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RESEARCH ARTICLE

Sesame (*Sesamum indicum* L.) response to drought stress: susceptible and tolerant genotypes exhibit different physiological, biochemical, and molecular response patterns

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Abstract

Drought is one of the main environmental stresses affecting the quality and quantity of sesame production worldwide. The present study was conducted to investigate the effect of drought stress and subsequent re-watering on physiological, biochemical, and molecular responses of two contrasted sesame genotypes (susceptible vs. tolerant). Results showed that plant growth, photosynthetic rate, stomatal conductance, transpiration rate, and relative water content were negatively affected in both genotypes during water deficit. Both genotypes accumulated more soluble sugars, free amino acids, and proline and exhibited an increased enzyme activity for peroxidase, catalase, superoxide dismutase, and pyruvate dehydrogenase in response to drought damages including increased lipid peroxidation and membrane disruption. However, the tolerant genotype revealed a more extended root system and a more efficient photosynthetic apparatus. It also accumulated more soluble sugars (152%), free amino acids (48%), proline (75%), and antioxidant enzymes while showing lower electrolyte leakage (26%), lipid peroxidation (31%), and starch (35%) content, compared to the susceptible genotype at severe drought. Moreover, drought-related genes such as MnSOD1, MnSOD2, and PDHA-M were more expressed in the tolerant genotype, which encode manganese-dependent superoxide dismutase and the alpha subunit of pyruvate dehydrogenase, respectively. Upon re-watering, tolerant genotype recovered to almost normal levels of photosynthesis, carboxylation efficiency, lipid peroxidation, and electrolyte leakage, while susceptible genotype still suffered critical issues. Overall, these results suggest that a developed root system and an efficient photosynthetic apparatus along with the timely and effective accumulation of protective compounds enabled the tolerant sesame to withstand stress and successfully return to a normal growth state after drought relief. The findings of this study can be used as promising criteria for evaluating genotypes under drought stress in future sesame breeding programs.

Keywords Antioxidant enzymes · Drought stress · Physiological and biochemical response · Sesame (*Sesamum indicum* L.) · Susceptible and tolerant genotypes

Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop grown in arid and semi-arid regions of the world. Sesame seeds contain about 25% protein, 50% oil rich in polyunsaturated fatty acids, and a high amount of antioxidant compounds such as sesamin, sesamolin, and tocopherol (Bedigian 2010). Sesame oil is of great importance in the food industry due to its stability, taste, and considerable nutritional value. Besides, various medicinal and pharmaceutical uses of sesame have been reported including antiulcer, antidiabetic, antitumor, cancer preventive, and cardioprotective (Cooney et al. 2001; Morris 2002; Yokota et al. 2007; Lin et al. 2014).

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Drought is a major abiotic stress that negatively affects the growth and productivity of crops including sesame, especially in arid and semi-arid regions. Osmotic adjustment is considered the main challenge plants face during drought. In osmotic stress conditions, growth is limited and a variety of physiological, molecular, and biochemical characteristics such as photosynthesis and metabolic processes are altered (Chintakovid et al. 2017). Photosynthesis is one of the first processes inhibited by drought stress. Reduced photosynthesis under water deficit is caused by a decrease in CO_2 availability and/or disruption of photosynthetic biochemical processes (Mo et al. 2016). Low availability of CO_2 is mainly caused by stomatal closure which limits CO_2 diffusion from the atmosphere to the leaves during water stress (Zhou et al. 2015). Decreased mesophyll conductance also limits CO_2 diffusion from sub-stomal cavities to carboxylation sites under drought conditions (Flexas et al. 2012).

Oxidative damage and subsequent production of reactive oxygen species (ROS) is another major negative effect of drought on plants. Normally, ROS are produced as a part of cell metabolism in plants (Verma et al. 2019). ROS production increases under drought stress conditions due to the disruption of CO_2 fixation and photosynthetic electron transport chain (Mattos and Moretti 2015). As long as this increase is under plant control at low levels, ROS functions as a component of the stress-signaling network and contributes to drought stress tolerance by activating defense responses (Verma et al. 2019). If ROS concentrations keep increasing, however, oxidative reactions start causing severe damage to the membranes, proteins, RNAs, and DNAs, ultimately leading to cell death (Verma et al. 2019).

Plants accumulate osmoprotectants such as soluble sugars, proline, and other free amino acids, in response to drought stress. These osmolyte compounds protect plant cells against perturbation caused by drought through adjusting osmotic balance, maintaining membrane fluidity, stabilizing cellular structures, and scavenging ROS (Ashraf and Foolad 2007). Moreover, these solutes facilitate the recovery of plant metabolism after stress relief (Abid et al. 2018). Soluble sugars were reported to play an important role in conferring drought tolerance in Cotton (Hasan et al. 2018). There are reports that high accumulation of proline was associated with enhanced drought tolerance in various plants under drought conditions (Khanna-Chopra et al. 2019).

Another strategy of plants to keep ROS under control and cope with oxidative damages during water stress is the employment of enzymatic and non-enzymatic antioxidants as a scavenging system. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) function as scavenging machines during water stress. The higher activity of antioxidant enzymes helped the tolerant cotton species to defend themselves more efficiently against ROS, under drought stress (Hasan et al. 2018).

Sesame is relatively tolerant to dehydration and drought due to its strong root system. However, drought stress restricts sesame growth and development and adversely affects the quality and quantity of its yield (Islam et al. 2016). It has been reported that drought stress, especially during flowering, considerably reduces the yield and quality of sesame seeds (Hassanzadeh et al. 2009; Dossa et al. 2017; Pandey et al. 2021). Therefore, it is necessary to study the protective mechanisms against drought stress and develop efficient ways to improve sesame tolerance. In recent years, studies on the physiological properties of sesame under drought stress have been growing in order to find the pattern of drought-response mechanisms and new approaches to improve its tolerance (Dossa et al. 2017; Gopika et al. 2022; Desoky et al. 2023). However, to the best of our knowledge, a comprehensive and comparative view of the sesame's molecular, physiological, and biochemical changes under drought stress considering the differences between susceptibility and tolerance responses is still lacking. Moreover, studies investigating the organization of the photosynthesis process, antioxidant activities, and osmolyte dynamics after relieving drought and during recovery are limited in sesame. Thus, the present study was conducted to explore the impact of progressive drought on photosynthesis and gas exchanges, cell and membrane stability, antioxidant activities, and accumulation of osmolytes and protective compounds during the stress and recovery periods, in two contrasting tolerant and susceptible genotypes of sesame.

Material and methods

Plant growth and drought treatments

Two contrasted *Sesamum indicum* L. genotypes named SaSiG004 (drought-susceptible) and SaSiG006 (drought-tolerant) were selected based on previous studies (Baghery et al. 2022a, 2022b) evaluating the effect of drought stress on a set of sesame genotypes collected from all over Iran and conserved at the plant breeding department of Sari Agricultural Sciences and Natural Resources University, Sari, Iran. The seeds were sown in pots (30 cm diameter and 45 cm depth) filled with 7 kg of silty-clay-loam soil (14% Sand, 51% Silt, 35% Clay) enriched with leaf compost and fertilizer. The experiment (2019–2020) was conducted as a completely randomized factorial design with two factors (two genotypes and five water treatments) and three replicates in the greenhouse. The mean temperature and relative humidity of the greenhouse were 35/26 °C day/night and 65–70%, respectively. The soil moisture of each pot was measured under volumetric water content (VWC) using a ProCheck sensor read-out with a 10HS moisture sensor (METER Group, Inc., USA) throughout the experiment. The

seedlings were well-watered until 35 days after sowing to keep their soil moisture at optimum condition. The seedlings were thinned at 14 and 21 days after sowing and finally, five uniform seedlings per pot were maintained for further experiments. Water stress treatments were initiated from the 35th day after sowing by stopping watering. Samples for the next steps were collected at 5 sampling times including control (C0) before stress with about 30% VWC soil moisture (corresponding to 35 days after sowing), low drought stress (D1) after 4 days of no watering when soil moisture reached about 15% VWC (corresponding to 39 days after sowing), moderate drought stress (D2) after 8 days of no watering when soil moisture reached about 9% VWC (corresponding to 43 days after sowing), severe drought stress (D3) after 12 days of no watering when soil moisture reached about 6% VWC (corresponding to 47 days after sowing) and recovery of water stress (R4) after re-watering for 2 days to reach about 30% VWC (49 days after sowing).

