SCAP
SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

Ecophysiology of banana seedlings grown in different water regimes

Ecofisiologia de mudas de bananeira submetida a diferentes regimes hídricos

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A B S T R A C T

Banana is one of the most cultivated fruits in tropical countries, however, the banana tree is susceptible to water deficit, with considerable physiological responses. Thus, the objective of this work was to evaluate ecophysiological parameters of seedlings of two banana cultivars grown in different water stress levels. The experiment was conducted at the State University of Maranhão in a completely randomized design with four replications, using a 2×3 factorial arrangement consisting of two banana cultivars (Pacovan-Ken, and Fhia-18) and three water regimes (0%, 67%, and 100% of the field capacity). The cultivar Pacovan-Ken showed better adaptation to the water stress conditions, and presented less damages to the photosynthetic apparatus of the plants, which may be related to the higher canopy cooling capacity of plants of this cultivar.

Keywords: *Musa* spp., water deficit, chlorophyll, fluorescence.

R E S U M O

A bananeira, uma das frutíferas tropicais mais cultivadas, é uma espécie suscetível ao déficit hídrico e apresenta considerável resposta fisiológica à escassez de água. Dessa forma, este estudo objetivou avaliar o comprometimento ecofisiológio de mudas de duas cultivares de banana sob diferentes níveis de estresse hídrico. O experimento foi conduzido na Universidade Estadual do Maranhão, em delineamento inteiramente casualizado, em esquema fatorial 2 x 3 com quatro repetições. Os tratamentos consistiram de duas cultivares de bananeira: Pacovan Ken e Fhia 18 e três regimes hídricos (0%, 67% e 100% CC), totalizando seis tratamentos. A cultivar Pacovan Ken mostrou ser melhor adaptada às condições do estresse hídrico aplicado, com danos mais tardios à integridade do aparato fotossintético das plantas, o que pode estar relacionado ao fato dessa cultivar apresentar maior capacidade de refrigeração do dossel.

Palavras-chave: *Musa* spp., déficit hídrico, clorofila, fluorescência

INTRODUCTION

The banana tree (*Musa* spp.) is one of the most cultivated perennial fruit species in tropical countries; its fruits have good organoleptic characteristics and are the fifth most consumed and traded product from agricultural crops (Singh *et al.*, 2016). Banana farming has been important for the economy, generating direct and indirect jobs, and is a relevant source of income for both large and small farmers (Salomão *et al.*, 2016).

According to Faostat (2014), 72% of world banana production is from India, followed by China, the Philippines, Ecuador, Indonesia, and Brazil. In Brazil, banana crops produce approximately 6.9 million Mg per year, in an area of 481 thousand hectares (yield of 14.35 Mg ha^{-1}). Banana is present all over Brazil, with a consumption per capita of approximately 25 kg per year (Gasparotto & Pereira, 2010; Mendonça *et al.* 2013).

Banana crops need high amount of water; their growth and productivity tend to increase proportionately with increasing transpiration, which depends on water availability. In environments with water deficits, the plant growth reduces due to reduced leaf area and stomatal conductance, which limits photosynthetic assimilation of CO_2 (Oliveira *et al*., 2013).

The amount of water transpired by leaves of banana, and most crops, in hot days with intense solar radiation is higher than the amount absorbed by the roots and transported by the xylem, causing a temporary water deficit, even when there is water available in the soil (Marenco and Lopes, 2007).

Partial closure of stomata avoids excessive dehydration and water imbalances in the epidermis of the leaves, reducing photosynthetic CO_2 assimilation (Levy, 1980; Medina and Machado, 1999; Machado *et al*., 2002; Ribeiro and Machado, 2007; Ribeiro *et al*., 2009).

According to Ravi *et al*. (2013), considering the forecasted increase in drought and thermal stresses, increasing banana production is feasible only through improvements in technology and cultivars for environments with limited water availability, using genotypes that are more efficient in water use and present improved physiological functions, such as osmotic adjustment.

