INFLUENCES OF TWO CO_2 CONCENTRATIONS AND WATER AVAILABILITY ON BEAN CROP

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ABSTRACT: This study was carried out to determine some physiological and phenological responses of the bean under high [CO_2] and drought stress. The experiment was conducted from April to July 2009 in Viçosa, Brazil. The open-top chambers were used to enrich the air with CO_2, whereas the drought stress was applied between the flowering and the ripening. The randomized block design was used, with four replicates in the subplots. The following plots were [CO_2] at 700ppm (F1) and [CO_2] environmental (F2) and the subplots were well watering (S1) and drought stress (S2). The results were subjected to Anova and the Tukey test ($P < 0.05$). For the treatments F1S1 and F1S2 the photosynthetic rate showed increments of 59% and the transpiration reduction of 12%. The yield, leaf temperature and stomatal conductance were not significant different to high [CO_2], different from the dry matter, who showed increment of 20% (F1S1) and the water use efficiency who showed increase of 90% for high [CO_2]. The osmotic potential was lower in plants under drought stress (F2S2 and F1S2), followed by plants under high [CO_2] (F1S1). Despite the increment in photosynthesis, high [CO_2] does not guarantee higher yield.

KEYWORDS: photosynthesis, *Phaseolus vulgaris* L., climate changes.

INFLUÊNCIAS DE DUAS CONCENTRAÇÕES DE CO_2 E DA DISPONIBILIDADE HÍDRICA NA CULTURA DO FEIJÃO

RESUMO: Neste trabalho, analisaram-se respostas fisiológicas e fenológicas do feijoeiro cultivado sob alta [CO_2] e estresse hídrico. O experimento foi realizado em Viçosa-MG, entre abril e julho de 2009, utilizando-se de câmaras de topo aberto equipadas com sistema de injeção de CO_2. O estresse hídrico foi aplicado durante o período da floração à maturação. O delineamento experimental foi em blocos casualizados, tendo nas parcelas plantas cultivadas em [CO_2] a 700 ppm (F1) e [CO_2] ambiente (F2), e nas subparcelas plantas sem (S1) e com estresse hídrico (S2). Os resultados foram submetidos à Anova e ao teste de Tukey ($P < 0,05$). Nas interações F1S1 e F1S2, a taxa fotossintética aumentou 59%, e a transpiração reduziu 12%. A massa de grãos, a temperatura foliar e a condutância estomática não diferiram significativamente a [CO_2]. Por outro lado, a matéria seca apresentou incremento de 20% para (F1S1), e o uso eficiente da água aumentou em 90% para alta [CO_2]. O potencial osmótico apresentou valores menores nas plantas sob estresse hídrico (F2S2 e F1S2) e naquelas sob CO_2 no ar a 700 ppm (F1S1). Apesar do incremento na fotossíntese sob alta [CO_2], a produtividade não aumentou.

PALAVRAS-CHAVE: fotossíntese, *Phaseolus vulgaris* L., mudanças climáticas.

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INTRODUCTION

After the industrial revolution, the atmospheric CO$_2$ concentration ([CO$_2$]) has increased, especially after the second half of the last century, when it went from 315ppm in 1958 to 345ppm in 1985 (STRECK, 2005). Experimentally, it is observed increments of 30 to 70% photosynthetic rate of C$_3$ plants with an increase of two to three times of the current atmospheric [CO$_2$] (380ppm). This effect is mainly due to the increase of the gradient of [CO$_2$] from the air to the leaf, favoring the higher carboxilative efficiency of ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco) by the greater availability of CO$_2$ and also by reduction between 20 to 40% of photorespiration (ZISKA & BUNCE, 2006; AINSWORTH & ROGERS, 2007).

