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Response of Maize to Temperature, Carbon Dioxide and Irrigation Levels under the Conditions of Greenhouse

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Abstract: Maize is one of the major cultivated crops in Turkey. There has been a significant increase in studies of maize under interactive effects of elevated CO_2 concentration and other factors, but the interactive effects of elevated CO_2 and increasing irrigation and temperature on maize has remained unclear. In this study, the effects of different temperature regimes (16/30 °C and 22/36 °C day/night), CO_2 (ambient CO_2 and elevated CO_2) conditions and irrigation treatments (full irrigated and reduced irrigated) on early growth characteristics of maize were studied as pot experiments under greenhouse conditions. The plant height (PH), chlorophyll content index (CCI), leaf area (LA), leaf fresh weight (FW), leaf dry weight (DW), relative water content (RWC), paraquat sensitivity index (PSI) and relative cell injury (RCI) were examined. Results suggested that the temperature was a primary important factor because of the direct influence on all observed characteristics in maize. Water stress-associated with high temperature was often considered to be a limiting factor in maize production. In addition, it was observed that CO_2 and irrigation also influential climate factors depending on the temperature.

Keywords: Climate change, CO_2, maize production, temperature, irrigation.

Sera Koşullarında Sıcaklık, Karbon Dioksit ve Sulama Seviyelerine Mısırın Tepkisi

Öz: Mısır Türkiye'de kültürü yapılan en önemli bitkilerden biridir. Artan CO_2 ve diğer koşulların interaksiyonu ile ilgili mısır üzerine yapılan çalışmalarda önemli bir artış bulunmaktadır, fakat artan CO_2 artan sulama ve sıcaklığın mısır üzerine birlikte etkisi belirsiz kalmıştır. Bu çalışmada, farklı sıcaklık rejimleri (16/30 °C ve 22/36 °C gündüz/gece), CO_2 koşulları (normal CO_2 ve yüksek CO_2) ve sulama uygulamasının (tam sulama ve azaltılmış sulama) mısırın erken gelişim parametreleri üzerine etkisi sera koşullarında saksı denemesi olarak yürütülmüştür. Bitki boyu (BB), klorofil içeriği (Kİ), yaprak alanı (YA), yaprak yaş ağırlık (YYA), yaprak kuru ağırlık (KA), nisbi nem içeriği (NNİ), paraquat hassasiyet indeksi (PHİ) ve nisbi hücre zararı (NHZ) incelenmiştir. Sonuçlar sıcaklığın mısırda gözlenen tüm özellikler üzerine doğrudan etkisi nedeniyle birincil önemli faktör olduğunu göstermiştir. Su stresi yüksek sıcaklık ile birlikte mısır üretimini sınırlayıcı bir faktör olarak kabul edilmiştir. Ayrıca, CO_2 ve sulamanında sıcaklığa bağlı etkili bir faktör olduğu gözlenmiştir.

Anahtar Kelimeler: İklim değişikliği, CO_2, mısır üretimi, sıcaklık, sulama.

