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Drought and Elevated CO_2 Impacts Photosynthesis and Biochemicals of Basil (*Ocimum basilicum* L.)

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Abstract: Drought-induced reduction in crop growth and productivity can be compensated by increasing atmospheric carbon dioxide (CO_2), a significant contributor to climate change. Drought stress (DS) affects crops worldwide due to dwindling water resources and irregular rainfall patterns. The experiment was set up under a randomized complete block design within a three-by-two factorial arrangement. Six SPAR chambers represent three blocks (10 replications each), where each chamber has 30 pots in three rows. Each chamber was maintained with 30/22 (day/night) °C temperature, with either ambient (aCO_2; 420 ppm) or elevated CO_2 (eCO_2; 720 ppm) concentrations. This experiment was designed to address the impact of DS on the physiological and biochemical attributes and study how the eCO_2 helps alleviate the adversity of DS in basil. The study demonstrated that DS + eCO_2 application highly accelerated the decrease in all forms of carotene and xanthophylls. eCO_2 positively influenced and increased anthocyanin (Antho) and chlorophyll (LChl). eCO_2 supplementation increased LChl content in basil under DS. Furthermore, DS significantly impeded the photosynthetic system in plants by decreasing CO_2 availability and causing stomatal closure. Although eCO_2 did not increase net photosynthesis (Pn) activity, it decreased stomatal conductance (gs) and leaf transpiration rate (E) under DS, showing that eCO_2 can improve plant water use efficiency by lowering E and gs. Peroxidase and ascorbate activity were higher due to the eCO_2 supply to acclimate the basil under the DS condition. This study suggests that the combination of eCO_2 during DS positively impacts basil's photosynthetic parameters and biochemical traits than aCO_2.

Keywords: chlorophyll; metabolites; carotenoids; antioxidants; phenotype

Citation: Barickman, T.C.; Adhikari, B.; Sehgal, A.; Walne, C.H.; Reddy, K.R.; Gao, W. Drought and Elevated CO_2 Impacts Photosynthesis and Biochemicals of Basil (*Ocimum basilicum* L.). *Stresses* **2021**, *1*, 223–237. https://doi.org/10.3390/stresses1040016

Academic Editor: Cecilia Brunetti

Received: 22 September 2021
Accepted: 19 October 2021
Published: 22 October 2021

1. Introduction

Over the last few decades, the influence of global climate change on agricultural productivity has emerged as a critical research issue [1]. With continued projected modifications in climate and the need to ensure future food supply, understanding the effects of climate change on the productivity of major agricultural crop species has become critical [1,2]. Water resources are responsible for 80–95% of the fresh biomass of nonwoody plants and have a fundamental role in plant growth, development, and metabolism [3–5]. However, water resources are the most affected due to the rising linear temperature trend of 0.74 °C (1906–2005) over 100 years [5]. Plants are often subjected to numerous environmental stresses in natural and agricultural settings [6]. Since drying up water resources and erratic rainfall patterns are evident globally, this situation leads to drought stress (DS) to the crops [7]. Moisture storing capacity of the soil, rainfall distribution, and natural disasters are other factors that cause DS, and these factors also make the severity of DS

unpredictable [8]. DS is multifaceted stress that affects plants' physiological, morphological, biochemical, and molecular properties [9,10]. Although plants have evolved specialized acclimation mechanisms to respond to short and long-term DS to some extent [11], it is undeniable that DS affects plants in several ways. For example, a study in maize by Earl and Davis [12] stated that DS affected crop productivity in three different ways: (1) lowering the absorption of photosynthetically active radiation by crop canopy; (2) reducing the radiation use efficiency, and (3) limiting the harvest index.

On the other hand, DS increased the formation of reactive oxygen species (ROS), which includes superoxide and hydrogen peroxide (H_2O_2) [11,13,14]. These ROS are combatted by the plant antioxidant system (ascorbate, glutathione, and superoxide dismutase (SOD)) [13,14]. These antioxidants scavenged ROS in the following steps: (I) SOD converts O_2^- into H_2O_2, and (II) ascorbate and glutathione remove H_2O_2 from the plant system [15].

On the other hand, rising atmospheric carbon dioxide (CO_2) may change the precipitation pattern in the long run, which is crucial for climate change. The change in rainfall pattern can directly impact soil temperature level and soil moisture content, significantly decreasing crop yield in the next 50 years [16]. Furthermore, the expected rise in drought conditions resulting from increased CO_2 and temperature in the atmosphere will also affect crop growth and production of basil (*Ocimum basilicum*, L.) [17]. Thus, it is crucial to evaluate essential climate drivers, the drought conditions, and the role of eCO_2 in basil growth and development to identify successful drought mitigation and adapt the crop to that climate.

Basil is the most widespread warm-season aromatic and medicinal herb, and it belongs to the subfamily Nepetoidae under the *Lamiaceae* family [18,19]. It is used as an ingredient for commercial fragrances and improves the shelf life of food products [20,21]. Since ancient times, it has been used for medicinal purposes, including treating headaches, lowering cholesterol, sugar, blood pressure, and kidney failure [22,23]. Basil contains several essential oils (1.5%), phenolic compounds, flavonoids, glycosides, and organic acids [24–27]. Basil thrives well under an optimal temperature range of 25–30 °C [28,29]. However, for commercial basil production to be efficient, supplemental water is needed. Plants react to DS via a series of physiological and biochemical responses [30,31]. Under DS, leaf water potential diminishes due to a higher transpiration rate than its absorption rate. These changes can result in the closure of the stomatal opening and a decrease in cell enlargement and growth [32].

