

Water relations and gas exchange in *Theobroma cacao* var. Guasare under periods of water deficit

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Abstract

Water relations and gas exchange of *Theobroma cacao* var. Guasare under periods of water deficit were evaluated. Microclimatic characteristics (air temperature, relative humidity and photosynthetically active radiation), leaf gas exchange (stomatal conductance, transpiration and CO₂ assimilation rates) and leaf water potential were measured throughout the day in plants subjected to 3, 12 and 25 days without water. Pressure-volume curves were used to determine osmotic potential at turgor loss. A significant decrease in stomatal conductance was observed as water stress increased. As a consequence, gas exchange characteristics were also affected. An evident osmotic adjustment was recorded in plants subjected to 3 and 12 days without water, while no additional adjustment occurred 25 days later. Water use efficiency increased as water availability decreased. CO₂ assimilation rates decreased 25% in severely stressed plants, while water loss was reduced in 39% indicating that stomatal closure affects water loss to a greater degree.

Key words: criollo type, water stress, CO₂ assimilation, stomatal conductance, osmotic adjustment.

Introduction

The quantity and distribution of precipitation constitute important environmental factors that affect *Theobroma cacao* L. production under field conditions (3). As Alvim (1977) pointed out, hydro periodicity is the

principal climatic factor that induces opening of vegetative buds as well as enhances flowering in cacao. Under field conditions, many periods without rain may occur. The length, especially of the drier season, may affect

physiological processes that reduce cacao production (4, 6).

In general, studies on the responses of cacao to water deficit have been carried out on juvenile plants under controlled environments. Under these conditions, assimilation and transpiration rates generally decrease linearly as the stress intensifies (5, 6). For instance, Joly (1987) and Joly and Hahn (1989a), working with Amazonian and Trinitario cacao types, have reported significantly reduced assimilation rates when leaf water potentials drop below $-0,8$ MPa. Additionally, a delay and a reduction in leaf area expansion rates occur under conditions of water deficit (14). Assimilate translocation has also been reported to be affected to a large degree by leaf water potential reductions (7). Frimpong *et al.* (8), working under greenhouse conditions in Amazonian cacao types, found drought tolerant seedlings had higher specific leaf weights, capacity to retain apical buds and a larger root dry weight. However, there were no differences in photosynthesis rates, leaf water content and epicuticular

wax content between tested cultivars. In contrast, Joly and Hahn (1989a) found differences with respect to assimilation rate and water use efficiency during dry periods among the different cacao types previously mentioned.

Besides the scarce information on cacao responses to different stresses in adult stages, most described aspects have been evaluated, basically, on Forastero and Trinitario cacao (2, 6, 13). Very little is known about the physiological responses of the Criollo type under periods of water deficit and how these responses differ in relation to the other types. It is interesting to note that very little research has been directed towards understanding the mechanisms of cacao trees to survive different climatic stress conditions in the field. Furthermore, cacao responses to water stress reported in the literature are confusing and contradictory (10). Taking all this into account, the objective of the present work was to evaluate gas exchange and water relations of Guasare cacao (criollo type) trees subjected to periods of water deficit under field conditions.

Materials and methods

The study was carried out with four-year old Guasare cacao plants. The site was located at the INIA experimental station in San Juan de Lagunillas, Mérida, Venezuela ($08^{\circ} 31'N$, $71^{\circ} 71'W$), 1100 m above sea level. The soil has been classified as Cambortid, with an effective depth of 21 cm (18). This is a semi-arid region

with mean annual rainfall of 570 mm, mean annual evaporation of 2007 mm and a mean annual temperature of $22^{\circ}C$. Cacao trees planted at 3×3 m were interspersed with plantain plants (3×3) m and *Erythrina poeppigiana* trees (20×20) m. Cacao plantation was fertilised twice a year with 14-14-14 commercial NPK

fertiliser according to soil nutritional needs. Fertilisation was done at the beginning of the rainy seasons (April and September). Before this study began, cacao plants had been regularly watered every 3 days using the furrow method.

