

CO₂ AND IRRIGATION IN RELATION TO YIELD AND WATER USE OF THE BELL PEPPER CROP

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ABSTRACT: Greenhouse production of vegetables is widely used throughout the world. Elevated carbon dioxide (CO₂) concentrations in these closed environments can increase net photosynthesis and yield. The objective of this study was to determine the effects of atmospheric CO₂ enrichment and water supply on the growth of potted bell pepper (*Capsicum annuum* L.) plants, cultivated under controlled environmental conditions. CO₂ was applied daily, and its distribution was monitored above plant rows through micro pipes located at 3.0 m height. A drip irrigation system with one dripper per plant was used to irrigate the plants. Different volumes of irrigation water, representing fractions of the water volume (V_{et}) consumed by pot plants growing under no water stress conditions (0.5V_{et}, 0.65V_{et}, 1.0V_{et}, and 1.35V_{et}) with four replications, were evaluated under four different CO₂ levels (atmospheric concentration of 367, 600, 800, and 1000 μmol mol⁻¹). Total fresh fruit mass, average number of fruits, and water use efficiency were recorded. For the water deficit treatments, the greatest fresh fruit mass was obtained for the highest CO₂ level environment. However, for treatments that received water volumes equal or greater than the evapotranspiration rate, the greatest total fresh fruit mass was observed at the 600 μmol mol⁻¹ of CO₂ environment. The yield increase due to CO₂ was represented by increase in fruit weight and not in fruit number. Water use efficiency increased in relation to the amount of water applied and it was highest at 600 μmol mol⁻¹ CO₂ concentration.

Key words: fruit mass, number of fruits, water use efficiency

CO₂ E IRRIGAÇÃO NA PRODUÇÃO E USO DA ÁGUA PARA CULTURA DO PIMENTÃO

RESUMO: O cultivo de hortaliças em ambiente protegido é amplamente utilizado e, nesses ambientes, o enriquecimento da atmosfera com gás carbônico (CO₂) pode aumentar a produtividade pois a fotossíntese líquida normalmente aumenta. Este trabalho avalia o efeito do enriquecimento do ambiente com CO₂ e do volume de água aplicado em plantas de pimentão (*Capsicum annuum* L.), cultivadas em vasos, em ambiente protegido. O experimento foi conduzido em Piracicaba, SP. O CO₂ foi aplicado diariamente e distribuído através de microtubos instalados a 3 m de altura, sobre a linha de plantas. A irrigação foi por gotejamento com um gotejador por planta e frequência de dois dias. Foram adotadas quatro concentrações de CO₂ (concentração normal da atmosfera, aproximadamente de 367, 600, 800 e 1000 μmol mol⁻¹) e quatro volumes de água determinados pelo volume evapotranspirado (V_{et}) por planta (0,5V_{et}; 0,65V_{et}; 1,0V_{et} e 1,35V_{et}), com quatro repetições. Analisaram-se a massa fresca total dos frutos, o número médio de frutos e a eficiência de uso da água. Nos tratamentos com restrição de água a maior massa fresca de frutos foi obtida nos ambientes com maior concentração de CO₂, entretanto nos tratamentos que receberam volume igual ou maior que o volume evapotranspirado, a maior massa fresca de fruto foi verificada no ambiente com 600 μmol mol⁻¹. O CO₂ promoveu o aumento da massa fresca e não do número de frutos. A eficiência de uso da água aumentou em relação ao volume de água aplicado, sendo maior no ambiente com concentração de 600 μmol mol⁻¹.

Palavras-chave: massa de fruto, número de frutos, eficiência de uso da água

INTRODUCTION

The use of carbon dioxide to enrich the atmosphere in greenhouses has been studied since the beginning of the 20th century. According to Schaffer et al. (1999), due to the global carbon dioxide emissions, the atmospheric CO₂ concentration is expected to reach

600 μmol mol⁻¹ in the year 2050. Undoubtedly, this level will affect agriculture in a near future. Due to higher net assimilation rate, atmospheric CO₂ enrichment, in most cases, increases biomass production mainly for C₃ plants. However, due to differences among plant species and environmental factors that limit plant responses to CO₂ there is no agreement about the quantitative effects of

CO₂ enrichment on plant growth (Morison & Gifford, 1984b). Water, nutrients, temperature and light affect carbon intake and metabolism and, therefore, plant yield (Pimentel, 1998).