Furthermore, DS inhibits cell elongation by reducing the turgor pressure and impairs cell division by reducing metabolism [33]. The photosynthetic rate of a crop is affected by DS as it causes a decrease in gas exchange activity and carbon assimilation [17,34,35]. The respiration and ion uptake are also reduced under DS, resulting in changes in the metabolic process and crop growth patterns resulting in crop failure [36]. For example, the reduction in photosynthetic rate, transpiration, and water use efficiency due to DS is also reported in several crops like basil [17], cowpea [31], maize [37], and mungbean genotype [38].

Furthermore, DS can also escalate reactive oxygen species responsible for oxidative stress that cause lipid peroxidation and alteration of both chlorophylls a (Chla) and b (Chlb) [9,39]. Chla and Chlb decreased in basil leaf tissue when basil plants were under DS and high-temperature stress for 14 days [40,41]. Similarly, superoxide dismutase (SOD), ascorbate, peroxidase, and antioxidant glutathione control the oxidative damage caused by reactive oxygen species (H_2O_2 anions) [13,42,43]. Previous research from Heidari and Golpayegani [42] demonstrated that hydrogen peroxide and superoxide anions increased in basil plants when subjected to DS.

According to a previous study, basil production increased by up to 80% in response to CO_2 levels rising from 360 to 620 ppm [44]. Studies have shown that eCO_2 enhanced the photosynthetic process and enriched the metabolites and the antioxidant activity in basil, parsley, and peppermint [44,45]. Al Jaouni et al. [44] also reported that eCO_2 (620 ppm) improved photosynthetic products and biomass accumulation by 40%. eCO_2 also promotes the proliferation of phenolics, flavonoids, glutathione, and several other antioxidants

to help combat damages from ROS during DS [44]. These dietary, physiological, and biochemical advantages of eCO_2 in basil may benefit human health concerns. Furthermore, screening stomatal conductance, photosynthetic rate, and water use efficiency under DS might help identify resistant genotypes. Thus, the current study's primary purpose is to understand the effect of DS coupled with eCO_2 on several physiological parameters, photosynthetic rates, carotenoids, chlorophylls, and several antioxidant concentrations in basil.

2. Results and Discussion

2.1. Physiological and Gas Exchange Measurements

Drought is one of the significant factors for damaging the photosynthetic pigments and thylakoid membranes [46]. DS inhibits the photosynthetic apparatus in plants by declining CO_2 availability and stomatal closure [35,47]. Several basil compounds have health benefits to humans, including chlorophylls, anthocyanins, flavonoids, and phenolics [48]. Leaf chlorophyll (LChl) content, epidermal flavonoids (flav), epidermal anthocyanin (antho), and nitrogen balance index (NBI) were measured as shown in Table 1. Antho and flav compounds together are responsible for antioxidant activity in the plants [49]. Flav are ubiquitous secondary metabolites in plants, which help protect the plant from abiotic and biotic stresses, while antho reduces the damage caused by free radical activity. [50]. Both antho and flav are reported to increase in different crops when subjected to DS + eCO_2 conditions [51–53]. However, flav content in the present findings contradicts many studies as flavonoid level was indifferent to the control under DS + eCO_2 condition [46,54,55]. Antho, on the other hand, decreased under DS + aCO_2 but increased under DS + eCO_2 as reported earlier [51,56]. Furthermore, NBI increased under DS + aCO_2 by 26.2% compared to control. NBI is the ratio of chlorophyll and epidermal flavanol [57]. DS decreases the nitrogen isotope composition and increases the accumulation of antho coupled with a transient decrease in LChl and NBI [54]. In the present study, LChl increased by 20% and 16% under DS + aCO_2 and DS + eCO_2, respectively, compared to control (Table 1). It is determined that eCO_2 positively impacts and increases the NBI and LChl by alleviating the adverse effect of DS.

Table 1. The mean of chlorophyll (Chl), flavonoids (Fla), anthocyanin (Antho), and nitrogen balance index (NBI) of basil plants grown without drought stress (control) and with drought stress at two levels of CO_2 (420 and 720 ppm) after 17 days of treatment.

Treatment	Chlorophyll [1]	Flavonoids	Anthocyanin	NBI
	[µg/mL]	[mg/g DM]	[mg/g DM]	
		420 ppm		
Control	21.468 bc	0.685 ab	0.114 b	32.415 b
Drought	25.744 a	0.645 b	0.102 c	40.890 a
		720 ppm		
Control	18.978 c	0.704 ab	0.113 bc	28.062 c
Drought	22.027 b	0.739 a	0.127 a	30.391 bc
Treatment [2]	***	ns	***	***
CO_2	**	*	**	***
Trt*CO_2	ns	ns	ns	*

[1] Mean separation within the column by Duncan's multiple range test; ns, *, **, *** indicate non-significant or significant at $p \leq 0.05$, 0.01, 0.001, respectively; values followed by the same letter are not significantly different. Data are presented as means ± SE (n = 10). [2] SE: standard error of the mean, Chl = 0.900; Fla = 0.03; Antho = 0.004; NBI = 1.600.

