Gas exchange alteration caused by water deficit during the bean reproductive stage

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ABSTRACT

The purpose of the present study was to analyze gas exchanges in leaves and the parameters of productivity of beans (*Phaseolus vulgaris*, L.) submitted to two water deficiency periods during which three water regimes were employed: W_1 (1,0 ET_o during the entire plant cycle); W_2 (1,0 ET_o up to the flowering period and irrigation interruption from the 37 to the 51st day following sowing, and W_3 (in addition to the reproductive phase, water deficit was also applied during the vegetative stage). Photosynthesis was one of the main physiological factors affected by water deficit. This was not only caused by the stomata closure, but also by carboxilation reduction due to metabolic damage. This effect was, however, offset 24 h after rehydration. During flowering, the water deficit caused crop productivity to drop significantly, reducing the number of pods and the number of seeds per pod, independently of the water deficit during the vegetative stage. The weight of 100 seeds however, was the same regardless of treatment. These results suggest that the water deficit caused the reduction of photo-assimilates, which affected grain productivity. Nevertheless, once properly formed, seeds developed totally; a strategy of the plant to produce less seeds under stress, but viable to perpetuate the species.

Key words: photosynthesis, transpiration, *Phaseolus vulgaris* L.

Alterações das trocas gasosas na fase reprodutiva e produtividade do feijão sob déficit hídrico

RESUMO

Neste trabalho, se analisaram as trocas gasosas das folhas e parâmetros de produtividade de feijão (*Phaseolus vulgaris*, L.) submetido a dois períodos de deficiência hídrica e se empregaram três regimes hídricos, a saber: W_1 (1,0 ET_o durante todo o ciclo da cultura); W_2 (1,0 ET_o até a floração e suspensão da irrigação do 37 ao 51º dia após a semeadura (DAS), correspondendo à fase de floração) e W_3 (além da fase reprodutiva também se aplicou um déficit hídrico durante a fase vegetativa). A fotossíntese foi um dos principais fatores fisiológicos afetados pelo déficit hídrico devido não só ao fechamento estomático, mas, também, à redução da eficiência de carboxilação, resultante de um dano metabólico; este efeito, contudo, foi neutralizado 24 h após reidratação. Durante a floração o déficit hídrico causou redução sensível da produtividade da cultura, com redução também do número de vagens e número de sementes por vagem, independentemente do déficit durante a fase vegetativa, porém a massa de 100 sementes não se alterou em nenhum dos regimes; esses resultados sugerem que o déficit hídrico leva a uma redução dos fotoassimilados prejudicando a produção final; no entanto, uma vez formada, a semente se desenvolve plenamente, sendo esta uma estratégia da planta em produzir poucas sementes, mas viáveis para perpetuar a espécie, mesmo sob condições de estresse.

Palavras-chave: fotossíntese, transpiração, *Phaseolus vulgaris* L.

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INTRODUCTION

In semi-arid areas, where the deficit of pluvial precipitation is one of the main factors limiting agricultural production, efforts have been directed towards the development of productive systems that has been well accepted by the local population (Smolikowski et al., 2001). In some areas of the Northeastern Brazil, bean is one of the main subsistence crops for small farmers. Most of the bean production in the Northeastern Brazil is accomplished under severe water deficiency, causing staggering losses at some time of the crop cycle. This problem is still greater for those small farmers who have no means to set up irrigation systems for their crops. To make matters worse, some modern varieties of beans have been less tolerant to drought stress (Singh, 2001).

The influence of meteorological conditions on human activities and on the environment as a whole is a fact well known by most researchers (Monteith & Unsworth, 1990; Rosenberg et al., 1983; Pereira et al., 1997). A far more comprehensive study of environmental issues, mainly those involving interactions between the atmosphere and the so-called surface processes (vegetation, for instance), constitutes basic prerequisites for further rational exploration of the region's natural resources. This will help subsidize researches in areas such as: meteorology, farming, engineering, energy and environmental sciences, among others.

