

Photosynthetic metabolism and growth of pineapple (*Ananas comosus* L. Merr.) cultivated *ex vitro*

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Abstract Pineapple (*Ananas comosus* L.) is a bromeliad with constitutive CAM metabolism. However, facultative C₃/CAM metabolism in the first 2 months of growth is a characteristic of this species. Thus, young pineapple plants can exhibit C₃ photosynthesis when the environmental conditions, such as the diurnal temperature range and water supply, are

favorable to growth. However, high temperature diurnal amplitude, soil/air moisture alterations and changes in light intensity in association with air humidity have caused facultative C₃/CAM metabolism. The objective of the study was to assess the physiology and growth of *ex vitro* ‘Vitória’ and ‘IAC Fantástico’ pineapple genotypes during acclimatization from the in vitro environment. The research was carried out in a greenhouse using the split plot design used in the in vitro culture (Couto et al, Rev Bras Frutic 36(2):459–466, 2014). The explants were planted in trays with Vivatto[®] substrate. The net photosynthesis rate, growth and photochemical efficiency were measured at 60 and 75 days of acclimatization (DA). The greater growth (canopy diameter and height, shoot dry mass, total dry mass and leaf area) of ‘IAC Fantástico’ was associated with CAM metabolism. CAM metabolism was likely induced by the stressful environmental micrometeorological variables inside the greenhouse. In contrast, ‘Vitória’ exhibited C₃ metabolism and reduced growth at 60 and 75 DA. ‘Vitoria’ had greater photosynthate partitioning to the root system and high photochemical efficiency 75 DA. We conclude that the stress imposed by the environmental conditions induced CAM metabolism in ‘IAC Fantástico’ and increased growth gain characteristics at 60–70 DA.

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1 Introduction

According to the estimates of the *Food and Agriculture Organization* (FAO), in 2014 world tropical fruit production was 82 million tons. This scenario represents a 1.7 % increase compared to the 2004 base period and 78 % of this increase refers to the more important fruits such as mango, pineapple, avocado and papaya. About 90 % of all tropical fruits are produced in countries considered to be developing. In these countries, including Brazil, this production contributes to job creation, increased producer income, greater food safety and reduced poverty levels (FAO 2015).

It is estimated that pineapple fruit production (*Ananas comosus* L.) reached 2.48 million tons in 2013 in Brazil, representing 10 % of the world production (FAO 2015). The pineapple is the third most important tropical fruit after bananas and oranges. Costa Rica produces the greatest quantity of tropical fruits, with 11 % of the world production. The United States is the largest world pineapple importer with about 38 % of the world production (FAO 2015).

The main characteristic of pineapple is its ability to adapt to regions with low rainfall. This capacity is related to the induction of crassulacean acid metabolism (CAM) (Carr 2012) and differs from other fruit species that utilize C_3 metabolism. The CAM expression is characterized by photosynthetic carbon assimilation at night, when the air temperature and humidity are lower than during the day. At night, stomata opening has little effect on the total water balance of the plant. When there is a soil water deficit, this metabolism increases the water use efficiency in pineapple. However, pineapple can present the facultative C_3 /CAM metabolism (Nievola et al. 2005; Aragón et al. 2012; Rodríguez-Escriba et al. 2015).

In CAM metabolism, the stomata of the pineapple leaves open at night and maximum stomata conductance is observed at dawn (Phase I). At the start of the light period, stomata closure starts due to the high CO_2 concentration, and under this condition, CO_2 can be assimilated (Phase II). In the light period, there is complete stomata closure when CO_2 assimilation does not occur (Phase III). At the end of the light period, stomata opening begins and carbon assimilation returns (Phase IV) (Bartholomew and Kadzimin 1977; Zhu et al. 2005).

