

# Gas exchanges, $\delta^{13}\text{C}/^{12}\text{C}$ isotopic ratio and enzymatic activity in bell pepper under different irrigation sheets

Intercambio de gases, relación isotópica de  $\delta^{13}\text{C}/^{12}\text{C}$  y actividad enzimática en pimiento morrón bajo diferentes láminas de riegos

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## ABSTRACT

The cultivation of bell pepper demand irrigation practices to guarantee fruit production and quality. The objective of this work was to assess the physiological and biochemical changes, as well as the variation in isotopic discrimination of  $\delta^{13}\text{C}$  in bell pepper plants submitted to different irrigation sheets. The experimental design was organized in a completely randomized design (CRD) with three treatments (irrigation sheets) and five replicas, sheet 1 (S1):  $\Psi_m = 10$  to 15 kPa, sheet 2 (S2):  $\Psi_m = 34$  to 40 kPa and sheet 3 (S3):  $\Psi_m = 54$  to 60 kPa. Four evaluations were performed at 50, 65, 80 and 95 days after transplantation (DAT), measuring Leaf water potential ( $\Psi_{lw}$ ), net  $\text{CO}_2$  assimilation ( $A$ ), stomatal conductance ( $g_s$ ), transpiration ( $E$ ), isotopic discrimination ( $\delta^{13}\text{C}/^{12}\text{C}$ ), enzyme activity of nitrate reductase (NR), superoxide dismutase (SOD) and catalase (CAT). Finally, fruits were collected to determine productivity. Bell pepper plants with less water availability and depending on their development, the leaf water potential was more negative, resulting in greater stomatal closure which caused a considerable decrease in the net  $\text{CO}_2$  assimilation, transpiration, enzyme activity of nitrate reductase and fruit production, and greater activity of antioxidant enzymes. The increase and duration of water restriction in bell pepper plants induced less isotopic discrimination of  $\delta^{13}\text{C}$ .

**Keywords:** *Capsicum annuum* L., carbon assimilation, hydric stress, metabolism productivity.

## RESUMEN

El cultivo de pimiento morrón demanda prácticas de riego para garantizar la producción y calidad del fruto. El objetivo de este trabajo fue evaluar los cambios fisiológicos y bioquímicos, así como la variación en la discriminación isotópica de  $\delta^{13}\text{C}$  en plantas de pimiento sometidas a diferentes láminas de riego. El diseño experimental consistió en un diseño completamente al azar (DCA) con tres tratamientos (láminas de riego) y cinco repeticiones, lámina 1 (L1):  $\Psi_m = 10$  a 15 kPa, lámina 2 (L2):  $\Psi_m = 34$  a 40 kPa y lámina 3 (L3):  $\Psi_m = 54$  a 60 kPa. Se realizaron cuatro evaluaciones a los 50, 65, 80 y 95 días después del trasplante (DDT), midiendo el potencial hídrico foliar ( $\Psi_{lw}$ ), la asimilación neta de  $\text{CO}_2$  ( $A$ ), la conductancia estomática ( $g_s$ ), la transpiración ( $E$ ), la discriminación isotópica ( $\delta^{13}\text{C}/^{12}\text{C}$ ), actividad enzimática de nitrato reductasa (NR), superóxido dismutasa (SOD) y catalasa (CAT). Finalmente se recolectaron frutos para determinar la productividad. En plantas de pimiento morrón con menor disponibilidad de agua y, dependiendo de su desarrollo, el potencial hídrico foliar fue más negativo, resultando en un mayor cierre de estomas lo que provocó una disminución considerable en la asimilación neta de  $\text{CO}_2$ , transpiración, actividad de la enzima nitrato reductasa y producción de frutos y mayor actividad de las enzimas antioxidantes. El aumento y la duración de la restricción de agua en las plantas de pimiento morrón indujeron una menor discriminación isotópica de  $\delta^{13}\text{C}$ .

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**Palabras clave:** *Capsicum annuum* L., asimilación de carbono, estrés hídrico, productividad del metabolismo.

