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Physiological Parameters and Development of Passion Fruit Subjected to Water Stress and Propagation Methods

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HIGHLIGHTS

- Irrigation intervals over 8 days compromise the physiology of passion fruit.
- Passion fruit seedlings grafted into seedlings obtained by cuttings or seeds have greater vigor.
- Passion fruit seedlings propagated by seeds show greater efficiency in the use of water.
- Propagation methods influence the development of passion fruit.

Abstract: The aim of this work was to evaluate the physiological parameters and development of passion fruit submitted to water stress and propagation methods. The experiment was conducted in a completely randomized design (DIC), in a 3x3 factorial arrangement, using methods of propagation (PM) of passion fruit, which are: the species *Passiflora edulis* propagated with seeds (PS); *Passiflora edulis* grafted on the *Passiflora gibertii* species propagated by seed (E x PS) and *Passiflora edulis* grafted on the *Passiflora gibertii* species propagated by cuttings (E x PE) and interacting with three irrigation intervals (I), which are irrigated with intervals of 4, 8 and 12 days, with four repetitions. The suspension of irrigations for eight days reduces the rate of CO₂ assimilation, transpiration and stomatal conductance in passion fruit seedlings propagated by seeds and grafting. Passion fruit plants propagated by seeds show greater efficiency in the use of water than those grafted on *P. gibertii*. Passion fruit seedlings grafted onto seedlings obtained by cuttings or seeds have vigor to be propagated commercially.

Keywords: Passion fruit; *Passiflora edulis*; *Passiflora gibertii*; irrigation; grafting.

INTRODUCTION

Brazil is the world's largest producer and consumer of passion fruit [1], however, factors such as water stress, nutritional deficiencies [2], phytosanitary problems and inadequate cultivation techniques [3] result in a productivity of 14.1 t ha⁻¹ which can be considered low for the culture. Soft moisture stress levels can already limit the vegetative growth and production of the passion fruit, which should maintain the moisture profile close to its field capacity [4].

One way to increase the tolerance to water stress in passion fruit is to use grafting as a propagation form, which can also be used to increase resistance to diseases caused by pathogens that live in the soil, increasing the useful life of the orchard [5]. The use of wild passion fruit species as rootstock has been successfully adopted in recent years, becoming an alternative for producers in commercial areas [6,7].

Some grafting techniques were shown to be viable for the formation of yellow passion fruit seedlings, aiming to control the premature death of plants, being able to mention the hypocotyledon grafting by full slit fork [8] and conventional grafting by full slot-type fork [9].

However, the diameter of the very thin stem of some species obtained by seeds and used as rootstocks has hampered the grafting operation, since the diameter of the yellow passion fruit forks has been larger in the nursery stage [10]. *Passiflora edulis* plants grafted on *Passiflora gibertii* showed lower average fruit mass and productivity due to less vigor of the plants [7].

The grafting in seedlings obtained by cuttings, in addition to having a larger diameter, give more uniformity to the seedlings obtained [11]. Thus, the use of vegetative propagation, combining cutting and grafting methods, can be used in the multiplication of yellow passion fruit plants, guaranteeing uniformity and quality to the orchards [12,13].

This work aimed to evaluate the physiological parameters and development of passion fruit submitted to water stress and propagation methods.

MATERIAL AND METHODS

The experiment was carried out from May to July 2019 at the Universidade Estadual Paulista (Unesp), Faculty of Agricultural and Technological Sciences, located in the municipality of Dracena, state of São Paulo and conducted in a greenhouse covered with plastic light diffuser film of 1200 microns, with a right foot of 4.0 meters, with its sides closed with a Sombrite® type screen with 50% light passage.

The experimental design was completely randomized (DIC), in a 3x3 factorial arrangement, using methods of propagation of passion fruit (MP), being: the species *P. edulis* propagated with seeds (PS); *P. edulis* grafted on the species *P. gibertii* propagated by seed (E x PS) and *P. edulis* grafted on the species *P. gibertii* propagated by cuttings (E x PE) and interacting with three irrigation intervals (I), they are irrigated with intervals of 4, 8 and 12 days, with four replications totaling 36 plots.

