Ciência e Agrotecnologia
SCIENCE AND AGROTECHNOLOGY

Ciência e Agrotecnologia, 44:e015620, 2020
http://dx.doi.org/10.1590/1413-7054202044015620

Agricultural Sciences
eISSN 1981-1829

Physiological and biochemical responses of apple trees to irrigation water depth in a semiarid region of Brazil

Respostas fisiológicas e bioquímicas de macieiras sob lâminas de irrigação no semiárido Brasileiro

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Received in June 2, 2020 and approved in September 22, 2020

ABSTRACT

The present study evaluated the physiological and biochemical parameters of apple trees under different irrigation water depths in a semiarid region of Brazil. The experiment had a split-plot randomized block design. Irrigation levels (60, 80, 100, and 120% of the reference evapotranspiration - ET0) and two apple cultivars (Julieta and Princesa) were analysed in the experimental plots and the phenological stages (vegetative, flowering and fruiting) were analysed in the experimental subplots. The physiological characteristics evaluated included the chlorophyll index, relative water content, net photosynthesis, stomatal conductance, transpiration, internal CO_2 concentration, leaf temperature and instant carboxylation efficiency. The biochemical characteristics evaluated included the leaf contents of reducing sugars, total soluble sugars, and non-reducing sugars and proteins. The best biochemical responses were observed in the Princesa cultivar under 80% ET0 during the flowering and vegetative stages and under 120% ET0 during the fruiting stage. The best physiological responses for all phenological stages were observed under irrigation levels of 120% ET0 for both cultivars. The cv. Princesa demonstrated osmotic adjustment under water stress conditions during the phenological stages evaluated.

Index terms: *Malus domestica*; gas exchange; leaf carbohydrates; physiological stages.

RESUMO

O presente estudo avaliou os parâmetros fisiológicos e bioquímicos de macieiras sob diferentes lâminas de irrigação na região semiárida brasileira. O experimento foi delineado em blocos ao acaso, em parcelas subdivididas. O fator irrigação (60; 80; 100; e 120% da evapotranspiração de referência (ET0), combinado com duas cultivares de macieira (Julieta e Princesa); arranjado e analisado nas parcelas experimentais e os estádios fenológicos (vegetativo, floração e frutificação) que foram analisados nas subparcelas experimentais. As características fisiológicas avaliadas foram o índice de clorofila, conteúdo relativo de água, fotossíntese líquida, condutância estomática, taxa de transpiração, concentração interna de CO_2, temperatura foliar, eficiência instantânea de uso da água, eficiência intrínseca no uso da água. As variáveis bioquímicas avaliadas foram os teores foliares de açúcares redutores, de açúcares solúveis totais, de açúcares não redutores e de proteínas. As melhores respostas bioquímicas foram para a cv. Princesa, sob 80% da ET0 aplicado no estádio de floração e vegetativo e de 120 % da ET0 na de frutificação. A melhor resposta fisiológica durante os estádios fenológicos avaliados foi sob a lâmina de 120% da ET0 para ambas cultivares. A cv. Princesa, durante os estágios fenológicos avaliados apresentou indicativo de ajuste osmótico em condições de estresse hídrico.

Termos para Indexação: *Malus domestica*; trocas gasosas; carboidratos foliares; estágios fenológicos.

INTRODUCTION

Among temperate climate crops, apples have achieved good yield in the semiarid regions of Brazil due to their adaptability to the edaphoclimatic conditions; examples include the cultivars ‘Princesa’ and ‘Julieta’ (Lopes; Oliveira; Cavalcantem, 2012). Several studies have demonstrated the feasibility of apple production in Brazilian semiarid regions (De Oliveira et al., 2017; Oliveira et al., 2017).

For efficient irrigation, it is essential to understand the water relations of plants and their interactions with other physiological processes (Palheta et al., 2017). According to Bolat et al. (2014), water monitoring in

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apples via physiological and biochemical characteristics has proven to be a good indicator of the response of plants to water deprivation. According to Francescatto et al. (2015), the phenological stages corresponding to flowering and fruiting are the most sensitive to abiotic factors and are the most critical factors determining apple production.

Thus, given that water deficits affect multiple physiological processes in plants, studies on water relations in plants and interactions between water deficit effects and physiological processes are extremely important (Nascimento; Nascimento; Gonçalves, 2019). The responses of apple trees to water deficit include reductions in production, leaf area and stomatal opening (Jie et al., 2010), and these processes may result in the acceleration of leaf senescence and abscission (Taiz et al., 2017).

Thus, the aim of this study was to evaluate the physiological and biochemical parameters of apple cultivars subjected to different irrigation water depths during one cultivation cycle in a semiarid region of Brazil.

MATERIAL AND METHODS

The study was conducted in an experimental orchard of the *Corcino Frutas* farm, which is located at the *Senador Nilo Coelho* Irrigation Project, Unit 5, Petrolina, PE. The study was conducted using two-year-old apple (*Malus domestica*) trees that were propagated by grafting ('Maruba' rootstock with 'M9' interstock) and cultivated at a spacing of 4.0 m x 1.25 m.

The experiment was designed in randomized blocks with subdivided plots. Three factors were evaluated: irrigation, cultivar and phenological stage. The irrigation factor was divided into four levels (60, 80, 100, and 120% of the reference evapotranspiration, ET0) and the cultivar factor was divided into two levels (Julieta and Princesa) for a total of eight treatments, which were randomly arranged in the experimental plots. The phenology factor was divided into three phases (vegetative, flowering and fruiting) and their interactions were analysed in the experimental subplots.

The irrigation system consisted of two driplines containing emitters with a flow rate of 2,1 Lh^{-1} during the daily irrigation interval. Irrigation was conducted based on ET0, which was calculated using the Penman-Monteith method based on climatic data collected at the meteorological station located closest to the experimental site. Crop evapotranspiration (ETC) was not used because there was no cultivation coefficient for irrigated apple trees under semiarid conditions.

Climatic data were recorded during the experimental period; temperature, reference evapotranspiration and precipitation are shown in Figure 1.