1. Introduction

Maize (*Zea mays* L.) is the C4 species which is the most extensively grown in the world. Maize production and productivity varies constantly depending on environmental changes. In scenario of global climate changes, air temperature may increase between 1.4 -5.8 ^{o}C in association with rises in atmospheric CO_2 (Cubasch et al. 2001). These changes may increase the frequency of extremes, including drought conditions, which will have significant consequences for crop growth and food supply in the future (Easterling et al. 2007). We focus on maize because of the importance of this crop to smallholders and have a wide range of uses than other cereal such as human food, as a feed grain, fodder crop, and for hundreds of industrial purposes. The reason for this it broad global distribution, its low price relative to other cereals, its diverse grain types and its wide range of biological and industrial properties (Ammani et al. 2012). By 2050 demand for maize will double in the developing world, and maize is predicted to turn out to be the crop with the greatest production globally, and in the developing world by 2025 (Cairns et al. 2012). Due to the C4 plant, maize is capable of using solar energy more effectively and can tolerate relatively high temperature up to a critical value. Heat stress can be described as temperatures above a threshold level that results in irreversible loss to crop growth and development (Cairns et al. 2012). Both high and low temperatures have a negative effect on the growth and development of maize (Nguyan et al. 2009; Chen et al. 2012; Ur Rahman et al. 2013). High temperatures can induce an array of morphological, anatomical, physiological and biochemical changes within maize (Cairns et al. 2012). Low temperature also has adverse effect on germination, seedling growth, early leaf development (Nguyan et al. 2009). The early growth stage of maize coincides with in May and June. Long term average minimum and maximum temperatures are 16 °C and 30 °C respectively in Aydin. Demir et al. (2008) revealed that day/night temperatures may increase 5-6 ^{o}C during growth period according to 2071-2100 years scenario in Turkey. Maize is usually disposed to present little or no additional growth in reaction to elevated CO_2. At elevated CO_2 conditions, maize showed an increase in CO_2 assimilation during periods with less rain. These increases are based on the high intracellular concentration of CO_2 at low stomatal conductance and decreased transpiration (João et al. 2012; Bunce 2014). The influence of high CO_2 depends on temperature. Based on the differential temperature susceptibility of the solubility of CO_2 and O_2, it could be expected that increasing temperature would increase the affinity of ribulose bisphosphate carboxylase/oxygenase for CO_2, causes a raise in the CO_2 stimulation of photosynthesis with temperature (Cairns et al. 2012). Maize is the most important crops in irrigated semiarid areas of the world. Thus it is very sensitive to water stress which affects growth and decreases the conversion of radiation into biomass (Rimski-Korsakov et al. 2009; Cavero et al. 2009).

There are very few studies about temperature, CO_2, irrigation and interaction with each other. High temperature and rising CO_2 level caused by global climate change and water stress which may be a consequence of these were investigated.

2. Materials and Methods

2.1. Greenhouse experiment

The pot experiment was conducted at the greenhouse of the Faculty of Agriculture, Adnan Menderes University, Aydin-Turkey in 2011-2012 and 2012-2013. Maize cultivar (*Zea mays* L. cv. PR31G98) was utilized in this study. The long term average minimum and maximum temperature (16/30 ^{o}C) (12 h/12 h) was used as present-day temperature values at early growth stage (May-June) in Aydin (Anonymous 2009).

Table 1. The temperature, humidity and CO_2 values at the greenhouse in 2011/12

Temperature regime	CO_2 regime	Temperature (°C)		CO_2 (ppm)		Relative hum.(%)	
		Night	Day	Night	Day	Night	Day
16/30 °C	Ambient CO_2	17	30	592	519	85	68
	Elevated CO_2	17	30	787	792	87	72
22/36 °C	Ambient CO_2	21	35	515	472	77	58
	Elevated CO_2	22	36	783	799	82	66

Night regime: 18:00-06:00; Day regime: 06:00-18:00

Table 2. The temperature, humidity and CO_2 values at the greenhouse in 2012/13

Temperature regime	CO_2 regime	Temperature (^{0}C)		CO_2 (ppm)		Relative hum. (%)	
		Night	Day	Night	Day	Night	Day
16/30 ^{0}C	Ambient CO_2	17	30	549	427	82	58
	Elevated CO_2	16	29	800	753	88	71
22/36 ^{0}C	Ambient CO_2	23	36	492	405	82	63
	Elevated CO_2	22	36	834	772	84	69