Each plot was composed of a plant; where the seedlings were obtained in a commercial nursery in the region of the municipality of Adamantina, state of São Paulo and had an average size of 20±3 cm; with 6 ± 1 definitive leaves and aged 60 days. The seedlings were planted in plastic pots with a volumetric capacity of 9.0 dm³ filled with Ferric Red Latosol [14] and had the following chemical attributes as shown in Table 1.

Table 1. Chemical attributes of the soil used in the implementation of the experiment.

pH	OM	P	K	Ca	Mg	SO ₄ ²⁻	H+Al	Al	SB	CEC	V	m
	mg dm ⁻³	--- (mmolc dm ⁻³) ---				mg dm ⁻³	----- (mmolc dm ⁻³) -----				--- (%) ---	
4.5	4.5	6.0	5.6	10	4.0	7.0	18	1.0	18.6	36.6	46.4	6.0

* OM - Organic matter, SB - Sum of bases, CTC - Cation exchange capacity, V - Base saturation and m - Aluminum saturation.

The soil was corrected and fertilized according to the requirements of the crop and was irrigated to determine the field capacity, where it was saturated and allowed to drain, naturally. The estimates of evapotranspiration and volume of water to be replaced in the irrigation intervals, were determined according to the methodology described by [15].

At 60 days after the beginning of the experiment, the values of CO₂ assimilation rate, expressed by area (A – μmol CO₂ m⁻² s⁻¹), transpiration (E – mmol H₂O m⁻² s⁻¹), stomatal conductance (Gs – mol H₂O m⁻² s⁻¹), the internal CO₂ concentration in the substomatic camera (Ci – μmol mol⁻¹), o efficient use of water (EUW – mol CO₂ mol H₂O⁻¹), determined through the formula:

$$EUW = \frac{A}{E}$$

Where a portable gas exchange device was used Infra-Red Gas Analyzer - IRGA, marca ADC BioScientific Ltd, model LC-Pro, where five measurements were made per plant and each plot was represented by the average. The following variables were also determined: the plant height (PH) through the use of a ruler graduated in millimeters; the diameter of the stem (DS) measured at 1.0 cm from the stem of the plant, with the aid of a 0.1 mm precision digital caliper, the number of leaves (NL) determined by direct counting at the plant, the dry mass of the aerial part (DMAP) and the dry mass of the root (DMR) where all the material developed was dried in an oven with circulation and air renewal at 65°C until they reach constant weight.

All variables were subjected to normality tests, where the Shapiro-Wilk, after meeting the precepts of the test, analysis of variance was performed using the F test ($p < 0.05$) and their averages compared by the Tukey test at 5% probability [16], Pearson's correlation was also performed where the statistical program was used RStudio [17].

RESULTS AND DISCUSSION

There were no differences between the different types of propagation for CO₂ assimilation rates as shown in Table 2. Passion fruit plants irrigated at four-day intervals had higher CO₂ assimilation rates than plants irrigated every eight and twelve days. These results agree with those of [18], when they found that six days after the suspension of irrigation, there was a decrease in the availability of water in the soil, causing a reduction in the water potential of the soil, in the water potential of the leaves and in the photosynthetic rate of passion fruit plants propagated by seeds and grafted. The results also corroborate those of [19] who verified that with the suspension of irrigation for seven days in yellow passion fruit plants inoculated with arbuscular mycorrhizal fungi, interfered with diffusive resistance and leaf temperature in non-inoculated passion fruit seedlings.

Table 2. Average values of CO₂ assimilation rate (A – $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration (E – $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance (GS – $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and internal CO₂ concentration in the substomatic camera (Ci – $\mu\text{mol mol}^{-1}$) and efficient use of water (EUW – $\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$) of passion fruit grafted plants when grown at different irrigation intervals. Dracena, 2019.