Leaf samples for physiological and biochemical analyses were collected between 9:00 and 11:00 am; mature, fully expanded leaves exposed to the sun and located in the middle third of the plants were selected. Data were collected every 85 days after the application of the slides (DAAS); 85 DAAS corresponded to the phenological stage of full bloom, 170 DAAS corresponded to the phenological stage of fruiting and 255 DAAS corresponded to the phenological stage of vegetative rest.

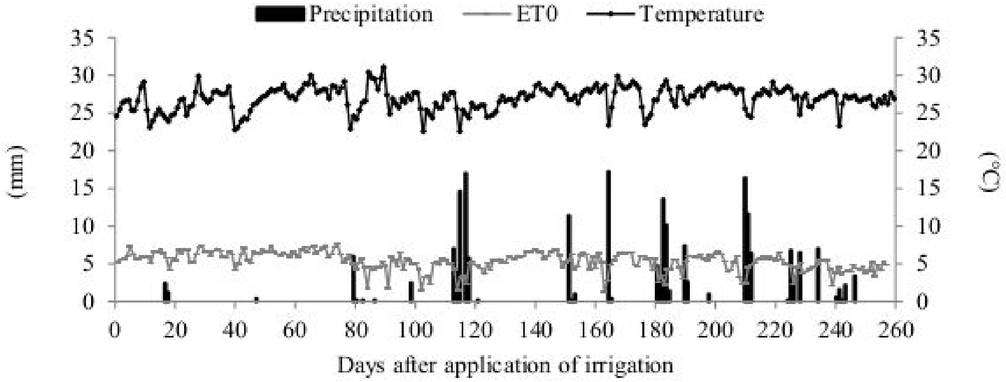

Figure 1: Average temperature (T), reference evapotranspiration (ET0, mm) and precipitation (mm) during the periods of flowering, fruiting and vegetative rest.

The following physiological characteristics were evaluated with a portable photosynthesis analyser using infrared radiation (Infrared Gas Analyser, Li6400, Licor): net photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), internal CO_2 concentration (*Ci*), leaf temperature (T_L), and instantaneous carboxylation efficiency (*CEi*). The physiological characteristics related to chlorophyll a, chlorophyll b and total chlorophyll were also evaluated using a chlorophyll meter (Brand: Falker, Model: ClorofiLOG) and were expressed using the Falker chlorophyll index (FCI). The leaf relative water content (RWC %) was estimated in grams from the determination of fresh mass (FM), turgid mass (TM) and dry mass (DM) based on the methodology described by Marenco and Lopes (2005).

The biochemical characteristics evaluated included the leaf contents of reducing sugars (RS), quantified by the dinitrosalicylic acid (DNS) method (Miller, 1959); total soluble sugars (TSS), using the methodology described by Yemm and Willis (1954); and total soluble proteins (TSP), following the method described by Bradford (1976) using bovine serum albumin (BSA, Sigma, USA) as the standard protein. The data on reducing sugars and total soluble sugars were used to estimate the content of non-reducing sugars (NRS).

The data were subjected to an analysis of variance, and the means of the varieties were compared using a Tukey test at 5% significance. When the effect of irrigation water depth was significant, a regression analysis was performed at the 5% significance level using the statistical program SISVAR (Ferreira, 2019).

RESULTS AND DISCUSSION

For the variables related to gas exchange, the statistical analysis did not show any interactions among the evaluated factors. However, a significant isolated effect was found between irrigation water depth and phenological stage for *A*, *E*, T_L and *CEi*. Moreover, significant effects were observed for the interactions between phenological stage and irrigation water depths for g_s and *Ci* and for the interaction between phenological stage and cultivar for *Ci* (Table 1).

A three-way interaction was observed among RWC, TSP and RS. Interactions were also observed between phenological stage and cultivar for the variables LCI, NRS and TSS, and between cultivar and irrigation water depth for the variables NRS and TSS (Table 2).

A significant effect ($p < 0.05$) of irrigation water depth was observed on the determinants of gas exchange (Figure 2). These variables (*A*, *E*, T_L and *CEi*) showed good regression fits with non-significant deviations from the regression ($p > 0.05$); the linear model was chosen among the significant regressions (based on the F test) because it exhibited a higher correlation coefficient (R^2).

Table 1: Mean squares and significance for net photosynthesis (*A*), transpiration (*E*), stomatal conductance (g_s), internal CO_2 concentration (C_i), leaf temperature (T_L), and instant carboxylation efficiency (CE_i).

Source Variation	Medium squares					
	A	*E*	$g_{s\,(x\,10^2)}$	$C_{i\,(x\,10^2)}$	T_L	$CEi_{(x10^{-3})}$
Block (B)	6.66^{ns}	1.77^{ns}	0.60^{ns}	1.05	0.34	0.20
Irrigation Water Depth (ID)	272.27^{**}	13.22^{**}	29.0^{**}	2.80^{ns}	1.62^{**}	4.29^{**}
Cultivar (C)	0.55^{ns}	0.04^{ns}	0.01^{ns}	5.43^{*}	0.17^{ns}	0.06^{ns}
C x ID	0.76^{ns}	0.89^{ns}	0.10^{ns}	0.11^{ns}	0.38^{ns}	0.01^{ns}
Phenological stage (PS)	85.74^{**}	221.82^{**}	0.20^{ns}	170.13^{**}	342.95^{**}	6.55^{**}
PS x ID	13.54^{ns}	0.72^{ns}	1.61^{**}	2.67^{**}	0.17^{ns}	0.20^{ns}
PS x C	0.50^{ns}	0.16^{ns}	0.10^{ns}	5.52^{**}	0.81^{ns}	0.07^{ns}
PS x ID X C	6.79^{ns}	1.32^{ns}	0.2^{ns}	0.39^{ns}	0.12^{ns}	0.12^{ns}
Overall mean	18.49	7.37	46.45	2.36	34.02	79.0
VC Plots (%)	15.58	10.88	14.13	4.35	1.41	17.2
VC Subplots (%)	14.69	14.17	15.00	3.14	1.75	15.44

**, * and ns indicate significance at 1%, significance at 5% and non-significance, respectively, for the F Test. DF: Degrees of Freedom.