Growth and physiological parameters

The fresh weight of shoot and root tissues (g) was measured at each sampling time. The samples were placed in an oven for 24 h at 80 °C and then their dry weights were measured. The root/shoot ratio (%) was calculated based on the dry weight. The root length (cm) of each sample was also measured at sampling times.

The relative water content (RWC) of leaf samples was determined using the following formula proposed by Ritchie et al. (1990): RWC (%) = (FW—DW) / (SW—DW) × 100. Where FW is the fresh weight, DW is the dry weight, and SW is the saturated weight in water. The dry weight of leaves was measured after drying samples for 24 h at 80 °C. To measure the saturated weight, the leaf samples were transferred to the test tube containing distilled water and after 24 h the swollen leaves were weighed.

The electrolyte leakage rate was calculated as described by Lutts et al. (1995), using the following equation: EL (%) = EC1 / EC2 × 100. The leaf samples were placed in test tubes containing 1ml of distilled water. After 24 h, the electrical conductivity of each sample (EC1) was measured using the AZ-8302 conductivity meter with RS232 interface (AZ Instrument Corp., Hong-Kong). Afterward, the tubes were covered with plastic caps and placed in a water bath at 90 °C for 20 min to destroy all cell membranes and measure the total electrolyte leakage of each sample. Then, samples were cooled to 25 °C and the electrical conductivity was measured (EC2). The assimilation rate (photosynthesis rate, A, µmol $CO_2/m^2.s$), stomatal conductance (G_s, mmol/$m^2.s$), transpiration rate (E, mmol/$m^2.s$), intercellular CO_2 concentration (C_i, ppm), and instantaneous carboxylation efficiency (A/C_i) of individual leaves were measured using GFS-3000-FL, a portable gas exchange fluorescence system (Walz, Germany). The measurements were conducted under controlled conditions between 8:00 am and 10:00 am, at 25 °C and 65% relative humidity with a reference CO_2 concentration of 500 µmol (CO_2) mol^{-1} using an artificial light source with a photon flux of 600 µmol m^{-2} s^{-1}.

Biochemical parameters

The extraction of free amino acids, soluble sugars, and starch from sesame leaves was carried out according to the method of Ndoumou et al. (1996). Fresh leaves (0.1 g) were crushed in a mortar with 5 ml of 80% ethanol and transferred to a tube. The mixture was incubated in the water bath for 10 min at 70 °C. Then, the ethanolic supernatant containing amino acids and soluble sugars was transferred to another tube. The extraction step was repeated four times by adding 5 ml of 80% ethanol to the bottom residues of the first tube. To remove chlorophyll, the obtained mixture was mixed with chloroform at a ratio of 1:5. The supernatant of the extract was centrifuged at 12,000 × g for 10 min. The transparent supernatant of the extract was used to measure amino acids and soluble sugars concentration and the pellets were used to determine the amount of starch.

The free amino acid concentration was measured using the ninhydrin solution as described by Yemm et al. (1955). The extract (100 ml) was mixed with 1 ml of 80% ethanol, 1 ml of 0.2 M citrate buffer, 2 ml of ninhydrin solution, and 1 ml of distilled water. The mixture was incubated in the water bath for 15 min at 100 °C. After cooling, 5 ml of distilled water was added to the mixture and then the absorbance was measured at 570 nm using WPA Biowave II UV/Visible Spectrophotometer (Biochrom Ltd., UK). The soluble sugars and starch content were determined using the anthrone reagent at 620 nm as described by McCready et al. (1950).

Free proline content was determined according to the method described by Bates et al. (1973). Fresh leaf samples (0.5 g) were cut into pieces smaller than 5 mm and were homogenized in 10 ml of 3% (*m/v*) aqueous sulfosalicylic acid for 3 min. In a tube, 2 ml of Acetic acid (96%) and 2 ml of ninhydrin reagent were added to 2 ml of solution. The mixture was incubated in a water bath for 1 h at 90 °C. After cooling, 4 ml of toluene was added to the experiment tube and was shaken for about 15–20 s. The absorbance of the supernatant was measured at 520 nm.

Protein quantification and antioxidant enzymes assay

To extract total soluble protein and for the antioxidant enzymes assays, fresh leaves of sesame plants (0.1 g) were powdered using liquid nitrogen in a mortar and then homogenized in 2 ml of 0.1 M phosphate buffer (pH 7.0). The

mixture was centrifuged at 13,000 × g for 15 min at 4 °C (Kar and Mishra 1976). The total soluble protein content was determined by the Bradford (1976) method.

Catalase activity was measured according to the method of Aebi (1984). The reaction mixture consisted of 1.5 ml of 100 mM potassium phosphate buffer (pH = 7), 0.5 ml of 7.5 mM hydrogen peroxide, and 50 µL of enzyme extract, which was raised to 3 ml by adding distilled water. The reaction was initiated by the addition of hydrogen peroxide and a decrease in absorbance of the mixture was recorded at 240 nm. The catalase activity was measured based on hydrogen peroxide destruction per minute with an extinction coefficient of 40 mM^{-1} cm^{-1}.

The peroxidase activity was determined by adding 1 ml of 100 mM phosphate buffer (pH = 7), 250 µL of 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 ml of 5 mM guaiacol, 1 ml of 15 mM peroxide to 50 µl of enzyme extract (Kim and Yoo 1996). The increase in absorbance was recorded at 470. The peroxidase activity was measured using an extinction coefficient of 26 mM^{-1} cm^{-1} based on the amount of tetraguaiacol formed per minute.

The superoxide dismutase (SOD) activity was measured by determining the ability of the enzyme to inhibit the photochemical reduction of nitroblue-tetrazolium (NBT) according to the Giannopolitis and Ries (1977) method. The reaction mixture consisted of 935 µl of 50 mM phosphate buffer containing 0.1 mM EDTA, 13 mM methionine, and nitroblue-tetrazolium (75 mM NBT and 15 µl of 0.12 mM Riboflavin) and 50 µl of extract. The mixture tubes were placed under the light of 4000 flux for 20 min. The absorbance was measured at 560 nm and the SOD activity was defined as the amount of enzyme that caused 50% inhibition of the NBT reduction.

