

Morphological, Phytochemical, Biochemical, and Enzymatic Evaluation of Pepper Species (*Capsicum annuum* L. and *Capsicum chinense* Jacq.) at Developmental Stages Under Drought Stress

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Research Article

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Abstract

Drought is an important abiotic stress factor that severely affects plant growth, especially in arid and semi-arid regions. The effect of limited irrigation on plant growth and its response depending on growth stages is critical for agriculture in these regions. This study was conducted to understand how different pepper species (*C. annuum* L. and *C. chinense* Jacq.) respond to drought conditions. Plants were subjected to four different irrigation regimes (100% field capacity (FC), 75% FC, 50% FC, and 25% FC) and three developmental stages (S1: 20 days after flowering, S2: 40 days after flowering, and S3: 60 days after flowering). The effects of drought on plant morphological growth, photosynthetic pigment content in leaves, phytochemical components [total phenolics (TPh), total flavonoids (TFv), and total antioxidant activity (TAa)], proline (PRL), protein (PRO), malondialdehyde (MDA), and activities of major antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD)] were investigated. According to the results, drought had a negative effect on plant morphology and physiology of both species, and these effects differed between plant species. The amounts of phytochemicals, MDA, PRO, PRL, and antioxidant enzymes increased in higher percentages with increasing drought severity (especially at S2 and S3 growth stages) in *C. annuum*. Moreover, antioxidative enzyme activities were significantly higher in *C. annuum* with increasing stress severity, helping the species to overcome oxidative stress under drought conditions. In conclusion, the findings showed that *C. annuum* is a drought-tolerant species with much more stable morphological, physiological, and biochemical performances compared to *C. chinense*.

Introduction

Agriculture is a vital sector aimed at meeting humanity's basic food needs and ensuring food security (Swaminathan and Bhavani 2013). Understanding how plants can survive under variable and sometimes harsh environmental conditions has become a critical factor in shaping the future of agriculture. Effects such as climate fluctuations and diminishing water resources can yield significant outcomes in crop production. Therefore, comprehending the impacts of environmental factors is of utmost importance for regulating agricultural practices and preparing for future agricultural challenges (Epa 2017). When plants are exposed to various environmental factors, they activate adaptation mechanisms called stress (Zuo et al. 2023). Stress is the effort of plants to adapt to changing conditions to maintain their normal physiological functions (Bhargava and Sawant 2013). Abiotic stress factors are environmental conditions that can significantly affect plant growth, development, and productivity. Notably, drought stress is among the most noteworthy of these factors (Akula and Ravishankar 2011). This stress occurs when plants face insufficient water resources, leading to a situation where they are stressed due to water scarcity. It can have a negative effect on plant growth and development processes (Mangena 2018). This, in turn, results in various stress symptoms, such as leaf wilting, curling of leaf edges, and leaf shedding, among others. Additionally, physiological changes like reduced photosynthetic activity slowed growth rate, and decreased crop yield can be observed. These symptoms reflect the physiological challenges that plants face under water stress conditions (Fahad et al. 2017). Water scarcity affects plants in various

ways. First, it affects the absorption of nutrients and minerals carried by water in plants (Gul et al. 2023). Additionally, water supports the cellular structures of plants and aids in regulating cellular pressure. Water scarcity can lead to damage to cellular structures, compression of cell membranes, and disruption of cellular integrity (Bista et al. 2018). To cope with situations like drought and water stress, plants develop various adaptation mechanisms. They attempt to minimize water loss by mechanisms like stomatal closure. Moreover, they change root morphology to enhance water uptake from deeper soil layers. Plants may also prefer different photosynthetic pathways that allow more efficient water use (Seleiman et al. 2021).

The pepper plant (*Capsicum spp.*) is a highly sought-after product worldwide due to its economic potential and nutritional value (Parisi et al. 2020). This plant boasts a wide range of species offering various colors, shapes, sizes, and flavors, making it appealing to a diverse consumer base. Peppers are widely used to add flavor and color to dishes, and their trade volume is continuously increasing (Olatunji and Afolayan 2018). However, pepper plants can exhibit high sensitivity to various abiotic stress factors. Particularly, stress conditions like drought can lead to physiological and biochemical changes in plants due to water deficiency (Bae et al. 2021). Efforts to minimize water loss in plants are supported by mechanisms such as stomatal closure, adversely affecting the photosynthetic activities of plants. Furthermore, under drought conditions, the cellular structures and metabolisms of plants can be significantly affected (Naawe et al. 2021). Limited irrigation can also have adverse effects on the growth and development of pepper plants (Yildirim et al. 2012). The absence of sufficient irrigation limits water and nutrient intake by plants, inhibiting the expansion of plant cells and affecting plant size and structure. Therefore, irrigation management emerges as a critical factor in pepper production (Alattar et al. 2022).

Plant developmental processes and stress conditions have pronounced effects on the morphological structure, chemical composition, pungency levels, biochemical contents, enzymatic activities, and hormonal balance of fruits and vegetables (Anjum et al. 2017). The developmental process of pepper plants comprises four main stages starting from flowering. These are flowering, green fruit stage, green-red fruit stage, and mature red fruit stage. Each stage brings about distinct changes in the plant's physical appearance and chemical composition. These alterations have a significant impact on fruit taste, aroma, color, and nutritional value (Oh and Koh 2019). Fruit developmental stages and environmental stress factors exhibit similar effects on pepper plants (Batista-Silva et al. 2018). For instance, as fruit color changes, sugar content increases, and acidity levels decrease. Likewise, pungency levels (especially about the capsaicin component) may vary depending on the ripening process. Enzymatic activities are crucial factors that dictate the speed and quality of plant and fruit development (Palma et al. 2020; Du et al. 2020). Water scarcity can disrupt the plant's water balance, reduce photosynthetic efficiency, and consequently adversely affect the plant's overall health and yield. Such stress conditions have significant effects on the growth and development of pepper plants (Pagamas and Nawata 2008).

This research primarily aims to investigate the impact of drought on the development of pepper plants. Specifically, it examines the effects of this critical abiotic stress factor, which holds particular importance in arid regions, on plant growth and responses at different growth stages using species such as *C. annuum* and *C. chinense*. Various irrigation levels and growth stages have been assessed. The study meticulously explores the effects of drought on plant morphology, photosynthetic pigment content, phytochemical components, oxidative stress indicators, and antioxidant enzyme activities. By elucidating the influence of this stress factor on plant health and productivity, the acquired knowledge is anticipated to make significant contributions to the improvement of agricultural practices and the optimization of pepper cultivation in arid regions.

Materials and Methods

Plant Materials and Growth Conditions

The study was conducted between March and September 2022 in the greenhouses of Kilis 7 Aralık University Agricultural Application and Research Center, located in Kilis, Turkey. Plant materials belonging to *Capsicum annuum* L. and *Capsicum chinense* Jacq. species were utilized in the study. Seeds of these species were sown in 150-cell trays containing a mixture of peat and perlite in a 3:1 ratio. The plants were grown in plastic greenhouse conditions until they developed four true leaves. In May, the seedlings, ready for transplanting, were placed in 10-liter pots filled with the cultivation soil, with only one plant per pot. The physicochemical properties of the cultivation soil were as follows: pH: 6.20, EC (dS/m): 0.26, texture: loamy, organic matter (%): 2.07, nitrogen (%): 0.36, phosphorus (mg kg⁻¹): 3.40, potassium (mg kg⁻¹): 349.08, calcium (mg kg⁻¹): 2942.83, magnesium (mg kg⁻¹): 429.91, sodium (mg kg⁻¹): 49.02, copper (mg kg⁻¹): 1.079, iron (mg kg⁻¹): 3.10, manganese (mg kg⁻¹): 18.24, and zinc (mg kg⁻¹): 0.98. The plants were regularly irrigated and subjected to cultural practices throughout the growing season. Furthermore, the climatic characteristics of the region where the material was grown during the growing period are given in Figure S1.

Treatments and Study Design

The study was conducted under stress conditions in three different growth stages for two different pepper species with four different irrigation regimes. Each irrigation regime was designed to include 3 replicates with 10 plants per replicate, following a randomized complete block design. After the plants had developed four true leaves, they were maintained under 75% FC irrigation conditions for two weeks. Control groups for all species were grown under 75% FC irrigation conditions. While applying water stress, field capacity was calculated by subtracting the weight of the dry soil-filled pot from the weight of the pot when saturated with water and free drainage occurred. Irrigation regimes were adjusted based on this calculation, and irrigation procedures continued until the S3 growth stage.

Plant Morphology and Growth Measurements

The measurements of stem diameter (SD) and length (SL), leaf number (LN) and area (LA), and root length (RL) were taken using a caliper (Mitutoyo, Japan) for all developmental stages, and the mean values were calculated and expressed numerically for each plant. Root fresh weight (RWW) and dry weight (RDW) were determined using a digital balance (Mettler Toledo, USA). After separating the upper parts of the stem from the roots, the fresh weights of the remaining root portions were recorded. Subsequently, these portions were dried at 60°C until their weights were stabilized and then weighed.

