



## **Assessing Different Irrigation Regimes Regarding Chlorophyll Content of the Sweet Bell Pepper**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors HK and AT designed the study. Authors AT, GEA and HK performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MC, AS, UG and HE managed the analyses of the study. Authors AT and GEA managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Effective use of water is critically significant in the agriculture where water is most used. Many researchers indicate that at present and in the future, irrigated agriculture will have to take place under limited irrigation water available. Deficit irrigation, which aims efficient use of water, is becoming more predominant. Due to all these reasons, it is very important to find out the effect of the deficit irrigation on the plant growth. Photosynthesis is one of the physiological events that affect plant development. Chlorophyll plays an important role in photosynthesis process by absorbing sunlight and uses its energy to synthesise carbohydrates from CO<sub>2</sub>. The aim of this study is to determine the effect of different irrigation regimes on chlorophyll a and b contents of the sweet bell pepper. For this purpose, this study was carried out in a controlled 1 da glasshouse environment in the research area at Faculty of Agriculture, Akdeniz University. In the experiment four irrigation regimes (I<sub>100</sub>, I<sub>80</sub>, I<sub>60</sub> and I<sub>40</sub>) were examined. Chlorophyll analysis of the sweet bell pepper was carried out according to the method of acetone extraction before and after irrigation following the

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fruit formation period. According to the experiment results, it was found out that chlorophyll degradation increases due to the aging of the plant leaves, therefore, the amount decreased. It was also found that this decrease in  $I_{40}$ , which is the most stressful treatment, is much more evident than in other treatments.

**Keywords:** *Capsicum annuum*; chlorophyll a; chlorophyll b; pepper; water deficit.

## 1. INTRODUCTION

Pepper of the *Solanaceae* family is one of the most produced vegetables in the world. According to the FAOSTAT [1] data, Turkey is the third largest pepper producer in the world with 2457822 tonnes. The most consumed pepper variety is *Capsicum annuum* L. Water is considered to be the most important factor limiting plant growth and yield [2,3]. Today, water-saving irrigation techniques must be developed in agriculture due to the fact that water resources are scarce in the world [4]. Deficit irrigation is one of these strategies that affect the yield at minimal level and water-saving by increasing the water use efficiency [5,6].

Water stress during the most critical periods such as vegetative, flowering or fruit setting causes substantial yield loss [7,8,9]. Limitation of water inhibits the plant growth by creating an adverse effect on cell growth and division [10]. Drought is one of the factors affecting photosynthesis and chlorophyll content [11]. Chlorophyll plays an important role in photosynthesis process by absorbing sunlight and uses its energy to synthesise carbohydrates from  $CO_2$  [12]. Chlorophyll fluorescence is a measure of the efficiency of photosynthesis and can be used, therefore, as an indicator of vegetation health and vitality [13]. Indeed, a decrease in leaf water potential (lower LWP) slows chlorophyll synthesis rate and accelerates chlorophyll degradation

[10]. The most important types of chlorophyll in plants are chlorophyll a and b [14].

The aim of this study is to determine the effects of different deficit irrigation treatments and dripper locations on chlorophyll a and b content of the sweet bell pepper plant.

## 2. MATERIALS AND METHODS

This study was conducted at the Research and Application Land of Agricultural Faculty in Akdeniz University. The geographic coordinates of the experimental area are  $36^{\circ}54'15''$  N and  $30^{\circ}38'30''$  E (Fig. 1). In the research area where the Mediterranean climate is dominant, summers are warm and dry, winters are cool and rainy. The average annual temperature is  $18.0^{\circ}C$ , the coldest is January by  $9.2^{\circ}C$  and the warmest at  $28.2^{\circ}C$  in July. Annual mean relative humidity, total precipitation and evaporation were 63%, 1063.5 and 1886.3 mm respectively [15]. In addition, average temperature and relative humidity values inside the greenhouse during the experiment were  $20.1^{\circ}C$  and 56.2%, respectively.

The soil of the experimental area is from Gölbasi territory series. These soils, developed on massive travertines, are included in the Entisol order. They have a clay texture in all profiles and almost flat topography. Their permeability is good and there is no drainage problem.



**Fig. 1. Experiment area**

The 'Doğanay F1' cultivar of the sweet bell pepper (*Capsicum annuum* L.) was used in the experiments. Doğanay F1 variety is thin-skinned, vigorous and very early. Growing periods of the pepper are spring and autumn seasons. The reason for choosing this pepper variety is that it is also cultivated widely in the region.