Mitochondrial pyruvate dehydrogenase enzyme assay

The extraction and isolation of mitochondrial pyruvate dehydrogenase (MPDH) was done according to the method of Millar et al. (1998) with some modifications. 100 mg of leaf samples were homogenized in the ice-cold extraction buffer composed of 50 mM potassium phosphate buffer (pH = 7.5), 0.1% (*v*/*v*) Triton X-100, 1mM EDTA, 0.3 M sorbitol, 0.77% (*v*/*v*) polyvinylpolypyrrolidone (PVPP), and 0.1% (*v*/*v*) 2-mercaptoethanol and stored at −20 °C for 24 h. Then, the enzyme extract was centrifuged at 13,000 × g for 15 min at 4 °C, and the supernatant was used to assay the enzyme activity based on the method of Hinman and Blass (1981) with some modifications. The final 1 ml volume of reaction mixture contained 100 µl of enzyme extract, 1 mM $MgCl_2$, 0.001% (*m*/*v*) bovine serum albumin (BSA), 0.1% (*v*/*v*) Triton X-100, 0.6 mM iodonitrotetrazolium chloride (INT), 0.2 mM thiamine pyrophosphate (TPP), 0.1 mM coenzyme A sodium salt hydrate (CoA), 2.5 mM β-Nicotinamide adenine dinucleotide (NAD^+), 6.5 µM phenazine methosulfate (PMSF), and 5 mM sodium pyruvate in 50 mM potassium phosphate buffer (pH = 7.5). The enzyme reaction was started by adding pyruvate to the reaction mixture and the increase in absorption at 500 nm was measured for 3 min. The MPDH activity was measured using an extinction coefficient of 12.4 mM^{-1} cm^{-1} based on the amount of INT reduced per minute.

Lipid peroxidation assay

Extracts for malondialdehyde (MDA) measurement were prepared according to the method of Dhindsa (1982). The leaf samples (0.05 g) were homogenized with 1 ml of 0.1% trichloroacetic acid in a mortar placed on an ice bath. The mortar was washed with another 1 ml of 0.1% trichloroacetic acid and the resultant mixture was transferred to a tube. The mixture was centrifuged at 20,000 × g for 15 min. The supernatant (1 ml) was added to 4 ml of 0.5% thiobarbituric acid (TBA) made in 20% trichloroacetic acid (TCA) and the mixture was heated in a water bath at 100 °C for 25 min. After cooling, the mixture was centrifuged at 20,000 × g for 15 min (Kuk et al. 2003). The absorbance of the supernatant was recorded at three wavelengths of 532 (specific), 440, and 600 (nonspecific) nm to determine MDA content based on Du and Bramlage (1992) formula.

RNA extraction and qRT-PCR

Total RNA of leaf samples was extracted using TRIzol Reagent (Thermo Fisher Scientific, US) based on the manufacturer's instructions. The contaminating DNA was removed from extracted RNA by DNase I treatment (Thermo Fisher Scientific, US). Then, the cDNA synthesis was carried out using the RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific, US) according to the instructions provided by the manufacturer. The qRT-PCR was carried out using the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., US) and SYBR Green Master Mix (Thermo Fisher Scientific, US). The primers for studied genes (MnSOD1, MnSOD2, and PDHA-M) were designed using the Primer3 software and as much as possible in the exon junction regions (Untergasser et al. 2012) (Supplemental Table S1). The relative expression (fold change, FC) of each gene was calculated using two housekeeping genes (Actin97 and EF1A) according to the method described by Vandesompele et al. (2002).

Statistical analysis

The data was statistically analyzed using XLSTAT (Addinsoft, France), a Microsoft Excel (Microsoft, USA) add-on.

The significance of the differences was determined by the Analysis of Variance (ANOVA) followed by Duncan's multiple range test ($P < 0.05$).

Results

Growth parameters analysis

Significant changes were observed in the morphological and developmental characteristics of sesame genotypes under drought stress and re-watering (Table 1 and Supplemental Fig. S1) such as the shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), root/shoot ratio (RSR), and root length (RL). The SFW and RFW of both genotypes were significantly decreased under drought stress and, as the drought level increased, they decreased more. However, the decrease in SFW and RFW of the susceptible genotype (SG) was significantly higher than the tolerant genotype (TG). After re-watering the SFW and RFW were significantly increased in both genotypes but the TG recovered more efficiently. The SDW was decreased under drought stress in both genotypes but with a lower rate in the TG. A significant increase in SDW was recorded in the TG after re-watering while it was not changed in the SG. The RDW also decreased in both genotypes, but unlike the SG, the TG did not show a significant decrease with progressing drought level. Moreover, the TG showed a lower decrease rate in RDW. The RDW of both genotypes was significantly increased after re-watering. The RSR was reduced in both genotypes under water stress. However, the ratio of TG was higher than the SG at severe drought. After the re-watering, the RSR returned to its normal state. The RL was significantly increased in both genotypes during low drought stress. The RL of TG kept increasing at higher drought levels (moderate drought) but the SG didn't. After re-watering, no significant change was observed in both genotypes compared to the last drought condition. Leaf RWC recorded an incremental decrease with the increase of drought levels in both SG and TG. However, the reduction in RWC was 36–77% higher in the SG than in the TG throughout drought stress. The RWC of both genotypes was increased with re-watering.

Changes in photosynthetic characteristics

In both genotypes, the transpiration rate (E) was decreased under drought stress and then significantly recovered by re-watering (Fig. 1a). The E of SG was higher than of TG at the low and moderate drought levels (27 and 151%, respectively). The stomatal conductance (G_s) was reduced in both genotypes under all drought levels (Fig. 1b). Under low drought levels (D1) SG showed a lower G_s than TG. On the other hand, the G_s was increased in both genotypes after re-watering. However, the TG had a higher G_s than the SG after recovery. The assimilation rate (A) was declined under drought stress in both genotypes (Fig. 1c). However, the TG showed a lower relative decrease in A compared to the SG under low drought levels (51%). The A of TG was recovered to the same level as recorded in the control sample, after re-watering. However, the recovery rate of assimilation was lower in the SG. The intercellular CO_2 (C_i) was decreased under low to moderate drought but was increased at severe drought in the TG (Fig. 1d). However, it was elevated at all drought levels in the SG. After re-watering, the C_i was decreased to control level in the TG but no significant change was observed in the SG. The carboxylation efficiency (A/C_i) was decreased in both genotypes during water deficit, but it was significantly higher (up to 96%) in the TG compared to the SG (Fig. 1e). After re-watering, A/C_i recovered

Table 1 Effect of drought stress and re-watering on the growth parameters of sesame genotypes