Photosynthetic efficiency is affected by air temperature, and there is an optimum temperature range for each species. Temperatures above this optimum cause a rapid reduction of the photosynthesis process. Optimum temperature for photosynthesis also changes with the vegetative growth stage (Acock et al., 1990). For the bell pepper, optimum temperature values lie between 20°C and 30°C, and tend to decrease as the plant matures. Both, carbon dioxide and temperature act upon carbohydrate production, while temperature is the most influential variable on carbohydrate mobilization and/or use. Pimentel (1998) pointed out that respiration rates increase with temperature up to the point where the high temperatures cause damage to the protoplasm, when increases in respiration do not affect plant growth anymore.

The experimental design inside of each greenhouse consisted of four randomized blocks with four irrigation treatments and four replications. Two parameters were varied along the experiment: the ambient CO₂ concentration ($\mu\text{molCO}_2 \text{ mol}^{-1}$): C1=1000; C2=800; C3=600; and C4= CO₂ concentration of the air and the volume of water applied through irrigation determined by the evapotranspiration rates of each day (Vet): V1=0.5Vet; V2=0.65Vet; V3=1.0Vet e V4=1.35Vet. A complete analysis was carried out considering the four CO₂ concentrations. The contrast between the two means was calculated by the Tukey test at 5% and plant parameters as a function of water volumes and CO₂ concentration levels was characterized by fitting data to equations.

Stomatal opening affects CO₂ and water vapor exchanges between leaf mesophyll and the atmosphere simultaneously (Raschke, 1986). The increase in CO₂ concentration leads to stomatal closure. This reduces crop transpiration, increases leaf temperature, and reduces nutrient translocation by transpiration mass flow (Huluka et al., 1994). Morison & Gifford (1984a) reported a 21% transpiration rate reduction in crops under CO₂ enriched conditions. Kimball & Idso (1983) reviewing 46 experiments with 18 different species verified that the average transpiration rate reduction was 34%. On the other hand, except under conditions of very low light intensity, Nederhoff & De Graaf (1993) were not able to detect CO₂ effect on transpiration.

Increase in crop yield in CO₂-enriched environments has been recorded in several experiments. Enoch et al. (1976) studied strawberry plants growing under different levels of CO₂ enrichment and noticed that under CO₂ levels of 900, 1500, and 3000 $\mu\text{mol mol}^{-1}$, yields increased respectively 31, 43 and 51% in relation to the control treatment (300 $\mu\text{mol mol}^{-1}$ CO₂ concentration). Caporn (1989) reported that a three-fold

CO₂ enrichment raised both leaf emergence rate and growth rate of individual leaves, resulting in an increase of 37 and 51% in the yield of 30 and 36 day-old lettuce plants, respectively. Reinart et al. (1997) reported increments in tomato yield of approximately 24% with atmospheric CO₂ enrichment.

Bell pepper is of great economical importance not only in Brazil, but also in many other countries. It is an important source of vitamins and minerals, especially iron and phosphorus. It is one of the most suitable crops for cultivation in controlled environments due to the large yield increase and high fruit quality that can be achieved when protected from insects, sun burn, and heavy rainfall. Irrigation management in protected environment is very similar to irrigation management in arid region conditions, where all the water received by the crop is provided by irrigation. Bell pepper is very sensitive to both, lack and excess of water and, in order to have higher productivity, an adequate water supply and a relatively moist soil during the entire development process are needed.

The average yield for field-cultivated bell pepper varies from 18,000 kg ha⁻¹ (Correia, 1984) to 30,000 kg ha⁻¹ (Caixeta et al., 1981). The maximum reported yield for bell peppers cultivated in a greenhouse using drip irrigation is 54,000 kg ha⁻¹ and the minimum 35,700 kg ha⁻¹, for three harvests (Braga, 2000).

Different moisture levels applied through drip irrigation were studied by Teodoro et al. (1993). Who verified that the highest yields were obtained with smaller uptake of available. The authors found that plants irrigated when 30% of the available irrigation water was consumed, yielded more than plants irrigated when 10, 50 or 70% of the available water was consumed. They also observed that plants submitted to a higher water stress (irrigated when 70% of the available water was consumed) had a higher number of defective fruits. Frizzone et al. (1997) observed that an average matric potential of -32 kPa drastically reduced bell pepper productivity under greenhouse conditions, although plant height was not affected by soil water potential.

