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Impacts of Ascorbic Acid and Alpha-Tocopherol on Chickpea (*Cicer arietinum* L.) Grown in Water Deficit Regimes for Sustainable Production

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Abstract: Drought is a major abiotic stress forced by the changing climate that affects plant production and soil structure and functions. A study was conducted to explore the impacts of ascorbic acid (AsA) and α-tocopherol (α-toc) on the agro-physiological attributes and antioxidant enzymes of chickpea grown in water deficit regions. The results of the soil analysis showed that the electrical conductivity (EC) and pH were decreased from 521 mS/m and 7.08 to 151 mS/m and 6.6 in 20-day drought regimes, respectively. Agronomic outcomes showed that exogenous application of AsA and α-toc increased the germination rate index (GRI), mean germination time (MGT), germination energy (GE), water use efficiency (WUE), germination percentage (GP), and seed vigor index (SVI). However, all the above attributes experienced a decline under 10- and 20-day drought stress. Similarly, the Chl. a, Chl. b, carotenoids, proline, protein, sugar, glycine betaine, and hydrogen peroxide contents were significantly increased. Meanwhile, malondialdehyde, glutathione reductase, and enzymatic antioxidants (APOX, SOD, and POD) increased during 10- and 20-day drought, except CAT, which decreased during drought. The exogenous fertigation of these growth regulators improved the photosynthetic pigments and enzymatic and non-enzymatic antioxidants in stressed plants. The current research concludes that simultaneous dusting of AsA and α-toc could be an efficient technique to mitigate the antagonistic impacts of drought, which might be linked to the regulation of antioxidant defense systems.

Keywords: agronomic characteristics; alpha-tocopherol; ascorbic acid; drought tolerance; secondary metabolites

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1. Introduction

Chickpea (*Cicer arietinum* L.; Leguminosae) is an essential and protein-rich leguminous crop with a cosmopolitan distribution, constituting part of the staple diet of more than five billion people [1]. Chickpeas contribute considerably to global food security by providing amino acids, calories, unsaturated fats, and vitamins to millions of people throughout the world. In the production of legumes in agricultural systems, it ranks third after beans and peas, which is a clear indication of the bulk quantity of chickpeas that is consumed [2].

After decades of substantial production, chickpea has experienced a steady decline due to the adverse effects of climate change in developing countries. These effects include prolonged drought, flooding, fluctuation in seasonal temperatures, rains, and moisture [3]. Adverse changes in the climate make it difficult to achieve the increasing demand for pulses in the world [3]. Among them, the water deficit problem is the most common adverse limiting factor affecting chickpea vegetative phase, pollen viability, pod filling, and yield [4]. Drought stress at the vegetative stage and terminal drought reduce yield by around 50%. In most countries, chickpea is regularly cultivated for rotation purposes in pulse farming systems that have some residual soil moisture. This frequently results in moisture stress at the end of the cropping season, along with subsequent drought stress episodes [4]. As a result, during the vegetative stage, the crop is exposed to stress, resulting in yield loss. Around 52% of the earth's scorched and semi-scorched regimes are vulnerable to short or prolonged drought stress conditions [5].

Water acts as a precursor for several primary and secondary metabolites responsible for growth and yield; therefore, its deficiency will affect all agronomic, physiological, and biochemical attributes [1]. Chickpea crops grown in water-limited regimes experience a nutritional imbalance, ion exchange, impairment in cell division, and changes in primary and secondary metabolism [6]. Plants' cellular membranes are vulnerable to the scarcity of water owing to their complex oxidative stress, which can easily break the biomolecules and lipids embedded in these membranes. In plants, the adverse consequences of drought stress are mitigated by several molecular and physiological practices, including maintenance of cell turgidity, water use efficiency, development of the deep rooting system, inhibition of transpiration, biosynthesis of osmolytes, and stomatal and osmoregulation. The enzymatic and non-enzymatic system includes defensive antioxidants such as ascorbate peroxidase (APOX), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), tocopherols, and ascorbic acid (AsA), which are effective in countering the drought-induced overaccumulation of reactive oxygen species (ROS) [5,7]. However, this response is different from crop to crop and mainly depends on the external environment and genetic makeup of the plant [6]. To alleviate the injurious effects of a drought environment, leaf foliar approaches such as the exogenous application of growth-stimulating substances have been efficiently applied by various researchers due to their cost-effective and efficient nature [7,8].

The literature revealed that several exogenous non-enzymatic compounds are used to alleviate the impacts of drought and recover crop agronomy and physiology [8]. AsA and α-toc are believed to be the primary growth buffers which guard plant tissues against scavenging free oxygen radicals [6]. Ascorbic acid acts as a helper for catalysts engaged in mitigating the biogenesis of phytohormones, regenerating enzymatic antioxidants, and regulating anabolism and catabolism [8]. Alpha-tocopherol also has a strong antioxidant function as an inhibitor of lipid peroxidation and is useful in maintaining the integrity of cellular membranes against oxidative stress. It is mostly present in actively dividing plant cells and all cellular compartments, including cell walls [9]. The growth and development of pulses having minimal AsA and α-toc content were expressly affected by abiotic stressors, especially drought. In a plant's cellular processes and defense mechanisms, it acts as a strong metabolite and signaling modulator by detoxifying hydrogen peroxide during water shortages [10]. Foliar application of these growth regulators has shown efficient results in boosting cell division and the development of crops through maintaining various physiological processes such as ionic transport, cell expansion, and phytohormone signaling and the defense system during stress conditions [11]. The literature revealed the mitigating impacts of AsA and α-toc foliar spraying on the morphology, physiology, and biochemistry of various crops, such as in [12–15].

The current research is an attempt to (i) assess the agronomic, molecular, and physio-biochemical responses of the chickpea variety NIFA-1995 to different levels of exogenously sprayed AsA and α-toc grown in water-limited regimes; (ii) perform comparative identification of the strongest and most effective drought stress regulator for the economically

important chickpea; and (iii) test the hypothesis that chickpea crops which receive exogenously applied AsA and α-toc simultaneously might perform better under drought stress.