Table 2: Mean squares and significance for relative water content (RWC), leaf chlorophyll index (LCI), reducing sugars (RS), non-reducing sugars (NRS), total soluble sugars (TSS), and total soluble protein (TSP).

Source of Variation	Medium squares					
	RWC	LCI	RS$_{(x10^3)}$	NRS$_{(x10^3)}$	TSS$_{(x10^3)}$	TSP$_{(x10^3)}$
Block (B)	10.16	2.96	0.61	10.67	14.20	0.12
Irrigation Water Depth (ID)	987.06**	161.82**	179.68**	1240.41**	2300.51**	20.68**
Cultivar (C)	8992.36**	1820.44**	631.82**	199.75*	1542.09**	98.27**
C x ID	60.42*	1.96^{ns}	12.28**	284.77**	354.77**	2.23**
Phenological stage (PS)	1082.89**	1986.94**	153.41**	863.61**	1701.08**	6.34**
PS x ID	20.06*	2.96^{ns}	0.73*	28.28^{ns}	25.21^{ns}	1.54**
PS x C	397.55*	81.79**	2.50**	932.99**	862.10**	14.82**
PS x ID X C	12.96**	1.07^{ns}	1.33**	30.44^{ns}	30.48^{ns}	0.79**
Overall mean	58.60	68.52	0.468	1.21	1.67	0.11
VC Plots (%)	4.50	3.90	3.13	12.36	8.68	9.34
VC Subplots (%)	4.13	2.77	3.85	10.84	7.85	7.99

**, * and ns indicate significance at 1%, significance at 5% and non-significance, respectively, for the F Test. DF: Degrees of Freedom.

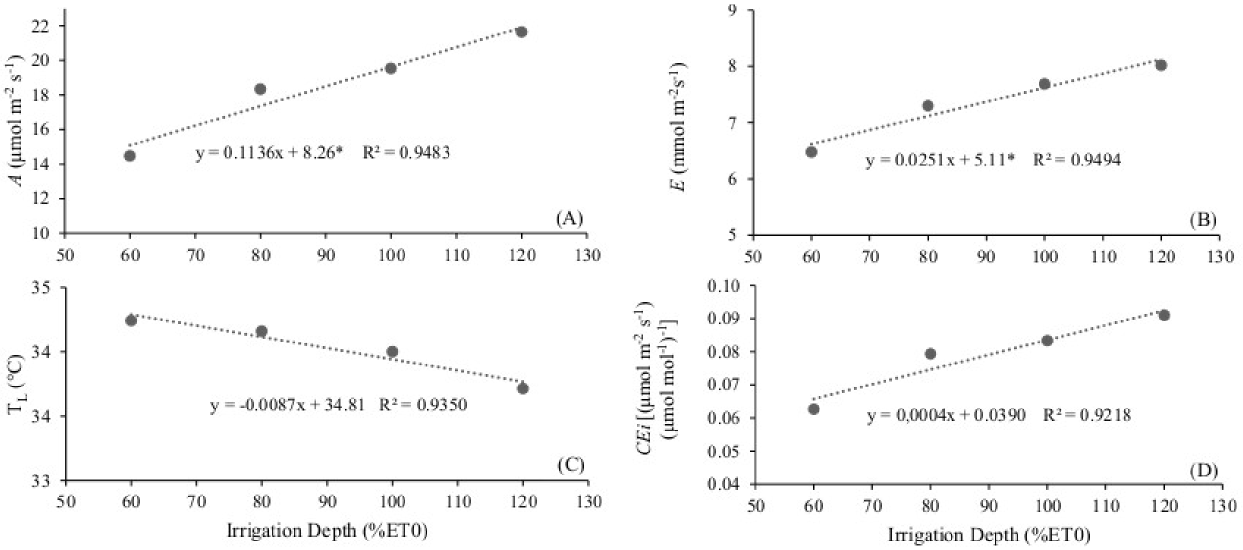

Figure 2: Net photosynthesis (*A*), transpiration (*E*), leaf temperature (T_L) and instant carboxylation efficiency (*CEi*) in apple leaves as a function of irrigation water depth.

The values of *A* and *E* decreased with the reduction in irrigation depth. The highest *A* (21.64 µmol m^{-2}s^{-1}) was observed for 120% ET0, whereas the lowest (14.47 µmol m^{-2}s^{-1}) was observed under 60% ET0 (Figure 2A). The highest *E* (8.01 mmol m^{-2}s^{-1}) was observed for 120% ET0, whereas the lowest *E* (6.48 mmol m^{-2}s^{-1}) was observed under 60% ET0 (Figure 2B).

A similar result was observed by Jie et al. (2010), who evaluated the influence of soil water stress on the physiological characteristics of Fuji apple leaves and stems, and demonstrated that drought stress led to decreases in *A* and *E*.

Wright, Cline and Earl (2019) evaluated different physiological responses in four genotypes of apple rootstocks under water deficit soil conditions, and verified that only one of the genotypes maintained transpiration under water reduction. In other genotypes as well those evaluated in this study, tolerance to water stress was not observed.

The decreases in *A* and *E* with decreasing irrigation depth observed in this study are associated with stomatal closure (Figure 3A) and limitations on CO_2 assimilation and transpiration, which reduced the rate of photosynthesis. A similar result was found by Silva et al. (2010). According to Quintão Scalon et al. (2011), when a plant experiences low soil water availability, several metabolic processes can be influenced, resulting in the alteration of various physiological events such as stomatal closure and the consequent reduction of stomatal conductance and photosynthesis as well as transpiration, which leads to a decrease in growth rate.

The T_L increased with the reduction in irrigation water depth; the highest T_L (34.24 °C) was observed under 60% ET0 and the lowest (33.71 °C) was observed under 120% ET0 (Figure 2C). Alizadeh et al. (2011) evaluated the impacts of water stress on the physiology of different apple rootstocks under arid conditions in Iran and observed an increase in leaf temperature as the level of stress increases, which corroborates the results of the present study.

The increase in leaf tissue temperature is a result of the decrease in transpiration, which is caused by the reduction in stomatal opening (Figueiredo et al., 2019). In this way, the increase in T_L may be related to the decrease in transpiration observed with the reduction in water depth (Figure 2B).