The overall photosynthetic process is affected by several stress-induced stomatal limitations or metabolic impairment [55]. Under DS, stomatal conductance (gs) tends to be reduced temporarily, which affects the leaf transpiration rate (E) and intercellular CO_2 (Ci) assimilation [58,59]. In this study, no interaction effect ($p > 0.05$) was observed in net photosynthesis (Pn), gs, electron transport rate (ETR), and leaf temperature (Tleaf)

(Table 2). However, the treatment effect was observed in gs, where gs reduced significantly under DS in both CO_2 levels compared to control. Similarly, Ci lowered under DS + eCO_2, and DS + aCO_2 application decreased by 32.8% and 45.1%, respectively, compared to control. This decrement in Ci is due to reduced gs to prevent leaf water loss (wilting), as Saibo et al. [55] reported. Additionally, a treatment effect ($p < 0.001$) on Pn, was observed where there was a significant reduction of Pn under DS + eCO_2 (Table 2). A previous study reported that in the presence of eCO_2, Pn could increase along with the more activity of the rubisco enzyme and reduced photorespiration [60,61]. Therefore, in this study, eCO_2 cannot alleviate the negative effect of DS through Pn's increment. This decrement in Pn under DS is reported due to a reduction in gs by Saibo et al. [55]. Although eCO_2 could not increase Pn activity, the gs and E decreased under DS + eCO_2 compared to control (Table 2). This result is supported by several reports where eCO_2 application increases plants' water use efficiency by reducing the E and gs [62–64]. The intercellular/ambient CO_2 (Ci/Ca) ratio decreased significantly by 33% ($p < 0.001$) and 45% ($p < 0.001$) under DS followed by aCO_2 and eCO_2 application respectively. The reduction in Ci/Ca under DS suggests that the reduction in Pn could also be due to decreased Ci/Ca. This result was further supported by a report by Rajasekaran and Blake [65]. Even though eCO_2 cannot increase Pn in this study, eCO_2 application fulfills the gap created due to reduced Ci due to DS and decreased E in leaves to maintain water loss stated by Acock [66].

Table 2. The mean of net photosynthesis (Pn), stomatal conductance to water vapor (gs), intercellular CO_2 concentration (Ci), electron transport rate (ETR), leaf transpiration rate (E), leaf temperature (Tleaf), and intercellular/ambient CO_2 ratio (CiCa) of basil plants grown under without drought stress (control) and with drought stress at two levels of CO_2 (420 and 720 ppm) after 17 days of treatment.

Treatment	Pn	gs	Ci	ETR	E	Tleaf	CiCa [1]
			420 ppm				
Control	24.475 b	0.375 a	295.090 b	187.340 a	6.788 a	30.483 c	0.704 a
Drought	20.123 b	0.159 b	198.400 c	182.140 a	4.578 b	31.710 ab	0.473 b
			720 ppm				
Control	31.513 a	0.312 a	530.710 a	184.980 a	6.670 a	31.263 b	0.737 a
Drought	24.475 b	0.083 b	291.480 b	193.460 a	2.498 c	32.400 a	0.406 b
Treatment [2,3]	**	***	***	ns	***	***	***
CO_2	ns	*	***	ns	*	**	ns
Trt*CO_2	ns	ns	***	ns	*	ns	*

[1] The measured intercellular CO_2/ambient CO_2 of LI-6400XT leaf cuvette. [2] Mean separation within the column by Duncan's multiple range test; ns, *, **, *** indicate non-significant or significant at $p \leq 0.05$, 0.01, 0.001, respectively; values followed by the same letter are not significantly different. Data are presented as means ± SE ($n = 10$). [3] SE-Standard error of the mean, Pn = 2.100; gs = 0.030; Ci = 13.200; ETR = 15.6; E = 0.400; Tleaf = 0.200; CiCa = 0.020.

The effect of DS on the fluorescence parameters is shown in Table 3. There was no interaction effect ($p > 0.001$) in all the fluorescence parameters. Furthermore, minimal fluorescence (Fo), maximal fluorescence (Fm), the quantum yield of photosystem II (ΦPSII), and photochemical quenching (qP) were not significantly affected under stress under both CO_2 levels, contradicting the previous report on basil and beech saplings [67,68]. The last statement also demonstrated that severe drought conditions decreased ATP and NADPH in photosynthetic metabolism and photorespiration and subsequently reduced the maximal quantum yield of photosystem II (FvFm) [69]. DS causes the suppression of photosynthesis activity which, in turn, reduces ΦPSII and increases the non-photochemical quenching (qN) [68]. On the contrary, qN FvFm, and ΦPSII decreased under the DS + eCO_2 in the present study when eCO_2 was applied. FvFm, qN, and ΦPSII showed a similar trend with Pn, Ci/Ca, Ci, and Tleaf. FvFm, ΦPSII, and qN are sensitive to DS and are not impacted significantly by the combination of eCO_2.

Table 3. The mean of light-adapted, minimal fluorescence (Fo'), dark-adapted, maximal fluorescence (Fm'), steady-state fluorescence (Fs), the maximal quantum yield of photosystem II photochemistry (Fv'/Fm'), the effective quantum yield of photosystem II photochemistry (ΦPSII), the effective quantum yield of gas exchange measurements (ΦCO_2), photochemical quenching (qP), and non-photochemical quenching (qN) of basil plants grown under without drought stress (control) and with drought stress at two levels of CO_2 (420 and 720 ppm) after 17 days of treatment.

