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High Light Intensity Increases the CAM Expression in "MD-2" Micro-Propagated Pineapple Plants at the End of the Acclimatization Stage

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Abstract

This work describes the evaluation of morpho-physiological and biochemical changes in "MD-2" micro-propagated pineapple plants (*Ananas comosus* (L.) Merr.) grown after 30 days under low light intensity (LL, greenhouse light conditions at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or high light intensity (HL, field light conditions at 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Gas exchange, leaf pH, protein content and superoxide dismutase activity (SOD) (EC 1.15.1.1) were measured every 3 h during one day. Chlorophylls content and succulence index (SI) were determined at 9 h. Results showed significant differences in CO_2 exchange rates, with a maximum occurring at 6 h (3.00 and 8.25 $\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$ for leaves under LL and HL conditions respectively). Plants under HL conditions had higher CO_2 uptake and lower pH values between 0 h and 6 h respective to LL plants. The maximum pH value was attained 3 h before in HL plants. Leaf SI was increased and chlorophyll content decreased by HL conditions. SOD activity was higher in plants under HL conditions, near doubling those of LL plants at 18 h (2.8 versus 1.5 $U \cdot mg^{-1}$ Protein respectively). Both groups showed a typical CAM phenotype, but it was stronger in HL conditions, which may confer these plants with a better acclimation to transfer to the field.

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Keywords

Crassulacean Acid Metabolism, Hardening Stage, *Ex Vitro*, Vitroplants, Gaseous Exchange

1. Introduction

Pineapple (*Ananascomosus* (L.) Merr.) is a perennial monocot plant belonging to the family Bromeliaceae, subfamily Bromelioideae, in the order Bromeliales. Pineapple is one of the most economically important tropical fruits [1] and the most of CAM plants [2]. After bananas and mangoes, it is the third most important tropical fruit produced in the worldwide [1]. Pineapple is propagated vegetatively, using crowns, slips, shoots or suckers, but these planting materials have their limitations including less uniformity, transmission of diseases, and inadequacy for commercial production. *In vitro* propagation is a crucial technique for disease free, rapid and mass production of pineapple plantlets [3]. In micro-propagation techniques, the pineapple multiplication rate can be considered low and it would take some years to obtain enough propagules [4]. The use of Temporary Immersion System (TIS) techniques can solve this problem, by increasing the number of pineapple plantlets with higher quality produced over short time, as a result of a better handling of the *in vitro* atmosphere and the nutrition [5]. Large-scale production of *in vitro* pineapple plantlets started in Cuba in some years ago [6]. After *in vitro* massive propagation, it is often necessary to adapt plantlets to the harsher and uncontrolled *ex vitro* environment. This may occur in a greenhouse or field during a phase called acclimatization or hardening [7]. Many authors have studied the factors affecting acclimatization in pineapple, such as light intensity [8], CO_2 enrichment, light and relative humidity controlled in *in vitro* conditions [5], or addition of bioferilizers during this phase [9]. Very often acclimatized plants do not have the appropriate size for direct planting into the field, which results in poor survival after transferral. To attain a better survival rate and establishment on field, these plants need to be further acclimatized in greenhouses using different pots, substrates, fertilizer programs, controlled light intensity and temperature and other important factors until plants reach the appropriate size [10]. Micro-propagated pineapple plants have a plastic morphology and physiology that can change according to the growth conditions. Temperature determines the occurrence of C_3 or CAM photosynthesis in pineapple plantlets grown under *in vitro* conditions [11], this behavior is also modulated by nitric oxide and water availability [12]. Besides C_3 or CAM metabolism in pineapple plantlets was conditioned by a combination of low relative humidity, and high temperatures and light intensity during the 4 first weeks of *ex vitro* growth [13] [14]. It is known that adult macro-propagated pineapple plants are classified like "strong constitutive CAM plants" [15]-[18]. For this reason, we conjecture that micro-propagated pineapple plants don't have the CAM expression degree and the defensive or adaptive mechanisms prepared like naturally grown pineapple plants in their natural habitat. Not much is known about the C_3 to CAM metabolism transitions in micro-propagated young pineapples plants, during or at the end of acclimatization phase. The aim of this work was to examine some morphological, physiological and biochemical changes in micro-propagated pineapple, 5-month old plants, grown during thirty days under low or high photosynthetic photon flux, in a greenhouse or under field light conditions respectively.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Pineappleplants (*Ananascomosus* (L.) Merr.) "MD-2" were micro-propagated as described by [19]. Plants were selected from plastic culture vessels according tothe following morphological characteristics: plants around of 4.0 cm height, 7.0 to 8.0 g fresh weight (FW), with 5 or 6 leaves on average [3]. Plants were dipped in 3.0 ml·L^{-1} of Previcur Energy$^®$ (Bayer Crop Science) during 5 min and planted in plastic 250 cm^3 glasses on a mixture of 1:1 (v:v) red ferralytic soil and filter cake (derived from sugarcane bagasse) [10]. Plants were acclimated in a greenhouse during five month under 80% ± 3% relative humidity, 25.5°C ± 2°C temperature and 250 ± 25 μmol m^{-2}·s^{-1} photosynthetic photon flux (PPF). Irrigation consisted of a 30 minmist at 9:00 am daily. A foliar fertilizer mix containing 16.0 g of crystalline N-P-K plus 1.0 g of Multimicro Comb (Haifa Chemicals Ltd., Haifa Bay 26,120, Israel) into 16 L of water was applied every ten days. Five months old plants were divided in two groups and cultivated during 30 days under different environmental conditions differing basically in illumination (**Table 1**). Fertilization and irrigation was similar to the acclimatization conditions described previously.