The *CEi* (Figure 2D) showed a pattern similar to that of net photosynthesis (Figure 2A) with values decreasing linearly with the gradual reduction in irrigation water depth. According Taiz et al. (2017), the decrease in *CEi* concomitant with the decrease in net photosynthesis occurs as a result of the reduction in rubisco activity, which is induced by the low availability of RuBP and consequently produces a low rate of CO_2 fixation in the Calvin-Benson cycle through the use of ATP and NADPH.

A significant effect ($p < 0.05$) of phenological stage was recorded for the determinants of leaf gas exchange (Table 3). The *E* observed during both the fruiting stage (6.02 mmol $m^{-2}s^{-1}$) and the vegetative stage (6.00 mmol $m^{-2}s^{-1}$) were sufficient to guarantee a significant (p <0, 05) rate of *A* (18.71 µmol $m^{-2}s^{-1}$ and 19.83 µmol $m^{-2}s^{-1}$, respectively).

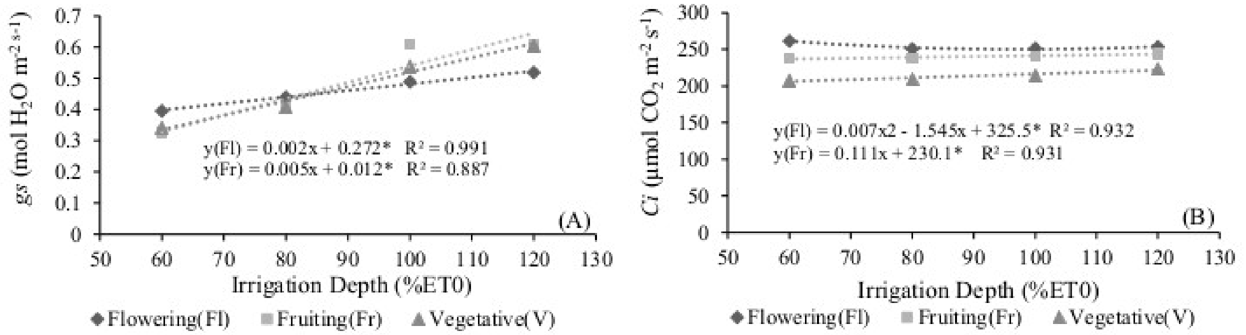

Figure 3: Effect of the interaction between irrigation water depth and phenological stage on stomatal conductance, g_s (A) and internal CO_2 concentration, *Ci* (B) in the leaves of apples cultivated under semiarid conditions.

Table 3: Net photosynthesis (*A*), transpiration (*E*), leaf temperature (T_L), and efficiency of instant carboxylation (*CEi*) on apple leaves at different phenological stages.

	A	E	T_L	CEi
	(µmol $m^{-2}s^{-1}$)	(mmol $m^{-2}s^{-1}$)	(%)	[(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$]
Flowering	16.93b	10.08a	37.40a	0.06c
Fruiting	18.71a	6.02b	32.50b	0.07b
Vegetative	19.83a	6.00b	32.18c	0.09a
DMS	1.45	0.55	0.32	0.006

Means followed by the same letters do not differ based on the F test at 5% significance.

The high *A* rate obtained during fruiting was related to the greater production and translocation of carbohydrates from the source leaves to nourish the fruits. The presence of fruit on apple trees can stimulate and prolong photosynthesis due to the increased demand for assimilates (Lenz, 1980).

Mohawesh and Al-Absi (2009) evaluated the physiological responses of two apple genotypes to different water regimes under semiarid conditions in Jordan and observed variation in *E* during the crop cycle, which indicated that gas exchange in apples can be regulated by changes in atmospheric parameters or plant developmental stage.

However, at the flowering stage, the high rate of *E* (10.08 mmol $m^{-2}s^{-1}$) did not influence *A* (16.93 μmol $m^{-2}s^{-1}$) (Table 3), but may have promoted leaf cooling given that this stage exhibited the highest T_L (37.40 °C). Thus, an increase in leaf temperature with possible damage to the photosynthesis apparatus (Silva et al., 2015) justifies the reduction in photosynthesis during this stage.

The increase in T_L inhibits the PSII reaction centre, leading to dissociation of the protein-pigment complex in the antenna complex that is linked to the central unit of the light-harvesting apparatus of PSII (Taiz et al., 2017). This behaviour may be associated with air temperatures greater than 30 ºC (Figure 1), which were recorded at this stage of the experiment. According to Sheriff and Muchow (1984), a leaf that is receiving light and/or transpiring is affected by air temperature differences caused by differential absorption of radiation.

Table 3 shows that the highest value of *CEi* occurred during the vegetative stage. This result may be associated with carbohydrate production in photosynthetically active tissues such as mature leaves that exceed the amount needed for maintaining metabolism and growth. Under these conditions, the surplus is exported in the form of sucrose to tissues that are photosynthetically less active or inactive such as young leaves, roots, fruits or branches (Fischer; Ulrichs; Ebert, 2015).

Figure 3 shows the significant interaction between irrigation water depth and phenological stage; the model had a good fit with a non-significant regression deviation ($p > 0.05$). The linear model was chosen among the significant regressions based on the F test because it possessed a higher correlation coefficient (R^2). Thus, for g_s, the linear model was fit for all the evaluated stages, whereas for *Ci* it was only fit for the fruiting and vegetative stages because during the flowering stage, the quadratic model showed the best fit. The g_s of all phenological stages decreased proportionally with the reduction in irrigation depth (Figure 3A).

According to Nascimento, Nascimento and Gonçalves (2019), when a plant experiences low soil water availability, several metabolic processes can be influenced, resulting in the alteration of various physiological events such as stomatal closure with a consequent reduction in g_s, *A*, and *E*, which leads to a decrease of growth rate; this sequence of events is consistent with the results obtained in this study.

However, the reduction in stomatal opening under limited water availability can be a strategy of apple trees to prevent an increase in *E* as well as to avoid embolism and cavitation in the xylem of plants (Nascimento; Nascimento; Gonçalves, 2019).