Treatments	Genotypes	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)	Root shoot ratio (%)	Relative water content (%)
C0	Tolerant	270 ± 4.67^{a}	57 ± 1.86^{a}	83 ± 2.65^{a}	17.7 ± 0.31^{a}	35 ± 2^{cd}	32 ± 0.56^{a}	69 ± 4.63^{a}
	Susceptible	262 ± 2.41^{a}	55 ± 1.86^{a}	79 ± 4.06^{a}	17.1 ± 0.89^{a}	33 ± 0.58^{d}	32 ± 0.73^{a}	69 ± 1.72^{a}
D1	Tolerant	228 ± 5.05^{b}	43 ± 1.16^{b}	53 ± 1.46^{c}	10 ± 0.37^{b}	42 ± 1.77^{b}	24 ± 0.29^{de}	55 ± 2.61^{b}
	Susceptible	196 ± 4.71^{c}	37 ± 0.67^{c}	41 ± 0.89^{de}	7.8 ± 0.35^{cd}	39 ± 1.34^{bc}	22 ± 0.68^{e}	41 ± 3.01^{cd}
D2	Tolerant	152 ± 6.49^{d}	33 ± 2^{cd}	40 ± 1.77^{de}	8.7 ± 0.54^{bcd}	48 ± 1.46^{a}	27 ± 0.19^{c}	39 ± 1.78^{cd}
	Susceptible	105 ± 6.99^{f}	20 ± 0.67^{f}	27 ± 1.21^{f}	5.1 ± 0.43^{e}	40 ± 1.74^{bc}	26 ± 1.77^{cd}	24 ± 1.29^{e}
D3	Tolerant	127 ± 4.17^{e}	26 ± 1.46^{e}	36 ± 2.91^{e}	7.4 ± 0.24^{d}	47 ± 2.09^{a}	30 ± 0.75^{ab}	34 ± 2.63^{d}
	Susceptible	76 ± 1.86^{g}	17 ± 1.21^{f}	13 ± 1.74^{g}	3.1 ± 0.45^{f}	40 ± 1.86^{bc}	19 ± 1.49^{f}	19 ± 1.15^{e}
R4	Tolerant	218 ± 5.7^{b}	31 ± 0.89^{d}	65 ± 0.34^{b}	8.9 ± 0.35^{bc}	51 ± 0.89^{a}	30 ± 0.36^{ab}	52 ± 0.89^{b}
	Susceptible	131 ± 1.53^{e}	19 ± 1.21^{f}	44 ± 0.89^{d}	5.1 ± 0.28^{e}	41 ± 1.53^{b}	28 ± 0.96^{bc}	44 ± 0.66^{c}

Each Value represents the mean (n = 3) ± standard error of the mean; Values followed by the same letter(s) within each column are not significantly different ($P < 0.05$)

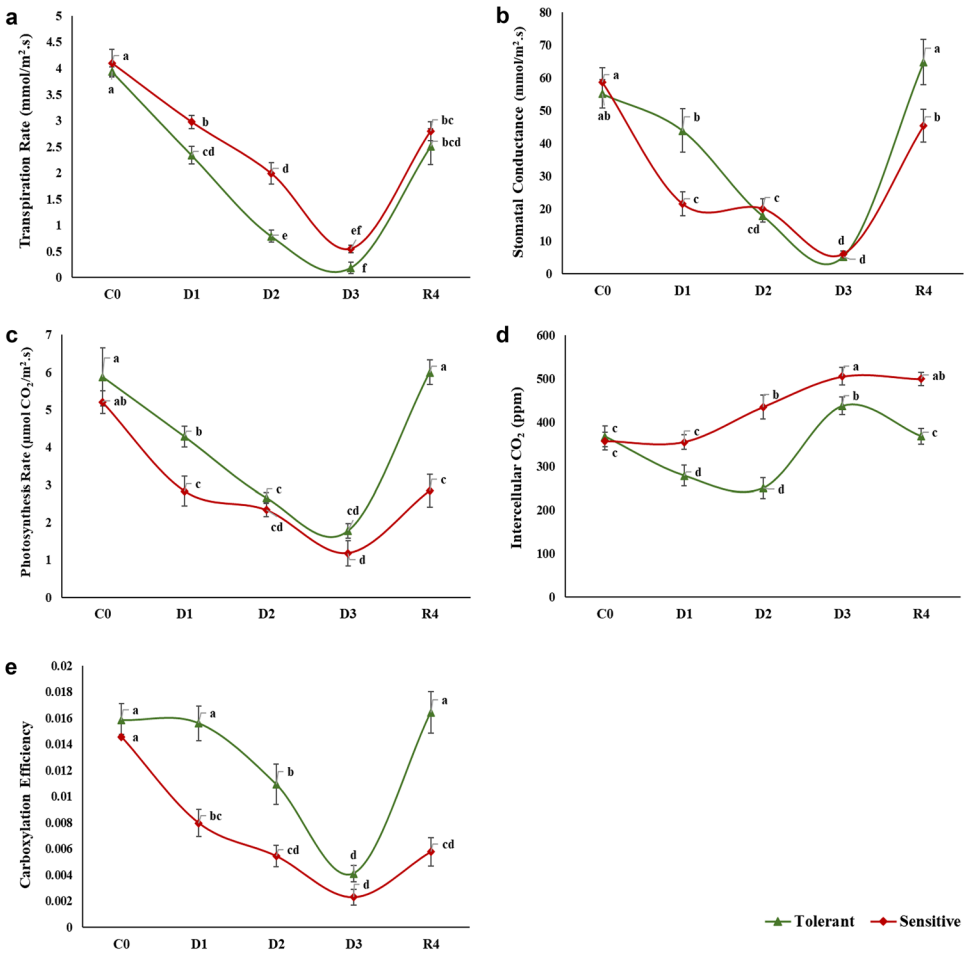

Fig. 1 Effect of drought stress on **a** transpiration rate (E), **b** stomatal conductance (Gs), **c** photosynthesis rate (A), **d** intercellular CO_2 concentration (Ci), and **e** instantaneous carboxylation efficiency (A/Ci) of leaves in two sesame genotypes. Samples were measured at 5 stress points including control (C0), low drought stress (D1), moderate drought stress (D2), severe drought stress (D3), and re-watering after severe drought stress (R4). The error bars indicate the SEs (n = 3). The different letters indicate significant differences among the samples at $P < 0.05$

to the control value in TG, while it showed a slight and non-significant rise in SG, compared to the D4 treatment.

MDA content and electrolyte leakage of leaves

Under drought conditions, electrolyte leakage (EL) was raised in both genotypes (Fig. 2a). The TG has shown a lower rate of increase in the EL under low and moderate drought. In the aftermath of severe drought, the EL of the TG increased even further. Similar increases in EL were observed in SG samples, but at a much higher level (up to 26% more). After re-watering, the EL was decreased significantly in the TG but no significant change was observed in the SG. Increased MDA content was observed in the samples of both genotypes under drought stress (Fig. 2b). The SG showed a relatively higher increase (31%) in MDA content

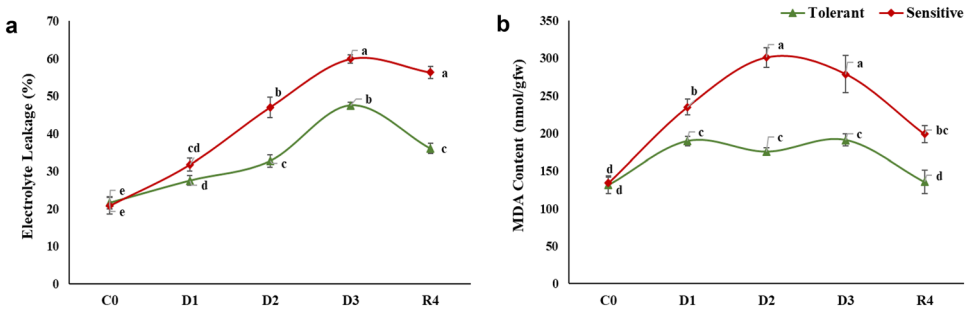

Fig. 2 Effect of drought stress on **a** electrolyte leakage and **b** MDA content of leaves in two sesame genotypes. Samples were collected at 5 stress points including control (C0), low drought stress (D1), moderate drought stress (D2), severe drought stress (D3), and re-watering after severe drought stress (R4). The error bars indicate the SEs (n = 3). The different letters indicate significant differences among the samples at $P < 0.05$

than the TG. The MDA content dropped significantly in both genotypes, after re-watering. It went back to the control level in TG but not in SG.