A – $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$				
	4 days	8 days	12 days	Mean (PM)
PS	6.43	6.94	3.00	5.46
E x PS	6.84	2.80	1.52	3.72
E x PE	6.40	3.41	2.44	4.08
Mean (I)	6.56 a	4.38 b	2.32 b	
	DMS: 2.15	CV(%): 52.50	MG: 4.33	
	F (PM): 2.21 ns	F (I): 11.81 **	F (PMxl): 1.36 ns	
E – $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$				
	4 days	8 days	12 days	Mean (PM)
PS	3.83	3.20	1.87	2.97
E x PS	4.80	1.57	2.43	2.94
E x PE	4.73	2.33	1.89	2.98
Mean (I)	4.46 A	2.37 B	2.06 B	
	DMS: 1.17	CV(%): 39.31	MG: 2.96	
	F (PM): 0.005 ns	F (I): 14.96 **	F (PMxl): 1.55 ns	
GS – $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$				
	4 days	8 days	12 days	Mean (PM)
PS	0.11	0.08	0.04	0.08
E x PS	0.13	0.03	0.06	0.07
E x PE	0.12	0.04	0.03	0.07
Mean (I)	0.12 A	0.05 B	0.04 B	
	DMS: 0.03	CV(%): 47.65	MG: 0.07	
	F (PM): 0.34 ns	F (I): 15.21 **	F (PMxl): 1.34 ns	

Cont. Table 2

Ci – $\mu\text{mol mol}^{-1}$				
	4 days	8 days	12 days	Mean (PM)
PS	289.41	246.00	261.66	265.69
E x PS	287.33	353.83	329.58	323.58
E x PE	287.00	280.33	280.33	282.55
Mean (I)	287.91	293.38	290.52	
	DMS: 73.13	CV(%): 24.89	OA: 290.61	
	F (PM): 2.03 ns	F (I): 0.01 ns	F (PMxl): 0.61 ns	
EUW				
	4 days	8 days	12 days	Mean (PM)
PS	1.67	2.22	1.68	1.86 a
E x PS	1.42	1.99	0.72	1.38 ab
E x PE	1.30	1.35	1.30	1.32 b
Mean (I)	1.47 AB	1.85 A	1.23 B	
	DMS: 0.52	CV(%): 33.95	OA: 1.52	
	F (PM): 3.91 *	F (I): 4.41 *	F (PMxl): 1.58 ns	

** - significant at the level of 1% probability ($p < 0.01$); * - significant at the level of 5% probability ($0.01 \leq p < 0.05$); ns - not significant ($p \geq 0.05$). Same lowercase letters in the column do not differ statistically. Same capital letters on the line do not differ statistically. The Tukey test was applied at the level of 5% probability. PS – propagated by seed; E x PS – grafted onto seed-propagated plants e E x PE – grafted on plants propagated by cutting. AO = overall average. PM = Propagation method e I = Irrigation interval in days.

In the Figure 1 Pearson correlations are presented between the variables analyzed in the grafted passion fruit plants when grown at different irrigation intervals, where significant positive correlations between the CO_2 assimilation rate and transpiration were evidenced (Figure 2A) with one $r = 0.79$ ($p < 0.01$) and with stomatal conductance (Figure 2B) with one $r = 0.79$ ($p < 0.01$) and it also correlated, however, in a negative way with the internal concentration of CO_2 (Figure 2C) with one $r = 0.51$ ($p < 0.01$).