MATERIAL AND METHODS

This study was carried out in Piracicaba, SP, Brazil (23°42'S, 47°38'W and average altitude of 520 m), where the Köppen climatic classification is Cwa. Bell pepper response to four irrigation levels was evaluated inside four greenhouses submitted to four CO₂ concentrations.

The greenhouses, 8.75 m long, 7 m wide and 3 m tall, were built along the east-west orientation, with arch shaped covers. Sides and fronts were covered with a shade net. The roof consisted of a 150 μm treated anti-UV polyethylene sheet. The sides were also protected with the same material, in such a way that they could be rolled up and down for air circulation.

Two 4 x 0.54 x 0.7 m (length, width, height) wood tables were placed in each greenhouse, leaving a 2 m gap between them. Between the tables and the greenhouse laterals a 2 m. gap was also left. The gap between the tables and the greenhouse fronts was 2.3 m. Each table supported eight pots with one plant each, and the gap between pots was 0.5 m. In one of the greenhouses another 2 x 0.54 x 0.70 m table was installed to support three pots used to measure the irrigation water volume (Vet) corresponding to no plant water stress.

The experimental design inside of each greenhouse consisted of randomized blocks with four irrigation treatments and four replications. The treatments represent four fractions of Vet: V1= 0.5Vet; V2= 0.65Vet; V3= 1.0Vet and V4= 1.35Vet. Following the variance analysis inside each greenhouse, a complete analysis was made considering the mean of the four CO₂ concentrations ($\mu\text{mol mol}^{-1}$): C1= 1000; C2= 800; C3= 600; C4= non-enriched atmosphere, approximately 367. The contrast between two means was measured by the Tukey test at the 5% probability level, and plant parameter behavior as a function of applied water volumes and CO₂ levels was characterized by fitting data to equations.

In order to measure volumes of water percolation, the bottom of all 67 pots had a 7 mm diameter tube connected to a 2 L flask. In order to avoid soil loss, a drainage layer of number one gravel, covered with a geotextile fabric (Bidim), was placed at the bottom of each pot. The drainage layer was uniformly placed, having a constant 2.5 kg mass. The empty volume inside each pot was completed with 2 mm sieved and fertilized sandy soil (Quartzipsamentic Haplorthox). Based on soil fertility analysis, each liter of soil received 0.64 g of lime, 10 g of manure, and 10 g of simple super phsophate. The total mass of the pots reached 20 kg. Initially, water was added to the pots until percolation started, and thereafter they were covered with plastic sheets. Three days before planting the plastic covers were removed.

The selected bell pepper cultivar was the hybrid Zarco, of yellow and greenish colored fruits, rectangular shaped, 12 to 16 cm long, 8 to 10 cm in diameter, with 200 to 260 g (Tivelli, 1998). The seedlings were obtained from a local producer, in cell packs containing as growing medium a commercially prepared seedling mixture (sown on April 17, 2000) and transplanted to pot on May 30, 2000 at the stage of two pairs of true leaves. All lateral sprouts below the first branch were eliminated in order to grow the plants with four shoots. To avoid frutification during the initial vegetative growth stage, the first flower that appeared on the first bifurcation was eliminated.

During planting 0.45 g of urea and 0.1 g of KCl per liter of soil were added. On August 4 0.22 g of urea and 0.017 g of KCl, diluted in water, were also added per liter of soil. According to leaf analysis, seventeen additional applications of 0.030 g of urea and 0.007 g of

KCl per liter were made on a weekly basis. A liquid mixture containing 2.57% of Ca, 0.52% of Bo, 52% of Cu, 2.1% of Fe, 2.57% of Mn, 0.13% of Mo, and 0.53% of Zn was diluted in a proportion of 1.0 g L⁻¹ and sprayed three times on the leaves.