## INTRODUCTION

Water deficiency (WD) is one of the most important environmental factors, which causes large losses in agriculture worldwide. Vegetables are the most sensitive to the lack of water compared to other crops (Kumar *et al.*, 2012). Several studies confirm that the reduction of water in the soil during both the vegetative and the reproductive phase of bell peppers affects the final productivity (Ferrara *et al.*, 2011; González-Dugo *et al.*, 2007). Delfine *et al.* (2001) report that the growth of plants and the production of the first fruits, under dry farming conditions and in the vegetative phase, were not affected. However, as the WD increases and as the plants age, photosynthetic activity and stomatal conductance were reduced, affecting their growth and decreasing the final fruit production when compared to plants without water restriction.

The isotopic discrimination of carbon  $\delta^{13}\text{C}$  (calculated as the proportion  $\delta^{13}\text{C}/^{12}\text{C}$ ) in leaves of  $C_3$  plants correlates with the photosynthetic gas exchange, and its control depends on the concentration ratio of  $\text{CO}_2$  in the intercellular spaces of leaves and the atmosphere (Farquhar *et al.*, 1982). Therefore, the lower discrimination of plants between the two isotopes (more positive  $^{13}\text{C}/^{12}\text{C}$ ) is due to the lower  $\text{CO}_2$  pressure in their interior, when compared to environmental pressure (Ehlers and Goss, 2003). Thus, the  $^{13}\text{C}$  isotope has been used as a criterion for crop improvement programs under conditions of water and salt stress (Yousfi *et al.*, 2012; Araus *et al.*, 2013).

In unfavourable environmental conditions, the rates of photosynthetic activity decrease, with a considerable increase in photorespiration, which induces the formation of reactive oxygen species (ROS) according to Miller *et al.* (2010). The ROS are more reactive molecules than molecular oxygen, which reacts with other macromolecules and can generate cascade reactions. ROS are extremely toxic, which can cause oxidative damage to proteins, DNA, and lipids that make up membranes (Apel and Hirt, 2004). The most common forms of ROS are superoxide radicals ( $\text{O}_2^-$ ), hydroxyl ( $\text{OH}^\cdot$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and singlet oxygen ( $^1\text{O}_2$ ).

Plants are able to adapt to the decrease in water potential and unfavorable climatic changes. This is done through the induction of antioxidative and non-enzymatic systems, which acts for the dismutation of ROS. Therefore, the essential enzymes of the antioxidative system are the superoxide dismutase (SOD), ascorbate peroxidase (APX) catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PrxR). Other non-enzymatic antioxidants are ascorbic acid (AsA) and glutathione (GSH) (Wang *et al.*, 2024). Another important enzyme for mineral metabolism in plants is the nitrate reductase (NR), responsible for the assimilative reduction of nitrate ( $\text{NO}_3^-$ ). Several studies have corroborated that plants under WD decrease the enzymatic activity of NR, probably due to the lower flow of nitrate from the soil to the root (Dubey *et al.*, 2021). Thus, the objective of this work was to assess the physiological and biochemical changes, as well as the variation in isotopic discrimination of  $\delta^{13}\text{C}$  in bell pepper plants submitted to different irrigation sheets.

## MATERIAL AND METHODS

The experiment was carried out in greenhouse with an area of 63 m<sup>2</sup> at the Department of Chemistry and Biochemistry of the IB/UNESP in Botucatu-SP - Brazil, between January and May 2017 during the summer and autumn seasons. Gaston Bell pepper seedlings (*Capsicum annum* L.), which present a red coloring when ripe, were used. The transplant was performed 40 days after sowing (plants with 4 to 6 expanded leaves), in 22-litter pots. The planting mineral fertilization was as recommended by Freire *et al.* (2013), and the covering fertilization was based on the recommendations of Trani *et al.* (2011) which performs a balance of nutrients for each growth stage.

