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WATER EFFECT DEFICIT ON MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN 'MD-2' MICRO-PROPAGATED PINEAPPLE PLANTS AT THE END OF ACCLIMATIZATION STAGE

Efecto del déficit hídrico sobre cambios morfo-fisiológicos y bioquímicos en plantas micropropagadas de piña 'MD-2' en la etapa final de aclimatización

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ABSTRACT. Current technology of pineapple micropropagation has problems with the plants transition to field, these problems are associated to drastic changes of environmental conditions linked to poor hardening plant for such transit. A possibility of preparation is the induction of defense mechanisms to drought stress and get modulating CAM, so that the object of this paper was study plants grown for 30 days in two water status (well-watered plants and non-watered plants) after 30 days both groups were full-watered during 15 days. Measurements were made on D-leaf, in which were determinates the gas exchange, water-efficiency use (WEU), night CO_2 uptake percentage, chlorophyll content, organic acids levels, succulence index (IS) and superoxide dismutase activity (SOD) (EC 1.15.1.1). After 15 days of drought, plants had the best response to field transfer, with increase in CAM expression, supported by the decline in total chlorophyll content and increases in the night CO_2 uptake percentage, WEU, SI, and SOD activity. After 30 days of drought plants had a CAM strong response, with 100 % of CO_2 uptake during night, but its rapid recovery with the establishment of irrigation to saturation of the substrate, showing high drought tolerance and great metabolic plasticity.

Key words: CAM cycle, photosynthesis, vitroplants

RESUMEN. La tecnología actual de micropropagación de piña presenta problemas con la transición de las plantas al campo, dichos problemas se asocian al drástico cambio de las condiciones ambientales unido al pobre endurecimiento de las plantas para su tránsito. Una posibilidad de su preparación es la inducción de mecanismos defensivos mediante el déficit hídrico y consigo la modulación de CAM (Metabolismo Ácido de las Crasuláceas, siglas en inglés) por lo que en este trabajo se estudiaron plantas crecidas durante 30 días en dos regímenes hídricos (plantas con riego y sin riego), después de este momento ambos grupos se regaron hasta la saturación del sustrato durante 15 días. Las determinaciones se realizaron en la hoja "D" con frecuencia quincenal. Se determinó el intercambio gaseoso, eficiencia en el uso del agua (EUA), porcentaje de captación de CO_2 en la noche, contenido de clorofilas, niveles de ácidos orgánicos, índice de suculencia (IS) y actividad de superóxido dismutasa (SOD) (EC 1.15.1.1). A los 15 días las plantas sin riego tuvieron la mejor respuesta para su traslado a campo, con el aumento de la expresión de CAM, sustentado por la disminución del contenido de clorofilas totales y por el incremento del porcentaje de asimilación de CO_2 durante la noche, EUA, IS y actividad SOD. A los 30 días las plantas sin riego tuvieron una respuesta CAM de mayor intensidad, con el 100 % de captación de CO_2 durante la noche, pero su rápida recuperación con el establecimiento del riego hasta la saturación del sustrato, lo cual demostró alta tolerancia a la sequía y elevada plasticidad metabólica.

Palabras clave: ciclo CAM, fotosíntesis, vitroplantas

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INTRODUCTION

The hybrid 'MD-2' possesses characters of high agronomic importance with high yields and fruit quality compared to the rest of the pineapple cultivars (1).

The need to introduce 'MD-2' in Cuban plantations requires large production of propagation material in the shortest possible time, which is possible with the use of in vitro cultivation techniques as tools of micropropagation, technology that allows to obtain large number of plants in a short time and with better quality; However, micropropagation laboratories have limitations, this technological process induces in the plants a physiological state disadvantageous to the transition to ex vitro conditions and, consequently, the obtaining of low growth rates and insufficient percentages of survival during the acclimatization (2).

Research has been carried out to determine the best chemical or physical in vitro culture to obtain plants capable of acclimatization to ex vitro conditions (3, 4) and in the acclimatization phase (5, 6). However, the protocol used for acclimatization presents problems with the transition of the plants to the field, once they are planted they show burns in the leaves and senescence in some tissues with a consequent low survival.

These problems are associated with the stress caused by the drastic change between the environmental conditions of the final phase of acclimatization and the field (7), and it is necessary to develop research aimed at reducing the negative effect of the environment on pineapple plants 'MD-2' in the transition to the field.

