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Effects of Ascorbic Acid and/or α-Tocopherol on Agronomic and Physio-Biochemical Traits of Oat (*Avena sativa* L.) under Drought Condition

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Abstract: Water stress is notably a critical environmental condition restricting plant growth and economic outputs in semi-arid and arid environments. In a pot experiment, we explored the potential function of α-tocopherol (α-toc) and/or ascorbic acid (AsA) on the agronomic and physio-biochemical features of oat grown in water-scarce conditions. Drought duration significantly reduced the soil electrical conductivity and pH but increased the soil temperature, influencing the nutrient availability and uptake. For example, post-drought (25 days) soil analysis indicated that electrical conductivity decreased from 597 to 306 mS/m, total dissolved solids from 298 to 153 mg/L, and pH from 7.5 to 6.3 in 25 days of drought. Further, the drought-stressed leaves also contained significantly lower metabolites, such as proline, protein, sugar, and glycine betaine, than the control leaves, indicating impaired plant defense mechanisms. Significantly increased enzymatic antioxidants in leaves (e.g., superoxide dismutase, ascorbate peroxidase, and peroxidase) suggested the inability of oat plants to overcome drought-induced oxidative damage. In contrast, AsA and/or α-toc significantly amplified the seed germination rates and plant growth. Taken together, our results demonstrate that AsA and α-toc have the capability to mitigate adverse effects of drought conditions on oat plants by improving leaf relative water contents, photosynthetic pigments, and the antioxidant defense system.

Keywords: water stress; cereal crops; secondary metabolites; growth mediators; antioxidant properties

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1. Introduction

Avena sativa L. (Gramineae), commonly known as oat, is a cereal grain grown for its seed and fodder. Oats have been globally cultivated for medicinal and food products for two thousand years [1]. Based on world cereal production statistics, oats rank sixth in production, after wheat, maize, rice, barley, and sorghum. Oats are an excellent source of protein, fiber, and minerals, but as agricultural mechanization progressed in the 1930s and 1950s, the global oat production declined [2]. In some ecosystems of developing and developed nations, oats are a significant crop for people with specific usage. In many parts of world, oats are grown for grain use, including feed, bedding straw, hay, silage, and husks. Grain feed for livestock remains the main use of oat crops, accounting for an average of approximately 74% of total global consumption between 1991 and 1992 [3]. For example, oats are cultivated throughout Pakistan and are now the primary source of

winter and spring feed from the plains up to the highlands (1000–2300 m). In Pakistan, oats occupy more than 35% of the feed area [4]. After decades of mass production, oats are steadily declining as a result of the climate change consequences in emerging nations. These impacts comprise long-term droughts, floods, high temperatures, rain, and seasonal fluctuations in humidity. Unfavorable climate change makes it more difficult to meet the growing global demand for oats. Among them, water scarcity is primarily an unfavorable restrictive factor distressing the nutritional stage, pod filling, pollen viability, and oat yield.

Because water is a vital compound for various metabolites, it is important for yield and growth and its absence impacts all physiological, agronomic, and biochemical characteristics [5]. Oat harvesting under water-stressed conditions exhibits cell division restriction, nutritional disparity, ion exchange, and variations in primary and secondary metabolism. Due to complicated oxidative stress, plant cellular membranes are prone to water shortages that may readily damage the lipids and biomolecules incorporated in these membranes. Drought disrupts various molecular and physiological processes in plants, including the suppression of transpiration, cell turgidity, stomatal and osmoregulation, water consumption efficacy, growth of the deep roots system, and manufacture of osmolytes [6]. Defense antioxidants help to capture unregulated reactive oxygen species (ROS) mediated by drought. This reaction, however, varies from crop to crop, and is primarily determined by the plant's genetic composition and external environment. Various studies using a leaf foliar spraying method to alleviate harmful impacts of drought stress, including the exogenous administration of growth-stimulating chemicals, have been proposed [7].

Alpha-tocopherol (α-toc) and/or ascorbic acid (AsA) are the key growth mediators that protect plant tissues from free oxygen radicals in plants. For example, AsA acts as a cofactor for enzymes regulating hormones biosynthesis, renewing enzymatic antioxidants, and modulating metabolism in plants [8]. Similarly, α-toc has a significant antioxidant effect as a suppressor of lipid peroxidation, and it is beneficial in protecting cellular membranes from oxidative stress. The most abundant of them are actively proliferating plant cells or whole cellular divisions, and even cell walls. Abiotic stresses, particularly dryness, influences the development and growth of cereals with a low α-toc and/or AsA content. It works as a powerful metabolite or signaling modulator in a plant's cellular activities or defensive systems by detoxicating hydrogen peroxide or H_2O_2 amid water scarcity [9]. The previous research presented the intensifying effects of foliar spraying with α-toc and/or AsA on the morphological, physiological, and biochemical characteristics of various crops, including faba bean, mung bean, and maize [10–12].

Thus, with this background, the present research work is an effort to (i) evaluate the agronomic, physio-biochemical and antioxidant properties of oat subjected to the foliar application of AsA and/or α-toc under drought conditions, and (ii) test the hypothesis that, under drought stress, better results can be achieved in oat crops that receive exogenously applied AsA and α-toc.

2. Materials and Methods

2.1. Experiment Layout and Treatments

In growing season of 2021 (October–December), a randomized block design with three replicates in each treatment or each replicate, comprising ten plants, was arranged in the net house. The research area had a sub-humid environment and was located 450 m above sea level, with a mild winter of 18.35 °C and hot summer of 40.8 °C [13]. The oat seeds were obtained from the Cereal Crops Research Institute Persabaq, Nowshera. After surface sterilizing with ethanol (95%), the seeds were sown in earthen pots (18 cm lower or upper inside diameter, 20 cm height, or 2 cm thickness) filled with 2 kg of air-dried soil and silt (2:1). After emergence of seedling, the plants were subjected to exogenous growth mediators with continuous 15 and 25-day drought. After, those treatments were started according to the following manner:

(T0) control: normal watering + 0 mg AsA and α-toc

(T1): 15 days drought + 0 mg AsA and α-toc

(T2): 15 days drought + 200 mg AsA
(T3): 15 days drought + 200 mg α-toc
(T4): 15 days drought + 200 mg AsA and α-toc
(T5): 25 days drought + 0 mg AsA and α-toc
(T6): 25 days drought + 200 mg AsA
(T7): 25 days drought + 200 mg α-toc
(T8): 25 days drought + 200 mg AsA and α-toc

Agronomic aspects of vegetative development, including data on germination, were noted. After reaching a five-leaf stage, plants along with roots were collected and the external materials along with soil particles adhering to the roots were removed. Half of the plants were harvested after 15 days, and the remaining were after 25 days of drought stress for further analysis. Fresh leaves were used to find out enzymatic activities while the remaining plants were stored in the freezer at −4 °C to evaluate their physio-biochemical traits.

2.2. Post-Experiment Soil Analysis

After the experiment, the soil samples were analyzed for total dissolved solutes, electrical conductivity, pH, dissolved oxygen, resistivity, soil moisture content, oxidation–reduction potential, salinity, and field capacity. In a small glass with 50 mL of distilled water, 10 g of air-dry soil was weighed in a 1:5 soil water suspension. The multiparameter Bluetooth portable water quality meter HI98494 was utilized to quantify soil characteristics.

2.3. Agronomic Analysis

Following this, agronomic and germination characteristics were calculated via the procedures of Nafees et al. [14] and Shah et al. [15] using following formulas.

$$\text{Mean germination time (MGT)} = (\in fx)/(\in f) \quad (1)$$

Here, f is the frequency of seeds that emerged on day X.

$$\text{Germination rate index (GRI)} = G1/1 + G2/2 + G3/3 \ldots \ldots Gx/x \quad (2)$$

Here, G1 or G2 are emergence rates on first and second days after propagating, and Gx is final emergence rate on final day.

$$\text{Germination energy (GE)} = X1/Y1 + ((X2 - X1)/Y2) + ((Xn - Xn - 1)/Yn) \quad (3)$$

Here, X1, X2, and Xn are number of seeds germinated on the 1st day, 2nd, and so on, Whereas Y1, Y2, and Yn are time from plating to 1st, 2nd, or up to day ten.

$$\text{Timson's germination index (TGI)} = (\in G)/T \quad (4)$$

G represents the overall percent of emerging seeds for respective day, whereas T denotes the emergence day.

$$\text{Water use efficiency (WUE)} = (\text{Water used overall for the experiment (mL)})/(\text{Total Biomass (g)}) \quad (5)$$

$$\text{Mean germination rate (MGR)} = 1/MGT \quad (6)$$

$$\text{Seed vigor indice I (SVI-I)} = (\text{Mean root length} + \text{Mean shoot length}) \times \% \text{ Germination} \quad (7)$$

$$\text{Seed vigor indice II (SVI-II)} = \text{Dry root} + \text{shoot weight (mg)} \times \% \text{ Germination} \quad (8)$$

$$\text{Root moisture content (RMC)} = (\text{Wet root weight} - \text{Dry root weight})/(\text{Wet root weight}) \quad (9)$$

$$\text{Time to 50\% germination (T50\%)} = (ti + (N/2 - ni)(tj - ti))/((nj - ni)) \quad (10)$$

Here, ni and nj are number of seeds germinated at time ti and tj, whereas N shows the final number of germinated seeds.