The beneficial effects of elevated [CO$_2$] in the growth of plants also depend on the water availability (ROGERS et al. 2009). Plants under drought stress showed a reduction in stomatal conductance, limiting gas exchanges and, hence, CO$_2$ uptake (DAVIES, 2006). However, high [CO$_2$] concentrations generally cause decrease in stomatal aperture, resulting in less water lost by transpiration and drought stress tolerance. In this atmosphere enriched with CO$_2$, plants under drought stress are able to maintain for a longer time the same absorption rate of CO$_2$ (LARCHER, 2006).

Depending on the supply of fertilizer nitrogen (N), beans under high [CO$_2$] show increments between 40 and 80% in photosynthesis and between 26 and 40% in dry matter, while the stomatal conductance is 35% lower, however the reduction decreases with advancing the plant cycle (JIFON & WOLFE, 2002). The production of grains is 17% greater in an environment with high [CO$_2$] (BUNCE, 2008).

Because the beans have high nutritional and economic value for Brazil, the study of crop responses subjected to environments enriched with CO$_2$ and drought stress is relevant, in view of the need for input experimental data in the models to predict climate scenarios for agronomic crops in general. Thus, this study aimed to analyze some physiological and phenological responses of bean yield (*Phaseolus vulgaris* L.) subjected to drought stress and to the environment enriched with CO$_2$.

MATERIAL AND METHODS

The experiment was conducted at the lysimetric station on the campus of the Federal University of Viçosa, Viçosa city – state of Minas Gerais (MG), in Brazil (20°45' S, 42°45' W), between April and July, 2009. Lysimeters, with section of 1.0m x 1.4m and 0.8m deep, were divided in half by a metal plate septum to perform the experimental design in split plots. The substrate used to fill the lysimeters was a Dystrophic Red Yellow Latosol, very clayey textural class, where was performed the acidity correction by liming. There were two fertilizations during the experiment, one of planting with NPK 8-28-16, 650kg ha^{-1}, plus 300kg ha^{-1} of magnesium sulfate, and other with coverage, with 200kg ha^{-1} of urea, plus 250g ha^{-1} of sodium molybdate via foliar fertilization. The bean cultivar used was Majestoso-UFV, carioca type, of short cycle. The plant spacing was 0.05m and 0.50m between rows, totaling 54 plants per lysimeter (plot) and 27 per subplot.

All plants were grown in open-top chambers consisted of mobile rectangular modules, added to follow the development of plants (Figure 1), with internal air distribution system composed of fans and drilled PVC pipes. It was used two [CO$_2$] in the chambers, 380 (ambient) and 700ppm. Plants cultivated under [CO$_2$] of 700ppm had a daily exposure from 6:00 to 18:00 from 9 DAS (days after planting), with control of CO$_2$ injection performed by a solenoid valve, with concentration monitored and adjusted daily in the morning with a meter of ambient [CO$_2$], 535 Model from Testo. The temperature inside the chamber was, on average, 2°C above the external environment, monitored by a portable weather station, Vantage Pro Model from Davis.

The plants were irrigated by drip tapes to maintain the soil at field capacity (FC = 33.62%). In plants subjected to drought stress, it was performed total suppression of irrigation between flowering and maturation. The water content in the soil was monitored hourly by TDR (Time Domain Reflectometry), with probes installed at 0.20m deep in the soil and automatic readings with the Campbell Scientific TDR100.
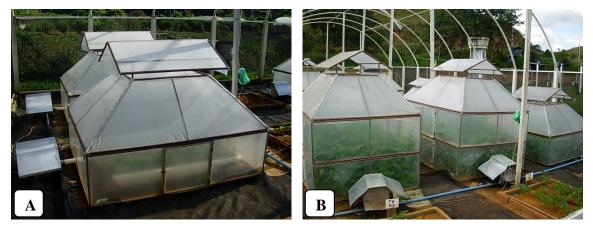

FIGURE 1. Open-top chambers composed of modules, added to follow the growth of plants: A – chambers with one module, between 1° to 40° DAS (days after sowing); and B – chambers with two modules, between 41° to 90° DAS.