Table 1. Experimental conditions of six month old micro-propagated pineapple plants, measured under greenhouse and field light conditions.

Growth conditions	PPF (12 h) (μmol m^{-2}·s^{-1})	Relative Humidity (%)		Temperature (°C)	
		Day	Night	Day	Night
Greenhouse light conditions	250 ± 30	68 ± 3	78 ± 3	33 ± 2	22 ± 2
Field light conditions	800 ± 30	71 ± 3	82 ± 3	32 ± 2	25 ± 2

PPF—Photosynthetic photon flux.

All measurements and determinations were carried out at 30din the D-leaf of plants (defined like the tallest leaf on the plant [15]). The experiment was done between September and October of 2013.

2.2. Methods Used

Morphological measurements: D-leaf length and weight were taken using a graduate ruler. Leaf water content was determined as the difference between the fresh weight (FW) and the weight after drying for 72 h at 60°C [dry weight (DW)] in the D-leaf of nine plantstaken at 9 h.

Gas exchange measurements: Samples were collected each 3 h during whole day and fifteen determinations were done on six D-leaves of plants for each treatment and day time. Gaseous exchange was quantified using an infrared gas analyzer (PP Systems CIRAS-2 Portable Photosynthesis System) coupled ata universal cuvette: PLC6 (U), 1.7 cm^2. CO_2 exchange (μmol CO_2 m^{-2}·s^{-1}) and transpiration rates (mmol H_2O m^{-2}·s^{-1}) were measured underphotosynthetic photon flux of 600 μmol m^{-2}·s^{-1}. CIRAS-2 was automatically calibrated before each measurement. Water-use efficiency (WUE) was calculated as ratio between CO_2 exchange and transpiration rates and defined as amount μmol CO_2 exchange per mmol H_2O transpired (μmol CO_2 mmol^{-1} H_2O).

Chlorophyll content determination: Samples of three D-leaves were collected at 9 h, and stored under liquid nitrogen until each determinations were made. Samples (0.1 g) were ground to a fine powder using liquid nitrogen with a pestle and mortar. The powder was diluted into 500 μl of 2.5 mM potassium phosphate-KOH/80% acetone, pH 7.8, and centrifuged at 12,000 g during 5 min at 4°C. The supernatant was used for spectrophotometric determination. Chlorophyll concentration was calculated by the equations described by Porra (2002) [20], using a Pharmacia Bio-spectrophotometer. Chlorophyll contents were expressed as μg·g^{-1} FW.

Leaves pH determination: Six fresh D-leaves of plants were collected every 3 h for 24 h. Samples were crushed and 0.5 ml of the resulting fluid were collected, clarified by addition of activated charcoal and centrifuged at 12,000 g during 5 min. Supernatant pH was immediately quantified with pH indicator strips (Merck KGaA 64,271) according to Aragón *et al.* (2012) [13].

Succulence index (SI): The succulence index was calculated using the values of chlorophyll content, fresh weight (FW) and dry weight (DW) to calculate in the formula: SI = (FW-DW)[Chl($a + b$)]$^{-1}$ [21].