The bell pepper plants were irrigated by keeping the soil  $\Psi_m$  in the 10 to 15 kPa range until 50 DAT (at the beginning of flowering). The differentiation of the irrigation sheets was initialized at 51 DAT establishing the sheet 1 (S1):  $\Psi_m = 10$  to 15 kPa, sheet 2 (S2):  $\Psi_m = 34$  to 40 kPa and sheet 3 (S3):  $\Psi_m = 54$  to 60 kPa. The drip irrigation system was used. Before the test, the water distribution uniformity test in the system was carried out using the distribution uniformity coefficient (DUC) method. For the monitoring of soil moisture, the tensiometry technique was used, previously determining the soil water retention curve (volumetric humidity as a function of the matric potential,  $\text{cm}^3 \text{cm}^{-3}$ ), using the Richards pressure camera method. For the soil water retention curve modeling, the SWRC (soil water retention curve) software, version 3.0, was utilized, described by Dourado-Neto *et al.* (2000) and adjusted to the Van Genuchten (1980) model. The irrigation sheets were calculated with the matric potential values of the soil ( $\text{cm}^3 \text{cm}^{-3}$ ), considering the concept of available water capacity (AWC) and irrigation efficiency. Finally, the irrigation time (in minutes) for each treatment was determined according to Equation 1:

$$It = \left( \frac{Ab \times A}{n \times q} \right) * 60 \quad \text{Eq. (1)}$$

In which: It=irrigation time (min); Ab = applied sheet (mm); A = area occupied by plant (m<sup>2</sup>); n = number of emitters per plant (1); q = dripper flow (L h<sup>-1</sup>).

To maintain the established tension ranges, six tensiometers were installed per treatment, at a distance of 5 cm from the plant stem and 15 cm deep in the soil. Daily, from 8:00 to 8:30 a.m., the reading of the soil water tension was recorded with the aid of a digital tensiometer, and depending on the voltage value, irrigation was proceeded or not.

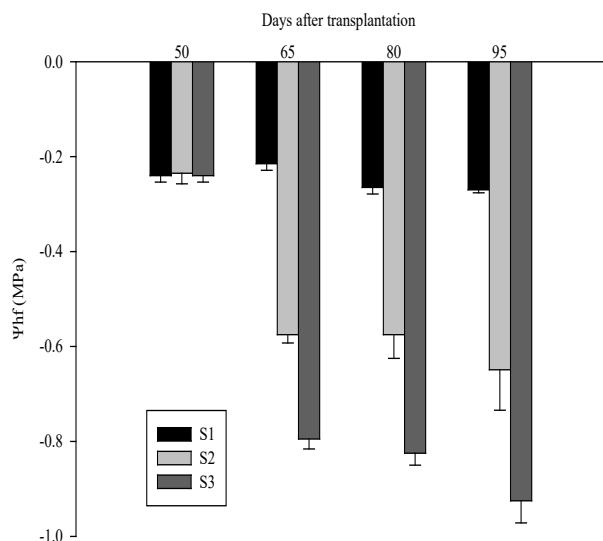
For all the evaluated variables, leaves from the middle third of the plants were used, except for fruit production. Leaf water potential ( $\Psi_{lw}$ ) was measured from the same plants used for biometric determination (data not shown). Leaves were collected for the analysis of the isotopic ratio, total soluble protein content and determination of enzymatic activity. In the crops, the leaves were frozen with liquid nitrogen and kept in an ultra-freezer at -80 °C. Measurements of the water potential in the leaf xylem at 5:00 a.m. (predawn), were performed with the aid of a Scholander pressure chamber (Scholander *et al.*, 1965), from Soil Moisture Equipment Corp., Santa Barbara, California-USA as described by Kramer and Boyer (1995). The net assimilation of  $\text{CO}_2$  (A), stomatal conductance (gs) and transpiration (E) were evaluated. For the measurements of photosynthesis a portable IRGA LI-COR 6400 was used between 9:00 and 11:30 a.m.