2. Materials and Methods

2.1. Site Description and Experiment Layout

A complete randomized block design layout with three pot replicates was accomplished in the net house of the Department of Botany, University of Peshawar, Khyber Pakhtunkhwa, Pakistan, in the 2021 growing season. The experiment site had a sub-humid climate, about 450 m above sea level, with extreme weather conditions (hot summer: 40.8 °C; mild winter: 18.35 °C) [16]. To investigate agronomic performance and physiological aspects under vegetative stage drought stress, chickpea seed var. NIFA 1995 was taken from the Nuclear Institute of Food and Agriculture (NIFA). Seeds were sowed in earthen pots of 20 cm length, 2 cm thickness, and 18 cm upper-lower diameter filled with 3 kg soil and sand (2:1) after being surface-sterilized with 95% ethanol. After seedling emergence, the plants were subjected to exogenous growth mediators at a concentration of 150 mg/L with continuous 10- and 20-day vegetative stage drought. Three pots were kept controlled and normally irrigated after 5-day intervals. Plants were uprooted from some pots after ten days of drought and the rest were uprooted after a continuous 20-day drought period. Data of germination and agronomic parameters of vegetative growth were recorded. The leftover plants were kept at −4 °C in the freezer to assess physio-biochemical and enzymatic characteristics.

2.2. Soil Assessment

The pot soil before and after the research was examined for different soil attributes, including temperature (T), electrical conductivity (EC), pH, total dissolved solutes (TDS), dissolved oxygen (DO), oxidation reduction potential (ORP), resistivity (R), and salinity (NaCl). A 1:5 soil:water suspension was arranged by weighing 10 g air-dry soil in a disposable glass containing 50 mL distilled water. The Multiparameter Bluetooth portable Water Quality Meter HI98494 was used to calculate the soil properties.

2.3. Germination and Agronomic Characteristics

The mentioned germination and agronomic indices were analyzed, including germination rate index (GRI), mean germination time (MGT), germination energy (GE), and Timson germination index (TGI), via the methods of Nafees et al. [17]. Water use efficiency (WUE), germination percentage (GP), seed vigor indices (SVIs), root moisture content (RMC), and time to 50% germination (T50%) were determined through the protocol of Shah et al. [18] using the following formulas:

$$\mathrm{MGT} = \frac{\in fx}{\in f} \tag{1}$$

where f is the frequency of seeds emerged on day X.

$$\mathrm{GRI} = \frac{G1}{1} + \frac{G2}{2} + \frac{G3}{3} \ldots \frac{Gx}{x} \tag{2}$$

where $G1$ and $G2$ are the emergence rates on the first and second days after propagating, respectively, and Gx is the final emergence rate on the final day.

$$\mathrm{GE} = \frac{X1}{Y1} + \left(\frac{X2 - X1}{Y2}\right) + \left(\frac{Xn - Xn - 1}{Yn}\right) \tag{3}$$

where X_1, X_2, and X_n are the number of seeds germinated on days 1, 2, etc., and Y_1, Y_2, and Y_n are the time from plating to days 1, 2, etc., up to day 10.

$$\mathrm{TGI} = \frac{\in \mathrm{G}}{\mathrm{T}} \tag{4}$$

where G is the grand percentage of emergence on each day and T is the day of emergence.

$$\text{WUE} = \frac{\text{Total Water during Experiment (ml)}}{\text{Total Biomass (g)}} \quad (5)$$

$$\text{GP} = \frac{\text{Number of seedlings emerged}}{\text{Total number of seeds sown}} \times 100 \quad (6)$$

$$\text{SVI-I} = \text{Seedlings length} \times \text{Germination \%age} \quad (7)$$

$$\text{SVI-II} = \text{Seedling dry weight (mg)} \times \text{Germintion \%age} \quad (8)$$

$$\text{RMC} = \frac{\text{Wet weight of root} - \text{Dry weight of root}}{\text{Wet weight of root}} \quad (9)$$

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

where N is the final frequency of seeds that emerged and nj and ni are the aggregate frequency of seeds that germinated after contiguous counts during tj and ti, respectively, when ni < N/2 > Nj.

2.4. Physiological and Biochemical Attributes

2.4.1. Leaf Photosynthetic Pigment

The various types of chlorophyll (Chl. a and Chl. b) were assessed by the method of Sonobe et al. [19]. The carotenoid (CAR) contents were assessed by following the protocol of Ahmad et al. [20]. The quantities were computed using the following equations:

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

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

$$\text{Carotenoid} = \text{DA480} + (0.114 \times \text{DA663}) - (0.638 \times \text{DA645}) \quad (13)$$

where DA is the optical density at a mentioned wavelength, V is the level of the extract (mL), and W is the fresh leaf weight.

2.4.2. Total Proline Content (TPC) and Soluble Protein Content (SPC)

The total proline content (TPC) of leaves was found through the method of Brugière et al. [21]. Meanwhile, the protocol of Zhang et al. [22] was used for the quantification of soluble protein. Both contents were computed using Equation (14).

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

where Cp is the protein concentration (mg L^{-1}), V is the volume of the buffer lysis, DF is the dilution factor, and wt is the weight of leaves (mg).

2.4.3. Soluble Sugar Content (SSC) and Hydrogen Peroxide (H_2O_2)

The leaves' sugar content was quantified using the technique of Johnson et al. [23]. Similarly, the H_2O_2 activity was quantified by following the protocol of Velikova et al. [24]. The OD of sugar and H_2O_2 was recorded at 420 and 390 nm, respectively.

2.4.4. Malondialdehyde (MDA) and Glycine Betaine (GB) Assay

The MDA content was assessed according to the assay of Zhang and Kirkham [25] and the OD was recorded at 530 nm. Meanwhile, the Khan et al. [26] method was used to determine glycine betaine content. The MDA and GB contents were computed via the following equations:

$$\text{MDA (nmol)} = \text{D (A532 nm} - \text{A 600 nm)}/1.56 \times 105 \quad (15)$$

$$\text{Glycine betaine (GB)} = [A \times DF \times MW \times 1000/\varepsilon \times L] \quad (16)$$

where DF is the dilution factor, MW is the molecular weight, and ε is the extinction coefficient.

2.4.5. Antioxidant Enzymatic Assays

The typical protocol of Flohe [27] was used for the estimation of superoxide dismutase (SOD) activity at 560 nm on a spectrophotometer. Similarly, the activity of peroxidase (POD) and glutathione reductase (GR) was analyzed via the technique of Ahmad et al. [28] at 420 and 340 nm, respectively. Leaf extract was also used for the quantification of ascorbate peroxidase (APOX) and catalase (CAT) enzymes via the method of Livingstone et al. [29].

$$\text{Enzyme Activity} = \Delta A \times \text{Total assay} \frac{\text{volume}}{\Delta t} \times \in \times i \times \text{Enzyme sample volume} \quad (17)$$

where ΔA is the change in absorbance, Δt is the time of incubation, and E is the absorbance coefficient of the substrate.