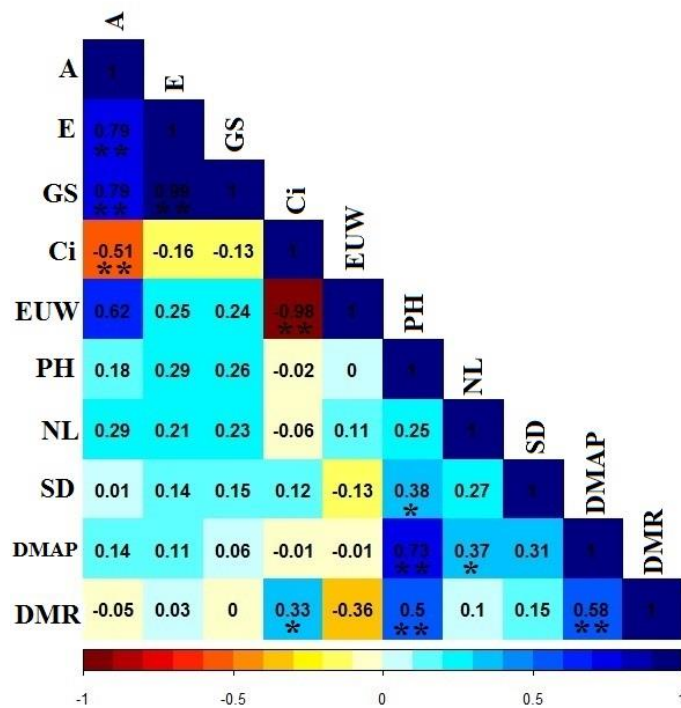


Figure 1. Pearson's correlations between the variables analyzed in grafted passion fruit plants when grown at different irrigation intervals. A = CO_2 assimilation rate, E = transpiration, GS = stomatal conductance, Ci = internal CO_2 concentration in the substomatic camera, EUW = efficient use of water, PH = plant height, NL = number of leaves; DS = diameter of the stem, DMAP = dry mass of the aerial part e DMR = dry mass of the root. **($p < 0.01$) e *(<0.05). Dracena, 2019.

As transpiration and stomatal conductance increase, there is an increase in the rate of CO₂ assimilation, proving that the stomata were active which enabled gas exchange with the environment, which allows a higher photosynthetic rate of the plant even under water stress. However, this efficiency can be compromised with a water restriction for long periods, as it compromises the photolysis of water in the oxygen evolution complex in photosystem II (PSII) and the release of NADH, for the subsequent process of photosynthesis [20,21]. In this way, the CO₂ assimilation rate is compromised due to water stress, which starts to compromise the stomatal opening (Figure 2C).