2.4. Physio-Biochemical Features

2.4.1. Leaf Pigment Contents

Chlorophyll (Chl. a and b) concentration was assessed by using procedure of Sonobe et al. [16], and carotenoids concentration was determined through Ahmad et al. [17]. Fresh leaves of 0.2 g were grounded in 80% acetone with a pestle and mortar or incubated in dark for 24 h then centrifugated for 10 min. Absorbance value of 645 nm was recorded for Chl. a, 663 nm designed for Chl. b, and 470 nm for carotenoids via spectrophotometer against 80% acetone blank. The following formula was used to calculate the parameters.

$$\text{Chl. a} = [12.7\ (OD_{663}) - 2.69\ (OD_{645})] \times V/1000 \times W \quad (11)$$

$$\text{Chl. b} = [22.9\ (OD_{645}) - 4.68\ (OD_{663})] \times V/1000 \times W \quad (12)$$

$$\text{Carotenoid} = OD_{480} + (0.114 \times DA_{663}) - (0.638 \times DA_{645}) \quad (13)$$

Here, OD is optical density at specified wavelength, V is the extract level (in mL), and W is weight of fresh leaves.

2.4.2. Soluble Sugar Content (SSC), Glycine Betaine (GB), Total Proline Content (TPC), and Soluble Protein Content (SPC)

Sugar content of leaves was quantified using technique of Buysse and Merckx [18]. A total of 0.5 g of plant material along with 10 mL of distilled water were ground and then centrifuged at 2500 rpm for 5 min. One milliliter of phenol 80% (*w*/*v*) was mixed in 0.1 mL of supernatant followed by addition of 3.0 mL concentrated H_2SO_4 kept at 25 °C for a few hours, and optical density was recorded at 420 nm, while the Hitz and Hanson [19] method was used to determine glycine betaine content. Proline content of leaves was determined through procedure of Bates et al. [20]. A total of 0.25 g of leaves was crushed in 5 mL of 3% aqueous sulpho-salicylic acid tailed by filtration. Then, 2 mL of acid ninhydrin or 2 mL of glacial acetic acid were reacted with 2 mL of filtrate in test tube for 60 min and heated at 100 °C in water bath followed by addition of 4 mL toluene to the mixture. The chromophore with toluene was extracted from aqueous part and warmed to 25 °C, and sample was observed at 520 nm against toluene as blank, while protocol of Bradford [21] was used to assess the amount of soluble protein. Through mortar and pestle, 0.2 g of fresh leaves was ground in 1 mL of phosphate buffer (pH 7.5). A total volume of 1 mL was made by adding 0.1 mL of the above-prepared extract and distilled water. A total of 3.0 mL of reagent was added, it having 3 g sodium carbonate (Na_2CO_3) and 0.6 g sodium hydroxide (NaOH) (0.1 N), while 150 mL distilled water was used to dissolve 1.5 g Na-K tartrate and $CuSO_4.5H_2O$ (0.125 g) dissolved in 25 mL of distilled water. After shaking for 10 min, 0.1 mL of Folin phenol reagent was added. After 30 min incubation, absorbance of each sample was recorded at 650 nm. Their contents were computed using Equation (14).

$$\text{Protein\% (W/W)} = Cp \times V \times DF/wt \quad (14)$$

where wt is weight of the leaves (mg), DF represent dilute factor, and Cp shows protein concentration (mg/L).

2.4.3. Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA)

The methodology by Velikova et al. [22] was followed to determine H_2O_2 activity. The MDA content was assessed according to assay of Sakaki et al. [23] or their OD was recorded at 530 nm. The fresh leaf (0.25 g) was triturated in TCA (3 mL; 1%) or subsequently centrifuged. Then, aliquot (1.0 mL) was homogenized with 4 mL of TBA (0.5% thiobarbituric acid). Samples were incubated for 50 min at 95 °C.

2.4.4. Antioxidant Enzymatic Assays

The procedure of Ukeda et al. [24] was applied for the approximation of SOD level at 560 nm using instrument spectrophotometer. Similarly, the activity of glutathione

reductase (GR) and peroxidase (POD) was analyzed at 420 nm and 340 nm, respectively, by the technique of Ahmad et al. [25]. The leaf tissue extract was furthermore utilized for measurement of catalase (CAT) or ascorbate peroxidase (APOX) enzymes via the method of Zhao et al. [26].

2.5. Analysis through Statistical Software

Microsoft Excel Software 2010 (Redmond, DC, USA), US was applied to compute standard and mean error for obtained data. Using Co-Stat Window version 6.3, UK analysis of variance (ANOVA) was performed to find significant differences between treatments. Using XLSTAT software, the principal component analysis (PCA) was performed for investigated parameters. Using R-Studio 8.1 software (Boston, MA, USA), US correlation analysis was performed.

3. Results and Discussion

Recent years have seen a substantial increase in the effect of water shortage conditions on agriculture, affecting the soil structure, function, plant physiology, and related metabolism. In this study, long-term drought significantly changed the soil attributes, impacting the soil organic matter, decomposition, and the release of excess CO_2 [27]. However, the oat plants treated with AsA and/or α-toc could sustain their agronomic, physiological, and biochemical properties under the stressed environments

3.1. Effect on Soil Physicochemical Properties

Electrical conductivity reduced to a maximal extent compared to the control, i.e., from 605 to 252 mS/m with the treatment of AsA for 15 days, pH declined from 7.6 to 6.3 after 25 days of drought. Total dissolved solutes decreased from 303 to 126 mg/L after 15 days of dryness supplemented with AsA, according to the physicochemical examination of the soil before and after seeding (Table 1). Furthermore, the soil temperature changed from 17.8 °C under control (T0) to 23.7 °C after 25 days of drought (T5). The alternations in the soil's physicochemical characteristics under drought and different treatments could considerably influence the soil traits. In this context, the study by Rojas and Huang revealed that the reductions in soil water cause a drop in crop nutrient absorption, which directly impacts all water precursor activities and turgor pressure [28]. Still, drought has no substantial alterations in resistivity or oxidation–reduction potential (Table 1). The study's findings revealed that extreme drought had negative impact on the structure, function, and productivity of agricultural soil [29]. This section may be divided by subheadings. It should provide a concise and precise description of experimental results and their interpretation, as well as experimental conclusions that can be drawn.

3.2. Agronomic Features

Results from the growth and germination parameters demonstrated that different levels of drought stress (15 days and 25 days) considerably reduced the morphological performance of the oat plants (Tables 2 and 3). Under 15 and 25 days of drought stress, the MGT, GE, GRI, FGP, TGI, CVG, SVI-I, SVI-II, and RMC were significantly reduced ($p \leq 0.05$). These negative consequences might result from stomatal closure and increased reactive oxygen species (ROS) generation, usually mediated by drought stress in plants. Even the reduced stomatal conductance and cellular membrane integrity can progressively slow the rate of CO_2 assimilation, disrupting water relations by lowering the plants' water use efficiency [30]. The highest MGT and CVG were observed in 15-day drought regimes treated with α-toc (T3), while the highest GRI and TGI were observed at α-toc + 25 days drought (T7) (Table 2). Furthermore, the foliar application of α-toc significantly boosted WUE and GE in 25-day drought regimes (T5). Table 3 describes that SVI-I, SVI-II, RMC, and T50% were recorded as maximum after 15 days of drought at T1, T4, T2, and T3, respectively, while MGR at 25 days of drought (T5). These parameters decrease as the drought level increases in the growth medium. Comparable results from the previous studies show the

same decline in agronomic characteristics under water-deficit conditions in carrot [31], maize [32], and common vegetables [33]. The AsA and/or α-toc can mitigate inhibiting the oat growth performance and development by enhancing antioxidant enzymes and osmolytes formation. In contrast, these parameters were recorded as maximum in plants subjected to foliar sprays of AsA and/or α-toc (200 mg/L). The synergistic impact of AsA and/or α-toc demonstrates that these PGRs have a favorable influence on oat agronomic characteristics. However, AsA is found to be more effective than α-toc in alleviating drought stress-induced adverse effects in oat. Exogenously administered AsA and/or α-toc were successfully employed to improve tolerance against drought stress in several crops, such as sunflower [34] and wheat [35].

Table 1. Impact of drought stress on soil physicochemical parameters after experiment.

Treatments	T (°C)	pH	ORP (mV)	R (Ω·m)	EC (mS m^{-1})	TDS (mg L^{-1})	Salinity	DO	SDW (g)	SMC	FC
T0	17.8	7.6	109.2	1653	605	303	0.29	10.2	6.81	31.9	46.8
T1	20.5	7.1	96.1	2688	372	148	0.18	11.1	6.89	31.1	45.1
T2	19.6	7.1	82.5	3984	252	126	0.12	11.2	7.13	28.7	40.3
T3	19.6	7.2	88.2	3367	296	148	0.14	11.2	7.43	25.7	34.6
T4	19.6	7.0	83	3704	270	135	0.13	11.2	7.18	28.2	39.3
T5	23.7	6.3	108.7	1675	597	298	0.29	10.2	7.82	21.8	27.9
T6	19.4	7.2	103	2857	350	175	0.17	10.1	6.53	34.7	53.1
T7	19.4	7.5	87.9	3268	306	153	0.15	11.2	6.3	37	58.7
T8	19.4	7.2	91	3012	332	166	0.16	10.4	6.83	31.7	46.4

T = temperature, pH = power of hydrogen ion concentration, EC = electrical conductivity, TDS = total dissolved solute, ORP = oxidation reduction potential, DO = dissolved oxygen, SDW = soil dry weight, R = resistivity, SMC = soil moisture content, and FC = field capacity. (T0) control: normal watering + 0 mg AsA and α-toc, (T1): 15 days drought + 0 mg AsA and α-toc, (T2): 15 days drought + 200 mg AsA, (T3): 15 days drought + 200 mg α-toc, (T4): 15 days drought + 200 mg AsA and α-toc, (T5): 25 days drought + 0 mg AsA and α-toc, (T6): 25 days drought + 200 mg AsA, (T7): 25 days drought + 200 mg α-toc, (T8): 25 days drought + 200 mg AsA and α-toc.