Changes in soluble sugars and starch contents

The soluble sugar content was elevated under drought stress in both genotypes (Fig. 3a). However, the TG significantly accumulated more (up to 152%) soluble sugars compared to SG. In addition, the SG under severe drought showed a decrease in soluble sugar content compared to moderate drought. The starch content was reduced under low stress and kept on decreasing at higher drought levels in both genotypes (Fig. 3b). However, the rate of starch reduction in the TG was more than the SG (up to 35%). Re-watering increased the amount of starch in the TG, but in the SG, the starch content continued to decrease.

Free amino acids, proline, and soluble protein content

The free amino acid content showed an increase in both genotypes under drought stress (Fig. 4a). In the SG, free amino acids were raised (up to 42.5%) under low drought and stayed at the same level during moderate drought. A slight decline was observed under severe drought but, no significant change was recorded after re-watering. The free amino acid content was significantly increased (up to 114%) under low drought, in the TG. Then the amino acid content remained at the same level under moderate to severe drought. After the re-watering, a decrease was observed in free amino acids. The TG had always accumulated more amino acids (up to 48%) than the SG throughout drought stress. The proline content was elevated in both genotypes under drought stress (Fig. 4b). As the drought level increased, the proline content kept increasing (up to 4.7-fold) in the TG but

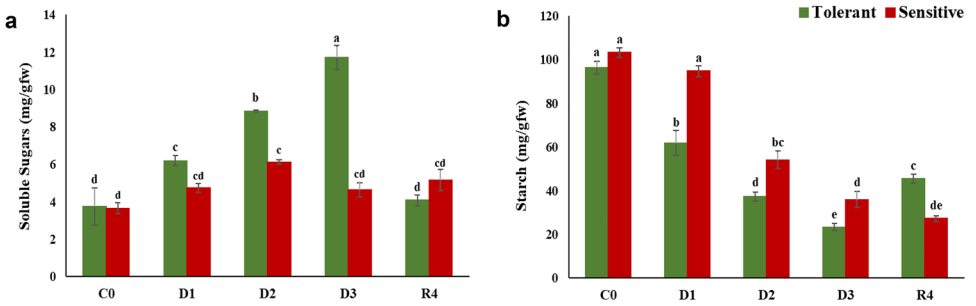

Fig. 3 Effect of drought on **a** soluble sugars, and **b** starch content of leaves in two sesame genotypes. Samples were collected at 5 stress points including control (C0), low drought stress (D1), moderate drought stress (D2), severe drought stress (D3), and re-watering after severe drought stress (R4). The error bars indicate the SEs (n = 3). The different letters indicate significant differences among the samples at $P < 0.05$

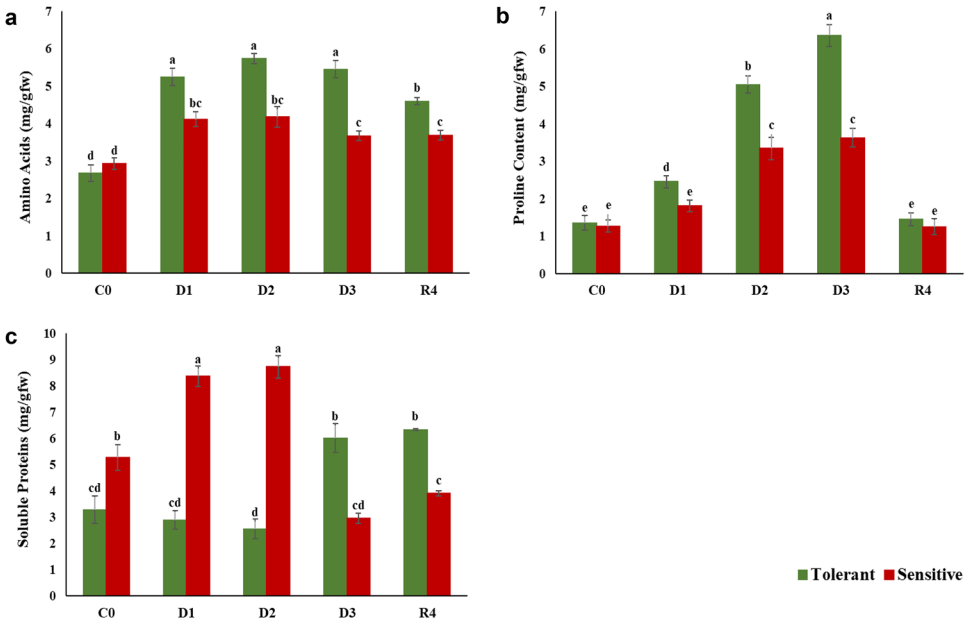

Fig. 4 Effect of drought on **a** free amino acids, **b** proline, and **c** soluble proteins content of leaves in two sesame genotypes. Samples were collected at 5 stress points including control (C0), low drought stress (D1), moderate drought stress (D2), severe drought stress (D3), and re-watering after severe drought stress (R4). The error bars indicate the SEs (n = 3). The different letters indicate significant differences among the samples at $P < 0.05$

stopped at the severe drought in the SG. Furthermore, the proline levels in the TG were significantly higher than those in the SG (up to 75%). After re-watering, the proline content of both genotypes returned to its normal level. The soluble protein content significantly increased (up to 65%) under low and moderate drought in the SG (Fig. 4c). However, under severe drought, the amount of soluble protein significantly declined and then after re-watering recovered to the control level. On the other hand, no significant change in soluble protein content was observed under the low and moderate drought, in the TG. However, the soluble protein content was significantly increased (up to 83%) under severe drought and remained at the same level after re-watering. Overall, the soluble protein content was higher (up to 100%) in the TG compared to the SG under severe drought.

Changes in antioxidant enzymes activities

The superoxide dismutase (SOD) activity was increased significantly under low drought in both genotypes and reached the highest level under moderate stress (up to 280% and 218% increase compared to control in TG and SG, respectively) (Fig. 5a). A significant decline in SOD activity was observed under severe drought. After re-watering, a great decrease in the SOD activity of the TG was recorded, but it was increased in the SG. In general, the SOD activity of TG was higher (up to 24%) than the SG under drought stress. The increase in catalase (CAT) activity was observed in both genotypes (Fig. 5b). However, a significant rise in CAT activity was first seen at the moderate drought level in the TG, while it was increased under the low drought level in the SG. The CAT activity reached its highest level (42.36 µmol/min.g fw) under moderate stress in SG. Thereafter, a significant decrease in CAT activity was observed under severe drought and the activity remained at the same level after re-watering. On the other hand, the highest CAT activity (50.13 µmol/min.g fw) was recorded at the severe drought level in the TG. After re-watering, the CAT activity dropped back to the control level. Overall, under drought stress, higher CAT activity (25-fold) was observed in the TG compared to the SG under severe drought. The peroxidase (POD) activity was increased first at the moderate drought level in the TG and then, a great increase was observed under severe drought (Fig. 5c). Although the POD activity of SG was also increased under moderate drought, a slight decrease was observed at the severe drought level. Overall, POD exhibited more activity (fivefold) in the TG under severe drought stress. Both genotypes showed a decrease in POD activity after re-watering.