For Carbon isotope ratio analysis ( $^{13}\text{C}/^{12}\text{C}$ ), the samples were analyzed at the Center for Stable Isotopes of the UNESP Biosciences Institute - Botucatu campus, state of Sao Paulo, Brazil. Part of the material (leaves) kept in the ultra-freezer at -80 °C was dried in a forced ventilation oven, at 50 °C for 48

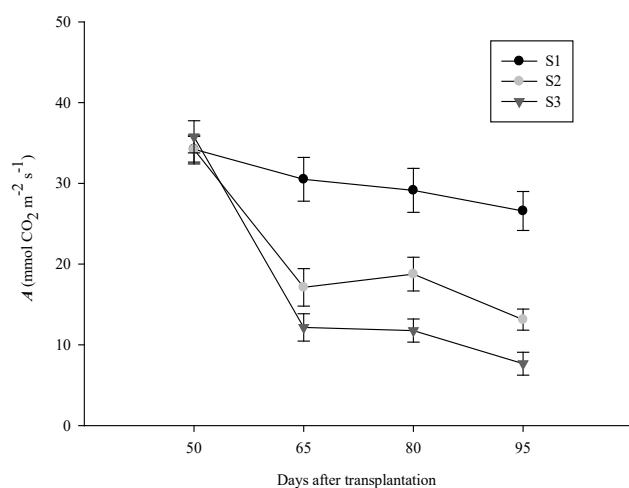




the reduction of  $\Psi_{\text{wf}}$  caused by the closure of stomata (Nardini *et al.*, 2001), which was demonstrated in this test (Figure 3). Figure 1 show that the plants in sheet 1 maintained water potential values between -0.24 MPa and -0.27 MPa during the performed evaluations. After the imposition of WR, the plants from irrigation sheets 2 and 3 showed values between -0.58 MPa and -0.65 MPa, and -0.80 MPa and -0.93 MPa, respectively. These results prove that, in bell pepper plants, the more negative the water potential is, the values of the components of gas exchange (Figures 2, 3 and 4) and fruit production (Table 5) are considerably reduced.



**Figure 1.** Leaf water potential ( $\Psi_{\text{wf}}$ ) measured before dawn in bell pepper plants grown under three irrigation sheets. S1:  $\Psi_{\text{m}} = 10$  to 15 kPa, S2:  $\Psi_{\text{m}} = 34$  to 40 kPa and S3:  $\Psi_{\text{m}} = 54$  to 60 kPa. Bars indicate standard deviation ( $n=5$ ).  
**Figura 1.** Potencial hídrico foliar ( $\Psi_{\text{wf}}$ ) medido antes del amanecer en plantas de pimiento morrón cultivado bajo tres láminas de riego. L1:  $\Psi_{\text{m}} = 10$  a 15 kPa, L2:  $\Psi_{\text{m}} = 34$  a 40 kPa y L3:  $\Psi_{\text{m}} = 54$  a 60 kPa. Las barras indican la desviación estándar ( $n=5$ ).

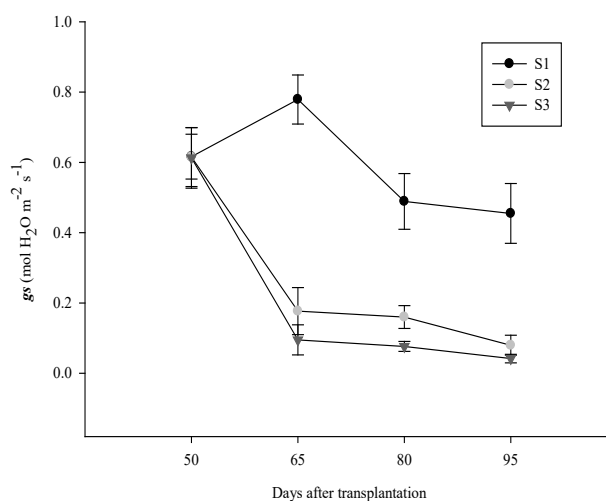


**Figure 2.** Liquid CO<sub>2</sub> assimilation ( $A$ ) in bell pepper plants grown under three irrigation sheets. S1:  $\Psi_{\text{m}} = 10$  to 15 kPa, S2:  $\Psi_{\text{m}} = 34$  to 40 kPa and S3:  $\Psi_{\text{m}} = 54$  to 60 kPa. Bars indicate standard deviation ( $n=5$ ).  
**Figura 2.** Asimilación de CO<sub>2</sub> líquido ( $A$ ) en plantas de pimiento morrón cultivadas bajo tres láminas de riego. L1:  $\Psi_{\text{m}} = 10$  a 15 kPa, L2:  $\Psi_{\text{m}} = 34$  a 40 kPa y L3:  $\Psi_{\text{m}} = 54$  a 60 kPa. Las barras indican la desviación estándar ( $n=5$ ).

