

Ethylene Production and Physiological Parameters in Common Bean Plants Under Water Deficit

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Received: October 22, 2018

Accepted: December 14, 2018

Online Published: February 15, 2019

doi:10.5539/jas.v11n3p301

URL: <https://doi.org/10.5539/jas.v11n3p301>

The research was financed by São Paulo Research Foundation (Fapesp—grant number 2014/13442-4).

Abstract

Common bean is a plant species cultivated around the globe. The objective of this study was to demonstrate how the physiological parameters associated with transpiration, photosynthesis and ethylene production vary in common bean plants under different periods without irrigation. Three experiments were carried out to assess leaf water potential, stomatal conductance, transpiration, carbon assimilation, and ethylene production as a function of different irrigation intervals. A fourth experiment with exogenous application of abscisic acid (ABA) in plants with daily irrigation was also carried out. In all experiments there was a reduction in stomatal conductance at the very early period without irrigation, which consequently influenced transpiration and carbon assimilation. It was also verified that the exogenous application of ABA caused a reduction on ethylene production in common bean plants. The relationship between increased ABA production and the reduction of ethylene production is discussed.

Keywords: *Phaseolus vulgaris*, C₂H₄, photosynthesis, irrigation, water stress

1. Introduction

A large amount of world's common bean production is made under conditions susceptible to water deficit along the crop life cycle. In a study on the *in vivo* photosynthetic parameters characterization, Pimentel et al. (2011) concluded that at 25 °C, the BRS-Pérola cultivar of common bean plants at the phenological stage R₅ and in optimal water conditions presented a maximum carbon assimilation rate (A_{max}) of 20 μmol[CO₂] m⁻² s⁻¹ at an atmospheric concentration of carbon dioxide equal to 380 μmol[CO₂] mol⁻¹ of air, and was saturated at the rate of 25 μmol[CO₂] m⁻² s⁻¹ at an atmospheric concentration of carbon dioxide between 600 and 800 μmol[CO₂] mol⁻¹ of air, with a maximum stomatal conductance (g_s) of 0.4 mol[H₂O] m⁻² s⁻¹. The authors also pointed out that the values recorded for these parameters in such conditions were close to the values presented by varieties used in the United States, which have a different center of origin.

Reductions in carbon assimilation over the course of the day in common bean plants under optimal soil water conditions are mainly due to stomatal closure. Ramalho et al. (2013) demonstrated this fact by exposing three different bean varieties to atmosphere with enhanced CO₂ concentration during the light period of the day, which led the plants to maintain A_{max}, indicating that the increase of the CO₂ concentration in the atmosphere exceeded the low conductance and that the reduction in photosynthesis during this period was only due to stomatal closure and not due to biochemical inhibition processes.

Ramalho et al. (2013) also pointed to the fact that reductions in stomatal conductance in plants under moderate drought conditions were followed by a large increase in internal CO₂ concentration. Therefore, in addition to the

low stomatal conductance, the reduction of the photosynthesis values may be due to low mesophyll conductance and, photochemical and biochemical limitations (reduction in the amount and/or activity of ribulose-1,5-bisphosphate carboxylase/oxygenase [RuBisCO] and other enzymes, and decrease in ribulose-1,5-bisphosphate [RuBP] regeneration due to reducing the supply of adenosine triphosphate [ATP] and nicotinamide adenine dinucleotide phosphate [NADPH] to the photosynthetic cycle of carbon reduction).

One of the topics responsible for many questions in literature regarding water deficit may be related to the production of ethylene (C₂H₄) in plants that are under this condition (Hsiao 1973; Morgan & Drew, 1997). A good example of the complexity of the subject can be observed in experiments conducted by Xu and Qi (1993), where the gradual and slow reduction of soil water content in soybean plants did not cause an increase in the levels of ethylene or its precursor—1-aminocyclopropane-1-carboxylic acid (ACC)—while the rapid and acute reduction of soil water promoted an increase of both. Thus, it can be deduced that one of the determining factors in the production of ethylene is the rapid decrease of the water potential of the plant (Morgan & Drew, 1997).

When analyzing the ethylene contents produced by common bean plants exposed to water deficit, Morgan et al. (1990) observed that the gas values in these plants were much lower than those obtained in plants that were submitted to optimal conditions of soil water. The authors also measured hormone levels after irrigation of pots under water deficit and did not observe an increase in C₂H₄ production.

According to Narayana, Lalonde, and Saini (1991), in studies with wheat plants, there were also no increase in ethylene production in plants under stress due to water deficit. The authors also pointed out that there may even be a reduction in ethylene production in plants submitted to low water potentials and that the results vary greatly according to the methodology used.

On the other hand, Apelbaum and Yang (1981), using the methodology described by Wright (1977)—in which the detached leaves are exposed to rapid dehydration—observed an increase in ethylene production in wheat plants, peaking at a maximum of 9% loss of fresh leaf mass and then declining. Such behavior was accompanied by the increase in the concentration of ACC in the leaves. However, they did not observe increase in the concentrations of S-adenosylmethionine (SAM), which is the precursor of the ACC, which led the authors to believe that the increase of the ethylene quantity was due to the increase in the production of ACC that was caused by a *de novo* synthesis of ACC synthase, responsible for the conversion of SAM into ACC.