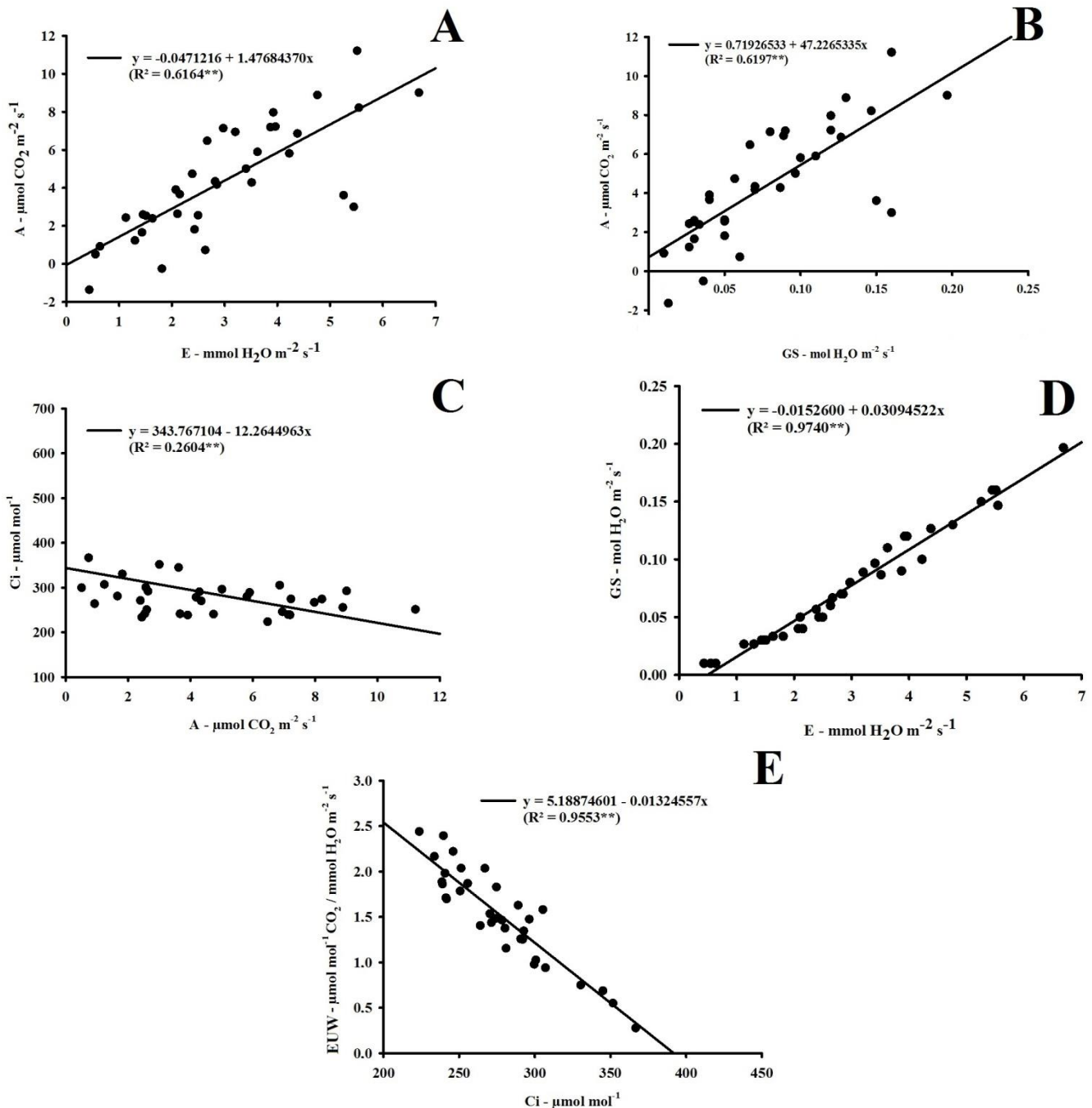


Figure 2. Significant linear regressions after significant Pearson correlations between the physiological variables of the grafted passion fruit plants when grown at different irrigation intervals A = CO₂ assimilation rate, E = transpiration, GS = stomatal conductance, Ci = internal CO₂ concentration in the substomatal camera and EUW = efficient use of water. Dracena, 2019.

The transpiration rates did not differ between the propagation methods, however, plants irrigated at intervals of eight and twelve days had significantly less transpiration than those irrigated every four days. This result agrees with those of [19] when they verified lower rates of transpiration in passion fruit seedlings submitted to water stress. According [22] when plants are under some type of stress they reduce transpiration

and consequently the rate of photosynthesis. Correlating the transpiration data with stomatal conductance, a correlation coefficient of $r = 0.99$ ($p < 0.01$) as shown in Figure 2D. This result was already expected, given that when the stomata are open, there is a greater passage of water through the stomatal cleft, which consequently adds this loss of water to the water transpired by the epidermal cells of the leaf, then it rises. leaf sweating [23].

The different propagation methods did not interfere in the stomatal conductance of yellow passion fruit seedlings, however, plants irrigated at intervals of eight and twelve days had their stomatal conductance affected, differing from those irrigated at intervals of four days. These results agree with those of [18] who verified that water stress reduced the values of stomatal conductance in passion fruit plants propagated by seeds and by grafting. The reduction of stomatal conductance can limit the rate of CO_2 retention and, consequently, the internal concentration of CO_2 decreases in the intercellular spaces due to the consumption of CO_2 by photosynthetic activity [24].

The internal concentration of CO_2 in the substomatic chamber was not affected by the different methods of propagation or by the different water regimes. According [21] the internal concentration of CO_2 is important because the productivity of a plant can be analyzed as the product of intercepted solar energy and CO_2 fixed over a period. The internal concentration of CO_2 was negatively correlated with the efficient use of water, which presented a correlation coefficient of $r = -0.98$ ($p < 0.01$) as shown in Figure 2E. This result was not expected, because with the increase in the internal concentration of CO_2 , it would require a greater amount of water for its fixation, this demonstrates that the water restriction during water stress compromised the fixation of CO_2 in the plant mass [25].

It was found that propagation methods and water regimes influenced the efficiency of water use (Table 2). Plants propagated by seeds had better efficiency in the use of water than those grafted in plants propagated by cuttings. In the formation of passion fruit seedlings by seeds and grafted, irrigation intervals of eight days reduce the rate of CO_2 assimilation, transpiration and stomatal conductance.

