Photochemical efficiency in pineapple plants under saline water irrigation

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Cleiton Fernando Barbosa Brito1†; Varley Andrade Fonseca1†; Marcelo Rocha dos Santos2; Sérgio Luiz Rodrigues Donato2†; Alessandro de Magalhães Arantes2†; Aloísio José dos Santos2†

1Departamento de Ciências Agrárias. Universidade Estadual de Montes Claros (UNIMONTES), Caixa Postal 91, CEP: 39440-000, Janaúba, MG, Brazil. E-mail: varley.ibce@ig.com.br
2Setor de Agricultura. Instituto Federal de Educação, Ciência e Tecnologia Baiano (IFBaiano), Caixa Postal 09, CEP: 46430-000, Guanambi, BA, Brazil. E-mail: marcelo.rocha@ifbaiano.edu.br, sergio.donato@guanambi.ifbaiano.edu.br, alessandro.arantes@guanambi.ifbaiano.edu.br, aloisio.santos@ifbaiano.edu.br

†Corresponding author. E-mail: cleiton.ibce@hotmail.com

ABSTRACT

Studies determining physiological characteristics of field-grown pineapples irrigated with low-quality water are lacking. This work evaluated the photochemical efficiency of ‘Pérola’ pineapple irrigated with saline water in the semiarid region of Bahia, Brazil. The experiment was carried out in randomized blocks with five treatments consisting of the following irrigation depths: 100% of ETc using water with electrical conductivity (ECw) of 0.75 dS m⁻¹; and 50, 75, 100 and 125% of ETc using water with ECw of 3.6 dS m⁻¹. Chlorophyll a fluorescence measurements were made over the course of 13 months using a pulse-modulated fluorometer, in all treatments. Quantum efficiency (Fv/Fm) fluctuated throughout the cycle of the pineapple with values below the ideal, especially at the end of the crop cycle. Quantum yield of photosystem II (Yield), photochemical quenching (qP), non-photochemical quenching (NPQ) and chlorophyll fluorescence decrease ratio (Rfd) were not influenced by irrigation depths. Therefore, energy used for photosynthetic processes in pineapple plants is not affected by irrigation using saline water with electrical conductivity of 3.6 dS m⁻¹.

Keywords: Ananas comosus, CAM plants, chlorophyll fluorescence, salinity.

Eficiência fotoquímica em plantas de abacaxi sob irrigação com água

RESUMO

São escassos estudos que determinem as características fisiológicas do abacaxizeiro, em condições de campo, irrigadas com água de qualidade inferior. Assim, objetivou-se avaliar eficiência fotoquímica em abacaxizeiro ‘Pérola’ irrigado com água salina no semiárido baiano. O experimento foi conduzido em blocos casualizados com cinco tratamentos representados pelas lâminas de irrigação: 100% da ETc com água de condutividade elétrica (CEa) de 0,75 dS m⁻¹ e 50, 75, 100 e 125% da ETc com aplicação de água de CEa de 3,6 dS m⁻¹. As leituras da fluorescência da clorofila “a” foram realizadas durante 13 meses através de fluorômetro de luz modulada, em todos os tratamentos. Verificou-se que a eficiência quântica (Fv/Fm) variou ao
longo do ciclo do abacaxizeiro com valores abaixo do ideal, principalmente, no final do ciclo. As variáveis rendimento quântico do fotossistema II (Yield), dissipação fotoquímica (qP), dissipação não-fotoquímica (NPQ) e taxa de redução de fluorescência (RFd) não foram influenciadas pelas lâminas de irrigação avaliadas. Portanto, a utilização da energia nos processos fotossintéticos de plantas de abacaxizeiro não são influenciados pela irrigação com lâminas de água salina com condutividade elétrica de 3,6 dS m⁻¹.

**Palavras-chave:** *Ananas comosus*, Fluorescência da clorofila, plantas CAM, salinidade.

### 1. INTRODUCTION

Pineapples (*Ananas comosus* L. Merril) are economically exploited in most Brazilian states, making an important contribution to employment and income generation (Franco *et al.*, 2014), in which the states of Pará, Paraíba, Minas Gerais and Bahia are the biggest producers. In the state of Bahia, the municipality of Itaberaba stands out as the main producer (IBGE, 2020) and is located in the semi-arid region of the state.