Photosynthesis is perhaps one of the parameters most affected by environmental conditions (Oren et al., 1999; He et al., 2009; Zheng et al., 2009). Water deficiency limits photosynthesis because of the restriction of CO_2 diffusion from the external environment to the carboxilation site in chloroplast (Lawlor & Cornic, 2002; Chaves et al., 2003). Severe drought stress can also induce biochemical damage by reducing Calvin Cycle activity (Lawlor & Cornic, 2002).

Stomata conductance is one of the main factors affecting the photosynthesis of plants (Medrano et al., 2002). In most cases, the stomata close themselves in response to drought before any change in water potential or in leaf water content occurs (Socias et al., 1997). The stomata may also close when the vapor pressure deficit between the leaves and the air increases (Oren et al., 1999). The CO_2 and water vapor are exchanged through the same stomata pores; consequently, stomata conductance becomes most significant both through photosynthesis determination and through transpiration.

Alterations that take place in the environmental conditions affect photosynthetic activities, altering, as a result, crop productivity (Fleisher et al., 2008; Reynolds et al., 2007).

Consequently, where water availability is concerned, knowledge about the relation between photosynthesis, plant growth and productivity is required in order to better define agricultural growth standards for the adoption of self-sustainable production procedures.

The present work proposes an analysis of photosynthesis and transpiration in leaf and the examination of bean productivity under water deficiency during flowering.

MATERIAL AND METHODS

An experiment with bean (*Phaseolus vulgaris* L.) variety Pérola (seeds made available by the EMBRAPA) was conducted in the municipality of Rio Largo, AL (9° 28' 02" S; 35° 49' 43" W; 127 m) during the period from December 2004 to March 2005. The experimental design was entirely randomized and consisted of eight replications with plot size of 2.5 m wide and 5 m long at a distance of 0.5 m between lines with 15 seeds per linear meter, resulting in a total of 240,000 plants ha^{-1}.

The soil presented pH (H_2O) 5.9; consequently, no liming was required, and basal dose of fertilizers was applied based on the chemical analysis of the soil (Table 1) according to EMBRAPA (1997) and consisted of 450 kg ha^{-1} of the formula 04-10-13 (totalizing 100 kg of ammonium sulphate, 250 kg of simple superphosphate, and 100 kg of potassium chloride per hectare). Twenty-five days after sowing 100 kg ha^{-1} of urea was used, at one single time, as top dressing.

The climate of the region is hot and moist (B_1), megathermal (A') with moderate water deficiency in the summer along with water surplus in the winter (w_2) according to Thornthwaite-Mather classification (Carvalho, 2003). Mean air temperature varies from 19 °C (August) to 32 °C (January), with an annual mean of 25 °C; the mean monthly relative humidity is 70%, and the mean annual rainfall being 1,818 mm with total minimum values of 41 mm in December and maximum values of 294 mm in July. Annual rainfall concentrates (61%) in April-July period. The months between October-February have a mean rainfall of 300 mm

Table 1. Chemical composition of soil of 0.20 cm of the experimental site

pH H_2O	P	K$^+$	H+Al	Al^{3+}	Ca^{2+}	Mg^{2+}	CTC$_{total}$	V	M	MO
	mg dm^{-3}		cmol$_c$ dm^{-3}					%		
5.9	17	45	3.5	0.05	2.5	2.1	8.5	54.8	1.1	2.15

Analysis carried out by the Central Analítica Ltda, Maceió, AL

Table 2. The physical characteristics of soil of the experimental site

Horizon		Granulemetry composition (%)				Clay dispersed in water (%)	Degre of flocculation (%)	Relation Silt/Clay	Density - kg dm^{-3}	
Identification	Depth - cm	GS	FS	Silt	Clay				Particle	Soil
Ap1	0-11	51	10	7	32	13	59	0.32	2.60	1.37
Ap2	11-22	47	17	5	31	0	100	0.16	2.66	1.46
AB	22-47	43	14	6	37	0	100	0.16	2.71	1.60
Bw1	47-82	32	12	2	54	21	61	0.04	2.60	1.58
Bw2	82-153	33	13	1	53	33	38	0.02	2.60	1.50
Bw3	153-200+	22	10	15	53	14	74	0.28	2.60	1.40

GS Gross sand; FS Fine sand

and an evaporation rate of 972 mm (Souza et al., 2005).