Ananas comosus presents acid metabolism of crasulaceous (CAM). Plants with CAM can operate in different modes: "constitutive CAM" or "forced CAM", with high accumulation of acids (ΔH^+) and CO_2 uptake during the night; "Inducible CAM or" CAM facultative ", also known as" C3-CAM "with a form C3 of CO_2 fixation and null variation of ΔH^+ in the non-induced state; But in the induced state it presents small fixations of CO_2 in the day and high levels of ΔH^+ during the night; "Cyclic CAM" with CO_2 fixation during the day and ΔH^+ but does not show stomatal opening at night; CAM "Idling" or "Futile CAM", with stomatal closure and small ΔH^+ variations throughout the day and night in severely stressed plants (8).

These behaviors may exhibit different physiotypes within the metabolism itself, as in the case of "constitutive CAM" plants, which may be strong or weak, according to the magnitude of C4 expression. Pineapples in their habitat or in field production conditions respond as a "strong constitutive CAM" (9), but it has been shown that micropropagated plants may exhibit a different physiotype, these may be C3 or CAM according to the conditions of in vitro growth (3, 4, 10, 11); In addition, previous studies demonstrated that an increase in light intensity increases the magnitude of CAM expression in the acclimatization stage and that the treated plants acquired an advantageous morpho-physiological and biochemical state for the transition to the field (1).

Taking into account that CAM is expressed and modulated with changes in environmental variables such as light intensity, humidity, nutrition and temperature (12), from the practical point of view and from an economic perspective it is possible to carry out irrigation and frequency management for induce a better morpho-physiological and biochemical response of the plants in the final stage of acclimatization favoring the acclimatization-field transit and thus the increase of survival.

MATERIALS AND METHODS

This research was carried out between September and November 2014 in the area of acclimatization of the Bioplant Center. Pineapple plants Ananas comosus (L.) Merr., 'MD-2' micropropagated, according to the protocol established in the Bioplants center. Plants larger than 4,0 cm in length were selected with 7,0 to 8,0 g of fresh mass and five and six functional leaves and immersed for five minutes in a solution of Previcur Energy® (Bayer CropScience) [3 ,0 mL L^{-1}] (13). They were then planted in plastic containers with a volume of substrate of 256,26 cm^3 with a ratio of 1: 1 (v / v) between red ferralitic soil and filter cake (derived from sugarcane) (6).

The plants were acclimatized in a culture house with average conditions of 80 ± 3 % relative humidity, 25,5 ± 2 °C temperature (determined with a digital thermohygrometer "TECPEL® model DTM-303"), light and Natural photoperiod with a photoreactive photon flux (FFF) of 400 ± 25 µmol $m^{-2}s^{-1}$ and atmospheric CO_2 concentration conditions between 330 and 375 µmol mol^{-1} (determined using a portable system of photosynthesis "PP Systems CIRAS-2 Portable Photosynthesis System "). Irrigation was carried out by spraying for 10 minutes at 9:00 a.m. and 14:00 a.m. daily. Leaf fertilization was applied with a fumigating bag every 15 days with crystalline NPK [1,0 g L^{-1}] and Multimicro Combi (Haifa Chemicals Ltd., Haifa Bay 26120, Israel) [62,5 mg L^{-1}].

After five months in acclimatization, 400 plants of 34-36 g were harvested fresh with 11-12 leaves and 12-13 functional roots and moved to the exterior of the growing house with a light intensity of 800 µmol m^{-2} s^{-1}. The groups were submitted to different irrigation conditions according to Table I.

Table I. Irrigation conditions during growth of micropropagated pineapples Ananas Comosus (L.) Merr. 'MD-2' in the final stage of acclimatization

Treatments	Time Intervals Up to 0 days	to 0-30 days	to 30-45 díays
Irrigated plants (CR)	120 mL plant (1)	120 mL plant	Substrate saturation (3)
Plants without irrigation (SR)	120 mL plant	0 mL plant (2)	Substrate saturation

(1) Irrigation each 48 h (2) without irrigation (3) Daily irrigation at 9:00 h until saturation of the substrate

Foliar fertilization was applied at 9:00 h at the initial time of the experiment (crystalline NPK [1,0 g L^{-1}] and Multimicro Combi (Haifa Chemicals Ltd., Haifa Bay 26120, Israel) [62,5 mg L^{-1}]). On day 31 of the experiment foliar fertilization was applied at 9:00 h (crystalline NPK [1,0 g L^{-1}] and Multimicro Combi (Haifa Chemicals Ltd., Haifa Bay 26120, Israel) [62,5 mg L^{-1}]). The irrigation was done with a manual watering can, homogeneously distributing 100 L of water on the leaf surface of the plants every 24 h.

