

Physiological responses of oil palm (*Elaeis guineensis* Jacq.) seedlings under different water soil conditions

Respuestas fisiológicas de plántulas de palma de aceite (*Elaeis guineensis* Jacq.) a diferentes condiciones hídricas del suelo

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ABSTRACT

Water supply is the main yield-limiting factor of oil palm crops. A water deficit affects virtually all aspects of metabolism and plant structure, while a water excess leads to waterlogging, an abiotic stress that affects crop production because of a reduction of oxygen in the soil. To determine the effect of water stress on the physiology of oil palms (*Elaeis guineensis*) during the nursery stage, clones from three embryogenic lines of three different ortets (LE 33-7, LE 28-59 and LE 16-79) were subjected to four water conditions (moderate deficit, field capacity, partial and continuous waterlogging) for 60 days under a completely randomized split-plot design with four replications. The gas exchange, vegetative growth and nutrient content variables were evaluated. There was no interaction between the water conditions and embryogenic lines for any variable. The moderate water-deficit stress significantly reduced the stomatic conductance, photosynthesis, transpiration and growth. Under waterlogged conditions, the seedlings developed root aerenchyma and pneumatophores as adaptation mechanisms, and their gas exchange or their micronutrient reduction and assimilation processes (with the exception of copper) were not affected. However, under permanent waterlogging, the seedlings had poor growth because of higher leaf respiration rates and probably pneumatophore maintenance and imitations in the absorption and transport of macronutrients. While the plants under partial waterlogging exhibited a growth pattern similar to that observed under optimal soil moisture conditions.

Key words: abiotic stress, aerenchyma, flooding, pneumatophores, tolerance, waterlogging.

RESUMEN

El suministro de agua es el principal factor limitante del rendimiento de la palma de aceite. El déficit afecta prácticamente a todos los aspectos del metabolismo y estructura de la planta, mientras que el exceso de agua conlleva al anegamiento, un estrés que condiciona la producción de cultivos debido a la reducción del oxígeno en el suelo. Con el fin de determinar el efecto del estrés hídrico sobre la fisiología de la palma de aceite (*Elaeis guineensis*) en etapa de vivero, clones provenientes de tres líneas embriogénicas de tres ortets diferentes (LE 33-7, LE 28-59 y LE 16-79) se sometieron a cuatro condiciones hídricas (déficit moderado, capacidad de campo, anegamiento parcial y permanente), durante 60 días, bajo un diseño completamente aleatorizado en arreglo de parcelas divididas con cuatro repeticiones. Variables de intercambio gaseoso, crecimiento vegetativo y contenido nutricional fueron evaluadas. No hubo interacción entre las condiciones de humedad y las líneas embriogénicas para ninguna variable. El déficit hídrico moderado redujo significativamente la fotosíntesis, la transpiración y el crecimiento. Bajo condiciones de anegamiento, las plantas desarrollaron neumatóforos y aerénquima radical como mecanismos de adaptación, por lo que no afectaron su intercambio gaseoso ni los procesos de reducción y asimilación de micronutrientes (con excepción del cobre). Sin embargo, bajo anegamiento permanente, mostraron un reducido crecimiento, debido a las mayores tasas de respiración foliar y probablemente al mantenimiento de los neumatóforos, y a las limitaciones en la absorción y transporte de macronutrientes, mientras que las plantas sometidas a un anegamiento parcial exhibieron un crecimiento similar al registrado bajo condiciones óptimas de humedad edáfica.

Palabras clave: estrés abiótico, aerénquima, inundación, neumatóforos, tolerancia, anegamiento.

Introduction

Water supply is the main yield-limiting factor of oil palm crops. A water deficit stress causes stomatal closure, available water reductions and impaired physiological reactions,

reducing the photosynthetic rate, growth and yield in terms of the production of fresh fruit bunches and oil (Rivera *et al.*, 2012; Jazayeri *et al.*, 2015). Excess water or floods can negatively impact agricultural yields by delaying planting, reducing vigor, altering development and increasing