Gas exchange measurements, net photosynthesis (A), transpiration (E) and stomatal conductance (G_s), were registered using an infrared portable gas analyser system in the open mode (LCA4, ADC Ltd., Hoddington, England). Leaf water potential (Ψ_l) was measured with a pressure chamber. All measurements were simultaneously recorded, at two-hour intervals from 9:00 to 18:00 h, on plants that had been subjected to different water stress periods: three days, twelve days and twenty five days. Each treatment had 15 trees. Measurements were carried out, during the two following days after irrigation had been suspended, on the third or fourth leaf of the most recent flush in five plants selected randomly for each treatment. Leaf osmotic

potential at turgor loss (Ψ_π^0) was estimated in the laboratory using pressure-volume curves for six leaves from different individuals of each treatment (21, 22).

Microclimatic parameters such as photosynthetically active radiation (PAR), relative humidity and air temperature together with leaf temperatures were obtained simultaneously with gas exchange and leaf water potential measurements. Leaf and air temperature were determined using copper-constantan thermocouples and relative humidity with a digital hygrometer. Leaf and air temperatures, together with relative humidity were used to estimate vapor pressure difference between leaf and air (VPD). Integration of daily CO_2 assimilation and transpiration curves (from 9:00 until 18:00 h) were carried out in order to obtain total daily assimilation and transpiration (A_{tot} and E_{tot} , respectively) (17, 20). Total water use efficiency (WUE) was estimated as the ratio A_{tot}/E_{tot} .

Results

Both PAR and VPD values recorded were similar for all treatments throughout the day (figure 1.A). Maximum daily PAR ($750 \mu\text{mol m}^{-2}\text{seg}^{-1}$) occurred between 10:00-12:00 h decreasing to values between $50\text{-}200 \mu\text{mol m}^{-2}\text{seg}^{-1}$ late afternoon. Maximum VPD (2.45 KPa) were reached at midday (figure 1.B).

Plants unwatered for 25 days showed a marked reduction in G_s . In this treatment maximum G_s was

observed at 9:00 h ($35 \text{ mmol m}^{-2}\text{s}^{-1}$). G_s varied for the rest of the day between $15 \text{ mmol m}^{-2}\text{s}^{-1}$ and $30 \text{ mmol m}^{-2}\text{s}^{-1}$. Less stressed plants showed higher G_s , which ranged from 50 to $60 \text{ mmol m}^{-2}\text{s}^{-1}$ until 14:00 h declining gradually to $35 \text{ mmol m}^{-2}\text{s}^{-1}$ between 16:00-18:00 h (figure 1.C). Maximum E for plants watered every 3 and 12 days were obtained at 13:00 h ($2.5 \text{ mmol m}^{-2}\text{s}^{-1}$), thereafter E decreased progressively to less than 0.6 mmol

m^2s^{-1} . Severely stressed plants (25 days without water) showed the lowest E in each measurement ($1.4 \text{ mmol m}^{-2}\text{s}^{-1}$ and $0.5 \text{ mmol m}^{-2}\text{s}^{-1}$, maximum and minimum values, respectively) (figure 1.D).

Maximum A values were reached in early morning (between 1.5 and $2.2 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$) in all treatments, decreasing progressively the rest of day (figure 1.E). While, in general, lowest A were obtained for the 25 day treatment through out the daily course. Midday, Ψ_L of plants without watering for 25 days had dropped to -1.7 MPa while plants irrigated every 12 and 3 days decreased to approximately -1.4 and -1.2 MPa , respectively (figure 1.F).

Clear differences in soil moisture resulted in variations in water status for the three treatments, i.e. a decrease in minimum leaf water potentials (table 1). Additionally, a certain degree of osmotic adjustment was also observed, $\Psi\pi^{100}$ and $\Psi\pi^0$ decreased as water stress was more

severe (table 1).

As water stress increased, a significant decrease in leaf conductances was observed (table 2). This reduction in stomatal opening between treatments resulted in lower assimilation and transpiration rates for the stressed plants. The highest assimilation rates were obtained for plants watered every three days in spite of lower PAR values. It is interesting to note that CO_2 assimilation rates are significantly higher when only considering PAR values above $800 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$ (table 2).