Table 2. Effect of AsA and/or α-toc on *A. sativa* TGI, MGT, CVG, GE, MGT, GRI, and WUE under water-deficit conditions.

Treatments	TGI	CVG	GE	MGT	GRI	WUE
T0	64.33 ± 3.0^{a}	3.82 ± 0.4^{a}	3.83 ± 0.4^{ab}	4.5 ± 0.3^{a}	130.3 ± 11.5^{ab}	5824.2 ± 702.6^{a}
T1	50.33 ± 3.4^{ac}	5.19 ± 0.5^{ab}	3.04 ± 0.5^{b}	5.9 ± 0.3^{b}	96.8 ± 14.4^{c}	8295.7 ± 2748.4^{b}
T2	51.67 ± 2.6^{b}	5.06 ± 0.4^{a}	3.08 ± 0.3^{ac}	5.8 ± 0.2^{ab}	99.4 ± 8.2^{a}	5849.4 ± 527.4^{cd}
T3	49.33 ± 1.2^{a}	5.67 ± 0.3^{b}	2.65 ± 0.3^{c}	6.0 ± 0.1^{c}	88.4 ± 7.5^{d}	5479.2 ± 1370.4^{d}
T4	50.33 ± 2.0^{bc}	4.97 ± 0.3^{cd}	3.37 ± 0.3^{d}	5.9 ± 0.2^{d}	104.6 ± 9.5^{de}	8540.2 ± 1877.2^{cd}
T5	54.00 ± 3.7^{d}	4.54 ± 0.3^{c}	3.38 ± 0.2^{cd}	5.6 ± 0.3^{de}	107.0 ± 7.2^{cd}	$12{,}696.1 \pm 10{,}168.7^{de}$
T6	51.67 ± 4.1^{cd}	5.05 ± 0.4^{b}	3.21 ± 0.3^{e}	5.8 ± 0.4^{a}	102.5 ± 11.0^{b}	7873.6 ± 3189.1^{e}
T7	54.67 ± 2.4^{d}	5.00 ± 0.6^{a}	3.31 ± 0.3^{d}	5.5 ± 0.2^{b}	108.7 ± 9.2^{a}	6902 ± 3802^{d}
T8	51.00 ± 2.1^{e}	5.12 ± 0.3^{de}	3.14 ± 0.4^{de}	5.9 ± 0.2^{cd}	100.5 ± 11.4^{d}	7270.9 ± 1044.1^{b}

TGI = Timson germination index, CVG = coefficient of velocity of germination, GE = germination energy, MGT = mean germination time, GRI = germination rate index, and WUE = water use efficiency. Superscript letters (a–e) shows the significance in data, (T0) control: normal watering + 0 mg AsA and α-toc, (T1): 15 days drought + 0 mg AsA and α-toc, (T2): 15 days drought + 200 mg AsA, (T3): 15 days drought + 200 mg α-toc, (T4): 15 days drought + 200 mg AsA and α-toc, (T5): 25 days drought + 0 mg AsA and α-toc, (T6): 25 days drought + 200 mg AsA, (T7): 25 days drought + 200 mg α-toc, (T8): 25 days drought + 200 mg AsA and α-toc.

Table 3. Effect of AsA and/or α-toc on *A. sativa* RMC, MGR, T50%, SVI-I, and SVI-II under drought condition.

Treatments	RMC	MGR	T50%	SVI-I	SVI-II
T0	47.7 ± 5.08^{b}	0.22 ± 0.01^{a}	3.4 ± 0.46^{bc}	5516.6 ± 812.92^{ab}	3760.0 ± 371.57^{a}
T1	42.4 ± 8.33^{cd}	0.17 ± 0.02^{b}	3.9 ± 0.29^{c}	6110.0 ± 10.00^{b}	3100.0 ± 353.27^{ab}
T2	52.6 ± 8.73^{b}	0.17 ± 0.01^{bc}	3.9 ± 0.29^{bc}	5536.6 ± 609.29^{bc}	3793.3 ± 245.54^{c}
T3	51.8 ± 4.99^{c}	0.16 ± 0.00^{d}	4.2 ± 0.23^{c}	5713.3 ± 990.07^{d}	4130.0 ± 201.16^{bc}
T4	49.6 ± 9.46^{a}	0.17 ± 0.01^{c}	3.7 ± 0.29^{de}	5043.3 ± 1110.20^{de}	4196.6 ± 310.09^{cd}
T5	48.5 ± 10.88^{cd}	0.18 ± 0.01^{de}	3.7 ± 0.29^{d}	4243.3 ± 516.17^{e}	3603.3 ± 530.18^{b}
T6	30.6 ± 9.33^{d}	0.17 ± 0.00^{b}	3.9 ± 0.29^{ab}	4316.6 ± 343.85^{cde}	3603.3 ± 575.63^{c}
T7	36.9 ± 5.67^{dc}	0.18 ± 0.02^{ab}	3.9 ± 0.29^{a}	4680.0 ± 980.15^{b}	3346.6 ± 616.68^{de}
T8	38.8 ± 3.7^{c}	0.17 ± 0.01^{b}	3.9 ± 0.29^{bc}	4643.3 ± 914.40^{a}	3796.6 ± 244.99^{c}

RMC = root moisture content, MGR = mean germination rate, T50% = time to 50% germination, SVI-I = seed vigor index-I, and SVI-II = seed vigor index-II, Superscript letters (a–e) shows the significance in data, (T0) control: normal watering + 0 mg AsA and α-toc, (T1): 15 days drought + 0 mg AsA and α-toc, (T2): 15 days drought + 200 mg AsA, (T3): 15 days drought + 200 mg α-toc, (T4): 15 days drought + 200 mg AsA and α-toc, (T5): 25 days drought + 0 mg AsA and α-toc, (T6): 25 days drought + 200 mg AsA, (T7): 25 days drought + 200 mg α-toc, (T8): 25 days drought + 200 mg AsA and α-toc.

3.3. AsA and/or α-toc Mitigate the Effect of Drought Stress on Photosynthetic Pigments

Photosynthesis is the most vital mechanism that is disrupted by plants that grow in water-deficit environments. Impaired photosynthesis under drought is attributable to non-stomata components as well as stomata closure, which lowers the intercellular CO_2 concentration. After a 25-day drought, the leaf chlorophyll a content declined to 0.121 mg/L and chlorophyll b to 0.112 mg/L compared to the control (T0) (Figure 1). On the contrary, compared to the control, foliar applications of both AsA and α-toc increased the chlorophyll content in both 15 or 25-day drought stress situations (T1, T5). By reducing hydrogen peroxide production and raising phenolic levels, foliar applications were observed to modify the photosynthetic pigments in plants, enabling them to function more effectively under stressful conditions [36]. The predominant symptom of induced drought stress was the photosynthetic pigments deprivation of plant because of their sensitivity to it. In addition, another main contributing cue is the breakdown of the chloroplast thylakoid membrane, which could be stimulated by the deterioration of amino acids and photosystem 2 (PSII), which is connected to the chloroplast membrane. A comparative study revealed that α-toc improves all chlorophyll contents (T7) better than AsA. Drought significantly ($p \leq 0.005$) decreases carotenoid contents from 1.802 in the control (T0) to 1.028 mg/g in 25 days of drought stress (T5). The literature shows that the exogenous application of AsA and/or -toc has a vital role in the recovery of photosynthetic pigments i.e., Chl. a, b, or carotenoids under drought stress in wheat [37] or pepper [29].

3.4. Impact of AsA and/or α-toc on Soluble Protein, Sugar, Proline, and GB under Drought Stress

As the interval of drought stress was increased from 15 to 25 days, the soluble protein contents show a prominent reduction of 2.67 in the control to 0.777 in 25-day drought stress (Table 4, Figure 2). At the same time, all of the treatments experienced a comparatively high concentration of protein content that received the exogenous application of foliar sprays (AsA and/or α-toc) (Table 4). Soluble proteins play an important role in osmotic adjustment under drought stress and can provide a storage form of nitrogen [32]. However, the results of protein accumulation in plants under stressful circumstances vary. According to Parida et al., the protein content rises during drought [37], while Liu et al. revealed that it reduces in plants cultivated in water-stressed environments [38]. Soluble sugar contents were recorded as maximum (2.517 mg/g) under 15-day drought with the foliar application of α-toc (T3), while they were minimum (1.053 mg/g) at 25-day drought conditions (T5) (Figure 2). A considerable increase in proline content (2.597 mg/g) was detected in oat plants exposed to 25 days of continuous drought stress treated with AsA (T6), whereas the lowest was reported in 25-day drought plants (T5) (Figure 2). Many researchers have documented

proline accumulation during drought stress [39,40]. It provides nutrients and serves as an osmo-protectant by influencing membrane and protein structures. At the same time, under drought stress conditions, the foliar application of α-toc and/or AsA results in a tremendous decrease in its level. Water deficit conditions caused a considerable accumulation in GB content in both T1 and T5 treatments. The maximum content (0.793 mg/g) was observed in the T5 treatment whereas the minimum content was recorded in the T4 treatment (0.545 mg/g) (Figure 2). According to research, GB protects the photosynthetic process, inhibits the production of ROS, and activates stress-related genes. Additionally, it is well known that GB protects protein structures from the effects of abiotic stressors [41].