## Gas exchange

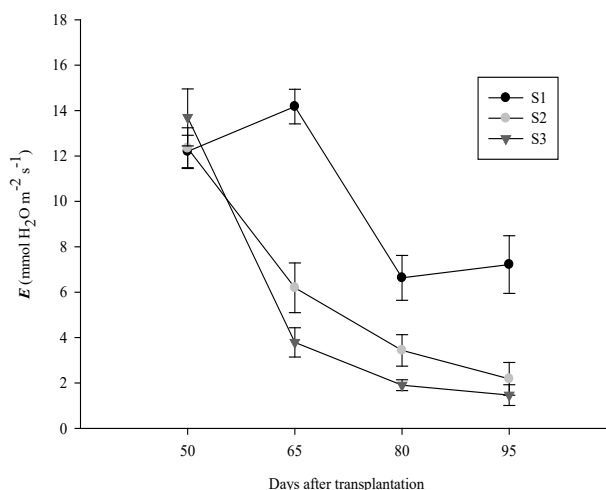
### Liquid CO<sub>2</sub> assimilation (A)

In figure 2, it can be observed that, when plants were submitted to WR, the photosynthesis gradually decreased as the water decreased in the soil. Similar results to this study, in which both the photosynthetic rate and the stomatal conductance decrease after the imposition of WR, were reported by Kulkarni and Phalke (2009). When working with different cultivars of hot pepper under conditions of protected environment and water restriction. Likewise, in seedlings (grafted and non-grafted) of mini-watermelon, these gas exchange components have decreased in parallel with the severity of water restriction (Rouphael *et al.*, 2008).



**Figure 3.** Stomatal conductance ( $g_s$ ) in bell pepper plants grown under three irrigation sheets. S1:  $\Psi_{\text{m}} = 10$  to 15 kPa, S2:  $\Psi_{\text{m}} = 34$  to 40 kPa and S3:  $\Psi_{\text{m}} = 54$  to 60 kPa. Bars indicate standard deviation ( $n=5$ ).

**Figura 3.** Conductancia estomática ( $g_s$ ) en plantas de pimiento morrón cultivadas bajo tres láminas de riego. L1:  $\Psi_{\text{m}} = 10$  a 15 kPa, L2:  $\Psi_{\text{m}} = 34$  a 40 kPa y L3:  $\Psi_{\text{m}} = 54$  a 60 kPa. Las barras indican la desviación estándar ( $n=5$ ).



**Figure 4.** Transpiration ( $E$ ) in bell pepper plants grown under three irrigation sheets. S1:  $\Psi_{\text{m}} = 10$  to 15 kPa, S2:  $\Psi_{\text{m}} = 34$  to 40 kPa and S3:  $\Psi_{\text{m}} = 54$  to 60 kPa. Bars indicate standard deviation ( $n=5$ ).

**Figura 4.** Transpiración ( $E$ ) en plantas de pimiento morrón cultivadas bajo tres láminas de riego. L1:  $\Psi_{\text{m}} = 10$  a 15 kPa, L2:  $\Psi_{\text{m}} = 34$  a 40 kPa y L3:  $\Psi_{\text{m}} = 54$  a 60 kPa. Las barras indican la desviación estándar ( $n=5$ ).



### Stomatal conductance (*gs*)

The *gs* is a parameter that indicates the opening and closing levels of the stomata, depending on the availability of water for the plant. Thus, WR promotes the opening reduction of stomatal pores, since the amount of water for the guard cells is reduced. In this test it was proved that the *gs* decreased considerably due to the severity of the WR (Figure 3), in response to the adaptation mechanisms of the plant itself to avoid dehydration, thus regulating its water status. Similarly, Paiva *et al.* (2005) reported that, in bean leaves, the *gs* decreased significantly in the flowering and grain filling stages in the treatment with less water availability.