In pineapple plants with facultative C_3 /CAM metabolism, the mechanisms associated with the transition between C_3 and CAM have not yet been fully elucidated. In some studies, it has been observed that temperature amplitudes, variation in soil and air moisture and variation in light intensity associated with air humidity have caused alteration in the pineapple C_3 /CAM transition metabolism (Nievola et al. 2005; Freschi et al. 2010; Aragón et al. 2012; Rodríguez-Escriba et al. 2015). Nievola et al. (2005) showed that pineapple plants cultivated in vitro with a diurnal temperature period of 28 °C light/15 °C dark presented CAM metabolism while plants cultivated at constant 28 °C temperature light/dark presented C_3 metabolism. Aragón et al. (2010, 2012) demonstrated that 50 % relative humidity and 260 $\mu\text{mol m}^{-2} \text{s}^{-1}$ induced CAM metabolism compared to an environment with 85 % and 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, that induced C_3 metabolism. According to Freschi et al. (2010) abscisic acid, cytokinins and nitric oxide are related to shift from the C_3 to CAM expression. It has been observed in the pineapple that the transition from the C_3 to CAM metabolism is associated with stressful factors in the environment (Nievola et al. 2005; Freschi et al. 2010; Aragón et al. 2012). Aragón et al. (2012) reported that in vitro pineapple plants induced C_3 metabolism but under *ex vitro*, they can present CAM type metabolism. However, these authors cultivated this species in *ex vitro* conditions under two systems that permitted the two metabolism types (CAM and C_3). CAM metabolism resulted in larger leaf area, greater dry and fresh mass and high ABA concentration compared to C_3 metabolism. In CAM expression these authors reported that in eight weeks *ex vitro* culture, there was greater maximum photosynthesis rate, reduced number of roots and lower leaf length/leaf width ratio. Under the same condition, after 4 weeks of *ex vitro* growth, the stomata conductance was higher. Further, in CAM metabolism, the pineapple plants developed more shoot than root system (Aragón et al. 2012). Thus, studying the type of metabolism of the pineapple plant under acclimatization conditions can help in management strategies and create mechanisms to optimize the physiological processes with possible positive effects on explant growth under *ex vitro* conditions. The objective of the present research was to assess the photosynthetic metabolism and growth of two

important commercial pineapple genotypes, ‘Vitória’ and ‘IAC Fantástico’ during the acclimatization phase.

2 Materials and methods

2.1 Plant material and growth conditions

This *ex vitro* analysis (acclimatization), were used explants of the pineapple cultivars ‘Vitória’ and ‘IAC Fantástico’ after 40 days in vitro culture (micropropagation) with about seven leaves.

Rooting medium for in vitro culture consisted of MS nutrients and the White vitamins (Murashige and Skoog 1962) with 100 mg L⁻¹ mioinositol, 2,7 µmol L⁻¹ naphthaleneacetic acid (ANA), solidified with 8 g L⁻¹ Vetec® bacteriological agar, pH adjusted to 5.7 and autoclaved for 20 min at 1.0 atm and 121 °C.

The explants from the in vitro culture were transplanted in 200-well expanded polystyrene trays with 15 cm³ Vivatto® substrate during the acclimatization and were acclimatized for 75 days.

The explants were irrigated two to three times a day, depending on the substrate moisture. Irrigation was applied until the substrate was saturated. Fifteen days after transplant, all the treatments were fertilized weekly with 5.0 mL of the nutritive solution proposed by Ramos et al. (2011), with the following composition (mg L⁻¹): N(NO₃⁻) = 112; N(NH₄⁺) = 3.5; P = 7.74; K = 156.4; Ca = 80; Mg = 24.3; S = 32.0; Cl = 1.77; Mn = 0.55; Zn = 0.13; Cu = 0.03; Mo = 0.05; B = 0.27; Fe-EDTA = 2.23, pH 5.5.

2.2 Growth, chlorophyll fluorescence, and gas exchange measurements

At 60 and 75 days acclimatization (DA), growth, maximum photochemical efficiency of PSII (Fv/Fm) and photosynthetic carbon assimilation were assessed. Leaf area (LA) was assessed using a LI-3100 leaf area meter (LI-COR, Lincoln, NE, USA). The root dry mass (RDM), shoot dry mass (SDM) and total dry mass (RDM + SDM) (TDM) were measured. Shoots and roots were then separately placed in paper bags, labeled, and placed in a forced air circulation oven at 70 °C for 72 h. At 60 and 75 DA, the canopy diameter (CD) and canopy height (CH) were assessed using a millimeter ruler.