In this context, the pineapple can become an alternative crop for other semiarid regions (Mota *et al.*, 2016) since it has the potential of maintaining its yield under hotter and drier climates (Borland *et al.*, 2014) owing to its crassulacean acid metabolism (CAM) (Zhang *et al.*, 2014; Couto *et al.*, 2016). CAM plants save water by closing their stomata during the day and opening them during the night with CO₂ fixation, resulting in better water-use efficiency in dry conditions (Carr, 2012).

Studies were developed for irrigated pineapple grown in semiarid regions (Franco *et al.*, 2014; Pegoraro *et al.*, 2014; Maia *et al.*, 2016); however, information about physiological characteristics of this crop irrigated with saline water is lacking, making field studies with pineapple subjected to saline conditions necessary (Elhag and Elzain, 2012).

Chlorophyll a fluorescence is a non-invasive analysis that allows collecting data from the efficiency of the photochemistry stage of photosynthesis (Light or hill reaction) and hence is a reliable source of information about plant condition, especially under abiotic stress conditions (Murchie and Lawson, 2013; Goltsev *et al.*, 2016).

Studies about physiological characteristics of pineapple in regard to fluorescence were mainly carried out in controlled environments such as greenhouses and laboratory, in vitro, using only plantlets (Vieira *et al.*, 2010; Cruz *et al.*, 2014; Couto *et al.*, 2016). Thus field research into chlorophyll fluorescence comprising the whole pineapple cycle is much needed.

Fluorescence determination under field conditions can be useful for studies on physiological behavior of CAM plants (Díez *et al.*, 2017), such as the pineapple, thereby shedding some light on the crop adaptability in context of the semiarid region. Therefore, this study evaluated photochemistry efficiency in ‘Pérola’ pineapple submitted to irrigation with saline water and water stress in the semiarid region of Bahia, Brazil.

### 2. MATERIAL AND METHODS

The study was carried out in an experimental area at the Federal Institute Baiano, Guanambi campus, located in the Irrigated Perimeter of Ceraíma, municipality of Guanambi, Bahia, Brazil. The mean annual rainfall depth of the region was 680 mm, and the mean annual temperature was 26°C. During the experimental period, there was a maximum average temperature (Tₘₐₓ) of 32.07°C and a minimum average temperature (Tₘᵟₐᵝ) of 21.95°C, relative humidity (RH) of 55.65%, gust wind of 46.70 km h⁻¹, reference evapotranspiration (ETₒ) of 5.68 mm dia⁻¹ and a rain total of 728 mm (Figure 1).
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Pineapples were cultivated in a typic dystrophic Red Yellow Latosol, with weak A horizon, on flat to gently undulating relief. The soil chemical characteristics (Tedesco et al., 1995) in the layer of 0-20 cm before installing the experiment were: pH (in water) = 5.7; P (Mehlich extractor) = 23.5 mg dm$^{-3}$; K (Mehlich extractor) = 108 mg dm$^{-3}$; Ca = 1.4 cmol$_{c}$ dm$^{-3}$; Mg = 0.6 cmol$_{c}$ dm$^{-3}$; Al = 0.0 cmol$_{c}$ dm$^{-3}$; H+Al = 1.7 cmol$_{c}$ dm$^{-3}$; SB = 2.4 cmol$_{c}$ dm$^{-3}$; t = 2.4 cmol$_{c}$ dm$^{-3}$; T = 4.1 cmol$_{c}$ dm$^{-3}$; V = 58%; B = 0.3 mg dm$^{-3}$; Cu = 0.4 mg dm$^{-3}$; Fe = 16.0 mg dm$^{-3}$; Mn = 32.5 mg dm$^{-3}$; Zn = 2.1 mg dm$^{-3}$; EC$_{e}$ = 0.7 dS m$^{-1}$. Soil textural class was sandy loam clay (Sand = 68 dag kg$^{-1}$; Silt = 11 dag kg$^{-1}$ and Clay = 21 dag kg$^{-1}$) (Embrapa, 2017).

‘Pérola’ pineapple seedlings (slips) were planted in April 2015 in a single row, at a spacing of 0.25 m between plants and 1.2 m between rows (33,300 plants ha$^{-1}$). Soil correction and basal and top-dressing fertilizations were performed according to the soil analysis (Souza et al., 2007). During the experiment, cultivation and phytosanitary practices established for the crop were employed and there was low incidence of pests and diseases.