### Transpiration (*E*)

When plants have good water availability in the soil (field capacity) they have high transpiratory rates, facilitating water movement between the soil-plant-atmosphere. However, as the water content in the soil decreases as a result of the treatments, the plants decrease their transpiratory rate to reduce the loss of water in the form of steam. In this test (Figure 4) plants under water restriction showed lower values of *E* when compared to plants without water restriction. At 65 and 80 DAT, the plants of irrigation sheets 2 and 3 decreased their transpiratory rate respectively. At 95 DAT, the sheet 3 irrigation plants achieved transpiration rates similar to those of the irrigation sheet 2. This behavior, a result of WR, maintained a direct relationship with what happened in stomatal conductance (Figure 3), resulting in the closure of stomata in plants with less water availability as a strategy to avoid the excessive loss of water from plant tissues. Results similar to those in this trial were reported by Ferrara *et al.* (2011), who observed that when submitting bell pepper plants to WR the physiological variables, such as liquid  $\text{CO}_2$  assimilation, stomatal conductance and transpiration significantly decreased in the vegetative and reproductive phases when compared to the control plants.

### Isotopic carbon breakdown ( $\delta^{13}\text{C}$ )

In bell pepper plants, irrigation sheets 2 and 3 caused a decrease in the isotopic discrimination values of  $\delta^{13}\text{C}$  to 80 DAT. In the last evaluation (95 DAT), only irrigation sheet 3 showed statistically less discrimination of  $\delta^{13}\text{C}$  in relation to other treatments. These results agree with those reported in cotton crops (Brito *et al.*, 2014) and sunflower (Adiredjo *et al.*, 2014), in which the isotopic discrimination of  $\delta^{13}\text{C}$  decreased in parallel with the severity of WR. That is, plants with scarce water availability in the soil have less isotopic discrimination of carbon. In this sense, the lower discrimination of  $\delta^{13}\text{C}$  in plants with less water availability in the soil after 80 DAT may have been influenced by the lower  $\text{CO}_2$  pressure (Figure 2) due to stomatal closure (Figure 3). That is a factor contributes to the discrimination during diffusion and carboxylation (Farquahr *et al.*, 1982).

### Activity of the nitrate reductase enzyme (NR)

The activity of the nitrate reductase enzyme has been used as an indicator of stress and changes linked to factors that

modulate plant growth (Dubey *et al.*, 2021; Carelli *et al.*, 1996). Table 2 shows that NR activity was also reduced in pepper plants after the imposition of water treatments (65 and 80 DAT). In the last evaluation (95 DAT), the irrigation 3 sheet plants differed statistically from irrigation 1 and 2 sheets plants. These results of the lower NR values under WR treatments are possibly related to the decrease in the photosynthetic rate (Figure 2), with nitrogen participating in the formation of chlorophyll, which is essential in the process of photosynthesis. Similarly, cotton plants (*Gossypium hirsutum* L.) under water stress have been reported to decrease NR activity in parallel with the decrease in leaf water potential (Marur *et al.*, 2000), a fact that was also evidenced in the present trial (Figure 1).

### Activity of the superoxide dismutase enzyme (SOD)

The increase in the enzymatic activity of superoxide dismutase is a defense response of plants under stress conditions, since it is the first response line of the antioxidative system (Del Río *et al.*, 2018). In Table 3 can be observed that, at 80 DAT, the plants in sheet 3 showed the highest SOD activity. In the last evaluation (95 DAT) the plants of sheets 2 and 3 had a significantly similar behavior, but with higher values to plants without water restriction. On the other hand, Yuan *et al.* (2016) worked with tomatoes under four soil moisture levels during two cycles under protected environment con-

**Table 1.** Carbon isotopic discrimination in bell pepper plants grown under three irrigation sheets.

**Tabla 1.** Discriminación isotópica de carbono en pimiento morrón cultivado bajo tres láminas de riego.

Treatment	50 DAT	65 DAT	80 DAT	95 DAT
	Carbon isotopic discrimination ( $\delta^{13}\text{C}$ )			
S1	-29.3 a	-30.1 a	-29.6 b	-29.3 b
S2	-29.3 a	-30.2 a	-28.4 a	-28.6 b
S3	-29.7 a	-29.8 a	-27.7 a	-27.1 a
P	0.4702	0.6332	0.0002	0.0020
CV (%)	2.0	2.4	1.7	2.7

Means followed by the same letter in the column do not differ statistically from each other by the Tukey test, at the level of 5 % probability. DAT = days after transplant; S1:  $\Psi_m = 10$  to 15 kPa, S2:  $\Psi_m = 34$  to 40 kPa and S3:  $\Psi_m = 54$  to 60 kPa. CV (%) = Percentage of the coefficient of variation.