Morphological determinations: 30 plants were taken to determine the number of leaves and length and width of D leaf. In addition, the fresh and dry mass of 10 leaf discs of 0,85 cm2 were determined on four D leaves collected at 3:00 p.m. The water content was calculated.

Quantification of gaseous exchange in the leaves: Gaseous exchange was quantified every three hours until a day. An infrared gas analyzer (IRGA) was used as part of a portable photosynthesis system (PP Systems CIRAS-2 Portable Photosynthesis System). Three D leaves were taken and 30 measurements were taken for each time and treatment. The determinations were performed under ambient temperature and relative humidity conditions, with an FFF of 600 µmol $m^{-2}s^{-1}$ (14). For the analysis of the results, the average was taken between 9:00 pm and 9:00 am. Efficiency in water use was calculated by dividing the CO_2 assimilation rate and the transpiration rate. In addition, the percentage represented by the assimilation of CO_2 between these hours and the whole day was determined.

Quantification of chlorophyll content: Three D leaves were collected at 3:00 p.m. and stored in liquid nitrogen (NL) until the time of the determinations. 0,1 g of leaves were taken and macerated in a mortar with NL until a fine powder was obtained. 500 µl of 80 % acetone was added with 2,5 mM Sodium Phosphate pH 7,8, then centrifuged at 12 000 g for 15 minutes at 4 °C with a Beckman vacuum-cooled centrifuge (JB-21). The supernatant was collected and the absorbance was measured at 664 nm and 647 nm. Concentrations of a, b and total chlorophylls were calculated (15). A "Pharmacia Bio-Spectrophotometer" spectrophotometer was used. The results were expressed as a function of fresh mass and were converted from µg Cfls g^{-1} MF to µg Cfls cm^{-2} as described (3).

Calculation of Succulency Index: Succulency Index (SI) was calculated using the values of the total chlorophyll content [Cfls (a + b)] and the water content (AC) of the "D" leaf discs. The equation IS = CA [Cfls (a + b)] - 1 (16) was used.

Determination of organic acid levels: 1g of leaves were taken, 10 mL of 50 % Ethanol was added and incubated in a 90 °C bath for 20 minutes. The liquid phase was separated and acid-base titration was performed with NaOH [20 mM] and phenolphthalein [1 mg L^{-1}] as indicator (17). The results were expressed in µmol H + h-1g-1 MF.

Protein quantification and enzyme activity Superoxide dismutase: 0,5 g of plant material were weighed and NL-macerated to a fine powder, 2 mL of 50 mM potassium phosphate buffer pH 7.8 was added with 1 mM EDTA ; 0,1 % Triton X-100 (v / v); 1 % PVP; 1 mM DTT and 1 mM PMSF), then centrifuged at 15 000 g for 20 minutes at 4 °C with a Beckman (JB-21) vacuum-cooled centrifuge (16). The supernatant was collected and the protein content (18) was quantified using a standard curve with "Bobine Sero Albumin" (BSA) as a standard protein. The protein content was expressed in mg Prot. G-1 MS. The activity of the enzyme Superoxide dismutase (SOD) (EC 1.15.1.1) was determined by the cytochrome C oxidoreduction method as described (16). The enzymatic activity was expressed as U mg-1 MS. A unit of SOD activity was defined as the amount of enzyme capable of inhibiting the 50 % reduction rate of cytochrome C.

Statistical analysis: all physiological and biochemical determinations were replicated three times in three different biological samples for each time of evaluation and treatment. Statgraphics Centurion XV software (19) was used. The results were processed with "ANOVA factorial" followed by "Tukey's Multiple Range test" for multiple ranges, or "Kruskal-Wallis" followed by "Student-Newman-Keuls" where necessary. All statistical tests were performed with a significance level of 5 %.

RESULTS AND DISCUSSION

Effect of water deficit on morphological indicators

After 15 days in plants subjected to water deficit (SR) a cessation of leaf D elongation was observed (Figure 1A), however, after 30 days, when both groups were irrigated until the saturation of the substrate was observed an increase in the length of this leaf in this same treatment. On the other hand, during the period of water deficit (up to 30 days) leaf emission was not observed in the SR plants (Figure 1C), but with the restoration of the irrigation.