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susceptibility to diseases. Flooding can lead to waterlogging stress by substituting the soil gas phase with a liquid phase, and thus cause a decrease of oxygen in the airspaces (O_2 poor availability or hypoxia with total absence or anoxia), in natural and man-made ecosystems worldwide (Voesenek and Bailey-Serres, 2015). During floods, plants endure environmental perturbations such as a restricted access to atmospheric O_2 and CO_2 , hampered outward diffusion of plant evolved ethylene (C_2H_4), and electrochemical soil changes resulting in higher concentrations of toxic elements including manganese (Mn^{2+}), iron (Fe^{2+}) and sulfide (H_2S , HS^- , S^{2-}) (Bailey-Serres and Voesenek, 2008). As a consequence, cells and tissues are exposed to pronounced internal variations in O_2 and CO_2 , and elevation in ethylene as well as reactive nitrogen and reactive oxygen species (ROS) (Voesenek and Bailey-Serres, 2015). Despite this, waterlogging-tolerant plants are characterized by a continuum of survival strategies of which the low- O_2 escape syndrome (LOES) and low- O_2 quiescence syndrome (LOQS) are extremes (Voesenek and Bailey-Serres, 2013). During escape, various induced and/or constitutive traits interact in such a way that the rates of gas exchange between cells and the atmosphere above the water level increase. Escape phenotypes, not necessarily all present in one species, include upward bending of leaves (hyponasty), enhanced shoot elongation, formation of interconnected air-filled voids (aerenchyma), induction of barriers to radial O_2 loss in roots, development of adventitious roots, formation of gas films on leaf surfaces, modifications of leaf anatomy and pressurized gas flow through porous tissues (Voesenek and Bailey-Serres, 2015). In contrast to the energy-consuming LOES, LOQS manages metabolism and constrains growth. Generally, to maintain cellular homeostasis under low- O_2 stress, starch catabolism, glycolysis, ethanolic fermentation and a bifurcated tricarboxylic acid cycle are used to fuel substrate-level ATP production and enable the synthesis of proteins involved in metabolite transport, ROS protection and chaperone activity (Bailey-Serres and Voesenek, 2008; Bailey-Serres and Colmer, 2014). This is accompanied by a reduction in bioenergy-consuming processes associated with cell division and growth, including DNA, protein, ribosome and cell wall synthesis (Voesenek and Bailey-Serres, 2015).

Oil palms are well adapted to a high-water table, but do not tolerate continuous waterlogging: roots are unable to carry out respiration when submerged in water, and if flooding is prolonged, the roots may die, water uptake may be impaired, with effects similar to those of a drought (stomatal closure), nitrogen uptake may also be limited,

and waterlogged palms often appear nitrogen deficient (Corley and Tinker, 2015). Nevertheless, there have been few physiological studies on the effects of waterlogging on oil palm, and this stress is detrimental for most terrestrial plants because it hampers growth, development and productivity and can result in premature death (Colmer and Voesenek, 2009), especially because the Intergovernmental Panel on Climate Change (IPCC) reported that climate change will increase the frequency of heavy precipitation and tropical cyclone activity of up to 30% for the 2011-2100 period in all of Colombia, as compared to the 1971-2000 reference cycle, with the possibility of stronger “La Niña” phenomenon events in terms of magnitude and duration, ie lower temperatures and stronger rains in non-coastal areas of the Pacific (Ruiz, 2010). This is likely to engender more frequent flooding events in flood plains and arable farmland (Bailey-Serres and Colmer, 2014), particularly affecting the Amazon, the Pacific region, the Magdalena Medio, and the valleys of the Sinú, San Jorge, Arauca and Meta Rivers (Ruiz, 2010), where oil palm cultivation is developed, such as happened between mid-2010 and most of 2011, when the percentage of rainfall was well above the local historical average and was even higher than during “La Niña” events, flooding approximately 40,000 ha of oil palm, causing a drop in the national average yield of 11.6% (from 3.4 to 3.0 t of crude oil per hectare) and the spread of diseases in some areas (Fedepalma, 2012).

Therefore, the Biology and Breeding Research Program of the Colombian Oil Palm Research Center (Cenipalma), as an initial approach to the analysis of the vulnerability of oil palms to climate change, conducted this study to determine the effect of deficit and excess water stresses on the physiology of *Elaeis guineensis* seedlings during the nursery stage.

Materials and methods

Location

The experiment was carried out under mesh-house conditions in the “Palmar de la Vizcaína” Experimental Field located in Barrancabermeja, Santander (Colombia) at an altitude of 125 m a.s.l., which has a 32°C average temperature, 70% relative humidity and 2,852 mm of annual rainfall.

Plant material

Oil palm (*E. guineensis*) plants from three embryogenic lines (EL) of three different ortets obtained in the Cenipalma’s Tissue Culture Laboratory, codes LE 16-79, LE 28-59 and LE 33-7, were evaluated during the nursery stage.