**Table 2.** Nitrate reductase enzyme activity in bell pepper plants grown under three irrigation sheets.

**Tabla 2.** Actividad de la enzima nitrato reductasa en pimiento morrón cultivado bajo tres láminas de riego.

Treatments	50 DAT	65 DAT	65 DAT	95 DAT
	Nitrate reductase enzyme activity ( $\text{nM NO}_2\text{ g}^{-1}\text{ MF h}^{-1}$ )			
S1	2309.2 a	2127.8 a	1878.4 a	1038.9 a
S2	2385.6 a	1686.2 b	1491.1 b	833.3 a
S3	2306.2 a	1554.8 b	1365.1 b	247.1 b
P	0.7219	0.0004	0.0098	0.0001
CV	7.45	9.42	14.37	17.31

Means followed by the same letter in the column do not differ statistically from each other by the Tukey test, at the level of 5% probability. DAT = days after transplant; S1:  $\Psi_m = 10$  to 15 kPa, S2:  $\Psi_m = 34$  to 40 kPa and S3:  $\Psi_m = 54$  to 60 kPa. CV (%) = percentage of the coefficient of variation.

**Table 3.** Superoxide dismutase enzyme activity in bell pepper plants grown under three irrigation sheets.

**Tabla 3.** Actividad de la enzima superóxido dismutasa en pimiento morrón cultivado bajo tres láminas de riego.

Treatment	50 DAT	65 DAT	80 DAT	95 DAT
	Superoxide dismutase enzyme activity (IU $\mu\text{g protein}^{-1}$ )			
S1	1.2 a	1.2 a	1.3 b	1.6 b
S2	1.1 a	1.6 a	1.5 b	2.2 ab
S3	1.0 a	1.9 a	2.5 a	2.7 a
P	0.779	0.147	0.0003	0.0149
CV (%)	18	13	20	11

Means followed by the same letter in the column do not differ statistically from each other by the Tukey test, at the level of 5% probability. DAT = days after transplant; S1:  $\Psi_m = 10$  to 15 kPa, S2:  $\Psi_m = 34$  to 40 kPa and S3:  $\Psi_m = 54$  to 60 kPa. CV (%) = percentage of the coefficient of variation.

ditions, noting that the SOD values increased according to the severity of WR in the growth stages.

However, in the fruit maturation stage, the plants of the control treatment (75 to 80 % of field capacity) and those of the treatment considered as mild water deficiency (55 to 60 % of the field capacity), showed significantly similar SOD values. Thus, according to the results reported by Yuan *et al.* (2016) and those of this test in bell pepper plants, the SOD values depend on the time of application of WR, severity, and phenological stage of the plants.

### Catalase enzyme activity (CAT)

Several studies show that plants under stress conditions (biotic and abiotic) present accelerated production of reactive oxygen species (ROS), which can be toxic to the metabolism of plants or inhibit their growth. The dismutation of reactive oxygen species is performed by the antioxidative system, which comprises numerous enzymes and low molecular weight compounds (Banerjee and Roychoudhury, 2017). The catalase enzyme is the main enzyme that acts in the decomposition of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) to water ( $\text{H}_2\text{O}$ ) and molecular oxygen ( $\text{O}_2$ ). In this trial, CAT activity (Table 4) was activated at 80 and 95 DAT in plants under water restriction, which showed significantly similar values between treatments, however, with values higher than those of irrigation sheet 1. Similar results to those obtained in this study were reported by Abid *et al.* (2017) in two greenhouse cul-