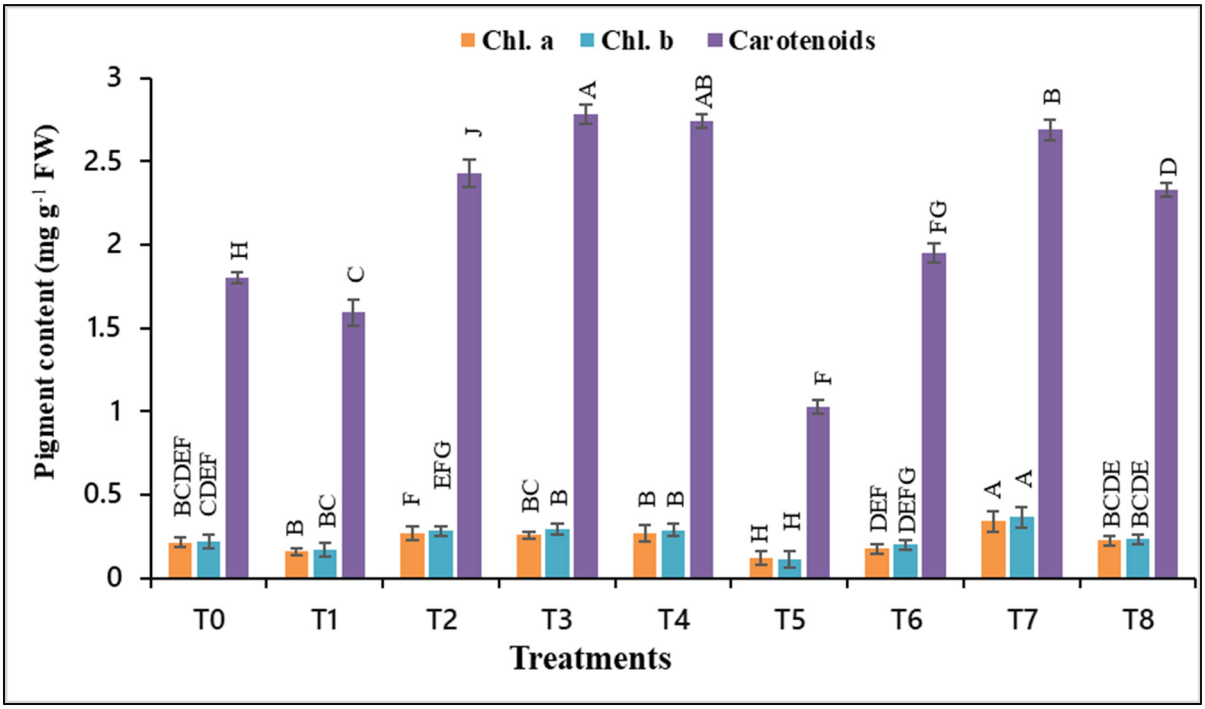

Figure 1. Effect of AsA and/or α-toc on chlorophyll (Chl. a, b) or carotenoid contents in fresh leaves of *A. sativa* under drought stress. Different alphabetical letters indicate significant differences among treatments at $p < 0.05$, according to the LSD test. (T0) control: normal watering + 0 mg AsA and α-toc, (T1): 15 days drought + 0 mg AsA and α-toc, (T2): 15 days drought + 200 mg AsA, (T3): 15 days drought + 200 mg α-toc, (T4): 15 days drought + 200 mg AsA and α-toc, (T5): 25 days drought + 0 mg AsA and α-toc, (T6): 25 days drought + 200 mg AsA, (T7): 25 days drought + 200 mg α-toc, (T8): 25 days drought + 200 mg AsA and α-toc.

3.5. H_2O_2 and MDA

The accumulation of H_2O_2 results from a reduction in soil water quantity or integration of CO_2. Antioxidants liquefy superoxide ions with H_2O_2, producing other enzymes in water particles. Malonaldehyde formation in plants rose as a result of the plant's loss of capacity to control ROS, whereas hydrogen peroxide induced membrane damage through the creation of hydroxyl radicals or lipid peroxidation. Figure 3 showed that water deficiency results in a substantial accumulation in the H_2O_2 level in the 15 and 25-day stress conditions. H_2O_2 has a maximum level of 2.27 mg/g observed in 25 days of drought and a minimum of 1.672 mg/g in 15-day drought sprayed with both AsA and α-toc. Thus, augmentation is related to AsA and/or α-toc's stress tolerance role. A substantial increase ($p \leq 0.005$) in MDA levels was detected in all plants under drought conditions (Figure 3). The MDA contents increased from 0.099 (T7) to 0.282 OD/mint/g in 25 days of drought. The upsurge in the MDA level under drought conditions in oat plants is comparable to maize [42] and wheat [43] reported previously.

Table 4. Summary of analysis of variance (ANOVA) based on the physiological characteristics of *A. sativa* grown under drought conditions.

Variables	Source of Variation	SS	DF	MS	F	*p*
Chl. a	Treatment	0.169	12	0.435	4.374	0.001 ***
	AsA + α-toc	0.125	5	0.767	3.685	0.002 **
	Treatment × AsA + α-toc	0.182	12	1.434	7.574	0.000 **
	Error	0.154	57	1.314	-	-
Chl. b	Treatment	0.672	12	0.985	14.335	0.002 ***
	AsA + α-toc	0.212	5	1.085	7.465	0.001 **
	Treatment × AsA + α-toc	0.169	12	1.442	10.905	0.000 ***
	Error	0.191	57	1.404	-	-
TCC	Treatment	0.483	12	2.338	3.575	0.005 ***
	AsA + α-toc	0.284	5	0.918	3.01	0.002 *
	Treatment × AsA + α-toc	0.218	12	1.095	3.575	0.002 **
	Error	0.769	57	0.318	-	-
SSC	Treatment	0.938	12	1.047	5.908	0.005 ***
	AsA + α-toc	0.882	5	0.545	3.344	0.001 **
	Treatment × AsA + α-toc	0.303	12	1.553	9.124	0.011 **
	Error	0.154	57	1.424	-	-
TPC	Treatment	0.548	12	2.193	13.214	0.002 ***
	AsA + α-toc	0.845	5	0.435	4.012	0.005 **
	Treatment × AsA + α-toc	0.992	12	1.432	9.125	0.000 ***
	Error	0.622	57	0.435	-	-
SPC	Treatment	0.769	12	0.871	9.453	0.005 ***
	AsA + α-toc	0.313	5	1.767	10.454	0.002 **
	Treatment × AsA + α-toc	0.422	12	1.435	3.901	0.000 ***
	Error	0.622	57	1.993	-	-
H_2O_2	Treatment	0.391	12	2.656	12.465	0.011
	AsA + α-toc	0.313	5	1.765	3.015	0.002 ***
	Treatment × AsA + α-toc	0.958	12	2.096	4.01	0.016 **
	Error	0.154	57	0.757	-	-
GB	Treatment	0.213	12	1.079	8.123	0.002 ***
	AsA + α-toc	0.311	5	0.371	4.901	0.011 **
	Treatment × AsA + α-toc	0.902	12	1	4.125	0.002 **
	Error	0.877	57	0.435	-	-
MDA	Treatment	0.655	12	0.427	10.224	0.002 **
	AsA + α-toc	0.32	5	0.394	6.015	0.018 ***
	Treatment × AsA + α-toc	0.146	12	1.435	4.224	0.001 **
	Error	0.664	57	1.885	-	-
APOX	Treatment	0.341	12	1.194	4.121	0.011 *
	AsA + α-toc	1.02	5	1.434	6.105	0.002 **
	Treatment × AsA + α-toc	0.904	12	1.076	2.325	0.016 ***
	Error	0.123	57	0.868	-	-
SOD	Treatment	0.324	12	0.536	4.232	0.005 **
	AsA + α-toc	0.283	5	0.217	4.105	0.002 **
	Treatment × AsA + α-toc	0.374	12	0.327	8.123	0.000 **
	Error	0.755	57	0.975	-	-
POD	Treatment	0.662	12	0.214	19.104	0.002 **
	AsA + α-toc	0.332	5	0.655	14.995	0.001 **
	Treatment × AsA + α-toc	0.182	12	0.291	9.125	0.014 ***
	Error	0.154	57	0.375	-	-
CAT	Treatment	0.623	12	1.075	5.885	0.005 ***
	AsA + α-toc	0.359	5	0.98	5.215	0.001 **
	Treatment × AsA + α-toc	0.973	12	2.085	4.225	0.002 **
	Error	0.335	57	1.965	-	-
GR	Treatment	0.893	12	1.114	22.434	0.002 **
	AsA + α-toc	0.372	5	0.538	14.034	0.005 **
	Treatment × AsA + α-toc	0.182	12	0.212	-	-
	Error	0.553	57	0.634	5.104	0.002 ***

* Significant, ** More significant, *** Most significant.

