PSII reaction centers are closed (oxidized),that is, in the dark-adapted state; Fv = (Fm - FO):maximum variable fluorescence obtained in dark adapted leaves.

The chlorophyll content was measured in the first fully expanded leaf after 1, 2 and 40 days of treatment with paraquat, with SPAD-502. The readings taken by a portable chlorophyll meter correspond to the relative chlorophyll content present in the plant leaf. The measures are calculated by the equipment based on the amount of light transmitted through the leaf in two wavelengths with different absorbances by chlorophyll (MINOLTA 1989). The values obtained are proportional to the amount of chlorophyll present in the leaf.

The fresh biomass was determined 40 days after treatment, which is equivalent to 160 days after planting. All plants were cut manually in the basal portion, close to the substrate, and weighted on a precision scale.

Results and discussions

The plant material produced by this experiment allowed the characterization of the oxidative response of each variety for the imposed oxidative stress. Parts of this study were presented in other works (FERREIRA et al., 2010). In their study, FERREIRA et al. (2010) observed differential tolerance of sugarcane to stress caused by herbicides.

Relative chlorophyll content (CC)

The analysis of variance showed significant effects on the chlorophyll content according to variety after 48 hours ($p<0.01$) and after 40 days ($p<0.001$) of treatment and according to dose after 24 and 48 hours ($p<0.001$).

The degradation of chlorophyll is one of the consequences of oxidative stress and is shown by the loss of green color by the leaves (LONG et al., 1994). The technique used here is widely accessible and has been proven a reliable tool to differentiate tolerant and sensitive plants to water stress (O'NEILL et al., 2006).

After 24 hours of paraquat application, none of the studied varieties had its chlorophyll content affected, regardless of the dose used. The comparison between varieties also showed no significant difference for any given dosage.

The analysis within varieties showed that, after 48 hours, all varieties had a reduction in the chlorophyll content comparing the doses D2, D3 and D4 to D0. The varieties CTC2 and SP80-3280 showed no difference between doses D0 and D1. VarietyCTC2 had no significant difference between the doses D2, D3 and D4. SP83-SP83 -2847 and 5073 showed no difference in the values of relative chlorophyll content between the doses D3 and D4 (Table 1).

In the comparison within doses, CTC2 and SP80-3280 showed higher SPAD index values than those of SP83-2847 and SP83-5073 with dose D1. Even without statistically significant difference, within dose D3, CTC2 and SP80-3280 showed different responses from SP83-2847 and SP83-5073, with higher values. Considering all doses, the varieties with the smallest average reduction in chlorophyll content were CTC2 and SP80-3280in the measurements after 24 and 48 hours, while those with the largest average reduction were SP83-2847 and SP83-5073.

Regardless of the dose, after 40 days of paraquat application, none of the varieties had its chlorophyll content affected.

Potential quantum efficiency of photosystem II (Fv/Fm)

According to the analysis of variance, there were significant effects on the variable Fv/Fm in relation to variety after 48 hours ($p<0.01$) and dose after 24 and 48 hours ($p<0.001$). The effect of the interaction between the factors variety and dose was not significant in any of the assessments. After 24 hours of the paraquat application, based on the Fv/Fm variable, there was no significant difference between varieties within each dose.

The analysis within dose shows that, after 48 hours, with D3, CTC2 and SP80-3280 presented higher values of Fv/Fm than SP83--2847 and SP83-5073. On the other hand, SP80-

-3280 showed statistically equivalent values for doses D2 and D3 (Table 2).

After 24 and 48 hours, both chlorophyll content (CC) and Fv/Fm were higher for CTC2 and SP80-3280 than for SP83-2847 and SP83-5073 at the dose D3, but the difference was statistically significant only for Fv/Fm after 48 hours (Table 2).

Table 1 - Relative chlorophyll content (CC) expressed in SPAD units, for the four varieties of sugarcane after 48 hours of paraquat application.

Varieties*	D0 (0 g ha^{-1})	D1 (25 g ha^{-1})	D2 (50 g ha^{-1})	D3 (100 g ha^{-1})	D4 (200 g ha^{-1})
CTC2	38.2 Aa	38.2 Aa	20.3 Ba	14.8 Ba	11.2 Ba
SP80-3280	43.8 Aa	36.5 Aa	21.1 Ba	14.2 Ba	4.5 Ca
SP83-2847	47.6 Aa	22.2 Bb	16.8 Ba	9.6 Ca	4.7 Ca
SP83-5073	46.8 Aa	26 Bb	16.6 Ca	3.1 Da	2.3 Da

*The averages followed by the same lowercase letter within a column are not different by the Scott-Knott test at 5% significance level. The averages followed by the same capital letter within a line are not different by the Scott-Knott test at 5% significance level.