The photosynthetic carbon assimilation was measured with a portable photosynthesis system (LI-6200, LI-COR, USA). The measurements were performed on the central part of the most developed leaf on each explant, between 7:00 am and 10:00 am, at mean light intensities of 1170 µmol m⁻² s⁻¹ (60 days) and 1400 µmol m⁻² s⁻¹ (75 days). At 75 DA, measurements were taken at night on the same leaves used in the daytime measurements at 8:30 pm and 11:30 pm. The chlorophyll fluorescence was assessed using a pocket PEA fluorimeter (Hansatech, England) on the same leaves as the photosynthetic carbon assimilation. The environmental conditions in the greenhouse such as temperature, photosynthetic active radiation (PAR), and relative humidity were monitored and the data collected at 30 min intervals throughout the experiment (Fig. 1). The Data Logger HOBO® (Spectrum Technologies, USA) was used to assess temperature and relative humidity and Data Logger WatchDog® was used to measure PAR (Spectrum Technologies, USA).

The data were submitted to analysis of variance, and the means for the interaction were compared by Tukey test at 5 % probability. The statistical analyses were made using the software SAS® (Statistical Analysis System 2003).

3 Results

During the experimental *ex vitro* condition, microclimatological variables such as air vapor pressure deficit (VPD_{air}), air temperature, relative humidity fluctuated widely (Fig. 1). Maximum temperature was 45 °C, and the value of the VPD_{ar}, maximum PAR were 8 kPa and 1500 µmol m⁻² s⁻¹, respectively. Minimum relative humidity was 20 %. For example, in one day temperature amplitude was observed of up to 25 °C, relative humidity amplitude of 80 % and VPD_{air} amplitude of up to 7 kPa (Fig. 1).

On the assessment dates (60 and 75 DA), except for the RDM/SDM at 60 DA, growth variables were higher for the ‘Vitória’ genotype (Table 1), since the explants of this genotype from in vitro culture presented greater growth (Couto et al. 2014). ‘Vitória’ genotype had highest values of the canopy diameter (CD), canopy height (CH), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM) and the RDM/SDM ratio at 75 DA (Table 1). However,