Data collected

Data were collected from daily mean leaf temperature, photosynthetic rate, transpiration, stomatal conductance, osmotic potential, leaf water potential, yield and dry matter. The mean leaf temperature was collected daily, four times a day, at 8:00, 11:00, 14:00 and 17:00, during the induction of drought stress (48 days), with the aid of an infrared thermometer with laser pointer, 62Mini Fluke model.

The photosynthetic rate (A), transpiration (E) and stomatal conductance (g_s) were measured in the morning, between 8:00 and 11:00, with portable meter for gas exchanges (IRGA - Infra Red Gas Analyser) LCi model, with system of external radiation source, 1,200µmol m^{-2}s^{-1} and environmental conditions of inside the chambers (temperature, [CO_2] and relative humidity). There were four measurements (Figure 2): 1st measurement, 20 DAS (vegetative stage); 2nd measurement, 41 DAS (pre-flowering); 3rd measurement, 67 DAS (grain filling); and 4th measurement, 82 DAS (maturation). The water use efficiency (WUE) was determined by the ratio A/E (LARCHER, 2006).

The osmotic potential was determined before the end of the drought stress, at the 70th DAS, using a cryoscope, ITR MK540 model, and the results of the equipment were correlated to the osmotic potential. The leaf water potential was measured on the day of measurement of osmotic potential, however during predawn, using the Scholander chamber, 3005F01HGP model from Soil Moisture. At the end of the crop cycle, 100th DAS, productivity was determined by the following variables: total mass of grains; mass of a hundred grains; grain number; and number of pods. For the determination of dry matter, all aerial parts of the plant were dried in a ventilated oven at 70ºC for three days.

Experimental design and statistical analysis

The experimental design was in randomized blocks with four replications (blocks), and split plots. The plots constituted the primary treatment (F), or levels of [CO_2]: F1 – plants grown with [CO_2] at 700ppm; and F2 – plants grown with ambient [CO_2] (380ppm). The subplots constituted the secondary treatment (S), or the availability of water for crop: S1 – plants grown without drought stress, and S2 – plants grown under drought stress. The induced drought stress by total suppression of irrigation consisted from the 22nd DAS, seven days before flowering, to the 70th DAS, early

maturation, lasting 48 days (Figure 2). With the levels of $[CO_2]$ of the plot and the levels of water availability in the subplot, interactions resulted in four treatments: F1S1; F1S2; F2S1 and F2S2.

The variables were analyzed using simple descriptive statistics (mean, standard deviation and coefficient of variation) for subsequent analysis of variance (ANOVA). For comparison of the different levels of the plots, subplots and treatments, it was applied the Tukey test ($P < 0.05$). The analyses were conducted in the SAS 9.3 statistical package (SAS, 2011).
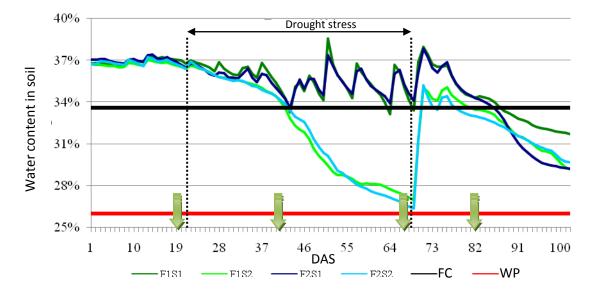

FIGURE 2. Water content in soil (%) during the experiment for each treatment, field capacity (FC), permanent wilting point (WP), induction period of drought stress and the four measurements of gas exchanges (green arrows). Where: F1 – plants growing in $[CO_2]$ at 700ppm; F2 – plants growing in ambient $[CO_2]$ (380ppm); S1 – plants growing without drought stress; S2 – plants growing with drought stress.