Treatment	Fo	Fm	Fs	Fv/Fm	ΦPSII	ΦCO_2	qP	qN
				420 ppm				
Control	448.300 a	840.500 a	622.100 a	0.466 b	0.261 a	0.0195 b	0.558 ab	1.875 b
Drought	453.000 a	815.400 a	599.900 a	0.444 b	0.264 a	0.0160 b	0.593 a	1.800 b
				720 ppm				
Control	440.800 a	907.900 a	674.100 a	0.513 a	0259 a	0.0248 a	0.507 b	2.058 a
Drought	457.600 a	854.100 a	638.900 a	0.462 b	0.249 a	0.0163 b	0.538 ab	1.865 b
Treatment [1,2]	ns	ns	ns	*	ns	**	ns	*
CO_2	ns	ns	ns	*	ns	ns	ns	*
Trt*CO_2	ns	ns	ns	ns	ns	ns	ns	ns

[1] Mean separation within the column by Duncan's multiple range test; ns, *, ** indicate non-significant or significant at $p \leq 0.05$, 0.01, respectively; values followed by the same letter are not significantly different. Data are presented as means ± SE (n = 10). [2] SE-Standard error of the mean, Fo = 9.9; Fm = 34.5; Fs = 25.9; Fv/Fm = 0.01; ΦPSII = 0.01; ΦCO_2 = 0.001; qP = 0.03; qN = 0.05.

2.2. Carotenoid and Chlorophyll Analysis

Carotenoids contribute to photosynthesis and photoprotection in plants and are key metabolites for the proper functioning of photosynthetic apparatus during light intensity fluctuations [70–73]. Carotenoids (β-car) transfer the photochemical energy to chlorophyll to facilitate photosynthesis [74,75]. The basil plant subjected to DS shows a reduction in the β-car [41]. In our study, the individual major carotenoid pigments (Neoxanthin (Neo), Antheraxanthin (Anth), and Lutein (Lut)) were modulated under the DS, followed by aCO_2 and eCO_2 application (Figure 1). However, the application of CO_2 under DS did not modulate β-car (Figure 2). Neo showed the linear decreasing trend under DS + eCO_2. Neo concentrations of the control plant under aCO_2 were 276.4 ppm, which decreased to 206.9 ppm when applied under DS + eCO_2. On the other hand, there was no effect of DS and both CO_2 applications on Vio. However, Anth ($p < 0.001$) and Zea ($p < 0.001$) indicated a significant reduction in concentration under DS under both CO_2 applications compared to control. On the other hand, Lut showed a significant ($p < 0.001$) reduction in DS concentration with increased CO_2 concentration from aCO_2 to eCO_2. Thus, drought, on the one hand, reduced the concentrations of the pigments. On the other hand, the application of CO_2 accelerated the reduction of carotene pigments as the highest reduction was observed in Neo, Anth, Zea, and Lut under drought + eCO_2. It is reported that the effect of eCO_2 on carotenoids in leaves is different in different plants [73]. Some plants like *Solanum lycopersicum* and *Gyanura bicolor* increased while *Glycine max*, *Zea mays*, *Brassica napus*, and *Lactuca sativa* showed decreased carotenoids level under eCO_2 [73]. The present study also demonstrated that DS + eCO_2 application highly accelerated the decrease in all forms of carotene and xanthophylls, supported by the study by Dhami et al. [73]. Although Xanthophylls was unaffected by both CO_2 applications in control, its concentration reduced with increased CO_2 application (from aCO_2 to eCO_2) under DS, i.e., 370.2 to 314.1 ppm. Overall, Xanthophylls was reduced significantly ($p < 0.001$) under DS + eCO_2 (Table 2). The Za/Zav ratio decreased by 14.5–38.1% under the drought condition compared to control. Based on the result, we suggest that eCO_2 application caused the prominent decrease in carotenoids and xanthophylls, mainly under abiotic stress conditions [76–78].