Banana tolerance to abiotic stress, normally found in producing areas, is important when adopting genetic improvement and management strategies to increase crop productivity (Donato and Arantes, 2009). The use of cultivars that are more tolerant to water deficit is essential to reduce water and nutrient losses, considering the current agricultural production systems (Donato *et al*., 2015).

In this context, the objective of this work was to evaluate ecophysiological parameters of seedlings of two banana cultivars grown in different water stress levels, considering the plants' photochemical efficiency and leaf greenness index responses.

MATERIAL AND METHODS

Plant material and growing conditions

The experiment was conducted in a greenhouse of the Agronomic Biotechnology Center of the State University of Maranhão (UEMA), in São Luís MA, Brazil, in July and August 2017. Seedlings of two banana (*Musa* spp.) cultivars (Pacovan-Ken, and Fhia-18) from a six-year old banana crop were used.

The seedlings were transplanted to 20-liter plastic pots, containing 15 kg of soil from the 0-20 cm layer of an arenic dystrophic Red Yellow Argissolo (Ultisol) of sandy-loam texture (Santos *et al*., 2013). The plants were irrigated daily, and the water regimes of 0%, 67%, and 100% of the field capacity (FC) were established using different water depths from 13 days after transplanting of the seedlings to each pot.

The water regimes were controlled by monitoring the weight of the pots (soil, plant, and pot) of the replication 1 of each treatment. Soil moisture was monitored using two moisture sensors connected to an automatic station (dataloggers).

The weights of the pots were uniformized after transplanting the seedlings to keep the soil at a constant field capacity. The seedlings were irrigated manually and the weights of the pots were adjusted daily to the gravimetric water content established as field capacity due to the growth of the plants. The pots were weighed daily and the soil moisture was reestablished on a weight basis to the established moisture.

The experiment was conducted in a completely randomized design with four replications, using a 2×3 factorial arrangement, consisting of two banana cultivars (Pacovan-Ken, and Fhia-18) and three water regimes (0%, 67% and 100% FC). Analyses were carried out with measurements repeated over time every seven days in a seven-week period.

The experimental plot consisted of one pot, totalizing 24 plots in the experiment.

The data were subjected to analysis of variance by the F test. Means were compared by the Tukey's test at 5% probability level, using the SAEG 9.1 program.

Evaluation of Ecophysiological parameters

The ecophysiological parameters in the intermediate region of fully expanded leaves grown in full sun were evaluated weekly for seven weeks.

Photochemical efficiency of three leaves per plant was evaluated three times a day (8:00 a.m., 12:00 p.m. and 4:00 p.m.). The photochemical efficiency was evaluated using a non-modulated Pocket-PEA Fluorometer (Hansatech Instruments Ltd, King's Lynn, Norfolk, UK).

The saturation pulse method was used for measuring the initial (F_0) and maximum (Fm) fluorescence after a dark period; variable fluorescence (Fv), which is equal to Fm-F_0; and the potential quantum efficiency of photosystem II (Fv/Fm).

Simultaneously, the greenness index (SPAD value) of the leaves of the same plants was evaluated using a SPAD-502 chlorophyll meter (Minolta, 1989). Five points of each leaf were read, according to the method of Swiader and Moore (2002).

RESULTS AND DISCUSSION

The initial fluorescence (F_0) of the Fhia-18, and Pacovan-Ken cultivars presented no statistical difference, although it was high in both cultivars. According to Baker and Rosenqvist (2004), a high F_0 denotes destruction of the photosystem II (PSII) reaction center, or decrease in transfer capacity of the excitation energy from the antenna to the reaction center (Table 1).