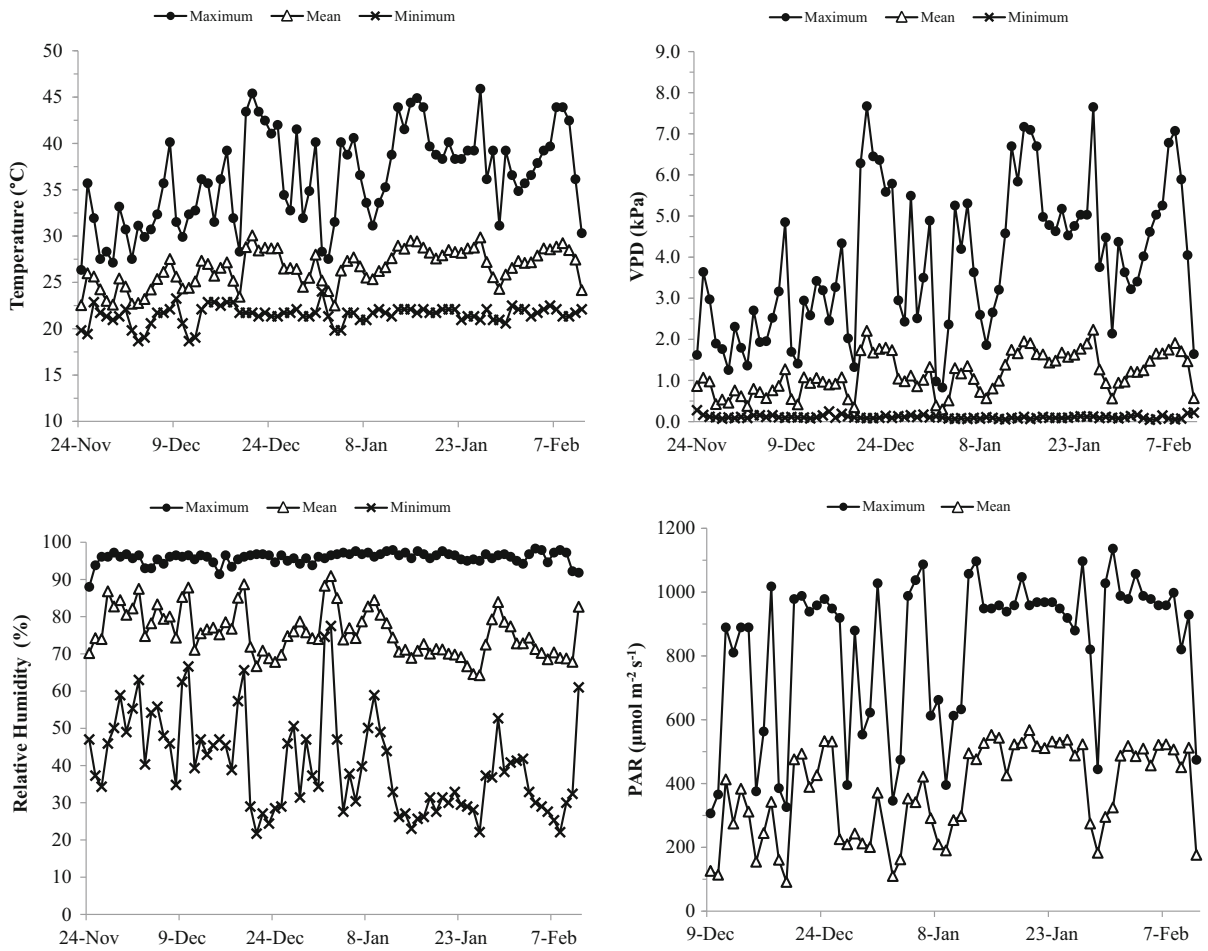


Fig. 1 Maximum, mean and minimum temperature, relative humidity, photosynthetic active radiation (PAR) and air vapor pressure deficit (VPD) inside the greenhouse. The *dots* on the *curves* represent the daily mean over time

Table 1 Canopy diameter (CD), canopy height (CH), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), root dry mass and shoot dry mass ratio (RDM/SDM), leaf area

(LA) and maximum photochemical efficiency of PSII (Fv/Fm) 'Vitória' and 'IAC Fantástico' pineapple cultivars at 60 and 75 days acclimatization

	IAC 'Fantástico'		Change (%)	Vitória		Change (%)
	60	75		60	75	
CD (mm)	94.3b	105.9b	12	117.5a	130.3a	11
CH (mm)	35.2b	44.1b	25	50.1a	59.5a	19
SDM (g)	0.204b	0.332b	62	0.502a	0.543a	8
RDM (g)	0.037b	0.067b	79	0.102a	0.247a	140
TDM (g)	0.242b	0.399b	65	0.605a	0.790a	30
RDM/SDM	0.183a	0.202b	10	0.204a	0.455a	122
LA (cm ²)	38.6b	54.7b	42	71.2a	87.4a	23
F _v /F _m	0.72b	0.75b	3	0.76a	0.77a	1.3

Means followed by the same letter did not differ statistically by the Tukey test at 5 % probability

Percentage of change (increase) between 60 and 75 DA are indicated in bold

although the ‘Vitória’ genotype presented greater values for the growth variables at 75 DA than at 60 DA, ‘IAC Fantástico’ genotype had a greater increase with 25 % in CH, 62 % in the SDM, 79 % in the RDM, 65 % in the TDM, 42 % in the LA and 707 % in the respiratory rate in the morning (Table 1; Fig. 2). For these variables, the ‘Vitória’ genotype had an increase of 11 % (CD), 19 % (CH), 8 % (SDM), 30.5 % (TDM), 22.8 % (LA) and a 143 % increase in the carbon assimilation metabolism (Table 1; Fig. 2). ‘Vitória’ genotype at 75 DA had 140 % increases in the RDM, 122 % in RDM/SDM ratio and 4.5 % and in Fv/Fm ratio (Table 1).