Weeds were controlled manually. Phytosanitary control was restricted to a few applications of sulphur-based fungicides which controlled *Oidiopsis sicula*. Insects such as thrips, leaf miner fly and broad mite, were controlled as they appeared using commercial insecticides. On July 8, more than 50% of the plants presented open flowers and on July 20 there were fruits on all plants. Harvest started on August 13, and the ripe fruits were picked at 8 to 15 day intervals. Seven harvests were made until the end of the crop-growing period which was 169 days long. A drip irrigation system composed by 67 emitters was used, having 98.17% emission uniformity, applying water at 98.1 kPa operating pressure, and a rate of 4.0 L h⁻¹ per plant.

In each greenhouse, irrigation treatments were applied to four pots. A two-day irrigation interval was used and the applied water volume was computed according to the evapotranspiration water volume measured on pots growing under no water stress conditions. The no water stress evapotranspiration volume was estimated daily based on the average mass difference of the three control pots located inside the greenhouse without CO₂ enrichment. This volume was calculated using the following equation:

$$\text{Vet} = [(m_j - m_i) / \gamma] 1000 \quad (1)$$

where: Vet is the no water stress evapotranspiration volume (liters), m_j is the average mass (kg) of the three pots on day j, m_i is the average mass (kg) of the three pots on day i, and γ is the density of water (kg m^{-3}).

Any percolation volume accumulated in the flask during an irrigation interval was reapplied to the respective pot (in order to avoid losing nutrients) and subtracted from the water volume to be applied during next irrigation.

CO₂ from a pressurized cylinder of 25 kg capacity was applied in the atmosphere of the greenhouses. A valve allowed the control of gas discharge rate and pressure. Inside the greenhouses the gas was distributed through two pipes located 3.0 m above the center of each wood table supporting the pots. Gas from these pipes was released to the greenhouse atmosphere through small diameter pipes that were inserted on the distribution line. In each greenhouse with CO₂ enrichment, two valves were installed: one for discharge control and other to control the application time.

CO₂ application started on June 14, after plants overcame the transplant stress. One hour long applications were made every morning, due to the higher photosynthetic efficiency of the plants during this period. During applications the greenhouses were closed, and kept closed for one hour after applications. After that, the

greenhouse sides were opened because the internal temperature raised above 40°C. Measured values of CO₂ concentration indicated that CO₂ levels inside the greenhouses became equal to the atmospheric level just after the opening of greenhouse sides.

For each combination of CO₂ concentration and water volume applied, the associated total fresh fruit mass value is the result of the sum of the mass of fruits picked from four plants along seven harvests. The total number of fruits per plant was obtained by counting the number of fruits picked from each plant. The water efficiency was calculated by the ratio between average total fruit fresh mass and water volume consumed during the crop growth period.

RESULTS AND DISCUSSION

Total fresh fruit

Total fresh fruit mass increased as water volume applied increased and also when CO₂ concentration increased (Table 1). For the two lowest water volumes applied, there was no difference at the 5% level in relation to the total fresh fruit mass between the C4 and C2 environments. For the greatest water volume applied there was no difference among all CO₂ concentration levels. When the applied water volume was increased from 0.65Vet to 1.00Vet (a 52% increase), the crop yield increased for all CO₂ concentrations (67% on the C4 environment, 170% on the C3 environment, 118% on the C2 environment, and 35% on the C1 environment).

For the C4 and C1 environments, an increase of the water volume applied was always followed by a bell pepper yield increase. A similar behavior was reported by Caixeta et al. (1981), Gil (1987), and Teodoro et al. (1993). However, for the C2 and C3 treatments, under increased water volumes yield increased up to a point, and decreased just after, in a similar behavior as reported by Ferreyra et al. (1985).

For the 0.5Vet water volume, in a C2 environment the yield was 28% higher than the C4 environment, and for the other CO₂ concentrations there was a yield reduction. In the C1 environment with the 0.65Vet water volume, the yield was 59% higher in relation to the C4 environment, and for the other the yield was lower. For

Table 1 - Total fresh fruit mass for different combinations of applied water volumes and CO₂ concentrations.