High water stresses can be caused by nonstomatal factors, such as reduction of activity and concentration of the ribulose 1,5-bisphosphate carboxylase/ oxygenase (RuBisCO) enzyme, photoinhibition, electron transfer rate, and reduction of the photochemical efficiency of PSII, which may decrease or inhibit photosynthesis (Flexas and Mendrano, 2002; Lawlor, 2002; Lawlor and Cornic, 2002; Grassi and Magnani, 2005).

The F_0 varied over time, however, with no significant effects of the different water regimes tested on either of the two cultivars (Table 1).

Plants of the Fhia-18 cultivar that were not irrigated had increasing F_0 over time, with a total increase of 70%, indicating a higher water stress when compare to those irrigated with 67% or 100% FC. The Pacovan-Ken cultivar was more tolerant to water stress than the Fhia-18 cultivar (Table 1).

Table 1 - Initial fluorescence (F_0) as a function of water regimes of 0%, 67%, and 100% of the field capacity (%FC) for banana cultivars (Pacovan-Ken, and Fhia-18)

Water Regime (%FC)		Weeks						
		1	2	3	4	5	6	7
					F_0			
Fhia-18	0	6302 aC	6389 aC	6600 aBC	7092 aBC	7396 aABC	7720 aAB	8706 aA
	67	6852 aA	6867 aA	7649 aA	7534 aA	7245 aA	7394 aA	7550 bA
	100	6963 aA	6204 aA	6981 aA	7127 aA	6898 aA	6945 aA	7216 bA
	CV%				8.68			
Pacovan-Ken	0	6742 aA	7328 aA	7165 aA	8134 aA	7435 aA	7431 aA	7524 aA
	67	6028 aA	6321 aA	7022 aA	7562 aA	7421 aA	7152 aA	7359 aA
	100	6488 aA	6284 aA	6419 aA	7055 aA	7178 aA	7119 aA	7104 aA
	CV%				9.15			

Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ statistically by the Tukey's test at 5% probability.

Plants developed in soils of sandy texture, such as the arenic dystrophic Red Yellow Argisoil (Ultisol) used, do not require low water potential to exhaust the soil available water, as shown by the Fhia-18 cultivar. Sandy soils may provide higher conductivity and soil moisture loss because water is less retained in larger capillaries (Hacke *et al*., 2000).

The Pacovan-Ken cultivar presented the highest mean maximum fluorescence (Fm) in the water regimes of 0% and 67% FC (Table 2). Strasser *et al.* (2010) evaluated the fluorescence of chlorophyll *a* in plants under stress and found a decrease in Fm in plants subjected to UV-C radiation, indicating the weakness of the PSII in reducing plastoquinone A (QA), which is the primary electron acceptor.

The Fm results indicated that the two cultivars had different responses to the water regimes used (Table 2). The Fm of the Fhia-18 cultivar was more stable over time in the treatment with 100% FC, while the Pacovan-Ken cultivar had the best results in the treatment with 67% FC, statistically differing from the other treatments, which showed a decrease in Fm.

Plants subjected to water deficit presented reductions in Fm, which were more expressive in plants subjected to water regime of 0% FC (Table 2). Reductions in Fm indicate problems of the PSII in reducing QA (Strasser *et al*., 2004).

The Fm of the Fhia-18 cultivar in the treatment with 100% FC differed from the other treatments (0, and 67% FC) from the third day evaluated. The Fm of plants of the Pacovan-Ken cultivar in the treatment with 67% and 100% FC differed from that of plants in the treatment with 0% FC, and remained more constant, varying less over time, showing better adaptation to water stress conditions (Table 2).

According to Larcher (2004), the plant can undergo a repair period depending on its potential for response, and intensity and duration of stress, and can normalize even under continuous stress due to a better stability.

Fv/Fm was higher for the water regimes of 67% and 100% FC; plants in the water regimes of 0% FC presented Fv/Fm below 0.75 (Table 3), indicating damages in their photosynthetic apparatus due to stress caused by low water availability. Both banana cultivars presented Fv/Fm from 0.77 to 0.80 in the treatments with 67% and 100% FC (Table 3), denoting a good functioning of the photosynthetic apparatus.