As seen in Figure 3B, *Ci* decreased linearly with the reduction in irrigation water depth during the fruiting and vegetative stages. According to Silva et al. (2015), the normal flow of CO_2 towards the carboxylation site is reduced by stomatal closure with a consequent decrease in photosynthesis.

However, there was an increase in *Ci* under 60% ET0 at the flowering stage (Figure 3B), which is an indication that the CO_2 fixation process during the carboxylation stage was compromised. A high value of *Ci* combined with low g_s (Figure 2A) indicates a reduction in *CEi*, which may be related to degradation of the photosynthetic apparatus (Silva et al., 2013).

A significant interaction ($p < 0.05$) was observed between phenological stage and cultivar for the variables *Ci*, LCI, NRS and TSS (Table 4). For *Ci*, the amount of CO_2 accumulated in the intercellular spaces at the flowering stage was statistically higher than the other stages and did not differ between the cultivars (255 μmol CO_2 $m^{-2}s^{-1}$ for cv. Julieta and 254 μmol CO_2 $m^{-2}s^{-1}$ for cv. Princesa). During the vegetative stage, the *Ci* was statistically lower for the cv. Princesa (208 μmol CO_2 m^{-2} s^{-1}).

The increase in *Ci* during flowering suggests that there was no limitation of CO_2 availability for the photosynthetic process (Taiz et al., 2017), but it does indicate that the high leaf temperatures recorded at this stage may have reduced CO_2 assimilation. According to Oliveira, Fernandes and Rodrigues (2005), temperatures above 30 ºC inhibit the assimilation of CO_2 in C3 plants by reducing rubisco activation through the inhibition of rubisco activase and by reducing photochemical activity.

Šircelj et al. (2007) detected different levels of drought stress in apple trees using biochemical and physiological parameters and observed that the increase in *Ci* with the reduction in net photosynthesis in apple trees under severe water stress could not be attributed to a CO_2 limitation but were instead related to damage or downregulation of the photosynthetic apparatus.

Table 4: Internal concentration of CO_2 (*Ci*), leaf chlorophyll index (LCI), non-reducing sugars (NRS) and total soluble sugars (TSS) in apple tree leaves grown under semiarid conditions.

Phenological stage	*Ci*		LCI		TSS		NRS	
	(μmol CO_2 m^{-2} s^{-1})		(%)		(mg g^{-1})		(mg g^{-1})	
	Jul	Prin	Jul	Prin	Jul	Prin	Jul	Prin
Flowering	255aA	254aA	66aC	56bC	1496bA	1389bB	1173aA	910bB
Fruiting	240bA	241bA	73aB	68bB	1609abB	2042aA	1134aB	1456aA
Vegetative	220cA	208cB	78aA	70bA	1594aB	1947aA	1204aB	1390aA
SMD^1 (5%)	5.60		1.39		86.43		87.20	
SMD^2 (5%)	4.67		1.43		99.63		99.17	

Means followed by the same lowercase letter in a row and uppercase letter in a column do not differ by the Tukey test ($p < 0,05$). Jul = cv. Julieta and Prin= cv. Princesa.

The reduction in *Ci* during the vegetative stage for the cv. Princesa was associated with an increase in *A* rate during this phase (Table 3); this may have been a strategy to stimulate stomatal opening, the entry of CO_2 and consequently carbon fixation for carbohydrate production (Rocha et al., 2019), given that this phase demonstrated the highest *CEi* for the production of photoassimilates in the growth and development of apple trees (Table 3).

The ICF observed in Table 4 varied with both cultivar and phenological stage. According to Silva et al. (2015), given the relationship between energy absorption and transfer, the levels of photosynthetic pigments can be directly related to net photosynthesis as well as the growth and adaptation of plants to stress and environmental changes, which corroborates the results obtained in this study. The flowering stage exhibited the lowest rate of *A* (16.93 μmol $m^{-2}s^{-1}$, Table 3) and consequently lowered LCI by 66% and 56% for the cvs. Julieta and Princesa, respectively (Table 4). The vegetative stage, with a greater rate of *A* (19.83 μmol $m^{-2}s^{-1}$, Table 3), exhibited an LCI of 78% and 70% for the cvs. Julieta and Princesa, respectively (Table 4).

The high values of LCI in the cv. Julieta and the low values of LCI in the cv. Princesa, (Table 4) may be related to the genetics of each cultivar. Da Silva et al. (2017) reported that the increase or decrease in LCI may be interpreted as an adaptive response to stressors. According to these authors, increases in total chlorophyll content under adverse conditions may indicate the activation of a protective mechanism in the photosynthetic apparatus. Reductions in chlorophyll content cannot be interpreted only as a deleterious effect inherent to stressful conditions, but may also represent an acclimatization response to the action of stressors in an attempt to conserve energy and consequently capture less light energy, thus possibly avoiding photooxidative stress.

Thus, it can be seen in Table 4 that the that the increase in CLI in the cv. Julieta resulted in an increase in NRS at flowering (1173 mg g^{-1}) but did not positively influence net photosynthesis at this stage, whereas the reduction in CLI in the cv. Princesa provided both an increase in SST during the fruiting (2042 mg g^{-1}) and vegetative (1456 mg g^{-1}) stages and an increase in NRS during the fruiting (1947 mg g^{-1}) and vegetative (1390 mg g^{-1}) stages. Thus, the strategy of the cv. Princesa to reduce CLI is more efficient for cultivation under semiarid conditions, given that it positively influenced net photosynthesis.

A significant interaction ($p < 0.05$) between irrigation water depth and cultivar was identified. The models had a good fit with a nonsignificant regression deviation ($p > 0.05$). The chosen models among the significant regressions based on the F test were those with the highest correlation coefficient (R^2). Thus, for TSS and NRS, the best fit was obtained with the linear model for cv. Julieta, whereas the quadratic model showed the best fit for cv. Princesa.

Figures 4A and 4B show a gradual increase in NRS and TSS contents for both cultivars with a reduction in irrigation depth. A similar result was observed by Alizadeh et al. (2011), who evaluated the effect of water stress on apple rootstock under arid conditions in Iran, and observed that water stress caused an increase in the leaf content of soluble sugars.

