

MORPHOPHYSIOLOGICAL ANALYSIS OF PASSION FRUIT PLANTS FROM DIFFERENT PROPAGATION METHODS AND PLANTING SPACING¹

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ABSTRACT – The passion fruit (*Passiflora cincinnata* Mast.) is a perennial and drought resistant species that represents a new alternative crop for small farmers in rainfed conditions. This study aimed to evaluate the vegetative and physiological development of passion fruit plants derived from two propagation methods and grown at varied planting spacing. The experiment was conducted from January to June of 2012, in the Universidade Estadual do Sudoeste da Bahia (State University from Southwestern Bahia), in Brazil. It was carried out in a randomized block design under a 2 x 3 factorial scheme, which consisted of two propagation methods (cutting and seeds) and three planting spacing distances within a row (1.5; 3.0 and 4.0 m), however, at same distance between rows (3.0 m), with four replicates and four plants per plot. Cuttings and seeds were sampled from adult plants pre-selected in native areas from Vitória da Conquista - BA, Brazil. Growth (stem diameter and leaf area) and physiological parameters (leaf chlorophyll content, leaf water potential before dawn, relative water content and leaf gas exchange) were assessed on the 90th, 120th and 150th day after transplanting of seedlings into the field (DAT). Based on our results, we concluded that despite plants propagated via cuttings showed most favorable water status, vegetative growth and photosynthetic capacity were lower whether compared to plants obtained from seeds.

Keywords: *Passiflora cincinnata* Mast. Growth. Photosynthesis. Gas exchange.

MORFOFISIOLOGIA DO MARACUJÁ-DO-MATO SOB DIFERENTES CONDIÇÕES DE PROPAGAÇÃO E ESPAÇAMENTOS

RESUMO – O maracujá-do-mato (*Passiflora cincinnata* Mast.) é uma espécie perene, resistente à seca, podendo representar uma nova alternativa de cultivo para o pequeno agricultor em condições de sequeiro. O presente trabalho teve como objetivo avaliar o desenvolvimento vegetativo e fisiológico de plantas de maracujá-do-mato, sob diferentes condições de propagação e espaçamentos. O experimento foi conduzido no período de janeiro a junho de 2012, na Universidade Estadual do Sudoeste da Bahia, com o delineamento em blocos casualizados, em esquema fatorial 2 x 3, constituído por dois métodos de propagação e três espaçamentos de plantio na linha (3,0 x 1,5; 3,0 x 3,0 e 3,0x 4,0 m) permanecendo constantes as distâncias entre as linhas de plantio de 3,0 m, com quatro repetições e quatro plantas por parcela. As estacas e sementes foram coletadas de plantas adultas selecionadas em áreas nativas de Vitória da Conquista-BA. Foram realizadas avaliações de crescimento (diâmetro do caule e área foliar) e fisiológicas (teor de clorofila na folha, potencial hídrico foliar antemanhã, teor relativo de água e trocas gasosas foliares) aos 90, 120 e 150 dias após transplante das mudas no campo (DAT). Nas condições em que o trabalho foi conduzido conclui-se que apesar da manutenção de status hídrico mais favorável das plantas propagadas via estaquia, o desenvolvimento vegetativo e a capacidade fotossintética são reduzidas quando comparadas aos de plantas obtidas por meio de sementes.

Palavras-chave: *Passiflora cincinnata* Mast. Crescimento. Fotossíntese. Trocas gasosas.

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¹Received for publication in 09/03/2014; accepted in 01/21/2016.

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INTRODUCTION

Crato passion fruit (*Passiflora cincinnata* Mast.) has great importance as a genetic resource, owning a great production potential because of its adaption under semiarid weather conditions in Northeastern Brazil, besides its fruit nutritional characteristics (SANTOS et al., 2012a).

According to Queiroz (2011), *P. cincinnata* has two main uses, one as a source of pathogen-resistance for same-genus species grown in irrigated fields, and another in farming for fruit production aside from ornamental or medicinal purposes, among which superior types can be grown under rainfed conditions.