2.5. Statistical Analysis

Microsoft Excel 2010, US, was used to estimate the mean and standard error from the collected data. Analysis of variance (ANOVA) was performed using Co-Stat Window version 6.3 to find significant differences between treatments. The mean and standard error were calculated using standard techniques; a least significant difference (LSD) test was performed at the ±0.05 significance level and was shown in letters (AE). Correlation analysis was performed using R Studio 8.1 software.

3. Results and Discussion

Drought stress has become a major agricultural constraint in recent decades, affecting soil structure and function, plant agronomy and physiology, and allied metabolism. Drought stress is accountable for amendments in soil properties, such as alteration in soil temperature due to drought affecting soil organic matter, which leads to decomposition and the release of excess CO_2 [30]. However, when α-toc was exogenously sprayed on drought-stressed plants, their agronomic, physiological, and biochemical attributes were dramatically changed and further enhanced (Figures 1–3).

Figure 1. Morphological response of *C. arietinum* to AsA and α-toc under induced (**A**) 10-day drought and (**B**) 20-day drought stress.

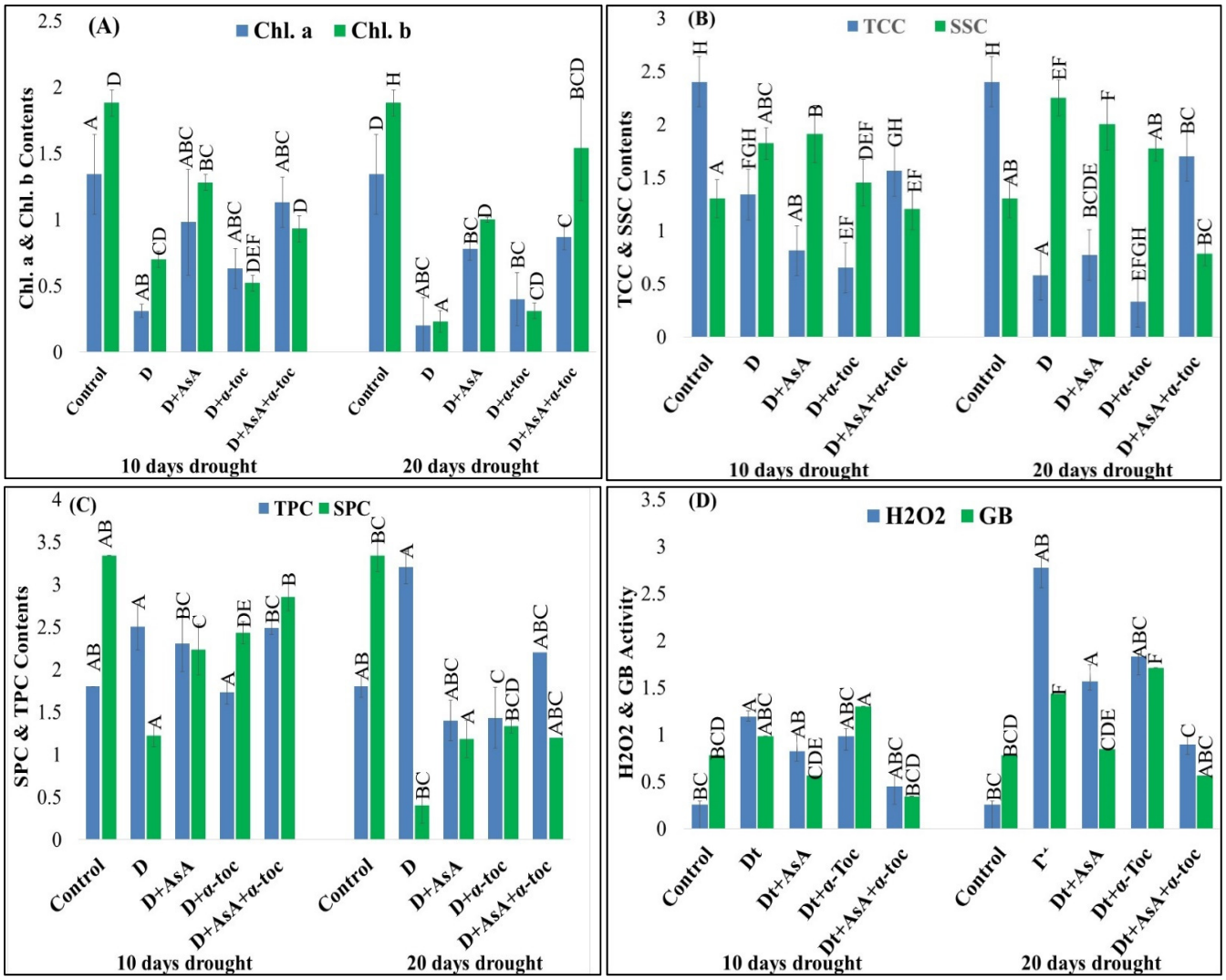

Figure 2. Effect of AsA and α-toc on *C. arietinum* (**A**) Chl. A and Chl. b, (**B**) carotenoid and sugar content, (**C**) protein and proline, and (**D**) H_2O_2 and GB under induced 10- and 20-day vegetative drought stress. Letters A–H represent significant and non dignificant difference in data in mean values.

3.1. Effect on Soil Physicochemical Properties

Physicochemical analysis of the soil before and after the sowing showed decreases in electrical conductivity from 521 to 151 mS/m, pH from 7.08 to 6.6, and the total dissolved solutes from 260 to 129 mg/L after 20 days in drought-affected soil (Table 1). Moreover, the temperature of the soil also altered from 18.6 in the control (T_o) to 24.4 °C after 20 days in drought soil (T5). Ojuederie et al. reported that decreases in soil water led to a decrease in the uptake of nutrients by crops, which directly affects turgor pressure and all the water precursor processes [31]. However, there were no significant differences noticed in the other properties of soil such as oxidation reduction potential and resistivity (Table 1). Results obtained from the study conducted by Ali and his colleagues documented that extreme drought severely affect the structure, function, and productivity of agricultural soil [32]. This leads to disruption in soil nutrients, soil aggregate stability, and porosity.