Extraction and quantification of proteins and SOD assay: Six D-leaf were collected every 3 h for 24 h and stored under liquid nitrogen until each determinations were made. Frozen samples were ground into a fine powder using liquid nitrogen. FW-buffer ratio was 0.25 g into 2 mL of extraction buffer containing 50 mM potassium phosphate pH 7.8, 1 mM EDTA, 1% (v/v) Triton X-100, 1% (w/v) polyvinyl-pyrrolidone (PVP), 1 mM dithiothreitol (DTT) and 1 mM phenyl-methylsulfonyl fluoride (PMSF). The resulting material was centrifuged at 15,000 g during 20 min at 4°C and the soluble fraction was collected for SOD (EC 1.15.1.1) activity determination. Total soluble proteins were determined according to Bradford, (1976) [22], using as reference Bovine seroalbumin (BSA). SOD was assayed by the ferricytochrome C method, using xanthine/xanthine oxidase as the source of superoxide radicals [23], in 1 mL of a reaction mixture containing 50 mM potassium phosphate-KOH buffer pH 7.6, 0.1 mM EDTA, 0.01 mM cytochrome C, 0.05 mM xanthine, 0.03 units xanthine oxidase (EC 1.1.3.22, Sigma Aldrich) and the soluble crude extract (80 μL). All measurements were made using a Pharmacia Bio-spectrophotometer, and one SOD unit was defined as the enzyme amount which caused 50% inhibition of the initial rate of reduction of cytochrome C.

Statistical analysis: The experiment was conformed in completely random blocks. At each sampling time and treatment, six plants were randomly selected to measure of gas exchange (with fifteen measurements to each one) and for determination of leaf pH and SOD activity (with two repetition to each one), nine samples for fresh

and dry weight, thirty for length and width of D-leaf, and six (with two repetition to each one) for chlorophyll content determinations. All analysis statistical analyses were carried out using SPSS version 12 [24]. Statistical significance was assessed where indicated through two-way analysis of variance (ANOVA) followed by Tukey's Multiple Range Test for bi-factorials comparisons or Student's t-test for comparison of two conditions, both at 5% significance level.

3. Results & Discussion

3.1. Morphological Changes

Pineapple plants grown under HL conditions increased the D-leaf width at thirty days, while D-leaf length did not show significant differences (**Table 2**). *In vitro* cultured pineapple leaves are longer and narrower than pineapple macro-propagated plants, which could be related to the low light of the culture room [13]. D-leaf width increment, could be a response to high light intensity conditions, also it is know that the D-leaf width of micro-propagated pineapple plants was increased to 3.5 cm at 60 days of planted in field [6]. Plants under HL conditions every 30 days increased the fresh and dry weight including water content of D-leaf. The succulent leaf and stem of CAM plants have very high water-storage capacitance in their above-ground tissues [2]. In different CAM plants formation of water storage tissues could be observed after exposure to high light intensity, perhaps as a heat dissipation strategy [13], this could be the answer to differences between both groups, which could be related to synthesis of water-storage tissues being higher in plants under HL conditions.

3.2. Gas Exchange

Night CO_2 fixation is initiated by phosphoenolpyruvate carboxylase (PEPC) (E.C. 4.1.1.3 l), in a light-independent way, whereas daytime assimilation is light-dependent and fixed by ribulose-l.5-bisphosphate carboxylase-oxygenase (RubisCO) (EC 4.1.1.39). The distinctive pattern of CO_2 exchange (**Figure 1(A)**) and transpiration rate (**Figure 1(B)**), are consistent with a well-established CAM mode. Net CO_2 uptake was occurred predominantly at night, while daytime CO_2 exchange at 15 h and 18 h was substantially negative in both groups (**Figure 1(A)**). CAM-functioning of micro-propagated pineapple plants is intrinsically related to the expression of PEPC coding genes [14]. Besides PEPC activity in CAM plants is controlled by a phosphorylation-dephosphorylation process by phosphoenolpiruvate carboxylase-kinase (PPCK) in which the phosphorylated enzyme, that abounds during the night period, is less susceptible to inhibition by malate and more responsive to the allosteric activator glucose-6-phosphate (Glc-6-P) [25]. The maximum CO_2 exchange rate was recorded at 6 h in both groups, although in plants under LL conditions it was significantly lower than in the HL treatment (**Figure 1(A)**). The CO_2 exchange rate at 6 h (8.25 and 3.0 µmol CO_2 m^{-2}·s^{-1}) show that HL plants fixed approximately 64% more CO_2 than plants under LL conditions. CAM plants immediately after dawn (phase II) can increase CO_2 uptake, since both carboxylating enzymes (Rubis CO and PEPC) are active [26]. Light saturation (photon photosynthetic flux) of pineapple plants occur at about 500 µmol·m^{-2}·s^{-1} [13]. However for plants grown under greenhouse, PPF was about to 250 µmol·m^{-2}·s^{-1} at 12 h, and much lower than that 6 h. This could account for the observed differences y maximum CO_2 uptake at this time. The observed values could also be the result of less PEPC activity due to lower expression under low light. This could also explain that plants under HL conditions increased their D-leaf dry weight (**Table 2**), since better CO_2 uptake means more substrate available for anabolic processes such as tissue growth. In CAM plants, phases II and IV (dawn and