According to Araújo (2007), *P. cincinnata* has market potential for being unique due to its distinctive flavor when compared to yellow passion fruit (*P. edulis* Deg.). Santos et al. (2012b) reported this fruit marketed *in natura* in the Municipal Market and Supply Center from Aracaju-SE, Brazil.

Despite the efforts to build a knowledge base on *P. cincinnata*, little information concerning its morphological, reproductive biology, breeding and physiological aspects are available, limiting thus the creation of management strategies for further production chain.

Cutting is one of the vegetative propagation techniques used in passion fruit plants which contributes to uniform orchards, besides mitigating, in a short-term, pathogen symptoms (VAZ et al., 2009) as well as reducing crop earliness (LIRA JÚNIOR, 2012). In contrast, this technique is absent of genetic segregation, which is drawback to passionfruit farming according to Rezende et al. (2005), since high rates of self-incompatibility require maintenance of genetic variability. Because of such limiting factor, passiflora propagation should be carried via seeds, once this type of reproduction would decrease viral infections (CORREA et al.,

2010) and promote a less complex and low-cost seedling production.

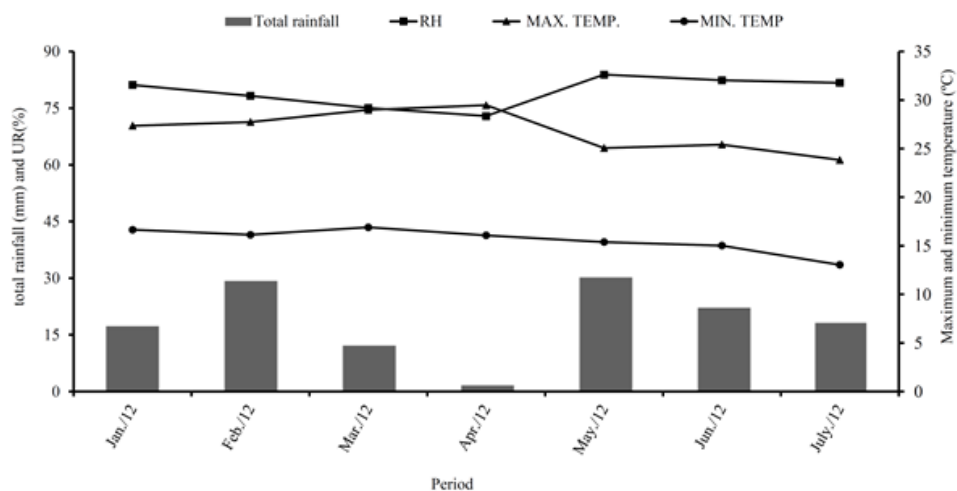
The seminiferous spread of *P. cincinnata* becomes unfeasible since this species has low germination rate, whose cause derives from its seed dormancy. According to Meletti et al. (2002), seed storage for more than two years plus heat treatment are ways of reaching acceptable rates of germination. In addition, pretreating seeds stored for one year with regulators GA4 +7+N-(phenyl-methyl)-aminopurine is also effective for raising the emergency *P. cincinnata* seeds (ZUCARELI et al., 2009).

The information on propagation methods and crop management are important to widen the knowledge on initial vegetative development of this species in the field, specially grounded on plant morphological and physiological parameters. Researches have shown that plants with higher initial vegetative vigor provide enhanced production yields.

Given the above, this study's goal was to evaluate the vegetative and physiological development of passion fruit plants (*P. cincinnata* Mast.) under varied propagation methods and planting spacing.

MATERIAL AND METHODS

The experiment was carried out at the Universidade Estadual do Sudoeste da Bahia (State University from Southwestern Bahia) from January to June of 2012, in Vitoria da Conquista, Bahia state, Brazil. The area is located at 14° 53' South latitude and 40° 48' West longitude and at an altitude of 941 meters. Local annual average temperatures vary between 19.5 to 20.5 °C (SANTOS et al., 2011). Figure 1 shows the total rainfall, relative humidity as well as maximum and minimum monthly average temperatures during the experimental period.