Morgan and Drew (1997), based on what has been described by Apelbaum and Yang (1981), Wright (1977), Aharoni (1978) and Morgan et al. (1990), argued that it is clear that rapid dehydration of leaves leads to an increase in ethylene biosynthesis.

Based on the above information, the objective of this study was to evaluate the water status of plants, the change in gas exchange variables (stomatal conductance, transpiration and carbon assimilation) and the ethylene production in common bean plants in response to water deficit, as well as the influence of abscisic acid (ABA) on the production of ethylene.

2. Method

Four experiments were carried out in the years 2014, 2015 and 2016 in greenhouses in Piracicaba-SP, Brazil (22°42' S, 47°30' W). Three of them related to the effect of water deficit on ethylene production and gas exchange variables (stomatal conductance, transpiration and carbon assimilation) and one experiment about the influence of exogenous application of ABA on the production of ethylene.

2.1 Experiment I

In experiment I, common bean plants of the Carioca group (variety IAC-Imperador) were grown in 9 L pots. The experiment was composed of seven treatments organized in a completely randomized design with six replicates, where each plot was represented by one pot. Treatments were distributed in a way to make combinations between three different irrigation intervals (2, 4 and 6 days without irrigation) and two different phenological stages (V₄ and R₆) (Fernández, Geptz, & López, 1982).

Treatment T1 (control) was maintained at the soil water content corresponding to field capacity with daily irrigation. Treatments T2, T3 and T4 were also maintained at field capacity until the phenological stage V₄ and then submitted to 2, 4 and 6 days, respectively, without irrigation, and treatments T5, T6 and T7 were also maintained at field capacity until phenological stage R₆ and then submitted to 2, 4 and 6 days, respectively, without irrigation.

The following parameters were measured: (i) leaf water potential, (ii) stomatal conductance, (iii) transpiration, (iv) carbon assimilation and (v) ethylene production.

To evaluate leaf water potential, a SAPS II 3115 equipment (Soil moisture-Santa Barbara, CA, USA) was used (Scholander et al., 1965). A trifoliolate leaf was collected at each replicate of each treatment, for a total of six replicates. The measurements were performed at noon.

The evaluations of stomatal conductance, transpiration and carbon assimilation were performed with IRGA (infrared gas analyzer—ADC Scientific LCPro+, Hertfordshire, UK) equipment. Measurements were made between 11:00 a.m. and 12:00 p.m. The PPFD (photosynthetic photon flux density) used for IRGA evaluation was $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$, which corresponds to PPFD of Piracicaba (SP) at noon of a summer day (Ribeiro et al., 2004). In the six pots of each treatment, readings were performed on a photosynthetically active and fully expanded trifoliolate leaf and five readings were recorded per trifoliolate leaf. The equipment was stabilized at each reading of a new trifoliolate leaf.

The methodology for evaluation of ethylene production used was described by Narayana, Lalonde and Saini (1991) and is an adaptation of the methodology developed by Wright (1977). The procedure consisted in the removal of a trifoliolate leaf from each of the six replicates of each treatment, which were stacked to remove a total of thirty-six discs (six replicates of six discs). Soon after, the discs were stored in 15-mL conical bottom centrifuge tubes with silicone-sealed lids for 2 hours. Determinations of the amounts of ethylene were made by gas chromatography. Ethylene standard gas was used to draw the standard curve and the carrier used for the analysis was nitrogen. After two hours storage of the leaf discs inside the tubes, 1 mL of the gas atmosphere present in the tubes was injected into the chromatograph equipment (TraceGC Thermo Scientific, Waltham, USA).

2.2 Experiment II

In experiment II, common bean plants of the Carioca group (variety IAC-Imperador) were grown in 3 L pots. The experiment was composed of seven treatments organized in a randomized complete block design with four replicates, where each plot was composed by four pots. Treatments consisted of six different irrigation intervals (1, 3, 5, 7, 9 and 11 days without irrigation) at the beginning of the phenological stage R_5 (pre-flowering) (Fernández, Geptz, & López, 1982), besides a control treatment with daily irrigation. All treatments were maintained at soil water content corresponding to field capacity of the soil used when not at the moment of submission to the water deficit. Treatments are described by numbers from 1 to 7: control (T1) (daily irrigation), and 1 (T2), 3 (T3), 5 (T4), 7 (T5), 9 (T6) and 11 (T7) days without irrigation starting at the phenological stage R_5 .

The following parameters were evaluated: (i) leaf water potential (at 05:00 a.m., 11:00 a.m., 07:00 p.m. and 01:00 a.m.); (ii) stomatal conductance (at 07:00 a.m., 11:00 a.m. and 03:00 p.m.); (iii) transpiration (at 07: a.m., 11:00 a.m. and 03:00 p.m.); and (iv) ethylene production. The methodologies used in the evaluations were the same as described for experiment I, with the exception that the IRGA equipment was replaced by a LiCor 6400 with a PPFD of $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$.

2.3 Experiment III

In experiment III, common bean plants of the Carioca group were grown in 3 L pots, using the varieties IAC-Imperador and IAC-Milênio. The experiment was composed of ten treatments in a 2×5 factorial scheme, organized in a randomized complete block design with four replicates, where each plot was composed by four pots. The first factor was the varieties used and the second factor was the six different irrigation intervals (4, 8, 12 and 16 days without irrigation) at the beginning of the phenological stage R_5 (pre-flowering) (Fernández, Geptz, & López, 1982), besides a control treatment with daily irrigation for both varieties. All treatments were maintained at a soil water content corresponding to field capacity of the soil used when not at the moment of submission to the water deficit.