Table 2. Morphological characteristics of D-leaf of six month old micro-propagated pineapple plants grown during thirty days under greenhouse light conditions (PPF = 250 µmol·m^{-2}·s^{-1}) and field light conditions (PPF = 800 µmol·m^{-2}·s^{-1}).

Growth conditions	Length (cm)	Width (cm)	FW (g)	DW (g)	WC (g)
Greenhouse light conditions	21.67 a	2.50 b	25.23 b	2.28 b	22.95 b
Field light conditions	21.59 a	2.83 a	27.35 a	2.51 a	24.84 a
SE	0.38	0.15	0.95	0.12	0.84

PPF—Photosynthetic photon flux, FW—fresh weight; DW—dry weight, WC—water content, SE—mean's standard error. Different letters indicate significant statistical differences after Student's t-test $p < 0.05$. Data represent means to n = 9 in case of FW, DW and WC, and n = 30 for D-leaf length and width.
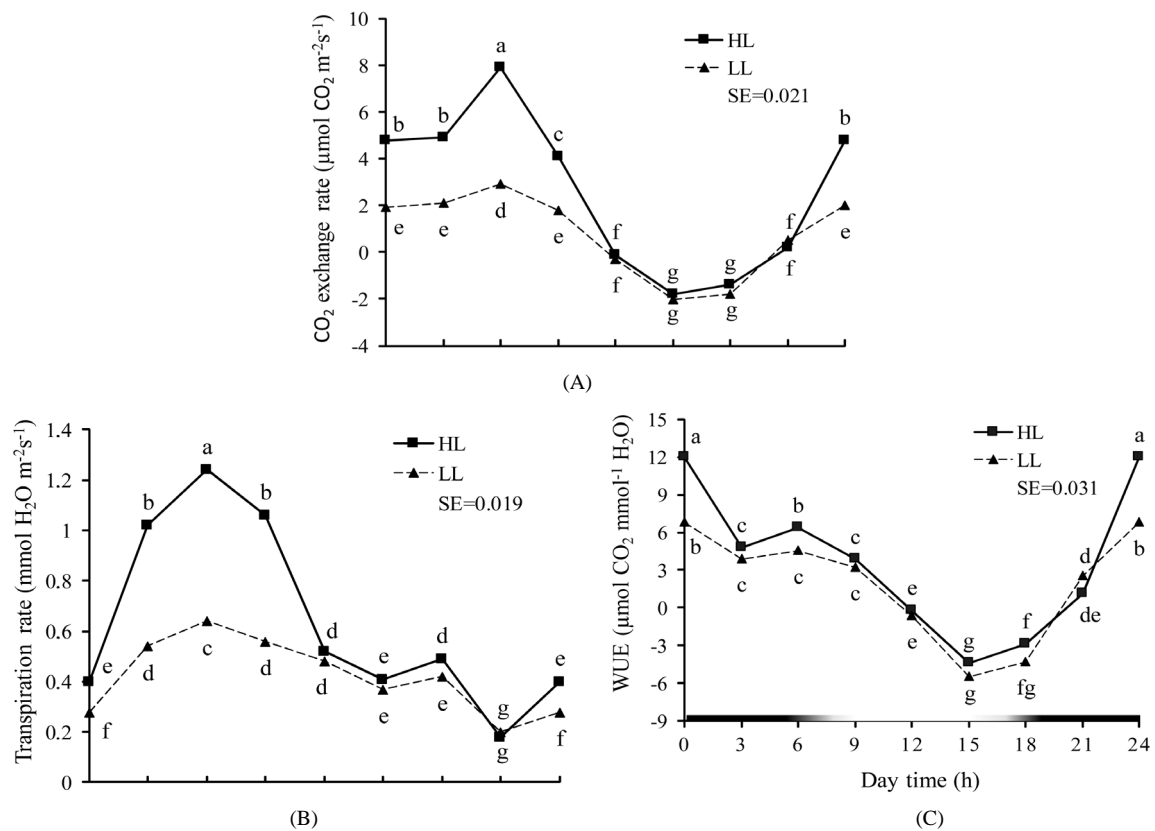
Figure 1. CO_2 exchange rate (A) Transpiration rate (B) and water-use efficiency (WUE) (C) in leaves of six month old micro-propagated pineapple plants (*Ananascomosus* (L.) Merr.) "MD-2" grown during thirty days under greenhouse light conditions (low light intensity (LL), with a photosynthetic photon flux, PPF = 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and field light conditions (high light intensity (HL), PPF = 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Data are the mean of six plants and fifteen measures to each one. The values recorded at 0 h and 24 h were *idem*. Different letters indicate significant statistical differences after two-way ANOVA, Tukey's test, $p < 0.05$ to n = 90. SE-mean's standard error. The section black on x-axis bar denotes the hours of time night.