Source: National Institute of Meteorology-INMET

Figure 1. Monthly average values of total rainfall (mm), maximum and minimum temperature (°C) and relative humidity (RH%) within the experimental period.

Passiflora cincinnata Mast. fruit were sampled from Vitória da Conquista-BA to produce seed-grown seedlings. First, we removed the seed mucilage using tap water and manual rubbing against a plastic mesh sieve, being placed to shade dry on paper towels for 72 hours and then being stored at 10 °C. At sowing time, seeds were heated in water bath for 5 minutes at 50 °C to break dormancy (OLIVEIRA JR et al., 2010).

We selected mother-plants in native areas from Vitoria da Conquista to produce the cutting-grown seedlings. From these matrices, we removed herbaceous branches from plant middle part. Cuttings were, on average, 15 cm long and with 4.5 mm diameter, containing two to three internodes and a half leaf (SANTOS et al., 2012a). The cuttings were dipped in indole-butyric acid (IBA) at 1,000 ppm for 10 seconds, and immediately planted. Seedlings propagated by both seed and cutting were grown in polythene bags with dimensions of 11x18 cm, filled with substrate constituted by agricultural soil and cow manure (200 L/ m³), which had been submitted to solarization for one week covered by clear plastic. The seedlings were grown in a greenhouse with transparent plastic cover and shade cloth sides (50% light restriction), being irrigated manually every day.

A randomized block design was adopted with a 2 x 3 factorial scheme, consisting of two propagation methods and three planting spacing within a row (1.5, 3.0 and 4.0 m) at same row distance (3 m), four replications and four plants per plot; only the two central plants were evaluated. Cropped lines were remained around the experimental area to avoid external interferences.

Seed- and cutting-grown seedlings were selected for planting when they had five to six leaf pairs and at the appearance of the first tendril. The area was previously plowed and harrowed; rows were furrowed out, having about 0.40 m depth and 0.30 m width. Liming was carried out throughout the area on the basis of soil analysis (Table 1) and using limestone with 29% CaO, 21% MgO and a PRNT at 90.5%, aiming to raise soil base saturation up to 80%, as recommended for *P. edulis* (CATI, 1992). Phosphate, nitrogen and potassic fertilizations followed recommendations proposed by Souza et al. (1999) for *P. edulis*. At planting time, fertilizer was added together with 10 L hardened cattle manure per plant, plus 50 g of micronutrients in chip form - FTEBR 12. Seedlings were placed above soil level and had their lap covered by soil, aiming to retain water. Irrigation was performed throughout the experiment by manual watering twice a week applying five liters of water per plant.

Table 1. Soil chemical analysis of the experimental area at a depth of 0-20 cm. UESB.

pH	P	K ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺	H	BS	t	T	V	m	OM
H ₂ O	mg dm ⁻³				cmol _c dm ⁻³					%		g Kg ⁻¹
5.0	1	0.13	1.6	0.7	0.2	3.2	2.4	2.6	5.8	42	8	19

Source: Soil Laboratory of the Universidade Estadual do Sudoeste da Bahia.

Seedlings were trained in espalier to a single stem through a string attached from soil up to a 2-m high trellis wire. Thinning pruning was carried to remove side shoots (suckers) and lopping at trellis wire line. In addition, two lateral branches were trained in opposite directions, growing curtain-shaped tertiary and quaternary branches at regular growth until plant formation.