Photosynthetic Pigment Contents

The concentrations of leaf photosynthetic pigments [total chlorophyll (TChl), chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (TCar)] were assessed according to the protocol by Lichtenthaler and Buschmann (2001). Pepper species under drought stress were sampled for TChl, Chl a, Chl b, and TCar measurements during the S1, S2, and S3 stages. All sampling procedures were conducted at the same time and under identical conditions. The samples were stored in a liquid nitrogen atmosphere and preserved at -80°C until analysis. The extraction of photosynthetic pigments from leaves was carried out by incubating 100 mg of ground leaf tissue in a 1:1 (v/v) ethanol/acetone mixture for 3 hours. The absorbance of the extracts at 470, 645, and 662 nm was measured using a UV spectrophotometer (Biochrom, UK), and the content of TChl, Chl a, Chl b, and TCar was calculated using the following formulas:

$$\text{Chla} \left(\frac{\text{mg}}{\text{g}} \right) = 12.70 \times A_{662} - 2.69 \times A_{645}$$

1

$$\text{Chlb} \left(\frac{\text{mg}}{\text{g}} \right) = 22.90 \times A_{645} - 4.68 \times A_{662}$$

2

$$\text{TChl} \left(\frac{\text{mg}}{\text{g}} \right) = 20.21 \times A_{645} + 8.02 \times A_{662}$$

3

$$\text{TCar} \left(\frac{\text{mg}}{\text{g}} \right) = \frac{1000 \times A_{470} - 2.27 \times \text{Chla} - 81.4 \times \text{Chlb}}{227}$$

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Phytochemical Contents

Leaf samples from different developmental stages of different plant species subjected to different irrigation regimes were cleaned, dried in the shade at room temperature, and then turned into a powder. For the extraction of total phenolics (TPh), total antioxidant activity (TAa), and total flavonoids (TFv), 2

grams of each leaf sample were mixed with 10 mL of methanol at room temperature in closed-neck flasks on magnetic stirrers until the extraction solvent became colorless. This process was repeated three times. The obtained extracts were filtered through the Whatman filter paper (No:1), and the filtrate was collected. Subsequently, the methanol was removed using an evaporator (Buchi, Switzerland) at 40°C. The residues formed at the bottom of the flask were dissolved in methanol and used for analysis. The obtained extracts were stored at -20°C until analysis.

The determination of total phenolic (TPh) contents followed the method outlined by Castro-Concha et al. (2014). Extracts were kept in the dark at room temperature for 90 minutes with the addition of Na₂CO₃ and Folin-Ciocalteu reagent. Subsequently, the absorbance of the samples was measured at 765 nm using a spectrophotometer. Gallic acid (GA) served as the standard, and the results were expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh weight of leaf material. The TPh content of leaf samples was calculated using a calibration curve established with standard GA solutions.

For total flavonoid (TFv) analysis, 100 µL of methanol plant extracts at a concentration of 10 mg mL⁻¹ were placed in a tube, to which 100 µL of AlCl₃ was added. Subsequently, 2–3 drops of acetic acid were introduced into the tube, and the volume was adjusted to 5 mL with methanol. The prepared solutions were incubated in the dark at room temperature for 30 minutes, and the absorbance of the resulting yellow color was measured at 415 nm against a blank sample. Methanol was utilized as the blank. The TFv content was quantified as quercetin equivalent (QE). Standard quercetin equivalent (QE) solutions were prepared, and a calibration curve was constructed by measuring against the blank sample. TFv content was expressed as mg equivalent QE per gram of fresh weight (FW) (Owoade et al. 2018).

Total antioxidant activity (TAa) measurements were carried out using the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method. The analysis followed the procedure developed by Re et al. (1999), based on the reduction of the ABTS radical cation by antioxidants, monitored by the decrease in absorbance values at 734 nm. A 7 mM ABTS solution was reduced and diluted over 12 hours using a 2.45 mM potassium persulfate solution. This diluted ABTS solution was added to the extracted pepper leaf extracts, and the absorbance value was recorded. Diluted ABTS solution served as the blank sample, and Trolox was employed as the standard solution. The TAa results were calculated as µmol Trolox equivalent antioxidant capacity (TEAC) per gram of fresh weight (FW) using the Trolox standard calibration curve.

Biochemical Analyses

MDA content was assessed in various pepper species subjected to different stress conditions at the S1, S2, and S3 developmental stages. This process involved the use of 200 mg of fresh leaf material, which was then extracted with 2 mL of 80% (v/v) methanol. The samples were agitated overnight in a flask and subsequently subjected to centrifugation to collect the upper phase. The obtained extracts were combined with a 0.5% (w/v) solution of thiobarbituric acid (TBA) prepared in 20% (w/v) trichloroacetic acid (TCA) and incubated at 95°C for 20 minutes. Following this, the samples were rapidly cooled on ice and centrifuged at 5000 rpm for 10 minutes at 4°C. Each sample was accompanied by a control sample,

examined in parallel, containing the extract and TCA but lacking TBA. The absorbance of the supernatants was measured at 532 nm after subtracting the non-specific absorbance at 600 and 400 nm. The concentration of MDA was determined using the equations established by Heath and Packer (1968). The MDA content was expressed as $\mu\text{g g}^{-1}$ fresh weight (FW).

The proline (PRL) content was quantified using the colorimetric method with acidic ninhydrin reagent (Ou et al. 2015). In brief, 200 mg of leaf material was digested in 2 mL of a 3% (w/v) sulfosalicylic acid solution. The samples were then mixed with the acid ninhydrin reagent and incubated in a water bath at 100°C for one hour. After cooling to room temperature, they were extracted with toluene. The absorbance of the organic phase was measured at 520 nm against toluene, and known PRL concentrations were determined using standard curves. Leaf PRL contents were expressed as $\mu\text{g g}^{-1}$ fresh weight (FW).

Total soluble proteins (PRO) were extracted from leaf samples of stressed plants following the procedure outlined by Kurkela et al. (1988). The resulting supernatant was preserved at -20°C for subsequent analysis. The spectrophotometric Bradford method, as detailed by Marks et al. (1988), was employed as the analytical technique. The protein content of the extracts was determined by measuring the binding of unknown protein solution to known standards using Coomassie Brilliant Blue G-250 dye. The absorbances of standard protein solutions were determined at 595 nm, and a standard curve of absorbances against protein concentration was plotted. Sample absorbances were also measured at 595 nm using a spectrophotometer, and protein concentrations were determined from the calibration curve. PRO contents were expressed in units of mg per g of FW.

Antioxidant Enzyme Activities

Stressed pepper leaves sampled at different developmental stages were used for the extraction of SOD, POD, and CAT enzymes following the protocol of Stagner and Popovic (2009). Concisely, 100 mg of leaf samples were extracted in 4 mL of 50 mM phosphate buffer at pH 7.0, with the inclusion of 1 mM EDTA and 1% polyvinyl polypyrrolidone (PVP). The supernatant was obtained through centrifugation at 5000 rpm for 30 minutes at 4°C and subsequently preserved at -20°C for further analysis.

The superoxide dismutase (SOD) enzyme activity was determined following the procedure developed by Stagner and Popovic (2009). The measurement involved evaluating the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) salt. To achieve this, 100 μL of supernatant was combined with phosphate buffer (500 μL), methionine (200 μL), riboflavin (100 μL), and d-H₂O (800 μL). This mixture was exposed to UV light for 15 minutes. Subsequently, the absorbance at 560 nm was recorded using a microplate reader (Thermo Fisher Scientific, USA), and SOD activity was expressed as U g^{-1} protein fresh weight (FW) based on the protein content.

Catalase (CAT) and peroxidase (POD) enzyme activities were determined according to Maechly and Chance (1954). For CAT activity, 100 μL of the extract was mixed with 500 μL of 300 mM phosphate buffer and 100 μM H₂O₂, and the change in absorbance was monitored at 240 nm for 2 minutes. CAT

activity was expressed as $\text{nmol min}^{-1} \text{g}^{-1}$ protein fresh weight (FW). For peroxidase (POD) activity, 200 μL of 40 mM H_2O_2 , 500 μL of 20 mM guaiacol, and 100 μL of the extract were added to a 500 μL solution containing 50 mM phosphate buffer at pH 5.0. Changes in the solution's absorbance were recorded at 470 nm in 20-second intervals. POD activity was expressed as $\text{nmol min}^{-1} \text{g}^{-1}$ protein FW.

Statistical Analysis

Mean values and standard deviations for all measurements were calculated. Data were analyzed using analysis of variance and multiple comparison tests with JMP 14 software (SAS, Malaysia). Tukey's HSD test was utilized for multiple comparisons, and significance was considered for p-values below 0.05. Pearson correlation analysis was performed using OriginPro software (OriginLab, USA).

Results

Effect of Drought Stress on Plant Growth

According to the results of the variance analysis (ANOVA) for morphological characteristics during all three growth periods, the effect of stress levels on leaf number (LN), stem length (SL), root length (RL), stem diameter (SD), root wet weight (RWW), and root dry weight (RDW), as well as the effect of pepper species, were found to be significant at a minimum level of 1% (Table 1).