The water deficit affected both the elongation of the SR plants and the emission of leaves, which is related to a better acclimatization to adverse environmental conditions. The growth rate and hence the biomass productivity of terrestrial CAM plants are very low compared to C3 and C4 plants under low stress conditions; however, within the same CAM species, biomass productivity is favored by favorable environmental conditions (12, 20, 21) and vice versa.

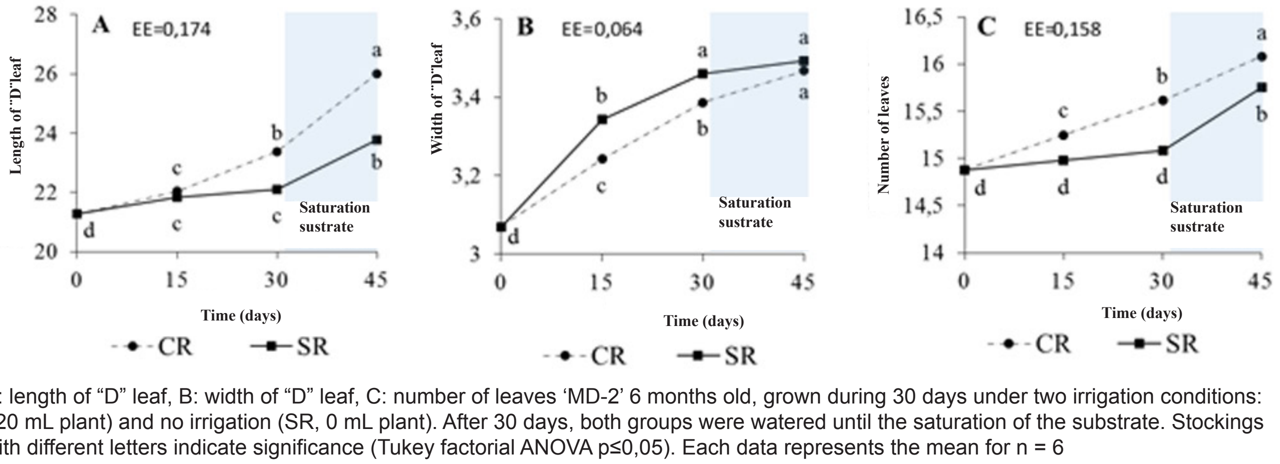
In contrast, a continuous increase in the width of leaf D was observed during the period of water deficit (Figure 1B), followed by a cessation of its increase after the establishment of irrigation (at 45 days). Pineapple micropropagated plants under acclimatization conditions have a direct relationship between the increase in the width of said leaf and the expression (3) and modulation (16) of CAM under stressful conditions.

It is important to note that there was also a continuous increase although to a lesser degree in the CR plants, this could be associated with the transfer of the plants outside the house of cultivation, together with the increase in the physiological age of the plants. Both groups were exposed to direct sunlight for 30 days before starting the experiment, thus increasing "D" leaf width in CR plants might be related to a gradual increase in CAM expression as a response to the high light intensity and to the increase of the physiological age of plants. In addition, the evapotranspiration rate outside the house of cultivation had to be higher than in the interior of the house, considering that there was a lower relative humidity, higher wind speed and high levels of irradiation, which favors the diffusion of water in the air from both the soil and the leaf surface, so that the irrigation established inside the house of cultivation could be insufficient in the exterior, and therefore the CR plants could be exposed to a light water deficit that would cause the Increment of the expression quantity CAM, and in response the increase of the width of "D" leaf during the experimental period.

Effect of water deficit on water content, total chlorophylls and succulence index