At 90 (April / 2012), 120 (May / 2012) and 150 (June / 2012) days after transplanting the seedlings to the field (DAT), we evaluated stem diameter (SD), leaf area (LA), leaf chlorophyll content (SPAD), leaf water potential (Ψ_w), relative water content (RWC). We also measured leaf gas exchange, stomatal conductance (Gs), transpiration (T), carboxylation efficiency (A/C_i) and net photosynthesis (A_{net}). The SD was measured by caliper at 15 cm above ground. For foliar analysis, two fully expanded leaves were collected from first order stems on the 90th DAT (primary sector), from second order on the 120th DAT (secondary branches) and from third order on the 150th DAT (tertiary branches). The analysis was performed with

LI-3100C area meter (LIQUOR, Inc., Lincoln, Neb., USA) and expressed in cm². Chlorophyll content analysis was done by chlorophyll meter in fully expanded mature leaves taken from the primary (90 DAT), secondary (120 DAT) and tertiary (150 days DAT) branches. The same leaves were used for gas exchange measurements, which were performed by means of infrared gas analyzer (IRGA) (model LCpro ADC Bioscientific Ltd., UK), to which was coupled a source of actinic light, resulting in a photosynthetic active radiation of 1,000 $\mu\text{m photons m}^{-2} \text{s}^{-1}$.

Measurements of Ψ_w were taken with the aid of a PMS 1000 pressure pump (PMS Instrument Company, EUA), according to Scholander (1965), at five in the morning, withdrawing two fully expanded leaves from the middle third plant branch.

To determine the RWC, we used the same leaves from Ψ_w measurements. Eight 16-mm diameter leaf discs were cut out, weighed on an analytical scale (fresh mass - FM). Afterwards, they were placed on Petri dishes, submerged in deionized water and taken to refrigerator, where they remained

for 24 hours. Then, water excess was removed with absorbent paper for turgid mass (TM) determination. The discs were again weighed and dried in a forced air circulation oven, remaining at 65 °C for 48 hours until constant weight for dry matter (DM) measurement. RWC was then calculated based on the measurements of fresh mass, turgid mass and dry mass, as described in the following equation: $RWC = \{(FM - DM) / (TM - DM)\} \times 100$.

Data underwent variance analysis and means compared by the F test at 5% probability. The degrees of freedom of the treatments were broken down through mean comparisons using the Tukey's test at 5% probability, with the aid of a System for Statistical and Genetic Analyses (SAEG, 2007).

RESULTS AND DISCUSSION

There was no effect of propagation methods, planting spacing on SPAD index and T. In addition, there was no effect of the interaction between both factors on all variables.

Through Figure 2, we can observe larger values of SD and LA in seedlings propagated via seed compared to cutting-grown ones on the 90th, 120th and 150th DAT in the field. The juvenile period of several fruit species is reduced in propagation by cuttings or grafting (ISUTSA, 2006); therefore, differences in vegetative characteristics were related to youth shortening of cutting-grown seedlings, which resulted in lower vegetative growth compared with seed-grown ones.

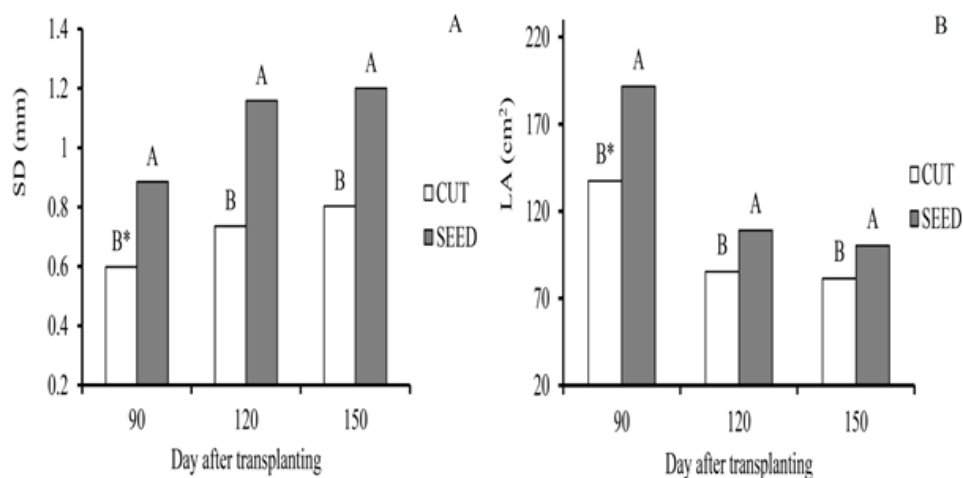


Figure 2. Stem diameter (SD) and leaf area (LA) of *Passiflora cincinnata* Mast. plants propagated by cuttings (CUT) and seeds (SEED). Vitória da Conquista-BA 2012. *Different letters differ from each other by the Tukey's test at 5% probability.