Under control conditions (75% FC), gradual increases were observed in SL, SD, LN, RL, RWW, and RDW values across the S1, S2, and S3 developmental stages for the examined species. For *C. annuum* and *C. chinense*: SL (from 32.02 cm to 74.81 cm and from 12.46 cm to 55.91 cm), SD (from 3.44 cm to 9.66 cm and from 2.27 cm to 5.39 cm), LN (from 17.82 cm to 49.48 cm and from 20.61 to 49.92), RL (from 3.19 cm to 11.04 cm and from 1.99 cm to 4.30 cm), RWW (from 2.00 g to 7.35 g and from 1.23 g to 3.89 g), and RDW (from 0.26 g to 0.92 g and from 0.14 g to 0.38 g) ranged respectively (Table 1, Figure S2, S3, S4, S5, S6 and S7). In the S3 stage, SL, SD, RL, RWW, and RDW values reached their highest levels in *C. annuum*, while LN exhibited the highest values in *C. chinense*.

Table 1

Mean comparison of the effect of drought stress on plant growth for different developmental stages and pepper species. Stem length (SL), stem diameter (SD), leaf number (LN), root length (RL), root wet weight (RWW), and root dry weight (RDW)

Drought Treatments	Ripening Stage	Species	SL (cm)	SD (cm)	LN (pcs)	RL (cm)	RWW (g)	RDW (g)
25% FC	S1	<i>C. annuum</i>	21.38 _d	2.26 _{ef}	10.82 _h	1.88 ^f _d	1.75 _d	0.22 _{cd}
50% FC			25.28 _c	2.96 _c	11.84 _g	2.73 _c	1.65 _c	0.24 _c
75% FC (C)			32.02 _b	3.44 _{ab}	17.82 _d	3.19 _{ab}	2.00 _b	0.26 _b
100% FC			32.93 _a	3.46 _a	19.97 _c	3.49 _a	2.44 _a	0.30 _a
25% FC		<i>C chinense</i>	12.46 _h	1.46 _h	13.15 _f	1.09 _h	0.79 _h	0.09 _h
50% FC			16.40 _g	1.93 _g	16.58 _e	1.26 _g	1.13 _{fg}	0.13 _g
75% FC (C)			19.32 _{ef}	2.27 _{de}	20.61 _b	1.99 _{de}	1.23 _f	0.14 _f
100% FC			19.92 _e	2.31 _d	21.71 _a	2.08 _d	1.47 _e	0.17 _e
25% FC	S2	<i>C. annuum</i>	48.82 _d	5.13 ^f _d	24.04 _h	3.84 ^f _d	3.57 _d	0.44 _d
50% FC			57.06 _c	6.04 _c	28.53 _{fg}	5.16 _c	3.78 _c	0.47 _c
75% FC (C)			74.17 _{ab}	7.82 _{ab}	39.58 _d	6.52 _b	4.04 _b	0.54 _b
100% FC			74.85 _a	7.87 _a	44.39 _c	7.12 _a	4.98 _a	0.62 _a
25% FC		<i>C chinense</i>	28.33 _h	3.33 _h	29.22 _f	2.23 _h	1.62 _h	0.19 _h
50% FC			37.91 _g	4.39 _g	36.85 _e	2.78 _g	2.31 _g	0.27 _g
75% FC (C)			43.91 _f	5.26 _{de}	45.60 _b	4.07 _{de}	2.51 _f	0.29 _f

Drought Treatments	Ripening Stage	Species	SL (cm)	SD (cm)	LN (pcs)	RL (cm)	RWW (g)	RDW (g)
100% FC			44.29 _e	5.32 _d	47.25 _a	4.24 _d	2.70 _e	0.35 _e
25% FC	S3	<i>C. annuum</i>	60.28 _d	6.34 _e	30.04 ^h	6.51 _e	6.06 _d	0.75 _d
50% FC			70.94 _c	7.46 _c	35.61 _g	8.86 _c	6.40 _c	0.80 _c
75% FC (C)			91.81 _{ab}	9.66 _b	49.48 _c	11.04 _b	7.35 _b	0.92 _b
100% FC			92.41 _a	9.72 _a	55.50 ^b	12.07 _a	8.44 _a	1.05 _a
25% FC			<i>C. chinense</i>	34.98 _h	4.11 _h	36.52 _f	3.78 _h	2.75 _h
50% FC	46.06 _{fg}	5.49 ^f		46.07 _{de}	4.46 ^f	3.97 _f	0.46 _f	
75% FC (C)	46.22 _f	5.39 _{fg}		46.92 _d	4.30 _{fg}	3.89 _g	0.38 _g	
100% FC	49.91 _e	6.58 _d		60.32 _a	7.19 _d	5.08 _e	0.59 _e	
<i>Significance</i>								
<i>Species (S)</i>			***	***	***	***	***	***
<i>Development Stage (DS)</i>			***	***	***	***	***	***
<i>Drought Treatments (DT)</i>			***	***	***	***	***	***
<i>S x DS</i>			***	***	***	***	***	***
<i>S x DT</i>			**	**	**	**	**	**
<i>DS x DT</i>			***	***	***	***	***	***
<i>S x DS x DT</i>			**	**	**	**	**	**

Values for each parameter indicate the results of the Tukey HSD test between species and drought stress for each development stage (S1, S2, and S3); ** $p \leq 0.01$, *** $p \leq 0.001$.

The impact of different drought stresses applied at different developmental stages (S1, S2, and S3) on SD, SL, LN, RL, RWW, and RDW of two *Capsicum* species are presented in Table 1. Under limited irrigation conditions, a significant decrease ($P \leq 0.001$) in all growth parameters was observed when comparing

100% field capacity (FC) irrigation with 50% FC and 25% FC irrigation conditions for both species. In *C. annuum* and *C. chinense*, under 100% FC irrigation conditions, SL, SD, LN, RL, RWW, and RDW showed less significant changes compared to other irrigation conditions ($p \leq 0.01$) when compared to control (75% FC). However, under 25% FC and 50% FC irrigation conditions, all growth parameters exhibited significant reductions compared to the control conditions ($p \leq 0.001$).

Effect of Drought Stress on Photosynthetic Pigments

The impact of drought stress on *C. annuum* and *C. chinense* species was evaluated in terms of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (TChl), and total carotenoid (TCar) contents during the S1, S2, and S3 stages. The responses of *C. annuum* and *C. chinense* species to different irrigation levels were thoroughly examined.

According to the ANOVA table of the photosynthetic traits in this study, the effects of drought treatments and developmental stages on Chl a, Chl b, TChl, and TCar were found significant at $p \leq 0.001$ level for both species (Table S1). Throughout the S1 to S3 period, a significant decrease in Chl a, Chl b, and TChl values was observed when the irrigation level of plants was reduced from 100% FC to 25% FC. According to the obtained data, Chl a, Chl b, and TChl contents significantly decreased in the 25% FC and 50% FC irrigation treatments compared to the control groups (Fig. 1). In the S1 developmental stage, the highest Chl a, Chl b, and TChl contents were recorded under 100% FC irrigation conditions. For *C. annuum*, these values were measured as 0.97, 0.48, and 1.50 mg g^{-1} FW, while for *C. chinense*, they were recorded as 0.82, 0.44, and 1.29 mg g^{-1} FW. During the S2 stage, a pronounced decrease in Chl a, Chl b, and TChl contents was observed under 100% FC irrigation conditions. In this stage, Chl a, Chl b, and TChl were measured as 0.78, 0.39, and 1.29 mg g^{-1} FW for *C. annuum* and 0.70, 0.37, and 1.10 mg g^{-1} FW for *C. chinense*. In the S3 stage, the decrease in Chl a, Chl b, and TChl contents continued. For *C. annuum*, Chl a, Chl b, and TChl contents decreased by approximately 72.9%, 61.2%, and 71.7%, respectively, compared to the S1 stage, while for *C. chinense*, these percentages were approximately 70.7%, 71.1%, and 76.4%. In both species and developmental stages, the application of 100% FC irrigation increased all photosynthetic pigment levels compared to the control groups to some extent. However, as the irrigation regime changed from 50% FC to 25% FC, significant decreases in photosynthetic pigment levels were observed. Furthermore, *C. annuum* displayed higher Chl a levels during all developmental stages and stress applications, whereas in the S3 stage of *C. chinense*, higher Chl b levels were observed under 25% FC irrigation conditions. The lowest TChl content (0.28 mg g^{-1} FW) was determined during the S3 developmental stage in *C. chinense* under the 25% FC treatment.