Night regime: 18:00-06:00; Day regime: 06:00-18:00

High temperature regimes are set at 22/36 ^{o}C (a temperature rise of 5.8 degrees as the upper limit given) (IPCC 2002). CO_2 values were 400 ppm which is present rate and 700-800 ppm which is expected to reach at the beginning of this century. Thus, different temperature regimes, CO_2 concentrations and irrigation levels were studied. Studies were conducted in 4 chambered greenhouses where temperature and CO_2 was controlled. Plants were grown in growing chamber at 12 h (06:00-18:00) light conditions and 200 micromol $m^2.sn^{-1}$ photosynthetic active light with 12 lamp. Plants divided into two irrigation groups in each chamber. The irrigation applications were full and reduced irrigated. Desired temperatures were provided through cooling/heating units at each chamber. The manifold which had 12 CO_2 tubes (out of the greenhouse) and pipe (in a greenhouse) were used for CO_2 applications. The temperature, humidity and CO_2 values in the greenhouse were measured through Hobo data recorder. Each pots (18x18x15 cm) filled with field soil/peat/sand/perlite mixture (1:1:1:1) with volume 3.8 liter. Maize was sown 4 seeds per pot and thinned to 1 plant per pot after emergence. There were no fertilizer applications. Full and reduced irrigated applications were started at the 2-4 leaf stage of maize. All plants were irrigated at required amount until this period. Drip irrigation system was used in greenhouse. The calculation of full irrigated amount was measured as; firstly the water was given until the water drained from the bottom of the pots and then irrigation time was measured for this application. In reduced irrigated application, water was provided as half of that time. Amount of water in different treatment was full irrigated (395 mm) and (406 mm) in 2012 and reduced irrigated (197.5 mm) and (203 mm) in 2013. Harvest was done 10.06.2012 and 10.06.2013 (V6 growing stage). In the study, five plants were selected for observations in each application. The temperature, humidity and CO_2 values at the greenhouse in 2011/12 and 2012/13 were given at Table 1 and Table 2. The experiment was laid out using Randomized Complete Block Design with three factors and three replications. Factors were CO_2 concentrations (main plot), temperature regimes (subplot) and irrigation levels (sub-sub plot), respectively.

2.2. Crop measurements

Plant height (cm): One week after starting of irrigation applications, measurements were made. Plant height was measured from the soil surface to top of leaf with the help of a meter rod and average height was calculated in the greenhouse.

Chlorophyll content index: For the portable Apogee, CCM-200 plus meter, readings were taken on the fully expanded leaf from the top of the plant and about halfway between the tip and the base of the leaf. Readings were taken from each pot.

Leaf area (cm^2): Leaf area was measured from 4th leaves with the help of area meter (model CI-202) by averaging the leaf value taken from five plant samples from each application.

Leaf fresh and dry weight (g): After the leaves were harvest from each pot, above-ground leaf weights were weighed. And then, leaves in an oven set to heat (65 degrees) two days.

Relative water content (RWC) (%): It was calculated according to Barr and Weatherley (1962): RWC = (FW-DW) / (TW-DW) x 100 (FW: Fresh weight, DW: Dry weight, TW: Turgor weight).

Paraquat sensitivity index (PSI): Firstly leaves were floated for 24 h within sterilized water containing 100 µM Paraquat under 1200 lux light conditions for paraquat applications. After that chlorophyll contents were measured. These measurements were used to determine percent chlorophyll loss of plants. After paraquat treatment, values were divided by control condition values and in this was Paraquat Sensitivity Indexes (PSI) were determined Cakmak (1994). PSI<1 (sensitive to photochemical injury); PSI=1 (tolerant to photochemical injury); PSI > (resistant to photochemical injury)

Relative cell injury (RCI) (%): It is highly correlated to yield (Farooq et al. 2011). RCI, an indicator of cell membrane thermostability (CMT) was measured according to Sullivan (1972) from the formula: RCI (%) = 1-[{1-(T1/T2)} / {1-(C1 / C2)}] × 100. T1: EC of sap of treated discs (50 °C) before autoclaving; T2: EC of sap of treated discs (50 °C) after autoclaving; C1: EC of sap of treated discs (25 °C) before autoclaving; C2: EC of sap of treated discs (25 °C) after autoclaving

2.3. Statistical analysis

All data were statistically analyzed with the SPSS (1999). Probabilities equal to or less than 0.05 were considered significant. Differences between treatments were performed with LSD test to separate them.