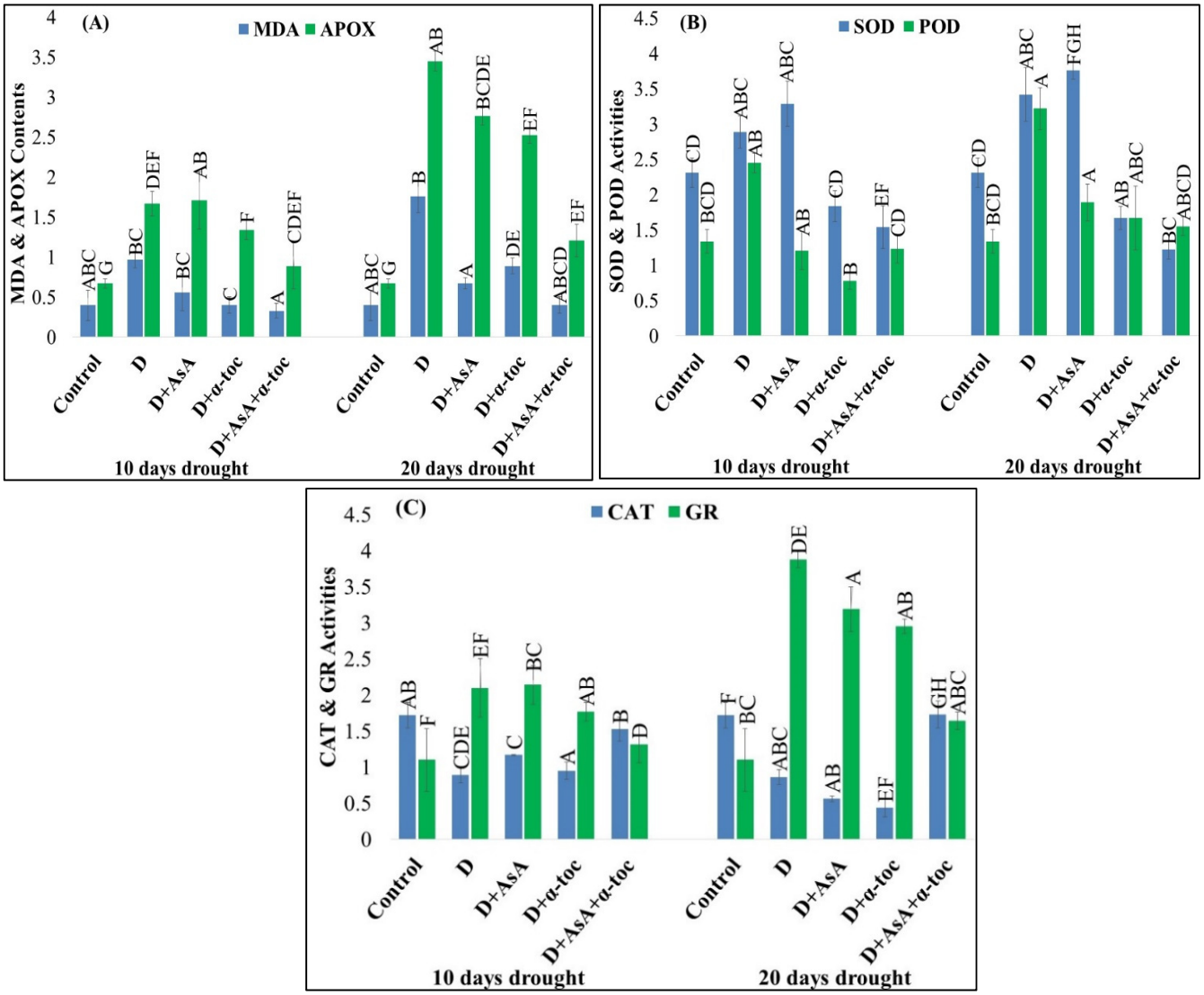

Figure 3. Effect of AsA and α-toc on *C. arietinum* (**A**) MDA and APOX, (**B**) SOD and POD, and (**C**) CAT and GR under induced drought stress. Letters A–H represent significant and non dignificant difference in data in mean values.

Table 1. Effect of drought stress on the physicochemical properties of soil before and after 10 or 20 days of drought under the application of treatments.

Treatment	T (°C)	pH	ORP (mV)	Resistivity (Ω·m)	EC (mS/m)	TDS (mg/L)	Salinity	DO
Control	18.6	7.96	99.5	1923	521	260	0.25	11.2
10-day drought	21.3	6.6	90.9	2953	290	256	0.25	11.2
D + AsA	19.6	7.2	88.1	2984	151	129	0.24	11.2
D + α-toc	19.6	7.0	86.9	2681	595	298	0.29	11.2
D (AsA + α-toc)	19.6	6.8	85.2	2890	529	264	0.26	11.2
20-day drought	24.4	7.15	82.2	1100	522	160	0.11	11.2
D + AsA	19.4	7.4	82.4	1900	429	129	0.15	11.2
D + α-toc	19.4	8.33	82.9	1789	586	133	0.17	11.2
D (AsA + α-toc)	19.4	7.13	92.3	1823	501	145	0.14	11.2

D: drought; pH = power of hydrogen ion; ORP = oxidation reduction potential; EC = electrical conductivity; DO = dissolved oxygen; T = temperature, TDS = total dissolved solutes.

3.2. Morphological Characteristics

Data from the germination and growth attributes revealed that varying levels (10 and 20 days) of drought stress significantly decreased the chickpea crop's morphological performance (Figure 1 and Tables 2 and 3). A significant decrease at $p \leq 0.05$ was experienced in the MGT, GRI, TGI, GE, CVG, FGP, SVI-I, SVI-II, and RMC after 10 and 20 days of induced drought stress (Tables 2 and 3). The negative effects are due to the higher production of reactive oxygen species (ROS) and stomatal closure due to drought stress conditions [31]. This condition progressively decreases the CO_2 acclimatization rate by decreasing stomatal conductance and the stability of the cellular membrane, which leads to disruptions in water relation by degrading water use efficiency [31].

Table 2. Effect of AsA and α-toc on *C. arietinum* mean germination time, germination rate index, germination energy, Timson germination index, coefficient of velocity of germination, and water use efficiency under induced drought stress.