One month after planting, plants received foliar application of urea, zinc sulfate and potassium chloride (KCl). After that, every two months, urea (5 g plant$^{-1}$) and KCl (2.5 g plant$^{-1}$) were broadcasted.

Irrigation was applied through a drip system, using pressure-compensating emitters with nominal flow rate of 8 L h$^{-1}$, spaced at 0.75 m apart, forming a continuous wet strip along plant rows. Until the fourth month after planting, irrigations were applied daily similarly in all plots, in order to maintain uniform soil water content and favor the initial growth of the seedlings and crop establishment. Then, irrigation depths started to be applied, and irrigation time was calculated based on crop evapotranspiration (ET$_{c}$) (Santos et al., 2015) obtained using the reference evapotranspiration (ET$_{o}$), determined daily through the Penman-Monteith method using data from a weather station installed approximately 200 m away from the experiment, and crop coefficient (Kc), which was 0.8 at the initial stage of crop establishment and 1 for both the vegetative stage and after flower induction (reproductive stage), according to Santana et al. (2013).

The experiment was conducted in randomized blocks with five treatments consisting of the following irrigation depths: 100% ET$_{c}$, using water with electrical conductivity (EC$_{w}$) of 0.75 dS m$^{-1}$ and C2S1 classification, and 50, 75, 100 and 125% ET$_{c}$, using water from a tubular well with EC$_{w}$ of 3.6 dS m$^{-1}$, classified as C4S1 according to Ayers and Westcot (1985). Treatments had four replicates and the experimental unit consisted of four 8-m-long rows. Evaluations were made in plants from the central 4 m of the two central rows, totaling 26
evaluated plants in the plot.

The water from tubular well has pH of 6.4, 11.90 mEq L$^{-1}$ of calcium, 9.54 mEq L$^{-1}$ of magnesium, 0.48 mEq L$^{-1}$ of potassium, 30.40 mEq L$^{-1}$ of sodium, 0.00 mEq L$^{-1}$ of carbonate, 4.10 mEq L$^{-1}$ of bicarbonate and 34.80 mEq L$^{-1}$ of chloride.

Pineapple flowering was artificially induced 13 months after planting, by applying ETHREL (240 g L$^{-1}$ of Ethephon), a synthetic growth regulator, precursor of the synthesis of ethylene, using a 20-L backpack sprayer. The backpack sprayer received 40 mL of ETHREL + 400 g of urea (2%) to apply an estimated volume of 50 mL of the mixture inside the leaf rosette.

Chlorophyll $a$ fluorescence readings were made from August 2015 and September 2016 using a pulse-modulated fluorometer, model OS1-FL (OPTI-Sciences) in the morning (8 a.m.) and in the afternoon (2 p.m.). Clips used to measure chlorophyll fluorescence were positioned on the middle third of the ‘D’ leaf, and the measurement was made after 5 min of dark adaptation, with an emission of a 0.3-s-long saturation light pulse at a frequency of 0.6 KHz. Then, ground fluorescence ($F_0$), maximum fluorescence ($F_m$), variable fluorescence ($F_v$) and photochemistry efficiency ($F_v/F_m$) were recorded. During measurements, a clip for adapting chloroplast to the dark was used so that all photosystem II (PSII) reaction centers were “open” and heat loss was kept to a minimum (Strauss et al., 2006).

Additionally, readings were taken on light-adapted ‘D’ leaf, on which saturation pulses were applied to determine fluorescence under steady state ($F_s$), maximum fluorescence in the light-adapted state ($F_{ms}$), variable fluorescence in the light-adapted state ($F_{vs}$), and quantum yield of PSII (Yield). Three dark-adapted readings and one light-adapted reading were taken at each identified plant. According to Lichtenhaller et al. (2005), photochemical quenching ($qP$), non-photochemical quenching (NPQ), and chlorophyll fluorescence decrease ratio ($R_{Fd}$) were determined using the following Equations 1, 2 and 3:

$$q_P = \frac{(F_{ms} - F)}{F_{vs}} \quad (1)$$

$$NPQ = \frac{(F_m - F_{ms})}{F_{ms}} \quad (2)$$

$$R_{Fd} = \frac{(F_m/F_s) - 1} \quad (3)$$

It is worth noting that the abbreviations $qP$ (photochemical quenching), NPQ (non-photochemical quenching) and $R_{Fd}$ (fluorescence decrease ratio) will be used in accordance with the international standard widely used in studies on chlorophyll fluorescence (Lichtenhaller et al., 2005).

