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# Physiological and Biochemical Changes in Sugar Beet Seedlings to Confer Stress Adaptability under Drought Condition

**Md. Jahirul Islam [1,2], Ji Woong Kim [1], Mst. Kohinoor Begum [2], Md. Abu Taher Sohel [3] and Young-Seok Lim [1,*]**

1 Department of Bio-Health Convergence, Kangwon National University, Chuncheon 24341, Korea; jahirulislam213@gmail.com (M.J.I.); jiwoongki@naver.com (J.W.K.)
2 Physiology and Sugar Chemistry Division, Bangladesh Sugarcrop Research Institute, Ishurdi 6620, Pabna, Bangladesh; kohinoorbegum.bsri@gmail.com
3 Agronomy and Farming System Division, Bangladesh Sugarcrop Research Institute, Ishurdi 6620, Pabna, Bangladesh; atsohel@yahoo.com
* Correspondence: potatoschool@kangwon.ac.kr; Tel.: +82-33-250-6474

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**Abstract:** The present study was conducted to examine the adaptability of 11 sugar beet cultivars grown under drought stress in the controlled glasshouse. The treatment was initiated on 30-day-old sugar beet plants where drought stress was made withholding water supply for consecutive 10 days while control was done with providing water as per requirement. It was observed that drought stress expressively reduced plant growth, photosynthetic pigments, and photosynthetic quantum yield in all the cultivars but comparative better results were observed in S1 (MAXIMELLA), S2 (HELENIKA), S6 (RECODDINA), S8 (SV2347), and S11 (BSRI Sugarbeet 2) cultivars. Besides, osmolytes like proline, glycine betaine, total soluble carbohydrate, total soluble sugar, total polyphenol, total flavonoid, and DPPH free radical scavenging activity were remarkably increased under drought condition in MAXIMELLA, HELENIKA, TERRANOVA, GREGOIA, SV2348, and BSRI Sugar beet 2 cultivars. In contrast, activities of enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were significantly decreased in all, while the cultivars SV2347, BSRI Sugar beet 1 and BSRI Sugar beet 2 were found with increased ascorbate peroxidase (APX) activity under drought condition. In parallel, polyphenol oxidase (PPO) was increased in all cultivars except HELENIKA. Overall, the cultivars HELENIKA, RECODDINA, GREGOIA, SV2347, SV2348, BSRI Sugar beet 1, and BSRI Sugar beet 2 were found best fitted to the given drought condition. These findings would help further for the improvement of stress adaptive sugar beet cultivars development in the breeding program for drought-prone regions.

**Keywords:** sugar beet cultivars; drought; osmolytes; antioxidant enzymes; secondary metabolites; and antioxidant capacity

## 1. Introduction

To overcome the threats on the agriculture and ecosystem, top-most priority has been given to evaluate the plant response under hostile environments, such as heat, drought, cold, toxic metals, and nutrient deficiency [1,2]. These harsh conditions are collectively referred to as abiotic stress which directly interlinked with the plant growth, development, and overall crop productivity [3,4]. Amidst various detrimental factors, drought is the most repulsive one and extensively impinges on crop growth, yield, and production. The yield and quality of the crop greatly depend on environmental conditions including soil moisture and rainfall pattern [5]. It was estimated that drought caused a

potential yield reduction of sugar beet in Europe, ranging from 5 to 30% each year [6]. This event is more tremendous in other regions including arid and semi-arid [7], particularly, where rainfall is relatively low. One such region is South Korea, where drought is a common phenomenon due to the short summer monsoon season [8]. It has observed a prolonged drought from 2013 to 2015 where annual precipitation was below 35–50% of normal levels [9]. United Nations also has classified South Korea as one of the water-deficient countries in the world for its water scarcity problems [10].

Drought tolerant or resurrection plants are quite rare in nature due to the complexity of the adaptation process for the plant. It down-regulates cell enlargement (near about four times higher than cell division) [11] as well as impacts on cell growth by affecting different physiological and biochemical activities [12,13]. However, to enhance the adaptability of a plant when it comes to water scarcity, it is necessary to investigate the sensitivity, morpho-physiological, and biochemical response of a plant under drought stress.

Different morphological traits like relative water content (RWC), chlorophyll fluorescence, grain yield, plant biomass, and plant height have been used to evaluate drought stress [14–17]. Also, various physiological, morphological, and biochemical responses of plants to water stress have been well documented [18]. For example, growing and water supply condition affects the chlorophyll content of leaves and Fv/Fm which influence the photosynthesis of the varieties [19,20]. Water stress can bring in the change in concentration, composition, and distribution of both primary and secondary metabolites in plant cells which boost their survival capacity against abiotic and biotic stress by following a complex interaction among them [21]. During drought, the plant is subjected to oxidative stress resulting in a series of physiological and biochemical changes that may cause serious metabolic disorders [22]. In response to oxidative damage, plant accumulates different compatible solutes or osmolytes, like proline, sorbitol, phenol, ascorbic acid, glycine betaine, sugars, and polyamines to cope with water stress by maintaining their water relations [23,24]. A previous study showed significantly higher carbohydrates accumulation under severe drought conditions which were considered as stress indicators [25]. However, the response of metabolites to drought stress may be varied among the plant parts and even in genotypes [26]. Therefore, a detailed study on osmoprotectants and their variation of role in different plants to osmotic stress should be analyzed.

Plants under the abiotic stress produce a pool of reactive oxygen species (ROS) which causes an imbalance of component quantities and dysfunction of their normal defensive system [27]. It is stated that the production of reactive molecules and ROS in plants may be stimulated due to the water loss below 70% of RWC [28]. The over-production of such ROS consists of both non-radical ($H_2O_2$) and free radical ($O_2^{-\bullet}$, $OH^{\bullet}$, $OH_2^{\bullet}$) species, which are persistently detrimental to plants cells [29]. Several previous studies showed a positive relationship between water deficiency and ROS accumulation in *Lotus japonicus* [30] and *Arabidopsis thaliana* [31]. Other studies also mentioned an excessive ROS accumulation in young senescing leaf cells under abiotic stress, which are subject to abolish by a complex mechanism in enzymatic and non-enzymatic pathways [32]. Higher plants also follow a similar mechanism among antioxidant enzymatic and non-enzymatic pathways to protect their cellular and subcellular systems [33]. In general, elevated activity of antioxidant enzymes including ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) is required to quench the intense flux of ROS [34,35]. Therefore, ROS homeostasis by enzymatic and non-enzymatic antioxidants may be an important tool to evaluate plant species to be selected under osmotic stress.

Secondary metabolites are natural compounds produced by the plant and can perform several physiological roles [36]. Secondary metabolites produced by plant cells have not only a significant role in their growth and development process under normal conditions, but also their production under stress can be regulated and used as defensive and tolerance mechanisms [21,37,38]. Also, changing environment can bring in significant change in metabolism and yield [39]. There is little information on sugar beet regarding the effect of drought on secondary metabolites, and their role in stress acclimatization is also unclear. Along with significantly increasing antioxidant activity in plant tissues, secondary metabolites (like polyphenol, flavonoids, and callose) may take part in a defensive

role against both biotic and abiotic stress [40–42]. Therefore, evaluation of sugar beet seedlings based on antioxidant activity may play a pivotal role in selecting drought-tolerant germplasms for future breeding.

Generally, sugar beet is a biennial crop of temperate regions that requires proper vernalization followed by growing in a long day length (>14 h) for bolting [43–45]. The Republic of Korea lies in the temperate zone and geographically it is located in the middle latitudes of the Northern Hemisphere which last 30 years of the average temperature of winter was 0.1–1.1 °C [46]. The average day length of spring (March to May) ranges from 12–14 h and summer (June–August) more than 14 h [47]. So, the climatic position may help to introduce sugar beet breeding in Korea. Moreover, there is no research on sugar beet relates to drought stress was found in Korea as far as our concern. Therefore, the objective of this study was to evaluate sugar beet cultivars against drought stress based on their physiological and biochemical responses for future breeding purposes.

## 2. Materials and Methods

### 2.1. Experimental Design and Treatment

Sugar beet seedlings were grown in a semi-controlled greenhouse in the department of Bio-Health Convergence, Kangwon National University, Chuncheon, Korea. The environmental condition such as temperature, relative humidity (RH) and average photoperiod was recorded at 30/25 °C (day/night), 60–70% RH and 12 h respectively. Seeds of sugar beet (*Beta vulgaris* L. subs. vulgaris) were provided by Asia seed co., ltd, Seoul, Korea and Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi-6620, Pabna, Bangladesh. The collected 11 genotypes (Table 1) were sterilized [70% (*v*/*v*) ethanol, 0.1% (*w*/*v*) $HgCl_2$ and 0.2% (*w*/*v*) thiram] and placed in the 16 cells hole growing pot (27 × 27 × 6 cm) containing commercial horticultural soil (Bio-soil No. 1, Heungnong Agricultural Materials Mart, Pyeongtaek, Korea). The seedlings were irrigated daily using tap water to the field capacity level up to 30 days. Two treatments were imposed on morphologically uniformed and healthy seedlings, i.e., (i) seedlings were grown under normal growth condition with regular watering and (ii) seedlings were subjected to water deficit by withholding the water supply for 10 days. The sample (youngest completely formed leaves) was collected from each cultivar and immerged immediately in liquid nitrogen followed by freeze-drying and subjected to further analysis.

**Table 1.** Sugar beet cultivars used in the experiment.

| S.N. | Name of the Cultivar | Origin | Code Name |
|---|---|---|---|
| **1** | MAXIMELLA | Germany | S1 |
| **2** | HELENIKA | Germany | S2 |
| **3** | TERRANOVA | Germany | S3 |
| **4** | TOLERANZA | Germany | S4 |
| **5** | BORNITA | Germany | S5 |
| **6** | RECODDINA | Germany | S6 |
| **7** | GREGOIA | Germany | S7 |
| **8** | SV2347 | Belgium | S8 |
| **9** | SV2348 | Belgium | S9 |
| **10** | BSRI Sugarbeet 1 | Bangladesh | S10 |
| **11** | BSRI Sugarbeet 2 | Bangladesh | S11 |

S.N.: Serial number.

### 2.2. Determination of Plant Height, Fresh Weight, and Dry Weight

On the 10th day on the onset of drought treatment, five seedlings from each pot were randomly selected and measured the plant height and fresh weight. Afterward, plants were placed in an oven at 60 °C for 48 h, to determine the dry weight.