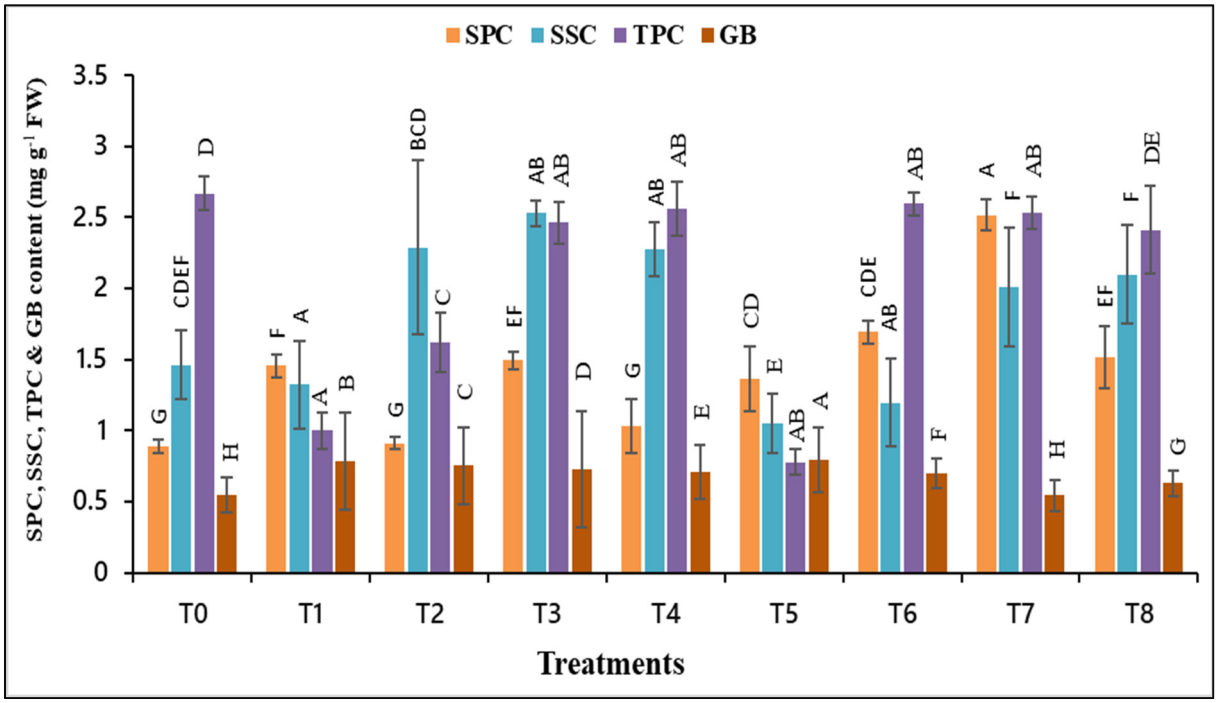

Figure 2. Effect of AsA and/or α-toc on soluble protein content (SPC), soluble sugar content (SSC), total proline content (TPC), and glycine betaine (GB) of *A. sativa* under drought stress. Different alphabetical letters indicate significant differences among treatments at $p < 0.05$, according to the LSD test. (T0) control: normal watering + 0 mg AsA and α-toc, (T1): 15 days drought + 0 mg AsA and α-toc, (T2): 15 days drought + 200 mg AsA, (T3): 15 days drought + 200 mg α-toc, (T4): 15 days drought + 200 mg AsA and α-toc, (T5): 25 days drought + 0 mg AsA and α-toc, (T6): 25 days drought + 200 mg AsA, (T7): 25 days drought + 200 mg α-toc, (T8): 25 days drought + 200 mg AsA and α-toc.

3.6. Antioxidant Enzyme Activities

All physiological traits quickly decreased under the water deficit, while the activity of protective antioxidant enzymes increased. GR also acts as a compatible molecule that aids in moderating the antagonistic result of water scarcity by regulating the cytosol water level or protecting biological cell membranes from ROS. A similar inclination was also detected for GR, which improved from 0.548 in the control (T0) to 0.793 OD/mint/g in 25-day drought stress (T5). The previous studies reported that the GB level amplified the drought tolerance in wheat [44] and cauliflower [45]. SOD is responsible for the dismutation of superoxide into H_2O_2 or is considered to be first line of defense against ROS. Moreover, the ability to adapt to drought conditions was linked to the maintenance of, or upsurges in, the ability to detoxify superoxide radicals by SOD, which play a key role in protecting plants from oxidative stress by increasing its activity. It is apparent that not only SOD but also H_2O_2 scavenging systems, as represented by CAT or APOX, are equally important in preventing oxidative stress induced by water stress in bitter gourd. Oat plants grown under water-deficit conditions showed pointedly high levels of APOX, POD, CAT, and SOD in plants with no exogenous application of AsA and/or α-toc (Figure 4).

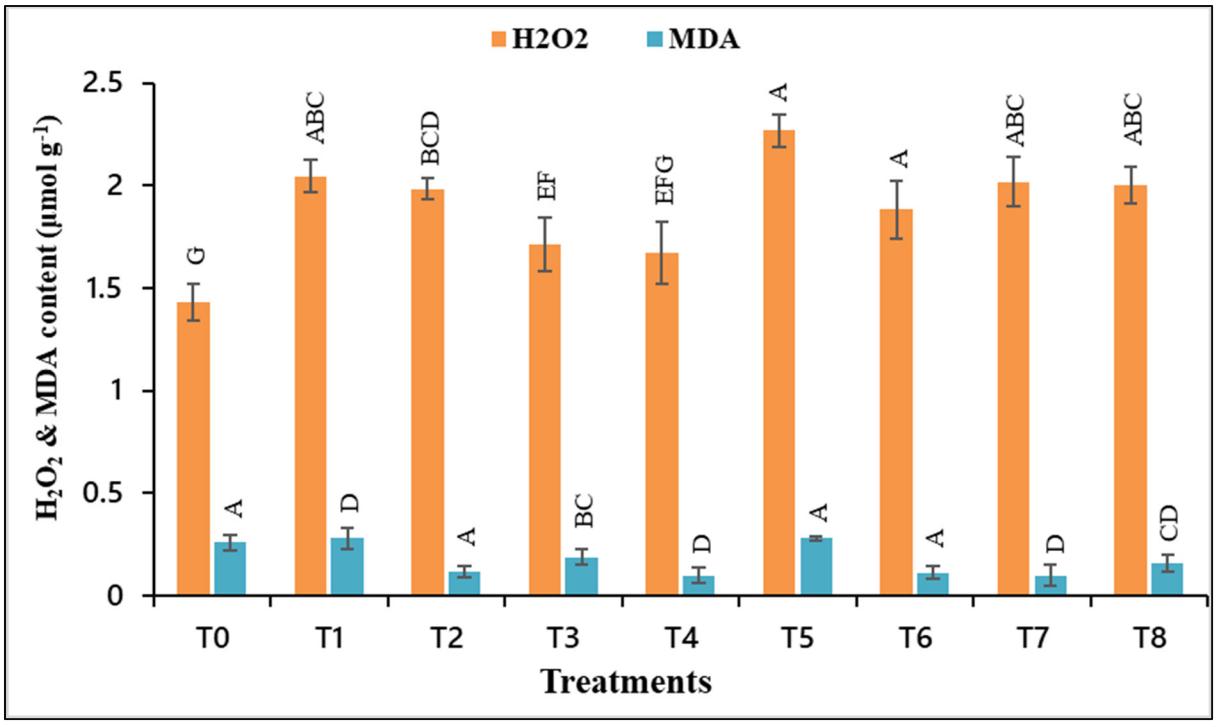

Figure 3. Effect of AsA and/or α-toc on hydrogen peroxide (H_2O_2) or malondialdehyde (MDA) of *A. sativa* under drought stress. Different alphabetical letters indicate significant differences among treatments at $p < 0.05$, according to the LSD test. (T0) control: normal watering + 0 mg AsA and α-toc, (T1): 15 days drought + 0 mg AsA and α-toc, (T2): 15 days drought + 200 mg AsA, (T3): 15 days drought + 200 mg α-toc, (T4): 15 days drought + 200 mg AsA and α-toc, (T5): 25 days drought + 0 mg AsA and α-toc, (T6): 25 days drought + 200 mg AsA, (T7): 25 days drought + 200 mg α-toc, (T8): 25 days drought + 200 mg AsA and α-toc.

The SOD was boosted from 0.193 in the control (T_0) to 0.253 OD/mint/g in 15 days of drought (T1) and finally to 0.275 in 25 days of drought (T5) (Figure 4). In tolerant plant species, antioxidant activities were found to be higher, enabling plants to protect themselves against oxidative stress [35], whereas such activities were not observed in sensitive plants [46–54]. Similarly, POD or APOX activities also increased significantly ($p \leq 0.05$) from 0.101 and 0.021 to 0.268 and 1.144 OD/mint/g in 25-day drought conditions (T5), respectively. The catalase activity was reduced in all plants subjected to foliar applications of AsA and α-toc and increased in plants subjected to drought stress with no foliar application (T1 and T5) (Figure 4). Figure 4 shows that, as the water deficit period extended to 15 and 25 days, the GR content increased dramatically. The exogenous administration of AsA and/or α-toc under induced drought stress significantly affected agronomic, physiological, and defense system activation.