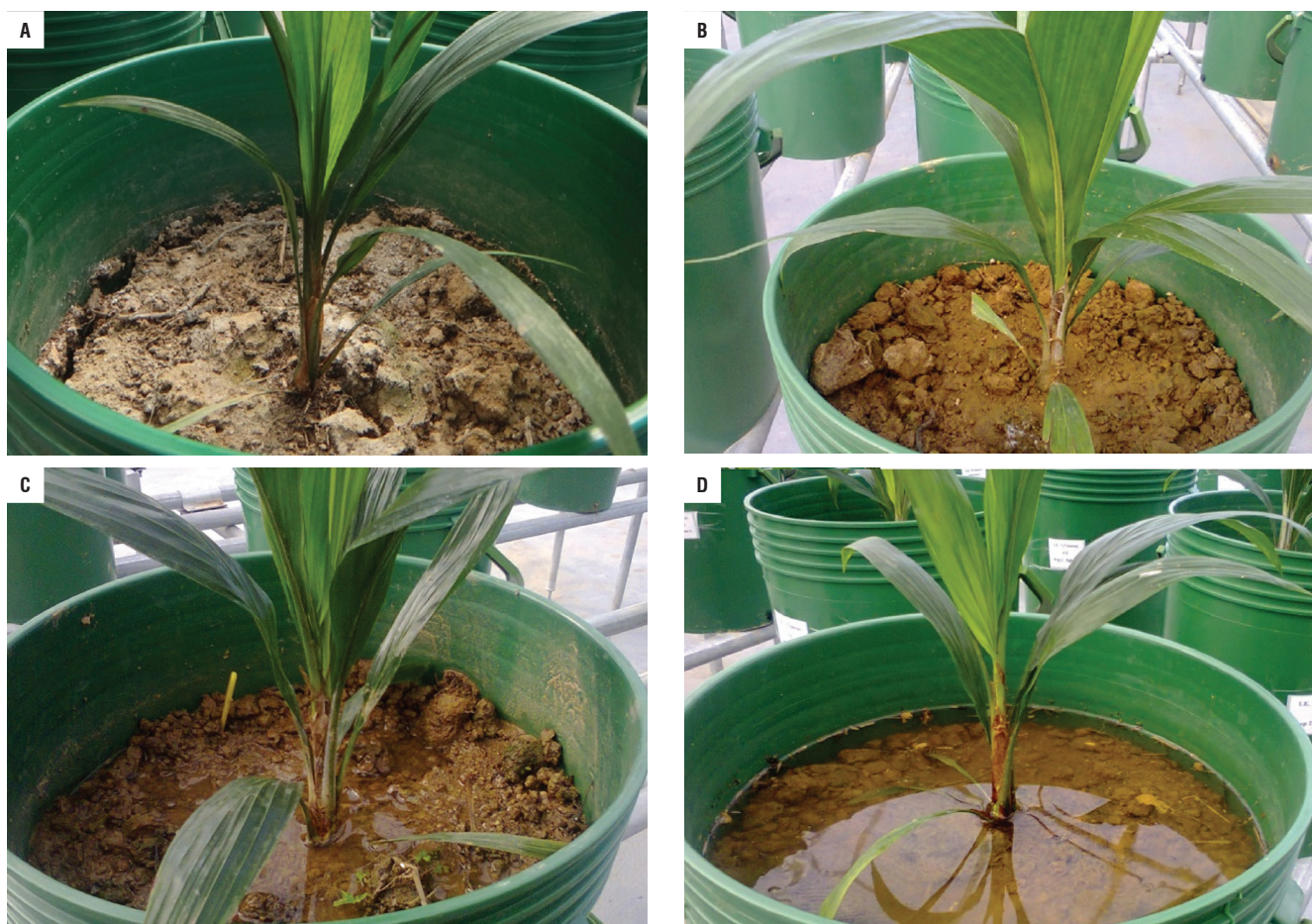


FIGURE 1. Soil water conditions evaluated in oil palm seedlings. A, deficit; B, field capacity; C, partial waterlogging; D, continuous waterlogging.

Experiment design

A completely randomized split-plot design was used, in which the main plot was the soil water condition (deficit, field capacity, partial waterlogging and continuous waterlogging), and the subplot, the embryogenic line (LE 16-79, LE 28-59 and LE 33-7) with four replications and three plants per experiment unit for a total of 144 evaluated seedlings.

Experiment conditions

Seedlings in the 102 phenological growth stage, with two fully developed lanceolate leaves (Forero *et al.*, 2011), were transplanted to pots with an approximate capacity of 0.02 m³ and were initially subjected to field capacity conditions for one month and later to different soil water stress conditions for 60 d:

- Deficit: moderate water deficit that was equivalent to a soil water potential of -0.5 MPa, as reported by Rivera *et al.* (2012) (Fig. 1A).
- Field capacity: amount of water the soil can hold against gravity when allowed to drain freely and

equivalent to a soil water potential of -0.058 MPa, as previously calculated (Fig. 1B).

- Partial waterlogging: no permanent waterlogged soil conditions with any presence of water above the soil surface, but over field capacity condition, equivalent to a soil matric potential close to 0 MPa (Fig. 1C).
- Continuous waterlogging: waterlogged condition with a permanent water of 2 cm above the soil surface, equivalent to a potential pressure of +2 cm (Fig. 1D).

The amount of dissolved oxygen was not measured in any soil water condition, but was kept constant through: (i) daily soil moisture monitoring using SM200 sensors attached to a manual datalogger HH2 (DELTA-T, Cambridge, UK) in the water deficit, field capacity and partial waterlogging treatments; (ii) the obstruction of drains with rubber plugs and replacing the water every 20 d in the continuous waterlogging treatment.

Measurements and analyses

Sixty days after starting the treatments, the gas exchange, vegetative growth and nutrient content were determined.