### *2.3. Photosynthetic Pigments Analysis*

#### 2.3.1. Chlorophyll (Chl) and Carotenoid (Car) Determination

For the determination of photosynthetic pigments, the freeze-dried (50 mg) leaves were extracted (10 mL of 80% acetone) and placed at room temperature for 15 min. The collected extract was transferred into a tube and centrifuged at 4000 rpm for 10 min. The absorbance was taken at 647, 663 and 470 nm, respectively using a spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). Chlorophyll a, Chlorophyll b, Total chlorophyll and Carotenoid were determined according to the formula proposed by Lichtenthaler (1987) [48] and expressed as mg $g^{-1}$ DW:

$$\text{Chl a} = 12.25 \times A_{663} - 2.79 \times A_{647}$$

$$\text{Chl b} = 21.50 \times A_{647} - 5.10 \times A_{663}$$

$$\text{TCh} = 7.15 \times A_{663} + 18.71 \times A_{647}$$

$$Car = \frac{1000 \times \text{A470} - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b}}{198}$$

#### 2.3.2. Determination of Photosystem II Quantum Yield

The photosynthetic quantum yield (Fv/Fm) of photosystem II (PSII) was measured using the Fluor Pen FP 100 (Photon system Instruments, Czech Republic) by measuring OJIP transient under the dark-adapted condition at least for 20 min [49].

### *2.4. Physiological Traits*

#### 2.4.1. Leaf Relative Water Content

Leaves from three seedlings of each genotype from control and water stress treatment were collected and immediately weighted (FW). Later, the leaves were put in deionized water and weighed (g) again after 48 h to determine the leaf turgid weight (TW). Finally, dry weight (DW) was determined by placing in an oven at 60 °C for 48 h. The relative water content (%) of the leaves was calculated according to the following formula [50]:

$$\text{RWC (\%)} = [(\text{FW} - \text{DW})/(\text{TW} - \text{DW})] \times 100$$

#### 2.4.2. Drought Tolerance Index

The drought tolerance index given by Fernandez [51] and adjusted for sugar beet by Ober et al., 2004 [52] was calculated according to the following formula:

$$\text{DTI} = (\text{PDMd}/\text{PDMw})/(\text{PDMTd}/\text{PDMTw})$$

where PDMd is the plant dry matter under drought stress, PDMw is the plant dry matter of the control plants for each genotype and PDMTd and PDMTw are the average values of the plant dry matter for all genotypes under drought stress and control condition, respectively.

#### 2.4.3. The Membrane Stability Index (MSI)

The membrane stability index (MSI) was taken by the methodology developed by Sairam et al. (2002) [53]. Leaf samples (0.1 g each) were cut into a uniform size (disk shape) and placed into the test tubes with double-distilled water (10 mL) in two sets. One set was kept in a hot water bath (40 °C for 30 min) and its conductivity was recorded ($C_1$) using a conductivity meter (HI 5321, HANNA Instruments Inc, Woonsocket, Rhode Island, USA). The second set was also placed in

hot water bath (100 °C for 15 min) to record its conductivity ($C_2$) again. The membrane stability index (MSI) was measured as: MSI = [1 − ($C_1$/ $C_2$)] × 100.

### 2.5. Determination of Lipid Peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in leaves of sugar beet seedlings. MDA was calculated by following the method of Heath and Packer (1968) [54] with slight modification. The freeze-dried leaf sample (25 mg) was macerated in 0.1% trichloroacetic acid (5 mL). The homogenate was centrifuged at 10,000× *g* for 5 min. After that, 4 mL of Trichloroacetic acid (TCA; 20%) containing thiobarbituric acid (0.5%) was added to 1 mL aliquot of supernatant in a test tube. The test tube was placed in a water bath and warmed at 95 °C for 30 min followed by cooled quickly on an ice bath. The resulting mixture was centrifuged again at 10,000× *g* for 15 min and the absorbance was taken at 532 nm. To avoid unspecific turbidity absorbance at 600 nm was subtracted from 532 nm and an extinction coefficient of 155 $mM^{-1}{\cdot}cm^{-1}$ was used to calculate the concentration.

### 2.6. Estimation of Hydrogen Peroxide ($H_2O_2$)

The estimation was done according to Singh et al. (2006) [55]. Freeze-dried leaves (25 mg) were extracted in an ice bath with 5 mL of 0.1% (*w*/*v*) TCA and centrifuged at 12,000× *g* for 15 min. For every 0.5 mL of the aliquot of the supernatant, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI were added followed by incubation in darkness for 1 h. The absorbance was measured at 390 nm where 0.1% TCA was used as blank prove in place of leaf extract. A standard $H_2O_2$ curve was prepared to calculate the concentration of $H_2O_2$ in the sample.

### 2.7. Determination of Osmolytes

#### 2.7.1. Free Proline Content

Proline concentration was determined by following the method of Bates et al. (1973) [56]. Approximately 25 mg of freeze-dried plant material was homogenized in 10 mL sulfosalicylic acid (3%) and filtered through Whatman's filter paper. Two milliliters of the filtrate was mixed with a similar amount (2 mL) of acid-ninhydrin and glacial acetic acid. The mixture was heated for 1 h at 100 °C and cooled immediately on an ice bath. The reaction mixture was extracted with toluene (4 mL). The upper chromophore layer (upper layer) was aspirated and cooled to room temperature. The absorbance was taken at 520 nm with a UV-Vis spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan) and calculations were done by using an appropriate proline standard curve.

#### 2.7.2. Glycine Betaine Content

A freeze-dried sample (25 mg) was extracted with methanol (80%) and sonicated for 2 h at room temperature. The solution was filtered through a syringe filter (0.45 µM, Millipore, Bedford, MA, USA). The stock solution of betaine was prepared in ultrapure water (10 mg/mL) and went under serial dilution with the mobile phase to prepare 10, 8, 6, 4, 2, and 1 $mg{\cdot}mL^{-1}$, respectively. An improved HPLC method developed by Xu et al. (2018) [57] was used to determined GB in plant leaves. The HPLC system (CBM 20A, Shimadzu Co, Ltd., Kyoto, Japan) with diode array detector (DAD) and a 5 µm C18 column (25 cm × 4.6 mm) were used. The mobile phase consisted of water and acetonitrile 10:90 (*v*/*v*) mixed with 0.2% phosphoric acid. Sample (2 µL) was injected with a flow rate of 0.5 $mL{\cdot}min^{-1}$ where oven temperature was 35 °C and the detection wavelength was 195 nm.

#### 2.7.3. Total Soluble Carbohydrate (TSC) and Sucrose Content

Each sample (25 mg, freeze-dried) was homogenized in 5 mL of ethanol (95%). The insoluble fraction of the extracts were washed with 5 mL of ethanol (70%) followed by centrifuging at 3500 rpm for 10 min and the supernatant was kept in a refrigerator (4 °C) for the determination of TSC and Sucrose content.

TSC content was determined according to Khoyerdi et al. (2016) [58]. Briefly, 0.1 mL of the aliquot was mixed with 1 mL anthrone (200 mg anthrone mixed with 100 mL of 72% sulfuric acid). The mixture was heated at 100 °C for 10 min and then cooled. Total soluble carbohydrate was estimated by using a standard curve, the detection wavelength was 625 nm and the results were expressed as $\mu g \cdot g^{-1}$ dry weight.

In the case of sucrose content, 0.2 mL of the supernatant was mixed with 0.1 mL of KOH (30%) and heated at 100 °C for 10 min. After cooling at room temperature 3 mL of anthrone (150 mg anthrone mixed with 100 mL 70% sulfuric acid) was added. Ten minutes later, the samples were cooled, and absorbance was read at 620 nm. Sucrose concentration was calculated using the standard curve and the results were expressed as $\mu g \cdot g^{-1}$ dry weight [59].

### *2.8. Determination of Antioxidant Enzyme Activities*

Leaves samples were collected and immersed immediately in liquid nitrogen followed by freeze-drying and stored at −80 °C until use. A 25 mg sample was homogenized in 5 mL 50 mM sodium phosphate buffer (pH 7.8) using a pre-chilled mortar and pestle, then centrifuged at 15,000× *g* for 20 min at 4 °C. The enzyme extract was stored at 4 °C for analysis [60]. The activity of superoxide dismutase (SOD; EC 1.15.1.1) was estimated by the method Giannopolitis and Ries (1977) [61] with slide modification. A 2 mL of reaction mixture contained 50 mM sodium phosphate buffer with 0.1 mM EDTA, 12 mM methionine, 75 µM Nitro blue tetrazolium chloride (NBT), 50 mM $Na_2CO_3$, and 100 µL enzyme extract, and in case of the blank reaction mixture, 100 µL buffer was used instead of enzyme extract. After that, 200 µL of 0.1 mM Riboflavin was added to each mixture. The tubes were shaken and irradiated under the fluorescent light (15 W) for 15 min. The absorbance of each solution was measured at 560 nm. One unit of SOD represented as the quantity of enzyme that caused 50% inhibition of NBT reduction under the experimental conditions.

The peroxidase (POD; EC 1.11.1.7) and catalase (CAT; EC 1.11.1.6) activities were determined according to Zhang (1992) [62]. The 3 mL reaction mixture for POD consisted of 100 µL enzyme extract, 100 µL guaiacol (1.5%, *v/v*), 100 µL $H_2O_2$ (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The increased rate of absorbance was measured spectrophotometrically at 470 nm ($\varepsilon$ = 26.6 $mM \cdot cm^{-1}$). The assay mixture for CAT contained 100 µL of enzyme extract, 100 µL of $H_2O_2$ (300 mM), and 2.8 mL of 50 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). CAT activity was assayed by observing decline absorbance reading at 240 nm ($\varepsilon$ = 39.4 $mM \cdot cm^{-1}$).

The activity of Ascorbate peroxidase (APX; EC 1.11.1.11) was assayed by the method developed by Nakano and Asada (1981) [63]. A 3 mL reaction mixture consisted of 25 µL enzyme extract, 100 µL ascorbate (7.5 mM), 100 µL $H_2O_2$ (300 mM), and 2.775 mL of 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation of ascorbate was determined by the decrease of absorbance at 290 nm ($\varepsilon$ = 2.8 $mM \cdot cm^{-1}$).