RESULTS AND DISCUSSION

In the photosynthetic rate measurements (*A*), it was observed increments between 40 and 80% in plants under high $[CO_2]$, 700ppm (Figure 3). These values were higher than those found by JIFON & WOLFE (2002), LAMBREVA et al. (2005), PRITCHARD & AMTHOR (2005), BUNCE (2008) and LEE et al. (2011), who reported increments between 13 and 60% for beans and other C_3 plants under $[CO_2]$ at 500ppm. The highest increase in the photosynthetic rate of the plants from the present study was due to the increase in the gradient of $[CO_2]$ between the atmosphere and the leaf, due to higher $[CO_2]$ in the atmosphere, increasing the availability of CO_2 at the site of carboxylation of Rubisco, which consequently limited more the photorespiration.
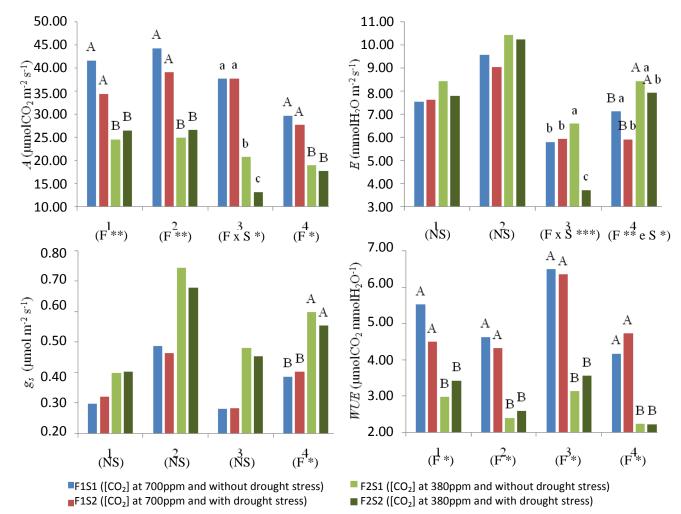
FIGURE 3. Means of the four measurements of gas exchanges, Anova summary and Turkey test (P < 0.05) between means of same measurement, where means followed by the same letter are statistically equal. Where: A – Photosynthesis rate; E – Transpiration rate; gs – Stomata conductance; WUE – Water use efficiency; F – [CO_2] levels; S – water availability; * significant at P < 0.05; ** significant at P < 0.01; *** significant at P < 0.001; and NS – not significant at P < 0.05; F1 – plants growing in [CO_2] at 700ppm; F2 – plants growing in ambient [CO_2] (380ppm); S1 – plants growing without drought stress; and S2 – plants growing with drought stress.

The photosynthetic rate (A) showed significant differences in the interaction between the levels of [CO_2] and water availability only after severe drought stress (3rd measurement; Figure 3). The increase in CO_2 availability was responsible for maintaining the plants photosynthetic rate, even under drought stress, similar to observations of LARCHER (2006) and LEAKEY et al. (2009), i.e., the plants exposed to high [CO_2] and under drought stress (F1S2) showed no decrease in photosynthetic rate in relation to the plants of treatment F1S1. Under ambient [CO_2], the effect of drought stress resulted in lower photosynthetic rate. Throughout the experiment, the plants under high [CO_2] had higher water use efficiency (WUE; Figure 3), increasing on average 90%, which may have contributed to maintenance of plants photosynthetic rate under high [CO_2] and drought stress (F1S2).

During the drought stress period, there were no significant differences in stomatal conductance (g_s), however, in numerical terms, the plants under high [CO_2] had lower values of g_s (between 20 and 40%) due to lower stomatal opening (Figure 3), similar to observations of AINSWORTH & ROGERS (2007) and LEE et al. (2011). Only after the end of the drought stress

(4th measurement), there were significant differences for g_s, due to the reduction in the coefficient of variation between readings.