The following parameters were evaluated: (i) leaf water potential (at 06:00 a.m.); (ii) stomatal conductance (at 10:00 a.m.); (iii) transpiration (at 10:00 a.m.); (iv) carbon assimilation (at 10:00 a.m.); and (v) ethylene production. The methodologies used in the evaluations were the same as described for experiment I, with the exception that the IRGA equipment that was replaced by a LiCor 6400 with a PPFD of $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$.

2.4 Experiment IV

Experiment IV was carried out with common bean plants of the Carioca group (variety IAC-Imperador), grown in 3 L pots. The experiment was composed of four treatments organized in a randomized complete block design with four replicates, each plot being represented by four pots. Treatments were based on the application of ABA (Sigma-Aldrich-purity $\geq 98.5\%$) at concentrations of 0 (T1: control-no ABA application), 1 (T2), 10 (T3) and

100 (T4) $\mu\text{M.L}^{-1}$ of ABA at the phenological stage R_5 (pre-flowering) (Fernández, Geptz, & López, 1982). All treatments were maintained at field capacity.

In this experiment, ethylene production was exclusively evaluated in plants 24, 48 and 72 h after the exogenous application of ABA, using the same methodology described in experiment I.

2.5 Statistical Analysis

In experiment I, as the vegetative and reproductive stages of the crop were not submitted at the same time to water deficit, the variables were statistically analyzed using the *t*-test ($\alpha = 0.05$), comparing each irrigation interval with the control treatment only. All data were submitted to the Arlington-Darling, Breusch Pagan and Bartlett tests to verify if the residues met the assumptions of the *t*-test. All analyses were performed using the R software (R Development Core Team 2013).

For all variables from experiments II, III and IV, ANOVA ($\alpha = 0.05$) was performed. Arlington-Darling normality test and Bartlett homogeneity test were performed to verify if the residues met the assumptions of the ANOVA. When not framed within normality or homogeneity, the data were transformed through the equation $[\sin(x)]^{1/2}$ for experiment II and the equation $(x)^{1/2}$ for experiment III. When treatments presented p -value < 0.05 , a Tukey test ($\alpha = 0.05$) was performed. All analyses were made using the R software (R Development Core Team, 2013).

3. Results

Experiment I represented a period of no irrigation for maximum of 6 days for both vegetative and reproductive phases of the crop. For the leaf water potential variable, a difference ($p < 0.05$) was observed for treatments T3, T5, T6 and T7 when compared to treatment T1 (Table 1).

Table 1. Experiment I: leaf water potential (ψ , MPa), stomatal conductance (gs, $\text{mmol}[\text{H}_2\text{O}] \text{m}^{-2} \text{s}^{-1}$), transpiration (T, $\text{mmol}[\text{H}_2\text{O}] \text{m}^{-2} \text{s}^{-1}$), carbon assimilation (A, $\mu\text{mol}[\text{CO}_2] \text{m}^{-2} \text{s}^{-1}$) and ethylene production (E, $\mu\text{L}[\text{C}_2\text{H}_4] \text{kg}^{-1}[\text{DM}] \text{h}^{-1}$) of common bean plants (variety IAC-Imperador) from the treatments of experiment I, measured on midday. The values of plants of each treatment under water deficit (T2 to T7) were compared with the values of the plants of the control treatment (T1)

Description	ψ	gs	T	A	E
T1: Daily irrigation	-0.9a ¹	199.0a	7.9a	8.4a	2.7a
T2: 2 days without irrigation starting at V_4	-0.9a	45.0b	2.7b	0.8b	3.0a
T1: Daily irrigation	-1.3a	282.7a	10.4a	11.2a	3.8a
T3: 4 days without irrigation starting at V_4	-1.0b	18.9b	1.1b	0.3b	2.2a
T1: Daily irrigation	-0.9a	936.0a	7.7a	18.9a	8.1a
T4: 6 days without irrigation starting at V_4	-0.7a	18.3b	0.5b	0.8b	1.3b
T1: Daily irrigation	-0.7a	603.7a	4.5a	14.6a	2.9a
T5: 2 days without irrigation starting at R_6	-1.0b	125.0b	1.3b	4.1b	2.3a
T1: Daily irrigation	-1.4a	46.7a	1.9a	3.0a	4.4a
T6: 4 days without irrigation starting at R_6	-0.9b	4.4b	0.1b	-0.1a	2.0b
T1: Daily irrigation	-1.2a	34.4a	1.5a	0.8a	3.1a
T7: 6 days without irrigation starting at R_6	-0.8b	5.9b	0.2b	-0.1a	1.5b

Note. ¹ Values followed by the same letter were not statistically different by the *t* test ($\alpha = 0.05$).

For the variables stomatal conductance and transpiration, there was a difference ($p < 0.05$) for all treatments compared to the control treatment, and in all cases, the highest values of the variables appeared in the daily irrigation treatment (T1). For the carbon assimilation variable, there was no difference ($p > 0.05$) for the T1 \times T6 and T1 \times T7 comparisons. Similar to stomatal conductance and transpiration, the highest values of carbon assimilation were also observed for the control treatment with daily irrigation (T1) (Table 1 and Figure 1).