Data were subjected to the analysis of variance and interactions were interpreted according to their significance. Variable means were compared by F-test and Tukey test (p<0.05) for the factors reading times and irrigation depths, respectively; then, they were grouped by the Scott-Knott criterion (p<0.05) for the factor evaluation season (months). Statistical analysis was performed with the statistical software ‘R’ (R Development Core Team, 2012).

3. RESULTS AND DISCUSSION

Quantum efficiency ($Fv/Fm$) of pineapple plants subjected to different irrigation depths with saline water over the months had a significant interaction between months and irrigation depths (Table 1). Nonetheless, significant differences were not observed across depths in all months of evaluation, except for November and December 2015. Irrigation depth 100% of ET$_c$ using water with EC$_w$ of 0.75 dS m$^{-1}$ differed from depths 50, 100 and 125% of ET$_c$ using water with 3.6 dS m$^{-1}$ in November; as for December, irrigation depth 100% of ET$_c$ using water of EC$_w$ of 0.75 dS m$^{-1}$ differed from depths 50 and 75% of ET$_c$.

Furthermore, Fv/Fm values generated two groups within each depth, except for the depth...
125% of ETc. In general, lower values were verified in the last three months (Table 1).

**Table 1.** Quantum efficiency (Fv/Fm) in ‘Pérola’ pineapple plants subjected to different irrigation depths with non saline water and saline water over the months.

| Month   | 100% ETc | 50% ETc | 75% ETc | 100% ETc | 125% ETc |
|---------|----------|---------|---------|----------|----------|
|         | 0.75 dSm⁻¹ | 3.6 dSm⁻¹ |
| Aug/15  | 0.57 A    | 0.62 A   | 0.56 A   | 0.52 B    | 0.56 A   |
| Sep/15  | 0.57 A    | 0.49 B   | 0.55 A   | 0.49 B    | 0.53 A   |
| Oct/15  | 0.57 A    | 0.52 B   | 0.54 A   | 0.49 B    | 0.51 A   |
| Nov/15  | 0.65 Aa   | 0.54 Bb  | 0.58Aab  | 0.55Ab    | 0.54Ab   |
| Dec/15  | 0.57 Aa   | 0.46 Bb  | 0.45 Bb  | 0.5 Bab   | 0.49Aab  |
| Feb/16  | 0.6 A     | 0.59 A   | 0.57 A   | 0.57 A    | 0.6 A    |
| Mar/16  | 0.56 A    | 0.59 A   | 0.61 A   | 0.57 A    | 0.53 A   |
| Apr/16  | 0.53 B    | 0.58 A   | 0.59 A   | 0.58 A    | 0.54 A   |
| May/16  | 0.52 B    | 0.57 A   | 0.58 A   | 0.58 A    | 0.5 A    |
| Jun/16  | 0.55 A    | 0.56 A   | 0.56 A   | 0.57 A    | 0.5 A    |
| Jul/16  | 0.48 B    | 0.51 B   | 0.52 B   | 0.5 B     | 0.52 A   |
| Aug/16  | 0.49 B    | 0.51 B   | 0.5 B    | 0.5 B     | 0.5 A    |
| Sep/16  | 0.46 B    | 0.49 B   | 0.5 B    | 0.5 B     | 0.49 A   |

CV (%) 40.27

*Means followed by the same uppercase letters, in the column for months, belong to the same group by the Skott-Knott criterion at 5% of significance level, and lowercase letters in the rows, for depths, do not differ from each other by the Tukey test at 5% of significance level.

Fluctuations in Fo, Fm and Fv contributed to the reduction in maximum quantum efficiency of PSII (Fv/Fm). Despite the difference between mean values resulting from the application of different irrigation depths, in the months of November and December, Fv/Fm in pineapple plants were below what is considered as optimal (Fv/Fm of 0.800 ± 0.5), according to Bolhàr-Nordenkampf et al. (1989). Accordingly, Fv/Fm ratios indicate that the photochemical system of pineapple plants was altered. These low values of Fv/Fm are possibly due to higher photochemical energy on the leaf than the capacity of using it to drive the photosynthesis, which decreases Fv/Fm, i.e., leads to higher non-photochemical quenching. This decrease below the ideal is perhaps a defense mechanism to reduce light energy absorption and thereby decreasing the electron flow within the electron transport chain (Willadino et al., 2011).