3. Results and Discussion

The results of the experiments were tested statistically to determine the effects of climate factors. The results showed that CO_2, irrigation and temperature levels were significantly affected maize agronomic and physiologic characters (Table 3 and Table 4). Mean values of maize were given at Table 5 and 6 in 2011/12, Table 7 and 8 in 2012/13. The highest plant height values were obtained from at increased temperature (22/36 °C) in 2011/12. The values of plant height were 84.8 cm and 107.7 cm at ambient CO_2 and elevated CO_2 conditions, respectively. Full irrigation levels showed that the highest values were observed with 99.5 cm at ambient CO_2 and 95.7 cm at elevated CO_2. Besides, high temperature and elevated CO_2 levels were increased the plant height. The results are in line with the findings of Koti et al. (2007), who revealed that there was CO_2's positive influence on plant height in soybean. Similarly, Odiyi (2013) stated that drought stress significantly reduced plant height of maize seedlings.

Table 3. Analysis of variance of some agronomic and physiological characters for maize in 2011/12

Source of variation	df	PH	FW	DW	PSI	RCI	RWC	LA	CCI
Replication	2	3.2	6.6	0.0	0.0	1.9	2.6	1.9	0.1
Factor A	1	661.5**	255.5**	14.3**	0.3**	422.5**	21.1	3975.8**	0.0
Factor B	1	192.7**	109.7**	0.1	0.5**	627.3**	2206.1**	7069.2**	1.7**
AxB	1	80.7**	113.1**	1.8**	0.2**	49.0**	2.9	5397.0**	0.0
Factor C	1	5280.7**	3021.8**	95.1**	0.4**	7815.7**	2055.4**	4985.3**	0.3**
AxC	1	912.7**	412.5**	17.7**	0.1**	24.2**	2.0	13286.9**	0.3*
BxC	1	0.2	19.6*	0.3**	0.2**	384.8**	3084.9**	2036.9**	3.3**
AxBxC	14	4.2	10.8*	0.7**	0.2**	36.8**	3.6	5224.5**	0.0
Error	23	6.8	2.2	0.2	0.0	2.4	13.0	7.04	0.0

* Significant at the 0.05 level, ** significant at the 0.01 level

Factor A: CO_2, Factor B: Irrigation, Factor C: Temperature, PH: Plant height, FW: Fresh weight, DW: Dry weight, PSI: Paraquat sensitivity index, RCI: Relative cell injury, RWC: Relative water content, LA: Leaf area, CCI: Chlorophyll content index

Table 4. Analysis of variance of some agronomic and physiological characters for maize in 2012/13

Source of variation	df	PH	FW	DW	PSI	RCI	RWC	LA	CCI
Replicat.	2	0.8	0.0	0.0	0.0	3.3	9.0	14.2	0.2
Factor A	1	35.0**	330.8**	3.6**	0.0*	108.4	490.5**	5244.8**	1.8**
Factor B	1	477.0**	100.5**	8.1**	0.07**	125.1	5627.3**	11042.0**	1.2**
AxB	1	35.0**	145.5**	4.6**	0.01**	2.9	86.3**	634.6**	26.3**
Factor C	1	108.4**	3320.6**	1.5**	0.02**	153.0	147.5**	4134.1**	84.8**
AxC	1	2.0**	490.5**	3.8**	0.01**	45.4	12.8	2173.8**	11.9**
BxC	1	0.4	13.1**	0.2**	0.00**	596.0*	437.8**	2790.5**	0.5
AxBxC	14	0.4	15.5**	3.6**	0.00	24.8	65.0**	4777.4**	22.6**
Error	23	0.2	0.0	0.0	0.00	111.0	4.7	4.7	0.1