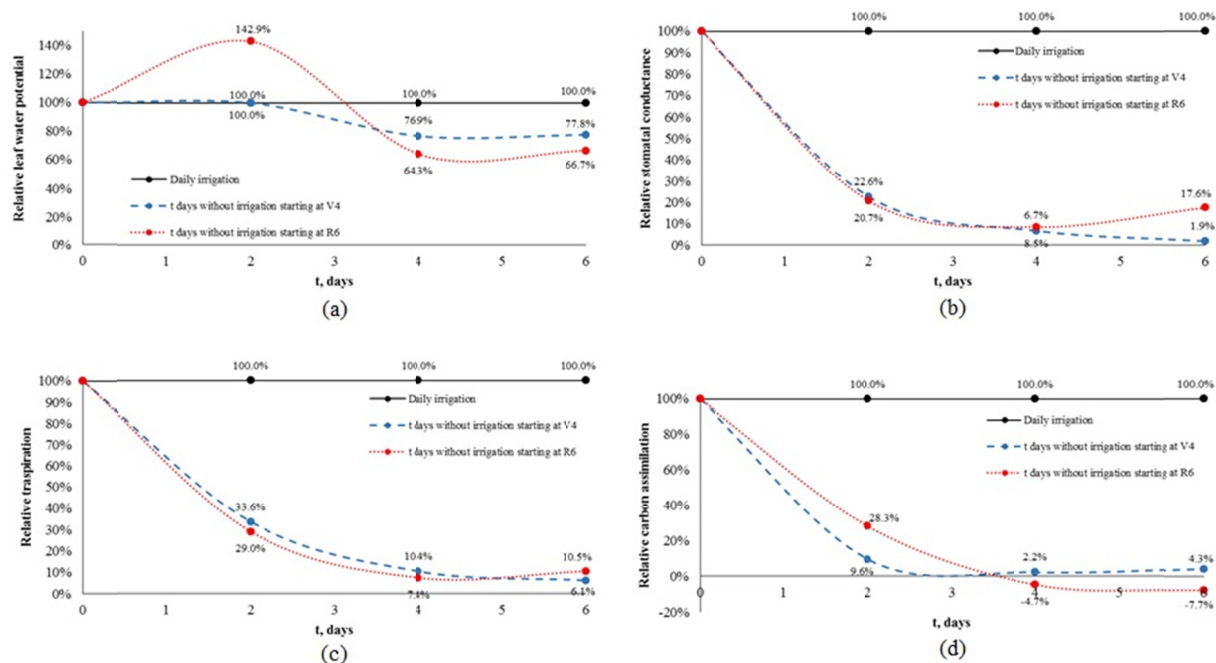


Figure 1. Relative values of (a) leaf water potential (%), (b) stomatal conductance (%), (c) transpiration (%) and (d) carbon assimilation (%) of common bean plants (variety IAC-Imperador) from the treatments of experiment I, measured on midday. The values of each treatment under water deficit were compared with the values of the plants of the control treatment. Experiment I

For the ethylene production variable, no increase in ethylene production as a function of the irrigation interval was observed in any of the comparisons (Table 1 and Figure 2). A difference ($p < 0.05$) was observed for treatments T4, T6 and T7 when compared to treatment T1 (daily irrigation), and this treatment was always higher than the others.

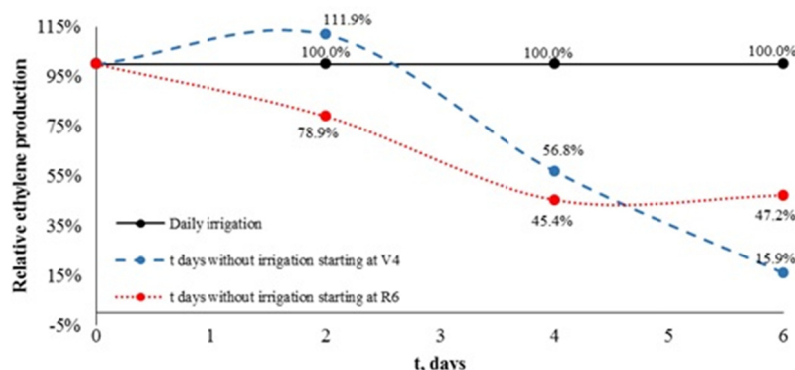


Figure 2. Relative ethylene production (%) of common bean plants (variety IAC-Imperador) from the treatments of experiment I, measured on midday. The values of each treatment under water deficit were compared with the values of the plants of the control treatment

Experiment II presented a maximum period without irrigation of 11 days imposed only during the reproductive phase of the crop. For the leaf water potential, no difference ($p > 0.05$) was observed between treatments (Table 2). In addition, analyzing the data of the control treatment (T1), it is noticed that the maximum value for the leaf water potential was obtained at 05:00 a.m., while the minimum value was found at 07:00 p.m. It is also noticed that in the period from 07:00 p.m. to 01:00 a.m. of the following day there was a recovery of the turgidity of the plants, increasing the value of the water potential (Figure 3).