The experiment was set up as a randomised design with four irrigation regimes ( $I_{100}$ ,  $I_{80}$ ,  $I_{60}$ ,  $I_{40}$ ) and two irrigation treatments replicated three times. There were 480 seedlings in total (4 irrigation regimes x 2 irrigation treatments x 3 replications x 20 seedlings) in the experiment area. Four water regimes: the control ( $I_{100}$ ), in which plants received 100% of the soil water consumption, and 80% ( $I_{80}$ ), 60% ( $I_{60}$ ), 40% ( $I_{40}$ ) of the control treatment and two irrigation treatments:  $T_1$ : drippers were located in the plant root zone,  $T_2$ : drippers were located among two plants.

The amount of water to be applied to the parcels in each irrigation is calculated according to equation 1 by using the measurements obtained from the Class-A evaporation pan which is located inside the greenhouse. The amount of irrigation water applied to the control treatments was 27.7 L/plant during the growing season.

$$I = E_p \times k_p \times k_c \times A \quad (1)$$

where  $I$  (L) is the amount of irrigation water,  $E_p$  (mm) is total evaporation from the A-Class pan corresponding to the irrigation range,  $k_p$  is the pan coefficient (0.80) and  $k_c$  is the crop coefficient (plant cover ratio was started from 0.30 and increased to 1.25 depending on plant development),  $A$  ( $m^2$ ) is the area to be irrigated.

Acetone extraction method was used to determine the chlorophyll content of the plant leaves [16]. Extraction was homogenised and prepared by adding 0.1 g  $CaCO_3$  and 25 ml acetone (80%) on 0.25 g fresh leaves taken from 3 healthy leaves in each replication. After the tissue is thoroughly disintegrated, it was filtered with filter paper. Extraction obtained was measured in 663 nm (for chlorophyll-a) and 645 nm (for chlorophyll-b) in UV-Vis T60 model spectrophotometer. Also, leaf water potential was measured at 13:00 (mid-day) when the stress was the most severe at the same time as chlorophyll was analysed at the beginning, middle and end of the growing season. Data was analysed by the general linear model procedure (GLM) using Statistical Analysis Software (SAS 9.2). Means are compared using Duncan's

Multiple Range Test, if necessary, to separate the means of the data at  $P < 0.05$  level of significance.

### 3. RESULTS AND DISCUSSION

In the study, it was aimed to determine the effect of different irrigation regimes and dripper locations on chlorophyll-a and b contents. Comparison of means of leaf water potential, chlorophyll-a and b content and dripper location before and after irrigation are given in Table 1. Results of analysis of variance showed that irrigation treatments had a significant effect on the leaf water potential, chlorophyll a and chlorophyll b. The highest means were found to be in the control ( $I_{100}$ ) treatment. It was found out that leaf water potential, chlorophyll a and b contents decreased by increasing water stress.

For chlorophyll a content before irrigation, it was determined that when irrigation regimes which were  $I_{80}$ ,  $I_{60}$  and  $I_{40}$  are compared with control treatment ( $I_{100}$ ), they decreased by the ratio of 4%, 6%, 9% according to the control treatment, respectively. It was also found out after irrigation that when  $I_{40}$  treatment were compared with the control treatment ( $I_{100}$ ), it decreased by the ratio of 14% according to the control treatment. In this study, it was obtained that before the irrigation chlorophyll b content of  $I_{60}$  and  $I_{40}$  treatments decreased by 6% and 9% compared to the control treatment respectively. After irrigation chlorophyll b content also decreased by 13% in  $I_{40}$  treatment. There were not any statistical differences in the leaf water potential values and after irrigation chlorophyll a and b content in dripper locations. However, before irrigation chlorophyll a and b content statistically differed in the leaf water potential values in dripper locations. These differences in before irrigation chlorophyll a content was caused by dripper location because drippers were exactly in the plant root zone in  $T_1$  treatment and among the two plants in  $T_2$  treatment. It was thought that the plants in  $T_2$  treatment were exposed to water stress faster than  $T_1$ .

It was shown that plants in all treatments were under stress conditions before irrigation, however, these stress conditions disappeared relatively after irrigation (Fig. 2). This can help understand that a similar situation was also observed with chlorophyll-a and b contents (Figs. 3 and 4). It was determined that chlorophyll-a and b contents increased after irrigation due to the stress conditions. As the level of stress

applied to the plants increases, the state of relief decreases after irrigation.