* Significant at the 0.05 level, ** significant at the 0.01 level

Factor A: CO_2, Factor B: Irrigation, Factor C: Temperature, PH: Plant height, FW: Fresh weight, DW: Dry weight, PSI: Paraquat sensitivity index, RCI: Relative cell injury, RWC: Relative water content, LA: Leaf area, CCI: Chlorophyll content index

This result is contradictory to the previous findings of Koti et al. (2007). However, Mulholland et al. (1997) found that elevated CO_2 had not significant effect on chlorophyll content relative to the control. Results were consistent with earlier results by Coskun et al. (2011), who reported chl a varied from 1.361 mg g $^{-1}$ to 1.839 mg g $^{-1}$ and it may be owing to high temperature stress conditions. Alberte et al. (1977) stated that the majority of chlorophyll lost in response to water stress occurred. Gholamin and Khayatnezhad (2011) also observed that drought stress was a negative effect on the chlorophyll parameters. In this study, it was found that elevated CO_2 conditions were reduced chlorophyll content but Mulholland et al. (1997) found that elevated CO_2 hadn't significant effect on chlorophyll content relative to the control.

Table 5. Mean values of some agronomic and physiological characters of maize in 2011/12

Treatments			FW (g)	DW (g)	PSI	RCI	LA (cm^2)
Ambient CO_2	Full irrigated	16/30 °C	9.3 a	1.0 a	0.94 a	10.9 b	208.8 a
		22/36 °C	26.6 b	3.4 b	0.98 a	34.5 b	121.9 b
	Reduced irrigated	16/30 °C	3.8 a	0.5 a	1.07 a	13.5 b	133.4 a
		22/36 °C	14.8 b	2.7 b	1.82 a	58.1 b	68.6 b
Elevated CO_2	Full irrigated	16/30 °C	4.5 b	0.7 b	0.85 b	17.7 a	76.5 b
		22/36 °C	35.7 a	5.8 a	0.98 a	50.3 a	142.7 a
	Reduced irrigated	16/30 °C	5.0 a	0.6 a	0.98 b	19.5 a	120.1 b
		22/36 °C	35.3 a	6.8 a	1.07 b	63.2 a	90.4 a
LSD (0.05)			2.6	0.2	0.09	2.7	4.7

FW: Fresh weight, DW: Dry weight, PSI: Paraquat sensitiviyty index, RCI: Relative cell injury, LA: Leaf area

The highest leaf area value was obtained from ambient CO_2, full irrigated and 16/30 °C with 208.8 cm^2. The second year the highest value (214.5 cm^2) was observed at ambient CO_2, full irrigation and 22/36 °C conditions. The values of leaf area were influenced by CO_2, irrigation and temperature applications. Full and reduced irrigated conditions gave the lower leaf area than that obtained from ambient CO_2 and full irrigated conditions. It has been reported by Human et al. (1990) that leaf area reduced proportionally with increased water stress. These results were also parallel to the findings of Prasad et al. (2008) who concluded that the drought and heat stress can negatively affect leaf area production and also green leaf area duration. However, Kim Soo et al. (2007) indicated that leaf area values were not changed in response to CO_2 enrichment.

The highest fresh weight was 35.7 g and 35.3 g at elevated CO_2 and high temperature in 2011/12. The second year the best values were observed at 22/36 °C conditions. Similarly the highest fresh weight was obtained from this application together with full irrigation. Because there is not difference each other as statistically. The results are supported by the finding of Maroco et al. (1999) in maize and Koti et al.