The polyphenol oxidase (PPO; EC 1.10.3.2) activity was determined following the method described by Tagele et al. (2019) [64]. The reaction mixture contained 2 mL of extract, 3 mL of 0.1M sodium phosphate buffer (pH 7.0), and 1 mL catechol (0.01M). The mixture was incubated for 5 min at 28 °C and immediately read the absorbance against a substrate blank at a wavelength of 495 nm at the one-minute interval. The activity of PPO was expressed as enzyme activity per mg dry weight per minute [65].

### *2.9. Determination of Total Phenolic Content, Total Flavonoid Content, and Antioxidant Capacity*

The freeze-dried (25 mg) sample was dissolved in 10 mL of ethanol (80%, *v/v* in water) followed by sonication at 35 °C for 60 min. Afterward, the extracts were filtered (Advantech 5B filter paper, Tokoyo Roshi Kaisha Ltd., Saitama, Japan) and kept in a refrigerator (4 °C for further analysis).

Folin–Ciocalteu method was performed to estimate the total phenolic content (TPC) of the sample [66]. A reaction mixture contained 1 mL of sample, 200 µL of phenol reagent (1N), and 1.8 mL of distilled water. The mixture was vortexed and 3 min later 400 µL of $Na_2CO_3$ (10%, *v/v* in water)

was added. After that, 600 µL of distilled water was added to get the final volume (4 mL) and left for 1 h incubation at room temperature. The absorbance was taken at 725 nm, and the phenolic acid was calculated from a standard calibration curve of Gallic acid and expressed as $\mu g \cdot g^{-1}$ dry weight.

The total flavonoid content (TFC) was estimated following the method described by Adnan et al. (2019) with modifications [67]. In brief, 500 µL of the extract was mixed with 100 µL of $Al(NO_3)_3$ (10%, *w*/*v*) and 100 µL of potassium acetate (1 M) solution, and finally, 3.3 mL of distilled water was added to adjust the volume up to 4 mL. The reaction mixture was vortexed and left at room temperature for incubation for 40 min and the absorbance was measured at 415 nm by a UV-Vis spectrophotometer. The total flavonoid was measured as mg/g of Quercetin equivalent on a dry weight basis.

DPPH (2,2-diphenyl-1 picryl hydrazyl) was used to assess the antioxidant capacity of sugar beet leaf extract following the method described by Braca et al. (2003) [68]. Firstly, DPPH powder (5.914 mg) was dissolved in methanol (100 mL) to prepare a stock solution, and the absorbance range was maintained between 1.1 and 1.3 by a spectrophotometer. After that, 1 mL of extract was mixed with 3 mL of DPPH solution followed by shaking vigorously and kept in a dark room for 30 min at room temperature. The blank sample was produced by mixing distilled water (1 mL) with DPPH solution instead of extract. The absorbance was taken at 517 nm by a UV-Vis spectrophotometer (UV-180 240 V, Shimadzu Corporation, Kyoto, Japan). The scavenging capacity of the samples was calculated by using the following formula and results were expressed as percentage (%):

$$\text{Inhibition (\%)} = [(\text{blank sample} - \text{extract sample})/\ \text{blank sample}] \times 100.$$

### *2.10. Statistical Analysis*

All results were expressed as mean ± SD (standard deviation) and mean ± SE (standard error) in tables and graphs respectively with 3 replications. All graphs were prepared by GraphPad prism 5 (San Diego, CA 92108, USA). A two-factor analysis followed by Duncan's multiple range test (DMRT) was done by Statistix 10 (Tallahassee, FL 32312, USA). The correlation test was done by SPSS statistical software package (Ver. 23.0, SPSS Inc., Chicago, IL, USA). The least significant differences (LSD) were calculated to compare the means of different treatments with 5% level of probability.

## 3. Results and Discussions

### *3.1. Response to Drought Stress on Plant Growth Characteristics*

Plant height, fresh weight, and dry weight significantly reduced ($p < 0.05$) under drought compared to the control condition in most of the genotypes (Table 2). In the case of all genotypes, plant height for control and drought conditions was found ranging from 27.67 to 18.33 cm and 21 to 16 cm, respectively. Higher plant height was recorded from S6 followed by S5, S7, S2, S3, S1, and S4 in control while S6 followed by S4, S3, S7, and S1 in drought condition. Plant fresh weight ranged from 5.94 to 3.38 g in control where the higher value was recorded for S2 followed by S9, S1, S5, and S8 genotypes. In comparison to control, drought stress condition revealed lower weight in all genotypes ranging from 2.5 to 1.11 g where S10 followed by S4, S2, S11 and S9 were recorded as a higher value. Besides, plant dry weight ranged from 0.37 to 0.17 g in control and 0.27 g to 0.13 g in drought condition. Higher plant dry weight was recorded from the genotype S2 followed by S9 and S5 in control but in case of drought, higher dry weight was recorded from the genotype S2 followed by S4, S9 and S10.

In the present experiment, cessation of watering for 10 days imposed severe water stress on all sugar beet genotypes resulted in a reduction of plant height, fresh weight and dry weight. Sugar beet is a well-known sensitive crop to water stress [6,69,70]. It is well described that drought stress can suppress seedling growth, leading to an ultimate reduction in biomass production [71–73]. Moreover, photosynthesis rate, partitioning coefficients and accumulations of photosynthetic products in different organs can be affected by the prolongation of drought [74]. It was also reported that drought has a

negative impact on sugar beet height and dry weight [75]. Our present investigation also found a negative impact of drought stress on sugar beet growth and dry matter accumulation especially in S1, S3, S5, and S7 which might be due to the lower production of plant biomass.

**Table 2.** Plant height, fresh weight and dry weight of 11 genotypes of sugar beet (*Beta vulgaris* L.) under control and drought conditions.

| Cultivars | Plant Height (cm) | | Fresh Weight/Plant (gm) | | Dry Weight/Plant (gm) | |
|---|---|---|---|---|---|---|
| | Control | Drought | Control | Drought | Control | Drought |
| **S1** | 23.33 ± 1.53 bc | 17.67 ± 0.58 de | 5.09 ± 1.52 abc | 1.72 ± 0.38 gh | 0.28 ± 0.09 bcd | 0.19 ± 0.03 eh |
| **S2** | 24.33 ± 1.53 bc | 17.17 ± 0.29 e | 5.94 ± 0.77 a | 1.99 ± 0.43 gh | 0.36 ± 0.04 a | 0.27 ± 0.09 b–e |
| **S3** | 23.83 ± 3.82 bc | 18.33 ± 1.15 de | 3.38 ± 1.20 ef | 1.10 ± 0.13 h | 0.20 ± 0.09 d–h | 0.13 ± 0.04 h |
| **S4** | 23.33 ± 3.79 bc | 18.43 ± 3.19 de | 4.00 ± 0.50 cde | 2.07 ± 0.41 gh | 0.25 ± 0.02 b–f | 0.23 ± 0.02 c–g |
| **S5** | 25.00 ± 1.00 ab | 17.17 ± 2.93 e | 4.72 ± 1.30 bcd | 1.38 ± 0.42 gh | 0.28 ± 0.06 abc | 0.14 ± 0.03 h |
| **S6** | 27.67 ± 0.76 a | 21.00 ± 3.04 cd | 4.55 ± 1.51 cde | 1.38 ± 0.30 gh | 0.26 ± 0.08 b–e | 0.15 ± 0.02 gh |
| **S7** | 24.67 ± 2.08 ab | 18.17 ± 2.75 de | 4.12 ± 1.12 cde | 1.71 ± 0.29 gh | 0.26 ± 0.08 b–e | 0.18 ± 0.05 fgh |
| **S8** | 18.67 ± 0.58 de | 17.00 ± 2.18 e | 4.71 ± 0.19 bcd | 1.79 ± 0.37 gh | 0.27 ± 0.005 b–e | 0.19 ± 0.02 e–h |
| **S9** | 18.33 ± 1.53 de | 17.33 ± 2.25 e | 5.88 ± 0.12 ab | 1.85 ± 0.06 gh | 0.33 ± 0.03 ab | 0.23 ± 0.01 c–g |
| **S10** | 18.10 ± 0.36 de | 16.67 ± 1.53 e | 4.18 ± 0.19 cde | 2.50 ± 0.29 fg | 0.23 ± 0.01 c–g | 0.23 ± 0.01 c–g |
| **S11** | 19.33 ± 2.02 de | 16.00 ± 1.73 e | 3.54 ± 0.53 def | 1.86 ± 0.37 gh | 0.18 ± 0.02 gh | 0.18 ± 0.03 fgh |
| **LSD $_{(0.05)}$** | 3.49 | | 1.18 | | 0.08 | |

Different letters indicate significant differences ($p < 0.05$) among the genotypes within each parameter. Values are expressed as mean ± SD ($n = 3$).

### *3.2. Influence of Drought on Photosynthetic Pigments and Fluorescence*

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid significantly decreased ($p < 0.05$) in drought compared to control condition (Figure 1). In control, the genotype S3 was recorded with significantly higher chlorophyll a (23.05 mg·g$^{-1}$ dry weight), chlorophyll b (10.22 mg·g$^{-1}$ dry weight), and total chlorophyll content (33.28 mg·g$^{-1}$ dry weight) while the genotype S11 was detected as notable for higher Carotenoid content (5.25 mg·g$^{-1}$ dry weight). However, under drought condition, S10 genotype demonstrated remarkable tolerant efficiency with the highest pigments content, such as chlorophyll a (5.27 mg·g$^{-1}$ dry weight), chlorophyll b (1.88 mg·g$^{-1}$ dry weight), total chlorophyll (7.15 mg·g$^{-1}$ dry weight), and carotenoid (1.48 mg·g$^{-1}$ dry weight). Also, other significant cultivars including S2, S5, S7, S10, and S11 for control whereas S1, S2, and S9 genotypes were noteworthy for the drought stress condition. In the case of photosynthetic quantum yield analysis, no significant differences were observed among the genotypes, while it was varied significantly in drought conditions ($p < 0.05$) where S11, S7, S6, S4, S2, and S5 performed better than others.