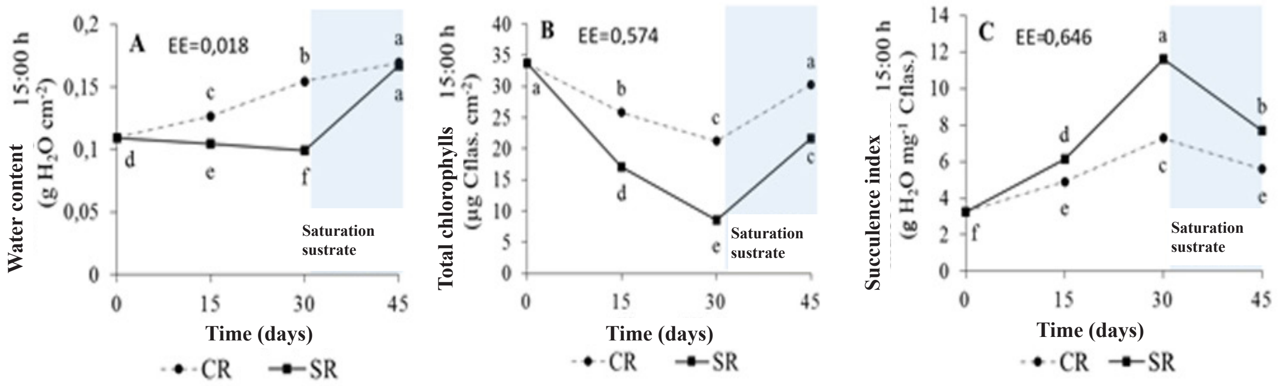
The water content of the leaves in the SR plants decreased during the period of water deficit at each evaluation time up to 30 days (Figure 2A); However, when both groups were irrigated until the saturation of the substrate, a significant increase in leaf water content was observed in the SR plants (45 days), at which point the water content of the leaves of the SR plants reached values similar to CR plants.

This could be associated to the synthesis of aquifer tissues in SR plants during the period of water deficit or to an increase in the osmotic potential of the cells associated with the influx of carbohydrates and malic acid into the tonoplast.



A: length of "D" leaf, B: width of "D" leaf, C: number of leaves 'MD-2' 6 months old, grown during 30 days under two irrigation conditions: 120 mL plant) and no irrigation (SR, 0 mL plant). After 30 days, both groups were watered until the saturation of the substrate. Stockings with different letters indicate significance (Tukey factorial ANOVA p≤0,05). Each data represents the mean for n = 6

Figure 1. Effect of water deficit on micropropagated pineapple plants (*Ananas comosus* (L.) Merr.)



A: water content, B: total chlorophyll content, C: 6-month-old mesophilic (MDS) '6-month-old mesophytic succulence index', grown during 30 days under two irrigation conditions: plants with irrigation (CR, 120 mL plant) and no irrigation (SR, 0 mL plant). After 30 days, both groups were watered until the saturation of the substrate. Stockings with different letters indicate significance (factorial ANOVA, Tukey p≤0.05). Each data represents the mean for n=6

Figure 2. Water deficit effect on leaflets of "D" leaves of micropropagated pineapples (*Ananas comosus* (L.) Merr)

On the other hand, in the total chlorophyll content, a similar trend was observed in both groups (Figure 2B), with a continuous decrease up to 30 days, followed by an increase in daily irrigation until substrate saturation (45 days). However, CR plants had higher values than SR plants at all times of evaluation.

In the case of the mesophilic succulence index both groups showed a similar trend throughout the experiment but a higher degree in SR plants (Figure 2C), with an increase in leaf succulence during the first 30 days and a decrease with establishment of the irrigation until the substrate saturation.

The index of mesophilic succulence represents the relationship between aquifer tissues and photosynthetic ones.

In this experiment a decrease of water content in the leaves of the SR plants caused by the severe water deficit was observed; however, a decrease in the total chlorophyll content was also observed. This indicates that the water deficit caused a decrease in the total chlorophyll content to a greater degree than the decrease in the water content of the leaves, which gives more succulence and Magnitude of CAM expression to SR plants.

A plant is considered CAM when this index is superior to the unit (8), and to greater succulence of stems and leaves greater magnitude of CAM expression and better acclimatization to adverse environmental conditions (22, 23); However, IS also increased in CR plants during the water deficit period, which supports the conjecture that in this group there was a gradual increase in the magnitude of CAM expression, as a response to the increase in physiological age and the transfer of the plants to the exterior of the cultivation house.

Effect of water deficit on gas exchange

SR plants had a decrease in CO_2 assimilation at 15 and 30 days of water deficit with respect to CR plants and the initial moment of the experiment (Figure 3A). The establishment of irrigation until the saturation of the substrate favored the uptake during the night in this same group.

A continuous decrease of transpiration in the SR plants was observed during all the evaluation moments in the period of water deficit, with significant differences among them and among treatments. In the establishment of irrigation until the saturation of the substrate, a considerable increase of transpiration was observed in this same group of plants, reaching values close to the CR plants, although statistically different. SR plants had an increase in the efficiency of water use during the period of water deficit, with significant differences with respect to CR plants at all times evaluated; However, with the restoration of irrigation, a significant decrease of this indicator was observed in both groups but with higher values in the SR plants.