Higher integrated WUE were obtained in stressed plants, 25 day treatment ($1.19 \text{ mmol mmol}^{-1}$). The values for the other treatments were lower (0.98 and $1.05 \text{ mmol mmol}^{-1}$). On the other hand, A_{tot} and E_{tot} values were lower as water stress increased (table 3). Total CO_2 assimilation decreased 11 and 26%, while an 18 and 39% reduction in water loss through transpiration was found for 12- and 25- day irrigation frequencies, respectively.

Discussion

Shade conditions given by *E. poeppigiana* trees and plantain plants lowered PAR between 700 and $1000 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$ compared to approximately $2000 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$ registered for the open field. Shade avoids high soil evaporation and reduces leaf transpiration rate, therefore a greater amount of water is available for longer periods. In the same area and soil type, Jaimez *et al.* (11) using a nine-day irrigation frequency in *Capsicum chinense*

plantations reported soil moisture water contents of 9-11%.

Reduced light intensity may explain the low CO_2 assimilation rates in our study, since assimilation rates between 2.4 and $3.5 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$ were obtained under PAR above $800 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$. CO_2 assimilation rates in non-stressed plants in our study were lower compared to those reported by Galyuon *et al.* (9) which ranged from 3.5 to $4 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$, Premachandra and Joly (19) from 4.3 to $5.2 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$.

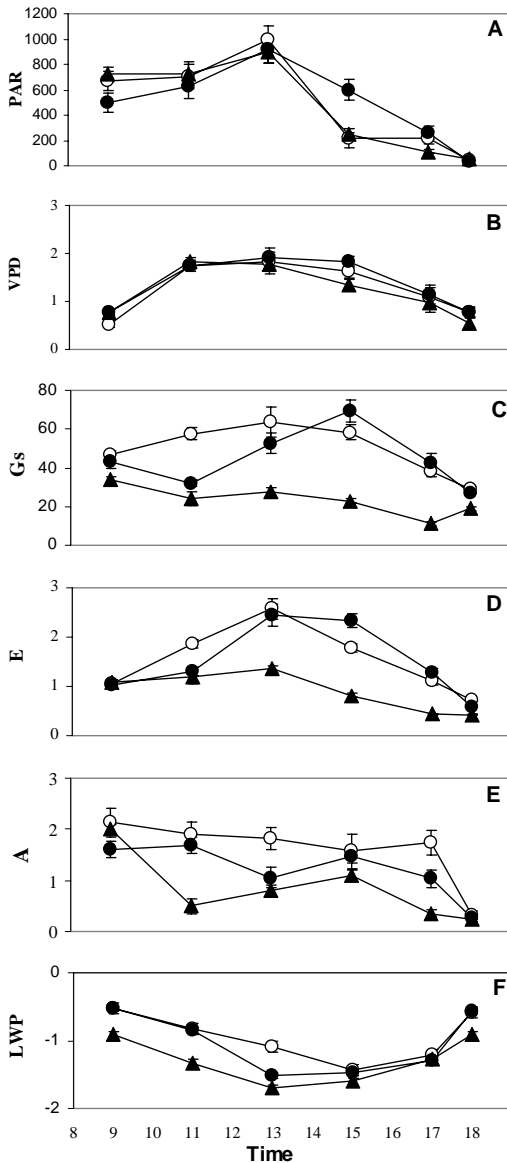


Figure 1. Daily cycle of: A. Photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2}\text{s}^{-1}$), B. Leaf-air vapor pressure difference (VPD, KPa), C. Stomatal conductance (G_s , $\text{mmol m}^{-2}\text{s}^{-1}$), D. Transpiration rate (E, $\text{mmol m}^{-2}\text{s}^{-1}$), E. CO_2 assimilation rate (A, $\mu\text{mol m}^{-2}\text{s}^{-1}$) and F. Leaf water potential (LWP, MPa) for four year old Guasare cacao trees irrigated every 3 (O), 12 (●) and 25 days (▲). Bars represent standad error.

Table 1. Minimum leaf water potential (Ψ_{\min} , MPa), osmotic potential at full turgor (Ψ_{π}^{100} , MPa), osmotic potential at turgor loss (Ψ_{π}^0 , MPa) and soil moisture (SM, %) for the three watering treatments in cacao plants. Values are means of six leaves \pm standard error.