Planting spacing had effect only on Ψ_w modulus on the 90th DAT, observing smaller values of Ψ_w for plants grown at farther spacing, on the 90th DAT. However, in subsequent evaluations (120th and 150th DAT), larger spacing had no effect on that variable (Figure 3A).

Spacing effect verified on the 90th DAT was related to a low precipitation rate within this period, as well as to a lower root absorptive capacity during initial development stage. In this context, on the 90th DAT, the lowest volume of available soil, given the narrowest spacing between rows, was associated with less water availability (MCCLYMONT et al., 2006). Another important consideration regarding plant vegetative stage, when there is leaf expansion and formation, is that these are key processes strongly sensitive to water restrictions if compared to the flowering and fruiting stages (CARR, 2013).

The spacing had no effect on Ψ_w on the 120th and 150th DAT. This has been associated to root vigor and hydraulic redistribution within the soil. Prieto et al. (2012) defined hydraulic redistribution as a passive movement of water throughout the soil and root system; such movement is guided by water

potential gradient at the plant-soil interface. Thus, a more intense hydraulic redistribution, induced by a root system with greater soil exploration capacity, even in closer planting spacing, could contribute to define maintenance of water status of plant shoot.

Nonetheless, water availability limitations may result in plant growth and vigor reductions without affecting parameters related to leaf water status or photosynthesis (TURNER et al., 1996). Srinivas et al. (2010) found out that despite the fertigation has resulted in increase in number and yield of *P. edulis*, the Ψ_w modulus indexes were elevated compared to the control. Earliness and increased sensitivity to water stress for cell elongation in relation to photosynthesis in the leaves occur across the board for plants, as described by Taiz and Zeiger (2013).

When comparing both propagation methods, Ψ_w obtained higher values for seed-grown plants on the 90th and 150th DAT, with no differences observed on the 120th DAT (Figure 3B). In this development stage, despite the maintenance of a larger LA for sexually propagated plants in detriment of cutting-grown seedlings, higher precipitation rates

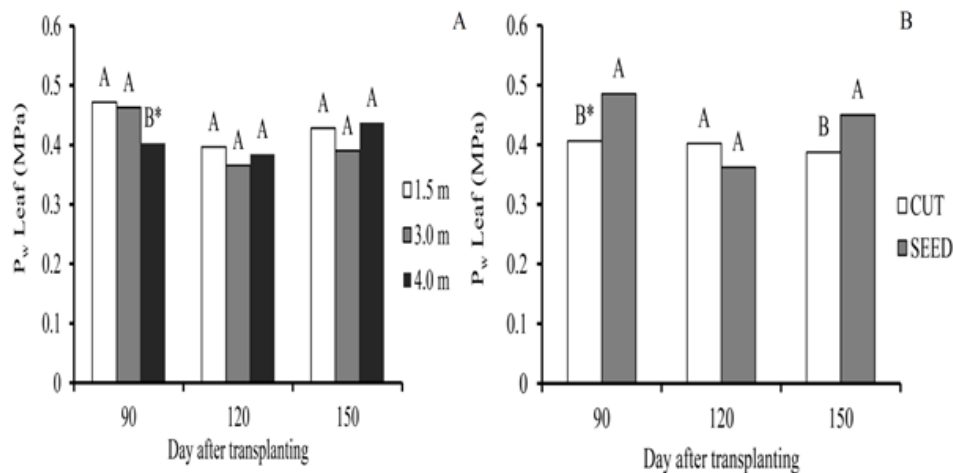


Figure 3. Leaf water potential modulus (P_w) in *Passiflora cincinnata* Mast. plants at different spacing (A) and propagation methods (cutting – CUT or seeds - SEED) (B). Vitória da Conquista-BA, 2012. *Different letters differ from each other by the Tukey's test at 5% probability.