Alvino et al. [17] find out that midday leaf water potentials of pepper plant were about twice as

low in the water-stressed treatment as in the controls. Delfine et al. [18] conducted a study in which bell pepper plants were grown under irrigated and rain-fed conditions. According to the research results, midday leaf water potentials in

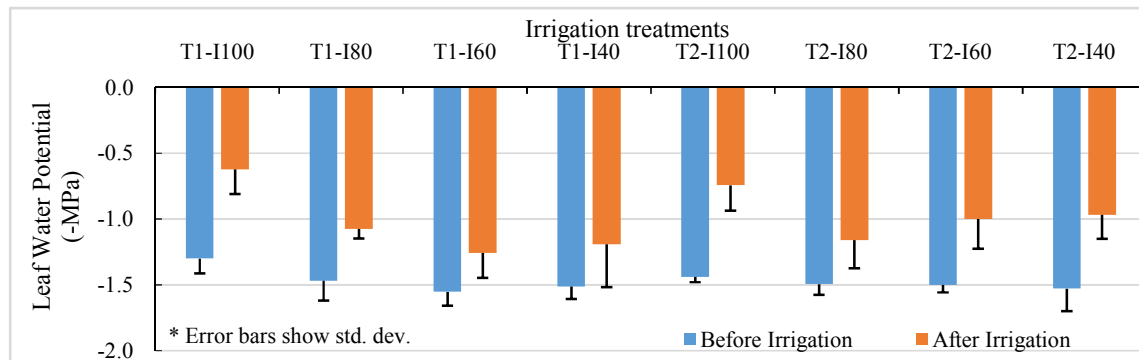
**Table 1. Comparisons of means of leaf water potential and chlorophyll-a and b at different irrigation regimes and dripper locations**

Treatments	Leaf water potential			Chlorophyll-a content			Chlorophyll-b content		
	Before irrigation	After irrigation	P>F	Before irrigation	After irrigation	P>F	Before irrigation	After irrigation	P>F
T1	-1.457	-1.040		0.720 a	0.820		0.305 a	0.326	
T2	-1.491	-0.971		0.683 b	0.828		0.280 b	0.330	
Dripper location (DL)	NS	NS	***	NS	NS	***	NS	NS	
I <sub>100</sub>	-1.370 Ba	-0.686 Aa	***	0.736 Ba	0.865 Aa	***	0.303 Ba	0.343 Aa	***
I <sub>80</sub>	-1.480 Bab	-1.120 Ab	***	0.709 Bb	0.850 Aa	***	0.308 a	0.343 a	NS
I <sub>60</sub>	-1.528 Bb	-1.131 Ab	***	0.689 Bbc	0.834 Aa	***	0.285 Bb	0.327 Aa	***
I <sub>40</sub>	-1.519 Bb	-1.084 Ab	**	0.673 c	0.747 b	NS	0.275 c	0.298 b	NS
Irrigation regime (IR)	*	***		***	**		***	**	

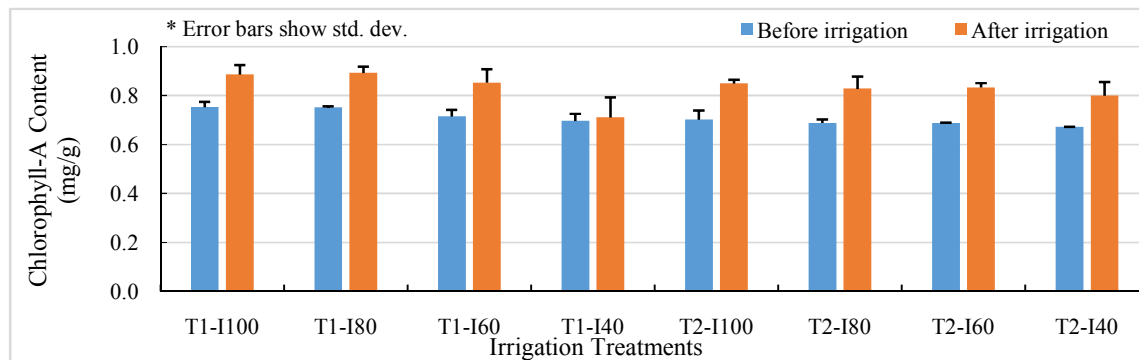
Means followed by the different capital letters in each row or small letters in each column are significantly different at 5% level by Duncan test.