nightfall, respectively) are often recognized like phases in which the CO_2 uptake via RubisCO (C3) and PEPC (C4) simultaneously is decisive for growth [26]. Co-ordination of carboxylases is essential for the efficient functioning of the CAM pathway, phase II is often characterized by a period of atmospheric CO_2 uptake which largely appear to be dominated by PEPC, while RubisCO remain at a low activation state until PEPC is dephosphorylated [27].

Transpiration rates in leaves of plants grown under HL conditions were significantly higher between 0 h and 9 h compared with plants in LL (**Figure 1(B)**). From 6 h on, transpiration rate declined steadily in both treatments to dawn without significant differences. Normally, light can induce stomatal opening, although for CAM species this happens at night [28]. This shows that under both treatment CAM is functioning, however, adult and macro-propagated pineapple plants shown a different behavior according to gasexchange patterns [15] [16] [18]. In fact pineapple is classified like a strong, constitutive CAM specie under field conditions, but micro-propagated pineapple plants can show the C_3 mode of photosynthesis [11] [12]. Stomatal re-opening of leaves in both treatments to allow significant night gas exchange after 21 h is also an indication of CAM functioning (**Figure 1(B)**).

Gas exchange-efficiency result was shown in **Figure 1(C)**. At 0 h and 6 h HL plants showed the highest WUE values with statistical differences compared with LL. It is known that an increase in WUE is an adaptive response of CAM plants terrestrials [29]. Both groups showed a similar water efficiency during the daily cycle, but plants under HL conditions were more efficient at 0 h and 6 h. On the other hand, between 12 h and 18 h WUE was substantially negative, which can be related to stomatal closure during day (phase III), where metabolic balance are inclined to respiratory processes, which is supported by negative values of CO_2 exchange during the same hours (**Figure 1(C)**).

3.3. Chlorophylls Content and Succulence Index

Leaves of pineapple plants grown in LL contained more chlorophyll (*a*, *b* and *a* + *b*), than plant leaves under HL conditions (**Table 3**). The chlorophyll *a/b* ratio did not show significant differences. Leaf chlorophyll content was affected by long-term high light exposition. The higher chlorophyll content in plants leaves under HL conditions during thirty days is an indicative of better acclimation to sun conditions. Higher values of total chlorophyll without a concomitant decrease in chlorophyll *a/b* ratio during acclimation of photosynthetic tissue to lower light levels, will occur if there is an increase in the number, but not size, of the "photosynthetic units" (PSU) in the tissue, as increases in PSU size in response to lower light levels typically reflect, in particular, an increase in the light-harvesting portions of the photosynthetic pigment/protein complex in both photo systems [30]. Also it can explain that pineapple plants under HL and LL conditions showed similar chlorophylls *a/b* ratio. Chloroplasts in leaves developing in the shade tend to have more photosynthetic pigments per unit volume, more thylakoids, a greater relative stacking of thylakoids into grana, and therefore a greater light-absorbing ability than do chloroplasts developing under high illumination [31]. Pineapple plants under HL condition, should be more acclimated to field condition, and should have better mechanisms to light uptake, to support the high energetic cost during phase III, which are required in the decarboxylation process during daytime [26]. SI values were significantly higher in plants grown in HL (**Table 3**). A clear difference is evident between treatments, which is the result of different degrees of CAM expression. The magnitude of CAM induction in facultative CAM plants tends not only to be influenced by water deficit, but also by associated environmental conditions such as temperature, light intensity, and humidity [29]. It is well established that high light intensity could enhance CAM.