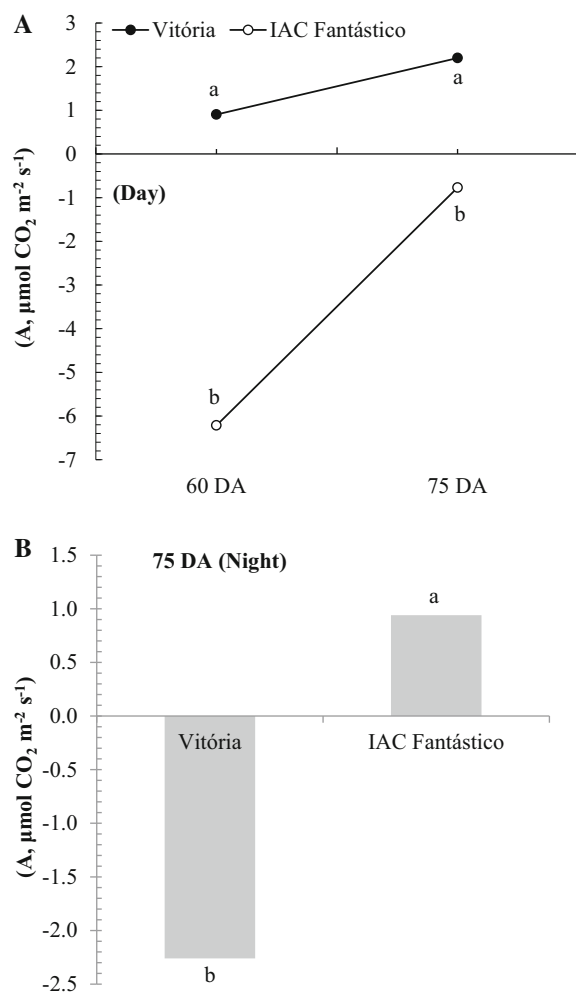


Fig. 2 Net photosynthesis rate (A) from 7 a.m. to 10 a.m. (a) and from 8:30 pm to 11:30 pm; (b) of pineapple cultivars ‘Vitória’ and ‘IAC Fantástico’ at 60 and 75 days acclimatization. Means followed by the same letter did not differ statistically by the Tukey test at 5 % probability

At 75 DA and at night, the ‘IAC Fantástico’ genotype had carbon photosynthesis assimilation of $0.94 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the ‘Vitória’ genotype had a respiratory rate of $2.25 \mu\text{mol m}^{-2} \text{s}^{-1}$. On this date in the day, the plants of the ‘IAC Fantástico’ genotype had a respiratory rate of $0.77 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the ‘Vitória’ genotype had photosynthetic carbon assimilation of $2.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 2).

The plant survival percentage was 100 % in the acclimatization phase (Couto et al. 2014).

4 Discussion

‘Vitória’ genotype presented greater growth compared to the ‘IAC Fantástico’ genotype regardless of the treatments applied during the in vitro culture (Couto et al. 2014). Under this condition, all the parameters analyzed presented significant difference for the genotype factor (Couto et al. 2014). That is, at the start of the present experiment in the acclimatization phase (*ex vitro*), the explants of the ‘Vitória’ genotype presented greater growth compared to the explants of the ‘IAC Fantástico’ genotype (Table 1).

Debergh (1991) stated that light and humidity in the in vitro environment should be carefully monitored for successful acclimatization. The adaptation of these in vitro plants depends on the adjustment of their photosynthesis mechanisms during *ex vitro* conditioning, so that the light is used in the most efficient manner possible. For this reason, the successful adaptation of a species to environments with low or high vapor pressure deficit (VPD_{air}) can be based on the efficaciousness and speed in the allocation patterns and adjusted photosynthesis metabolism to increase resource acquisition in the *ex vitro* environment.

Previous studies on the two pineapple genotypes cultivated in vitro showed that, regardless of the treatments applied ‘Vitória’ had greater growth compared to the ‘IAC Fantástico’ genotype (Couto et al. 2014). Indeed, in the present research, the plants of the ‘Vitória’ genotype started the acclimatization process with higher values in the growth characteristics (Table 1). However, during the 75 days of acclimatization (DA) the ‘IAC Fantástico’ genotype increased the canopy diameter (12 %), canopy height (25 %), canopy dry mass (62 %), root dry mass (79 %), total dry mass (65 %) and leaf area by 42 %. The ‘Vitória’ genotype presented greater increase in root dry mass

(140 %) and in the root dry mass (140 %), canopy dry mass (122 %) ratio, that is, during the acclimatization process, the 'Vitória' genotype allocated more dry mass to the root system. However, this increase in biomass production was not associated with canopy increase, shown by the smaller value of the leaf area in this genotype (23 % increase in this variable, compared to 42 % in the 'IAC Fantástico' genotype).