According to the research findings, under control conditions, the TCar concentrations in the leaves of the species varied between 1.05 to 1.94 mg g^{-1} FW and 0.80 to 1.25 mg g^{-1} FW for different developmental stages (Fig. 1). Under control conditions, *C. annuum* species exhibited the highest TCar concentrations during the S1 developmental stage, while *C. chinense* showed the lowest TCar concentrations during the S3 developmental stage. Drought conditions led to reductions in photosynthetic pigments and TCar content in the leaves. Specifically, under 25% FC irrigation conditions, TCar concentrations for *C. annuum*

and *C. chinense* species were reduced by approximately 24.8% and 18.5% (S1), 26.3% and 30.2% (S2), and 28.3% and 34.1% (S3), respectively, compared to the control conditions. According to the data, TCar contents significantly decreased in the 25% FC and 50% FC irrigation treatments compared to the control group ($p \leq 0.001$). However, TCar levels in the 100% FC treatment exhibited decreases with a lower level of significance compared to other irrigation application groups ($p \leq 0.01$). Considering the decreases in Chl a, Chl b, TChl, and TCar levels under different drought treatments for the species at the end of the S3 period, it can be suggested that *C. annuum* is more resilient to drought in terms of photosynthetic pigments and carotenoid content.

Effect of Drought Stress on Phytochemical Contents

According to the ANOVA table of the phytochemical contents in the study, the effects of the experimental factors on TPh, TFv, and TAa were significant in all three developmental stages (Table S2). Statistically significant effects of drought stress treatments on the phytochemical contents of pepper species were also observed (Fig. 2). It has been determined that different responses to stress applications were given by species at different developmental stages. During the S1 and S2 developmental stages, as the severity of drought stress increased, the amounts of TPh and TFv significantly increased, while in the S3 stage, the increases in TPh and TFv quantities were found to be insignificant when compared to the control group ($p > 0.05$). The TPh content was measured at its maximum value ($6.02 \text{ mg GAE g}^{-1} \text{ FW}$) in the S1 developmental stage under 25% FC stress conditions in the *C. annuum* species, while it was determined at the minimum level ($1.99 \text{ mg GAE g}^{-1} \text{ FW}$) in the *C. chinense* species under 50% FC stress conditions in the S2 stage. The maximum TFv value was determined in the *C. annuum* species in the S1 stage under 25% FC conditions ($8.24 \text{ mg QE g}^{-1} \text{ FW}$), while the minimum TFv ($2.88 \text{ mg QE g}^{-1} \text{ FW}$) was measured in the *C. chinense* species in the S3 stage under 100% FC stress conditions (Table S2). In all developmental stages, excessive irrigation conditions (100% FC) had no significant effect on the accumulation of phenolic and flavonoid compounds for both species. However, excessive irrigation conditions in the S2 developmental stage significantly ($p \leq 0.001$) reduced the TAa level.

TAa increased proportionally with the stress applied during the S1 and S3 stages, while it decreased under 50% FC irrigation conditions in the S2 stage. The maximum TAa value ($26.85 \text{ } \mu\text{mol TEAC g}^{-1} \text{ FW}$) was reached under 25% FC conditions where irrigation was minimal (Table S2). The S2 developmental stage had the highest TAa levels for the species, while in the S1 and S3 stages, the species did not show significant variations in stress groups other than 25% FC irrigation conditions. For both species and in all developmental stages, it was observed that both low (25% FC) and excessive irrigation (100% FC) had no significant effect on TAa values. The analysis of variance showed that drought stress had a significant effect on TPh, TFv, and TAa, and there was significant variation between species for both stress applications and developmental stages ($p \leq 0.001$).

Effect of Drought Stress on Biochemical Contents

The values of malondialdehyde (MDA), protein (PRO), and proline (PRL) were measured to be higher in both species and all development stages compared to the control group under drought stress conditions (Fig. 3). MDA, PRO, and PRL levels increased significantly ($p \leq 0.001$) as the stress conditions intensified from 100% FC stress to 25% FC stress. The maximum increase in all three parameters was observed during the S2 period for both species. While the application of 25% FC stress resulted in elevated MDA, PRO, and PRL levels, the lowest levels were measured in the control groups throughout all developmental stages. The change in MDA levels during the S1 period was found to be insignificant ($p > 0.05$) for both species.

Furthermore, the responses of both species to 100% FC stress increased MDA, PRO, and PRL levels compared to the control group throughout all developmental stages. In addition, in the case of *C. annuum* species, the change in MDA and PRO levels was found to be insignificant ($p > 0.05$) compared to the control group during the S1 and S3 developmental periods under 100% FC application. Additionally, for *C. chinense* species in this stress group, the change in MDA during the S1 period and PRL during the S3 period was insignificant compared to the control group. Furthermore, the changes in MDA for *C. chinense* in the S1 and S3 periods under 50% FC and 100% FC stress applications were also found to be insignificant. For *C. annuum* and *C. chinense* species, the highest PRO quantity was measured at 32.41–22.39 mg per g of FW, respectively, during the S3 stage. The highest PRL was measured at 68.83–46.14 μg per gram of FW, and the highest MDA was determined at 2.76–1.87 μg per gram of FW at the S2 stage (Table S3).

Effect of Drought Stress on Antioxidant Enzyme Activities

POD, SOD, and CAT are antioxidative enzymes that respond to stress and also indicate tolerance. This study investigated the enzyme activities exhibited by the examined pepper species, *C. annuum* and *C. chinense*, in response to applied drought stress. The ANOVA analysis of antioxidant enzyme activities reveals significant differences among the *Capsicum* species concerning the application periods at a 0.1% level of significance. Furthermore, it demonstrates that the levels of drought stress applied to the species are also significant at a 0.1% level of significance (Table S4). The data obtained indicate that *C. annuum* species exhibited more pronounced responses to stress conditions below (S1 and S2) and above (S3) field capacity.

Drought stress applications had a significant impact ($p \leq 0.001$) on the levels of POD, SOD, and CAT in pepper species. Various stress intensities led to highly significant increases in POD, SOD, and CAT levels in *C. annuum* species during all three developmental stages (Fig. 4). In contrast, in *C. chinense* species, while the POD and CAT values increased compared to the control in the S1 developmental stage under all drought applications, significant decreases in SOD values were observed in the S2 and S3 developmental stages under 25% FC treatment. In *C. annuum* species, the highest SOD, POD, and CAT values were found in the S3 developmental stage under 25% FC treatment, with values of 101.55 U g^{-1} Protein FW, 825.02 $\text{nmol min}^{-1} \text{g}^{-1}$ Protein FW, and 1098.37 $\text{nmol min}^{-1} \text{g}^{-1}$ Protein FW, respectively. In *C. chinense* species, these values for SOD were 77.83 U g^{-1} Protein FW under 50% FC treatment in the S3 stage, and for POD

and CAT, they were $620.48 \text{ nmol min}^{-1} \text{ g}^{-1}$ Protein FW and $870.57 \text{ nmol min}^{-1} \text{ g}^{-1}$ Protein FW under 25% FC treatment in the S3 stage. The activities of SOD, POD, and CAT showed a gradual increase in response to the intensity of applied stress throughout the developmental stages, with the most substantial proportional increase observed as both species transitioned from the S1 to the S2 stage. Additionally, the drought application that exhibited the least response compared to the control group was the 100% FC irrigation conditions. Furthermore, the lowest SOD (24.10 U g^{-1} Protein FW), POD ($195.31 \text{ nmol min}^{-1} \text{ g}^{-1}$ Protein FW), and CAT ($216.92 \text{ nmol min}^{-1} \text{ g}^{-1}$ Protein FW) activities were determined in the S1 developmental stage of *C. chinense* species (Table S4).

Discussion

Drought poses a significant threat to our natural environment, profoundly impacting plant life. It is imperative to conduct further research to gain a deeper understanding of the mechanisms through which it affects plants. The analysis of various physiological changes occurring in plants during their growth and development under drought conditions represents an approach rooted in the comprehensive evaluation of plant physiological parameters (Pandey and Singh 2015). In the case of *Capsicum* species, soil moisture content plays a vital role in supporting fundamental physiological functions. Nevertheless, various abiotic stress factors, such as water deficiency, can exert distinct effects on plant physiology and metabolic processes at various stages of growth and development (Ahmed et al. 2015).

The results of the study indicate that different levels of drought significantly reduce the morphological characteristics of pepper species. However, these reductions are not the same for both species. *C. annuum* was found to be a species that exhibits greater tolerance to severe drought conditions. Measurements reveal that this species has some special physiological advantages that enable its viability under limited irrigation conditions. Under severe stress, *C. annuum* displayed greater morphological development, such as increased stem length, stem diameter, leaf count, and root weight. Additionally, the findings demonstrate a significant decrease in the examined growth parameters for both species across all growth stages with increasing severity of drought stress. In this context, the study highlights the adverse effects of drought stress in conjunction with the severity of drought on the above-ground parts of both species. The difference in plant growth between the species, compared to the control, continuously varied throughout the developmental stages.