The increase in the amount of CAM expression is related to the increase in the percentage of CO_2 uptake during the night (24), for the CAM plants it is a benefit to capture more CO_2 at moments where the environmental conditions are less drastic and this way prevent an excessive loss of water (12, 25, 26).

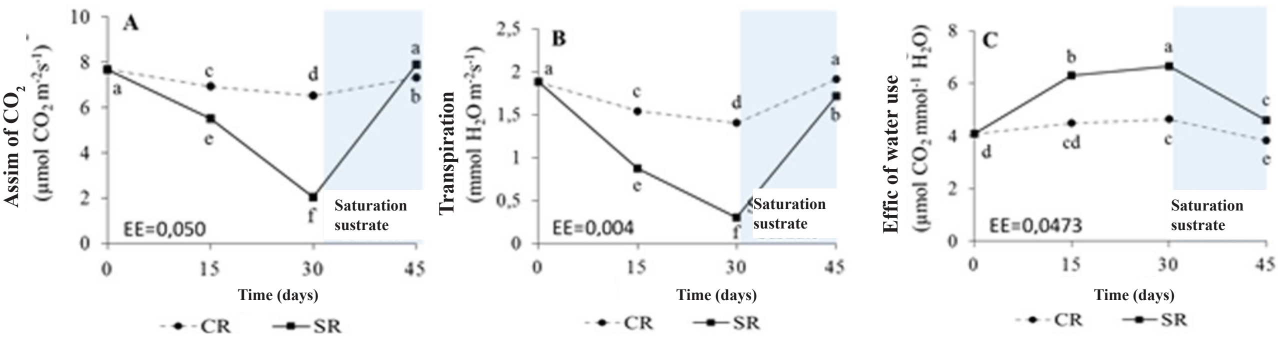
The water deficit caused a decrease in the uptake of CO_2 from the SR plants; however, the percentage of CO_2 assimilation between 21:00 and 9:00 h was continuously increased in SR plants (0-30 days) during the period of water deficit (Table II).

When CAM plants are exposed to water deficit, they present a decrease in CO_2 uptake during the day, especially in the hours near dawn and dusk, but when the water deficit is prolonged or severe, the CO_2 uptake ceases during most or all of the daily cycle (17, 27, 28, 29), in this state, CAM plants for their survival capture and therefore recycle all or a large part of the CO_2 released during respiration (30, 31), and this response to unfavorable conditions is called “CAM Idling” or “Futile CAM” (8).

It has been shown that under favorable growth conditions, CO_2 captured by pineapple overnight (phase I) represents more than 75 % of the CO_2 assimilated during the whole day; However, due to prolonged water deficit and high temperatures during the day, this percentage can increase, which gives it high tolerance to drought (32). This explains the increase in the percentage of CO_2 assimilation during the night in the SR plants (Table II) compared to the water deficit for 30 days, where this percentage represented approximately 100 % of the uptake throughout the day, which shows that the SR plants had a similar response to “CAM Idling” in the face of severe water deficit, with stomatal closure for most of the day and opening them only when the transpiration gradient was minimal.

Pineapple micropropagated plants under severe water deficit also responded similarly to “CAM Idling” plants, instead of “CAM facultative” (3, 4, 10, 11), or “constitutive CAM” (1) as demonstrated for pineapple plants from in vitro culture. Despite the water deficit for 30 days, 15 days after the irrigation restoration until the substrate saturation, SR plants showed a rapid recovery, demonstrating a high metabolic plasticity. In addition, an increase in leaf length “D” (Figure 1A) and number of leaves (Figure 1C) was also observed at this same time, which allows us to conjecture that with the rehydration of the SR plant tissues (Figure 2A) The carbohydrate partition had to favor the growth in greater proportion.

In the case of transpiration SR plants showed a decrease in transpiration with increasing water deficit up to 30 days (Figure 3B), from this moment a considerable increase in transpiration was observed with the establishment of irrigation.