The gas exchange (stomatic conductance, photosynthesis, transpiration and water use efficiency) and leaf respiration variables were measured with an infrared gas analyzer IRGA - LI-6400 (LI-COR, Lincoln, NE), analyzing the central area of leaf number 3, the first four variables under full sunlight conditions (8:30-11:30 A.M.), and respiration at dawn (2:30-4:30 A.M.). The vegetative growth was determined by measuring the plant height, number of leaves, total dry weight and leaf area. The plant height was measured with a ruler between the ground level and the insertion of leaf number 1; the number of leaves was determined by directly counting the fully expanded leaves at the time of sampling. The aerial part of the plant was separated from the root system and dried at 85°C for 24 h; the weight of each part was measured using an analytical scale with a 1-mg accuracy to determine the total dry weight. The leaf area of each plant was measured using a LI-COR 3100 leaf area meter (LI-COR Inc., Lincoln, NE) before drying. As for the nutritional content, after measuring the dry weight of leaf number 3, it was sent to Cenipalma's Soil and Leaf Analysis Lab to quantify the content of the macro and micronutrients by colorimetric (P, B) and atomic absorption (N, K, Ca, Mg, Cu, Fe, Mn, Zn) methods.

The values obtained for each variable were subjected to variance analysis and to a Tukey's mean comparison test ($P \leq 0.05$) using the statistical software SAS®, v. 9.1 (SAS Institute, Cary, NC).

Results

Changes in root morphology

The soil water conditions had a significant effect on the physiological response of the oil palm clones. The plants developed a series of epigeous roots with a negative geotropism that grew above the water level, called pneumatophores (Fig. 2), as well as wide intercellular spaces or aerenchyma in these structures and the submerged roots as tolerance mechanisms against the continuous and partial waterlogging (Fig. 3). Pneumatophores began to appear approximately 15 d after starting the treatments in plants under waterlogged soil conditions and some days later in plants that were not subjected to permanent waterlogging. Thirty days after starting the treatments, 86 and 78% of the plants under continuous and partial waterlogging, respectively, formed pneumatophores, while at the end of the evaluation period (60 d after starting the treatments), all of the plants growing under waterlogged conditions developed these structures. Most of the pneumatophores under partially waterlogged conditions were very superficial, while those developed under permanently waterlogged

conditions were longer and easier to count and measure. The plants kept permanently under waterlogged conditions formed between 8 and 79 pneumatophores, with a maximum diameter of 1.2 mm, while plants that were not subjected to permanent waterlogging showed fewer pneumatophores, with diameters (of the longer ones) not exceeding 1.3 mm (Tab. 1). These structures grew on the primary roots, their color varied from white to cream, and they were composed of two parts: the apical part and a body part with the presence of lenticels or openings that facilitated the entry and diffusion of oxygen to the waterlogged root system (Fig. 2).

TABLE 1. Minimum and maximum number (#) and diameter (Φ) of the pneumatophores developed by three oil palm embryogenic lines (LE 33-7, 28-59 LE, LE 16-79) subjected to waterlogging conditions (partial and continuous) after 60 d.

Embryogenic line	Partial waterlogging		Continuous waterlogging	
	#	Φ (mm)	#	Φ (mm)
LE 33-7	8 - 70	0.56 - 1.05	3 - 24	0.66 - 1.00
LE 28-59	11 - 79	0.61 - 1.20	4 - 27	0.61 - 1.32
LE 16-79	15 - 75	0.63 - 1.11	5 - 25	0.63 - 1.23

A cross-section of the body part and the primary root, cut using a Leica® vibratome (Leica Biosystems, Wetzlar, Germany), showed three different tissues: the root epidermis or rhizodermis with no root hairs, cortex or parenchyma, and central cylinder. In the case of the pneumatophores, the rhizodermis broke and the central cylinder and parenchyma came out; the rhizodermis proliferated and suberized around the edges. The parenchyma of the roots was kept under waterlogged conditions and the pneumatophores had a large proportion of aerenchyma to facilitate gas exchange, but there were no differences in the configuration of the central cylinder (endodermis, pericycle, phloem, xylem and medulla) (Fig. 3).

Gas exchange and vegetative growth

The waterlogged conditions did not limit the stomatic conductance and, therefore, did not affect the photosynthesis or transpiration of the evaluated clones, while the water deficit decreased these variables by 86, 87 and 67%, respectively, as compared to the field capacity condition after 60 d of treatment. The water use efficiency associated with photosynthesis was not altered by any condition of soil water stress. The leaf respiration of the plants subjected to the permanently waterlogged and drought conditions averaged 31 and 87%, respectively, higher than that recorded under field capacity or partial waterlogging. The field capacity and partially waterlogged conditions favored vegetative growth, as did the conditions of the total absence of oxygen,