For *C. annuum* and *C. chinense*, very significant ($p \leq 0.001$) changes were observed in the measurements of SL, SD, LN, RL, RWW, and RDW under 100% FC, 50% FC, and 25% FC irrigation conditions compared to the control (75% FC). Additionally, the effect of different developmental stages on growth parameters was found to be significant at the $p \leq 0.01$ level (Table 1). The developmental stages, different application rates, and interactions between pepper species showed positive and significant correlations for all growth parameters (Fig. 5). However, the degree of positivity was higher for RL and RWW, while it was lower for SL, SD, LN, and RDW. The results indicated that the physiological responses of species to the severity of drought stress were entirely negative. As reported by Kusvuran et al. (2020), Sahitya et al. (2018), Ismail (2010), Khan et al. (2008), and Katerji et al. (1992), physiological growth parameters were adversely

affected under drought stress conditions. Restricting water access during the development process of species has a negative impact on their metabolic and physiological functions (Din et al. 2011). Differences in the size of plant parts among species may indicate different water requirements for the physiological and biological processes of species (Zubaer et al. 2007). In a study investigating the response of different tomato varieties to drought stress, conducted on three different tomato varieties belonging to the *Solanum* genus, it was reported that the varieties responded in line with our study to drought stress. Significant reductions in the fresh and dry weights of plant parts were reported (Zhou et al. 2017). Moreover, in our study, the proportional decrease in the percentage change of plant growth parameters under 100% FC, 75% FC, 50% FC, and 25% FC irrigation applications in the S1, S2, and S3 periods makes the effect of the irrigation regime noticeable on plant growth. Furthermore, the changes in plant growth parameters in different developmental stages are in line with other studies (Poudyal et al. 2023; Dhaliwal et al. 2019; Khah et al. 2007).

Leaves are the most crucial part of a plant since photosynthesis occurs in this region. The nutrients produced as a result of photosynthesis are transported to different parts of the plant. Therefore, the growth, development, and productivity of the plant are closely related to the active growth of leaves (Hussain and Ali 2015). In the study, as the severity of drought increased, reductions in the quantity of chlorophyll components were observed. Chlorophyll content serves as a valuable indicator for assessing drought tolerance, as it reflects the photosynthetic efficiency under drought stress conditions. The reductions in photosynthetic pigments observed in *C. annuum* were notably less significant compared to those in *C. chinense*. Guo et al. (2016) proposed a connection between the decline in photosynthetic activity and the reduction in leaf chlorophyll content in seedlings subjected to drought stress. Furthermore, Pirzad et al. (2011) reported that increasing drought severity resulted in a decrease in chlorophyll concentration in leaves. Similarly, as with other plants, increased stress has restricted the photosynthesis rate of both *C. annuum* and *C. chinense*. Additionally, Farooq et al. (2009) stated that an increase in stress resulted in increased starch accumulation and a loss of enzyme activities involved in photosynthesis. Furthermore, it has been reported that stress directly hinders metabolism by restricting CO₂ entry into the leaf. Yusuf and Hamed (2021) reported a significant decrease in chlorophyll content at advanced stages of water stress for *C. frutescens* species. The content of Chl a and b exhibited a similar trend to TChl content. Significant correlations were found between growth parameters, including SL, SD, LN, RL, RWW, and RDW, and Chl a, Chl b, and TChl for both species in terms of developmental stages and different application rates (Fig. 5). It is said that drought stress can change the chlorophyll content and carotenoid amount (Hussein et al. 2008). High SL and LN values observed in *C. annuum* species (Figure S2 and Figure S4) contribute to enhanced photosynthetic activity and growth even under drought stress. The decline in chlorophyll and carotenoid content in species subjected to increasing drought stress may be linked to the reduction in plant growth parameters under such conditions. Farooq et al. (2009) proposed that this response could serve as a defense mechanism to mitigate the detrimental effects of drought stress. Studies on cotton and sunflower plants have also reported a decrease in photosynthetic pigment contents with escalating drought stress (Massacci et al. 2008; Jaleel et al. 2008). Carotenoids are terpenoid compounds that play a pivotal role in various biological processes in plants, including the

regulation of light energy absorption in photosynthesis and the protection of plants from excessive light exposure (Ko et al. 2022). Their involvement in photosynthesis, photoprotection, and photomorphogenesis reactions underscores the importance of carotenoids (Maoka, 2020). As evident in Figs. 1S, 2S, 3S, 4S, 5S, and 6S, the reduction in morphological characteristics under stress conditions is less pronounced in *C. annuum* compared to the other species. Furthermore, it is noteworthy that in all maturity stages where the intensity of stress increased (25% FC and 50% FC treatments), the morphological measurements of *C. annuum* surpassed those of *C. chinense*, implying its superior potential for physiological development compared to *C. chinense*. Additionally, in the experimental group where irrigation exceeded the control group (100% FC), it can be inferred that *C. annuum* also exhibits a high degree of tolerance. It was observed that carotenoid accumulation in leaves decreased with plant development, and significant quantitative changes occurred in the total carotenoid content. Changes in leaves with the time elapsed after flowering were similar for the studied species. For both species, with the increasing severity of drought stress in the S1, S2, and S3 developmental stages, significant and meaningful changes occurred in the TCar levels. The intensity of stress significantly reduced TCar levels. *C. annuum* species had higher leaf TCar value than other species with increasing stress intensity. TChl was also found to be higher than *C. chinense*. It is clear that this species is able to preserve more photosynthetic pigments depending on the intensity of stress. However, the effect of irrigation conditions above the control on TCar content was found to be insignificant in the S1 stage, while it was positive and significant in the S2 and S3 stages. A decrease in leaf photosynthetic pigment levels due to drought has been reported in many plants (Zulini et al. 2005; Ali et al. 2020). Drought stress induces oxidative damage by increasing ROS levels in chloroplasts and mitochondria (Bartoli et al. 2004; Selote and Khanna-Chopra 2006), leading to peroxidation and degradation of photosynthetic pigments. The higher levels of TChl and TCar in *C. annuum* (Table S1) can be attributed to enhanced photosynthetic activity and plant growth under escalating stress severity (Table 1). A positive and highly significant correlation between Chl a, Chl b, TChl, and TCar was observed at $p \leq 0.001$ for both species (Fig. 5). This highlights the substantial impact of metabolic events associated with drought stress on Chl and TCar levels in the plant.

Peppers offer numerous nutraceutical benefits attributed to their phytochemical content (Sánchez-Segura et al. 2015). Interest in the phytochemical compounds found in peppers has grown due to their antioxidant, anti-inflammatory, hypolipidemic, hypoglycemic, and health-promoting properties (Jia et al. 2017). Phenolic compounds can neutralize free radicals in vitro and in vivo, which are implicated in conditions like cancer, aging, and certain neurodegenerative diseases (Bogusz et al. 2018). Consequently, the presence of substances in fruits and vegetables that can counteract the effects of these radicals holds significant importance. Phenolic compounds, carotenoids, and flavonoids exhibit substantial antioxidant activity, rendering peppers a valuable source of antioxidant compounds (Materska and Perucka 2005; Zimmer et al. 2012).

It has been documented that phytochemical changes occurring during the ripening process have an impact on the composition and antioxidant activity of peppers (Howard et al. 2000). In this context, the present study revealed that the total phenolics (TPh) and total flavonoids (TFv) contents significantly increased in all developmental stages of the species as drought stress severity intensified, although no

significant changes were observed under control conditions (75% FC). Total antioxidant activity (TAa), conversely, exhibited an increase, reaching its maximum level during the S2 stage under control conditions, and then decreased in the S3 stage. Ghasemnezhad et al. (2011) reported an increase in antioxidant activity, measured using the DPPH and ABTS tests, in *C. annuum* plants as they progressed in growth. As the severity of drought stress escalated, TAa levels also displayed significant increases. In a study by Sarafi et al. (2018), it was noted that an elevation in the amount of flavonoids in various *C. annuum* varieties positively influenced antioxidant activity and played a significant role in augmenting the quantity of antioxidant compounds. Furthermore, it was reported that the increase in polyphenol biosynthesis, driven by plant growth, was associated with the accumulation of nitrate (NO_3^-) and phosphate (PO_4^{3-}) ions. The biosynthesis of these organic compounds was found to contribute to polyphenol production, with fluctuations in the concentration of these organic nutrients resulting in changes in the quantity of phenolic components during the plant's growth process. The decrease in polyphenol concentration during the plant's growth stages may be due to changes in the biosynthesis, translocation, or degradation of these metabolites (Bhandari et al. 2016). A study by Cho et al. (2020) compared the total phenols, antioxidants, and flavonoids in fruits and leaves of a variety of *C. annuum* peppers. The study reported that the biological activities of peppers are mostly studied in fruits, but according to the research results, phenolic, antioxidant, and flavonoid contents in leaves are higher than in other plant parts. It is worth noting that the composition of antioxidants can vary significantly, influenced by factors such as ripeness, variety, climate conditions, and the specific plant part analyzed (Rodriguez-Amaya, 2003). According to Fig. 2, the abrupt and severe changes in TPh, TFv, and TAa levels during drought applications (25% FC and 50% FC) of *C. chinense* throughout all examined developmental stages indicate that this species is more sensitive to drought. Conversely, *C. annuum*, under different stress intensities, exhibited lower percentages of change in TPh, TFv, and TAa. Thus, it can be concluded that *C. annuum* can tolerate drought stress better than *C. chinense*. Moreover, the correlation between TPh, TFv, and TAa in pepper plants under different drought stress and growth stages is significant, with at least a $p \leq 0.05$ level of significance. Positive correlations were found between TPh-TAa and TFv-TAa, while a negative correlation was observed between TPh and TAa. Moreover, a significant positive correlation at the $p \leq 0.01$ level exists between total carotenoids (TCar) and total flavonoids (TFv) (Fig. 5).