According to Bolhàr-Nordenkampf *et al.* (1989), when the photosynthetic apparatus of the plant is intact, the Fv/Fm ratio is 0.75 to 0.85. A decrease in this range means photoinhibition damage to PSII reaction centers.

According to Silva *et al.* (2007), the ability of a plant in maintaining a high Fv/Fm under water stress conditions indicates that it maintains high efficiency in using solar radiation and carbon assimilation, and a better water use efficiency.

Table 2 - Maximum fluorescence (Fm) as a function of water regimes of 0%, 67%, and 100% of the field capacity (%FC) for banana cultivars (Pacovan-Ken, and Fhia-18)

Water regime (%CC)		Weeks						
		1	2	3	4	5	6	7
					Fm			
Fhia-18	0	35894 aA	33491 aB	32921 bBC	32250 abBC	32199 aBC	31999 bBC	31689 bC
	67	33955 bA	32859 aAB	32682 bAB	32046 bB	32390 aAB	32230 bAB	32151 bB
	100	35297 abA	34157 aAB	34634 aAB	33596 aAB	33500 aB	34890 aAB	34232 aAB
	CV%				2.45			
Pacovan-Ken	0	38557 aA	37999 aAB	37250 aB	37293 aAB	36939 aBC	35718 aC	34217 abD
	67	35511 bA	35778 bA	35385 bAB	34114 bBC	34849 bABC	34681 bABC	33654 bC
	100	39347 aA	38248 aAB	37912 aB	37283 aBC	37274 aBC	36175 aCD	35165 aD
	CV%				1.64			

Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ statistically by the Tukey's test at 5% probability.

Table 3 - Photochemical efficiency (Fv/Fm) as a function of water regimes of 0%, 67%, and 100% of the field capacity (%FC) for banana cultivars (Pacovan-Ken, and Fhia-18)

Water regime (%CC)					Weeks			
	1	2	3	4	5	6	7	
				Fv/Fm				
	0	0.79 aA	0.79 aA	0.77 aAB	0.74 aABC	0.69 aBC	0.67 bC	0.45 bD
Fhia-18	67	0.80 aA	0.76 aA	0.76 aA	0.79 aA	0.76 aA	0.76 aA	0.73 aA
	100	0.83 aA	0.80 aA	0.79aA	0.77 aA	0.77 aA	0.76 aA	0.75 aA
	CV%				6.08			
	0	0.80 bA	0.78 bA	0.76 aA	0.68 bB	0.56 bC	0.60 bC	0.50 bD
Pacovan-Ken	67	0.80 bA	0.80 abA	0.79 aA	0.79 aA	0.78 aA	0.76 aA	0.74 aA
	100	0.86 aA	0.83 aAB	0.81 aABC	0.78 aBC	0.79 aBC	0.76 aC	0.77 aBC
	CV%				3.75			

Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ statistically by the Tukey's test at 5% probability.

The treatments with 67% and 100% FC resulted in the highest Fv/Fm for both cultivars (Table 3), which were not affected by the water stress. When the PSII is not affected by water stress, the plant has in general high efficiency in solar radiation use due to carbon assimilation reactions (Silva *et al.*, 2007).

Plants of the both cultivars in the treatments with 67% and 100% FC presented similar Fv/Fm (Table 3), with small reductions over time, which was probably due to their response to water stress. The photosynthetic apparatus of plants in the treatments with of 0% FC was damaged by the water stress, presenting Fv/Fm below 0.75. These plants were possibly on oxidative stress.

Decreases in Fv/Fm indicate photoinhibition damage when plants are subjected to environmental stresses, including water deficit and cold (Baker *et al.*, 1983; Ögren and Öquist, 1985; Campostrini, 2001). Variations in Fm denote variations in properties of PSII electron receptors due to stress-induced conformational changes in the main constituent of the protein complex that forms the PSII, the D1 protein (Bulkhov *et al.*, 1999).