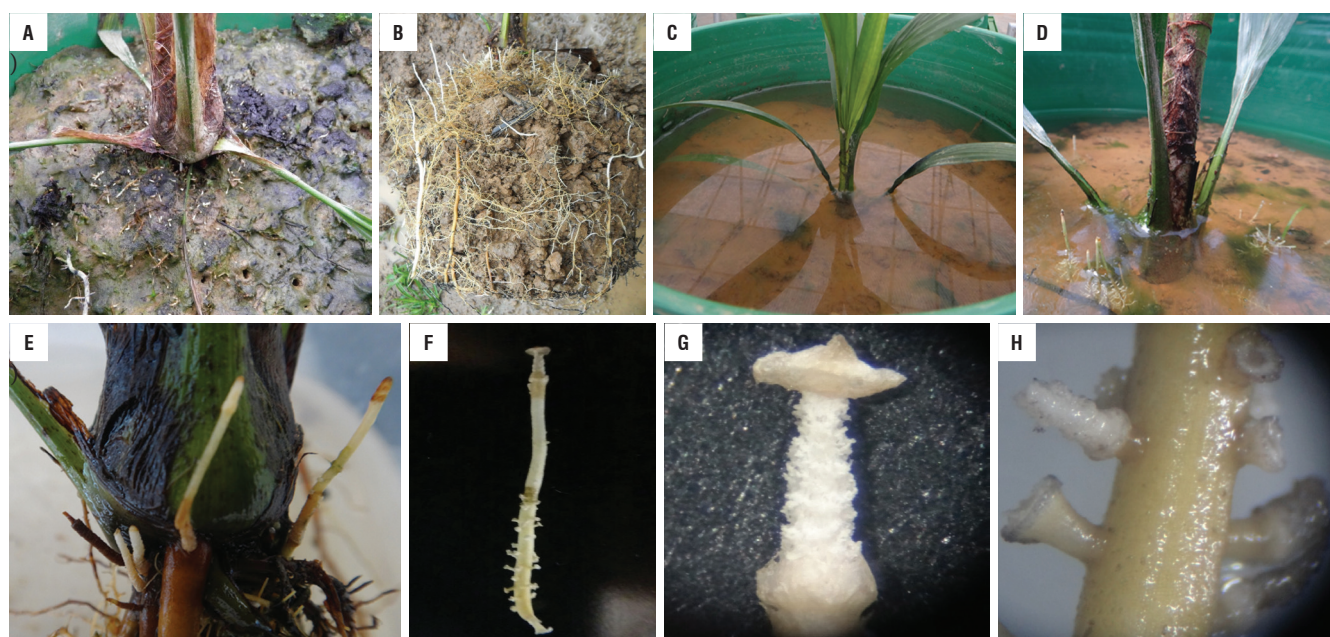


FIGURE 2. Appearance of the pneumatophores that grow under waterlogged conditions in oil palm (*E. guineensis*) seedlings: A, under partial waterlogging; B-H, under continuous waterlogging; C, water surface without the presence of pneumatophores one day after starting treatment; D, presence of pneumatophores 20 d after starting treatment; E, formation of pneumatophores from primary roots; F, individual pneumatophore; G, apex of the pneumatophore; H, lenticels on the surface of the pneumatophore; G and H, 10x stereomicroscopic image.

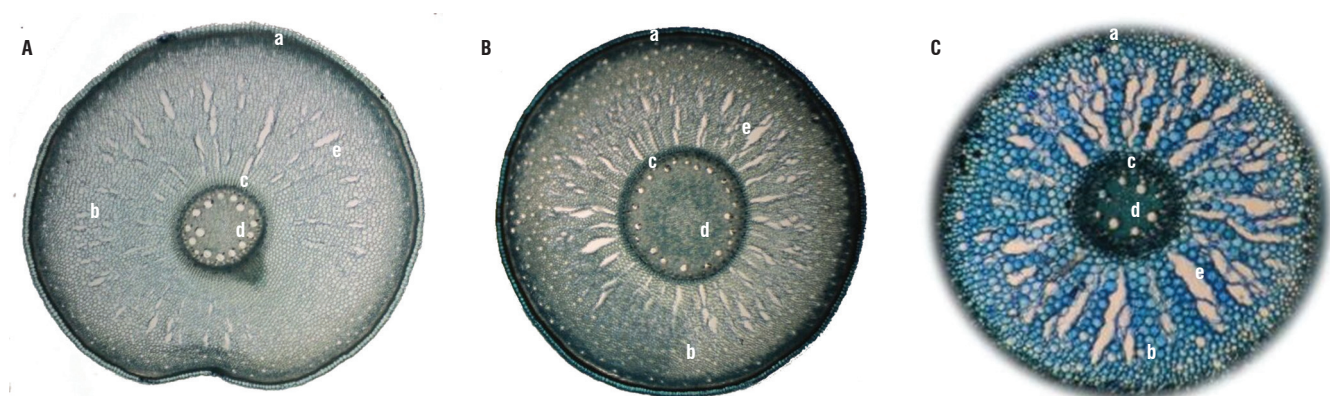


FIGURE 3. Optical microscope image (10x) of methylene blue stained cross-sections ($120\ \mu\text{m}$ thick and $3.5 \pm 0.5\ \text{mm}$ diameter) of primary roots oil palm (*E. guineensis*) grown under waterlogged conditions. A, partial waterlogging; B, continuous waterlogging; C, pneumatophore: a, rhizodermis (in the case of pneumatophores, it broke and the parenchyma became suberized on the edges); b, parenchyma; c, endodermis; d, vascular bundle; e, aerenchyma. A secondary root or pneumatophore appears from the endodermis of the root under partial waterlogging.

and the water deficit significantly affected the number of leaves, plant height, leaf area and total dry weight, while the water deficit conditions generated the most pressure to reduce these variables (Tab. 2).