The soil of area studied is classified as Cohesive Argisolic Yellow Latosol (Table 2). The water retention curve of the soil under analysis was obtained by means of Richards' method (Richards, 1948) at the Laboratory of CECA/UFAL, and revealed the available water capacity (AWC) of 24 mm in a depth of 0.30 m.

The irrigation method employed was that of conventional aspersion in a 12 x 12 m spatial intervals. The service pressure (Ps) and the average discharge (q) of aspersers was found to be 1.8 kPa and 1.10 m^3 h^{-1} respectively, uniformity coefficient of application of irrigation water application was 85%, as determined by means of mini-pluviometers. A two-fixed-day interval was set up to apply irrigation. The pressure of the irrigation system and discharge were monitored by means of manometers and hydrometers, respectively.

The reference evapotranspiration (ET_o), as determined by the Penman-Monteith model following the recommendation of FAO-56 (Allen et al., 1998) was determined and used as the main criteria in the water application. The climatic elements needed to determine the ET_o estimates were obtained from an automatic meteorological station (Micrologger – 21XL, Campbell Scientific, Logan, Utah). The soil water measurements were obtained by automatic water content reflectometers (CS616 Water Content Reflectometers, Campbell Scientific, Inc.).

Treatments consisted of three levels of irrigation: W_1 (water application equivalent to $1,0\,ET_o$ during the whole crop cycle); W_2 (water application equivalent to $1,0\,ET_o$ up to the flowering period and irrigation interruption from the 37th to 51st day after sowing (DAS) which corresponded to the flowering phase; and W_3 ($1,0\,ET_o$ up to the 12th DAS and 0.5 ET_o from the 15th to 28th day DAS, irrigation disruption between the 29th and 36th DAS, during the vegetative phase; application of 15.6 mm on the 36th DAS to reach field capacity and again the irrigation was suspended from the 37th to 51st DAS during flowering. During the period of irrigation interruption, rainfall was negligible, but from the 51st DAS, water application was equivalent to $1.0\,ET_o$ in all three treatments. Treatment W_1 corresponded to the control without water deficit; W_2, the water deficit was employed only during flowering; W_3, water deficit was applied during the vegetative and reproductive phases. Both the stages and phenological phases of bean crop were determined in accordance with a phenological scale of Fernandez et al. 1982 (Vieira 1991). The occurrence of phenological events was taken into account only when 50% of the plants attained determined stage.

Gas exchange measurements were made on plants located in the central part of the plot, and on fully expanded leaves, at 8:00 to 10:00 am by using a portable infrared CO_2 analyzer (IRGA), ADC, Lei model (Hoddesdon, UK) on the 35th, 37th, 42nd, 45th and 50th DAS during the crop productive phase, in which irrigation was suspended in W_2 and W_3 treatments (irrigation was interrupted on the 36th DAS). The following parameters were evaluated: photosynthesis rate (A), transpiration rate (E), internal CO_2 concentration (Ci), ambient CO_2 concentration (Ca) and the instantaneous efficiency of carboxilation given by the relation A/Ci.

In each experimental plot, harvesting was carried out on the 72nd DAS in 4.0 m of central row, leaving a boarder of 0.5 m on each side, determining the mass of 100 seeds, number of pods per plant, number of seeds per pod and productivity.

The statistical design was completely randomized and consisted of 8 replications. Three sub-samples were utilized to carry out gas exchange measurements; and to obtain the parameters of production, one sample was taken. The data were compared by Turkey test at 5% probability.

