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BIOCHEMICAL RESPONSE-BASED SCREENING OF SESAME GENOTYPES FOR DROUGHT TOLERANCE

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ABSTRACT

Sesame is an important crop in Bangladesh playing the role of an alternate cash crop. However, sesame productivity is highly influenced by drought. Water shortage is a major challenge to crop productivity under changing environmental conditions, especially in dry areas. Recently, different management approaches (agronomic, physiological biochemical, and molecular) have been used to overcome the problems associated to drought. Development of drought-tolerant sesame genotypes may be a sustainable choice to boost sesame productivity under drought conditions. Hence, the study was designed to screen out sesame genotypes based on the level of stress tolerance. Ten sesame genotypes (BD 6964, BD 6971, BD 6979, BD 6980, BD 6982, BD 6985, BD 6990, BD 6993, BD 7004, and BD 7014) were grown-up in pots filled with surface soil under water stress condition (40-45% FC). Treatments were arranged under a Randomized Completely Block Design (RCBD) with three replications. Results showed that drought stress significantly affected the biochemical attributes (*viz.*, chlorophyll, proline, $O2^{\bullet-}$, H_2O_2, MDA, and MG content) in all sesame genotypes. Among the genotypes, biochemical traits were found to be significantly higher in BD 6964 and BD 6971 genotypes, which was also proven to be more drought-tolerant than the other tested genotypes. The sesame genotype BD 6982 and BD 6980 were affected more than other genotypes and was classified as drought-sensitive genotypes. These results suggest that screening for drought-tolerant genotypes may be a more viable option to minimize drought-induced effects on sesame in drought-prone regions. Moreover, this study represents a reference for future research towards developing new varieties with improved drought tolerance in Bangladesh.

***Key words*:** sesame, drought tolerance, screening, biochemical attribute

Introduction

Sesame (*Sesamum indicum* L.), belongs to the Pedaliaceae family and is one of the most ancient oil crops (P. Wei *et al.*, 2022). It is considered to have both nutritional and medicinal value. Sesame seeds contain sesamolin, which converts to sesamol after roasting and has been recognized to have anti-oxidative effects. In recent years, the anti-photo oxidant capacity of sesamol has been reported due to the scavenging of reactive oxygen species (ROS), particularly of single singlet oxygen. In terms of acreage and production, it is Bangladesh's second largest edible oilseed crop after mustard. During 2019-20, sesame occupied 83.168 thousand acres of land with the production of 31.786 thousand tons (BBS, 2021). It can be cultivated in all districts of Bangladesh, but greater Khulna, Pabna, Faridpur, Barisal, Kumilla, Noakhali, Rajshahi, Jessore, Dhaka, Tangail, Patuakhali, Sylhet, Rangpur and Mymensingh districts are the major growing areas. Sesame is known as a drought-tolerant crop (Gloaguen *et al.*, 2021) and is grown between mid-February to mid-March in Bangladesh. However, severe scarcity of water during the growing period affects its production. As sesame has good tolerance against drought compared to other crops, sesame cultivation on fallow and underutilized lands can be an effective way of mitigating the prevailing drought conditions as well as the adverse impact of climate change. Moreover, high-yielding sesame variety development with substantial drought tolerance is essential to increase unit area production of oil to meet the increasing demand for edible oil. Thus, sesame cultivation could be a viable option during the *Kharif*-1 (March-June) season in the drought-affected areas of the country.

Drought is an important factor which limits crop production and becoming a serious problem in different regions of the world (Gwambene *et al*., 2023). From the 1970s to the early 2000s, drought-affected areas has more than doubled in the world (Abate *et al*., 2015). Growth of population and expansion of agricultural, energy, and industrial sectors, the water demand has increased many folds which causes water scarcity and this situation is getting worsen every year. Other factors, including climate change and contamination of water bodies, have also aggravated the water scarcity situation (DeNicola *et al*., 2015). To improve crop productivity in drought-prone areas with limited water, it is needed to understand the response of plant to drought. These responses are very much complex where different mechanisms are activated by plants when they encounter water stress (Fang & Xiong, 2015). However, plant responses influence on the growth stage, the intensity of stress, the rate and time of exposure to stress, plant species and cultivars within species (Ben Rejeb *et al*., 2014). All abiotic stresses, including drought, produce Reactive Oxygen Species (ROS) which degrade and oxidase cellular organelles leading to cell death (Ahsan *et al*., 2022). In plants, ROS are always generated by the inevitable electrons leakage onto O_2 through the electron transport activities of chloroplasts, mitochondria, and plasma membranes or as a consequence of various metabolic pathways in different cellular compartments (Mishra & Sharma, 2019). Oxidative stress occurs when plants are exposed to most unfavorable conditions, which badly affects plant growth because of the excessive generation of ROS (Garcia-Caparros *et al*., 2021). ROS are highly sensitive to membranes, DNA, and proteins and cause severe damage. Additionally, methylglyoxal (MG) which is both a mutagen and a genotoxic agent at high concentrations is also a potential cytotoxic compound produced under abiotic stress. A higher level of MG accumulation in cell is toxic because it impedes cell proliferation (Hoque *et al*., 2016) and consequences in several adverse effects, such as increasing the degradation of proteins and DNA and deactivating the antioxidant defense mechanism system (Nahar *et al*., 2016). However, cytotoxic and reactive properties of ROS and MG must be maintained under toxic levels for cellular survival. Maintaining GSH homeostasis and antioxidant enzyme levels, overexpression of enzymes (glyoxalase) in plants has been found to restrict the increase in ROS and MG levels under drought-stress conditions. Plants have evolved a complex antioxidant system to protect cells from ROS-induced cellular harm, which plays an important role in ROS scavenging in plants (Nadarajah, 2020) . Effective scavenging of ROS, which is formed in different environmental stresses including drought, requires several non-enzymatic action as well as enzymatic antioxidants exist in the tissue (Choudhury *et al*., 2013). The antioxidant defense enzyme of plants includes Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX), Glutathione S-Transferase (GST), Ascorbate peroxidase (APX), Monodehydroascorbate reductase (MDHAR), Monodehydroascorbate (DHAR), and Glutathione Reductase (GR) together with non-enzymatic components, *viz*., Ascorbate (ASA) and Reduced glutathione (GSH) (Rajput *et al*., 2021). Conversely, MG is detoxified and GSH homeostasis is maintained through the glyoxalase system (Li, 2016) which consists of two enzymes, namely, Gly-I and Gly-II. It was stated that the synchronized induction or regulation of the antioxidant and glyoxalase pathway enzymes is needed to obtain significant tolerance in plants against oxidative stress (Hasanuzzaman *et al*., 2017).