Nutrient content

The nutrient content in the leaves was affected by the water stress conditions, both deficit and excess (waterlogged), after 60 d of treatment. With regard to the field capacity condition, there was an overall reduction in

macronutrients, particularly under permanently waterlogged conditions, but this did not happen for micronutrients, with the exception of copper (Tab. 3). Furthermore, the water deficit caused a significant reduction in the absorption of nitrogen, phosphorus and potassium in *E. guineensis* plants. Additionally, the foliar content of these three macronutrients (N, P and K) was below the macronutrient reference value reported for *E. guineensis* in the nursery stage (Rincón *et al.*, 2012). However, it was not possible to make the same comparison for the

TABLE 2. Means and $\pm$ standard deviation of the gas exchange (above) and vegetative growth (down) of three oil palm embryogenic lines (LE 33-7, LE 28-59, LE 16-79) subjected to different soil water conditions (deficit, field capacity, partial waterlogging, and continuous waterlogging) after 60 d.

Factor	Level	Stomatic conductance (mmol H ₂ O m ⁻² s ⁻¹)	Photosynthesis (μ mol CO ₂ m ⁻² s ⁻¹)	Transpiration (mmol H ₂ O m ⁻² s ⁻¹)	Water use efficiency (mol CO ₂ mol ⁻¹ H ₂ O)	Leaf respiration (μ mol CO ₂ m ⁻² s ⁻¹)
Soil water conditions	Deficit	0.038 $\pm$ 0.009 b	2.80 $\pm$ 0.92 b	0.82 $\pm$ 0.20 b	0.0034 $\pm$ 0.0006 a	0.125 $\pm$ 0.015 a
	Field capacity	0.228 $\pm$ 0.048 a	9.69 $\pm$ 1.17 a	2.96 $\pm$ 0.50 a	0.0033 $\pm$ 0.0002 a	0.067 $\pm$ 0.004 b
	Partial waterlogging	0.219 $\pm$ 0.061 a	8.60 $\pm$ 1.93 a	2.80 $\pm$ 0.51 a	0.0031 $\pm$ 0.0002 a	0.080 $\pm$ 0.019 b
	Continuous waterlogging	0.204 $\pm$ 0.038 a	8.40 $\pm$ 0.79 a	2.48 $\pm$ 0.31 a	0.0034 $\pm$ 0.0002 a	0.088 $\pm$ 0.027 ab
Embryogenic line	LE 33-7	0.165 $\pm$ 0.052 a	6.94 $\pm$ 1.56 a	2.08 $\pm$ 0.50 a	0.0034 $\pm$ 0.0003 a	0.092 $\pm$ 0.014 a
	LE 28-59	0.175 $\pm$ 0.049 a	7.45 $\pm$ 1.26 a	2.33 $\pm$ 0.34 a	0.0032 $\pm$ 0.0003 a	0.098 $\pm$ 0.016 a
	LE 16-79	0.176 $\pm$ 0.031 a	7.72 $\pm$ 0.79 a	2.38 $\pm$ 0.30 a	0.0033 $\pm$ 0.0004 a	0.080 $\pm$ 0.019 a

Means with different letters in same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

Factor	Level	Leaves (#)	Height (cm)	Leaf area (cm ²)	Total dry weight (g)
Soil water conditions	Deficit	7.5 $\pm$ 0.5 c	10.4 $\pm$ 1.0 c	825.6 $\pm$ 223.7 b	13.9 $\pm$ 4.5 b
	Field capacity	9.8 $\pm$ 0.7 a	13.8 $\pm$ 1.5 a	1622.3 $\pm$ 399.0 a	25.8 $\pm$ 7.5 a
	Partial waterlogging	9.8 $\pm$ 1.1 a	14.5 $\pm$ 1.9 a	1729.4 $\pm$ 510.4 a	26.9 $\pm$ 9.5 a
	Continuous waterlogging	8.5 $\pm$ 0.9 b	12.2 $\pm$ 1.4 b	1113.9 $\pm$ 325.5 b	18.6 $\pm$ 5.8 b
Embryogenic line	LE 33-7	8.7 $\pm$ 0.8 a	12.5 $\pm$ 1.3 ab	1371.7 $\pm$ 326.6 ab	21.2 $\pm$ 6.9 b
	LE 28-59	9.0 $\pm$ 0.9 a	13.3 $\pm$ 1.6 a	1593.8 $\pm$ 512.3 a	27.9 $\pm$ 9.1 a
	LE 16-79	9.0 $\pm$ 0.7 a	12.4 $\pm$ 1.4 b	1002.4 $\pm$ 255.0 b	14.8 $\pm$ 4.4 b

Means with different letters in same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

TABLE 3. Means and $\pm$ standard deviation of the foliar nutritional content of macronutrients (above) and micronutrients (down) of three oil palm embryogenic lines (LE 33-7, 28-59 LE, LE 16-79) subjected to different soil water conditions (deficit, field capacity, partial waterlogging, and continuous waterlogging) after 60 d. Symbols indicate whether it is within (*) or below (†) the reference level (Rincón *et al.*, 2012).