Table 2. Experiment II: leaf water potential (ψ , MPa), stomatal conductance (g_s , $\text{mmol}[\text{H}_2\text{O}] \text{m}^{-2} \text{s}^{-1}$), transpiration (T , $\text{mmol}[\text{H}_2\text{O}] \text{m}^{-2} \text{s}^{-1}$) and ethylene production (E , $\mu\text{L}[\text{C}_2\text{H}_4] \text{kg}^{-1}[\text{DM}] \text{h}^{-1}$) of common bean plants (variety IAC-Imperador), as a function of number (n) of days without irrigation (treatment T1-Control (daily irrigation) to T7) starting at R_5 , measured along different times of the day

n	ψ				g_s			T			E
	05:00a.m.	11:00a.m.	07:00p.m.	01:00a.m.	07:00a.m.	11:00a.m.	03:00p.m.	07:00a.m.	11:00a.m.	03:00p.m.	12:00p.m.
0 (T1)	-0.2a ¹	-0.3a	-0.7a	-0.3a	9.8a	12.5a	8.0a	0.4a	0.7a	0.6a	1.5a
1 (T2)	-0.5a	-0.4a	-0.8a	-0.5a	4.2ab	4.4b	6.0ab	0.2ab	0.3b	0.5ab	0.5ab
3 (T3)	-0.5a	-0.5a	-0.7a	-0.6a	2.2b	2.0b	3.1ab	0.9b	0.1b	0.2ab	0.3bc
5 (T4)	-0.4a	-0.4a	-0.8a	-0.4a	2.6b	1.5b	2.8b	0.1b	0.1b	0.2ab	0.4ab
7 (T5)	-0.5a	-0.5a	-0.7a	-0.6a	2.9b	2.9b	3.2ab	0.1b	0.2b	0.2ab	0.3abc
9 (T6)	-0.5a	-0.5a	-0.7a	-0.6a	2.7b	2.4b	3.9ab	0.1b	0.1b	0.3ab	0.2bc
11 (T7)	-0.5a	-0.5a	-0.8a	-0.6a	2.0b	1.4b	2.2b	0.1b	0.1b	0.2b	0.1c

Note. ¹Values followed by the same letter were not statistically different by the Tukey test ($\alpha = 0.05$).

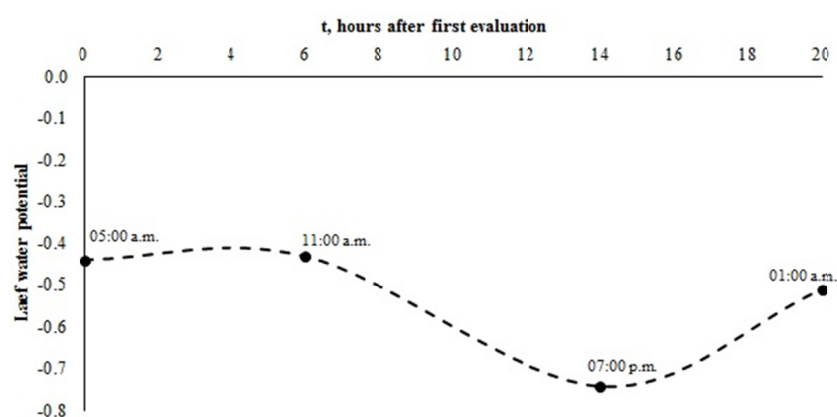


Figure 3. Mean values of leaf water potential (ψ , MPa), measured along different times of the day, of common bean plants (variety IAC-Imperador) under different number of days without irrigation (treatment T1-Control (daily irrigation) to T7) starting at R_5 . Experiment II

For the variables stomatal conductance and transpiration, it is observed that both presented similar statistical behavior throughout the three evaluations. For the 7 a.m. evaluation, treatment T1 was superior ($p < 0.05$) to treatments T3, T4, T5, T6 and T7 for both variables. For the 11:00 a.m. evaluation, the control treatment (daily irrigation) was superior ($p < 0.05$) to all other treatments for both stomatal conductance and transpiration. For the 03:00 p.m. evaluation, treatment T1 was statistically superior ($p < 0.05$) only to treatments T4 and T7 for stomatal conductance, whereas for treatment T1, transpiration was superior ($p < 0.05$) only in relation to treatment T7 (Table 2).

For the ethylene production variable, it was observed that there was no increase in the hormone production with longer periods without irrigation and that the control treatment with daily irrigation showed a higher value ($p < 0.05$) than treatments T3, T6 and T7 (Table 2).

Experiment III presented a maximum period without irrigation of 16 days imposed only during the reproductive phase of two varieties of different growth characteristics. For leaf water potential, no interaction or difference ($p > 0.05$) was observed between the irrigation interval and variety factors (Table 3 and Figure 4).

Table 3. Experiment III: leaf water potential (ψ , MPa), stomatal conductance (g_s , $\text{mmol}[\text{H}_2\text{O}] \text{ m}^{-2} \text{ s}^{-1}$), transpiration (T , $\text{mmol}[\text{H}_2\text{O}] \text{ m}^{-2} \text{ s}^{-1}$) and carbon assimilation (A , $\mu\text{mol}[\text{CO}_2] \text{ m}^{-2} \text{ s}^{-1}$) of common bean plants (mean values for IAC-Milênio and IAC-Imperador varieties)

Description	ψ	g_s	T	A
Control (daily irrigation)	-2.4a ¹	21.2a	0.8a	5.4a
4 days without irrigation starting at R ₅	-2.6a	14.1ab	0.5ab	3.9ab
8 days without irrigation starting at R ₅	-3.8a	14.9ab	0.6ab	3.3ab
12 days without irrigation starting at R ₅	-3.4a	11.1b	0.4b	2.8b
16 days without irrigation starting at R ₅	-3.2a	10.7b	0.4b	2.9b

Note. ¹Values followed by the same letter were not statistically different by the Tukey test ($\alpha = 0.05$).