It is well known that photosynthetic pigments considerably decrease under drought stress which causes impairment of plant growth and yield [76,77]. A low photosynthetic rate is a common issue under drought due to reduced green pigment synthesis [77]. In this study, chlorophyll a, b, total chlorophyll, and carotenoid contents significantly decreased in all the genotypes under drought conditions. However, the maximum reduction of pigments was observed in S3, S5, and S4 respectively. Importantly, activities of chlorophyllase and peroxidase are enhanced under stress conditions involved in chlorophyll breakdown that may have a crucial role in chlorophyll pigments reduction [78]. Apart from chlorophyll content and photosynthetic rate, drought has also a negative impact on different chlorophyll fluorescence parameters [79]. Concerning this principle in our study, quantum yield (Fv/Fm) was significantly reduced in most of the genotypes (except S3, S7, and S11) under 10 days' long drought condition. It was reported that the photochemical activity of photosystem II (PS II) and electron requirement for photosynthesis both might be influenced by the drought stress, resulting in an over-excitation and photo-inhibition damage to reaction centers of PS II [80,81]. An earlier study found a significant decrease of Fv/Fm under drought conditions with comparison to the well-irrigated condition [19]. The positive correlation (Table 3) among the photosynthetic pigments, Fv/Fm and growth parameters in the present study also comply with the previous findings.

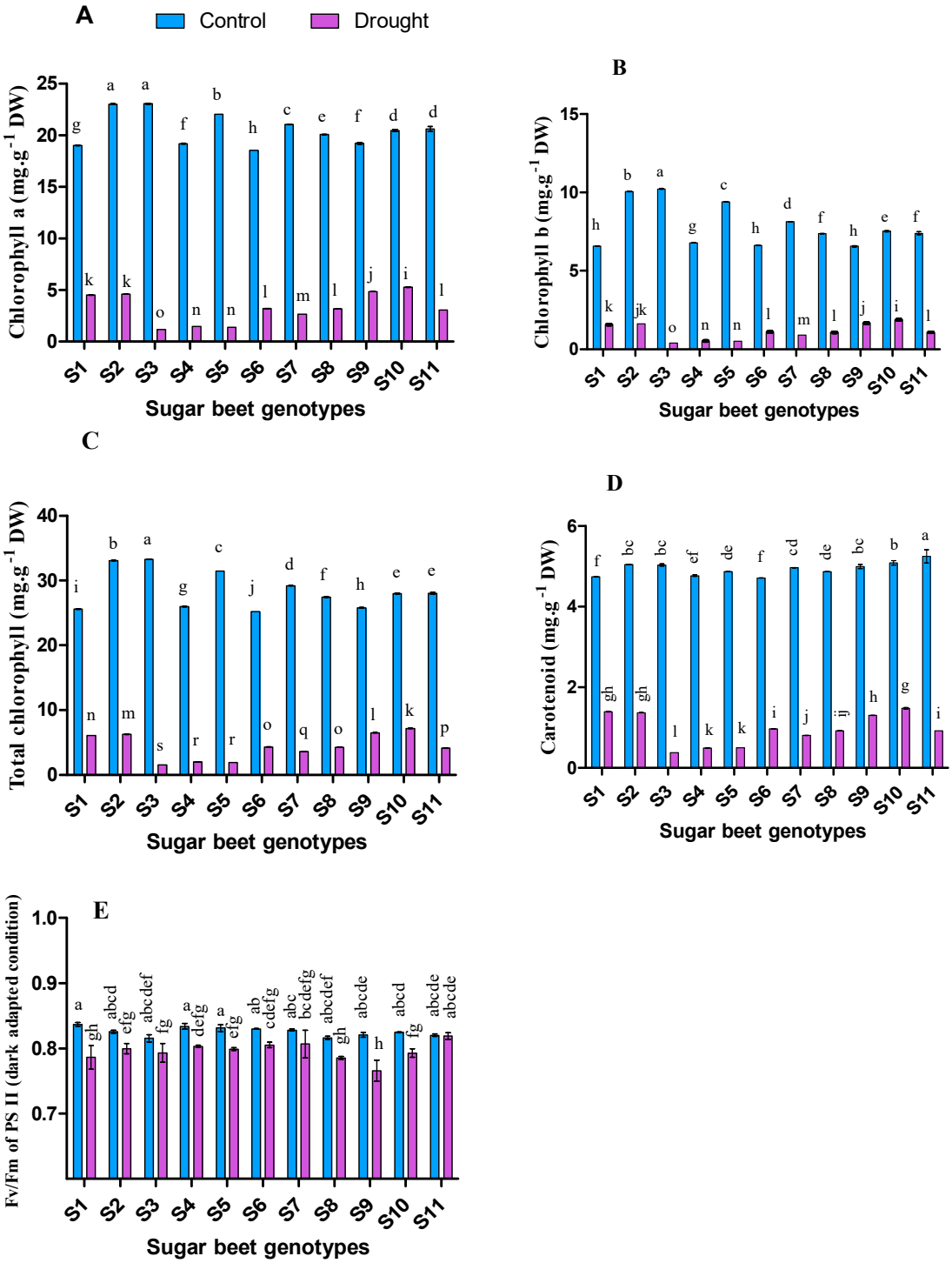


**Figure 1.** Chlorophyll a (**A**), Chlorophyll b (**B**), Total chlorophyll (**C**), Carotenoid (**D**), and Quantum yield (**E**) of 11 genotypes of sugar beet (*Beta vulgaris* L.) under control and drought conditions. Values are expressed as mean ± S.E. ($n = 3$). Different letters indicate significant differences ($p < 0.05$) among the genotypes within each parameter using Duncan's multiple range test.

**Table 3.** Correlations among the parameters in 11 genotypes of sugar beet (*Beta vulgaris* L.) under control and drought conditions.

| | DTI | Chl a | Chl b | Car | RWC | MSI | MDA | Fv/Fm | Plant ht. | Plant fr. Wt. | Plant dry wt. | H2O2 | SOD | CAT | APX | POD | PPO | Proline | GB | TSC | Sucrose | TPC | TFC | DPPH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DTI** | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| **Chl a** | 0.005 | 1 | | | | | | | | | | | | | | | | | | | | | | |
| **Chl b** | −0.036 | 0.988 ** | 1 | | | | | | | | | | | | | | | | | | | | | |
| **Car** | 0.038 | 0.994 ** | 0.967 ** | 1 | | | | | | | | | | | | | | | | | | | | |
| **RWC** | 0.025 | 0.926 ** | 0.893 ** | 0.932 ** | 1 | | | | | | | | | | | | | | | | | | | |
| **MSI** | −0.256 | 0.276 | 0.282 | 0.262 | 0.286 | 1 | | | | | | | | | | | | | | | | | | |
| **MDA** | −0.133 | −0.758 ** | −0.705 ** | −0.786 ** | −0.728 ** | −0.176 | 1 | | | | | | | | | | | | | | | | | |
| **Fv/Fm** | 0.073 | 0.767 ** | 0.739 ** | 0.772 ** | 0.762 ** | 0.065 | −0.463 * | 1 | | | | | | | | | | | | | | | | |
| **Plant ht.** | −0.333 | 0.699 ** | 0.721 ** | 0.669 ** | 0.570 ** | 0.239 | −0.444 * | 0.681 ** | 1 | | | | | | | | | | | | | | | |
| **Plant fr. Wt.** | −0.142 | 0.709 ** | 0.731 ** | 0.676 ** | 0.606 ** | 0.125 | −0.374 | 0.717 ** | 0.927 ** | 1 | | | | | | | | | | | | | | |
| **Plant dry wt.** | −0.202 | 0.391 | 0.42 | 0.356 | 0.315 | −0.026 | −0.105 | 0.495 * | 0.811 ** | 0.90 ** | 1 | | | | | | | | | | | | | |
| **H2O2** | −0.079 | −0.580 ** | −0.565 ** | −0.587 ** | −0.426 * | 0.015 | 0.498 * | −0.365 | −0.465 * | −0.429 * | −0.346 | 1 | | | | | | | | | | | | |
| **SOD** | 0.365 | 0.560 ** | 0.514 * | 0.582 ** | 0.595 ** | 0.415 | −0.643 ** | 0.342 | 0.197 | 0.265 | −0.05 | −0.174 | 1 | | | | | | | | | | | |
| **CAT** | 0.178 | 0.609 ** | 0.518 * | 0.659 ** | 0.668 ** | 0.257 | −0.693 ** | 0.465 * | 0.194 | 0.151 | −0.134 | −0.343 | 0.640 ** | 1 | | | | | | | | | | |
| **APX** | 0.18 | 0.391 | 0.38 | 0.392 | 0.285 | 0.450 * | −0.414 | 0.204 | 0.253 | 0.219 | −0.024 | −0.141 | 0.648 ** | 0.385 | 1 | | | | | | | | | |
| **POD** | −0.229 | 0.831 ** | 0.835 ** | 0.817 ** | 0.752 ** | 0.388 | −0.584 ** | 0.604 ** | 0.760 ** | 0.713 ** | 0.492 * | −0.356 | 0.332 | 0.36 | 0.406 | 1 | | | | | | | | |
| **PPO** | −0.197 | −0.596 ** | −0.558 ** | −0.614 ** | −0.461 * | −0.175 | 0.625 | −0.412 | −0.301 | −0.329 | −0.045 | 0.389 | −0.610 ** | −0.531 * | −0.642 ** | −0.429 * | 1 | | | | | | | |
| **Proline** | −0.054 | −0.867 ** | −0.840 ** | −0.873 ** | −0.744 ** | −0.282 | 0.835 ** | −0.530* | −0.594 ** | −0.590 ** | −0.293 | 0.606 ** | −0.589 ** | −0.561 ** | −0.484 * | −0.750 ** | 0.704 ** | 1 | | | | | | |
| **GB** | 0.094 | −0.366 | −0.301 | −0.404 | −0.443 * | −0.513 * | 0.42 | −0.231 | −0.177 | −0.071 | 0.108 | −0.059 | −0.461 * | −0.702 ** | −0.539 ** | −0.473 * | 0.347 | 0.295 | 1 | | | | | |
| **TSC** | −0.327 | −0.500 * | −0.480 * | −0.507 * | −0.494 * | −0.440 * | 0.355 | −0.583 ** | −0.266 | −0.275 | −0.023 | 0.205 | −0.590 ** | −0.470 * | −0.428 * | −0.243 | 0.364 | 0.33 | 0.221 | 1 | | | | |
| **Sucrose** | −0.402 | −0.425 * | −0.416 | −0.419 | −0.486 * | −0.268 | 0.278 | −0.535 * | −0.203 | −0.276 | −0.114 | 0.188 | −0.503 * | −0.276 | −0.288 | −0.185 | 0.226 | 0.296 | −0.011 | 0.801 ** | 1 | | | |
| **TPC** | −0.093 | −0.964 ** | −0.941 ** | −0.965 ** | −0.885 ** | −0.235 | 0.729 ** | −0.790 ** | −0.671 ** | −0.692 ** | −0.354 | 0.553 ** | −0.634 ** | −0.616 ** | −0.486 * | −0.769 ** | 0.634 ** | 0.842 ** | 0.375 | 0.533 * | 0.462 * | 1 | | |
| **TFC** | −0.132 | −0.863 ** | −0.855 ** | −0.853 ** | −0.832 ** | −0.275 | 0.534 * | −0.820 ** | −0.757 ** | −0.636 ** | −0.301 | 0.377 | −0.635 ** | −0.617 ** | −0.460 * | −0.645 ** | 0.501 * | 0.618 ** | 0.391 | 0.660 ** | 0.536 * | 0.903 ** | 1 | |
| **DPPH** | 0.003 | −0.506 * | −0.478 * | −0.517 * | −0.455 * | 0.274 | 0.403 | −0.391 | −0.245 | −0.275 | −0.088 | 0.291 | −0.221 | −0.436 * | −0.25 | −0.314 | 0.253 | 0.423 * | 0.217 | −0.031 | 0.035 | 0.549 ** | 0.506 * | 1 |