Factor	Level	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Soil water conditions	Deficit	2.60 $\pm$ 0.18 b [†]	0.17 $\pm$ 0.02 b [†]	1.31 $\pm$ 0.24 b [†]	0.84 $\pm$ 0.20 ab [†]	0.30 $\pm$ 0.04 a [†]
	Field capacity	2.73 $\pm$ 0.25 a [†]	0.20 $\pm$ 0.01 a [†]	1.39 $\pm$ 0.20 a [†]	0.91 $\pm$ 0.15 a [†]	0.31 $\pm$ 0.03 a [†]
	Partial waterlogging	2.34 $\pm$ 0.25 bc [†]	0.15 $\pm$ 0.01 bc [†]	1.27 $\pm$ 0.15 b [†]	0.65 $\pm$ 0.05 bc [†]	0.24 $\pm$ 0.02 b [†]
	Continuous waterlogging	2.24 $\pm$ 0.22 c [†]	0.13 $\pm$ 0.01 c [†]	1.24 $\pm$ 0.24 b [†]	0.61 $\pm$ 0.11 c [†]	0.24 $\pm$ 0.02 b [†]
Embryogenic line	LE 33-7	2.38 $\pm$ 0.19 a [†]	0.16 $\pm$ 0.01 a [†]	1.24 $\pm$ 0.22 a [†]	0.73 $\pm$ 0.12 a [†]	0.26 $\pm$ 0.03 b [†]
	LE 28-59	2.46 $\pm$ 0.20 a [†]	0.15 $\pm$ 0.01 a [†]	1.34 $\pm$ 0.19 a [†]	0.75 $\pm$ 0.11 a [†]	0.27 $\pm$ 0.03 ab [†]
	LE 16-79	2.59 $\pm$ 0.28 a [†]	0.18 $\pm$ 0.03 a [†]	1.31 $\pm$ 0.21 a [†]	0.77 $\pm$ 0.15 a [†]	0.29 $\pm$ 0.03 a [†]

Means with different letters in same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

Factor	Nivel	B (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)
Soil water conditions	Deficit	12.85 $\pm$ 1.26 a [†]	128.80 $\pm$ 23.03 a	7.08 $\pm$ 1.71 ab	129.90 $\pm$ 44.77 a	21.68 $\pm$ 2.73 a
	Field capacity	11.92 $\pm$ 0.94 a [†]	111.87 $\pm$ 24.21 a	9.41 $\pm$ 1.67 a	105.33 $\pm$ 46.83 a	22.23 $\pm$ 4.08 a
	Partial waterlogging	11.12 $\pm$ 1.45 a [†]	111.85 $\pm$ 11.64 a	5.87 $\pm$ 1.36 b	136.43 $\pm$ 26.49 a	18.99 $\pm$ 5.88 a
	Continuous waterlogging	12.21 $\pm$ 0.58 a [†]	119.47 $\pm$ 10.11 a	4.61 $\pm$ 1.45 b	139.53 $\pm$ 30.88 a	17.19 $\pm$ 4.14 a
Embryogenic line	LE 33-7	11.68 $\pm$ 1.32 a [†]	119.19 $\pm$ 22.59 a	7.08 $\pm$ 1.59 a	126.04 $\pm$ 42.39 a	21.20 $\pm$ 4.50 a
	LE 28-59	11.97 $\pm$ 0.88 a [†]	111.68 $\pm$ 10.33 a	6.66 $\pm$ 1.32 a	125.93 $\pm$ 39.69 a	19.57 $\pm$ 5.12 a
	LE 16-79	12.43 $\pm$ 0.97 a [†]	123.12 $\pm$ 18.83 a	7.49 $\pm$ 1.75 a	131.42 $\pm$ 29.64 a	19.30 $\pm$ 3.00 a

Means with different letters in same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

micronutrients iron, copper, manganese and zinc because of the lack of documented information.

Discussion

The water deficit and excess water affected the physiology of the oil palms during the nursery stage; the water deficit condition was the most limiting factor in terms of gas exchange and growth. In regards to growth, Rivera *et al.* (2012) and Jazayeri *et al.* (2015) reported that a water deficit disrupts all aspects of the metabolism of the oil palm, both *E. guineensis* and the OxG interspecific hybrid (*E. oleifera* × *E. guineensis*), causing stomatal closure and a significant reduction in the transpiration and photosynthesis rates, thus limiting the production of photoassimilates and growth regulators. Meanwhile, the waterlogged soil conditions did not affect the gas exchange or the micronutrient reduction and assimilation processes, but did affect growth, in terms of height, number of leaves, leaf area and total dry mass accumulation.