The increases observed in the contents of NRS and TSS in these cultivars with the reduction in irrigation depth may be related to disturbances caused by stressful conditions such as the reduction in cell water content. According to Moura et al. (2016), the accumulation of free sugars in leaves may result from the restriction of protein synthesis and the hydrolysis of starch reserves as well as disturbances caused by water deficit in phloem tissues, thereby reducing their translocation to other organs.

A three-way interaction ($p < 0.05$) was found among RS, TSP and RWC. A suitable model was found to represent the data only for cv. Julieta during the fruiting stage, as the regression deviation was not significant (P> 0.05). For the other variables, the fit of any model must be accepted with caution, because the regression deviation was significant (P> 0.05). In all cases, the linear regression model was chosen among the significant regressions based on the F test as it generated a higher correlation coefficient (R^2) (Table 5).

The linear model showed the best fit for all factors for the RS (Table 5 and Figure 5). For this variable, the contents during the fruiting stage were significantly higher than those of the other stages with no variation among the irrigation levels, but the levels of cv. Princesa were higher than those of cv. Julieta (Table 5).

The levels of RS in cv. Princesa during the vegetative stage at 80% ET0 did not differ from the levels during the fruiting stage for that same cultivar (Table 5). However, the levels of RS during the flowering stage were significantly lower than those during the other stages, primarily for cv. Julieta, presenting water depths variation with 80% of ET0 for cv. Princesa, where the levels were higher in relation to the other water depths for this cultivar (Table 5).

The increase in these sugars at the fruiting stage may have occurred due to the higher production of carbohydrates necessary for the accumulation and transport of excess photoassimilates in the form of sucrose to growing fruits, as described by Julius et al. (2017).

The reduction in the RS content at flowering may have occurred due to the low net photosynthesis observed during this stage. According to Taiz et al. (2017), when the photosynthesis rate is high, TSS and starch accumulate in the leaves, which is consistent with the results obtained in this study.

The higher RS content in cv. Princesa compared with cv. Julieta is a reflection of the intrinsic mechanisms of each cultivar (Table 5). According to Alizadeh et al. (2011), leaf physiological differences between apple cultivars are an indication of differences in tolerance.

The increase in RS at 80% ET0 is an indication that moderate stress in both the flowering and vegetative stages is sufficient to promote the probable hydrolysis of sucrose, which releases hexoses that would be utilized in anabolic or catabolic processes for plant growth and development (Morando et al., 2014).

There were significant differences in TSP between apple cultivars, among phenological stages and at different irrigation water depths (Table 5). The linear model provided the best fit for all factors for the total soluble proteins (TSP) (Table 5 and Figure 6).

Figure 6 shows that of the apple cultivars, 'Princesa' had a higher protein content than 'Julieta'. According to Horta et al. (2018), protein consumption or production is related to the gene expression of each species and is necessary for the plant to recover from stress in a less expensive for the maintenance of growth and development.

The highest TSP was observed during the vegetative stage in apple leaves, which is consistent with the results of Cheng and Robinson (2004), who also observed that during the apple vegetative cycle, reserves are accumulated primarily in the aerial parts of the plant in the form of proteins, free amino acids and non-structural carbohydrates. This increase in TSP may be associated with the demand for photoassimilates for the current cycle, but they can also be stored and used in metabolic activities at the beginning of the next cycle (Campoy; Ruiz; Egea, 2011).

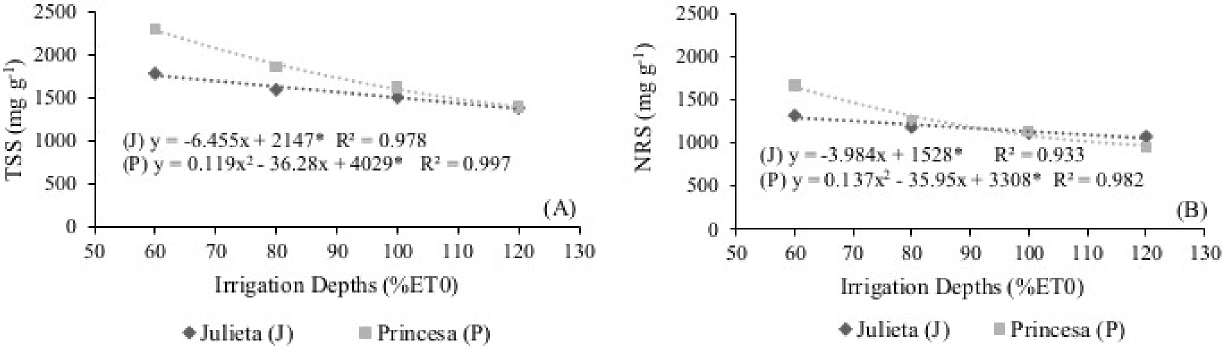

Figure 4: Effect of the interaction between irrigation water depth and cultivar for total soluble sugar, TSS (A) and non-reducing sugar, NRS (B) in the leaves of apples cultivated under semiarid conditions.

Table 5: Split of significant three-way interaction among reducing sugars (RS), total soluble proteins (TSP) and relative water content (RWC).