* Indicates statistical difference significance at $p < 0.05$ among the treatments by Duncan's multiple range tests; ** Indicates statistical difference significance at $p < 0.01$ among the treatments by Duncan's multiple range tests.

### *3.3. Effect of Drought on Drought Tolerance Index, Relative Water Content (%), and Membrane Stability Index (%)*

Drought tolerance index (DTI) varied among the sugar beet genotypes ranging from 1.55 to 0.69 (Figure 2). The highest DTI was recorded from S10 genotype followed by S4 and S11. Relative water content (RWC %) was found higher in control (70.01 to 85.26) than drought (43.47 to 57.88) (Figure 3). Despite low statistical dissimilarities among most of the genotypes, S10 followed by S8, S7, S4, and S9 from control, and S5 followed by S9, S11, S4, and S2 from drought were found with higher RWC. Besides, membrane stability index (MSI %) was higher in control than drought condition for all the cultivars, but significant ($p < 0.05$) genotypes were noted for S5, S10, and S11. Overall, S9 genotype was recorded as higher MSI (%) in the case of both control (92.24) and drought condition (88.17) (Figure 3).

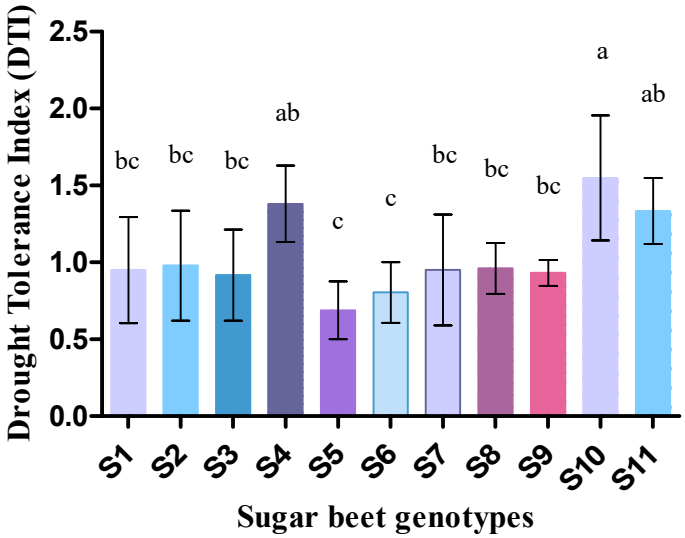


**Figure 2.** Drought tolerance index of 11 genotypes of sugar beet (*Beta vulgaris* L.) under control and drought conditions. Values are expressed as mean ± S.E. ($n = 3$). Different letters indicate significant differences ($p < 0.05$) among the genotypes within each parameter using Duncan's multiple range test.

Evidence found that the cultivars with higher DTI under drought have a better carbon assimilation rate than that of lower DTI, but they may grow slowly and their productivity also hampered [52]. But in our study, the genotypes with higher DTI manifested relatively higher fresh and dry weight at the seedling stage. However, in a strict sense, this phenomenon is treated as "drought delay" not "drought tolerance" but still may be considered as a good index for early selection of suitable sugar beet genotypes under drought stress.

RWC is one of the most important and reliable physiological indices by which assist to evaluate a particular genotype whether it is drought tolerant or not [69,82]. As shown in Figure 4, despite the reduction of RWC in all the genotypes under drought stress condition, the genotypes S2, S4, S5, S9, and S11 exposed as higher RWC with minimum loss (30%, 34%, 19.35%, 31.4%, and 25.4% respectively) compare to others. This higher RWC may be caused due to stomatal closure induced by stress, resulting reduction of transpiration [83,84]. To maintain cell integrity and proper functioning, the stability of cell membrane lipidome is an important index in a growing plant [85]. Like RWC, a higher membrane stability index (MSI) of tolerant genotypes may be due to reduced transpiration and efflux of solutes that are involved in the osmoregulation process of plant leaves [86]. In the present experiment the genotypes S3, S6, and S9 demonstrated strong MSI in drought conditions compared to control. Evidence showed that protecting the cell membrane helps from reducing sugar beet root yield under drought stress in different genotypes [87]. Thus, the genotypes with higher MSI under drought condition may be considered as tolerant plants.

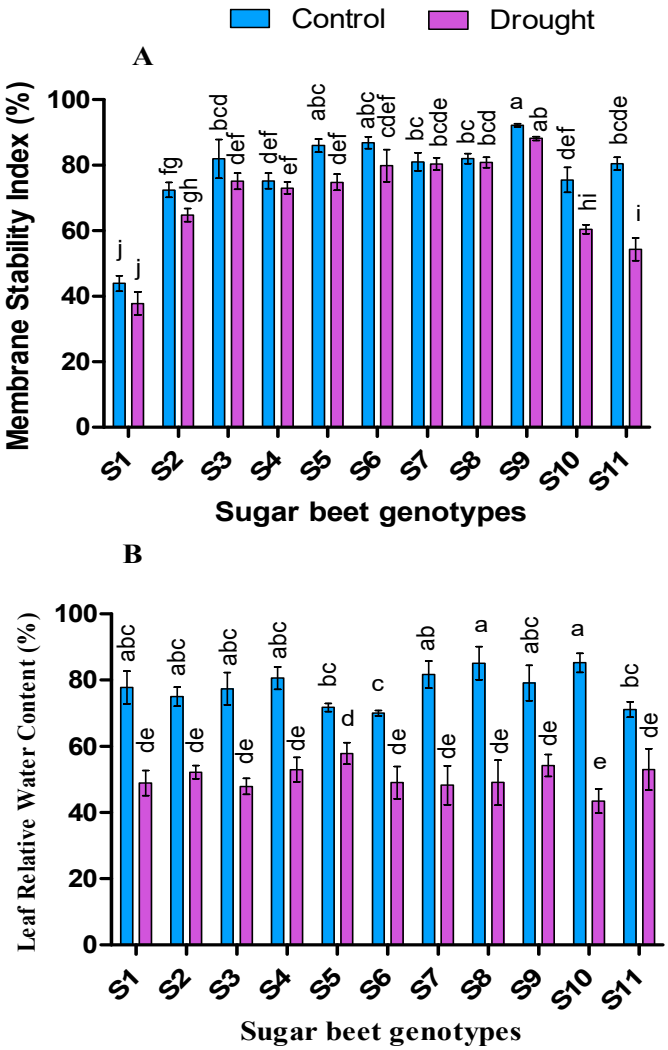


**Figure 3.** Membrane stability index (**A**) and leaf relative water content (**B**) of 11 genotypes of sugar beet (*Beta vulgaris* L.) under control and drought conditions. Values are expressed as mean ± S.E. ($n = 3$). Different letters indicate significant differences ($p < 0.05$) among the genotypes within each parameter using Duncan's multiple range test.