3.4. Leaf pH

The D-leaf pH was measured in leaves of plants under LL or HL at the same time points, during the day and night periods (**Figure 2**). The pH values started to rise in the early morning (9 h, starting at pH 3.0) in HL plants but only at 12 h (starting at pH 4.0) in LL, respectively. In both treatments there is a clear trend of nocturnal acidification versus daytime deacidification, typical of CAM. However, the degree of acidification and the time frame in which it changes are clearly different among treatments. The leaf pH of LL plants was high compared to HL plants at all times during the night period. This should be related to a better CAM expression, which is also supported by results of CO_2 exchange during the same time (**Figure 1(A)**). Organic acid levels-changes in pineapple plants is due fundamentally to changes of malic acid levels [32]. The lower pH levels of plants under HL can be related to a higher amount of malic acid (perhaps the result of higher PEPC levels/activity). LL plants started to deacidify 3 h later than those under HL conditions, although in both cases an identical maximum pH was attained. While it is evident that greenhouse conditions affect the behavior of CAM, the reason behind this effect is not clear at present. It could be related to low light intensity, because in CAM plants it has generally been demonstrated that day-time integrated PPF is positively correlated with the magnitude of nocturnal malic acid accumulation and its subsequent mobilization from the vacuole during the following day [33]. Also it may well be that milder conditions affect the tonoplast in such a way that malate efflux is delayed, and with it the whole deacidification process. This would not be unusual given that the tonoplast is probably the master switch governing acid fluctuations in CAM plants as well explained by Lüttge (2000) [34].

Table 3. Changes in chlorophyll content and succulence index in D-leaf of six month old micro-propagated pineapple plants grown during thirty days under greenhouse light conditions (PPF = 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and field light conditions (PPF = 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Growth conditions	Chlorophyll ($\mu g \cdot g^{-1}$ FW)			Chl. (*a/b*)	Succulence index (g H_2O mg^{-1} Chl.)
	Chl.*a*	Chl.*b*	Chl. (*a* + *b*)		
Greenhouse light conditions	18.57 a	10.31 a	28.89 a	1.80 a	3.4 b
Field light conditions	12.23 b	7.03 b	19.26 b	1.78 a	5.2 a
SE	0.52	0.29	0.81	0.021	0.53

PPF—Photosynthetic photon flux, Chl.—Chlorophyll, SE—mean's standard error. Different letters indicate significant statistical differences after Student's t-test $p < 0.05$. Data represent means to n = 12 in all cases.
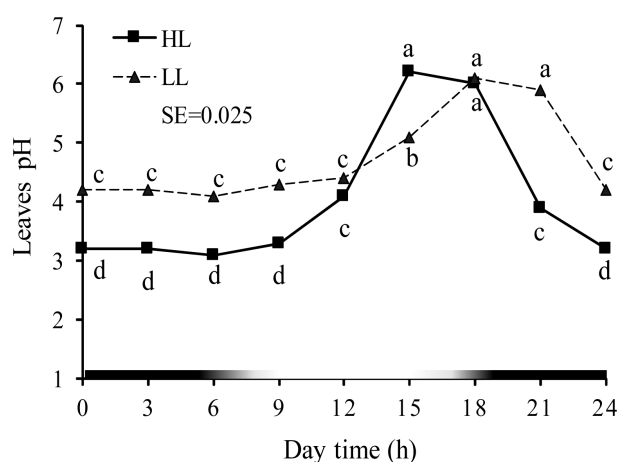
Figure 2. Leaves pH values in leaves of six month age micro-propagated pineapple plants (*Ananascomosus* (L.) Merr.) "MD-2" grown during thirty days under greenhouse light conditions (low light intensity (LL), with a photosynthetic photon flux, PPF = 250 µmol·m^{-2}·s^{-1}) and field light conditions (high light intensity (HL), PPF = 800 µmol·m^{-2}·s^{-1}). Data are the mean of six plants and two measures to each one. The values obtained at 0 h and 24 h were *idem*. Different letters indicate significant statistical differences after two-way ANOVA, Tukey's test, $p < 0.05$ to n = 12. SE-mean's standard error. The section black on x-axis bar denotes the hours of time night.