The height of yellow passion fruit plants grafted on the species *P. gibertii* propagated by seeds (E x PS) and by stakes (E x PE) were higher than those propagated by seeds (Table 3). [9] did not find differences between plants of *P. edulis* grafted on three different rootstocks (*P. edulis*, *P. alata* and *P. gibertii*). Plants irrigated at four-day intervals (Figure 3A, 3D, 3G) showed higher height than plants irrigated at intervals of eight (Figures 3B, 3E, 3H) and twelve days (Figures 3C, 3F, 3I). Irrigation intervals of eight days are already limiting the development of the passion fruit.



Figure 3. Passion fruit plants grafted in different grafting methods under different irrigation shifts. A – *P. edulis* advertisement with seeds with an interval of 4 days; B – *P. edulis* advertisement with seeds with an interval of 8 days and C – *P. edulis* advertising with seeds with an interval of 12 days; D – *P. edulis* grafted on the species *P. gibertii* propagated by seed with an interval of 4 days, E – *P. edulis* grafted on the species *P. gibertii* propagated by seed with an interval of 8 days; F – *P. edulis* grafted on the species *P. gibertii* propagated by seed with an interval of 12 days; G – *P. edulis* grafted on the species *P. gibertii* propagated by cutting with an interval of 4 days; H – *P. edulis* grafted on the species *P. gibertii* propagated by cutting with an interval of 8 days and I – *P. edulis* grafted on the species *P. gibertii* propagated by cutting with an interval of 12 days. Bar = 30 cm. Dracena, 2019.

There was no influence on the number of leaves for the different methods of propagation and for the different water regimes as shown in the Table 3. This result disagrees with [26] who verified that the increase in water availability resulted in a greater number of sweet passion fruit seedlings obtained by cutting.

Table 3. Average values of plant height (PH), number of leaves (NL); stem diameter (SD), dry mass of aerial part (DMAP) and dry mass of roots (DMR) of grafted passion fruit plants when grown at different irrigation intervals. Dracena, 2019.

PH (cm)				
	4 days	8 days	12 days	Mean (PM)
PS	81.50	52.25	49.75	61.16 b
E x PS	108.12	65.50	56.00	76.54 a
E x ES	87.75	74.50	67.62	76.62 a
Mean (I)	92.45 a	64.08 b	57.79 b	
DMS: 14.45		CV(%): 20.02	OA: 71.44	
F (PM): 4.64 *		F (I): 20.01 **	F (PMxl): 1.60 ns	
NL				
	4 days	8 days	12 days	Mean (PM)
PS	5.75	4.50	4.25	4.83
E x PS	6.00	5.00	1.75	4.25
E x PE	5.25	4.25	4.75	4.75
Mean (I)	5.66	4.58	3.58	
DMS: 2.31		CV(%): 49.65	OA: 4.61	
F (PM): 0.22 ns		F (I): 2.48 ns	F (PMxl): 0.98 ns	
SD (mm)				
	4 days	8 days	12 days	Mean (PM)
PS	2.97	2.58	2.93	2.83
E x PS	3.51	2.83	2.68	3.01
E x PE	3.25	2.70	2.69	2.88
Mean (I)	3.24	2.70	2.77	
DMS: 0.68		CV(%): 23.32	OA: 2.90	
F (PM): 0.22 ns		F (I): 2.26 ns	F (PMxl): 0.35 ns	
DMAP (g)				
	4 days	8 days	12 days	Mean (PM)
PS	12.73	11.11	10.12	11.32
E x PS	16.00	11.96	9.64	12.53
E x PE	13.69	14.71	11.29	13.23
Mean (I)	14.14 a	12.59 a	10.35 b	
DMS: 1.93		CV(%): 15.47	MG: 12.36	
F (PM): 3.06 ns		F (I): 11.91 **	F (PMxl): 2.33 ns	
DMR (g)				
	4 days	8 days	12 days	Mean (PM)
PS	5.34	3.72	4.42	4.49
E x PS	5.92	5.32	4.42	5.22
E x PE	5.68	6.00	4.63	5.44
Mean (I)	5.65 a	5.01 ab	4.49 b	
DMS: 1.09		CV(%): 21.51	OA: 5.05	
F (PM): 2.49 ns		F (I): 3.41 *	F (PMxl): 1.23 ns	

** - significant at the level of 1% probability ($p < 0.01$); * - significant at the level of 5% probability ($0.01 \leq p < 0.05$); ns - not significant ($p \geq 0.05$). Same lowercase letters in the column do not differ statistically. Same capital letters on the line do not differ statistically. The Tukey test was applied at the level of 5% probability. PS – propagated by seed; E x PS – grafted onto seed-propagated plants e E x PE – grafted on plants propagated by cutting. AO = overall average. PM = Propagation method e I = Irrigation interval in days.