In the pineapple, leaf mass production per time unit is extremely important to raise the total photosynthesis of the plant because a greater leaf area is associated with CO₂ gas exchanges, oxygen and water in the leaves. In parallel, greater gas exchange promotes greater carbon incorporation that increases plant biomass production. In the present experiment, the greater percentage growth gain of the 'IAC Fantástico' genotype can be associated with CAM metabolism during the acclimatization. 'IAC Fantástico' at 75 DA demonstrated CAM metabolism by respiring CO₂ between 7:00 am and 10:00 am and assimilating CO₂ between 8:30 pm and 11:30 pm and the the PSII photochemical efficiency (Fv/Fm) was not damaged in the plants of this genotype. As reported by Nievola et al. (2005), Freschi et al. (2010) and Aragón et al. (2010) (2012), environmental factors associated with light, temperature and relative humidity can promote CAM expression in the pineapple, because it has a facultative C₃/CAM metabolism (Nievola et al. 2005; Freschi et al., 2010; Aragón et al. 2010, 2012). In the present study, the micrometeorological variables varied greatly in the greenhouse during the 75 days of acclimatization (Fig. 1). Thus the environmental conditions in the greenhouse during the acclimatization, such as high day temperature, PAR of over 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$, low relative humidity and wide amplitude in the VPD values, may have stressed 'IAC Fantástico' genotype and induced CAM metabolism.

The variability in the micrometeorological values led to an alteration in the type of metabolism in 'IAC Fantástico', so that it demonstrated CAM, which was not observed in 'Vitória'. Between 60 and 75 DA, in the morning, 'IAC Fantástico' reduced CO₂ release from 6.6 $\mu\text{mol m}^{-2}\text{s}^{-1}$ to 0.77 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (706 % reduction) while 'Vitória' genotype increased CO₂ assimilation by 142 %. In the same period, at night, 'IAC Fantástico' assimilated 0.94 $\mu\text{mol m}^{-2}\text{s}^{-1}$ while 'Vitória' demonstrated respiratory carbon loss. At 75 DA, 'Vitória' released a greater quantity of CO₂ at night and assimilated less during the day, while

'IAC Fantástico' released less in the day and assimilated a greater quantity of CO₂ at night. Thus 'IAC Fantástico' had a greater net carbon gain that resulted in greater biomass gain between 60 and 70 DA.

According to Carr (2012), this type of CAM metabolism allows the pineapple to adapt in low rainfall areas and leads to more efficient water use when the species experiences a water shortage. Even when irrigating the plants during the 75 days of growth in the acclimatization phase, the intense water demand of the leaves in the 'IAC Fantástico' and 'Vitória' genotypes in the greenhouse that was observed by the VPD values in Fig. 1, was likely not met by the root system that occupied a reduced volume in the culture substrate (15 cm³), even though the 'Vitória' genotype presented greater root dry matter. The greater root dry mass of 'Vitória' visually demonstrated a greater quantity of old, and reduced number of thin roots. Aragón et al. (2012) related that the lower canopy growth and carbon assimilation rates of pineapple plants with C₃ metabolism can be compensated by an increase in root development and represent a crucial factor to the final yield of plants with this type of metabolism. However, Richards and Rowe (1977) showed that reduced root volumes restricted the physical space for new thin roots that have greater water and mineral nutrient absorption and phytohormone production. The old roots absorb less water and mineral nutrients because there is greater endoderm and exoderm formation in the root cortex.

The intense oscillation in the micrometeorological variables resulted in the induction of CAM metabolism in 'IAC Fantástico' but did not divert the metabolism to CAM in 'Vitória' plants so they maintained C₃ metabolism. The degree of maturity of the 'IAC Fantástico' tissue meant that the young pineapple plants had metabolic plasticity and alternated between the C₃ and CAM metabolisms. Depending on the environmental conditions, there is the genetic factor of each cultivar, that will influence in the predisposition to stress due to adverse environmental conditions and consequently there will be interference at the level of expression of the CAM metabolism (Winter et al. 2008). Thus future studies should elucidate the permanence of the C₃ metabolism in the 'Vitória' genotype under conditions of fluctuation in environmental factors such as light, temperature and air water availability, as was verified in the present experiment.

Aragón et al. (2012) reported that when pineapple plants present CAM metabolism *ex vitro*, they have greater leaf area, greater dry and total fresh mass and high ABA concentration. In the same study, Aragón et al. (2012) observed that after eight weeks of *ex vitro* culture, pineapple plants with CAM metabolism had reduced number roots, greater leaf area and a lower leaf length/width ratio. In CAM metabolism, pineapple plants partition more dry matter to the canopy than the root system (Aragón et al. 2012). We confirm these results in the present study. ‘IAC Fantástico’ demonstrated CAM metabolism and had lower root and greater canopy production compared to ‘Vitória’ that did not induce CAM. This indicates that in pineapple, CAM metabolism alters biomass allocation with greater investment in the canopy than in the root system.