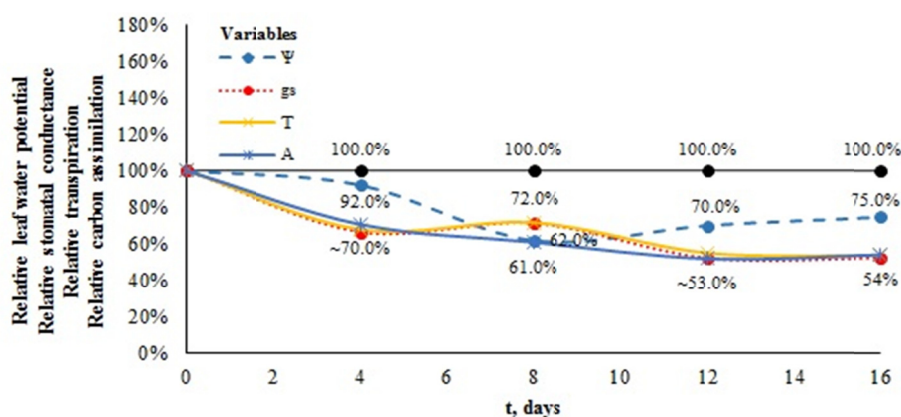


Figure 4. Relative values of leaf water potential (%), stomatal conductance (%), transpiration (%) and carbon assimilation (%) of common bean plants (mean values of varieties IAC-Milênio and IAC-Imperador) from the treatments of experiment III

For the variables stomatal conductance, transpiration and carbon assimilation, it was observed that there was no interaction ($p > 0.05$) between the factors. For the different irrigation intervals, a difference ($p < 0.05$) was found between the treatments, and the control treatment with daily irrigation was higher than treatments with 12 and 16 days of irrigation interval (Table 3 and Figure 4).

For ethylene production there was interaction ($p < 0.05$) between the factors irrigation intervals and varieties. For the variety IAC-Milênio, it was observed that the control treatment with daily irrigation was superior ($p < 0.05$) to the treatment with 16 days without irrigation, whereas for the variety IAC-Imperador, the control treatment was superior ($p < 0.05$) to all other irrigation intervals. In addition, IAC-Imperador presented a higher value ($p < 0.05$) than the cultivar IAC-Milênio for the treatment with daily irrigation (Table 4).

Table 4. Experimento III: ethylene production ($\mu\text{L}[\text{C}_2\text{H}_4] \text{ kg}^{-1}[\text{DM}] \text{ h}^{-1}$) of common bean plants (varieties IAC-Milênio and IAC-Imperador)

Description	Ethylene production	
	IAC-Milênio	IAC-Imperador
Control (daily irrigation)	1.6aB ¹	12.4aA
4 days without irrigation starting at R ₅	0.7abA	1.4bA
8 days without irrigation starting at R ₅	0.6abA	0.7bA
12 days without irrigation starting at R ₅	0.6abA	0.7bA
16 days without irrigation starting at R ₅	0.3bA	0.5bA

Note. ¹Values followed by the same lowercase letter were not statistically different in the columns by Tukey test ($\alpha = 0.05$); values followed by the same uppercase letter were not statistically different in the lines by Tukey test ($\alpha = 0.05$).

For experiment IV, there were differences in exogenous ABA concentration applied to the plants. For ethylene production it was observed that in the evaluation 24h after application, treatment T1 was superior ($p < 0.05$) to all treatments. For the evaluation of 48 hours after exogenous application of ABA, treatment T1 was superior ($p < 0.05$) to treatments T2 and T4. Finally, for the evaluation of 72 hours after ABA application, the control treatment was higher ($p < 0.05$) only in relation to the treatment with higher concentration of abscisic acid (Table 5).

Table 5. Experiment IV: ethylene production ($\mu\text{L}[\text{C}_2\text{H}_4] \text{ kg}^{-1}[\text{DM}] \text{ h}^{-1}$) of common bean plants from the treatments of experiment IV, after 24, 48 and 72 hours of exogenous application of ABA

Treatment	Description	Ethylene production		
		24 h	48 h	72 h
T ₁	Control (no ABA application)	11.7 a ¹	14.4 a	18.8 a
T ₂	1 μM of ABA in R ₅	6.8 b	8.3 b	19.1 a
T ₃	10 μM of ABA if R ₅	2.3 d	13.5 a	14.0 ab
T ₄	100 μM of ABA in R ₅	4.1 c	5.5 c	9.5 b

Note.¹ Values followed by the same letter were not statistically different by the Tukey test ($\alpha = 0.05$).

4. Discussion

Results show that for experiments I, II and III, the water deficiency caused by the different irrigation intervals did not result in an increase in the production of ethylene, and in many cases there was even a reduction in the production of this hormone when compared to the treatment with daily irrigation. These results corroborate with several published studies. Morgan et al. (1990) also demonstrated that there was no increase of the hormone in plants submitted to low levels of water in the soil. The authors evaluated the gas exchange of common bean plants in a closed system and measured the ethylene levels of the system's gaseous atmosphere. Neither during the stress period, nor after rehydration, had the plants showed an increase in ethylene levels.