3.7. Pearson's Correlation and Principal Component Analysis

With the exception of antioxidant activities, which were not interrelated with other parameters, the Pearson's correlation coefficient demonstrates that all physiological variables were positively associated with one another at $p \leq 0.05$ (Figure 5).

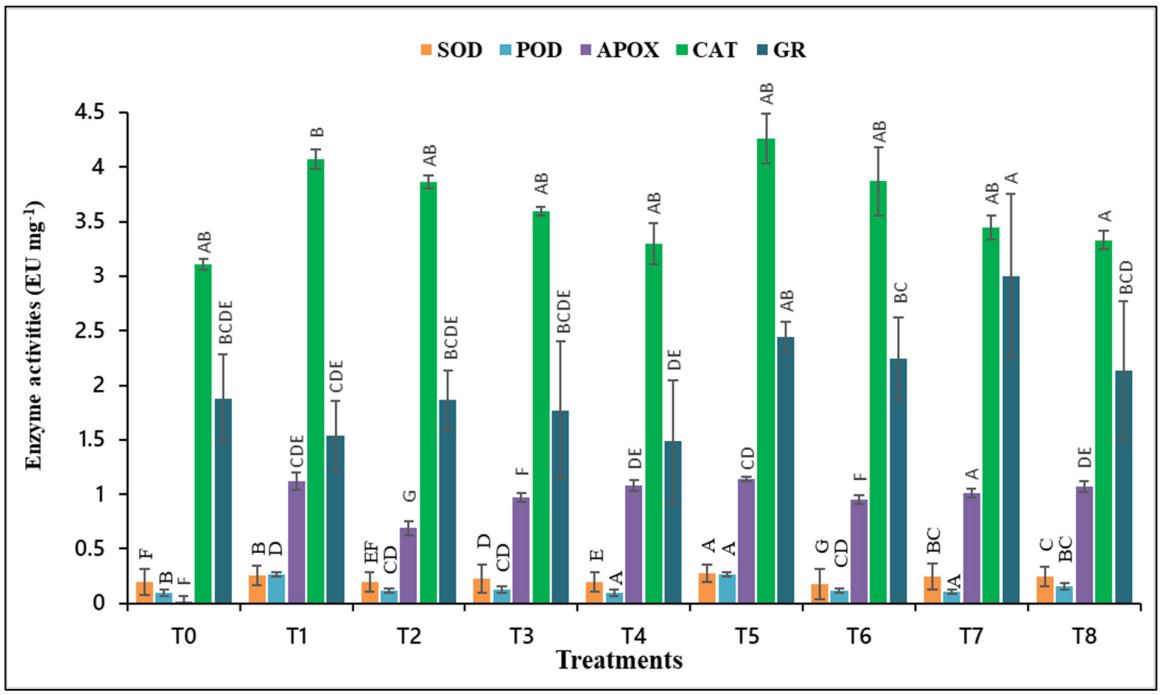

Figure 4. Effect of AsA and/or α-toc on superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APOX), catalase (CAT), or glutathione reductase (GR) of *A. sativa* under drought stress. Different alphabetical letters indicate significant differences among treatments at $p < 0.05$, according to the LSD test. (T0) control: normal watering + 0 mg AsA and α-toc, (T1): 15 days drought + 0 mg AsA and α-toc, (T2): 15 days drought + 200 mg AsA, (T3): 15 days drought + 200 mg α-toc, (T4): 15 days drought + 200 mg AsA and α-toc, (T5): 25 days drought + 0 mg AsA and α-toc, (T6): 25 days drought + 200 mg AsA, (T7): 25 days drought + 200 mg α-toc, (T8): 25 days drought + 200 mg AsA and α-toc.

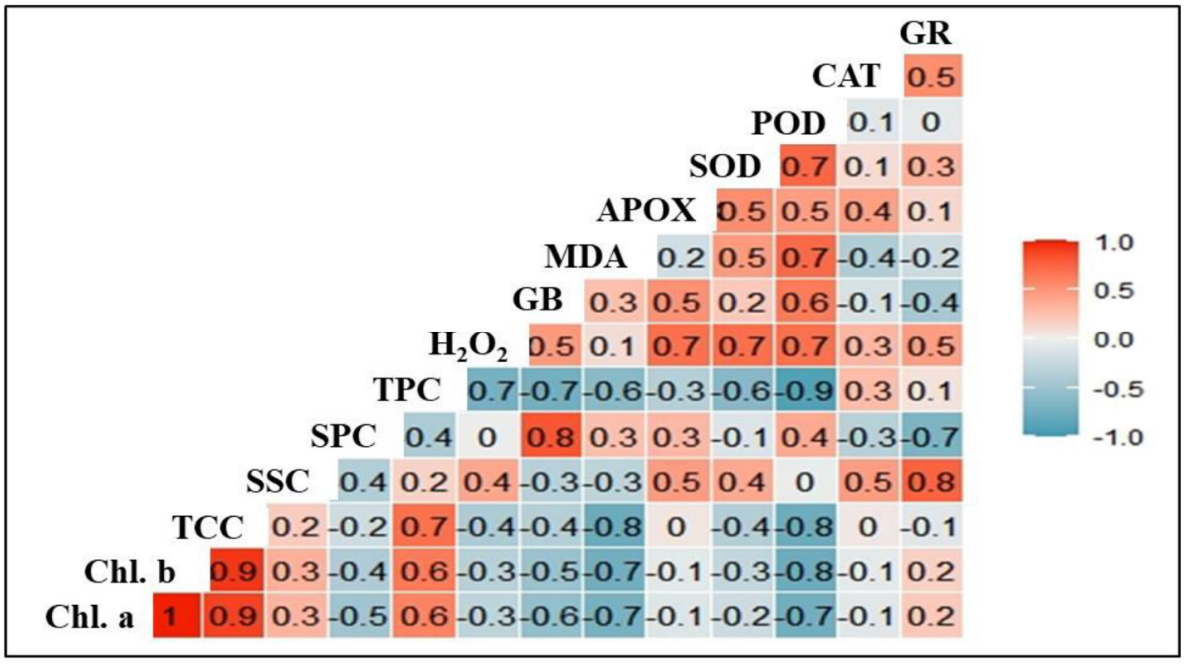

Figure 5. Correlation between various physio-biochemical activities of *A. sativa* under drought stress conditions.

This connection demonstrates how these characteristics are highly dependent on one another. The principal component analysis demonstrated the association of the physiological parameters under drought stress. Leaf pigments and proline contents were negatively correlated with most of the antioxidant enzymes, suggesting that oxidative stress reduced leaf physiological processes. The projections of the cases on a factor-plane with the two principal components (PC) were imaged as a consequence of the PCA, and the effectiveness of separation in the case of the major factors was tested (Figure 6). PC1 in the experiment demonstrated a distinct separation of AsA and/or α-toc treatments, accounting for 44.3% of the total variance. The treatments and controls were apart, with minimal overlapping.

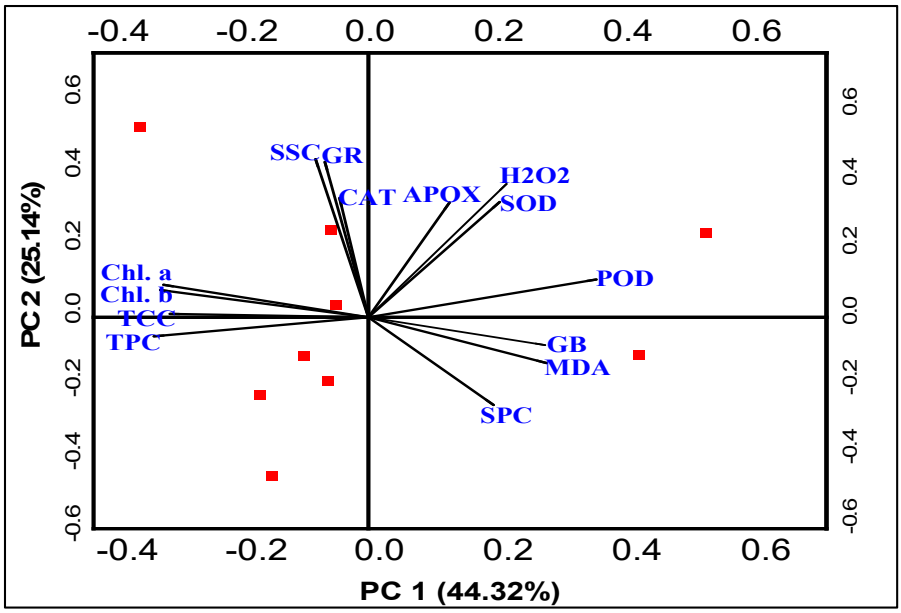

Figure 6. Principal component analysis of different physio-biochemical characteristics of *A. sativa* subjected to foliar sprays of AsA and/or α-toc under water deficit condition. Red squares indicate the treatments from T0 to T8.