Mitochondrial pyruvate dehydrogenase activity assay

In both TG and SG, an increase (up to 115 and 56%, respectively) in mitochondrial pyruvate dehydrogenase (MPDH) activity was observed under drought stress (Fig. 5d). Both genotypes showed a significant elevation in MPDH activity at the moderate drought level. Under severe drought,

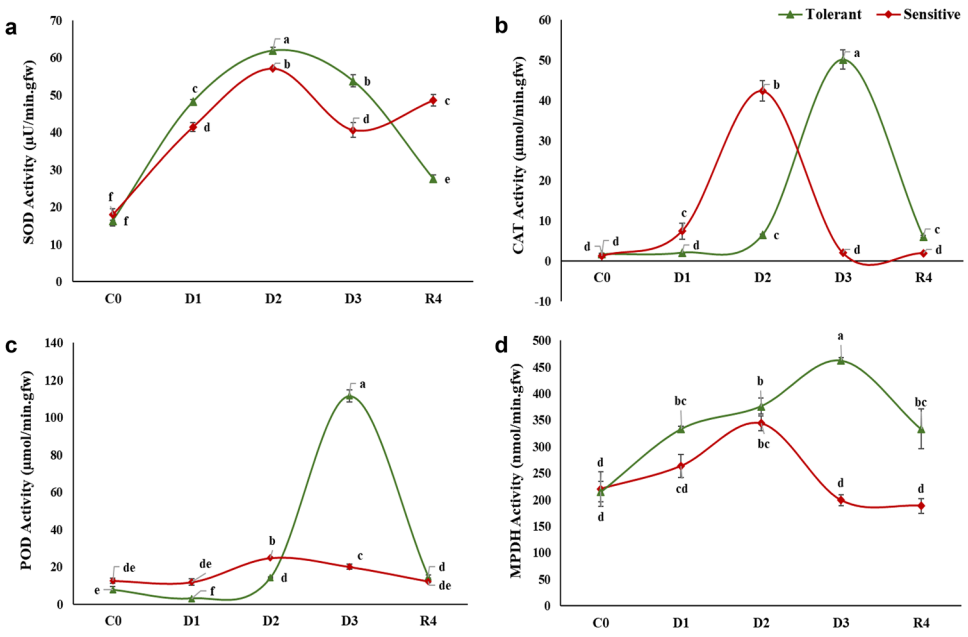

Fig. 5 Effect of drought stress on **a** SOD, **b** CAT, **c** POD, and **d** MPDH enzyme activities of leaves in two sesame genotypes. Samples were collected at 5 stress points including control (C0), low drought stress (D1), moderate drought stress (D2), severe drought stress (D3), and re-watering after severe drought stress (R4). The error bars indicate the SEs (n = 3). The different letters indicate significant differences among the samples at $P < 0.05$

MPDH activity continued to increase in the TG, while it was decreased to control level in the SG. Overall, the TG demonstrated higher MPDH activity than the SG at all stress levels, especially under severe drought (132%). Re-watering of the TG caused a significant reduction in MPDH activity, but no change was observed in the susceptible plants.

Gene expression analysis

The relative expression of the MnSOD1 was increased (0.92 log2FC) in the TG at low drought compared to the control, but it declined to the control level under moderate drought conditions (Fig. 6a). Under severe drought, a significant decrease (−0.65 log2FC) in MnSOD1 expression was observed compared to the control. Meanwhile, in the SG, the drop in MnSOD1 expression began at a low drought level and continued to decline as drought severity increased (−0.58 to −1.66 log2FC). The re-watering enhanced MnSOD1 expression to the control level in both genotypes.

On the other hand, the expression of MnSOD2 was increased in both genotypes in all drought levels compared to the control (Fig. 6b). The highest relative expression for both genotypes (7.98 and 4.90 log2FC for TG and SG, respectively) was observed in severe drought. Compared to severe drought, re-watering reduced MnSOD2 relative expression in the TG, while slightly increasing it in the SG. Overall, the relative expression of both MnSOD genes was higher in the TG than in the SG during drought stress.

PDHA-M expression was not changed significantly in the TG under low drought compared to control but increased (1.82 log2FC) during moderate drought (Fig. 6c). Then, it significantly rose even further (5.48 log2FC) following a severe drought. Meanwhile, PDHA-M expression declined in the SG under low drought. Following moderate drought, a significant rise (0.82 log2FC) in PDHA-M relative expression was recorded. Then, it remained at about the same expression level (1.53 log2FC) during severe drought. Both genotypes showed significant reductions in PDHA-M expression after re-watering.

Discussion

The plant's ability to tolerate drought is a complex process that involves various molecular, biochemical, and physiological mechanisms (Shah et al. 2020). The present study was designed to shed light on some of these mechanisms by comparing the response pattern to drought in two contrasting tolerant and susceptible sesame genotypes. We observed (Table 1) a significant decrease in growth parameters of both genotypes under drought stress, including SFW, RFW, SDW, RDW, and RWC. Similar results have been reported in various plants under drought stress, similar to our results (Hasan

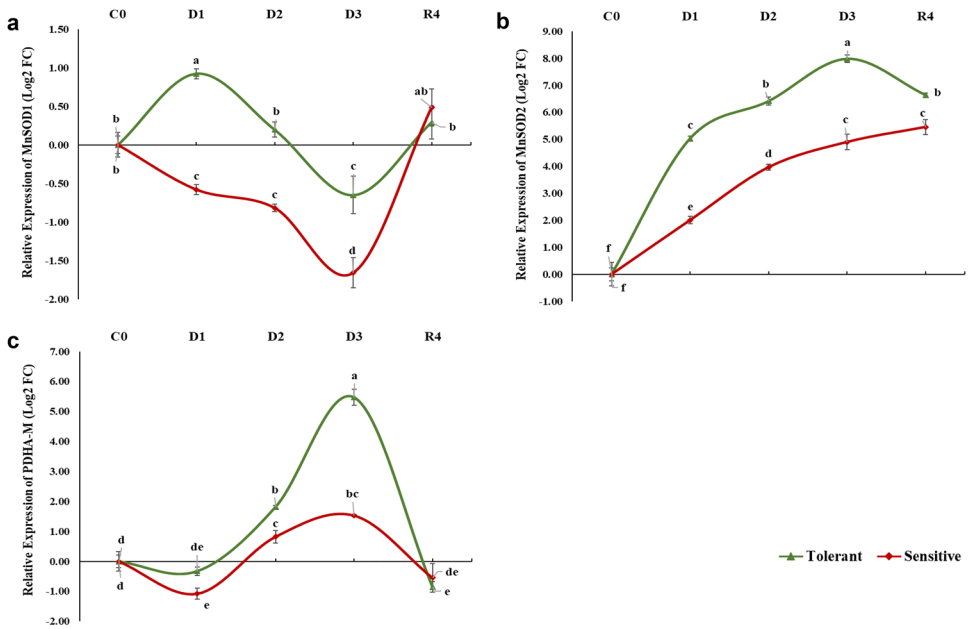

Fig. 6 Effect of drought stress on relative expression of **a** MnSOD1, **b** MnSOD2, and **c** PDHA-M genes in leaves of two sesame genotypes. Samples were collected at 5 stress points including control (C0), low drought stress (D1), moderate drought stress (D2), severe drought stress (D3), and re-watering after severe drought stress (R4). The error bars indicate the SEs (n = 3). The different letters indicate significant differences among the samples at $P < 0.05$

et al. 2018; Amoah et al. 2019; Yadav et al. 2022). Plant water availability and subsequently leave's humidity are significantly reduced during drought stress. Reduced growth and development rate are the most visible consequences of low water availability in plants (Da Silva et al. 2013). The leaf relative water content is usually considered a simple and reliable indicator of plant tolerance to drought (Zegaoui et al. 2017; Amoah et al. 2019). It was demonstrated that drought-tolerant plants have a higher RWC during water stress (Zegaoui et al. 2017). Reduced growth and multiple drought-induced disturbances resulted in decreased root and shoot biomass accumulation. However, it has been shown that tolerant plants exhibited a lower reduction of shoot and root weight during water deficit which is in accordance with our results (Hasan et al. 2018). Higher biomass accumulation in the drought-tolerant genotype demonstrates its ability to conserve the cell's water content and metabolic activities. Similar to our results (Table 1), a decline has been recorded in the RSR of cotton species under water deficit but the tolerant genotype had a higher RSR compared to other genotypes (Hasan et al. 2018). The higher RSR can be considered as an adaptation to drought, as preserving the root system efficiency contributes the plant to more water absorption and less water loss during the stress (Du et al. 2020). Under drought stress, plants typically increase their RL, as enhanced root growth helps the plant absorb more water from deeper soil areas (Kavas et al. 2013; Yadav et al. 2022). Following our results (Table 1), the RL of drought-tolerant cotton species was higher than that of the susceptible species (Hasan et al. 2018).