The accumulation of ROS compounds under stressful conditions is a natural occurrence in cellular metabolism and plays a pivotal role in transmitting intercellular signals (Noctor and Foyer 2016). Therefore, reducing the levels of ROS compounds during drought stress is essential for plants to cope with stressful conditions (Fang et al. 2015). In this study examining the impact of drought stress on *C. annuum* and *C. chinense* species, PRO and PRL levels significantly increased in all developmental stages with the intensity of drought stress. These increases elucidate a significant coping mechanism aimed at alleviating the adverse impacts of drought stress and facilitating the ongoing growth of the plant (Verbruggen and Hermans 2008). The accumulation of PRO and PRL increased to higher levels in *C. annuum* plants. This increase was observed in both species during the S2 stage, but in the S3 stage, these values showed a slight decrease compared to those in the S2 stage. On the other hand, the MDA

content exhibited significant variations during the S2 and S3 stages, whereas the changes in the S1 stage were less pronounced ($p \leq 0.05$). Notably, during the S2 and S3 developmental stages, the alterations in MDA, PRL, and PRO levels in *C. annuum* were found to be significantly higher in percentage compared to *C. chinense* (Table S3). This can be explained by *C. annuum* exhibiting more pronounced responses to ROS compounds and greater tolerance to drought. The fact that drought stress applied to the species in the S1 stage resulted in less change in MDA content compared to other stages is an important factor indicating that oxidative damage caused by stress was less in the S1 stage. In a study involving drought stress on leaf-edible vegetables, it was reported that root and leaf measurements decreased, chlorophyll content decreased, and MDA content increased (Assaha 2016). The increase in soluble PRO and PRL accumulation with drought severity and plant growth was observed to help maintain tissue hydration and avoid damage caused by drought (Nasser and Aal 2002). This contributed to protecting the plant from the active and reactive oxygen species caused by drought. Additionally, the accumulation of PRO, PRL, and MDA in tissues was reported to prevent water loss, as well as carboxylation and the inactivation and denaturation of various enzymes (Anjum 2012). In addition, *C. annuum*, in terms of the investigated biochemical contents, appears to increase PRO, PRL, and MDA levels more than *C. chinense* under drought conditions, indicating greater resistance to drought stress. In *C. annuum*, there were at least $p \leq 0.01$ levels of significant and positive correlations between PRO, PRL, and MDA, whereas in *C. chinense*, significant and positive correlations were determined at least at the $p \leq 0.05$ level (Fig. 5).

Plants employ enzymatic and non-enzymatic antioxidant molecules to combat oxidative stresses resulting from the buildup of reactive oxygen compounds (Farooq et al. 2009; Turk et al. 2014). It has been observed that all enzyme activities vary in response to drought stress. Drought stress significantly increased antioxidant enzyme activities compared to control conditions ($p \leq 0.001$). Intense drought treatment at 25% FC resulted in higher antioxidant enzyme activities in both species compared to 50% FC and 100% FC applications. The two species exhibited different enzymatic responses to different drought stress conditions. *C. annuum* responded by showing a higher level of antioxidant enzyme activity with increasing stress. The higher tolerance of *C. annuum* to drought stress was attributed to the proportional increase in CAT, SOD, and POD activities and the accumulation levels of PRO, PRL, and MDA, compared to *C. chinense*. In response to oxidative stress, ROS levels increase lipid oxidation and lead to MDA formation. Therefore, MDA is commonly used as a marker to assess oxidative damage caused by abiotic stress (Moller et al. 2007). In *C. annuum*, MDA is positively and significantly correlated with CAT, POD, and SOD enzyme activities, at least at the $p \leq 0.01$ level (Fig. 5). Significant decreases in SOD activity levels were observed during the S2 and S3 stages and under 25% FC drought conditions. Studies have reported that the decrease in SOD and CAT activities is due to the aging of leaves. The decrease in SOD activity with the development of wheat (Srivalli and Khanna-Chopra 2001) and blackberry (Wang and Jiao 2001) can be attributed to this.

Conclusions

The intensity of stress, plant growth stages, and the interaction between species have been shown to be important for morphological measurements, total phenolics, total flavonoids, total antioxidants, proline,

MDA, soluble proteins, catalase, peroxidase, and superoxide dismutase. Under control conditions (75% FC), the impact of plant growth stages on the examined parameters was similar for both species. Drought stress negatively affected the growth parameters of both species. In *C. annuum*, it was observed that photosynthetic pigments were less affected by drought. Changes in TPh, TFv, and TAa content varied depending on the severity of stress and the plant species' growth stage. Low (25% FC) and excessive (100% FC) irrigation conditions had a significant impact on the accumulation of TPh and TFv for both species. MDA, proline, and protein levels increased significantly as the stress severity increased (from 100% FC to 25% FC). With the increasing severity of drought stress, the biophysical and enzymatic measurement values of *C. annuum* were significantly higher than those of *C. chinense*. Different stress conditions led to significant increases in SOD, POD, and CAT levels in *C. annuum* during all three growth stages. Finally, this study reveals the significant effects of stress intensity, plant developmental stages, and interactions between species on a range of morphological, phytochemical, biochemical, and enzymatic parameters. Comparisons between the two species have shown that *C. annuum* exhibits a higher tolerance to drought stress. These findings hold great importance for pepper cultivation, as they indicate the potential for cultivating *C. annuum* under drought conditions. Furthermore, this study provides valuable insights into how pepper species respond to drought conditions and can guide future cultivation practices. Subsequent studies are planned to expand on these findings with more detailed investigations, especially focusing on potential changes in fruit characteristics under stress conditions. This way, a solid foundation will be laid for developing more effective and sustainable practices in pepper cultivation.

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Ethics declarations

Conflict of interest

Ü.H. Erol declares that he has no conflict of interest.

Additional information

Data availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Supplementary Information