(2007) in soybean. Similarly, it was observed that, the CO_2 enrichment increased the fresh weights of the mint and thyme by 3.1 and 5.8 fold, respectively (Anonymous 2014). Researcher stated that drought stress decreased fresh weight of shoot in maize (Hussain 2009). Bilgin et al. (2008) found that 75% available irrigation treatment caused in increasing the dry matter root and shoot. It was observed that dry matter was reduced with reduced irrigated conditions. Odiyi (2013) obtained similar results when he observed the fresh weight and dry weights of the plant of maize were significantly decreased with drought conditions. Reimer (2010) observed that maize plants were accumulated more leaf dry weight at optimum temperature and under heat stress. They found that the effects of CO_2 (500 ppm or 1000 ppm CO_2) and/or relative humidity (37% or 79%) on C3 plants and corn were accelerated by an elevated concentration of CO_2.

Table 6. Mean values of plant height, relative water content and chlorophyll content index of maize in 2011/12

Treatments		PH (cm)	RWC (%)	CCI
Ambient CO_2	Full irrigated	80.8 b	-	-
	Reduced irrigated	71.5 b	-	-
Elevated CO_2	Full irrigated	87.7 a	-	-
	Reduced irrigated	85.7 a	-	-
Ambient CO_2	16/30 °C	67.5 a	-	-
	22/36 °C	84.8 b	-	-
Elevated CO_2	16/30 °C	65.7 a	-	-
	22/36 °C	107.7 a	-	-
16/30 °C	Full irrigated	-	78.7 a	4.4 b
	Reduced irrigated	-	82.2 a	5.6 a
22/36 °C	Full irrigated	-	82.8 a	4.9 a
	Reduced irrigated	-	41.0 b	4.7 a
LSD (0.05)		3.2	4.5	0.2

PH: Plant height, RWC: Relative water content, CCI: Chlorophyll content index

The RWC decreased with increasing water deficit conditions. Leaf RWC is of the best growth indices indicating the stress intensity (Arjenaki et al., 2012). Jiang and Huang (2001) found that the leaf RWC was not affected by heat until 12 d for perennial ryegrass and tall fescue. These results were in confirmation with those of Farooq et al. (2011) who reported that leaf relative water content and leaf water potential were not influenced by heat stress when soil water content was close to field capacity, but relative water contents were sligltly affected by day/night temperatures of 40/35 °C. The sensitivity of maize to paraquat was especially observed at reduced irrigation and high temperature conditions in 2011/12 and at elevated CO_2 levels in 2012/13.

Table 7. Mean values of some agronomic and physiological characters of maize in 2012/13

Treatments			FW (g)	DW (g)	RWC (%)	LA (cm^2)	CCI
Ambient CO_2	Full irrigated	16/30 °C	9.5 a	5.7 a	48.8 a	210.0 a	5.4 a
		22/36 °C	27.1 b	6.4 a	50.0 a	214.5 a	6.1 b
	Reduced irrigated	16/30 °C	3.6 b	4.6 a	78.0 a	206.6 a	5.4 a
		22/36 °C	15.0 b	3.3 b	89.7 a	111.5 b	9.4 a
Elevated CO_2	Full irrigated	16/30 °C	4.6 b	5.6 a	48.3 a	179.3 b	3.6 b
		22/36 °C	37.0 a	6.3 a	40.0 b	165.5 b	1.0 a
	Reduced irrigated	16/30 °C	5.3 a	4.7 a	68.3 b	140.1 b	3.3 b
		22/36 °C	38.0 a	6.6 a	78.7 b	139.5 a	6.2 b
LSD (0.05)			0.2	0.2	3.8	3.8	0.6

FW: Fresh weight, DW: Dry weight, RWC: Relative water content, LA: Leaf area, CCI: Chlorophyll content index

Table 8. Mean values of plant height, relative cell injury and paraquat sensitivity index of maize in 2012/13