The number of leaves of the yellow passion fruit showed a positive correlation with the dry mass of the aerial part with the $r = 0.37$ ($p < 0.05$) (Figure 4E), result that was already expected, because with a larger number of leaves, the plant's active photosynthetic rate provides a greater carbon fixation in its Mass in the aerial part [27]. The height of passion fruit plants correlated significantly and positively with the stem diameter with a correlation coefficient of $r = 0.38$ ($p < 0.05$) (Figure 4B), with the dry mass of the aerial part with a correction coefficient of $r = 0.73$ ($p < 0.01$) (Figure 4C) and while, with the dry mass of the roots, the correlation coefficient was $r = 0.5$ ($p < 0.01$) (Figure 4D).

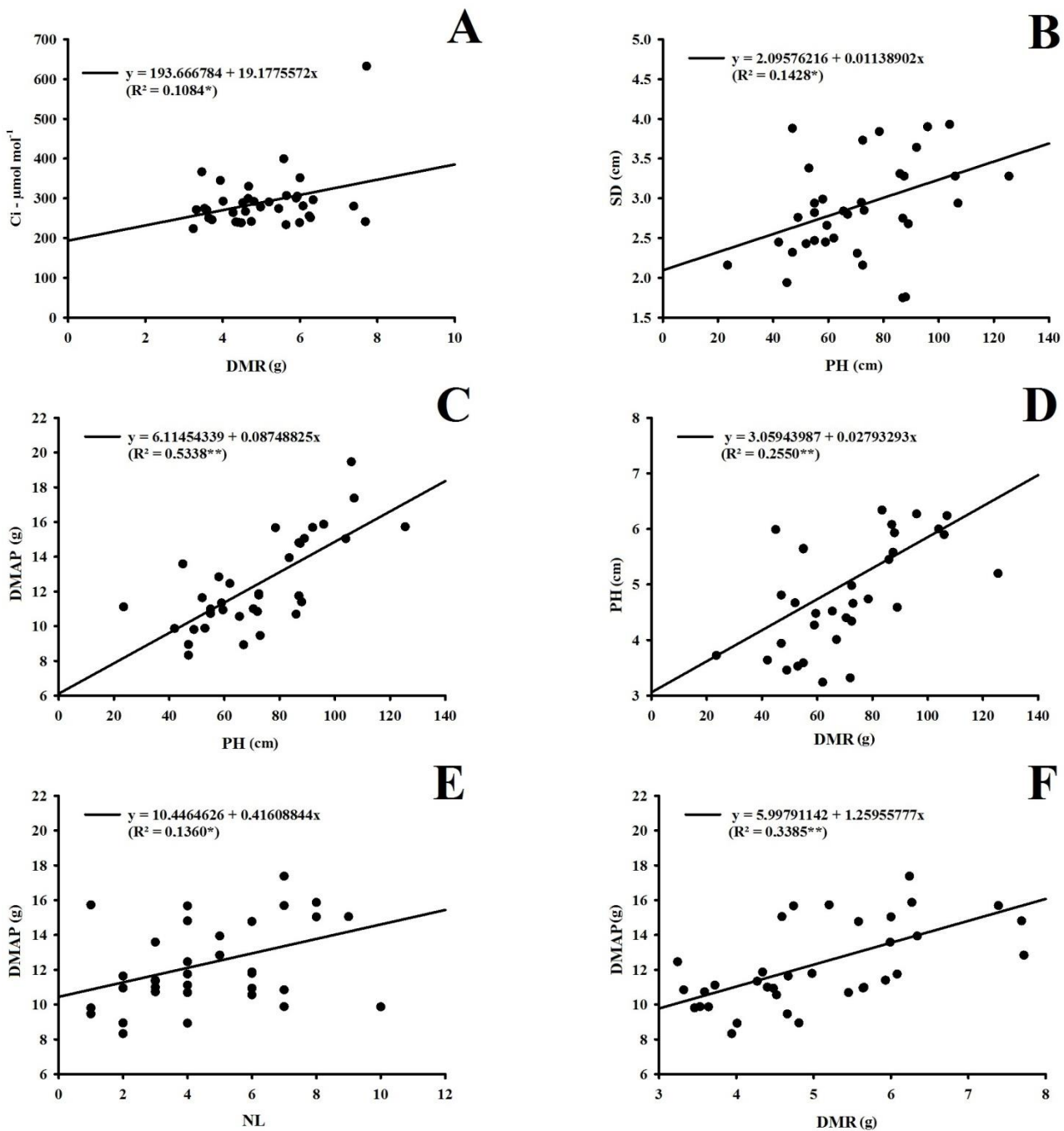


Figure 4. Linear regressions after observing the significant Pearson correlations between the development variables of the passion fruit grafted plants when grown at different irrigation intervals. Ci = internal CO₂ concentration in the substomatic camera, PH = plant height, NL = number of leaves; SD = stem diameter, DMAP = dry mass of aerial part and DMR = dry mass of roots. Dracena, 2019.

The diameter of the plant stem was not affected by different propagation methods and different water regimes. These data agree with those of [9] when they also did not observe differences for stem diameter between plants of *P. edulis* grafted on *P. edulis* and *P. gibertii*. Meantime, [8] studying hypocotyledonous grafting on yellow passion fruit, they observed a larger stem diameter in seedlings grafted on *P. gibertii* when compared with seedlings grafted on *P. edulis* at 70 days after grafting. This information is important because, according to [7] the stem diameter of the plant is indicative of vigor, so it can be said that yellow passion fruit plants grafted on *P. gibertii*, using cuttings or seeds, they can be used commercially, as they are vigorous.

No differences were observed for the dry masses of the aerial part and roots of passion fruit seedlings obtained by seeds and grafting, which disagrees with the results obtained by [18] that working with grafted plants on the *P. mucronata* observed lower values of root dry mass compared to seed propagated plants. However, when using twelve-day irrigation intervals, less dry mass of the aerial part was obtained than plants irrigated at intervals of four and eight days.