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Figures

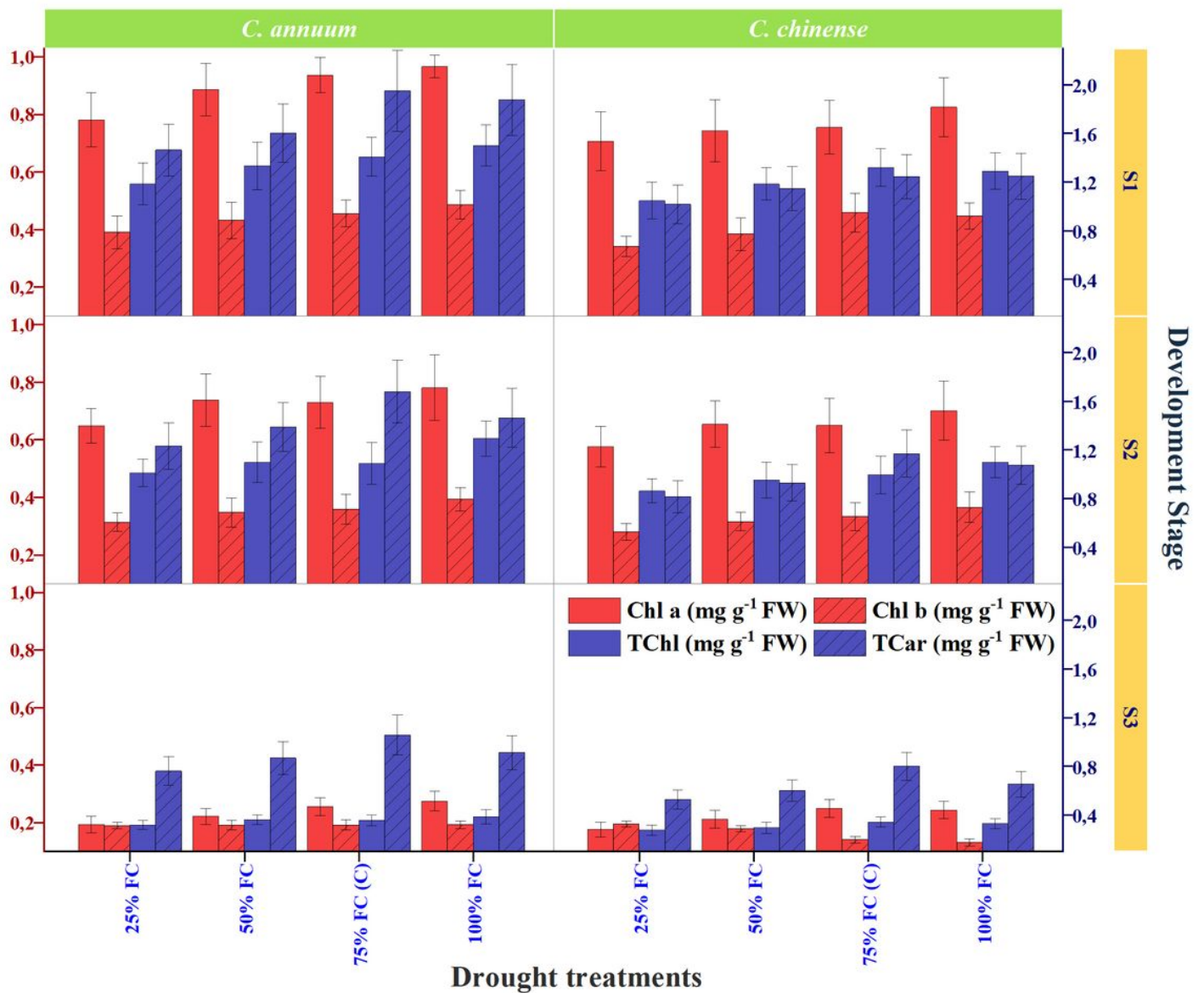


Figure 1

Chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (TChl), and total carotenoid (TCar) contents of pepper species about irrigation regimes and ripening periods. Values are mean $\pm$ standard deviation (SD). ^{ns} Non-Significant ($p > 0.05$), * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) *** Very significant ($p \leq 0.001$) while results depicted that for *Chl a*: S = ***, DS=***, DT = ***, S $\times$ DS =*, S $\times$ DT=ns, DS $\times$ DT=***, S $\times$ DS $\times$ DT=*; for *Chl b*: S = ***, DS=***, DT = ***, S $\times$ DS =**, S $\times$ DT=ns, DS $\times$ DT=***, S $\times$ DS $\times$ DT=ns; for *TChl*: S = ***, DS=***, DT = ***, S $\times$ DS =**, S $\times$ DT=ns, DS $\times$ DT=**, S $\times$ DS $\times$ DT=ns; for *TCar*: S = ***, DS=***, DT = ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=**, S $\times$ DS $\times$ DT=**

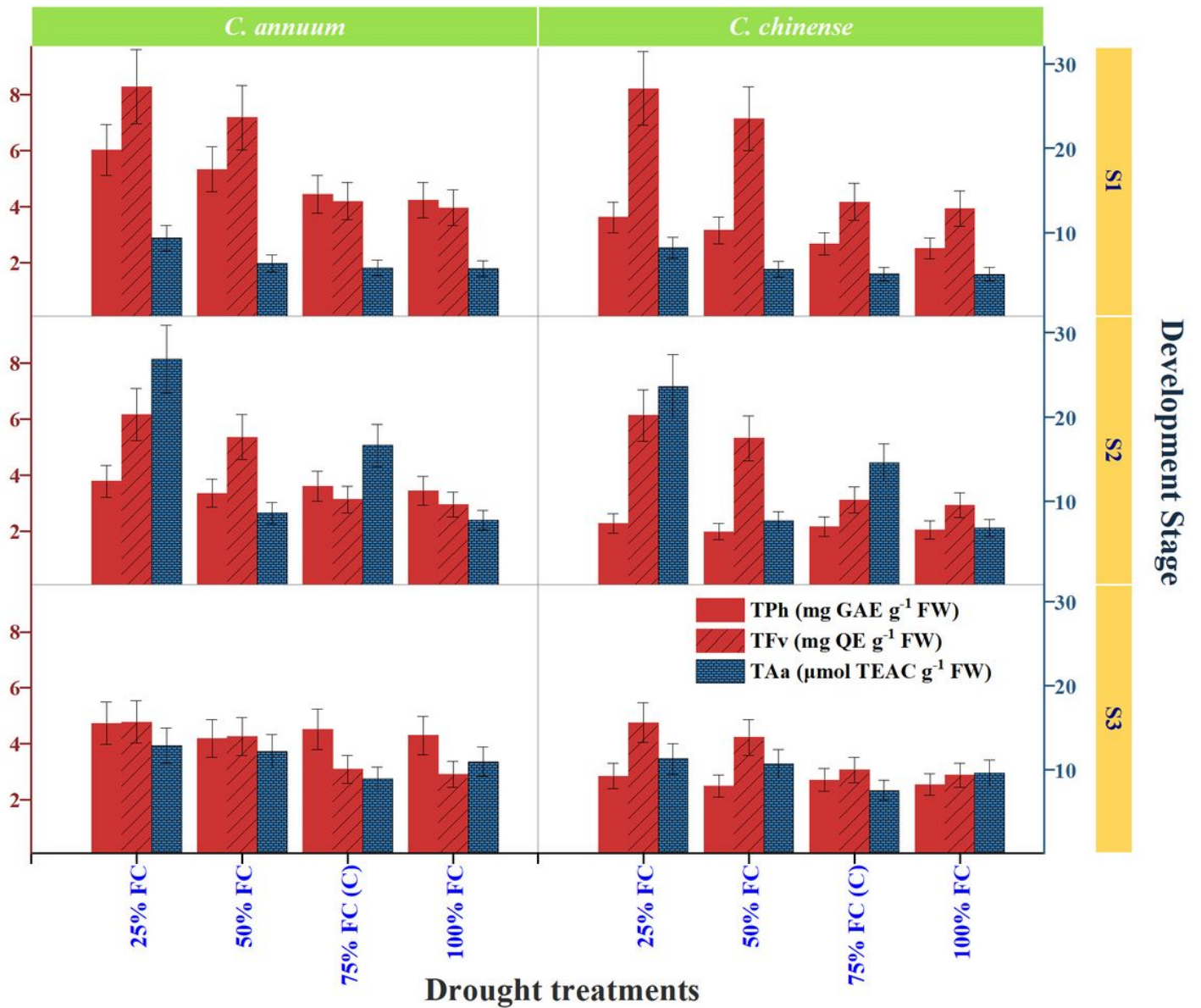


Figure 2

Total phenolic (TPh), total flavonoid (TFv), and total antioxidant (TAa) contents of pepper species about irrigation regimes and ripening periods. Values are mean $\pm$ standard deviation (SD). ns non-Significant ($p > 0.05$), * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) *** Very significant ($p \leq 0.001$) while results depicted that for *TPh*: S = ***, DS=***, DT = ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=***, S $\times$ DS $\times$ DT=**; for *TFv*: S = ***, DS=***, DT = ***, S $\times$ DS =*, S $\times$ DT=**, DS $\times$ DT=***, S $\times$ DS $\times$ DT=ns; for *TAa*: S = ***, DS=***, DT = ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=***, S $\times$ DS $\times$ DT=***