RESULTS AND DISCUSSION

Plant water status had a significant impact on gas exchange in leaves. Plants in W_3 treatment, on the 35th DAS, were under water deficiency. This affected enormously stomata conductance (Figure 1A), transpiration (Figure 1B) and photosynthesis (Figure 1C), with reductions of 96, 83 and 85% in relation to control, respectively. On the 36th day, all treatments were irrigated with a 15.6 mm water in order to achieve field capacity. Twenty-four hours later, the plants which were submitted to stress (W_3) had already recovered their water status and their photosynthesis capacity, as verified after measuring the stomata conductance and gas exchange. Bean is, therefore, a crop that responds well to irrigation when submitted to moderate water deficit. Similar results with bean (cv. Yamashirokurosanndosaitou) were obtained by Miyashita et al. (2005) who observed complete recovery of photosynthetic rate two days following rehydration after moderate water deficit; however, under severe water deficit total recovery did not occur.

Considering the period between the 37th and the 42nd DAS, the plants submitted to severe water deficit (W_3) showed larger reductions in their stomata conductance (Figure 1A), gas exchange (Figures 1B and 1C) and an increase in leaf temperature (Figure 1F) compared to the other treatments. Plants under treatment W_3 also went through a water deficit during vegetative phase, which may have contributed to a more immediate response to stress. The W_2, plants, for which irrigation was suspended only on the 37th DAS, started to suffer from water deficiency on the 42nd DAS.

A decrease in photosynthetic activity as a result of water deficiency may be due to the impairment of the CO_2 entry through the stomata, or it may be due to direct damages over the photosynthetic metabolism. On beans submitted to experimental trial conditions, in spite of the existence of restriction on photosynthesis (Figure 1C) because of a decrease in the internal concentration of CO_2 (Figure 1D) caused by reduction of stomata conductance (Figure 1A), there also occurred some metabolic damage which was confirmed by decrease in carboxilation rate (Figure 1E). According to Miyashita et al. (2005), under severe water deficit, it is impossible for the bean to recover its full photosynthetic activities after rehydration because of irreversible metabolic damage in chloroplast.