The same occurred in relation to the dry mass of the roots, however without differing from the plants irrigated every eight days, but lower than those irrigated every four days. [18] also verified a reduction in the dry mass of roots in plants subjected to water stress. It was observed that the dry mass of the roots correlated with the dry mass of the aerial part, presenting a correlation coefficient of $r = 0.58$ ($p < 0.01$) (Figure 4F) and with the internal concentration of CO_2 (Figure 4A), with a coefficient of $r = 0.33$ ($p < 0.05$). This demonstrates that the internal concentration of CO_2 provided a greater development of the plant, proving that the increase of the gas internally enhances the rate of photosynthesis (Figure 2C) that consequently a higher rate of carbon fixation in plant masses [21].

These correlations are important to note, given that the interpretation of the physiological condition of the passion fruit when exposed to water stress makes it an important factor in decision making regarding the management and choice of the best irrigation systems. Corrêa et al. (2010) did not find differences between the dry masses of the aerial part for plants of *P. edulis* grafted on *P. edulis* e *P. gibertii*, the same occurring for dry mass of the roots of the species *P. edulis* e *P. gibertii*. In addition to water availability, other factors such as auxins, light and temperature may be involved in the rooting of plants propagated by cuttings [28].

CONCLUSIONS

The suspension of irrigations for eight days reduces the rate of CO_2 assimilation, transpiration and stomatal conductance in passion fruit seedlings propagated by seeds and by grafting.

Passion fruit plants propagated by seeds show greater efficiency in the use of water than those grafted on *P. gibertii*.

Passion fruit seedlings grafted onto seedlings obtained by cuttings or seeds have vigor to be propagated commercially.

Conflicts of Interest: The authors declare no conflict of interest.

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