**Table 4.** Catalase enzyme activity in bell pepper plants grown under three irrigation sheets.

**Tabla 4.** Actividad de la enzima catalasa en cultivo de pimiento morrón cultivado bajo tres láminas de riego.

Treatments	50 DAT	65 DAT	80 DAT	95 DAT
	Activity of the catalase enzyme ( $\mu\text{Kat } \mu\text{g protein}^{-1}$ )			
S1	44.8 a	47.5 a	50.6 b	56.9 b
S2	46.7 a	60.3 a	64.5 ab	70.7 ab
S3	45.7 a	71.5 a	75.7 a	84.5 a
P	0.983	0.093	0.002	0.001
VC (%)	20	16	12	13

Means followed by the same letter in the column do not differ statistically from each other by the Tukey test, at the level of 5% probability. DAT = days after transplant; S1:  $\Psi_m = 10$  to 15 kPa, S2:  $\Psi_m = 34$  to 40 kPa and S3:  $\Psi_m = 54$  to 60 kPa. CV (%) = percentage of the coefficient of variation.

tivars of fava beans (*Vicia faba* L.) in the winter period under three irrigation sheets (90, 60 and 30 % of field capacity). The results obtained for both cultivars (tolerant and susceptible) under the treatment considered as severe water restriction, showed significantly higher CAT activity when compared to the plants of the control treatment.

### Bell pepper fruit production

On average, 7.4 fruits were collected per plant for irrigation sheet 1, with commercial productivity of 31.1 t  $\text{ha}^{-1}$  (Table 5). Plants of sheet 2 and 3 presented lower commercial productivity, in the order of 11.2 and 2.7 t  $\text{ha}^{-1}$ , respectively. It should be noted that the harvest period lasted 28 days. On the other hand, Carvalho *et al.* (2016) worked with different soil water tensions in the reproductive phase of red pepper cv. Konan, the authors reported production for the control treatment of 5.85 fruits per plant, equivalent to commercial production of 12.03 t  $\text{ha}^{-1}$  in protected environment conditions, with a 42-day harvest period. Thus, and according to the results obtained in this trial and those reported by other authors (Frizzone *et al.*, 2001; Dermitas and Ayas, 2009), it is concluded that the decrease in water in the soil strongly affects the yield of peppers. In addition, it is emphasized that this difference in fruit productivity may vary according to the cultivar, season and climatic conditions.

**Table 5.** Production of ripe bell pepper fruits grown under three irrigation sheets.

**Tabla 5.** Producción de frutos maduros de pimiento morrón cultivados bajo tres láminas de riego.

Treatments	NFP	AFM (kg)	TFMP (kg)	TP ( $\text{ha}^{-1}$ )	% NMF	PC (t $\text{ha}^{-1}$ )
S1	7.4	0.183	1.354	33.9	8	31.1
S2	5.4	0.128	0.691	17.3	35	11.2
S3	4.0	0.70	0.280	7.0	62	2.7

NFP = number of fruits per plant; AFM = average fruit mass; TFMP = total fruit mass per plant; TP = total production; % NMF = percentage of non-marketable fruits; CP = commercial production; S1:  $\Psi_m = 10$  to 15 kPa, S2:  $\Psi_m = 34$  to 40 kPa and S3:  $\Psi_m = 54$  to 60 kPa.

### CONCLUSIONS

Bell pepper plants with less water availability and according to their development, decreased relative to  $\Psi_{lw}$  resulting in a lower photosynthetic rate which may be related to stomatal closure, and therefore less discrimination of  $\delta^{13}\text{C}$ . Likewise, plants under water restriction showed a considerable reduction in the enzymatic activity of nitrate reductase (NR). Bell pepper plants have generated defense mechanisms to adapt to less water availability in the soil (decreased leaf water potential), increasing the activity of antioxidant enzymes in both superoxide dismutase (SOD) and catalase (CAT), a strategy used to decrease the production of ROS. Therefore, the physiological and the biochemical parameters evaluated were affected according to the severity of the water deficiency, resulting in less fruit production and quality.

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**CONFLICTS OF INTEREST**

The authors declare that there is no conflict of interest.

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