Treatments	MGT	GRI	GE	TGI	CVG	WUE
Control	6.4 ± 0.21 ^{a}	75.5 ± 3.5 ^{b}	10.1 ± 1.2 ^{a}	55.4 ± 3.4 ^{b}	6.3 ± 1.2 ^{a}	2.3 ± 0.5 ^{a}
10-day drought	6.1 ± 0.56 ^{ab}	66.2 ± 2.3 ^{ab}	7.4 ± 1.0 ^{bc}	54.2 ± 5.2 ^{a}	5.6 ± 0.6 ^{a}	3.5 ± 1.2 ^{cd}
D + AsA	5.5 ± 0.34 ^{cd}	61.2 ± 3.3 ^{ab}	4.6 ± 0.8 ^{cd}	54.6 ± 2.5 ^{c}	4.3 ± 0.8 ^{ab}	6.2 ± 0.5 ^{bc}
D + α-toc	5.8 ± 0.22 ^{a}	71.7 ± 1.9 ^{ef}	5.2 ± 0.5 ^{ac}	56.2 ± 4.3 ^{d}	4.6 ± 1.5 ^{bc}	5.1 ± 1.8 ^{ac}
D (AsA + α-toc)	5.7 ± 0.12 ^{c}	65.3 ± 2.7 ^{d}	6.1 ± 1.5 ^{ab}	56.8 ± 6.4 ^{cd}	5.4 ± 0.9 ^{bc}	5.2 ± 2.1 ^{ae}
20-day drought	5.9 ± 0.56 ^{de}	67.4 ± 3.5 ^{b}	11.3 ± 2.0 ^{b}	53.2 ± 3.4 ^{ab}	6.4 ± 0.4 ^{cd}	4.2 ± 0.5 ^{de}
D + AsA	6.2 ± 0.76 ^{cd}	64.2 ± 4.0 ^{a}	6.7 ± 1.1 ^{cd}	51.9 ± 7.3 ^{bc}	3.7 ± 1.1 ^{c}	3.2 ± 0.7 ^{bc}
D + α-toc	6.6 ± 0.23 ^{c}	79.4 ± 3.8 ^{cd}	5.9 ± 1.0 ^{a}	57.2 ± 4.9 ^{a}	6.2 ± 0.9d ^{e}	6.1 ± 0.3 ^{bc}
D (AsA + α-toc)	6.0 ± 0.33 ^{a}	77.3 ± 3.1 ^{a}	10.0 ± 2.3 ^{ab}	53.4 ± 4.5 ^{a}	5.4 ± 0.5 ^{a}	4.3 ± 0.8 ^{a}

D: drought; MGT: mean germination time; GRI: germination rate index; GE: germination energy; TGI: Timson germination index; CVG: coefficient of velocity of germination; WUE: water use efficiency, Single superscript letters indicate non-significant data while different letters next to mean values indicate significant difference in data.

Table 3. Effect of AsA and α-toc on *C. arietinum* germination percentage, seed vigor index I, seed vigor index II, root moisture content, and time to 50% germination under induced 10- and 20-day drought stress.

Treatments	GP	SVI-I	SVI-II	RMC	T50%
Control	96.1 ± 5.16 ^{ab}	2427 ± 458.7 ^{d}	11,493.3 ± 598 ^{ab}	86.4 ± 1.2 ^{a}	5.3 ± 0.2 ^{ab}
10-day drought	93.3 ± 4.71 ^{c}	937 ± 49.253 ^{c}	6616.9 ± 153.2 ^{ab}	83.8 ± 0.4 ^{de}	5.3 ± 0.2 ^{cd}
D + AsA	90.6 ± 4.71 ^{d}	1261 ± 107.0 ^{c}	6619.23 ± 57.1 ^{bc}	67.3 ± 0.4 ^{ef}	5.6 ± 0.3 ^{de}
D + α-toc	94.6 ± 4.71 ^{a}	1217 ± 160.0 ^{b}	7322.4 ± 150.4 ^{de}	88.7 ± 0.8 ^{d}	5.0 ± 0.9 ^{a}
D (AsA + α-toc)	96.3 ± 4.71 ^{c}	1599 ± 88.41 ^{c}	10761 ± 200.5 ^{ab}	87.3 ± 0.5 ^{c}	4.8 ± 0.2 ^{b}
20-day drought	96.6 ± 4.71 ^{ab}	1143 ± 50.70 ^{a}	7026 ± 161.90 ^{bc}	71.21 ± 0.8 ^{a}	4.3 ± 0.2 ^{cd}
D + AsA	93.3 ± 9.43 ^{ab}	1604. ± 112.2 ^{a}	11,967 ± 345.4 ^{ab}	82.08 ± 0.8 ^{bc}	5.1 ± 0.2 ^{ef}
D + α-toc	96.3 ± 9.43 ^{a}	1471 ± 17.58 ^{ab}	7529 ± 76.183 ^{bc}	83.6 ± 0.6 ^{a}	5.1 ± 0.4 ^{a}
D (AsA + α-toc)	86.3 ± 4.71 ^{bc}	3319 ± 37.66 ^{a}	12,531 ± 308.2 ^{ab}	79.8 ± 0.9 ^{ab}	4.6 ± 0.2 ^{cd}

D: drought; GP: germination percentage; SVI-I: seed vigor index I; SVI-II: seed vigor index II; RMC: root moisture content; T50%: time to 50% germination, Single superscript letters indicate non-significant data while different letters next to mean values indicate significant difference in data.

The highest MGT, GRI, and TGI were observed in the 10-day drought regime treated with AsA+ α-toc (T4) (Table 2). Moreover, the exogenous application of α-toc considerably enhanced the GE, CVG, FGP, and RMC in 10-day drought regimes (T3). Table 3 shows that the maximum RMC, SVI-I, and SVI-II values were recorded in the 20-day drought regime with foliar application of AsA and α-toc (T8). These features declined consistently with the increasing intensity of the drought level of the growth medium (Figure 1). Similar results in which drought stress had the same drastic effects were reported in maize [32], carrot [33], and common vegetables [34]. The chickpea growth performance and develop-

ment inhibiting effect can be mitigated by AsA and α-toc by accelerating osmolytes and antioxidant enzyme production. These attributes were reported at a maximum in plants treated with foliar application of ascorbic acid (150 mg/L) and α-toc as compared to the control and stressed plants (Tables 2 and 3). However, the variables WUE and T50% showed improvements with the increase in the interval of drought stress and had the highest values in plants grown in the 20-day water-deficient regimes. The interactive effect of AsA and α-toc exhibits that these PGRs have a positive effect on the germination and agronomic attributes of chickpea (Tables 2 and 3). However, comparatively, AsA is more efficient than α-toc for ameliorating the hostile effects of drought stress in chickpea. Exogenously applied AsA and α-toc were also commendably used to ameliorate the drought stress tolerance in various crops such as wheat [35] and sunflower [36].