Water Volume	CO ₂ Concentration (μmol mol ⁻¹)			
	C4 = 367	C3 = 600	C2 = 800	C3 = 1000
0.5Vet	177.30 aAB	144.97 aA	227.28 aB	162.62 aA
0.65Vet	248.29 aA	238.89 bA	240.86 aA	394.84 bB
1.00Vet	414.16 bA	645.00 cC	524.40 bB	534.65 cB
1.35Vet	466.10 bA	610.69 cC	525.54 bAB	559.90 cBC

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Values in the same row followed by the same upper case letter are not different according to a 5% Tukey test.

the two highest applied water volumes, 1.00Vet and 1.35Vet, yield increase was observed in all CO₂ enriched environments. The C3 environment resulted in the highest yield, with increase rates of 56% for 1.00Vet and 31% for 1.35Vet.

For treatments in which water was the limiting factor (0.5Vet and 0.65Vet) the highest yields were achieved for the environments with higher CO₂ concentrations. Idso & Idso (1994) report that there is a great diversity between results of studies conducted under CO₂ enriched environments. However, in most of the reports it becomes clear that, when water is the limiting factor, the response of water stressed plants to CO₂ concentration is considerably greater than from non-water stressed plants.

Enoch et al. (1970) carried out an experiment with bell pepper growing under plastic tunnels with and without CO₂ enrichment (10,000 μmol mol⁻¹). On this study, CO₂ applications were limited to that short part of the daytime in which the plastic tunnels were kept closed. These applications started 30 days after transplanting and ended 15 days before the first harvest. Over the complete growth period the yield increase in the CO₂ enriched treatments was 20% in relation to the control. Guri et al. (1998) applied CO₂ in a 400 μmol mol⁻¹ concentration through irrigation water and reported a 10% increase in total green peeper yield. However Storile & Heckman (1996) reported no bell pepper yield increase when CO₂ was injected into the irrigation water at 0, 0.33, 0.67, or 1.0 times the base rate of 0.0273 mol L⁻¹ (1.2 g L⁻¹).

Considering the difficulty in predicting crop response to CO₂-enriched environments, Kimbal (1983) predicted yield increase values between 14 and 61%. The causes of these variations (Peet, 1986) are: conditions in which the crop grows, CO₂ application technique (source, concentration and application regime), total carbon amount, crop specific response, source/sink ratio etc. Fruit production is related to many processes such as net assimilation rate, flowering and dry matter distribution. All these factors can be affected by climate conditions, water and fertilizer supplies, insects, diseases and physiological disturbances.

Average fresh fruit mass (MFF) values as function of water volume (Vet) and CO₂ concentration (C), (Table 1), were adjusted by multiple regression, resulting in a model with a determination coefficient (R²) of 0.8514. The model includes a square term associated to the applied water volume and linear terms associated to the applied water volume and to the CO₂ concentration. The parameters corresponding to the square of the CO₂ concentration and to the interaction between both variables were non-significant at 5% by t test. According to the regression analysis the average fresh fruit mass can be expressed by the following equation:

$$\text{MFF} = -616.51 + 0.112 C + 28.37 \text{ Vet} - 0.184 \cdot \text{Vet}^2$$

$$R^2 = 0.8514$$

Water use efficiency

In most cases water use efficiency was affected by both water and CO₂ (Table 2). For the C4 environment there was no effect of applied water volume upon water use efficiency. For treatments with water stressed plants (0.5Vet and 0.65Vet), the higher values of water use efficiency were observed in C2 and C1, which differ from the C4 values. For treatments 1.00Vet and 1.35Vet, water use efficiency was lower for the C4 environment in relation to the other CO₂ enriched environments. For plants irrigated with the 1.35Vet, the increase in water use efficiency, in comparison to the non-enriched CO₂ environment, was of 56% for the C3 environment, 27% for C2, and 29% for C1.

For the C4 and C1 environments, the fresh fruit mass increased for treatment 1.35Vet (Table 1), indicating that a greater water volume would induce a greater yield increase. It is, however, evident that the production factor viability should not be determined only by yield increase, but also by the plant use efficiency on this factor.

Stomatal opening necessary for CO₂ assimilation causes an unavoidable water loss as the transpiration control is directly associated to the CO₂ supply to leaves. The stomatal conductivity must show a time variation in such a way that water loss is minimized and CO₂ assimilation is maximized. Biomass increase associated to transpiration reduction induced by increase in CO₂ concentration has been observed in many experiments (Morison & Gifford, 1984b, Zarbi & Burrage, 1998; Centrito et al., 1999).