Due to the drought stress period and reduced g_s, transpiration rates (E) showed significant differences only in the last two measurements (Figure 3). For the third measurement, there was significant interaction between levels of $[CO_2]$ and water availability. The higher transpiration rates were for plants under ambient $[CO_2]$ and without drought stress (F2S1), while the lowest rates were for plants under ambient $[CO_2]$ and drought stress (F2S2). The lowest rates of F2S2 were due to the defense mechanism of the plant to minimize water loss. Similarly to photosynthesis, the plant transpiration under high $[CO_2]$, F1S1 and F1S2, did not differ among themselves, regardless of water availability. In the fourth measurement of transpiration (E), there was no significant interaction, since the effects of the treatments acted independently; the plants under high $[CO_2]$ (F1) showed lower transpiration, as well as the plants under drought stress (S2).

The daily mean leaf temperature (TF) throughout the experiment showed no significant differences for the levels of the treatments applied (Table 1). The opposite was reported on observations of DAVIES (2006) and KÖRNER (2006), who claim that due to high $[CO_2]$ and drought stress, there was an increase of leaf temperature as a result of reduced stomatal conductance (g_s). However, in the study of these authors, the leaf temperature was measured by spot metering mode, unlike this study, in which the mean leaf temperature represented the mean over the period of drought stress.

TABLE 1. Descriptive statistics, Anova summary of analyzed variables and Tukey test, where means in the same column followed by the same letters are equal.

		Ψ_w	Ψ_s	LT	$MG100$	TMG	NG	NP	TDM
Analysis of variance (Anova)									
Factors		Ψ_w	Ψ_s	LT	$MG100$	TMG	NG	NP	TDM
F		**	NS	NS	NS	NS	**	NS	NS
S		*	***	NS	NS	***	***	***	***
F × S		NS	*	NS	NS	NS	NS	NS	*
Mean		-1.26	-0.37	21.60	23.21	395.23	1291.61	292.19	796.47
Sd		0.48	0.02	0.08	2.76	58.91	232.36	49.67	59.26
Cv (%)		38.64	4.04	0.37	11.88	19.30	17.99	59.26	7.44
Tukey test (P < 0.05)									
F1	S1	-1.05	-0.36 [B]	21.64	23.48	478.23	2035.37	420.75	1030.87 [A]
F1	S2	-1.78	-0.41 [C]	21.68	21.95	154.96	701.76	209.75	692.98 [BC]
F2	S1	-0.85	-0.32 [A]	21.58	24.61	417.14	1697.08	332.25	825.79 [B]
F2	S2	-1.35	-0.41 [C]	21.49	22.78	170.59	732.22	206.00	636.24 [C]

Where: * significant at $P < 0.05$; ** significant at < 0.01; *** significant at < 0.001; NS – not significant at $P < 0.05$; F – levels of CO_2 (plot); S – water availability (subplot); Sd – standard deviation; Cv – coefficient of variation; Ψ_w - water potential (MPa); Ψ_s - osmotic potential (MPa); LT – leaf temperature (°C); $MG100$ – mass of 100 grains (g); TMG – total mass of grains (g); NG – number of grains; NP – number of pods; TDM – total dry matter (g); F1 – plants grown with $[CO_2]$ at 700ppm; F2 – plants grown with ambient $[CO_2]$ (380ppm); S1 – plants grown without drought stress; e S2 – plants grown under drought stress.

The increase in $[CO_2]$ or prolonged drought stress resulted in decrease of leaf osmotic potential (Ψ_s), which showed significant differences for interaction between $[CO_2]$ and water availability, F x S (Table 1). The decrease of Ψ_s, in environments with high $[CO_2]$ is related to the increase of the ratio between the number of moles of solute and the volume of the cell solution while the decrease of Ψ_s by drought stress is related to the loss of water in the cell. Thus, plants subjected to drought stress (F1S2 and F2S2) had the lowest values of Ψ_s, with a reduction of approximately 27% (Table 1). This reduction of Ψ_s due to drought stress, according to DAVIES (2006), consists of a defense mechanism of the plant, because it favors the absorption of water, even

in plants under drought stress. In general, plants not subjected to drought stress showed the highest values of Ψ_s.