3.3. Effects on Physiological Attributes and Antioxidant Activities

Drought stress caused a rapid decrease in all the physiological attributes and an increase in the activity of defense antioxidant enzymes. In comparison with the control (T_o), the leaf Chl. a and Chl. b contents were reduced to 0.10 and 0.23 mg/L, respectively, in the 20-day drought regime (Figure 2A). In contrast, the foliar application of both AsA and α-toc improved the chlorophyll contents in both the 10- and 20-day drought stress conditions (T1, T5). The foliar application regulates the plant's photosynthetic pigments by reducing the production of hydrogen peroxide and elevating the phenolic level, making the plant perform better under stress conditions [37]. Due to their sensitive nature, the degradation of plant photosynthetic pigments (Chl. a, Chl. b, and carotenoids) was the primary sign noticed under the induced drought stress conditions. It is due to the disintegration of the chloroplast thylakoid membrane [37], which occurs due to the corrosion of amino acids and photosystem 2 (PSII) linked with the chloroplast membrane [38]. The comparative study revealed that AsA showed a better performance in improving all the chlorophyll contents (T2) as compared to α-toc (Figure 1A). Drought significantly ($p \leq 0.005$) reduced the carotenoid contents from 2.4 in the control (T_o) to 0.58 under 20-day drought stress (T5). The induced drought stress quickly enhanced the concentration of total soluble sugar (1.3 to 2.25) in all the stressed treatments, and a further increase was observed with increasing the drought interval (Figure 2B). Plant growth regulators (AsA and α-toc) caused a substantial recovery in the photosynthetic system by decreasing the production of ROS [31]. Similar results were reported in wheat [38,39] and pepper [40].

Figure 2C shows that a significantly high proline content (2.25) was observed in chickpea plants exposed to 20 days of continuous drought stress (T5), while the lowest was in 20-day drought plants treated with AsA (T6). Proline accumulation under drought stress has been reported by many researchers [41–43]. It acts not only as a source of nutrition but also as an osmotolerant by determining protein and membrane structures. The application of AsA and alpha-tocopherol reduced its concentration under drought stress conditions. A prominent ($p \leq 0.005$) decrease of 3.34 in the control to 0.4 after 20 days of drought stress was observed in the content of protein as we increased the interval of drought stress (Table 4) (Figure 2C). However, all the treatments that received foliar application of AsA, α-toc, or both experienced a relatively high concentration of protein content (Table 4). This is a clear indication that protein plays an essential role in plant response to drought stress [44]. However, there have been different outcomes of protein accumulation in plants under stress conditions. For example, research conducted by Xu et al. reported that protein content increases under drought stress [44], while Savvides et al. documented that it decreases in plants grown in water deficit regimes [45].

Figure 2D shows that drought stress caused a considerable increase in the H_2O_2 and GB contents in all the 10- and 20-day stress treatments. The two had maximum concentrations of 2.77 and 1.43, respectively, observed in the 20-day drought stress regimes and a minimum of 0.45 and 0.34, respectively, in the 10-day water deficit regime sprayed with AsA and α-toc. The increase is due to the stress regulatory function of AsA and α-toc. The H_2O_2 accumulation is due to a decrease in the soil water content and assimilation

of CO_2. The antioxidants dissolve superoxide ions with H_2O_2, and other enzymes are produced into water molecules. MDA acts as a signaling indicator in response to ROS, which can be used as a signal for drought to assess the plasma membrane injury and the ability of crops to tolerate drought. A significant upsurge ($p \leq 0.005$) was observed in the content of MDA and GR in all the drought stress-treated plants (Figure 3A). The content of MDA increased from 0.12 (T8) to 1.76 in 20-day drought stress conditions. The increase in MDA content under drought stress in chickpea is similar to that reported in ornamental grass [46] and rice [47]. Glycine betaine also works as an attuned solute which helps to mitigate the adverse effect of drought stress by taming the cytosol water status and protecting the cell biological membranes from ROS. The same trend was also observed for GR, which increased from 1.1 in the control (T_o) to 3.88 under 20-day drought stress (T5). GB is believed to be a catalyst for photosynthesis in plants grown in water-deficient soil by increasing the Hill reaction speed and Ca^{2+}-ATPase in the thylakoid membrane system. The literature revealed that GB increased the drought stress tolerance threshold in wheat [48] and cauliflower [49].

Table 4. Analysis of variance calculated for the physiological attributes of *C. arietinum* grown in water deficit regimes.

Variables	Variation Source	SS	DF	MS	F	*p*
Chl. a	Treatment	0.067	9	0.231	3.140	0.005 **
	Genotype	0.023	2	0.563	2.451	0.000 ***
	Treatment × Genotype	0.080	9	1.230	6.340	0.000 ***
	Error	0.052	54	1.110	-	-
Chl. b	Treatment	0.570	9	0.781	13.101	0.005 **
	Genotype	0.110	2	0.881	6.231	0.002 **
	Treatment × Genotype	0.067	9	1.238	9.671	0.000 ***
	Error	0.089	54	1.200	-	-
TCC	Treatment	0.381	9	2.134	2.341	0.000 ***
	Genotype	0.182	2	0.714	1.776	0.015
	Treatment × Genotype	0.116	9	0.891	2.341	0.018
	Error	0.667	54	0.114	-	-
SSC	Treatment	0.836	9	0.843	4.674	0.011
	Genotype	0.780	2	0.341	2.110	0.000 ***
	Treatment × Genotype	0.201	9	1.349	7.890	0.010 **
	Error	0.052	54	1.220	-	-
TPC	Treatment	0.446	9	1.989	11.98	0.005 **
	Genotype	0.743	2	0.231	2.778	0.000 ***
	Treatment × Genotype	0.890	9	1.228	7.891	0.000 ***
	Error	0.520	54	0.231	-	-
SPC	Treatment	0.667	9	0.667	8.219	0.000 ***
	Genotype	0.211	2	1.563	9.220	0.005 **
	Treatment × Genotype	0.320	9	1.231	2.667	0.000 ***
	Error	0.520	54	1.789	-	-
H_2O_2	Treatment	0.289	9	2.452	11.231	0.001 *
	Genotype	0.211	2	1.561	1.781	0.000 ***
	Treatment × Genotype	0.856	9	1.892	2.776	0.000 ***
	Error	0.052	54	0.553	-	-
GB	Treatment	0.911	9	0.875	6.889	0.000 ***
	Genotype	0.909	2	0.167	3.667	0.005 **
	Treatment × Genotype	0.800	9	0.796	2.891	0.017 **
	Error	0.775	54	0.231	-	-