Although the plants growing under the partially waterlogged conditions showed similar growth behavior to that recorded under the optimal soil moisture conditions (field capacity), the plants that were kept permanently under waterlogged conditions had stunted growth, mainly because of higher rates of foliar respiration and maintenance for adaptation and the formation of a large number of pneumatophores. A situation like this is possibly compounded by the fact that waterlogged conditions limit the absorption and transport of nutrients, particularly nitrogen, phosphorus, potassium, calcium, magnesium and copper. This reduced production of dry matter of the permanently waterlogged plants can be explained in first instance by the fact that, although the total assimilation was not affected, this condition did cause higher foliar respiration rates and foliar respiration is a good indicator of plant respiration because the leaflets are the organs that contribute most to total respiration losses, with 4-5% of gross assimilation in growth respiration and 33-34% in maintenance respiration, followed by the reproductive organs and roots (Corley and Tinker, 2015). Furthermore, the total uptake of macronutrients usually decreases during waterlogging, while, with few exceptions, the total uptake of manganese and iron increases and the abilities to take up trace metals from the soil (organic or inorganic) can follow a selective pattern for cobalt, but not for zinc, copper or cadmium (Krog and Schierup, 1985). So, the formation of nitrate (NO_3^-) and regeneration of PO_4^- , which are the most common forms of nitrogen and phosphorus absorbed by plants, are reduced or become slower under permanently waterlogged conditions

(Ezin *et al.*, 2010). The above may result in a denitrification process, very common when there is flooding and waterlogging, resulting in strong nitrogen deficiency symptoms, mainly pale colors, that are reversible when this nutrient is supplied (Corley and Tinker, 2015). Additionally, plants may show morphological, biochemical and/or physiological changes that involve a continuous use of energy (ATP and NAD(P)H) for the maintenance, supply and transport of oxygen and ions to organs submerged in water, as well as protein regeneration and repair processes, as a tolerance mechanism against waterlogged conditions (Bailey-Serres *et al.*, 2012).

Whenever the roots of *E. guineensis* and other palms are kept underwater or in waterlogged conditions, particularly seedlings in the nursery stage, they form pneumatophores (Corley and Tinker, 2015). Thus, the plants in the nursery stage under the waterlogged conditions ensured an adequate concentration of oxygen in all of their structures, because the oxygen entered not only through the stomata of leaves and stems but also through the lenticels of the pneumatophores with suberized cell walls, which prevented the loss of O_2 to the atmosphere; from there, it spreaded to other less suberized parts of the root system, which also contained lysigenous formations of radially oriented aerenchyma and dead cells, which in turn reduced the number of oxygen-consuming cells (Bailey-Serres and Colmer, 2014). Therefore, the plants, particularly those under permanently waterlogged conditions, used reserve carbohydrates, starch and sucrose through aerobic respiration for the growth and maintenance of the pneumatophores and aerenchymatous tissue, which ensures the transport of oxygen to the waterlogged roots (Jackson *et al.*, 2009).

The oil palms seedlings in the nursery stage subjected to the stress conditions described above showed a tolerance response against waterlogged soil conditions, as compared to other plant species, such as the absence of premature senescence, no oxidation and Fe^{2+} or Mn^{2+} toxicity, maintenance of the stomatal openings for gas exchange, a reduction, but not inhibition, of vegetative growth only under the permanently waterlogged conditions, and changes in the root anatomy and morphology (Bailey-Serres and Colmer, 2014). It is likely that *E. guineensis* palms will have the same tolerance response in their definitive planting site (> 3 years) because palms develop a large number of roots growing towards the aerobic soil environment or to the surface under partial or permanent waterlogged conditions (Fig. 4). According to Bailey-Serres *et al.* (2012), tolerance of spermatophytes to wet conditions and the absence of oxygen involve anatomical and morphological adaptations



FIGURE 4. Pneumatophores observed in the plate of 3+ year-old *E. guineensis* palms clones under waterlogged soil conditions in their final planting site.

of the root system, such as the presence of pneumatophores and numerous adventitious roots that quickly replace dead roots. However, such a response must be validated under experiment conditions because the effect of excess soil water on plants varies depending on the species, stage of development and duration and frequency of the event (Ezin *et al.*, 2010).

These results are the first step in understanding the physiology of oil palms under waterlogged soil conditions. This is a subject of great interest because there is no history of it and waterlogging is a condition that contributes to the spread of bud rot disease, which is the most limiting disease of oil palms in the Americas because water creates an ideal medium for the production of sporangia and the release and mobility of zoospores of *Phytophthora palmivora*, the causal agent of bud rot disease (Sarria *et al.*, 2015). Therefore, there is an ongoing assessment of the effect of waterlogging stress on the physiology of oil palm genotypes (from commercial and clonal lines) in the nursery stage by further determining the presence of any waterlogging-tolerant traits (LOES or LOQS), such as aerenchyma or pneumatophores (Voeselek and Bailey-Serres, 2013; 2015), and the contents of

ethylene, malate, and lactic acid, and the activity of the antioxidant enzyme system (catalase, peroxidase, ascorbate peroxidase, glutathione reductase, alcohol dehydrogenase), which are compounds that accumulate and/or are activated under anaerobic conditions (Colmer and Voeselek, 2009; Jackson *et al.*, 2009; Bailey-Serres and Colmer, 2014). Thus, studies that integrate these physiological indicators with yield records and even genomic technologies (Bailey-Serres *et al.*, 2012) in the medium-to-long-term could form the basis for oil palm breeding materials (*E. guineensis*, *E. oleifera* and OxG interspecific hybrid) that are tolerant to water stress. A very important aspect for the oil palm sector includes establishing clear policies that reduce or mitigate exposure to this risk.

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