Plants close their stomata to reduce water loss and prevent evapotranspiration under drought (Agurla et al. 2018). Similar to our results (Fig. 1a), a reduced transpiration rate of the leaves under drought stress has been reported (Maheswari et al. 2021; Desoky et al. 2023). Besides, it has been shown that the G_s of the plants was reduced under water stress due to stomata closure, which is consistent with the results (Fig. 1b) of this study (Maheswari et al. 2021; Desoky et al. 2023). Reduced G_s decreases the available CO_2 in the plant leaves. As described earlier, the reduction in available CO_2 causes a decrease in the rate of photosynthesis. Accordingly, CO_2 assimilation (photosynthesis) rates were decreased in both genotypes under drought stress (Fig. 1c). However, a higher photosynthetic rate was recorded in the TG compared to the SG at low drought levels. The low growth rate and reduced biomass production (root and shoot weight) shown earlier might be due to the low CO_2 assimilation, which resulted in reduced photosynthetic products (Du et al. 2020).

On the other hand, although C_i was decreased at low and moderate drought levels in the TG, it was increased under severe drought as well as in the SG under moderate to severe drought (Fig. 1d). Lu et al. (2018) showed that C_i concentration in *Sophora japonica* seedlings was decreased in low to moderate drought conditions but increased under severe drought. The elevated concentration of C_i at severe drought levels indicated that a decrease in photosynthesis was not just caused by stomatal closure and insufficient CO_2 available to plant, but could be attributed to lower mesophyll conductance and/or inactivation of enzymes and molecular structures involved in photosynthesis (non-stomatal factors) under water deficit conditions (Ji et al. 2012; Yang and Li 2016). Indeed, at severe drought levels, disturbances in mesophyll conductance and/or the photochemical processes might resulted in reduced CO_2 fixation and carboxylation and eventually led to the accumulation of CO_2 at the cellular level. Accordingly, carboxylation efficiency (A/C_i) was high in the TG at early drought stages, but a considerable reduction was observed under severe drought (Fig. 1e). Whiles, A/C_i dropped in the SG at moderate to severe drought levels. It was suggested that a decline in C_i along with G_s could be considered as a stomatal limitation in photosynthesis while a decrease in A/C_i might be a sign of non-stomatal limitation (Zhou et al. 2013).

Limited CO_2 assimilation naturally results in ROS generation through either photoinhibition or photoprotective reactions like photorespiration (Fahad et al. 2019). As described previously, elevated ROS levels damage cellular structures and ultimately lead to cell death. MDA is produced upon lipid peroxidation of polyunsaturated fatty acids and is known to be a marker of oxidative activity (Davey et al. 2005). Electrolyte leakage is also a parameter that can be used to estimate the extent of cell membrane damage caused by oxidative damage (Bajji et al. 2002). The MDA content and electrolyte leakage were elevated under drought stress in various plants, including sesame (Abid et al. 2018; Erdal et al. 2021; Desoky et al. 2023Wang et al. 2023). It was shown that during drought stress, MDA content and electrolyte leakage were lower in the tolerant plants compared with the susceptible ones (Su et al. 2017; Hasan et al. 2018). Similarly, in the current study, the MDA content in the TG was lower than SG and was not changed significantly with raising drought levels (Fig. 2a). Electrolyte leakage also showed a lesser increase in the TG than the SG (Fig. 2b). Lower cellular damages of TG than SG are presumably an indication of a more appropriate tolerance mechanism under drought stress.

On the other hand, the soluble sugar content was increased in both genotypes during drought stress (Fig. 3a). However, the soluble sugars accumulation was higher in the TG and was further enhanced under severe drought. Similar to our results, it was revealed that the tolerant genotypes exhibited higher soluble sugar content under drought stress in sesame and many other crops (Abid et al. 2018; Hasan et al. 2018; Du et al. 2020; Wang et al. 2023). One of the widely known drought stress responses in crop plants is the accumulation of compatible solutes, such as soluble sugars (Abid et al. 2018; Hasan et al. 2018; Du et al. 2020; Wang et al. 2023). Soluble sugars not only are involved in the synthesis of metabolites and cellular constituents under drought stress but also act as osmoprotectants that positively regulate osmotic adjustment and cell membrane stability (Poonam et al. 2016). Moreover, studies have shown that these compounds act as signaling molecules under drought stress and have a crucial role in regulating the expression of drought-responsive genes (Poonam et al. 2016). A protective role against ROS and oxidative stress and even antioxidant activity for soluble sugars have been reported (Bolouri-Moghaddam et al. 2010).

We demonstrated that starch content was dropped during drought stress in both genotypes (Fig. 3b). However, its content in the TG was decreased from the low levels of drought and to a greater extent. Despite the decline in CO_2 assimilation, starch degradation is probably the major contributor to the accumulation of soluble sugars under drought stress (Du et al. 2020; Poonam et al. 2016). Starch is the main carbohydrate storage in the plants which is remobilized to supply energy and carbon under drought stress (Thalmann and Santelia 2017). Accordingly, it has been shown that tolerant genotypes decomposed more starch under drought stress (Du et al. 2020).

It was revealed that water stress raised the concentration of free amino acids in both genotypes, but this increase was higher for TG (Fig. 4a). Similarly, an increase in free amino acids was reported in wheat plants under drought stress (Abid et al. 2018). The accumulation of free amino acids as osmolytes contributes to the osmotic adjustment during water stress (Rai 2002). Amino acids also support the plant to overcome the destructive effects of osmotic stress by controlling ion transport, stomatal movement, synthesis and activity of enzymes, and gene expression (Rai 2002). Free amino acids can also function as precursors to polyamines which are involved in adaptation to drought stress (Sequera-Mutiozabal et al. 2017).

Our results also showed that proline content was elevated in both genotypes (Fig. 4b). However, proline accumulation in the TG was more than SG. In the same way, it was shown that tolerant cultivars had a higher accumulation of proline in their leaves under drought stress in sesame and other plants (Abid et al. 2018; Bayat and Moghadam 2019; Wang et al. 2023). It is well-established that proline accumulation contributes to improved crop tolerance under water deficit conditions through osmotic regulation as another compatible solute (Forlani et al. 2019; Khanna-Chopra et al. 2019). Moreover, proline has been considered as an energy sink and a source of carbon and nitrogen compounds (Khanna-Chopra et al. 2019). On the other hand, it plays an active role in ROS scavenging and maintaining the stability of protein, DNA, and cell membranes against oxidative damage caused by drought (Forlani et al. 2019).