Table 4. *Cont.*

Variables	Variation Source	SS	DF	MS	F	*p*
MDA	Treatment	0.553	9	0.223	8.990	0.000 ***
	Genotype	0.218	2	0.190	4.781	0.000 ***
	Treatment × Genotype	0182	9	1.231	2.990	0.000 ***
	Error	0.562	54	1.681	-	-
APOX	Treatment	0.239	9	0.990	2.887	0.000 ***
	Genotype	0.918	2	1.230	4.871	0.005 **
	Treatment × Genotype	0.802	9	0.872	1.091	0.000 ***
	Error	0.921	54	0.664	-	-
SOD	Treatment	0.222	9	0.332	2.998	0.000 ***
	Genotype	0.181	2	0.013	2.871	0.018
	Treatment × Genotype	0.272	9	0.123	6.889	0.080
	Error	0.653	54	0.771	-	-
POD	Treatment	0.560	9	0.010	17.870	0.010 **
	Genotype	0.230	2	0.451	13.761	0.019
	Treatment × Genotype	0.080	9	0.087	7.891	0.000 ***
	Error	0.052	54	0.171	-	-
CAT	Treatment	0.521	9	0.871	4.651	0.005 **
	Genotype	0.257	2	0.776	3.981	0.005 **
	Treatment × Genotype	0.871	9	1.881	2.991	0.004 **
	Error	0.233	54	1.761	-	-
GR	Treatment	0.791	9	0.910	21.2	0.001 *
	Genotype	0.270	2	0.334	12.8	0.000 ***
	Treatment × Genotype	0.080	9	0.008	-	-
	Error	0.451	54	0.430	3.87	0.005 **

Chl. a: chlorophyll a; Chl. b: chlorophyll b; TCC: total carotenoid content; SSC: soluble sugar content; TPC: total protein content; SPC: soluble proline content; APOX: ascorbate peroxidase; H_2O_2: hydrogen peroxide; MDA: malondialdehyde; GR: glutathione reductase; SOD: superoxide dismutase; POD; peroxidase; CAT: catalase. * Significant, ** More significant *** Most significant.

Induced drought stress increases the levels of ROS, lipid peroxidation, and oxidative damage due to the excitation and conversion of O_2 to OH^-, H_2O_2, or O^{-2}. The transfer of this restricted electron during photosynthesis and respiration makes the plant metabolically unstable. This overproduced ROS damaging effect can be mitigated by increased activities of antioxidant enzymes following foliar application of AsA and α-toc. Chickpea grown in water-limited conditions showed significantly high activities of SOD, POD, and APOX, while that of CAT decreased with drought stress (Figure 3). The upsurges in all three antioxidants are due to drought stress and exogenous application of growth regulators (AsA and α-toc). The SOD activity was enhanced from 2.31 in the control (T_o) to 2.89 in the 10-day drought regimes (T1) and finally to 3.45 in the 20-day drought conditions (T5) (Figure 3B). These antioxidant enzymes are a prime solution to rescue crops from the adverse effect of drought stress [50]. Similarly, the activities of POD and APOX also increased sharply ($p \leq 0.05$) from 1.34 and 0.67 to 3.22 and 3.45 following 20 days of drought stress (T5), respectively.

In contrast, the activity of catalase decreased in all the plants in the 10- and 20-day drought stress regimes and increased due to the foliar application of AsA and α-toc (Figure 3C). Figure 3 reveals that the GR content also sharply increased as the drought stress interval increased to 10 and 20 days. In the current study, exogenous application of AsA and α-toc under induced drought stress led to significant impacts on the agronomic and physiological attributes and activation of the plant's defense system (Figure 3).

3.4. Principal Components and Correlations

The Pearson correlation coefficient illustrates that all the physiological parameters were positively correlated at $p \leq 0.05$, except antioxidant enzymes which were not correlated with the other parameters (Figure 4).

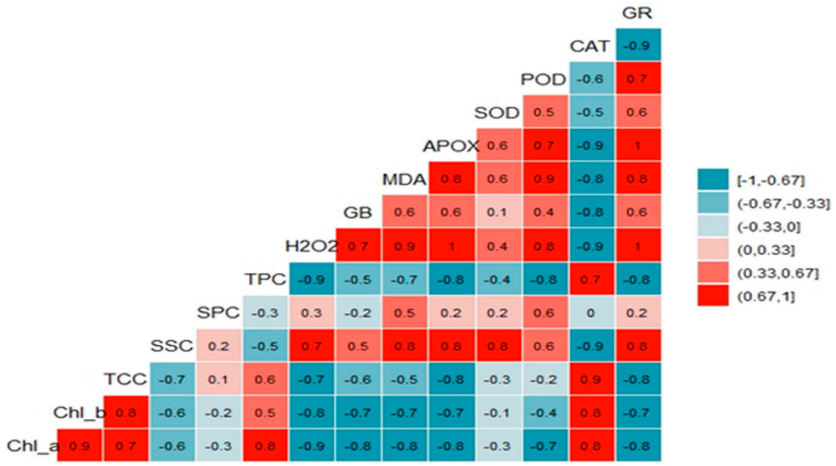

Figure 4. Correlation between different physiological attributes of *C. arietinum* under drought stress and applied AsA and α-toc.