4. Conclusions

Climatic change typified by global warming and drought is the primary impediment to the productivity of many cereal crops, including *A. sativa* L. Researchers attempted various traditional approaches and endogenous and exogenous applications to reduce these implications. The current study shows that AsA and/or α-tocopherol can protect the physiological functioning of oat crops from drought injury, with AsA being relatively more effective. However, further studies are required to determine the best method of AsA application. Furthermore, the present study carefully examined the physicochemical parameters of water-constrained agricultural soil since there was a lack of research on the effects of drought on soil properties.

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References

1. Ramzan, S. Oat: A novel therapeutic ingredient for food applications. *J. Microbiol. Biotechnol. Food Sci.* **2021**, *2021*, 756–760. [CrossRef]
2. Ahmad, M.; Jehangir, I.; Rizvan, R.; Dar, S.A.; Iqbal, S.; Wani, S.; Mehraj, U.; Hassan, R. Phylogenetic Relationship of Oats (*Avena sativa* L): A Guide to Conservation and Utilisation of Genetic Resources. *Int. J. Curr. Microbiol. App. Sci.* **2020**, *9*, 831–845. [CrossRef]
3. Mehraj, U.; Abidi, I.; Ahmad, M.; Wani, B.; Mir, S. Genotype x environment interaction for forage yield and its components in oats (*Avena sativa* L.). *Electron. J. Plant Breed.* **2017**, *8*, 157–162. [CrossRef]
4. Shoaib, M.; Khan, M.N.; Akhtar, N.; Ayub, M.; Ashraf, M.S.; Ghaffar, A.; Ullah, S. Productivity of oat (*Avena sativa* L.)-berseem (*Trifolium alexandrinum* L.) forage mixture in irrigated plain of Pakistan. *Pak. J. Agric. Sci.* **2018**, *55*, 303–312.
5. Teklić, T.; Paradiković, N.; Špoljarević, M.; Zeljković, S.; Lončarić, Z.; Lisjak, M. Linking abiotic stress, plant metabolites, biostimulants and functional food. *Ann. Appl. Biol.* **2021**, *178*, 169–191. [CrossRef]
6. Kapoor, D.; Bhardwaj, S.; Landi, M.; Sharma, A.; Ramakrishnan, M.; Sharma, A. The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. *Appl. Sci.* **2020**, *10*, 5692. [CrossRef]
7. Hossain, M.S.; Li, J.; Sikdar, A.; Hasanuzzaman, M.; Uzizerimana, F.; Muhammad, I.; Yuan, Y.; Zhang, C.; Wang, C.; Feng, B. Exogenous melatonin modulates the physiological and biochemical mechanisms of drought tolerance in tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn). *Molecules* **2020**, *25*, 2828. [CrossRef] [PubMed]
8. Malik, A.; Mor, V.S.; Tokas, J.; Punia, H.; Malik, S.; Malik, K.; Sangwan, S.; Tomar, S.; Singh, P.; Singh, N. Biostimulant-treated seedlings under sustainable agriculture: A global perspective facing climate change. *Agronomy* **2020**, *11*, 14. [CrossRef]
9. Verma, G.; Srivastava, D.; Tiwari, P.; Chakrabarty, D. ROS modulation in crop plants under drought stress. In *Reactive Oxygen, Nitrogen and Sulfur Species in Plants: Production, Metabolism, Signaling and Defense Mechanisms*; Wiley: Hoboken, NJ, USA, 2019; pp. 311–336.
10. Sadiq, M.; Akram, N.A.; Ashraf, M. Impact of exogenously applied tocopherol on some key physio-biochemical and yield attributes in mungbean [*Vigna radiata* (L.) Wilczek] under limited irrigation regimes. *Acta Physiol. Plant.* **2018**, *40*, 131. [CrossRef]
11. Ali, Q.; Tariq Javed, M.; Haider, M.Z.; Habib, N.; Rizwan, M.; Perveen, R.; Ali, S.; Nasser Alyemeni, M.; El-Serehy, H.A.; Al-Misned, F.A. α-Tocopherol foliar spray and translocation mediates growth, photosynthetic pigments, nutrient uptake, and oxidative defense in maize (*Zea mays* L.) under drought stress. *Agronomy* **2020**, *10*, 1235. [CrossRef]
12. Desoky, E.-S.M.; Elrys, A.S.; Mansour, E.; Eid, R.S.; Selem, E.; Rady, M.M.; Ali, E.F.; Mersal, G.A.; Semida, W.M. Application of biostimulants promotes growth and productivity by fortifying the antioxidant machinery and suppressing oxidative stress in faba bean under various abiotic stresses. *Sci. Hortic.* **2021**, *288*, 110340. [CrossRef]
13. Shah, S.M.; Sadaf; Amin, M.; Gul, B.; Begum, M. Ethnoecological, Elemental, and Phytochemical Evaluation of Five Plant Species of Lamiaceae in Peshawar, Pakistan. *Scientifica* **2020**, *2020*, 2982934. [CrossRef]
14. Nafees, M.; Ullah, S.; Ahmed, I. Morphological and elemental evaluation of biochar through analytical techniques and its combined effect along with plant growth promoting rhizobacteria on *Vicia faba* L. under induced drought stress. *Microsc. Res. Tech.* **2021**, *84*, 2947–2959. [CrossRef] [PubMed]
15. Shah, S.; Ullah, S.; Ali, S.; Khan, A.; Ali, M.; Hassan, S. Using mathematical models to evaluate germination rate and seedlings length of chickpea seed (*Cicer arietinum* L.) to osmotic stress at cardinal temperatures. *PLoS ONE* **2021**, *16*, e0260990. [CrossRef] [PubMed]
16. Sonobe, R.; Yamashita, H.; Mihara, H.; Morita, A.; Ikka, T. Estimation of leaf chlorophyll a, b and carotenoid contents and their ratios using hyperspectral reflectance. *Remote Sens.* **2020**, *12*, 3265. [CrossRef]
17. Ahmad, A.; Anis, M. Meta-topolin improves in vitro morphogenesis, rhizogenesis and biochemical analysis in Pterocarpus marsupium Roxb.: A potential drug-yielding tree. *J. Plant Growth Regul.* **2019**, *38*, 1007–1016. [CrossRef]
18. Buysse, J.; Merckx, R. An improved colorimetric method to quantify sugar content of plant tissue. *J. Exp. Bot.* **1993**, *44*, 1627–1629. [CrossRef]
19. Hitz, W.D.; Hanson, A.D. Determination of glycine betaine by pyrolysis-gas chromatography in cereals and grasses. In *Genetic Engineering of Osmoregulation*; Springer: Cham, Switzerland, 1980; p. 362.
20. Bates, L.S.; Waldren, R.P.; Teare, I. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
21. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]