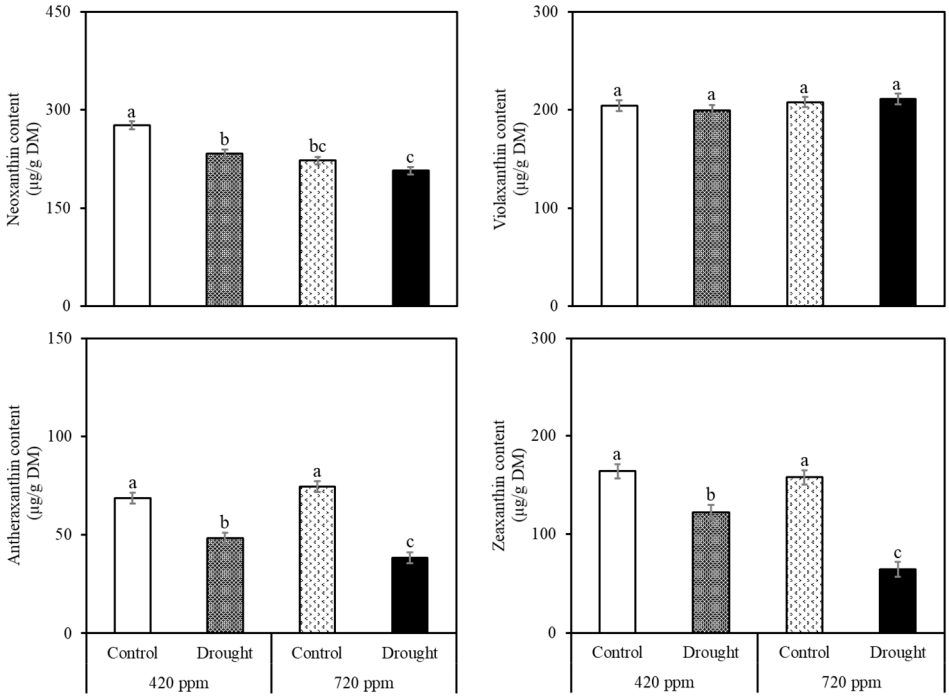

Figure 1. Neoxanthin (Neo), Violaxanthin (Vio), Antheraxanthin (Anth), and Zeaxanthin (Zea) estimation of basil plants grown without drought stress (control) and with drought stress at two levels of CO_2 (420 and 720 ppm) after 17 days of treatment. Data are presented as treatment means ± SE (n = 10). Different low case letters indicate a significant difference at $p < 0.05$ by the least significant difference. DM, Dry mass.

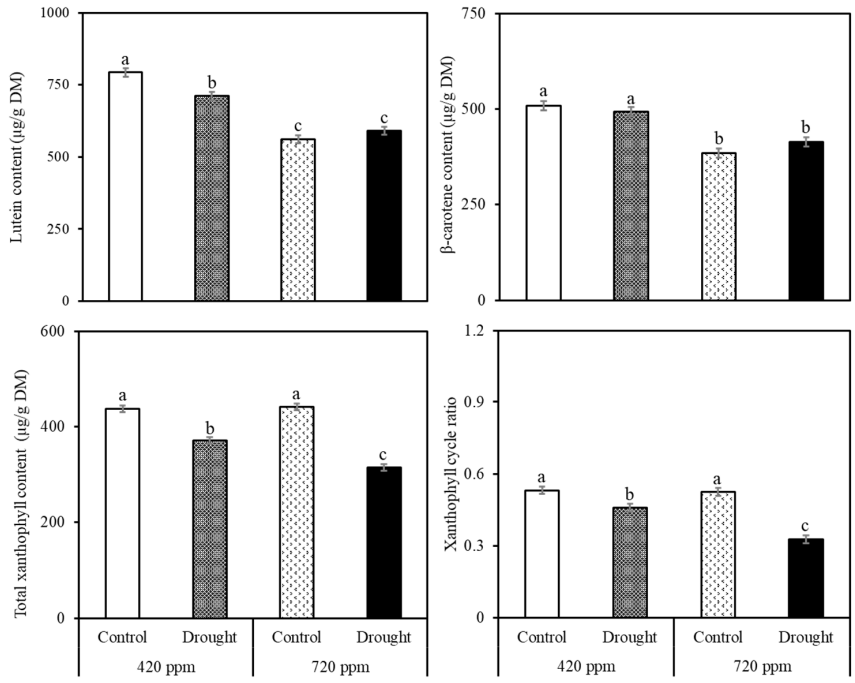

Figure 2. Lutein (Lut), β-carotene (β-car), Total xanthophyll, and Xanthophyll cycle ratio (Za/Zav) estimation of basil plants grown without drought stress (control) and with drought stress at two levels of CO_2 (420 and 720 ppm) after 17 days of treatment. Data are presented as treatment means ± SE (n = 10). Different low case letters indicate a significant difference at $p < 0.05$ by the least significant difference. DM, Dry mass.

Chlorophyll is an antioxidant and a signature pigment of photosynthetic organisms involved in photochemical activity [79,80]. Chlorophyll content in the plant is positively correlated to photosynthesis, and its reduction under abiotic stress like drought contributes to the inhibition of photosynthetic activity [41,81]. No interaction effect was observed in the present study on Chla, Chlb, and TChl (Figure 3). However, the treatment effect was observed on Chla and TChl, where Chla and TChl content significantly increased under DS, which contradicts the earlier reports [82,83], which could be because of CO_2 supplementation [84,85]. ChlB decreased in both drought and control conditions under eCO_2. The information further supports this result, with a significant decline of Chlb due to DS [86].