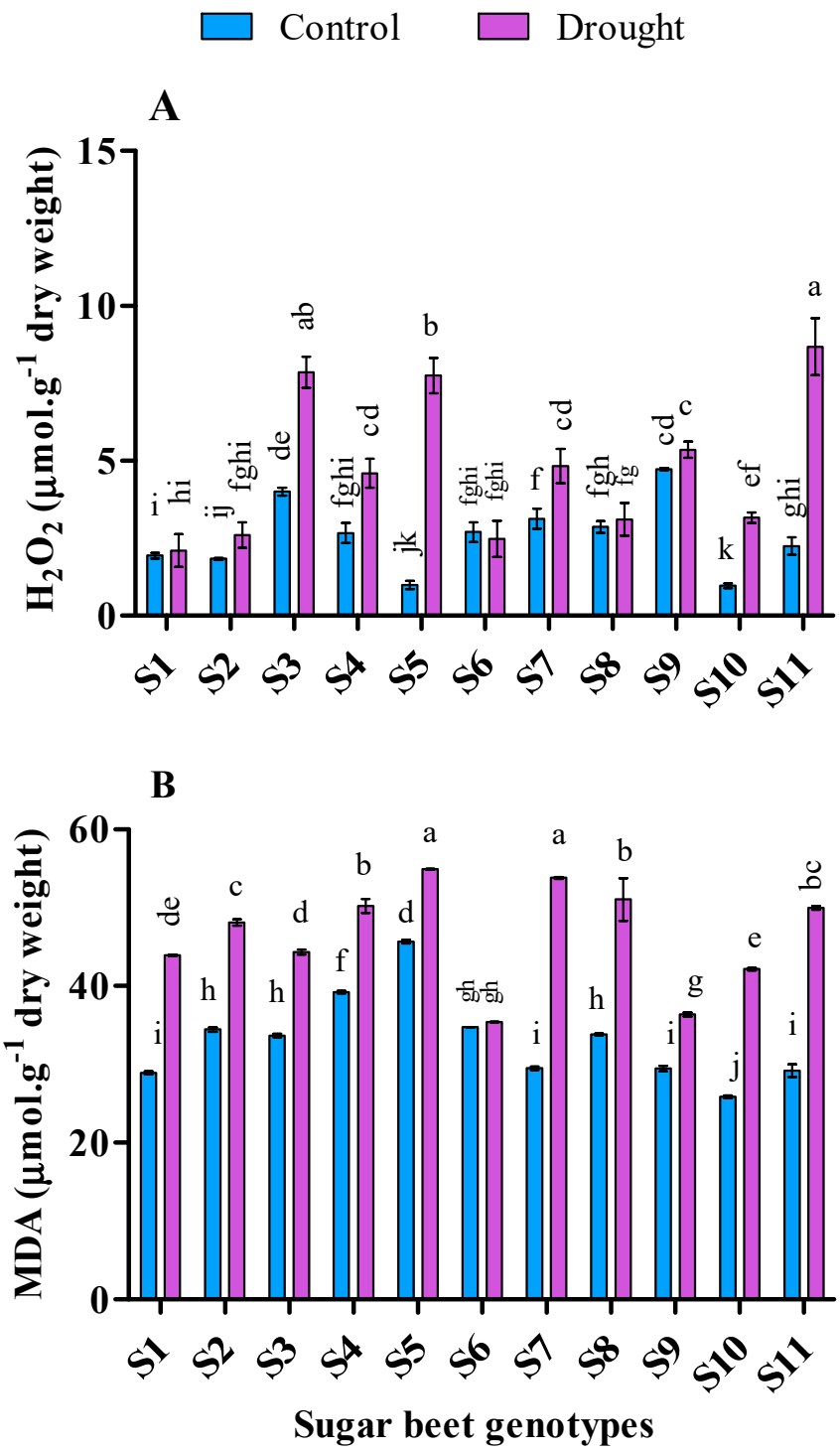


**Figure 4.** Hydrogen peroxide (**A**) and malondialdehyde content (**B**) of 11 genotypes of sugar beet (Beta vulgaris L.) under control and drought conditions. Values are expressed as mean ± S.E. ($n$ = 3). Different letters indicate significant differences ($p < 0.05$) among the genotypes within each parameter using Duncan's multiple range test.

### *3.4. Influence of Drought Stress on Lipid Peroxidation and Hydrogen Peroxide*

In this experiment, almost all the experimental genotypes contained higher malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) in drought compare to control ($p < 0.05$) (Figure 4). During drought stress, genotype S6 revealed top-most tolerance capacity confirmed by the lowest MDA (35.44 µmol·g$^{-1}$ dry weight) and $H_2O_2$ (2.48 µmol·g$^{-1}$ dry weight). Moreover, genotypes S9

(36.38 μmol·g$^{-1}$ dry weight), S3 (44.34 μmol·g$^{-1}$ dry weight), and S1 (43.91 μmol·g$^{-1}$ dry weight) in MDA as well as S1 (2.10 μmol·g$^{-1}$ dry weight), S2 (2.6 μmol·g$^{-1}$ dry weight), and S8 (3.1 μmol·g$^{-1}$ dry weight) in $H_2O_2$ demonstrated considerable drought tolerance in compare to control.

The value of the estimated MDA level is commonly used to measure the quantity of lipid peroxidation "an exponent of cell membrane damage" under drought [88]. MDA is a valuable indicator to determine the degree of damage at the cellular level caused by abiotic stress [89]. In the present study, the lower increment of MDA was recorded from S6 (1.98%) followed by S5 (20.24%), S9 (23.50%) and S3 (31.67%) and lower increment of $H_2O_2$ was recorded from S6 (−8.22%) followed by S8 (8.3%), S1 (8.7%), and S9 (13.15%). It was stated that oxidative burst induced by drought stimulate to increase MDA level, has been regarded as an index of lipid peroxidation, membrane permeability, and finally cell injury [90]. In a previous study, it was reported that, MDA folds two times higher than control in sugar beet under drought stress [91] which is in agreement with our present experiment. In contrast, over-expression of $H_2O_2$ may cause cell damage, but depend on the strong ROS scavenging mechanisms; it may act as a signaling molecule [92]. A similar experiment on *Amaranthus tricolor* [93] and fescue genotypes [29] showed that both MDA and $H_2O_2$ increased in drought conditions and they augmented progressively with the increment of drought stress. Thus, a higher generation of MDA and $H_2O_2$ can boost up the damage of cell membranes more in sensitive genotypes than tolerant genotypes. In our study, MDA and $H_2O_2$ showed a positive correlation with each other (Table 3). On the other hand, MDA manifested a positive correlation with proline, TPC and TFC, and a negative correlation with growth, photosynthetic pigments, Fv/Fm, MSI, and RWC. In this connection, higher MSI was observed in S2, S6, and S9, and higher RWC in S6 and S9 genotypes respectively with lower MDA and $H_2O_2$ accumulation.

### *3.5. Effect of Drought on Osmotic Adjustment Molecules*

The sugar beet genotypes varied concerning individual osmolytes in their leaves (Table 4). Proline was found significantly higher ($p < 0.05$) in all the genotypes in drought than the control condition. A similar result was also observed for glycine betaine (GB), total soluble carbohydrate (TSC), and sucrose in most of the case. It was observed that S4, S5, S7, and S11 produced higher proline than others ($p < 0.05$), while S1, S2, S4, S6, and S11 produced higher GB and S1, S5, and S10 produced both higher TSC and sucrose under drought condition.

**Table 4.** Osmolytes of 11 genotypes of sugar beet (*Beta vulgaris* L.) under control and drought conditions.

| Genotypes | Proline (μg·g$^{-1}$ DW) | | Glycine Betaine (mg·g$^{-1}$ DW) | | TSC (μg·g$^{-1}$ DW) | | Sucrose (μg·g$^{-1}$ DW) | |
|---|---|---|---|---|---|---|---|---|
| | Control | Drought | Control | Drought | Control | Drought | Control | Drought |
| **S1** | 12.99 ± 0.44 h | 159.45 ± 0.16 c | 7.35 ± 0.05 g | 8.37 ± 0.03 d | 471.33 ± 13.87 ghi | 670.67 ± 14.39 a | 151.17 ± 1.39 e | 205.59 ± 4.48 a |
| **S2** | 9.70 ± 0.44 h | 119.01 ± 12.87 d | 8.91 ± 0.06 b | 8.78 ± 0.06 c | 389.44 ± 63.57 jkl | 533.00 ± 2.17 de | 142.94 ± 0.30 f | 174.09 ± 3.12 c |
| **S3** | 8.89 ± 0.22 h | 118.36 ± 15.39 d | 5.91 ± 0.06 k | 7.66 ± 0.06 f | 456.22 ± 24.94 hij | 547.22 ± 18.17 cd | 149.72 ± 7.19 e | 172.77 ± 4.07 cd |
| **S4** | 18.92 ± 2.35 h | 217.99 ± 15.21 b | 6.78 ± 0.08 i | 9.10 ± 0.1 a | 444.11 ± 40.21 hij | 388.44 ± 38.83 l | 127.51 ± 1.29 g | 96.15 ± 2.79 j |
| **S5** | 12.41 ± 2.69 h | 235.43 ± 0.21 a | 8.34 ± 0.07 d | 6.14 ± 0.05 j | 445.00 ± 9.75 h–k | 579.67 ± 11.07 bc | 124.25 ± 1.59 gh | 206.98 ± 5.08 a |
| **S6** | 8.20 ± 1.22 h | 102.49 ± 2.30 e | 4.61 ± 0.05 o | 7.78 ± 0.07 e | 435.22 ± 17.25 h–k | 496.78 ± 2.17 fg | 173.37 ± 2.58 c | 154.44 ± 0.40 e |
| **S7** | 8.56 ± 0.71 h | 233.46 ± 3.30 a | 3.09 ± 0.04 q | 4.72 ± 0.05 n | 496.78 ± 26.34 efg | 416.11 ± 2.80 kl | 139.02 ± 2.99 f | 167.28 ± 0.41 d |
| **S8** | 16.25 ± 0.34 h | 90.73 ± 7.13 e | 5.36 ± 0.05 l | 7.12 ± 0.05 h | 501.67 ± 18.15 ef | 477.33 ± 8.27 fgh | 139.35 ± 0.60 f | 149.74 ± 2.78 e |
| **S9** | 12.70 ± 4.62 h | 75.75 ± 12.19 f | 4.31 ± 0.06 p | 6.16 ± 0.04 j | 394.56 ± 10.56 l | 527.89 ± 27.71 de | 140.28 ± 8.35 f | 172.04 ± 3.87 cd |
| **S10** | 10.52 ± 0.09 h | 59.07 ± 4.83 g | 5.84 ± 0.06 k | 7.29 ± 0.04 g | 347.22 ± 27.74 m | 585.78 ± 3.89 b | 117.06 ± 2.70 i | 195.93 ± 2.78 b |
| **S11** | 7.62 ± 0.61 h | 172.39 ± 22.07 c | 5.24 ± 0.05 m | 7.76 ± 0.06 e | 320.11 ± 10.24 m | 433.89 ± 25.32 ijk | 125.26 ± 8.38 g | 119.04 ± 1.79 hi |
| **LSD $_{(0.05)}$** | 13.43 | | 0.097 | | 34.16 | | 6.08 | |

Different letters indicate significant differences ($p < 0.05$) among the genotypes within each parameter. Values are expressed as mean ± SD ($n = 3$).

The accumulation of proline is a useful indicator of stress in sugar beet which can act as a signaling molecule for modulation of mitochondrial functions, influencing cell proliferation and expression of specific stress tolerant genes [94,95]. From our experiment, maximum proline increment (%) was observed from S7 (>2600%) followed by S11 (>2150%), S5 (1800%) and S3 (>1200%). Proline is considered the most important organic solute can play a key role to regulate ROS, Hydroxyl radicals, also can be an important component of cell wall proteins and that is why genotype having more proline is considered as more resistant to drought stress [58,96].

Glycine betaine also important osmoprotectants produced in response to abiotic stress which can contribute to metabolic pathways like osmotic adjustments and osmoregulation consequently reduce the negative impact [97,98]. Under osmotic stress, proline generally is accumulated in the cytosol and plays an important role in osmotic adjustment in the cytoplasm, whereas Glycine betaine is accumulated mainly in chloroplast and takes part in maintaining photosynthetic efficiency by adjustment and protection of thylakoid membrane [99,100]. Glycine betaine can also mediate osmoregulation, scavenging free radical, maintain membrane integrity, and protecting macromolecules [101]. In our study, the maximum GB increment (%) was recorded from S6 (67%) followed by S7 (51%), S11 (46%), and S9 (42%).