\*, \*\*, \*\*\* : significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

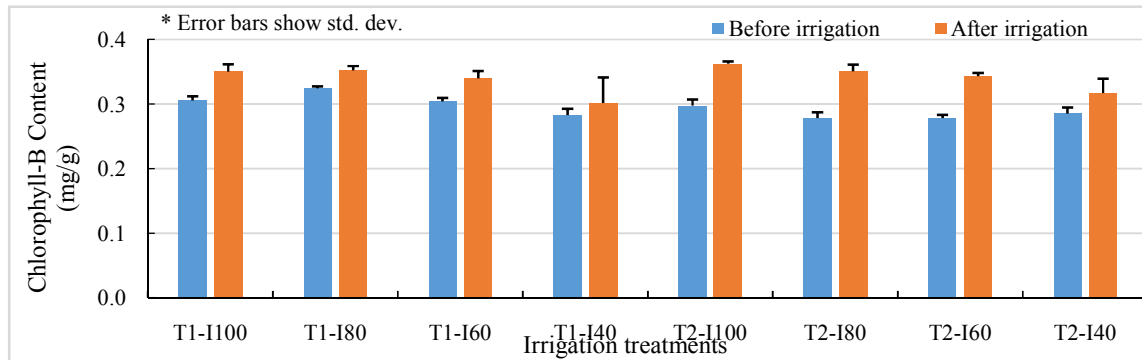
NS: non-significant



**Fig. 2. Comparisons of leaf water potential before and after irrigation**



**Fig. 3. Comparisons of chlorophyll-a content before and after irrigation**



**Fig. 4. Comparisons of chlorophyll-b content before and after irrigation**

the rain-fed plants decreased to -2.4 MPa, while in the irrigated plants ranged between -1.4 and -1.5 MPa. Leaf water potentials of pepper plants increased as the soil dried (at maximum water stress levels). QiuShi et al. [19] attempted to determine the effects of water stress on photosynthesis and associated physiological characteristics of pepper (*Capsicum annuum*). They subjected to two water levels: 75-80% (control) and 30-40% (water stress) of the field capacity. They found out that leaf water potential of pepper plant decreased under the water stress conditions. Kulkarni and Phalke, [20] grew pepper plant with two different water treatments, normal and 50% water application under water deficit condition. They found that predawn leaf water potential was lower in the water-stressed plants -0.42 over the season as compared to the control (-0.23). Drought treatment significantly reduced the mean leaf water potential to -0.79 throughout the growth period as compared to the control (-0.51) at noon, but LWP decreased markedly at the fruiting stage. Our findings were quite similar with these results.

Anjum et al. [21]; Farooq et al. [22] found that water stress changes the ratio of chlorophyll 'a' and 'b'. According to Khaleghi et al. [12], the amount of chlorophyll a was reduced by increasing the water deficit. In fact, the amount of chlorophyll a was higher in plants receiving 100%  $ET_{crop}$  than 65 and 40%  $ET_{crop}$ . Some researchers also reported that lower irrigation levels decreased chlorophyll index value [23], chlorophyll content [24,25] and chlorophyll a and b [26] significantly in pepper plant. Reduction in chlorophyll content was reported for drought stressed pepper, cotton, oriental lily, maize [23,27,28,29] Arzani and Yazdani [30] showed that the amount of chlorophyll a, b significantly decreased under drought stress. Our results

are in line with these findings regarding pepper plant.

#### 4. CONCLUSION

According to the research findings, application of water deficit during the growing season causes decrease in leaf water potential, chlorophyll-a and b contents in the sweet bell pepper plants. It was also found out that these stress conditions can be decreased with the irrigation for the pepper plant relatively. Chlorophyll-a and b contents of  $I_{40}$  irrigation regime were minimum before and after irrigation. Differences between before and after leaf water potential are more in  $I_{100}$  and  $I_{80}$  irrigation regimes. The reason for this is thought to be due to the fact that the stress level in other irrigation regimes is high. Differences between before and after leaf water potential in  $I_{60}$  and  $I_{40}$  irrigation regime is also lower because of continuously water stress. As a result, it can be stated that leaf water potential, chlorophyll-a and b contents decrease in relation to each other as water stress increases. According to the results,  $I_{80}$  irrigation regime can be suggested as there are no significant differences with  $I_{100}$  irrigation regime when these parameters such as leaf water potential and chlorophyll content which is used as an indicator of vegetation health and vitality are considered. In addition,  $I_{80}$  irrigation regime increases water use efficiency by saving water 20%.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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