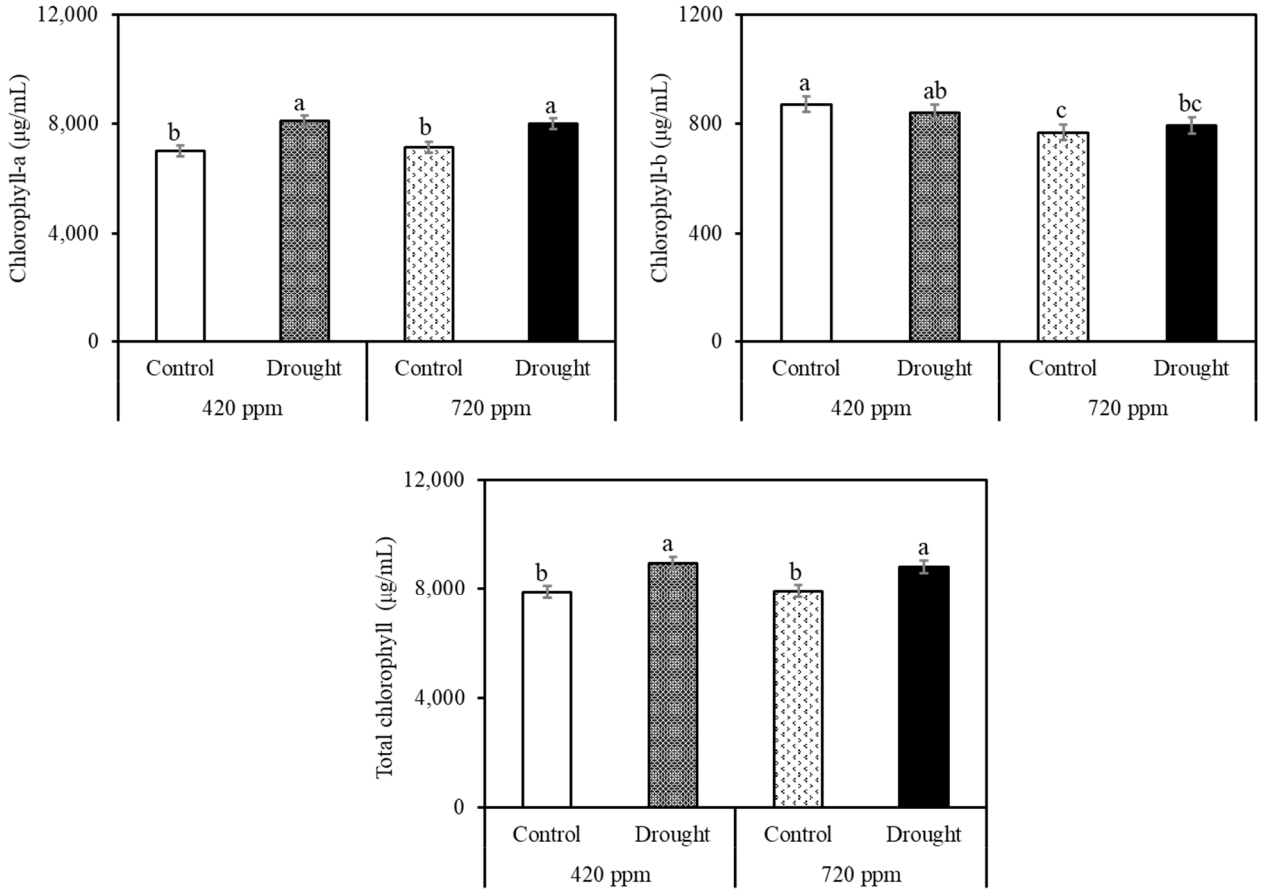

Figure 3. Chlorophyll-a (Chla), Chlorophyll-b (Chlb), and Total chlorophyll (TChl) estimation of basil plants grown without drought stress (control) and with drought stress at two levels of CO_2 (420 and 720 ppm) after 17 days of treatment. Data are presented as treatment means ± SE (n = 10). Different low case letters indicate a significant difference at $p < 0.05$ by the least significant difference.

2.3. Biochemical and Phytonutrient Analysis

The biochemical parameters measured are shown in Figure 4. There was no interaction effect in malondialdehyde (MDA), superoxide dismutase (SOD), trehalose, peroxidase, ascorbate, and glutathione under CO_2 levels. However, peroxidase and ascorbate increased under DS under both CO_2 groups compared to control. Peroxidase and ascorbate are crucial for acclimating a plant to any stress. Both peroxidase and ascorbate work together with SOD and catalase to protect the photosynthetic systems from oxidative damage by any environmental stress [87,88]. A previous report on CO_2 laser treatment revealed that eCO_2 enhances peroxidase and ascorbate, decreasing the MDA and H_2O_2, which strongly supports our study [89]. Furthermore, phenolic compounds are the metabolites responsible for antioxidant activity in basil, mainly produced in leaves and roots [90,91].

The present study demonstrated that there was a significant effect ($p < 0.001$) of CO_2 application on phenolics where it decreased under drought + eCO_2, which contradicted the result presented earlier in basil by Bekhradi et al. [92] and Al Jaouni et al. [44]. However, some metabolites tend to show species specificity, which might be the reason behind the decrease of phenolic compounds [44].