Table 2 - Potential quantum efficiency of photosystem II (Fv/Fm) for the four varieties of sugarcane, 48 hours after paraquat application.

Varieties*	D0 (0 g ha^{-1})	D1 (25 g ha^{-1})	D2 (50 g ha^{-1})	D3 (100 g ha^{-1})	D4 (200 g ha^{-1})
CTC2	0.779 Aa	0.604 Ba	0.288 Ca	0.175 Ca	0.092 Ca
SP80-3280	0.757 Aa	0.652 Aa	0.285 Ba	0.238 Ba	0.069 Ca
SP83-2847	0.785 Aa	0.518 Ba	0.195 Ca	0.033 Cb	0.044 Ca
SP83-5073	0.781 Aa	0.528 Ba	0.266 Ca	0.051 Db	0.030 Da

*The averages followed by the same lowercase letter within a column are not different by the Scott-Knott test at 5% significance level. The averages followed by the same capital letter within a line are not different by the Scott-Knott test at 5% significance level.

After 40 days of the paraquat application, none of the studied varieties presented alterations in the ratio Fv/Fm, regardless of the dose used.

It is likely that the oxidative stress imposed on the plants do not cause a long-term photoinhibition, since after 40 days of the paraquat application, there was no alterations in the Fv/Fm ratio, regardless of the dose used. This fact suggests the absence of severe damage in the light collector complex of PSII, as exposed by QUEIROZ et al. (2002) for mastic plants under water deficit.

In *Arabidopsis thaliana,* photosynthesis can be reduced before any changes in chlorophyll content. The combination of high chlorophyll content and low CO_2 assimilation can cause an imbalance between the capture and energy dissipation, resulting in photoinhibition and senescence (STESSMAN et al., 2002).

Evaluation of phytotoxicity (NF)

In the analysis of variance, there were significant effects on the phytotoxicity variable promoted by the factors variety and dose, after 24 hours ($p<0.001$) and dose after 48 hours and 960 hours (40 days) ($p<0.001$). There was no significant effect of the interaction between varieties and doses in any of the assessments.

The analysis within varieties after 24, 48 and 960 hours showed that all varieties had an increase in the values of phytotoxicity rating comparing the doses D1, D2, D3 and D4 to D0.

Within dosages, SP80 and CTC2 3280 showed lower phytotoxicity rating values than SP83-2847 and SP83-5073 after 24 hours for doses D1 and D2. In dose D3, CTC2 and SP83-2847 showed lower phytotoxicity rating values than SP80-3280 and SP80-5073 (Table 3).

Also, after 48 hours, CTC2 and SP80-3280 showed lower phytotoxicity note values than SP83-2847 and SP83-5073 for the dose D1. For doses D1, D2 and D3, the CTC2 showed the lowest values for phytotoxicity rating (Table 4).

In a more thorough analysis, within dose, after 40 days (960 hours) and in D1, CTC2, SP80-3280 and SP83-5073 showed lower phytotoxicity ratings than SP83-2847 (Table 5).

In a more thorough analysis, within dose, after 40 days (960 hours) and in D1, CTC2, SP80-3280 and SP83-5073 showed lower phytotoxicity ratings than SP83-2847(Table 5).

Considering all doses after 24 hours, 48 hours and 960 hours, the variety with the lowest average score of phytotoxicity (NF) was CTC2 (5.0). The other average ratings were SP80-3280 (5.4), SP83-5073 (5.4) and SP83-2847 (5.5).

Table 3 - Phytotoxicity rate for the four varieties of sugarcane after 24 hours of paraquat application.

Varieties*	D0 (0 g ha^{-1})	D1 (25 g ha^{-1})	D2 (50 g ha^{-1})	D3 (100 g ha^{-1})	D4 (200 g ha^{-1})
CTC2	0 Ea	2.2 Db	4.1 Cb	5.6 Bb	7.7 Aa
SP80-3280	0 Ea	2.4 Db	4.1 Cb	6.0 Ba	7.6 Aa
SP83-2847	0 Ea	2.7 Da	4.6 Ca	5.7 Bb	7.4 Aa
SP83-5073	0 Ea	2.8 Da	4.8 Ca	6.3 Ba	7.5 Aa

*The averages followed by the same lowercase letter within a column are not different by the Scott-Knott test at 5% significance level. The averages followed by the same capital letter within a line are not different by the Scott-Knott test at 5% significance level.

Table 4 – Phytotoxicity ratings for the four varieties of sugarcane after 48 hours of paraquat application.