(March of 2012) might have favored water availability and extinguished the changes in Ψ_w .

In June, on the 150th DAT evaluation, rainfall rate continued above than those measured on the 90th DAT. However, the values of Ψ_w in seed propagated plants were higher than the others due to achievement of reproductive stage, in which crop coefficient is increased by higher evapotranspiration (CARR, 2013).

According to studies carried by Souza et al. (2009) in Vale do Curu-CE, there was a higher requirement of water flowering-fruit stage of *P. edulis*. For the present study, on the 90th and 150th DAT, although the earliness of reproductive phase for cutting seedlings, the highest absolute values of Ψ_w before dawn were observed in plants grown from seeds due to their higher vegetative vigor derived from a larger LA (Figure 3B).

A positive correlation between LA and T (Pearson correlation = 0.6887 at 1% probability) indicates that plant internal water status became less favorable for seed-grown plants, since they have larger LAs that promotes more water vapor exchanges between plant and atmosphere. Such vapor loss to atmosphere can be maximized with stomatal density and by inducing stomata opening through relative humidity decreases. Sánchez et al. (2013) verified the stomatal density in leaves of *P. edulis* is of 106.53 stomata per millimeter square, leading to a negative correlation between stomatal opening and relative humidity.

Even though propagation method had no effect on T, Rodriguez-Garmin et al. (2010) pointed out that citrus seedlings with greater relations between shoot and root weights showed a reduced T. When related to total LA, we could observe the elevated impact of plant shoot biomass on T capacity of the seedlings.

On the 90th DAT, larger leaf RWC values were observed in plants propagated via cuttings

compared to plants grown from seeds (Figure 4A). These higher values were related to lower levels of Ψ_w , reaching values approximately five times lower than those observed by Srinivas et al. (2010) were. Studying water replacement proportional to evaporation and fertigation in plants of *P. edulis*, these authors found higher turgidity in leaf tissues (higher RWCs) with higher values of Ψ_w .

Gomes et al. (2012), studying *P. edulis* plants under water stress, verified a variation of Ψ_w from 0.16 to 3.25 MPa between control and plants under stress, respectively. This contrast consisted of a factor to be considered in future studies regarding the ability of *P. cincinnata* to withstand water stress when compared to *P. edulis*, concerning osmotic adjustment and hydraulic conductivity between root and shoot.

Higher values of A_{net} were observed on the 90th DAP in plants of *P. cincinnata* Mast. originated from seeds in comparison with plants propagated through cuttings (Figure 4B). Gama et al. (2013), studying five cultivars of *P. edulis* plant, observed values from 10.86 to 18.07 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Yet Vasconcellos and Martelleto (2012) verified that A_{net} levels of approximately, 13.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *P. cincinnata*.

There was no significant effect of Gs on *P. cincinnata* plants grown from seeds when compared to plants from cuttings (Figure 5A). However, there was a performance pattern on the 90th and the 150th DAT, being similar to those observed for Ψ_w . In studies made with grapevine, McClymont et al. (2006) verified that increased values of Gs and Ψ_w was associated with a larger volume of soil available for plants. Since the fibrous root system of plants propagated via cutting usually reaches only the superficial soil layers, these plants have less capacity of intercepting water when compared to the absorption range of a taproot system, which is characteristic of the plants obtained from seeds.