This relationship is a clear indication of the high dependency of these attributes on each other. Principal component analysis was performed to present the relationship of the physiological attributes under drought stress. Akhtar et al. stated that such statistical attributes as analysis of variance, principal component analysis, and Pearson correlation are the keys to calculating the correlation among drought, growth regulators, and agronomic and physiological attributes [51–58]. Figure 5 shows that the photosynthetic pigments, carotenoids, catalase, and proline contents showed a negative relation in the database. Lazic et al. also used PCA to assess the plant response to experimental queries for rapeseed [59]. PCA can condense a large number of original variables into a new compact set of principal components with minimal loss of information.

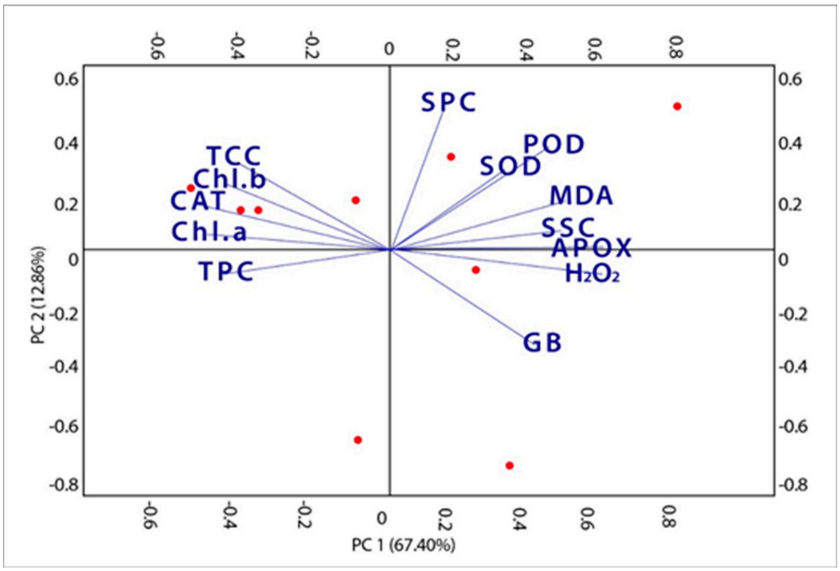

Figure 5. Principal component analysis of various physiological attributes of *C. arietinum* in response to foliar application of AsA and α-toc under induced drought stress. Red circles indicate the treatments from T0 to T8.

4. Conclusions

Drought stress induced by the changing climate and global warming is the main obstacle in increasing the agro-physiological growth and production of leguminous plants such as *Cicer arietinum* L. The results of the current study conclude that AsA and α-tocopherol are a prime solution for ameliorating the antagonistic effects of drought stress. Improved germination rate index, mean germination time, germination energy, water use efficiency, germination percentage, seed vigor index, and photosynthesis were among the positive effects. Moreover, AsA and α-toc reduced the ROS-induced oxidative damage by activating a wide spectrum of antioxidative defense systems (enzymatic and non-enzymatic). Due to this key role, the foliar application of AsA and α-toc was found to be important for making chickpea crops drought-tolerant by curbing various anabolic and catabolic activities. During the comparative investigation, it was observed that AsA was more applicable and effective than α-toc in ameliorating the hostile possessions of drought stress in chickpea (Figure 6). However, further research is needed to investigate the best route of AsA administration. Moreover, due to the unavailability of literature on the effects of drought on soil properties, the current study extensively investigated the physicochemical attributes of water-limited agricultural soil. The soil results provide a new insight to understand the extent of soil damage and chickpea response in water-limited regimes.

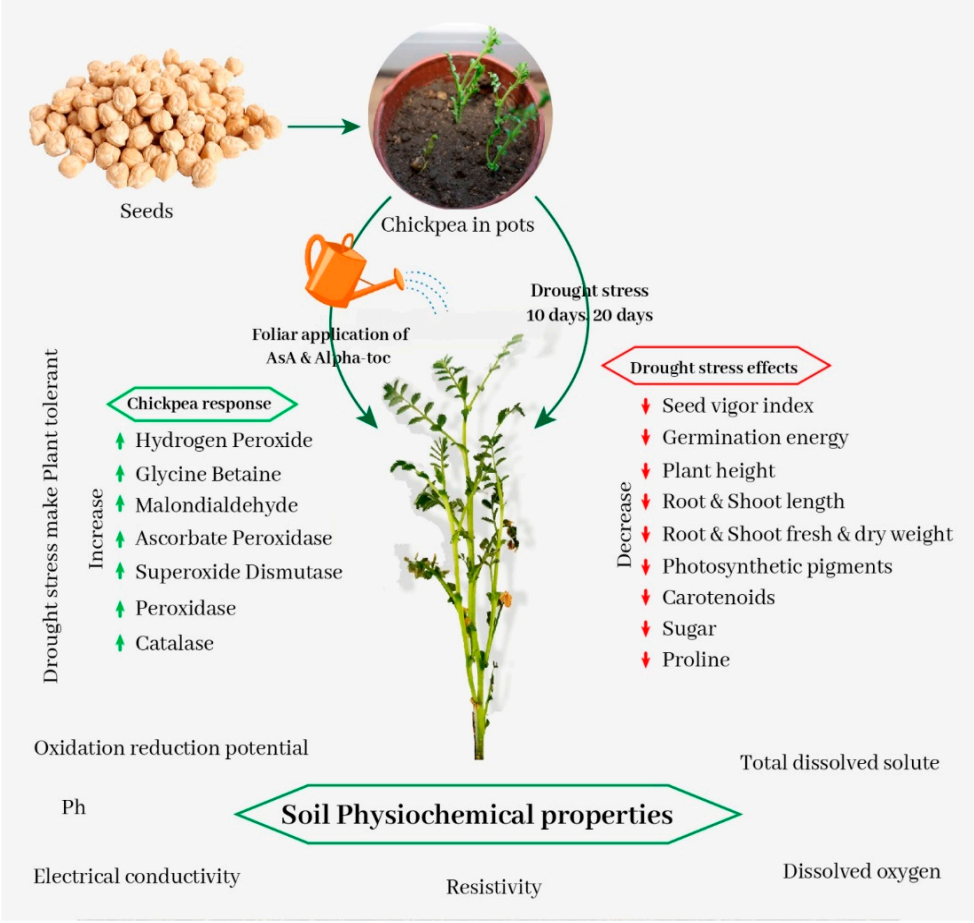

Figure 6. Model showing the regulation of drought stress tolerance and application of ascorbic acid and alpha-tocopherol.

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