22. Velikova, V.; Loreto, F.; Tsonev, T.; Brilli, F.; Edreva, A. Isoprene prevents the negative consequences of high temperature stress in Platanus orientalis leaves. *Funct. Plant Biol.* **2006**, *33*, 931–940. [CrossRef] [PubMed]
23. Shalaby, T.A.; Taha, N.A.; Rakha, M.T.; El-Beltagi, H.S.; Shehata, W.F.; Ramadan, K.M.A.; El-Ramady, H.; Bayoumi, Y.A. CanGrafting Manage Fusarium Wilt Disease of Cucumber and Increase Productivity under Heat Stress? *Plants* **2022**, *11*, 1147. [CrossRef]
24. El-Beltagi, H.S.; Shah, S.; Ullah, S.; Sulaiman; Mansour, A.T.; Shalaby, T.A. Impacts of ascorbic acid and alpha-tocopherol on Chickpea (*Cicer arietinum* L.) grown in water deficit regimes for sustainable production. *Sustainability* **2022**, *14*, 8861. [CrossRef]
25. Piechowiak, T.; Grzelak-Błaszczyk, K.; Sójka, M.; Balawejder, M. Changes in phenolic compounds profile and glutathione status in raspberry fruit during storage in ozone-enriched atmosphere. *Postharvest Biol. Technol.* **2020**, *168*, 111277. [CrossRef]
26. Shalaby, T.A.; El-Newiry, N.A.; El-Tarawy, M.; El-Mahrouk, M.E.; Shala, A.Y.; El-Beltagi, H.S.; Rezk, A.A.; Ramadan, K.M.A.; Shehata, W.F.; El-Ramady, H. Biochemical and physiological response of Marigold (*Tagetes Erecta* L.) to foliar application ofsalicylic acid and potassium humate in different soil growth media. *Gesunde Pflanz.* **2022**, *11*, 14. [CrossRef]
27. El-Beltagi, H.S.; Mohamed, H.I.; Aldaej, M.I.; Al-Khayri, J.M.; Rezk, A.A.; Al-Mssallem, M.Q.; Sattar, M.N.; Ramadan, K.M.A. Production and antioxidant activity of secondary metabolites in Hassawi rice (*Oryza sativa* L.) cell suspension under salicylic acid, yeast extract, and pectin elicitation. *Vitr. Cell Dev. Biol. Plant* **2022**, *58*, 615–629. [CrossRef]
28. Rojas, E.R.; Huang, K.C. Regulation of microbial growth by turgor pressure. *Curr. Opin. Microbiol.* **2018**, *42*, 62–70. [CrossRef] [PubMed]
29. Mohamed, H.I.; Akladious, S.A.; El-Beltagi, H.S. Mitigation the harmful effect of salt stress on physiological, biochemical andanatomical traits by foliar spray with trehalose on wheat cultivars. *Fresenius Env. Bull.* **2018**, *27*, 7054–7065.
30. Sharma, A.; Kumar, V.; Shahzad, B.; Ramakrishnan, M.; Singh Sidhu, G.P.; Bali, A.S.; Handa, N.; Kapoor, D.; Yadav, P.; Khanna, K. Photosynthetic response of plants under different abiotic stresses: A review. *J. Plant Growth Regul.* **2020**, *39*, 509–531. [CrossRef]
31. Hameed, A.; Akram, N.A.; Saleem, M.H.; Ashraf, M.; Ahmed, S.; Ali, S.; Abdullah Alsahli, A.; Alyemeni, M.N. Seed treatment with α-tocopherol regulates growth and key physio-biochemical attributes in carrot (*Daucus carota* L.) plants under water limited regimes. *Agronomy* **2021**, *11*, 469. [CrossRef]
32. Ali, A.; Beshir Issa, A.; Rahut, D.B. Adoption and impact of the maize hybrid on the livelihood of the maize growers: Some policy insights from Pakistan. *Scientifica* **2020**, *2020*, 5959868. [CrossRef]
33. Razi, K.; Muneer, S. Drought stress-induced physiological mechanisms, signaling pathways and molecular response of chloroplasts in common vegetable crops. *Crit. Rev. Biotechnol.* **2021**, *41*, 669–691. [CrossRef] [PubMed]
34. Kosar, F.; Akram, N.A.; Ashraf, M.; Ahmad, A.; Alyemeni, M.N.; Ahmad, P. Impact of exogenously applied trehalose on leaf biochemistry, achene yield and oil composition of sunflower under drought stress. *Physiol. Plant.* **2021**, *172*, 317–333. [CrossRef] [PubMed]
35. Ali, Q.; Ali, S.; Iqbal, N.; Javed, M.T.; Rizwan, M.; Khaliq, R.; Shahid, S.; Perveen, R.; Alamri, S.A.; Alyemeni, M.N. Alpha-tocopherol fertigation confers growth physio-biochemical and qualitative yield enhancement in field grown water deficit wheat (*Triticum aestivum* L.). *Sci. Rep.* **2019**, *9*, 12924. [CrossRef] [PubMed]
36. Farooq, A.; Bukhari, S.A.; Akram, N.A.; Ashraf, M.; Wijaya, L.; Alyemeni, M.N.; Ahmad, P. Exogenously applied ascorbic acid-mediated changes in osmoprotection and oxidative defense system enhanced water stress tolerance in different cultivars of safflower (*Carthamus tinctorious* L.). *Plants* **2020**, *9*, 104. [CrossRef] [PubMed]
37. Parida, A.K.; Dagaonkar, V.S.; Phalak, M.S.; Umalkar, G.; Aurangabadkar, L.P. Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. *Plant Biotechnol. Rep.* **2007**, *1*, 37–48. [CrossRef]
38. Liu, Y.; Wu, Q.; Ge, G.; Han, G.; Jia, Y. Influence of drought stress on afalfa yields and nutritional composition. *BMC Plant Biol.* **2018**, *18*, 1–9. [CrossRef] [PubMed]
39. Ozturk, M.; Turkyilmaz Unal, B.; García-Caparrós, P.; Khursheed, A.; Gul, A.; Hasanuzzaman, M. Osmoregulation and its actions during the drought stress in plants. *Physiol. Plant.* **2021**, *172*, 1321–1335. [CrossRef] [PubMed]
40. Maghsoudi, K.; Emam, Y.; Niazi, A.; Pessarakli, M.; Arvin, M.J. P5CS expression level and proline accumulation in the sensitive and tolerant wheat cultivars under control and drought stress conditions in the presence/absence of silicon and salicylic acid. *J. Plant Interact.* **2018**, *13*, 461–471. [CrossRef]
41. Hassan, A.; Amjad, S.F.; Saleem, M.H.; Yasmin, H.; Imran, M.; Riaz, M.; Ali, Q.; Joyia, F.A.; Ahmed, S.; Ali, S. Foliar application of ascorbic acid enhances salinity stress tolerance in barley (*Hordeum vulgare* L.) through modulation of morpho-physio-biochemical attributes, ions uptake, osmo-protectants and stress response genes expression. *Saudi J. Biol. Sci.* **2021**, *28*, 4276–4290. [CrossRef] [PubMed]
42. Moussa, H.R.; Abdel-Aziz, S.M. Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Aust. J. Crop Sci.* **2008**, *1*, 31–36.
43. Shalaby, T.A.; Taha, N.A.; Taher, D.I.; Metwaly, M.M.; El-Beltagi, H.S.; Rezk, A.A.; El-Ganainy, S.M.; Shehata, W.F.; El-Ramady, H.R.; Bayoumi, Y.A. Paclobutrazol improves the quality of tomato seedlings to be resistant to Alternaria solani blight disease:Biochemical and histological perspectives. *Plants* **2022**, *11*, 425. [CrossRef] [PubMed]
44. El-Beltagi, H.S.; Ahmad, I.; Basit, A.; Abd El-Lateef, H.M.; Yasir, M.; Shah, S.T.; Ullah, I.; Mohamed, M.E.M.; Ali, I.; Ali, F. Effect of azospirillum and azotobacter species on the performance of cherry tomato under different salinity levels. *Gesunde Pflanz.* **2022**, *74*, 487–499. [CrossRef]

45. El-Beltagi, H.S.; Ahmad, I.; Basit, A.; Shehata, W.F.; Hassan, U.; Shah, S.T.; Haleema, B.; Jalal, A.; Amin, R.; Khalid, M.A.; et al. Ascorbic acid enhances growth and yield of sweet peppers (*Capsicum annum*) by mitigating salinity stress. *Gesunde Pflanz.* **2022**, *74*, 423–433. [CrossRef]
46. El-Beltagi, H.S.; Mohamed, H.I.; Sofy, M.R. Role of ascorbic acid, glutathione and proline applied as singly or in sequencecombination in improving chickpea plant through physiological change and antioxidant defense under different levels ofirrigation intervals. *Molecules* **2020**, *25*, 1702. [CrossRef] [PubMed]
47. Kobeasy, M.I.; El-Beltagi, H.S.; El-Shazly, M.A.; Khattab, E.A.H. Induction of resistance in *Arachis hypogaea* L. Against Peanut mottle virus by nitric oxide and salicylic acid. *Physiol. Mol. Plant Pathol.* **2011**, *76*, 112–118. [CrossRef]
48. El-Beltagi, H.S.; Ismail, S.A.; Ibrahim, N.M.; Shehata, W.F.; Alkhateeb, A.A.; Ghazzawy, H.S.; El-Mogy, M.M.; Sayed, E.G. Unravelling the effect of triacontanol in combating drought stress by improving growth, productivity, and physiological performance in Strawberry plants. *Plants* **2022**, *11*, 1913. [CrossRef] [PubMed]
49. El-Beltagi, H.S.; Ali, M.R.; Ramadan, K.M.A.; Anwar, R.; Shalaby, T.A.; Rezk, A.A.; El-Ganainy, S.M.; Mahmoud, S.F.; Alkafafy, M.; El-Mogy, M.M. Exogenous postharvest application of calcium chloride and salicylic acid to maintain the quality of broccoli florets. *Plants* **2022**, *11*, 1513. [CrossRef]
50. Shah, S.; Khan, S.; Bussmann, R.W.; Ali, M.; Hussain, D.; Hussain, W. Quantitative ethnobotanical study of Indigenous knowledge on medicinal plants used by the tribal communities of Gokand Valley, District Buner, Khyber Pakhtunkhwa, Pakistan. *Plants* **2020**, *9*, 1001.
51. Mohamed, A.A.; El-Beltagi, H.S.; Rashed, M.M. Cadmium stress induced change in some hydrolytic enzymes, free radical formation and ultrastructural disorders in radish plant. *Electron. J. Environ. Agric. Food Chem.* **2009**, *8*, 969–983.
52. El-Beltagi, H.S.; Maraei, R.W.; Shalaby, T.A.; Aly, A.A. Metabolites, nutritional quality and antioxidant activity of red radish roots affected by gamma rays. *Agronomy* **2022**, *12*, 1916. [CrossRef]
53. Afify, A.M.M.R.; El-Beltagi, H.S.; Aly, A.A.; El-Ansary, A.E. Antioxidant enzyme activities and lipid peroxidation as biomarker for potato tuber stored by two essential oils from Caraway and Clove and its main component carvone and eugenol. *Asian Pac. J. Trop. Biomed.* **2012**, *2*, S772–S780. [CrossRef]
54. Ramadan, K.M.A.; Alharbi, M.M.; Alenzi, A.M.; El-Beltagi, H.S.; Darwish, D.B.; Aldaej, M.I.; Shalaby, T.A.; Mansour, T.A.; El-Gabry, Y.A.E.-G.; Ibrahim, M.F.M. Alpha Lipoic Acid as a Protective Mediator for Regulating the Defensive Responses of Wheat Plants against Sodic Alkaline Stress: Physiological, Biochemical and Molecular Aspects. *Plants* **2022**, *11*, 787. [CrossRef] [PubMed]