A: assimilation of CO2 B: transpiration C: efficiency in water use
MD-2¨ of 6 months of age, grown during 30 days under two irrigation conditions: plants with irrigation (CR, 120 mL plant) and without irrigation (SR, 0 mL plant). After 30 days, both groups were watered until the saturation of the substrate. Stockings with different letters indicate Significance (factorial ANOVA, Tukey p≤0,05). Each data represents the mean for n = 90

Figure 3. Water deficit effect of on “D” leaves of micropropagated pineapples (*Ananas comosus* (L.) Merr)

Table II. Percentage of assimilation of CO^2 between 21:00 and 9:00 h in relation to the total assimilation of plants during the whole day

Time of evaluation	Percentage of assimilation of CR Plants (%)	Percentage of assimilation of SR plants (%)
0	74,47 c	74,47 c
15	77,58 b	78,93 b
30	78,35 b	99,95 a
45	73,14 d	74,03 cd

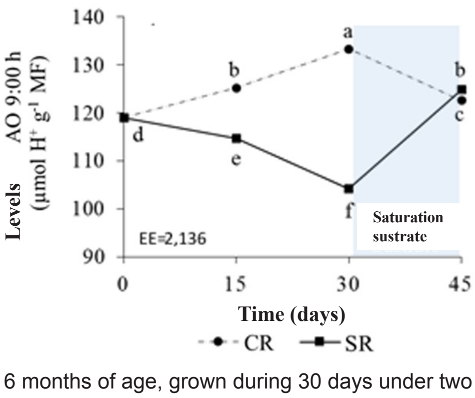
CR- plants with irrigation; SR- plants without irrigation. After 30 days both groups were watered daily until saturation of the substrate. The data were transformed according to X ‘= 2 arcsen [(x / 100) 0.5]. Socks with different letters indicate significance (ANOVA of double classification, Tukey test p≤0.05). Each data represents the mean for n = 90

The significant decrease in transpiration in SR plants at 15 and 30 days supports the hypothesis of increasing the magnitude of CAM; However, the considerable decrease at 30 days is also related to the decrease in CO_2 assimilation (Figure 3A) and water content of the leaves (Figure 2A), both indicators in close relation to the water deficit involving stomatal closure, which allows us to conjecture that at 30 days the SR plants responded similarly to “CAM Idling”. In addition, the water deficit increased the efficiency of water use at night in SR plants at 15 days (Figure 3C).

An increase in water efficiency in CAM plants is related to an increase in the magnitude of expression in terrestrial CAM plants (12). Regarding the size of the organs of the CAM plants, the stomata appear in low densities and present low conductivities to water vapor, reflecting a high capacity of water storage in the leaves and stems, decrease of the area-volume relation of the leaves and thus high water efficiency (12). This explains the increase in leaf water content in SR plants at 45 days (Figure 2A), and the increase in water efficiency of SR plants at 15 and 45 days of evaluation, further supports the conjecture that the considerable increase in water content in SR plants at 45 days could be related to the synthesis of water reserve tissues (Figure 2A) coupled with the increase in the osmotic potential of the cells.

Effect of water deficit on organic acid levels

A contrary trend in organic acid levels of both groups was observed in Figure 4. The water deficit caused a decrease in organic acid (AO) levels in the SR plants until the 30 days of drought, in contrast the levels of AO in the CR plants increased during



MD-2 of 6 months of age, grown during 30 days under two irrigation conditions: plants with irrigation (CR, 120 mL plant) and without irrigation (SR, 0 mL plant). After 30 days, both groups were watered until the saturation of the substrate. Stockings with different letters indicate significance (Kruskal-Wallis, Student-Newman-Keuls P≤0.05). Each data represents the mean for n=6

Figure 4. Water deficit effect of on organic acid (AO) levels on “D” leaves of micropropagated pineapples (*Ananas comosus* (L.) Merr

the period of water deficit and then decreased to 45 with the establishment of irrigation until saturation of the substrate.

In the CAM plants, the CO_2 assimilated overnight is fixed by the enzyme phosphoenolpyruvate carboxylase (PEPC) in phosphoenolpyruvate (PEP) which is converted to malate and transported into the vacuole where it is stored as malic acid (33); in addition, changes in AO levels correspond mainly to the malic, citric and isocitric acid levels; However, in Ananas comosus the changes in AO levels correspond mainly to malic acid levels, with citric acid levels remaining constant and isocratic (16) negligible, in addition the relationship between AO and malic levels responds to a 2: 1 stoichiometry (24).

In this way, for the pineapple, a direct relationship can be established between the levels of AO and the levels of malic acid product of the nocturnal assimilation, and therefore it can be said that the amount of malate accumulated during the period of water deficit in the plants SR was lower than the levels of the CR plants, which sustains the decrease of the nocturnal assimilation of CO2 (Figure 3A), the decrease of the elongation speed of the leaf D and the cessation of leaf emission during the period of Water deficit.