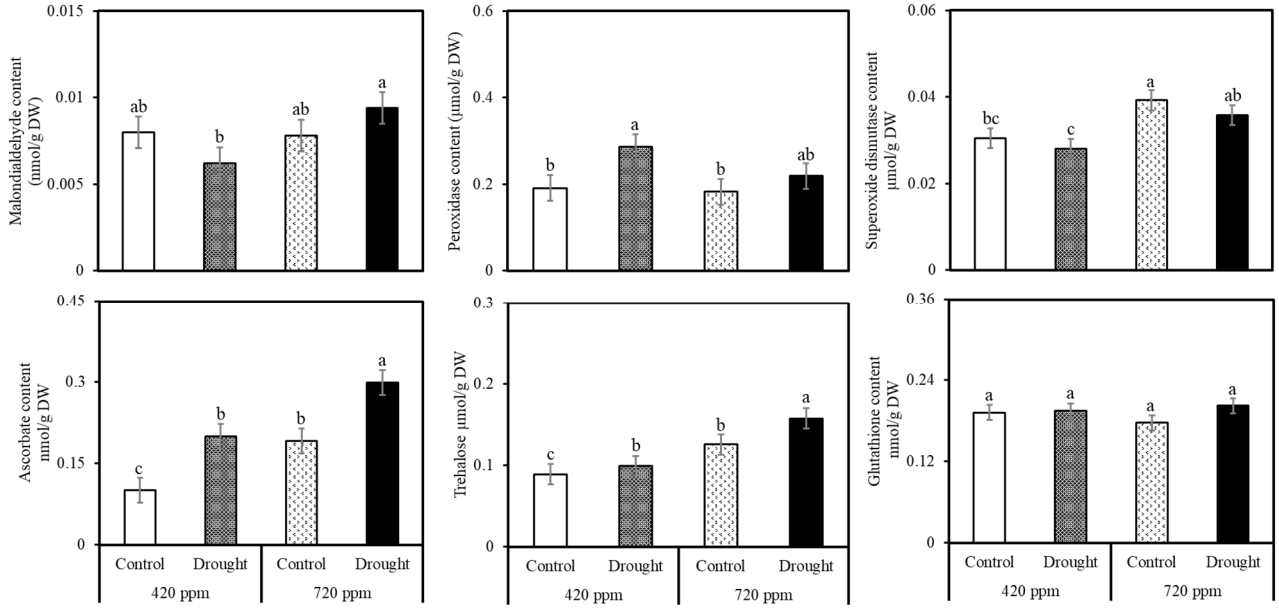

Figure 4. Malondialdehyde (MDA), peroxidase, superoxide dismutase (SOD), ascorbate, trehalose, and glutathione estimation of basil plants grown under without drought stress (control) and with drought stress at two levels of CO_2 (420 and 720 ppm) after 17 days of treatment. Data are presented as treatment means ± SE (n = 10). Different low case letters indicate a significant difference at $p < 0.05$ by the least significant difference.

3. Materials and Methods

3.1. Plant Materials and Growing Condition

Basil 'Genovese' seeds (Johnny's Selected Seeds, Winslow, ME, USA) were planted in polyvinyl chloride pots (15.2 cm diameter by 30.5 cm height) with a soil medium of 3:1 sand/soil classified as a sandy loam (87 percent sand, 2 percent clay, and 11 percent silt) and 500 g of gravel at the bottom of each pot. The experiment was set up under a randomized complete block design within a three-by-two factorial arrangement. Six SPAR chambers represent three blocks (10 replications each), where each chamber has 30 pots in three rows. More detailed information on the SPAR chamber was earlier elaborated by Reddy et al. [93] and Wijewardana et al. [94]. Basil plants were irrigated three times (700, 1200, and 1700 h) per day with full-strength Hoagland's nutrient solution via an automated computer-controlled drip system [95].

3.2. Treatments Application

Each chamber was maintained with 30/22 (day/night) °C temperature, with either ambient (420 ppm) or elevated (720 ppm) CO_2 concentrations. Temperature one hour after sunset was considered daytime temperatures, and one hour after sunset as nighttime temperatures. A full-strength Hoagland's solution [95] was applied to basil plants at 120 percent of evapotranspiration. For drought treatment, 50 percent of the full-strength Hoagland's solution was added to basil plants.

3.3. Physiological and Gas Exchange Measurements

The Dualex chlorophyll meter (FORCE-A, Orsay, France) was used to measure leaf chlorophyll (LChl), epidermal flavonoids (flav), epidermal anthocyanin (antho), and nitrogen balance index (NBI), with the device reader placed in the adaxial portion of the

leaf. Dualex is a hand-held leaf-clip tool that evaluates leaf quality using fluorescence and light transmission. For the OJIP fluorescence measurements on the second most completely developed leaf, a FluorPen FP 100 (Photon Systems Instruments, Drasov, Czech Republic) was utilized. At 17 DAT, the minimum fluorescence (Fo) was measured at 50 s when all PSII reaction centers were open, the maximum fluorescence (Fm) was measured when all PSII reaction centers were closed, and the steady-state fluorescence (Fs) was measured in each plant.

An LI-6400XT portable photosynthesis system (LiCor Biosciences, Inc., Lincoln, NE, USA) was used to measure photosynthesis and fluorescence parameters on the same leaf between 1000 and 1200 h at 18 DAT. The setup and regulation of relative humidity, the temperature of the chamber, and light intensity in the leaf chamber were followed as described by Barickman et al. [96]. Net photosynthesis (Pn) and quantum efficiency (Fv′/Fm′) were measured when the total coefficient of variation (percent CV) was less than 0.5 percent. The device calculates transpiration rate (E), stomatal conductance (gs), internal CO_2 concentration (Ci), and electron transport rate (E) based on incoming and outgoing flow rates and leaf area (ETR). The interior to exterior CO_2 ratio was estimated using the Ci/Ca relationship.

3.4. Carotenoid and Chlorophyll Analysis

Carotenoid and chlorophyll pigments were extracted from freeze-dried basil tissues, according to Kopsell et al. [97], with a few modifications adopted by Barickman et al. [98].

4. Biochemical and Phytonutrient Parameters

4.1. Malondialdehyde (MDA)

Lipid peroxidation of membranes was estimated from MDA content, a final lipid peroxidation product, using the method described by Heath and Packer [99] with few modifications as described by Barickman et al. [96]. The extinction coefficient of 155 mM^{-1} cm^{-1} was used to determine the MDA concentration, which was reported as nmol g^{-1} DW.