Treatment	Ψ_{\min}	Ψ_{π}^{100}	Ψ_{π}^0	SM
3 days	-1.4	-1.54 \pm 0.10	-1.85 \pm 0.13	17.31
12 days	-1.5	-1.95 \pm 0.35	-2.48 \pm 0.31	13.60
25 days	-1.7	-2.18 \pm 0.29	-2.53 \pm 0.42	9.71

$^2\text{s}^{-1}$ and Joly and Hahn (1989b) between 2.7 and 3.7 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

A rapid osmotic adjustment was observed between 3 and 12 days (0.63 MPa difference). This mechanism permits Guasare cacao maintain turgor under unfavorable conditions, mainly at midday when the air evaporative demand is greater. It is interesting to note that after 12 days, this species does not seem to be able to increase its capacity to osmotically adjust. These results contrast those studies with cacao juvenile plants where osmotic adjustment does not play a role as a physiological mechanism to adapt during water stress periods (19). On the other hand, although osmotic adjustment

increased as water stress was intensified from 3 to 12 days, CO_2 assimilation rates were not maintained to the same levels due to partial stomatal closure. Similar results have been obtained for coffee plants (1, 16). Moreover, severe water stress produces stomatal closure, which surely affects other physiological processes such as photosynthesis (7, 15).

Lower Ψ_{π}^{100} found with a decrease in irrigation frequency differs from that reported by Premachandra and Joly (1991). These authors found similar results after 14 days without watering cacao juvenile plants. However, a progressive decrease in Ψ_{π}^{100} was reported after

Table 2. Mean photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2}\text{s}^{-1}$), CO_2 assimilation rate (A, $\text{mmol m}^{-2}\text{s}^{-1}$), assimilation rate at PAR above 800 $\mu\text{mol m}^{-2}\text{s}^{-1}$ ($A_{>800}$), transpiration rate (E, $\text{mmol m}^{-2}\text{s}^{-1}$) and stomatal conductance (G_s , $\text{mmol m}^{-2}\text{s}^{-1}$) for the three watering treatments. Values are means of two days \pm standard error (n=13).

Treatment	PAR	A	$A_{>800}$	E	G_s
3 days	355.8 \pm 31.8	1.57 \pm 0.11	3.51 \pm 0.08	1.38 \pm 0.05	46.5 \pm 1.50
12 days	416.3 \pm 33.5	1.25 \pm 0.06	3.24 \pm 0.05	1.33 \pm 0.05	33.1 \pm 0.38
25 days	483.4 \pm 35.5	1.06 \pm 0.07	2.38 \pm 0.04	0.85 \pm 0.03	24.0 \pm 0.08

Table 3. Daily total assimilation (A_{tot} , mmol m^{-2}), transpiration (E_{tot} , mol m^{-2}) and water use efficiency (WUE, $\text{mmol CO}_2/\text{mol H}_2\text{O}$) for the three watering treatments in Guasare cacao plants.

Treatment	A_{tot}	E_{tot}	WUE
3 days	53.1	54.0	0.98
12 days	47.0	44.5	1.06
25 days	39.5	33.3	1.19

18 days. They also reported about 80% reduction in CO_2 assimilation after 14 days.

The reduction in CO_2 assimilation is also associated with partial stomatal closure, which was maintained throughout the day in plants without watering for 25 days. The reduction in stomatal aperture was related to severe water stress. Stomatal closure reduced water loss through transpiration to a much

greater degree compared to the effects on CO_2 assimilation rates. Joly and Hahn (1989a) reported no increase in WUE as water stress intensified even though CO_2 and water vapor exchange rates underwent large displacements. In our study, WUE increased, as drought was more severe. This supports the idea that water losses are more affected than CO_2 assimilation in these plants.

Acknowledgements

We thank the staff of the INIA Experimental Station in San Juan de Lagunillas (Mérida, Venezuela) for their support. Special thanks to Gladys Ramos, Director of the station,

for her assistance. This research was supported by a FONACIT grant (#96001-500) and a CDCHT-ULA grant (#FO-499-99B).