Another important factor is that in the months that correspond to the reproductive stage, after flowering in June 2016, Fv/Fm had the lowest values.

Thus, it is evident that biotic and abiotic conditions under which the experiment was carried out damaged the photosynthetic apparatus, impairing the PSII over the time of exposition to stress (Freire et al., 2014), so the ecophysiological behavior of pineapple over the growing season in the semiarid might be a result of several environmental factors.

Regarding the readings on the light-adapted ‘D’ leaves of pineapple, chlorophyll fluorescence under steady state (Fs), maximum fluorescence in the light-adapted state (Fms) and variable fluorescence in the light-adapted state (Fvs) were affected only by irrigation depth, regardless of reading time or evaluation season of these variables (Table 2). Fs, Fms and Fvs had the highest values for irrigation depth 100% of ETc using water with ECw of 0.75 dSm⁻¹.
Chlorophyll $a$ Fluorescence under steady state ($F_s$), maximum fluorescence in the light-adapted state ($F_{ms}$), variable fluorescence in the light-adapted state ($F_{vs}$) and quantum yield of photosystem II ($Yield$) in 'Pérola' pineapple plants subjected to different irrigation depths with non-saline water and saline water over the months.

| Depths  | $F_s$ | $F_{ms}$ | $F_{vs}$ |
|---------|-------|----------|----------|
| 100% ET, (EC$_w$ = 0.75 dS m$^{-1}$) | 403.9 a | 688.8 a | 284.8 a |
| 50% ET, (EC$_w$ = 3.6 dS m$^{-1}$) | 354.6 b | 547.9 b | 193.2 b |
| 75% ET, (EC$_w$ = 3.6 dS m$^{-1}$) | 380.1 ab | 589.5 b | 209.4 b |
| 100% ET, (EC$_w$ = 3.6 dS m$^{-1}$) | 363.6 b | 572.1 b | 208.5 b |
| 125% ET, (EC$_w$ = 3.6 dS m$^{-1}$) | 374.0 ab | 595.2 b | 221.3 b |
| CV (%) | 29.78 | 48.94 | 91.74 |

Means followed by the same letters, in the columns, do not differ from each other by Tukey test at 5% of significance level.

$F_s$, $F_{ms}$ and $F_{vs}$ were higher in plants irrigated using water with EC$_w$ of 0.75 dS m$^{-1}$ (Table 2), although Yield did not have the same tendency. It is noted that under field conditions, salinity stress is enhanced by other environmental adversities, such as high temperatures, low relative humidity and high wind speed (Figure 1).

Chlorophyll fluorescence results presented herein prove that pineapple plants subjected to abiotic stresses such as salinity, high temperatures and low relative humidity exhibit changes in the functional state of thylakoid membranes of chloroplasts. These changes have an impact on fluorescence and, consequently, on quantum efficiency ($F_v/F_m$) and on potential quantum yield ($Yield$) (Cha-Um and Kirmanee, 2011). In spite of $F_v/F_m$ being below the ideal, the pineapple proved to tolerate salinity since $Yield$, $q_P$, NPQ and RFd results indicate a high efficiency at converting light energy into chemical energy, without differences between using water with EC$_w$ of 0.75 and 3.6 dS m$^{-1}$ for irrigation.

Quantum photosynthetic yield of PSII ($Yield$), photochemical quenching, ($q_P$) non-photochemical quenching (NPQ) and fluorescence decrease ratio ($R_{FD}$) were not influenced by irrigation depths (Table 3). There was only the effect of the time factor. Yield and $R_{FD}$ were higher at 2 p.m. while $q_P$ was higher at 8 a.m. As for NPQ, no significant differences were observed.

Table 3. Quantum yield of photosystem II ($Yield$), photochemical quenching ($q_P$) non-photochemical quenching (NPQ) and fluorescence decrease ratio ($R_{FD}$) in 'Pérola' pineapple plants subjected to different irrigation depths with non-saline water and saline water.