In this study water use efficiency was computed based on the water volume applied per plant. If they had been computed based on plant transpiration, the values would have been higher for the CO₂ enriched environments, since the water consumption by plants was reduced in the CO₂ enriched environments. At the end of the experiment the leaf area was smaller for the CO₂ enriched environments. This may have contributed for the reduction in plant evapotranspiration rate for the CO₂ enriched environments.

Number of fruits per plant

Number of fruits per plant was higher in treatments (1.00Vet and 1.35Vet) when compared to

Table 2 - Average water use efficiency for different combinations of applied water volume and CO₂ concentrations.

Water Volume	CO ₂ Concentration (μmol mol ⁻¹)			
	C4 = 367	C3 = 600	C2 = 800	C3 = 1000
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0.50Vet	5.74 aAB	4.69 aA	7.36 abB	5.26 aA
0.65Vet	6.10 aA	5.87 bA	5.92 aA	9.70 cB
1.00Vet	6.70 aA	10.43 cC	8.48 bB	8.64 bcB
1.35Vet	5.63 aA	7.37 dC	6.34 aAB	6.76 abBC

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Values in the same row followed by the same upper case letter are not different according to a 5% Tukey test.

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smaller amounts of water applied (0.5Vet and 0.65Vet). For all CO₂ concentration treatments, no statistical differences were found at the level of 5% between number of fruits per plants for treatments 1.00Vet and 1.35Vet (Table 3).

In relation to the CO₂ concentration, for the 0.5Vet and 1.00Vet volumes, there was no difference in the number of fruits per plant. For the 0.5Vet volume, the number of fruits of the C4 environment was equal to the C2 environment. However, for the C2 environment, the fresh fruit mass was greater (Table 1). For 0.65Vet, in the C4, C2, and C1 environments, there was no significant difference between the numbers of fruits per plant. However, an increase of 59% on the fresh fruit mass was observed for the C1 environment.

In a CO₂ enriched environment, the yield increase is due to the increase of individual fruit mass and not to the number of fruits produced per plant. Similar results were obtained in studies with tomatoes (Calvert & Slack, 1975; Kimball & Mitchell, 1979; Islam et al., 1996). According to Islam et al. (1996), probably more carbohydrates were accumulated in the fruits, since the rate of photosynthesis is higher in CO₂ enriched environments.

For the 1.00Vet and 1.35Vet volumes, the highest number of fruits was obtained for the C3 environment (Table 3). Calvert & Slack (1975) obtained similar results for tomatoes cultivated in a 600 μmol mol⁻¹ CO₂ enriched environment.

Multiple regression analysis applied to the average number of fruits per plant (NF) showed that the quadratic effect of CO₂ concentration and the interaction between water volume and CO₂ concentration were not significant at 5%. The resulting model is:

$$NF = -8.42 + 0.0014 C + 0.55 V - 0.0034 V^2 \quad (R^2) = 0.9095$$

The values of fresh fruit mass were well below the expected for the selected hybrid (cv. Zarco). This was the same for all plants considered in the experiment, regardless of water volume or CO₂ concentration. Several factors could have induced to the development of small fruits. High soil and air temperatures and low air relative humidity prevailing

Table 3 - Average number of fruits per plant (NF), for seven harvests and for different combinations of applied water volumes and CO₂ concentrations.

Water Volume	CO ₂ Concentration (μmol mol ⁻¹)			
	C4 = 367	C3 = 600	C2 = 800	C3 = 1000
0.50Vet	6.25 aA	6.75 aA	6.25 aA	5.00 aA
0.65Vet	9.25 bAB	7.75 aA	10.00 aB	10.50 bB
1.00Vet	12.50 cA	13.75 bA	12.75 bcA	13.50 cA
1.35Vet	12.25 cA	15.75 bB	14.00 cAB	15.50 cB

Values in the same row followed by the same lower case letter are not different according to a 5% Tukey test.

Values in the same line followed by the same upper case letter are not different according to a 5% Tukey test.

during the crop growing period were important factors contributing to development of smaller fruits.

In conclusion, increases in CO₂ concentration of the atmosphere promote increases in both, yield and water use efficiency. The greatest increase was for the 600 μmol mol⁻¹ CO₂ (C3) concentration environment with the application of 1.00Vet of water.

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