4.2. Hydrogen Peroxide (H_2O_2)

The content of H_2O_2 was measured following the method of Mukherjee and Choudhuri [100] with few modifications as described by Barickman et al. [96]. The content of H_2O_2 in samples was obtained from a standard curve using pure H_2O_2 and expressed as µmol g^{-1} DW.

4.3. Superoxide Dismutase (SOD)

The activity of SOD was measured following the method of Dhindsa et al. [101] with few modifications by Awasthi et al. [102]. The reaction mixture (3 mL) used in this method has a mixture of 13 mM methionine, 25 mM nitro blue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM sodium bicarbonate, 50 mM phosphate buffer (pH 7.8) and 0.1 mL enzyme extract. The absorbance of the samples was measured at 560 nm, and their total SOD activity was determined by evaluating their capacity to block the photochemical reduction of NBT. One unit of SOD activity was defined as the quantity of enzyme that inhibits the photochemical degradation of NBT by 50%. It was represented as SOD activity mg^{-1} protein units.

4.4. Ascorbic Acid (ASC)

The estimation of ASC was done according to the combined method of Mukherjee and Choudhuri [100] and Awasthi et al. [102]. The ASC content was determined using a standard curve with a known ASC concentration and represented as nmoL g^{-1} DW.

4.5. Trehalose

Trehalose concentration was estimated according to the method of Trevelyan and Harrison [103] and the Anthrone method of Brin [104]. Further details regarding the assay of an

enzyme associated with trehalose metabolism were described by Barickman et al. [96]. Trehalase activity was measured by activating phosphorylation with cAMP (cyclic adenosine monophosphate) and monitoring glucose levels [105].

4.6. Glutathione

Reduced glutathione was estimated according to the method of Griffith [106], adopted by Awasthi et al. [102]. Glutathione content was calculated from a standard graph calibrated by Griffith [106], and it was expressed as nmol g^{-1} DW.

4.7. Data Analysis

SAS was used for statistical analysis on the data (version 9.4; SAS Institute, Cary, NC, USA), followed by PROC GLIMMIX analysis of variance (ANOVA) and mean separation. The study involved a randomized full block in a factorial arrangement with two water and two CO_2 treatments, three blocks, and ten replications. The standard errors were calculated using the ANOVA table's pooled error term. Duncan's multiple range test ($p < 0.05$) was used to distinguish between treatment classifications. Model-based values were reported rather than the unequal standard error from a data-based calculation because pooled errors reflect the statistical testing. To differentiate between treatment classifications, Duncan's multiple range test ($p < 0.05$) was used. Because pooled errors reflect statistical testing, model-based values were reported rather than the unequal standard error from a data-based calculation.

5. Conclusions

This study provided evidence that the interaction of DS and eCO_2 significantly impacts basil's overall physiological and biochemical aspects. eCO_2 positively impacted and increased the antho, and LChl by alleviating the adverse effect of DS. antho, on the other hand, decreased under DS + aCO_2 but increased under DS + eCO_2. LChl increased by 20% and 16% under DS + aCO_2 and DS + eCO_2, respectively. Furthermore, NBI increased under DS + aCO_2 by 26.2% compared to control. The application of CO_2 under DS did not modulate β-car. However, the individual primary carotenoid pigments (Neo, Anth, and Lut) were modulated under the DS, followed by aCO_2 and eCO_2 application. Neo showed the linear decreasing trend from under DS with the shift from aCO_2 (276.4 ppm) to eCO_2 (206.9 ppm). Anth and Lut decreased significantly under DS irrespective of both CO_2 treatments. Additionally, DS considerably inhibited the photosynthetic apparatus in plants by declining CO_2 availability and stomatal closure. Although eCO_2 could not increase Pn activity, gs and E under DS decreased with eCO_2 application, indicating that eCO_2 can uplift plants' water use efficiency by reducing the E and gs. FvFm, ΦPSII, and qN were sensitive to DS and were not impacted significantly by the eCO_2 application. Drought and eCO_2 accelerated most carotenes' reduction (Neo, Anth, Zea, and Lut) and Xanthophylls. It is worth addressing that eCO_2 supplementation can explain the increased chlorophyll content in basil under DS. Chlb decreased in both drought and control conditions under eCO_2. Peroxidase and ascorbate activity was higher due to the eCO_2 supply to acclimate the basil under the DS environment to withstand oxidative damage caused by H_2O_2. This study suggests that the application of eCO_2 during DS has a more significant impact on basil's photosynthetic parameters and biochemical traits than aCO_2.

Author Contributions: T.C.B.: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing—original draft, writing—review and editing, visualization, supervision, project administration, and funding acquisition. B.A.: formal analysis, writing—original draft, and writing—review and editing. A.S.: methodology, validation, and investigation. C.H.W.: methodology, validation, formal analysis, and investigation. K.R.R.: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing—review and editing, visualization, supervision, project administration, and funding acquisition. W.G.: conceptualization, methodology, validation, resources, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This material is based on the work supported by the USDA-NIFA Hatch Project under accession number 149210, and the National Institute of Food and Agriculture, 2019-34263-30552, and MIS 043050 funded this research.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We thank David Brand for technical assistance and graduate students at the Environmental Plant Physiology Laboratory for their help during data collection. We would also like to thank Thomas Horgan for his technical help.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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