| Depths  | Yield 8 a.m. | $q_P$ 8 a.m. | NPQ 8 a.m. | $R_{FD}$ 8 a.m. | Yield 2 p.m. | $q_P$ 2 p.m. | NPQ 2 p.m. | $R_{FD}$ 2 p.m. |
|---------|--------------|--------------|------------|----------------|--------------|--------------|------------|----------------|
| 100% ET, (EC$_w$ = 0.75 dS m$^{-1}$) | 0.26 Ab | 0.30 Aa | 0.44 Aa | 0.33 Ab | 0.30 Aa | 0.36 Aa | 1.48 Ab | 2.28 Aa |
| 50% ET, (EC$_w$ = 3.6 dS m$^{-1}$) | 0.24 Ab | 0.30 Aa | 0.44 Aa | 0.34 Ab | 0.31 Aa | 0.35 Aa | 1.50 Ab | 2.24 Aa |
| 75% ET, (EC$_w$ = 3.6 dS m$^{-1}$) | 0.24 Ab | 0.34 Aa | 0.45 Aa | 0.34 Ab | 0.30 Aa | 0.31 Aa | 1.39 Ab | 2.12 Aa |
| 100% ET, (EC$_w$ = 3.6 dS m$^{-1}$) | 0.26 Ab | 0.31 Aa | 0.45 Aa | 0.38 Ab | 0.27 Aa | 0.30 Aa | 1.38 Ab | 1.92 Aa |
| 125% ET, (EC$_w$ = 3.6 dS m$^{-1}$) | 0.27 Aa | 0.30 Aa | 0.43 Aa | 0.37 Ab | 0.29 Aa | 0.31 Aa | 1.43 Ab | 1.95 Aa |
| CV (%) | 38.40 | 28.56 | 51.54 | 53.51 |

Means followed by the same letters, in the rows, do not differ from each other by F-test at 5% of significance level. Means followed by the same uppercase letters in the column for months do not differ from each other by the Tukey test at 5% of significance level.

$R_{FD}$ is used as an indicator of the Calvin cycle activity and related processes. When $R_{FD}$ is below 1, CO$_2$ fixation is believed to be severely impaired (Lichtenthaler et al., 2005; Perera-
Castro et al., 2018). Thus, salinity did not have an effect on CO\textsubscript{2} fixation under the experimental conditions of this study. Moreover, the higher values of R\textsubscript{Fd} at 2 p.m. possibly indicate a high amount of internal CO\textsubscript{2} coming from the decarboxylation of malic acid, which favors Calvin cycle activity (Borland et al., 2014).

R\textsubscript{Fd} and q\textsubscript{p} results agree with CO\textsubscript{2} fixation stages described for CAM plants (Osmond, 1978; Borland et al., 2014). Stage II begins with the presence of light at which CO\textsubscript{2} is taken up from the atmosphere through the stomata still open. Then, at Stage III, as temperature and radiation rise, the stomata close (increasing stomatal conductance) and internal CO\textsubscript{2} uptake ceases, which explains the lower values of RFd at 8 p.m. At this stage, malate stored in vacuoles during Stage I is decarboxylated, internal CO\textsubscript{2} is released and assimilated, and starch is produced; hence, the increased values of R\textsubscript{Fd} at 2 p.m.

NPQ indicates light energy quenching as heat in the antenna complex of PSII. In this study, salinity had no effect on energy quenching, indicating ideal use of excitation energy for q\textsubscript{p}.

Salinity stress reduces photosynthetic yield in C3 plants as a result of closing stomata and inhibition of photosynthetic carbon fixation (Tatagiba et al., 2014). However, since the pineapple has characteristics of a CAM plant, as shown in this study by the values of Yield, R\textsubscript{Fd} and q\textsubscript{p}, it is suggested that applying saline water does not have a negative impact on fluorescence characteristics evaluated over the study. Decreases in yield observed when applying saline irrigation water under semiarid conditions (Brito et al., 2017) might be due to other salinity-related effects such as changes in nutrient uptake, transport, assimilation and distribution throughout the plant (Santos and Brito, 2016).

Findings shown herein are relevant because they broaden existing knowledge about pineapple physiology and enable the association with other research results, thereby improving management practices to increase yield of crops irrigated with low-quality water and grown under semiarid conditions.

4. CONCLUSIONS

Fluctuations in quantum efficiency (Fv/Fm) occur over the cycle of ‘Pérola’ pineapple grown under semiarid conditions.

Energy use for photosynthetic processes of ‘Pérola’ pineapple plants is not affected by irrigation using saline water with electrical conductivity of 3.6 dS m\textsuperscript{-1}.

The results of the evaluated physiological characteristics indicate resilience of the pineapple in cultivation under irrigation water salinity conditions. Thus, new research must be developed to adjust the cultivation of pineapple with saline water in the semiarid region of Bahia.

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