Varieties*	D0 (0 g ha^{-1})	D1 (25 g ha^{-1})	D2 (50 g ha^{-1})	D3 (100 g ha^{-1})	D4 (200 g ha^{-1})
CTC2	0 Ea	2.9 Db	4.1 Cb	6.2 Bb	7.8 Aa
SP80-3280	0 Da	2.9 Cb	5.3 Ba	7.9 Aa	8.1 Aa
SP83-2847	0 Da	3.9 Ca	5.8 Ba	7.4 Aa	7.4 Aa
SP83-5073	0 Da	3.4 Ca	5.5 Ba	7.3 Aa	7.6 Aa

*The averages followed by the same lowercase letter within a column are not different by the Scott-Knott test at 5% significance level. The averages followed by the same capital letter within a line are not different by the Scott-Knott test at 5% significance level.

Table 5 - Phytotoxicity ratings for four varieties of sugarcane 40 days (960 hours)after paraquat application.

Varieties*	D0 (0 g ha^{-1})	D1 (25 g ha^{-1})	D2 (50 g ha^{-1})	D3 (100 g ha^{-1})	D4 (200 g ha^{-1})
CTC2	0 Da	3.1 Cb	4.9 Ba	5.8 Aa	5.8 Aa
SP80-3280	0 Da	3.3 Cb	5.3 Ba	5.8 Ba	6.5 Aa
SP83-2847	0 Ca	4.1 Ba	5.2 Aa	6.0 Aa	5.8 Aa
SP83-5073	0 Ca	3.2 Bb	5.1 Aa	5.5 Aa	5.7 Aa

*The averages followed by the same lowercase letter within a column are not different by the Scott-Knott test at 5% significance level. The averages followed by the same capital letter within a line are not different by the Scott-Knott test at 5% significance level.

Fresh biomass (MF - kg $plant^{-1}$)

According to the analysis of variance, there were statistically significant effects on the fresh biomass variable promoted by the factors variety and dose after 40 days (960 hours) ($p < 0.001$). The interaction between variety and dose had no significant effect.

Looking within each variety, for 40 days (960 hours), the variety CTC2 showed weight loss only for D3, while SP83-5073 and SP83-2847 showed weight loss for D2 and SP80-3280 for D1 (Table 6).Withindose, SP80-3280 presented a larger fresh biomass than the other varieties (Table 6).

Considering all doses, the variety with the lowest average biomass weight loss was CTC2 (18%), followed by SP83-2847 (25.4%) and SP83-5073 (25.5%). The SP80-3280 decreased 30.3%.

Table 6 - Fresh biomass (kg $plant^{-1}$) for the four varieties of sugarcane 40 days (960 hours) after paraquat application.

Varieties*	D0 (0 g ha^{-1})	D1 (25 g ha^{-1})	D2 (50 g ha^{-1})	D3 (100 g ha^{-1})	D4 (200 g ha^{-1})
CTC2	0.52 Ab	0.49 Aa	0.49 Aa	0.36 Bb	0.37 Ba
SP80-3280	0.73 Aa	0.58 Ba	0.52 Ba	0.50 Ba	0.46 Ba
SP83-2847	0.58 Ab	0.61 Aa	0.45 Ba	0.37 Bb	0.39 Ba
SP83-5073	0.54 Ab	0.52 Aa	0.42 Ba	0.34 Bb	0.33 Ba

*The averages followed by the same lowercase letter within a column are not different by the Scott-Knott test at 5% significance level. The averages followed by the same capital letter within a line are not different by the Scott-Knott test at 5% significance level.

Conclusions

The response to oxidative stress caused by paraquat application in sugarcane varies according to the measured parameters-variety, herbicide doses and time of evaluation.

The periods of 24 and 48 hours after application of paraquat presented the best results to identify physiological differences in the response to oxidative stress among the varieties.

After 24 hours of paraquat application, although the chlorophyll content (CC) is unaltered, the potential quantum efficiency of photosystem II (Fv/Fm) is significantly affected in the varieties studied; however there are no differences between varieties for the same doses.

After 40 days of application, none of the varieties showed reduction on the parameters chlorophyll content (CC) and potential quantum efficiency of photosystem II (Fv/Fm), regardless of dose.

Visual phytotoxicity (VR) is the only parameter assessed with significant differences in all varieties, in all periods of evaluation and for all doses in comparison to D0.

The chlorophyll content estimated by SPAD, the technique of potential quantum efficiency of photosystem II (Fv/Fm), the visual phytotoxicity rating (VR) and the fresh biomass (FB) are negatively influenced by the treatment with paraquat.

The response patterns of the physiological aspects to the application of paraquat in the varieties considered tolerant to drought are not clearly distinct from the patterns expected from hydric deficit. This fails to corroborate the hypothesis that varieties tolerant to oxidative stress caused by herbicides would also be tolerant to drought. On the other hand, the parameters quantum efficiency and chlorophyll content need further attention, since this work indicated a potential for research in association with appropriate doses and seasonalities.

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