Literature cited

- Almeida A.A.F. and M. Maestri. 1997. Photosynthetic oxygen evolution by four *Coffea arabica* genotypes subjected to a dehydration/rehydration cycle. *J. Hort. Sci.* 72: 593-599.
- Alvim P. de T. 1977. Cacao. p. 279-313. *In*: P de T Alvim, and T.T. Kozlowski (Eds.), *Ecophysiology of tropical crops*. New York, Academic Press.
- Alvim P. de T. 1981. Recent studies on environmental physiology of cacao. p. 85-89. *Proceedings of the 7th International Cacao Research Conference*, Lagos, Kenya.
- Balasimha D. 1999. Stress physiology of cocoa. *J. Plantation Crops* 27: 1-8.
- Balasimha D. and V. Rajagopal. 1988. Stomatal responses of cocoa (*Theobroma cacao*) to climatic factors. *Indian J. Agric. Sci.* 58: 213-216.

6. Balasimha D., E.V. Daniel and P.G. Bhat. 1991. Influence of environmental factors on photosynthesis in cocoa trees. *Agric. Forest Meteorology* 55: 15-21.
7. Deng X., R. Joly and D. Hahn. 1990. The influence of plant water deficit on distribution of ^{14}C -labelled assimilates in cacao seedlings. *Ann. Bot.* 66: 211-217.
8. Frimpong E., Y. Adu-Ampomah and A. Karimu. 1996. Efforts to breed for drought resistant cacao in Ghana. p. 24-25. Proceedings of the 12th International Cacao Research Conference, Bahía, Brasil.
9. Galyoun I.K.A., C.R. McDavid, F.B. López and J.A. Spence. 1996. The effect of irradiance level on cacao (*Theobroma cacao* L.): II Gas exchange and chlorophyll fluorescence. *Trop. Agric.* 73: 29-33.
10. Hadley P and S. Pearson. 1996. A physiologist's view of cacao yield. p. 194-209. Proceedings of the Malaysian International Cacao Conference, Kuala Lumpur, Malaysia.
11. Jaimez R., F. Rada and C. García-Núñez. 1999. The effects of irrigation frequency on water and carbon relations in three cultivars of sweet pepper (*Capsicum chinense*, Jacq) in a tropical semiarid region. *Sci. Hortic.* 81: 301-308.
12. Joly R. 1987. Physiological adaptations for maintaining photosynthesis under water stress in cacao. p. 199-203. Proceedings of the 10th International Cacao Research Conference, Santo Domingo, Dominican Republic.
13. Joly R. and D. Hahn. 1989a. Net CO_2 assimilation of cacao seedlings during periods of plant water deficit. *Photosynth. Res.* 21: 151-159.
14. Joly R. and D. Hahn. 1989b. An empirical model for leaf expansion in cacao in relation to plant water deficit. *Ann. Bot.* 64: 1-8.
15. Kaiser W.M. 1982. Correlation between changes in photosynthetic activity and changes in total protoplasm volume in leaf tissue from hydro-, meso and xerophytes under osmotic stress. *Planta* 153: 430-435.
16. Kumar D. and L.L. Tieszen. 1980. Photosynthesis in *Coffea arabica*. II. Effects of water stress. *Exp. Agr.* 6: 21-27.
17. McCree K.J., C.E. Kallsen and S.G. Richardson. 1984. Carbon balance in sorghum plants during osmotic adjustment to water stress. *Plant Physiol.* 76: 898-902.
18. Ochoa G. and D. Malagón. 1979. Atlas de microscopía electrónica en suelos de Venezuela. ULA - CIDIAT. Mérida, 40 p.
19. Premachandra G. and R. Joly. 1991. Leaf water relations, net CO_2 assimilation stomatal conductance and osmotic concentration as affected by water deficit in cacao seedlings. p. 1-8. Proceedings of the International Cacao Research Conference, Kuala Lumpur, Malaysia.
20. Rada F., A. Azócar, B. Briceño, J. González and C. García-Núñez. 1996. Carbon and water balance in *Polylepis sericea*, a tropical treeline species. *Trees* 10: 218-222.
21. Tyree M. and H. Hammel. 1972. The measurement of the turgor pressure and the water relations of plants by the pressure bomb technique. *J. Exp. Bot.* 23: 267-282.
22. Tyree M. and H. Richter. 1981. Alternative methods of analyzing water potential isotherms: some cautions and clarifications. *J. Exp. Bot.* 32: 643-653.