ID	PS	RS		TSP		RWC	
		Cultivar		Cultivar		Cultivar	
		Julieta	Princesa	Julieta	Princesa	Julieta	Princesa
	FL	380.48cB	574.04cA	69.59aB	91.50bA	62.74aA	41.98bB
60	FR	541.58aB	679.74aA	76.96aB	94.88aB	66.39 A	50.65aB
	V	478.30bB	638.74bA	45.28bA	127.91aB	48.26bA	42.6bB
	FL	355.67cB	543.85bA	81.45aB	116.08bA	68.71aA	45.43bB
80	FR	475.18aB	641.62aA	92.83aB	120.25aB	69.61aA	54.93aB
	V	411.91bB	628.99aA	64.30bB	141.92aA	55.49bA	45.43bB
	FL	340.67cB	422.32cA	89.82abB	119.89cA	73.44 A	46.53bB
100	FR	438.47aB	542.28aA	99.77aB	158.36bA	72.91aA	56.12aB
	V	397.80bB	507.75bA	80.43bB	178.67aA	64.13bA	46.53bB
	FL	214.71cB	375.32cA	98.64bB	124.99cA	80.13aA	54.41bB
120	FR	381.69aB	481.16aA	127.94aB	194.19bA	77.04aA	60.20aB
	V	331.71bB	453.81bA	92.03bB	237.22aA	68.13bA	54.41bB
Regression							
	FL	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}
L	FR	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}
	V	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}
	FL	0.000^{*}	0.285^{ns}	0.75ns*	0.02^{*}	0.747^{ns}	0.04^{*}
Q	FR	0.540^{ns}	0.145^{ns}	0.14^{ns}	0.23^{ns}	0.683^{ns}	0.92^{ns}
	V	0.985^{ns}	0.006^{*}	0.37^{ns}	0.000^{*}	0.14^{ns}	0.02^{*}
	FL	0.001^{*}	0.000^{*}	0.83^{ns}	0.23^{ns}	0.51^{ns}	0.06^{ns}
D	FR	0.158^{ns}	0.006^{*}	0.42^{ns}	0.006^{*}	0.882^{ns}	0.22^{ns}
	V	0.004^{*}	0.000^{*}	0.92^{ns}	0.95^{ns}	0.22^{ns}	0.08^{ns}

Within the same level of a factor, averages followed by the same letter do not differ; lowercase letters between lines are based on the Tukey test (comparing phenological stages) and upper case letters between columns are based on the F test (comparing cultivars), both at 5% significance. ID, irrigation water depth; PS, phenological stage; L, linear; Q, quadratic; D, regression deviation; FL, flowering; FR, fruiting; and V, vegetative.

Table 5 and Figure 7 show the data on relative water content (RWC); the linear model showed the best fit for all factors for the cv. Julieta, whereas the quadratic model was the best fit for the cv. Princesa during the flowering and vegetative phenological stages.

Table 5 and Figure 7 shows the different behaviours of the cultivars; cv. Julieta reduced RWC gradually with the decrease in the water depths across all the phenological stages, whereas cv. Princesa showed little variation in RWC with water depth (45% and 42% at 80 and 60% ET0, respectively) for both the vegetative and flowering stages.

According to Gardner (1965), for the relationships between relative water content and leaf water potential to be manifested, it is necessary to consider the intrinsic characteristics of the species such as its genetic specificities.

Similar to the behaviour of cv. Julieta, Bolat et al. (2014) observed the effect of water stress on morphological, physiological and biochemical factors in apple rootstock leaves in Turkey, and found that the RWC decreased with increasing levels of water stress. The reduction in leaf RWC indicates that the metabolic activity in tissues has decreased significantly due to water stress (Bolat et al., 2014).

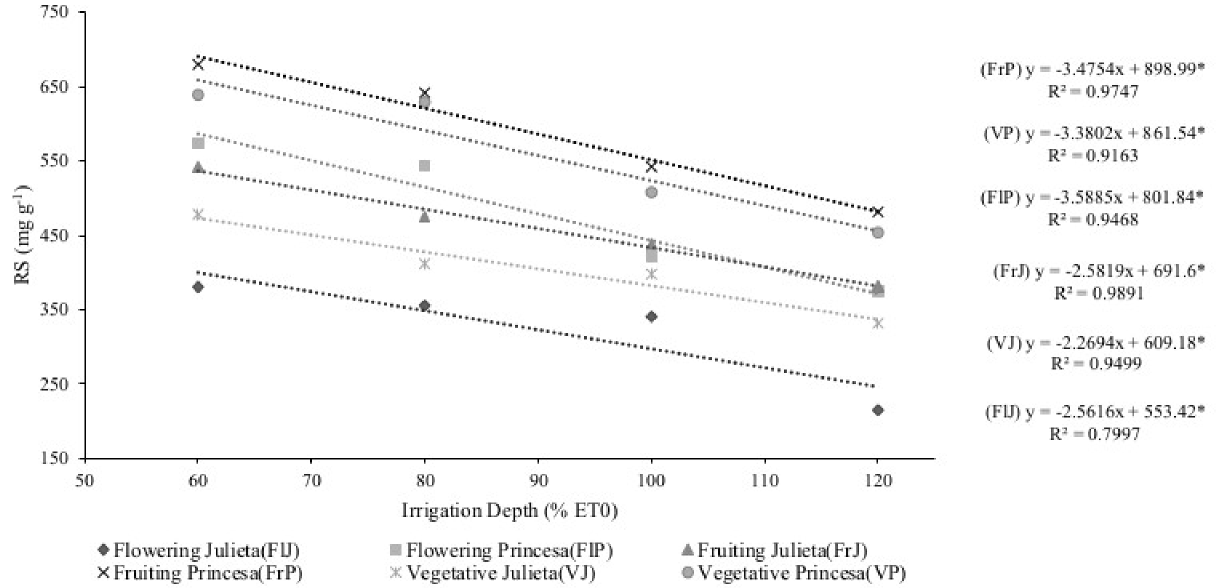

Figure 5: Regression models adjusted for RS in the leaves of apples cultivated under semiarid conditions.