The greater growth rate from 60 to 75 DA (canopy diameter and height, shoot dry mass, total dry mass and leaf area) of ‘IAC Fantástico’ was associated with CAM metabolism. CAM-induced plants were switched by stressful environmental micrometeorological variables (Fig. 1). The ‘Vitória’ genotype exhibited C₃ metabolism that reduced growth between 60 and 75 days of acclimatization compared to ‘IAC Fantástico’, although ‘Vitória’ genotype had greater dry matter partitioning to the root system and high photochemical efficiency 75 days after acclimatization. We conclude that the stress imposed by the environmental conditions induced CAM metabolism in ‘IAC Fantástico’ pineapple genotype and increased growth characteristics in greenhouse conditions.

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References

- Aragón CE, Carvalho L, González J, Escalona M, Amâncio S (2010) *Ex vitro* acclimatization of plantain plantlets micropropagated in temporary immersion bioreactor. *Biol Plant* 54:237–244. doi:10.1007/s10535-010-0042-y
- Aragón CE, Carvalho L, González J, Escalona M, Amâncio S (2012) The physiology of *ex vitro* pineapple (*Ananas*

- comosus* L. Merr. var MD-2) as CAM or C₃ is regulated by the environmental conditions. *Plant Cell Rep* 31:757–769. doi:10.1007/s00299-011-1195-7
- Bartholomew DP, Kadzimin SB (1977) Pineapple. In: Alvim PT, Kozłowski TT (eds) *Ecophysiology of tropical crops*. Academic, New York, pp 113–156
- Carr MKV (2012) The water relations and irrigation requirements of pineapple (*Ananas comosus* ver. *comosus*): a review. *Exp Agric* 48(4):488–501
- Couto TR, Silva JR, Torres Netto A, Carvalho VS, Camostrini E (2014) Photosynthetic efficiency and genotypes growth of pineapple cultivated in vitro in different qualities of light, growing jar types and concentration of sucrose. *Rev Bras Frutic* 36(2):459–466. doi:10.1590/0100-2945-167/13
- Debergh PC (1991) Acclimatization techniques of plants from in vitro. *Acta Hort* 289:291–300
- FAO FAOSTAT (2015) Agricultural statistics database. Home: world agricultural information center. Disponível em: <http://apps.fao.org>
- Freschi L, Rodrigues MA, Domingues DS, Purgatto E, Sluys MA, Magalhães JR, Kaiser WM, Mercier H (2010) Nitric oxide mediates the hormonal control of crassulacean acid metabolism expression in young pineapple plants. *Plant Physiol* 154(4):1971–1985. doi:10.1104/pp.109.151613
- Institute SAS (2003) *Sas user’s guide: statistics*. SAS Institute, Cary
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
- Nievola CC, Kraus JE, Freschi L, Souza BM, Mercier H (2005) Temperature determines the occurrence of CAM or C₃ photosynthesis in pineapple plantlets grown in vitro. *Vitro Cell Dev Biol Plant* 41:832–837. doi:10.1079/IVP2005694
- Ramos MJ, Monnerat PH, Pinhol LGR, Silva JA (2011) Macronutrients and boron deficiency in “Imperial” pineapple: mineral composition. *Rev Bras Frutic* 33(1):261–271. doi:10.1590/S0100-29452011005000032
- Richards D, Rowe RN (1977) Effects of root restriction, root pruning and 6-benzylaminopurine on the growth of peach seedlings. *Ann Bot* 41:729–740
- Rodríguez-Escriba RC, Rodríguez R, López D, Lorente GY, Pino Y, Aragón CE, Garza Y, Podestá FE, González-Olmedo JL (2015) High light intensity increases the CAM expression in “MD-2” micro-propagated pineapple plants at the end of the acclimatization stage. *Am J Plant Sci* 6:3109–3118. doi:10.4236/ajps.2015.619303
- Winter K, Garcia M, Holtum JAM (2008) On the nature of facultative and constitutive CAM: environmental and developmental control of CAM expression during early growth of *Clusia*, *Kalanchoe*, and *Opuntia*. *J Exp Bot* 59:1829–1840. doi:10.1093/jxb/ern080
- Zhu J, Bartholomew DP, Goldstein G (2005) Photosynthetic gas exchange and water relations during drought in ‘Smooth Cayenne’ pineapple (*Ananas comosus* (L.) Merr.) grown under ambient and elevated CO₂ and three day/night temperatures. *Acta Hort* 666:161–173