The production and accumulation of TSC and sucrose in the plant can be used to avoid the adverse effect of water stress [58]. In a previous study, a strong correlation was described as drought stress with the accumulation of these substances [102]. In our study, increment of TSC was found higher in S10 (68.7%), S1 (42.3%), S2 (36.9%), S11 (35.5%), and S9 (33.8%) while S10 (67.4%), S5 (66.6%), S1 (36%), S9 (22.6%), and S2 (21.8%) were found with higher increment of sucrose under drought stress.

In our study, we observed that the ROS (MDA and $H_2O_2$) were significantly increased under drought stress. Generally, the plant creates its defensive mechanism to scavenge the ROS which in this regard positively produces a higher level of osmolytes. All the osmolytes maintained a positive correlation among them (Table 3). Besides, proline mediates a positive correlation with MDA and $H_2O_2$. We also observed that GB, TSC, and sucrose have a negative correlation with photosynthetic pigments, Fv/Fm, and RWC, etc. In a previous study, it is believed that TSC and sucrose accumulation under stress depends on lower photosynthesis rate and carbohydrate consumption in plants [103]. From the overall results the genotypes S2, S6, and S11 produced higher osmolytes with higher photosynthetic traits, MSI, and RWC with minimum cell damaged by reactive oxygen species.

### *3.6. Effect of Drought Stresses on Antioxidant Enzymes Activities*

The activities of antioxidant enzymes varied within the variety and treatments (Figure 5). Superoxide dismutase (SOD) ranged from 2187.6 to 1888.1 (units·$g^{-1}$ dry weight) and 2237.5 to 1636.9 (units·$g^{-1}$ dry weight) in control and drought, respectively. Higher SOD was recorded from the genotypes S10 followed by S11, S4, S8, S9, and S7 in control, while genotype S9 was followed by S11, S10, S6, S8, and S4 in that of drought condition. SOD increased in S9 (8.38%) but decreased in all other genotypes after 10 days of water rescission. However, the minimum reduction of SOD under drought condition was recorded by 4.14%, 4.53%, 6.37%, and 9.57% from the genotypes S11, S5, S6, and S8, respectively.

Catalase (CAT) ranged from 1.79 to 0.57 (mM·$g^{-1}$ dry weight) and 1.13 to 0.28 (mM·$g^{-1}$ dry weight) in control and drought treatment, respectively. Higher CAT was recorded from S10 followed by S11, S1, S8, and S7 in control, while S7 was followed by S9, S8 in that of drought condition. The activity of CAT was reduced in all genotypes under drought stress compared to the control condition. However, the minimum reduction was recorded at 9.89%, 12.83%, 21.52%, 24.75%, and 36.5% from the genotypes S5, S7, S9, S3, and S6 respectively.

Ascorbate peroxidase (APX) ranged from 6.68 to 3.63 (mM·$g^{-1}$ dry weight) and 6.12 to 1.39 (mM· $g^{-1}$ dry weight) in control and drought condition, respectively. Higher APX was recorded from S9 followed by S6, S7, S3, S10, and S2 in control, while S10 followed by S9, S8, S7, and S11 in that of drought condition. It was also observed that, only S8, S10, and S11 genotypes were found with

increased APX in drought treatment compared to the control condition, while the opposite result was observed from others. Also, during peroxidase (POD) analysis, S3 (1.11 mM·g$^{-1}$ dry weight) followed by S6, S7, S9, S4, and S8 demonstrated higher enzyme activity in control whereas S2 (0.43 mM·g$^{-1}$ dry weight) followed by S3, S10, S8, and S6 in drought condition.

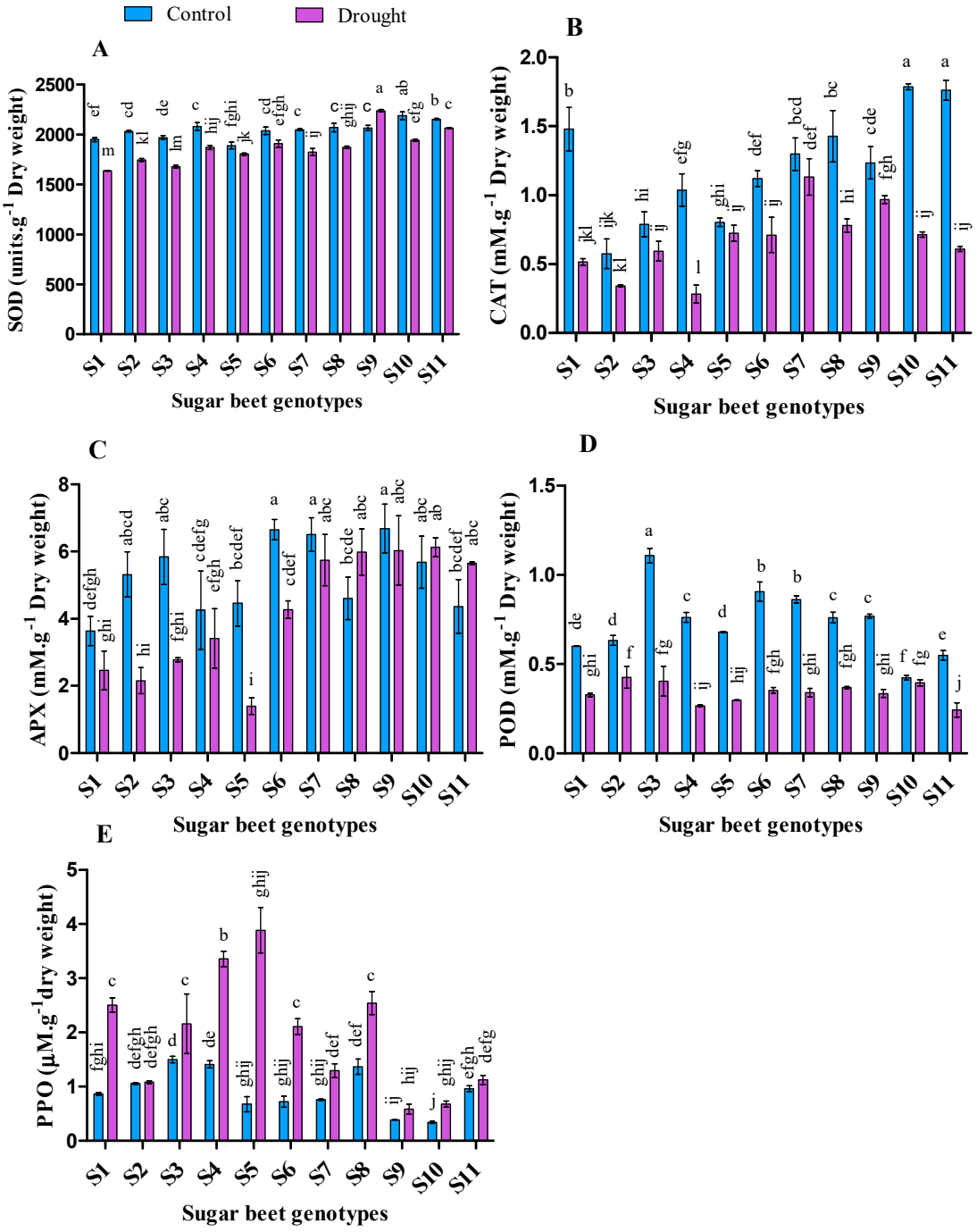


**Figure 5.** Activities of superoxide dismutase (SOD) (**A**), catalase (CAT) (**B**), ascorbate peroxidase (APX) (**C**), peroxidase (POD) (**D**), and polyphenol oxidase (PPO) (**E**) enzymes of 11 genotypes of sugar beet (*Beta vulgaris* L.) under control and drought conditions. Values are expressed as mean ± S.E. ($n$ = 3). Different letters indicate significant differences ($p < 0.05$) among the genotypes within each parameter using Duncan's multiple range test.

In the case of polyphenol oxidase (PPO), higher PPO was observed in all the genotypes in drought compared to the control condition. PPO ranged from 1.5 to 0.34 ($\mu M \cdot g^{-1}$ dry weight) and 3.89 to 0.59 ($\mu M \cdot g^{-1}$ dry weight) in control and drought conditions, respectively. Among all the genotypes, higher PPO was recorded for S3 followed by S4 and S8 in control, while S5 followed by S4, S8, S1, S3, and S6 in that of drought condition. Maximum augmentation in drought was observed 471.5% followed by 191.2%, 189.2%, and 138% from S5 followed by S6, S1, and S4 respectively.

Antioxidant enzyme defense activities had a high positive relation with increased abiotic stress [104]. Under drought stress, plants with higher antioxidant enzyme activities are considered to have a better free radical scavenging ability [105]. In this connection, cellular SOD acts as a first-line scavenger of ROS by catalyzing the superoxide radical ($O_2^{-\bullet}$) into oxygen and $H_2O_2$ [106,107]. It was stated that, prolong drought stress causes oxidative damage as exhibited by a general decline in antioxidant enzyme activities and an increase in lipid peroxidation [108]. In our study, higher lipid peroxidation was found with lower antioxidant activities in most cases. Generally, higher SOD activity may be exerted due to the consequences of excess superoxide radical generation [29] that can act as a signal for the induction of antioxidant enzyme, resulting in greater SOD induction [109]. This hypothesis indicated that the genotypes S9 followed by S11, S10, S6 and S8 with comparatively higher SOD might have a high capacity to catalyze superoxide radical under drought conditions.