The water potential (Ψ_w) showed high CV and significant differences only for the separate effects of [CO_2] and water availability (Table 1). Plants under high [CO_2] (F1) had lower water potential (Ψ_w), due to lower osmotic potential (Ψ_s). As for the plants under drought stress (S2), these showed lower water potentials due to low water availability of soil to the plant (Figure 2), as well as the lowest osmotic potential.

Plants under high [CO_2] (F1) and without drought stress (S1) showed higher number of grains (*NG*). However the mass of a hundred grains (*MG100*) showed no significant differences, indicating that the increase of [CO_2] and drought stress did not affect the weight of the grain itself, but rather the production of flowers and pods of the plants, as may be observed by the drop in the number of pods (*NP*) in plants subjected to drought stress (Table 1). This reduction in the number of pods (*NP*) for plants subjected to drought stress also resulted in the decrease of the total mass of grains (T*MG*), due to the abortion of flowers and fall of pods during drought stress. The reduction of 63.64% in the total mass of grains (T*MG*) of plants subjected to drought stress was greater than that found by SAUCEDO et al. (2006) and SILVEIRA & STONE (2008), approximately 30% and 42%, respectively, possibly due to the magnitude and period of drought stress in this experiment, because according to DAVIES (2006), when the drought stress occurs in important stages of the plant, such as flowering and pod formation, the productivity losses are considerable.

The increase of the number of grains (*NG*) in plants under high [CO_2], according to JABLONSKI et al. (2002); ZISKA & BUNCE (2006) and MIYAGI et al. (2007), arises from the increase of the number of flowers and, consequently, the number of pods (*NP*). The *NP*, despite not showing significant difference at high [CO_2], due to the high CV, showed numerical differences, 27% more than plants under ambient [CO_2] (Table 1). However, despite the higher photosynthetic rates (*A*), water use efficiency (*WUE*) and number of grains (*NG*) presented by plants under high [CO_2] (Figure 3 and Table 1), an increase of only 15% in total mass of grains (T*MG*) did not guarantee significant gain. Therefore, BUNCE (2008) questions the hypothesis that increases in photosynthetic rates should always result in higher grain yield, but in greater quantity in the plant dry matter (*TDM*), as in this study (Table 1) and the studies of JABLONSKI et al. (2002) and AINSWORTH et al. (2004). Thus, the dry matter of shoots was higher in plants under high [CO_2] and without drought stress (F1S1), demonstrating that the majority of photoassimilates, coming from the highest photosynthetic rates, went to the stems and leaves (Table 1). However, the plants under high [CO_2] and drought stress (F1S2) did not show the same response, indicating that the high [CO_2] retained only the photosynthetic rate, despite the higher water use efficiency (*WUE*), while the severe effects of drought stress were felt by plants, because they show reduction of dry matter (Table 1). The gain in dry matter in F1S1 was 24.83%, while JIFON & WOLFE (2002), NASSER et al. (2008) and LEAKEY et al. (2009) showed gains, on average, in the range of 35 to 49%.

CONCLUSIONS

The reduction in water content of the soil is not a limiting factor to photosynthetic rates of beans under high [CO_2], which show higher efficiency in water use and lower transpiration rates. However, the increase in [CO_2] provides an increase in the concentration of solutes in leaf cells, which results in reduced water and osmotic potential. The increase in [CO_2] provides a significant increase in total dry matter of the aerial part of the plant, as well as the number of grains, due to higher reproduction. However, higher [CO_2] does not cause greater productivity, because the grain mass shows no significant increases, unlike drought stress, which leads to significant reduction, especially if it occurs at important stages of the plant, as in the flowering and pod formation.

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