The same situation was observed for other plant species as well. In wheat, Narayana, Lalonde, and Saini (1991) showed that there was also no increase in ethylene production in plants under low soil water content and that in some situations there may even be a reduction in ethylene production in the plants submitted to low water potential.

According to Narayana, Lalonde, and Saini (1991), the methodology used for the evaluations greatly influences the result of ethylene production. When analyzing ethylene production in plants within a closed system or removing discs from leaves of plants under water deficit, the results did not indicate an increase in ethylene production by the plants. However, when using the methodology described by Wright (1977), which consists of the removal of leaf discs from plants under optimal conditions of soil water content and dehydrating them to simulate stress due to water deficit, there was an increase in the levels of ethylene production.

One of the possible explanations for the decrease of ethylene content in plants under stress due to water deficit is the inhibition of the production of ACC (1-aminocyclopropane-1-carboxylic acid, precursor of the hormone) by the production of abscisic acid (ABA) (Hoffman et al., 1982). Sharp (2002), in a review about the subject, pointed out that endogenous ABA could play an important role in inhibiting ethylene production, resulting in the maintenance of root and shoot growth under stress. Spollen et al. (2000), in an experiment with mutant corn seedlings that were deficient in ABA production, observed that they had higher rates of ethylene production when under stress due to water deficit, and had different morphology of the root system. On the other hand, the exogenous application of ABA in mutant seedlings restored root normality and inhibited the effect of ethylene. The results presented in experiment IV meet the above information, since the application of ABA, regardless of its concentrations, resulted in a reduction of ethylene production after 24 hours.

Therefore, reductions in ethylene production in plants under water deficiency may be associated with increased abscisic acid production under this condition, even at small intervals without irrigation (1 to 2 days). Thus, since this hormone is responsible for stomatal closure, a rapid increase in ABA concentrations at the first signal of water deficiency would result in reduction of stomatal conductance and transpiration (Tables 1, 2 and 3), and with this, a maintenance of the leaf turgidity, not resulting in the decrease of the water potential, according to results found in experiments I, II and III.

Also, as a consequence of the reduction of stomatal conductance, there was a reduction of CO₂ entry in the leaf, and thus a reduction on the carbon assimilation of plants under water deficiency, which can begin to occur after 2 days without irrigation (Table 1).

There are several studies that show the same results found in this experiment. Trujillo, Rivas and Castrillo (2013) in an experiment with two varieties and three irrigation levels, observed a reduction in the stomatal conductance of the common bean plants submitted to low soil water contents for both varieties. After rehydration, the two varieties presented recovery of the evaluated parameter. Santos et al. (2009), in an experiment with five different common bean varieties and two irrigation levels, observed reductions in stomatal conductance and photosynthetic rate for all varieties, with subsequent restoration of the parameters after rehydration of the plants.

Ramalho et al. (2013), in an experiment with three common bean varieties and two irrigation levels, observed a great reduction in plant photosynthesis, as well as stomatal conductance and transpiration for the three varieties. Also Kumar et al. (2006), in an experiment with five varieties and two irrigation levels, observed a great reduction in the same three variables, both in flowering and grain filling stages for all five varieties. Reductions in photosynthetic rates and stomatal conductance of two common bean varieties under water deficit were also verified by Lizana et al. (2006).

The reduction of net photosynthesis in plants under conditions of low soil water content can be explained by the low internal concentration of CO₂, which impairs the performance of RuBisCO (Cornic et al., 1992) or by direct inhibition of photosynthetic enzymes such as RuBisCO.

The limitation of photosynthesis by stomatal closure in stress situations due to water deficit is considered a primary effect, which is followed by changes in photosynthetic reactions and limitations in leaf mesophyll. However, stomatal conductance may be the most dominant factor in photosynthetic limitation under stress situations due to water deficit than non-stomatic limitations in some species (Medrano et al., 2002).

In conclusion, reduced leaf water potentials of plants under different levels of water deficit were not constant when compared to the control with daily irrigation. The water deficiency caused reduction of the stomatal conductance in the first days without irrigation, and as a consequence there was a reduction in transpiration and carbon assimilation levels. Water deficiency did not increase ethylene production in common bean plants and the exogenous application of abscisic acid caused a reduction in ethylene production.

Acknowledgements

The authors are grateful to the São Paulo Research Foundation (Fapesp—grant number 2014/13442-4) for granting the funds for this study.