Treatments		PH (cm)	RCI (%)	PSI
Ambient CO_2	Full irrigated	99.5 a	-	0.77 b
	Reduced irrigated	88.2 b	-	0.69 a
Elevated CO_2	Full irrigated	99.5 a	-	0.80 a
	Reduced irrigated	93.0 a	-	0.67 b
Ambient CO_2	16/30 °C	92.0 b	-	0.73 b
	22/36 °C	95.7 b	-	0.72 a
Elevated CO_2	16/30 °C	93.8 a	-	0.78 a
	22/36 °C	98.7 a	-	0.68 b
16/30 °C	Full irrigated	-	23.9 a	0.80 a
	Reduced irrigated	-	18.5 a	0.71 b
22/36 °C	Full irrigated	-	18.9 b	0.76 a
	Reduced irrigated	-	33.5 a	0.64 b
LSD (0.05)		0.6	13.1	0.01

PH: Plant height, RCI: Relative cell injury, PSI: Paraquat sensitivity index

The results showed that the relative cell injury values generally increased with high temperature. By only evaluating carbon dioxide, Baczek-Kwinta and Koscielniak (2003) determined that leaf membrane injury was significantly lower in elevated CO_2 than that of ambient CO_2 in maize. Naveed et al. (2014) stated that high temperature decreases cell membrane thermostability (CMT) a high values are considered as tolerant to high temperature stress which indicates lower value of cell membrane injury. The highest relative cell injury was 63.2 observed with elevated CO_2, reduced irrigation and high temperature conditions. The second year the values showed that the relative cell injury was increased with reduced irrigation and 22/36 °C.

4. Conclusions

CO_2 x irrigation x temperature interaction was significant in terms of fresh weight and dry weight in both years. The highest values were obtained from both full irrigation and reduced irrigation at elevated CO_2 and higher temperature conditions. According to future scenarios, this situation showed that elevated CO_2 and temperature brought about the increase in maize biomass. The paraquat sensitivity index (PSI) will increase at higher temperature and reduced irrigation regardless of ambient or elevated CO_2 conditions. However relative cell injury (RCI) is increasing at ambient and elevated CO_2 regardless of reduced irrigation and 16/30°C conditions. In this case, it was appeared that the main effective factor was irrigation in terms of both features regardless of the CO_2 and temperature. The highest leaf area values were observed at ambient CO_2 × full irrigation × normal temperature conditions in both years. On the other hand the lowest values were obtained from ambient CO_2 × reduced irrigation × higher temperature conditions in 2011/2012 and 2012/2013. However, it has not been established similarities between the other combinations and years. These results showed that reduced irrigation and temperature increase may affect leaf area values. Therefore, if research performs with less factors, it will give better results. There were important interaction between CO_2 and irrigation on plant height in both years. While the highest values were obtained from full irrigation application both ambient and elevated CO_2 conditions, the lowest values were from reduced irrigation. On the other hand, the interaction between temperature and CO_2 concentration were significant. The highest values observed with elevated CO_2 and 22/36°C but elevated CO_2 and 16/30 °C conditions caused a decrease in plant height. It was indicated that reduced irrigation may decrease the plant height at elevated CO_2 and higher temperature conditions. The interaction of temperature x irrigation was significant on relative water content (RWC) in 2011/2012 and CO_2 x irrigation x temperature in 2012/2013. While the highest values were observed at reduced irrigation and high temperature conditions, the other applications gave similar results. It was observed that the ambient CO_2, full irrigation and both temperature conditions. Similarly elevated CO_2, full irrigation with higher temperature conditions gave the highest values in 2012/2013. However, the lowest values were found at elevated CO_2, reduced irrigation and both temperature regimes. These results showed that the amount of irrigation may be more important factor than CO_2 and temperature application for RWC. The interaction of temperature x irrigation was significant on chlorophyll content index (CCI) in 2011/2012 and CO_2 x irrigation x temperature in 2012/2013. The highest values for CCI were obtained from ambient CO_2 and higher temperature conditions followed by elevated CO_2, reduced irrigation and higher temperature conditions. The lowest values for this parameter were obtained under elevated CO_2, full irrigation and higher temperature conditions. It can be said that reduced irrigation caused higher CCI values.

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118

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