Transpiration restriction caused by water deficit (Figure 1F) caused a rise in canopy temperature (Figure 1F). This may have contributed towards the development of thermal stress of the leaves, which may have decreased further the photosynthetic efficiency (Figure 1C) causing direct damage in the
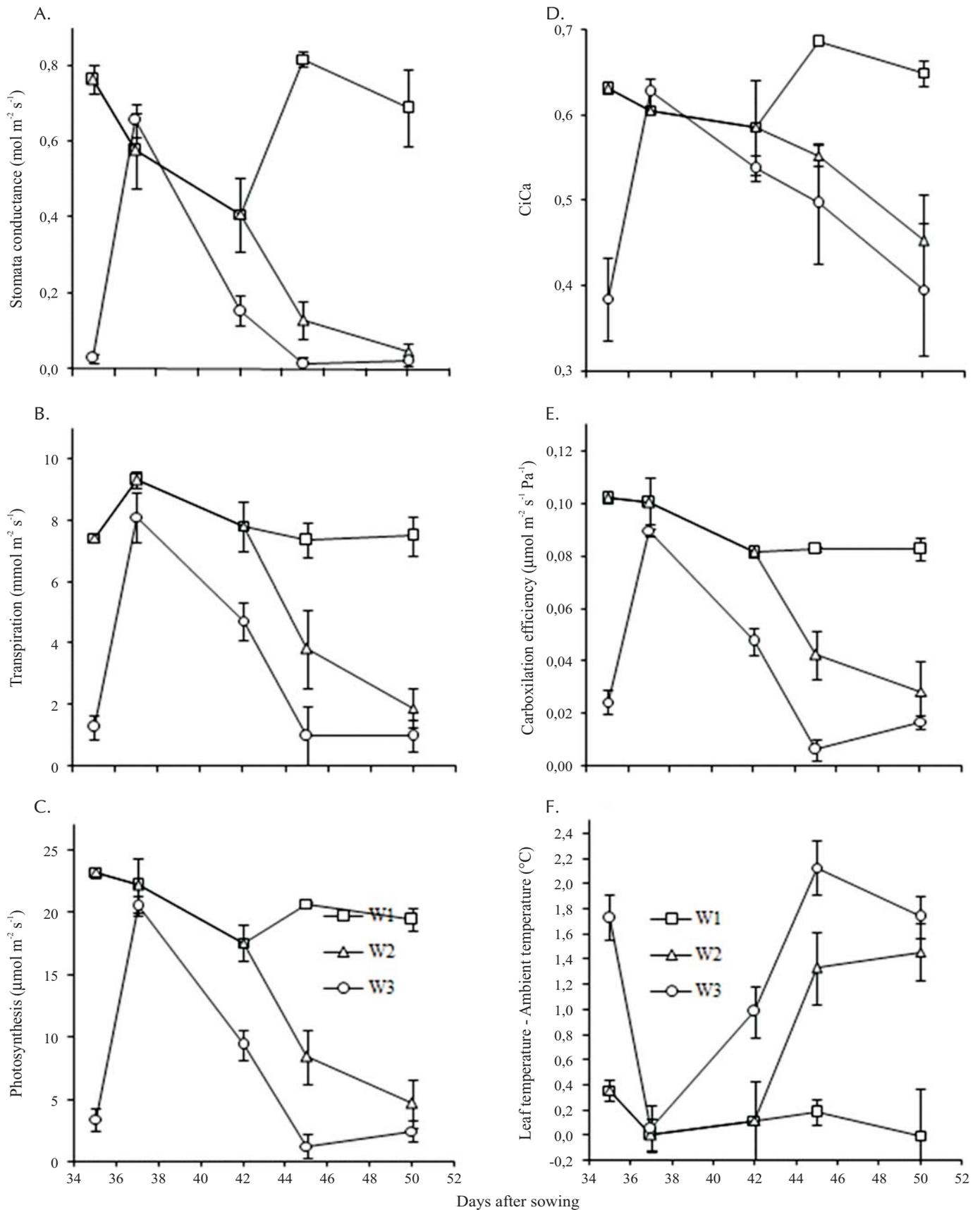
Observation: W_1 (water application equivalent to 1,0 ET_o during the whole crop cycle); W_2 (water application equivalent to 1.0 ET_o up to flowering and irrigation interruption from the 37th to the 51st day after sowing (DAS), corresponding to the flowering period); and W_3 (water application equivalent to 1.0 ET_o up to the 12th DAS and 0.5 ET_o from the 15th to the 28th DAS, irrigation interruption between the 29th and 36th DAS during the vegetative phase; 15.6 mm on the 36th DAS to attain field capacity and again irrigation interruption from the 37th to the 51st DAS during the flowering period).

Figure 1. Stomata conductance (A), transpiration (B), net photosynthesis (C), maximum internal/external concentration of CO_2 (Ci/Ca) (D), instantaneous carboxilation efficiency (A/Ci) (E) and $T_{leaf} - T_{air}$ (F) of bean plants cv. Pérola submitted to different water regimes

photosynthetic metabolism (Figure 1E). Maintenance of leaf temperature – either equal or slightly superior to the air temperature – attests for the plant's cooling capacity through transpiration so as to keep itself protected against higher thermal oscillations (Oliveira et al., 2005).