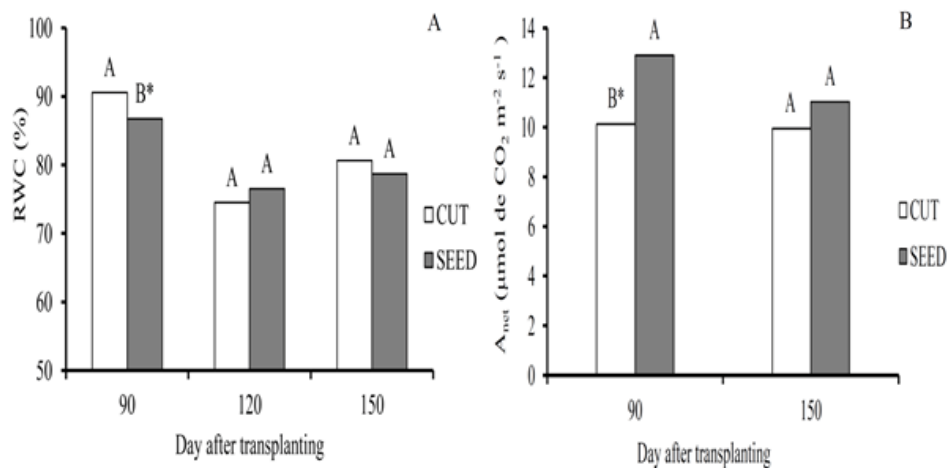


Figure 4. Leaf relative water content (RWC) and net photosynthesis (A_{net}) of *Passiflora cincinnata* Mast. plants grown from cuttings (CUT) and from seeds (SEED). Vitória da Conquista-BA, 2012. *Different letters differ from each other by the Tukey's test at 5% probability.

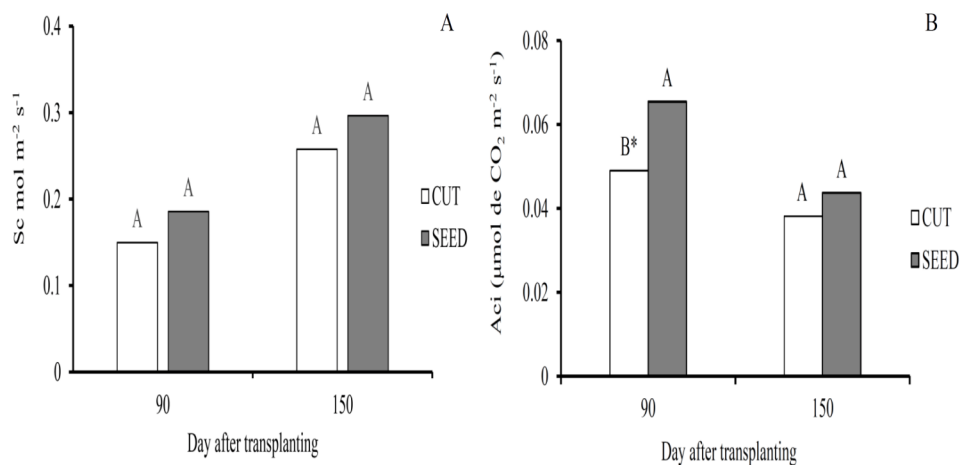


Figure 5. Stomatal conductance (Sc) and carboxylation efficiency (A/C_i) of *Passiflora cincinnata* Mast. plants grown from cuttings (CUT) and from seeds (SEED). Vitória da Conquista-BA, 2012. *Different letters differ from each other by the Tukey's test at 5% probability.

An increased A/C_i was observed in plants grown from seed on the 90th DAT (Figure 5B). The CO_2 concentration within the sub-stomatal chamber (C_i) reflects the termination of CO_2 that is a result of biochemical reactions of such gas incorporation through carboxylation enzymes. Freire et al. (2014) verified reduction in A/C_i due to a decrease in A_{net} and increase in C_i for *P. edulis* plants irrigated with saline water, revealing their limitation on carboxylation capacity compared to plants irrigated with fresh water. In this study, we may highlight that A/C_i is primarily related to A_{net} rate in *P. cincinnata* plants from seeds, as C_i values were similar in all treatments (data not illustrated).

CONCLUSION

Cutting-grown plants had lower vegetative development than those propagated by seeds.

Plants propagated via seeds exhibited higher gas exchange and lower leaf turgidity at the beginning of the vegetative development (90th DAT) compared to plants from cuttings.

Passiflora cincinnata plants spaced by 4 meters had the lowest leaf water potential on the 90th DAT.

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