3.5. SOD Activity

The high light intensity caused an over production of the Reactive Oxygen Species (ROS), suggested by the daily fluctuation in extractable SOD activity (**Figure 3**). SOD is one of the primary enzymatic components of the antioxidant system, which converts superoxide into H_2O_2 and oxygen, it acts like the first defensive response of enzymatic and non-enzymatic mechanisms into cell to remove ROS. Since ROS formation is extremely difficult to measure, the response of antioxidant system in plants is often used as a marker for ROS production [35] *In vitro* pineapple plants under stressing environmental conditions presented an oxidative stress pattern of response described by protein damage, altered enzymatic activities (SOD) and gene expression levels, strongly influenced by C3 or CAM-stressing conditions induction [14]. During Phase III of CAM plants the stomata are closed, which can lead to a parallel increase in both the intercellular partial pressure of O_2 (piO$_2$) and CO_2 (pi-CO$_2$). An increased O_2 level in Phase III behind closed stomata can promote overproduction of H_2O_2/ROS in CAM plants [30]. SOD activity in pineapple plants studied in this work showed a treatment-dependent response. Plants under HL always contained more SOD activity than those under LL, with a minimum ratio HL/LL of 1.25 at 12 h and 1.7 at 15 h (**Figure 3**). This difference is mainly due to the fact the HL plants show an unmistakable time-dependent profile that is only slightly paralleled by LL plants. This finding is in line with the above discussion that suggests that a stronger CAM promotes a stronger ROS generation with associated strong antioxidative response. In that sense LL plants did not produce much ROS and SOD activity was lower. If so, HL plants should be more resistant to oxidative stress and hence able to better cope with an aggressive environment.

4. Conclusion

CAM in pineapple plants from *in vitro* culture was stronger a natural environment (sun) than under greenhouse conditions (shade). The main differences could be related to an increase of CAM expression degree of plants under HL conditions. We argue that these results will expand our knowledge about micro-propagated pineapple plants on new ways to prepare pineapple plants during acclimatization-field transition, and at the same time open new investigations to study the biochemical and molecular mechanisms related with the photosynthetic pathways and oxidative stress in micro-propagated pineapple plants during hardening stage. Considering that micro-propagated pineapple plants could have different behaviors related to CO_2 uptake and greatest metabolic plasticity (C_3 or CAM), depending of both, physiological age and environmental conditions, we can conjectured that CAM degree expression on micro-propagated pineapple plants could be also modulated by other environmental factors, not only at the end of acclimatization stage rather also during it.
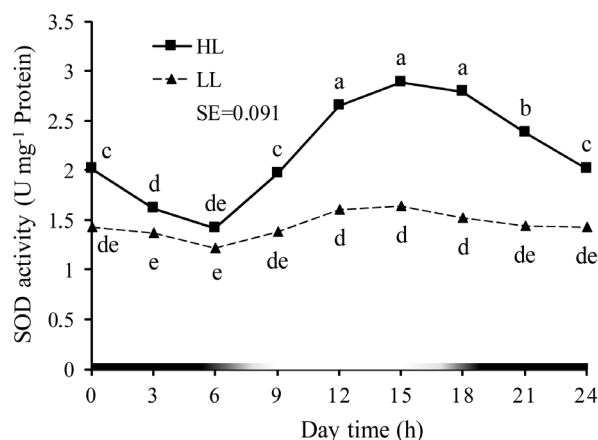
Figure 3. Specific enzymatic activity of Superoxide dismutase (SOD) (EC 1.15.1.1) in leaves of six month old micro-propagated pineapple plants (*Ananascomosus* (L.) Merr.) "MD-2" grown during thirty days under greenhouse light conditions (low light intensity (LL), with a photosynthetic photon flux, PPF = 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and field light conditions (high light intensity (HL), PPF = 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Data are the mean of six plants and two measures to each one. The values obtained at 0 h and 24 h were *idem*. Different letters indicate significant statistical differences after two-way ANOVA, Tukey's test, *p* < 0.05 to n = 12 and mean's standard error (SE). The section black on x-axis bar denotes the hours of time night.

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