It is worth noticing that the size of the seeds did not decrease on account of water deficit (Figure 2A); on the other hand, the number of pods decreased (Figure 1B) together with the number of seeds per pod (Figure 2C). Similar results were observed in bean by Lizana et al. (2006). Considering the evolution aspect of plants, one can say that it would be far more interesting for plants to produce fewer seeds under unfavorable conditions, but with enough reserves to sustain new seedling growth to guarantee the species preservation. Should this strategy prove true, yield will fall
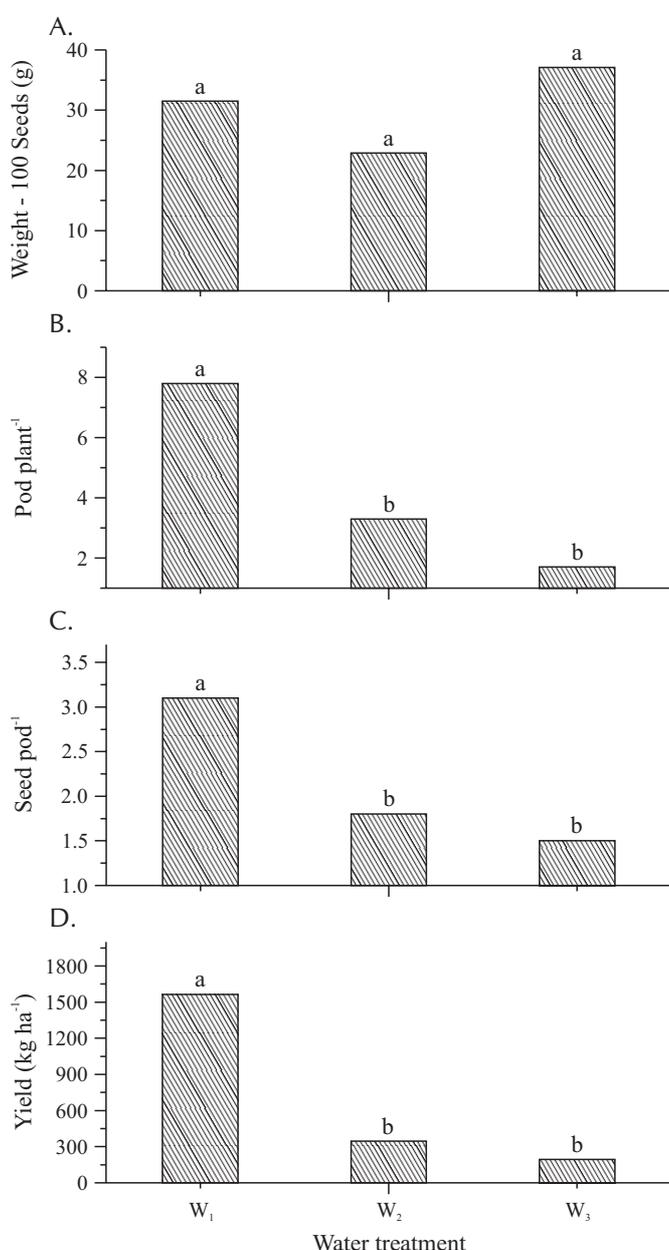
drastically (Figure 2D), mainly because the water deficit may have affected CO_2 assimilation with direct impact on flower abscission, one of the predominant factors of production (Clements & Atkins, 2001). According to Karam et al. (2005), the bean reproductive phase is most vulnerable to water deficit in the soil. Any reduction concerning normal water condition will reflect on productivity, making it drop; for instance, reduction in photosynthesis during pollination causes embryos to abort, causing an inevitable reduction in yields (Kramer & Boyer, 1995). This may be due to the fact that there were no significant differences between productivity parameters from the treatments W_2 and W_3, notwithstanding the fact that this last had suffered water deficit during reproductive period, which shows again that an adequate availability of soil water is extremely vital for grain production, and that a lack of photoassimilation may have a serious impact on the whole process.

Studies have demonstrated that many crops can resist water deficiencies with no damage to plant growth or to any other physiological processes (Turner, 1990). This, however, is not the case of bean crop, especially in its reproductive phase. Water availability is vital for bean production. Water deficit damages the plant's photosynthetic activity by closing the stomata and altering the photosynthetic metabolism. In case this deficit occurs during the reproductive phase, the outcomes of such effects are most disastrous for the plant, causing not only the abortion of embryos, but also the loss of leaf and fruit. The final result is fewer seeds per pod and drastic fall in productivity.

CONCLUSIONS

1. Photosynthesis was one of the main physiological parameter affected by water deficit.

2. Photosynthesis reduction aggravated by water deficit was caused by stomata closure and by reducntion in carboxilation efficiency.

3. Water deficit during flowering caused moderate reduction in the crop productivity due to a reduction in the number of pods and in the number of seeds per pod.

4. The weight of 100 seeds was not affected by water deficit.

ACKNOWLEDGMENTS

Figure 2. Weight of 100 seeds (A), pods per plant (B), seeds per pod (C) and bean productivity (D) cv Pérola submitted to different water regimes (details of the water regime may be seen in Figure 1 legend)

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