The increase of organic acid levels at 45 days reached similar values in both groups at 15 days of study; this implies a recovery of SR plants from prolonged water stress.

The gradual and continuous increase of the CR plants could be related to the increase of the physiological age, together with the transfer effect of the plants to the exterior of the cultivation house, this last one related to the exposure during prolonged time to conditions of high luminous intensity and a moderate water deficit, which is associated with the reduction of organic acid levels at 45 days in this same group.

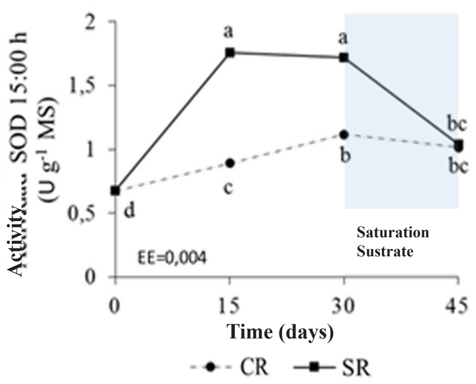
The terrestrial CAM plants present an increase in the water content of stems and leaves in response to moisture limiting conditions, this increase is related to the synthesis of water reserve tissues and the increase of the osmotic potential of the cells, the latter provoked by the influx of malic acid and carbohydrates that are stored inside the large and dominant vacuoles (12).

The increase in the osmotic potential explains the decrease in water content in SR plants during the period of water deficit (Figure 2A), the significant increase of SR plants with reestablishment of irrigation and the gradual increase of CR plants during the experiment, which is related to a decrease or increase of the levels of organic acids and therefore of malic acid as the case may be; although this conjecture is not consistent with the decrease of AO levels in the CR plants at 45 days, so that with the irrigation establishment until the saturation of the substrate said decrease could be associated with a lower magnitude of CAM expression.

Effect of water deficit on Superoxide dismutase activity

Figure 5 shows an increase in the SOD activity in the SR plants at 15 days of evaluation without significant differences between them at 30 days, from that moment showed a decrease in the SOD activity. The water deficit applied to the SR plants caused an increase in SOD activity, indicating that during the water deficit (15 and 30 days) SR plants had to increase the degree of expression since it has been shown that the expression of CAM is related to oxidative stress pathways (4).

With the restoration of irrigation to substrate saturation conditions a significant decrease in SOD activity was observed in SR plants; this implies a decrease in the magnitude of CAM expression in this group. The CR plants did not reach the SOD activity values of the SR plants at any of the water deficit moments, which implies that the SR plants presented a greater capacity for the removal of the superoxide radical compared to the CR plants, in addition indicates that the SR plants presented better preparation to attenuate the negative effect of the drastic field environmental conditions compared to the acclimatization environmental conditions.



MD-2 of 6 months of age, grown during 30 days in two irrigation conditions: plants with irrigation (CR, 120 mL plant) and without irrigation (SR, 0 mL plant). After 30 days, both groups were watered until the saturation of the substrate. Stockings with different letters indicate significance (Kruskal-Wallis, Student-Newman-Keuls
P≤0.05). Each data represents the mean for n = 3

Figure 5. Water deficit effect on the enzymatic activity of Superoxide dismutase (SOD, EC 1.15.1.1) (A), on “D” leaves of micropropagated pineapples (Ananas comosus (L.) Merr.)

CONCLUSIONS

- During the period of water deficit SR plants showed greater magnitude of CAM expression than CR plants, with better response at 15 days of experiment.
- At 30 days a similar behavior was observed in this same group as “CAM Idling”, which shows severe water stress in the SR plants.
- It was observed that the studied variables demonstrated a decrease in the expression of CAM with the reestablishment of the irrigation until the substrate saturation, so that the micropropagated pineapple plants in the final stage of acclimatization showed a high metabolic plasticity, firstly because in soil 15 days of water deficit had a response to such stress to maintain cellular homeostasis, and as a consequence the increase in the expression of CAM and increase in morphophysiological quality, 15 days after irrigation restoration until substrate saturation was observed increase in morphological indicators related to growth, so it can be said that at 15 days of water deficit SR plants presented a better preparation for the transition to field conditions.

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Note:

During the editing process it was not possible to access the work of retouching and improvement of images, so they have been inserted with the same quality as the ones sent by their authors.

The editorial