Besides, CAT, APX, and POD are also the major enzymes involved in the detoxification of $H_2O_2$ (produced through the dismutation of $O_2^{-\bullet}$ in peroxisomes and chloroplasts) to $H_2O$ and $O_2$ [29,110,111]. In the present experiment, genotypes S6, S7, S8, S9, and S10 were found with higher CAT, APX, and POD activity under drought stress. This may be attributed to the increased $H_2O_2$ and MDA accumulation, resulting in excitation of enzyme activities. A similar response was also observed in previous research on cotton [112]. Usually, high oxidative stress provokes the $H_2O_2$ generation under drought stress which also stimulates to produce high antioxidant enzyme activity. Subsequently, this enzyme activity may minimize the adverse effect of abiotic stresses either by acting as a bio-signal and/or modulating resistant gene expression [113]. Moreover, It has been reported that PPO can protect the over reduction of photosynthetic electron transport during environmental stress [114]. In a previous study, PPO activity was found significantly high in white clover after 7 days of drought treatment [115]. However, some conflict results were also reported, which showed that suppression of PPO activity increased drought tolerance in tomato [116]. In our study, we observed a positive correlation of PPO with TPC (Table 3). In this connection, the genotypes S1, S3, S5, S6, and S8 performed better in drought conditions. Besides, PPO can directly influence photosynthesis by acting as an oxygen buffer to facilitate reactive oxygen scavenging [114,117,118]. Although PPO regulates available oxygen to oxidize phenolic compounds to o-quinones and $H_2O$, but it can regenerate o-diphenol to o-quinones by providing a source of reducing power with the close association of photosystems during photosynthesis [118–120].

In general, under stress conditions plant physiological activities and performance decline with a decrease in antioxidant enzymes activities and an increase in ROS chemicals. These phenomena indicating an inability of plant cells against ROS chemicals. The activities and involvement of different antioxidant enzymes in ROS scavenging vary with some factors including plant species, stress severity and duration [108]. Sairam et al. (2000) observed a variation of antioxidant enzymes activities such as SOD, CAT, and APX among different wheat genotypes [121]. In the present study, we observed lower activities of SOD in S5, CAT in S2, S4, S5, S6, and S8, APX in S4 and S5, POD in S4, S5 and S11, and PPO in S7 and S11 genotypes along with a higher accumulation of MDA. From the overall observations the genotypes S6, S7, S8, S9, and S10 showed better stress response through redox homeostasis process by using different antioxidant enzymes under drought condition.

### *3.7. Influence of Drought Stresses on Antioxidant Activities*

Total phenolic content (TPC) was augmented in all the genotypes under drought stress ($p < 0.05$) in the present experiment (Figure 6). TPC ranged from 170.37 to 32.59 ($\mu g \cdot g^{-1}$ as GAE) and 690.37

to 340.74 (μg·g$^{-1}$ as GAE) in control and drought, respectively. Higher TPC was recorded from S4 followed by S3, S10, S8, S9, and S11 in control, while S3 was followed by S5, S6, S1, S2, and S7 in that of drought condition.

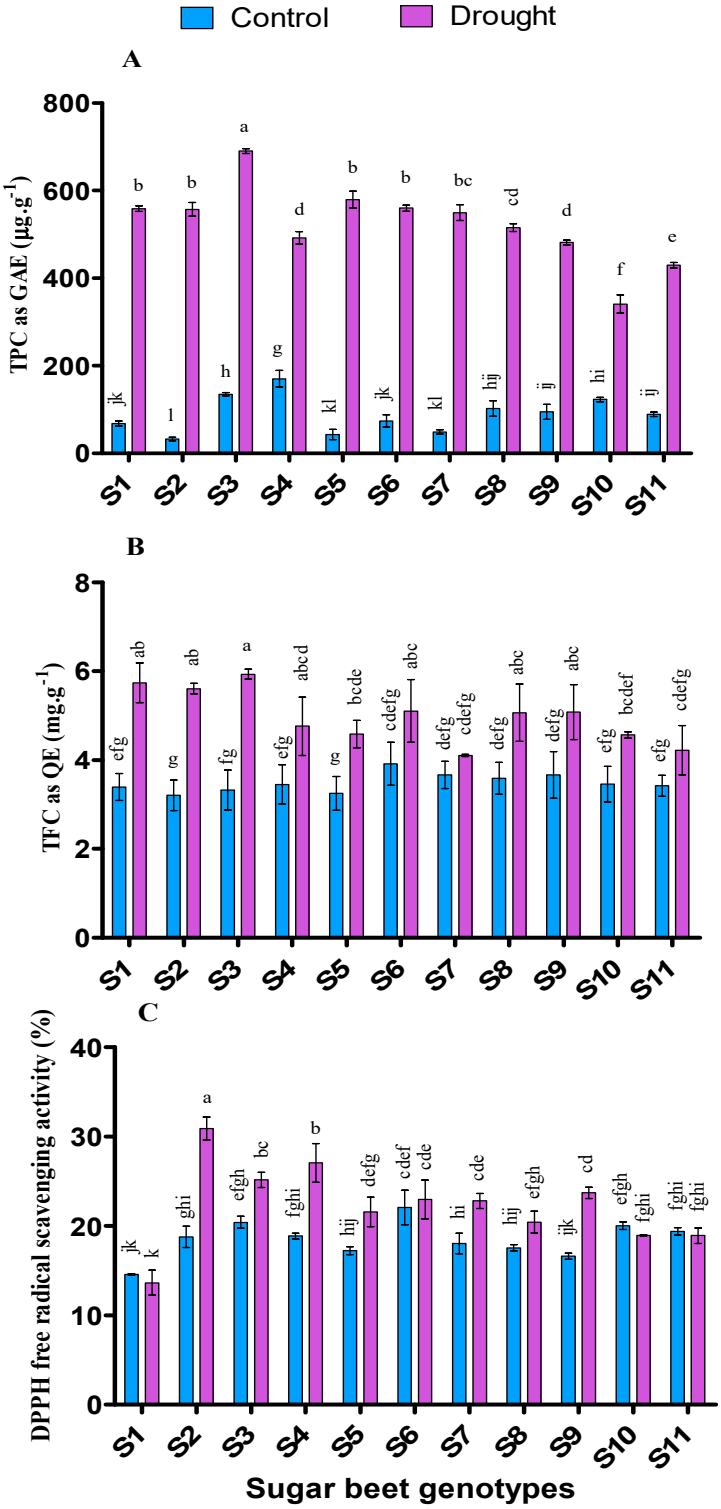


**Figure 6.** Total polyphenol content (TPC) (**A**), total flavonoid content (TFC) (**B**) and DPPH radical scavenging activity (%) (**C**) of 11 genotypes of sugar beet (*Beta vulgaris* L.) under control and drought conditions. Values are expressed as mean ± S.E. ($n$ = 3). Different letters indicate significant differences ($p < 0.05$) among the genotypes within each parameter using Duncan's multiple range test.

Total flavonoid content (TFC) is also influenced by drought stress in all the genotypes compared to control ($p < 0.05$) (Figure 6). TFC ranged from 3.92 to 3.21 ($mg \cdot g^{-1}$ as QE) and 5.93 to 4.11 ($mg \cdot g^{-1}$ as QE) in control and drought treatment. Higher TFC was recorded from S6 followed by S9, S7, and S8 in control, while S3 was followed by S1, S2, S6, S9, S8, and S4 in that of drought condition.

Antioxidant capacity (DPPH) was observed higher under drought treatment than the control condition ($p < 0.05$) in most of the genotypes (except S1, S10, and S11) (Figure 6). DPPH radical scavenging activity (%) ranged from 22% to 14.58% and 30.91% to 13.65% in control and drought, respectively. Higher activity was observed from S6 followed by S3, S10, S11, and S4 in control while S2 followed by S4, S3, S9, S6, S7, and S5 in drought condition.

Both TPC and TFC were found higher under drought stress compared to control in all the cultivars. In a previous experiment, it was observed that TPC, TFC, and total antioxidant activity were significantly increased with the severity of drought stress in Amaranthus leafy vegetables [122]. In our experiment, higher augmentation of TPC were observed in S2 (1600%), S5 (1250%), S7 (1025%), and S1 (720%); higher TFC augmented in S3 (78%), S2 (75%), and S1 (69%) while higher DPPH activity were found in S2 (64%) followed by S4 (43%), S9 (73%), S7 (26%), and S5 (25%) under drought stress. In general, the accumulation of ROS under biotic and abiotic stress stimulates to enhance the antioxidant properties of plants [123]. Polyphenols can scavenge reactive oxygen species and may modulate MDA by controlling lipid alkoxyl radical. On the other hand, peroxidase can oxidize flavonoids and phenylpropanoids, resulting in $H_2O_2$-scavenging in the phenolic/ASA/POD system [93].

Generally, total antioxidant activity is the combined activities of both enzymatic and non-enzymatic antioxidants in plants. In a previous experiment, total antioxidant activity along with total polyphenol and total flavonoid content significantly increased in *Achillea* species [124] and also in soybean [125] by drought stress. Tolerant plants can protect themselves by augmenting the antioxidant enzymes, molecules activity, and quantity under stress (biotic or abiotic) condition [93]. A plant having higher antioxidants can improve their defensive responses under oxidative stress by scavenging free radicals rapidly [126]. Since drought can trigger the increment of ROS, hence a higher amount of antioxidants are required to get acclimated by improving the tolerance level [127]. Antioxidant activity plays a vital role in plant defense mechanisms by keeping the balance between synthesis and scavenging of free radicals [128]. In our study, antioxidants have a positive correlation with osmolytes however, a vice-versa relationship was also observed with enzymes (Table 3). Overall, the genotypes S2, S3, S5, S6, S7, and S9 performed better regarding TPC, TFC, and antioxidant capacity.

## 4. Conclusions

Under drought conditions, seedlings growth and development, photosynthetic pigments and photosynthetic quantum yield (Fv/Fm) expressively reduced compare to control in all sugar beet genotypes. Significant reductions were also observed in membrane stability index, leaf relative water content, and activity of antioxidant enzymes. Positive correlations were noticed between growth parameters and photosynthetic traits, physiological parameters and antioxidant enzyme activities respectively. On the other hand, drought significantly increased the reactive oxygen species and osmotic adjusting molecules in sugar beet seedlings. A remarkable increment of secondary metabolites and antioxidant capacity was also observed in drought conditions. Although enzymes have a negative correlation with osmolytes and secondary metabolites, the reactive oxygen species maintained a positive correlation with them. Based on the results, higher activities of antioxidant enzymes, osmolytes, membrane stability index and relative water content with lower reactive oxygen species accumulation were the most important factors considering drought-tolerant sugar beet seedlings. After analyzing all the data, the cultivars S2 (HELENIKA), S6 (RECODDINA), S7 (GREGOIA), S8 (SV2347), S9 (SV2348), S10 (BSRI Sugarbeet 1), and S11 (BSRI Sugarbeet 2) were found best fitted under drought condition. The findings of the experiment can help further for better understanding of different mechanisms related to the adaptability of sugar beet in drought conditions to improve drought tolerance cultivars.

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