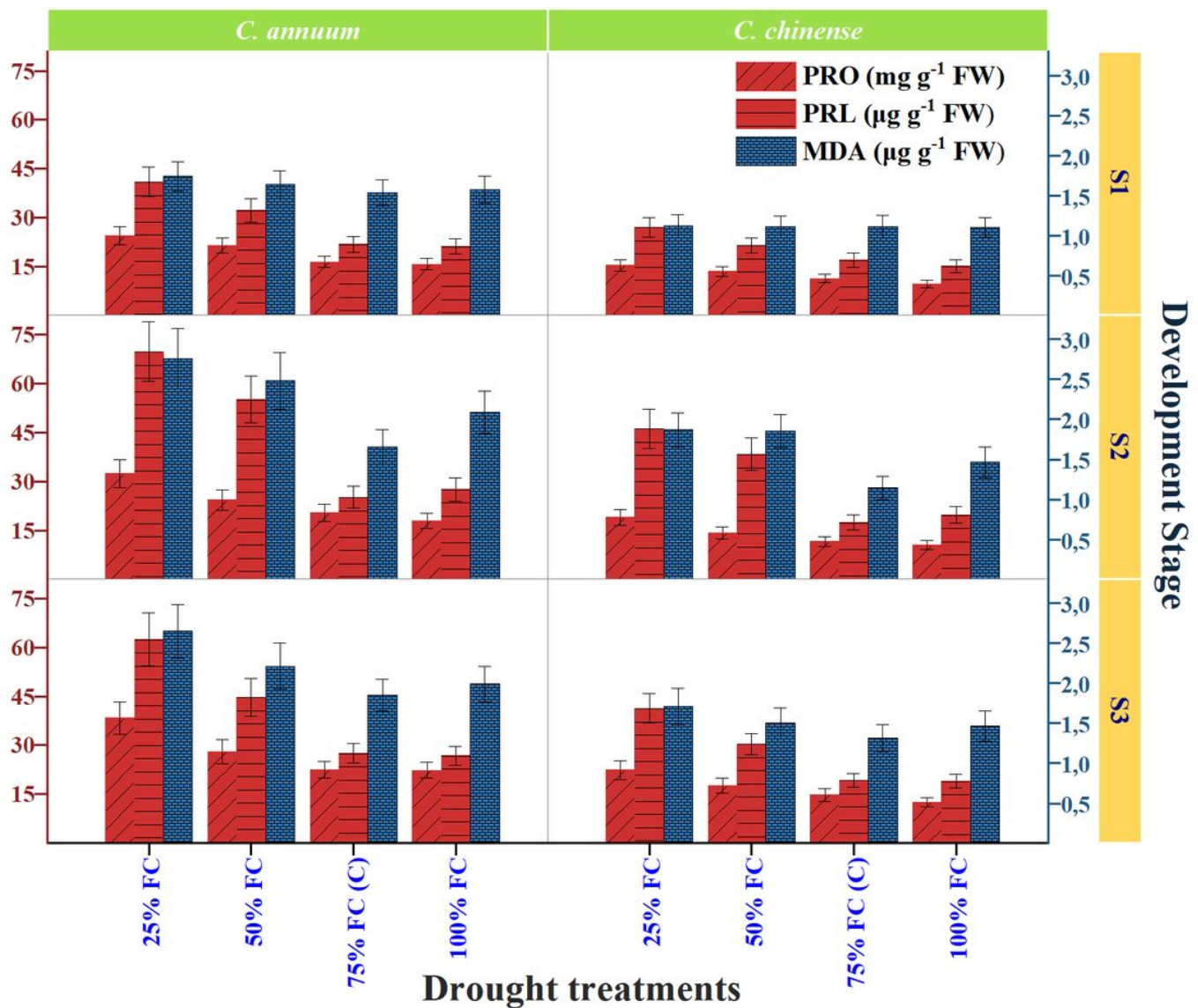


Figure 3

Malondialdehyde (MDA), proline (PRL), and protein (PRO) contents of pepper species about irrigation regimes and ripening periods. Values are mean $\pm$ standard deviation (SD). ns non-Significant ($p > 0.05$), * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) *** Very significant ($p \leq 0.001$) while results depicted that for *PRO*: S = ***, DS=***, DT = ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=**, S $\times$ DS $\times$ DT=**; for *PRL*: S= ***, DS=***, DT = ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=***, S $\times$ DS $\times$ DT=**; for *MDA*: S = ***, DS=***, DT= ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=***, S $\times$ DS $\times$ DT=**

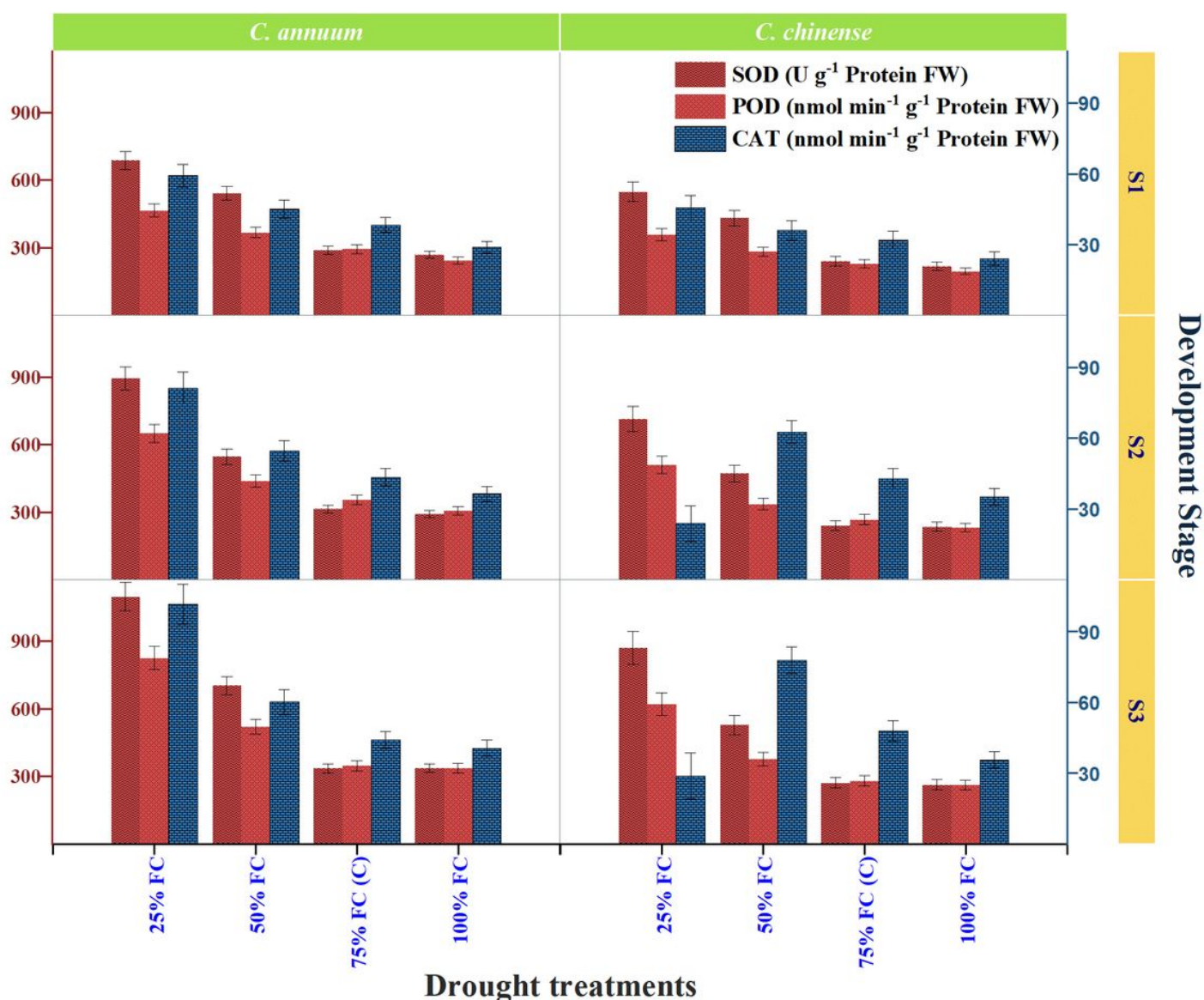


Figure 4

Peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) enzyme activities of pepper species about irrigation regimes and ripening periods. Values are mean $\pm$ standard deviation (SD). ns non-Significant ($p > 0.05$), * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) *** Very significant ($p \leq 0.001$) while results depicted that for *SOD*: S = ***, DS=***, DT = ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=**, S $\times$ DS $\times$ DT=**; for *POD*: S = ***, DS=***, DT = ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=***, S $\times$ DS $\times$ DT=**; for *CAT*: S = ***, DS=***, DT= ***, S $\times$ DS =***, S $\times$ DT=***, DS $\times$ DT=***, S $\times$ DS $\times$ DT=**

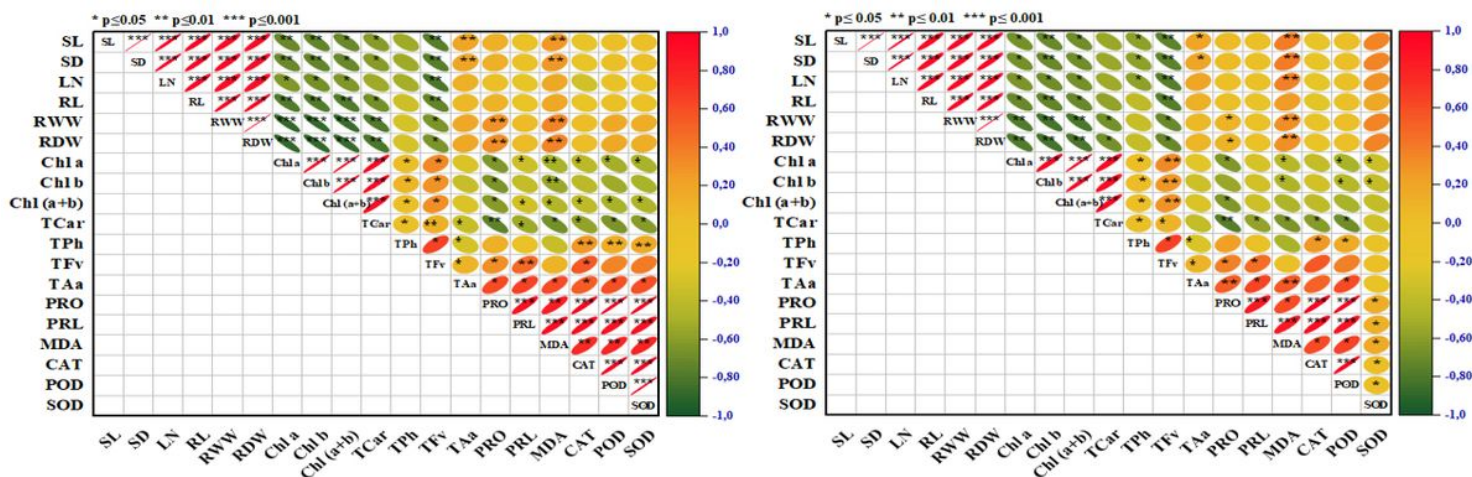


Figure 5

Pearson correlation between photosynthetic pigments, phytochemical contents, biochemical contents, and antioxidant enzyme activities in pepper plants for different drought stress and different growth stages. The figure represents (A) *C. annuum* and (B) *C. chinense*. Circles and ellipses in red and their shades show a positive correlation with the parameters. Circles and ellipses in green and shades of green are negatively correlated with the parameters. * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$), *** Very significant ($p \leq 0.001$)

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