According to Krause and Weis (1991), environmental stresses decrease potential quantum efficiency of PSII, which can be detected by decreases in Fv/Fm due to the lower photochemical efficiency. This can cause reduction in the activity of some Calvin cycle enzymes, and changes in biochemical pathways, such as accumulation of amino acids and organic acids (Wright *et al.*, 1995).

The Fhia-18, and Pacovan-Ken cultivars presented higher greenness index in the water regime of 100% FC; and the Fhia-18 cultivar had a greater chlorophyll degradation (Table 4). Chlorophyll degradation is a consequence of the water stress, which results in decreases in leaf greenness (Long *et al.*, 1994).

The highest greenness index of the Pacovan-Ken, and Fhia-18 cultivars were found in plants subjected to the water regime of 100% FC (Table 4). Plants in the treatments with 0% and 67% FC had marked degradation of photosynthetic pigments, and possible mobilization of mobile nutrients, especially nitrogen, because of the lower water availability, since they are necessary to maintain the turgidity, and biochemical processes of the leaf. However, the Pacovan-Ken cultivar always presented higher mean greenness index when subjected water stress.

Plants under water deficit are usually characterized by loss of chlorophyll and a progressive decrease in photosynthetic capacity of plants. Thus, the analysis of photosynthetic pigments is important to evaluate the health and integrity of the internal apparatus of the plant cells responsible for the photosynthesis process (Rong-Hua *et al.*, 2006).

Carvalho *et al.* (2005) evaluated the soil water availability and the growth of *Tanacetum parthenium* L. and found decreases in leaf chlorophyll contents (SPAD values) in plants subjected to water deficit over time, regardless of the water deficit level.

Table 4 - Greenness index (SPAD value) as a function of water regimes of 0%, 67%, and 100% of the field capacity (%FC) for banana cultivars (Pacovan-Ken, and Fhia-18)

Water regime (%CC)		Weeks						
		1	2	3	4	5	6	7
		SPAD value						
Fhia-18	0	37.5 bA	36.4 cA	34.3 cB	32.8 cBC	32.7 cBC	31.6 cC	29.9 cD
	67	38.4 bA	37.7 bAB	36.9 bAB	36.2 bBC	34.6 bCD	34.3 bD	32.1 bE
	100	42.4 aA	41.6 aA	40.9 aAB	39.8 aB	37.8 aC	36.6 aC	34.5 aD
	CV%				2.08			
Pacovan-Ken	0	39.9 cA	38.4 cB	37.3 cBC	37.2 cCD	36.2 bDE	35.2 bE	35.4 bE
	67	42.8 bA	41.7 bB	39.8 bC	38.4 bD	37.0 bE	35.8 bF	34.9 bF
	100	47.4 aA	46.1 aB	44.8 aC	43.8 aC	42.5 aD	41.2 aE	38.7 aF
	CV%				1.37			

Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ statistically by the Tukey's test at 5% probability.

Similarly, Nautiyal *et al.* (1996) found rapid reductions in the chlorophyll content in *Pongamia pinnata* plants subjected to water stress caused by irrigation intervals equal to or greater than 30 days. Thus, longer periods of drought can cause degradation of chlorophyll in plants, even when they are under a moderate water stress.

Silva *et al.* (2014) evaluated responses of sugarcane cultivars subjected to different water deficit intensities and found that the SPAD value can be used to differentiate cultivars and select tolerant cultivars to water stress in breeding programs.

CONCLUSIONS

Photochemical efficiency parameters, and greenness index are efficient for differentiating cultivars and selecting the more tolerant ones to a specific water stress.

Photochemical efficiency parameters, and greenness index are affected by water regimes.

Plants of the Pacovan-Ken cultivar are better adapted and more tolerant to water stress, maintaining longer the integrity of their photosynthetic apparatus.

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