References

- Aharoni, N. (1978). Relationship between leaf water status and endogenous ethylene in detached leaves. *Plant Physiology*, *61*, 658-662. <https://doi.org/10.1104/pp.61.4.658>
- Apelbaum, A., & Yang, S. F. (1981). Biosynthesis of stress ethylene induced by water deficit. *Plant Physiology*, *68*, 594-596. <https://doi.org/10.1104/pp.68.3.594>
- Cornic, G., Ghashghaie, J., Genty, B., & Briantais, J. M. (1992). Leaf photosynthesis is resistant to a mild drought stress. *Photosynthetica*, *27*, 295-309.
- Fernández, F., Gepts, P., & López, G. M. (1982). Etapas de desarrollo de la planta de frijole común. *Centro Internacional de Agricultura Tropical*, *26*, 1-5.
- Graham, P. H., & Ranalli, P. (1997). Common bean (*Phaseolus vulgaris* L.). *Field Crops Research*, *53*, 131-146. [https://doi.org/10.1016/S0378-4290\(97\)00112-3](https://doi.org/10.1016/S0378-4290(97)00112-3)
- Hoffman, N. E., Yang, S. F., & Mckee T. (1982). Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in higher-plants. *Biochemistry and Biophysics Research Communication*, *104*, 765-770. [https://doi.org/10.1016/0006-291X\(82\)90703-3](https://doi.org/10.1016/0006-291X(82)90703-3)
- Hsiao, T. C. (1973). Plant responses to water stress. *Annual Review of Plant Physiology and Plant Molecular Biology*, *24*, 519-570. <https://doi.org/10.1146/annurev.pp.24.060173.002511>
- Kumar, A., Omae, H., Egawa, Y., Kashiwaba, K., & Shono, M. (2006). Influence of irrigation level, growth stages and cultivars on leaf gas exchange characteristics in snap bean (*Phaseolus vulgaris*) under subtropical environment. *Japan Agricultural Research Quarterly*, *41*, 201-206. <https://doi.org/10.6090/jarq.41.201>

- Lizana, C., Wentworth, M., Martinez, J. P., Villegas, D., Meneses, R., Murchie, E. H., ... Pinto, M. (2006). Differential adaptation of two varieties of common bean to abiotic stress. I-Effects of drought on yield and photosynthesis. *Journal of Experimental Botany*, *57*, 685-697. <https://doi.org/10.1093/jxb/erj062>
- Medrano, H., Escalona, J. M., Bota, J., Giulias, J., & Flexas, J. (2002). Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of Botany*, *89*, 895-905. <https://doi.org/10.1093/aob/mcf079>
- Morgan, P. W., & Drew, M. C. (1997). Ethylene and plant responses to stress. *Physiologia Plantarum*, *100*, 620-630. <https://doi.org/10.1034/j.1399-3054.1997.1000325.x>
- Morgan, P. W., He, C. J., Degreef, J. A., & Deproft, M. P. (1990). Does water deficit stress promote ethylene synthesis by intact plants? *Plant Physiology*, *94*, 1616-1624. <https://doi.org/10.1104/pp.94.4.1616>
- Narayana, I., Lalonde, S., & Saini, H. S. (1991). Water-stress-induced ethylene production in wheat—A fact or artifact. *Plant Physiology*, *96*, 406-410. <https://doi.org/10.1104/pp.96.2.406>
- Pimentel, C., Oliveira, R. F., Ribeiro, R. V., Santos, M. G., & Machado, E. C. (2011). Photosynthetic characteristics of *Phaseolus vulgaris* L. *Hoehnea*, *38*, 273-279. <https://doi.org/10.1590/S2236-8906201100200007>
- R Development Core Team. (2013). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Ramvalho, J. C., Zlatev, Z. S., Leitao, A. E., Pais, I. P., Fortunato, A. S., & Lidon, F. C. (2013). Moderate water stress causes different stomatal and non-stomatal changes in the photosynthetic functioning of *Phaseolus vulgaris* L. genotypes. *Plant Biology*, *16*, 133-146. <https://doi.org/10.1111/plb.12018>
- Ribeiro, R. V., Santos, M. G., Souza, G. M., Machado, E. D., Oliveira, R. F., Angelocci, L. R., & Pimentel, C. (2004). Environmental effects on photosynthetic capacity of bean genotypes. *Pesquisa Agropecuária Brasileira*, *39*, 615-623. <https://doi.org/10.1590/S0100-204X2004000700001>
- Santos, M. G., Ribeiro, R. V., Machado, E. C., & Pimentel, C. (2009). Photosynthetic parameters and leaf water potential of five common bean genotypes under mild water deficit. *Biologia Plantarum*, *53*, 229-236. <https://doi.org/10.1007/s10535-009-0044-9>
- Scholander, P. F., Hammel, H. T., Brastreet, E. D., & Hemmingsen, E. A. (1965). Sap pressure in vascular plants. *Science*, *148*, 339-346. <https://doi.org/10.1126/science.148.3668.339>
- Sharp, R. E. (2002). Interaction with ethylene: Changing views on the role of abscisic acid in root and shoot growth responses to water stress. *Plant Cell Environment*, *25*, 211-222. <https://doi.org/10.1046/j.1365-3040.2002.00798.x>
- Spollen, W. G., Lenoble, M. E., Samuels, T. D., Bernstein, N., & Sharp, R. E. (2000). Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiology*, *122*, 967-976. <https://doi.org/10.1104/pp.122.3.967>
- Trujillo, I., Rivas, M., & Castrillo, M. (2013). Leaf recovery responses during rehydration after water deficit in two bean (*Phaseolus vulgaris* L.) cultivars. *Journal of Plant Interaction*, *8*, 360-369. <https://doi.org/10.1080/17429145.2012.754959>
- Wright, S. T. C. (1977). The relationship between leaf water potential and the levels of abscisic acid and ethylene in excised wheat leaves. *Planta*, *134*, 6. <https://doi.org/10.1007/BF00384969>
- Xu, C. C., & Qi, Z. (1993). Effect of drought on lipoxygenase activity, ethylene and ethane in leaves of soybean plants. *Acta Botanica Sinica*, *35*, 6.

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