As shown in the results, except for the susceptible samples under severe drought, not only no decrease in the amount of soluble protein was observed under drought stress, but there was also an increase in some samples (Fig. 4c). Interestingly, while soluble proteins content was increased in the TG under severe drought, it was decreased in the SG. Similarly, the increase in soluble proteins content was reported in several plants under drought stress (Li et al. 2010; Chen et al. 2019). The synthesis of protective and drought-related proteins is likely to increase during the water stress, although the overall rate of protein synthesis is expected to decrease (Li et al. 2010).

Elevated levels of free amino acids in this study can be attributed to the acceleration of their synthesis, inhibition of amino acid degradation, impaired protein synthesis, or increased protein degradation (Good and Zaplachinski 1994; Huang and Jander 2017; Hildebrandt 2018; Batista-Silva et al. 2019). However, based on the evidence that no reduction in soluble protein content was observed at most levels, this accumulation seems to be more due to de novo synthesis and/or inhibition of amino acid degradation under drought stress. Likewise, studies have shown that the elevated accumulation of amino acids under drought, especially high abundant amino acids, is often due to de novo synthesis (Hildebrandt 2018; Acevedo et al. 2019; Batista-Silva et al. 2019; Coutinho et al. 2019). In the case of proline, it has been shown that its accumulation was mainly due to increased de novo synthesis rather than reduced catabolism or elevated protein degradation (Hildebrandt 2018; Forlani et al. 2019). Besides, it has been proposed recently that starch degradation plays an important role in sustained proline biosynthesis under drought stress (Zanella et al. 2016). Such a function of starch may also be conceived for other amino acids.

The relative expression of PDHA-M, encoding the alpha subunit of MPDH, was increased in both genotypes under moderate to severe drought stress (Fig. 6c). In support of these findings, MPDH activity was enhanced in both genotypes during the stress, except for those susceptible plants under severe drought (Fig. 5d). Depleted MPDH activity despite the increased PDHA-M expression in the susceptible genotype may reflect the post-translational inactivation of its subunits by severe drought damages. Aside from this, MPDH activity and its subunit relative expression were higher in the tolerant genotype at almost all drought levels. Accordingly, upregulation of MPDH subunit genes has been reported in tolerant plants under drought stress (Guo et al. 2009; Silveira et al. 2015; Hu et al. 2018). This suggests that enhanced MPDH activity may contributes to drought tolerance. PDH plays a key role in carbon metabolism and energy production (ATP) by converting pyruvate to acetyl-CoA which is the starting point of the citric acid cycle (TCA). It was suggested that the use of pyruvate for acetyl-CoA production may even increase as glycolysis slowed down during drought stress (Fàbregas and Fernie 2019; Kreuzwieser et al. 2021). Acetyl-CoA serves as a precursor for the synthesis of many organic compounds, including fatty acids, amino acids, carotenoids, cuticle waxes, flavonoids, and hormones, such as abscisic acid (ABA), which are vital for drought tolerance (Fatland et al. 2005; Taiz et al. 2018). Moreover, the starch breakdown can potentially lead to the production of pyruvate. On the other hand, acetyl-CoA can ultimately result in the synthesis of amino acids, including proline, through the TCA cycle. Accordingly, our results also indicated consistent concordance between starch degradation, increased PDH activity, amino acid accumulation, and drought tolerance. Consequently, drought tolerance may be conferred via regulating this pathway.

There was no increase in MnSOD1 relative expression during drought stress except in the tolerant one under low drought (Fig. 6a). Meanwhile, Mn-SOD2 relative expression was increased in both genotypes at all drought levels (Fig. 6b). The TG, however, exhibited a higher relative expression of both MnSOD genes than the SG throughout the drought stress period. SOD is the first line of the antioxidant defense system that dismutates superoxide to H_2O_2 and O_2. In plants, SODs are classified into Fe–SOD, Mn-SOD, and Cu–Zn–SOD based on the metal at their active site. Plant Mn–SOD is typically found in mitochondria, although it has also been observed in peroxisomes (Schmidt and Husted 2019). An increase in the relative expression of the Mn-SOD gene under drought stress has been shown in previous studies (Berta et al. 2005; Zhu et al. 2020). However, there are also reports of Mn-SOD downregulation under drought stress in some plants (Song et al. 2018). Similar to our findings, it was shown that each Mn-SOD paralog gene of cotton had a different response pattern under various abiotic stresses (Wang et al. 2017). So that under drought stress, one of the four Mn-SOD paralogs was upregulated, while other genes were downregulated. It was revealed that overexpression of MnSOD enhances plants' drought tolerance (McKersie et al. 1996; Liu et al. 2013). Therefore, genotypes with higher Mn–SOD induction potential under drought stress are likely to be more tolerant, as seen in this study.

Accordingly, SOD enzyme activity was enhanced in both genotypes under drought stress, although it was higher in TG at all drought levels (Fig. 5a). Enhanced SOD activity results in more protection of the plants against oxidative damages and provides broader tolerance under drought (Del Río et al. 2018). However, the product of this enzyme is another type of ROS and so its activity is not sufficient alone under drought conditions. It was revealed that CAT activity was elevated in both studied genotypes while the highest activity was recorded under severe drought in the TG and moderate drought in the SG (Fig. 5b). On the other hand, the POD activity was increased in both genotypes although a drastic increase was just observed at severe drought in the TG (Fig. 5c). The high CAT activity significantly protects the plant from H_2O_2 oxidative damages and improves drought tolerance (Silva et al. 2019). However, CAT possesses a much lower affinity for H_2O_2 and poor activity against organic peroxides than POD although it has a high turnover frequency (Anjum et al. 2016; Moural et al. 2017; Jovanović et al. 2018). So, PODs also are of great importance in protecting against oxidative damage during drought. It has been shown that increased POD activity was associated with improved tolerance in plants under drought stress (Jovanović et al. 2018; Wu et al. 2019). Similar to our results, studies on sesame under drought stress showed that the TG exhibited higher POD, SOD, and CAT activity than the susceptible one (Dossa et al. 2017; Wang et al. 2023).

Conclusion

In this study, the effect of drought stress and subsequent re-watering on physiological and biochemical traits in two contrasted sesame genotypes (susceptible vs. tolerant) was evaluated. It was revealed that the TG was able to withstand drought thanks to its higher ability in moisture retention along with a developed root network and highly efficient photosynthetic system. Moreover, the TG developed a more robust defensive and protective system against damages caused by drought compared with

Fig. 7 Hypothetical model of sesame drought response and tolerance mechanisms, based on the different patterns of the changes in the measured parameters between the tolerant and the susceptible genotypes during drought stress and recovery

SG. High levels of soluble sugars, free amino acids, and proline, as well as increased activity of SOD, CAT, and POD enzymes, were observed as some of these protective mechanisms. Interestingly, at the first, the TG just highly accumulated osmolytes such as soluble sugars, proline, and free amino acids and then gradually enhanced the accumulation of antioxidant enzymes with the raise in drought severity. In contrast, the SG accumulated soluble proteins and antioxidant enzymes from mild drought levels while most of the protective mechanisms were stopped or slowed down under severe drought. Furthermore, the TG also displayed higher expression of drought-related genes such as MnSOD1, MnSOD2, and PDHA-M. All of these (Fig. 7) have enabled the TG to cope with stressful conditions with fewer damages (lower MDA and EL) and successfully recovered to normal growth conditions after drought relief. However, how the identified drought responses were regulated differently in the two genotypes remains unclear, which can be explored in future studies. Overall, observed findings well revealed some mechanisms of tolerance that can be used as effective criteria in sesame breeding for drought stress.

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Code availability Not applicable.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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