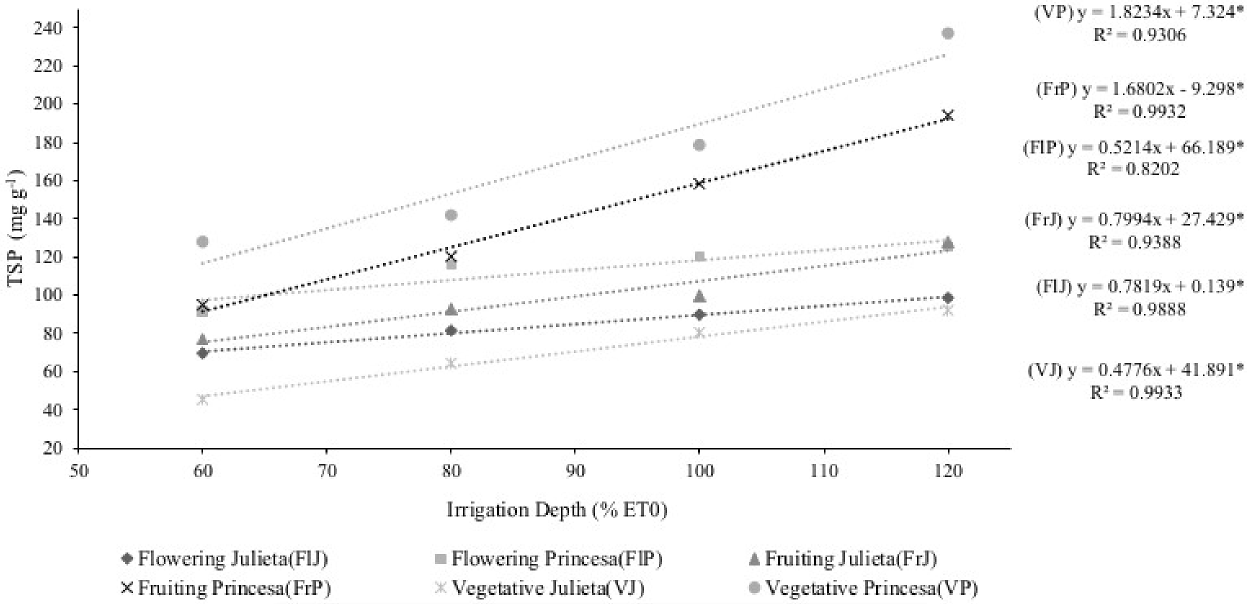

Figure 6: Regression models for TSP in the leaves of apples cultivated under semiarid conditions.

In contrast, the differentiated behaviour of cv. Princesa indicates that the cultivar did activate the mechanism of osmotic adjustment, because under stressful conditions, the RWC was maintained (Figure 7) and there was an accumulation of solutes (Figure 4A). According to Sharma et al. (2019), osmotic adjustment is one of the most effective physiological mechanisms for maintaining cell turgor under conditions of low soil water potential and is established through the accumulation of compatible solutes in the vacuole or cytosol.

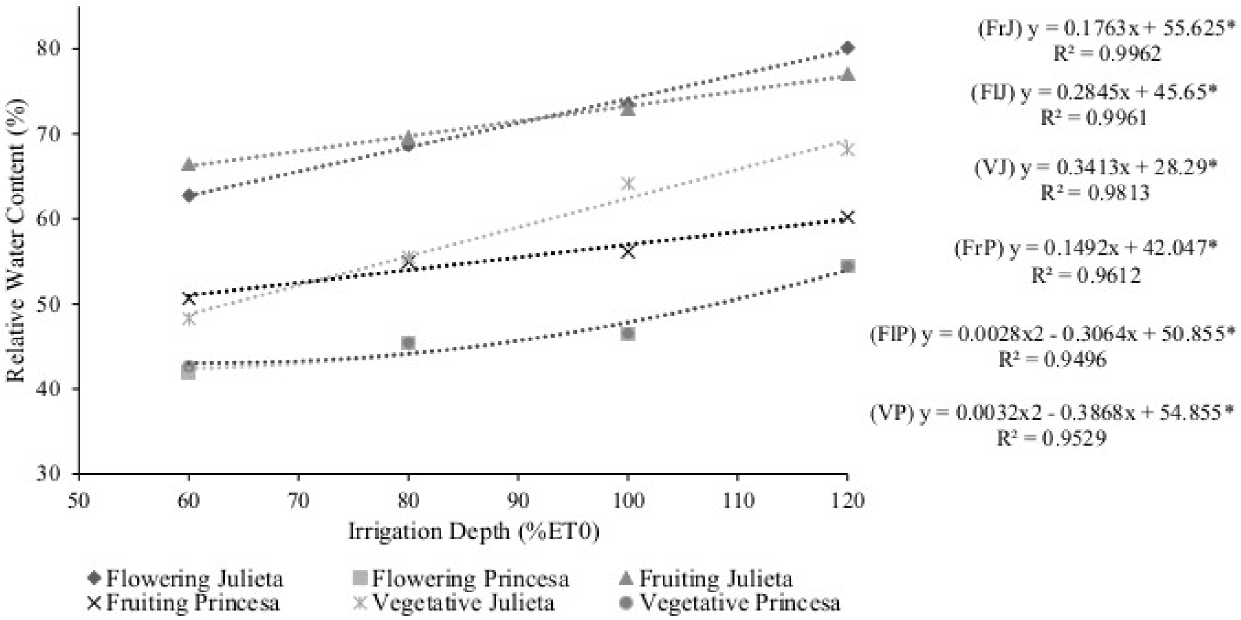

Figure 7: Regression models for RWC in the leaves of apples cultivated under semiarid conditions.

In Figure 7, the higher RWC at flowering and fruiting may be related to the greater water need during the development of flowers and fruits. According to Francescatto et al. (2015), in apple, the phenological stage of fruiting is stage that is the most sensitive to water deficit.

According to Dheilly et al. (2016), apple fruits must maintain cell turgor to promote cellular expansion in the tissues that form the fruits. Thus, the increase in RWC at this stage was probably due to the need to maintain cell turgor, a phenomenon that must have influenced the regulation of stomatal opening or closing in the leaf (Rucińska, 2016).

In this respect, the increase in RWC during fruiting corroborates observations by Oliveira et al. (2017), who evaluated the effect of different irrigation water depths on the quality of apple fruit under semiarid conditions, and found that 120% ET0 is recommended for the production of larger fruits without compromising post-harvest commercial quality.

CONCLUSIONS

The best biochemical responses were observed for cv. Princesa during the flowering and vegetative stages at 80% ET0 and during the fruiting stage at 120% ET0. The best physiological response for both cultivars during all phenological stages was under 120% ET0. Only cv. Princesa exhibited osmotic adjustment under water stress conditions (60% ET0).

ACKNOWLEDGEMENTS

Embrapa Semiárido, Federal University of Vale do São Francisco and Universidade Estadual Paulista provided scientific technical support; the Coordination for the Improvement of Higher Education Personnel (CAPES) provided a scholarship and Fazenda Corcino Frutas S.A. provided technical support for the implementation of experiences and studies.

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