Several studies reported that the ability of plants to scavenge ROS and reduce their detrimental effects is frequently correlated with drought tolerance (Hussain *et al*., 2019; Laxa *et al*., 2019; Shukla *et al*., 2015). The harmonized induction and regulation of the antioxidant and glyoxalase pathway enzymes are obligatory for obtaining considerable tolerance against oxidative stress (Alam *et al*., 2013). Detoxification of ROS and MG might be a tactic for drought tolerance (Nguyen *et al*., 2019).

The comparative mechanism can help better understand the abiotic stress tolerance mechanism. Considering the above discussion, research work was planned to find out drought-tolerant sesame genotypes for future breeding programme using specific biochemical indices.

Materials and Methods

Experimental set-up

The experiment was performed in the poly house of the seed technology division, Bangladesh Agricultural Research Institute (BARI), Gazipur ($23^0$59' latitude and $90^0$24’ longitude). The pots (28 cm × 28 cm) were filled with 12 kg air-dried soil (sandy loam having pH 7). Tthe pot was filled with a mixture of soil and well-decomposed cow dung at a ratio of 4:1. The final weight of the pot was 13.50 kg. The experiment comprised two factors, viz., duration of drought (30, 45, 60, 75, and 90 days after sowing) at 40-45% FC and sesame genotypes (10) namely, BD 6964, BD 6971, BD 6979, BD 6980, BD 6982, BD 6985, BD 6990, BD 6993, BD 7004 and BD 7014 those were selected based on their morphological characteristics, cluster analysis of qualitative traits, rank of mean and standard deviation of rank of different indices. At 30 days after sowing (DAS), stress was imposed and maintained up to 45, 60, 75, and 90 DAS. The field capacity of the pot was maintained by the TDR 300 machine and also with the gravimetric method. The study was executed in a Randomized Complete Block Design with three replications. The potting media was fertilized with NPKS @ 75, 25, 45, and 18 kg ha^{-1} in the form of urea, triple super phosphate (TSP), muriate of potash (MoP), and gypsum, respectively (FRG, 2012). Full amount of TSP, MoP, gypsum, and two-thirds of urea was applied before three days of seed sowing, while the remaining one-third urea was applied at 30 days after sowing corresponding to the pre-flowering stage. The genotypes were finally harvested at full maturity.

Chlorophyll determination

Determination of chlorophyll was performed according to the method followed by (Arnon, 1949). Five hundred milligrams of fresh leaf were ground in 10 mL of acetone (80%) at 4^{o}C and centrifuged for 10 minutes at 2500 rpm maintaining the same temperature. This process was repeated until the residue became colorless. The extract was transferred to a tube and filled up to 10 mL with 80% acetone to assay immediately. Approximately, 3 mL aliquots were poured to a cuvette and the absorbance of the sample was read at 645 and 663 nm with a spectrophotometer (UV-1800, Shimadzu, Japan) against blank (80% acetone). Chlorophyll content of the sample was calculated and expressed as mg g^{-1} FW by the following formula

$$\text{Chlorophyll a (mg g}^{-1}\text{)}=\{12.7(D_{663})-2.69(D_{645})\}\times V/(1000\times w)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{)}=\{22.9(D_{645})-4.68(D_{663})\}\times V/(1000\times w)$$

Determination of proline

Colorimetric determination of proline was proceeded according to (Ábrahám *et al.*, 2010) based on the reaction between proline and ninhydrin. For proline determination, a solution of proline, ninhydrin acid, and glacial acetic acid (1:1:1) was incubated at 100! for 1 hour. The reaction was halted in an iced bath and the chromophore was removed with 4 mL toluene and its absorbance was recorded with a UV-spectrophotometer (UV-1800, Shimadzu, Japan) at 520 nm wavelength.

Measurement of lipid peroxidation (Malondialdehyde)

Malondialdehyde (MDA), a breakdown product of the peroxidized polyunsaturated fatty acid of the membrane lipid, was estimated using thiobarbituric acid (TBA), as described by (Haque *et al.*, 2022). Approximately, 0.5 g leaf tissue was homogenized in 3 mL 5% (w/v) trichloro acetic acid (TCA), and centrifuged at 11,500 *g* for 10 min. The supernatant (1 mL) was poured in 4 mL of TBA reagent mixture (0.5% TBA in 20% TCA). The reaction solution was heated at 95! in a water bath for 30 minutes, quickly cooled in an ice bath and centrifuged at 11,500 *g* for 15 minutes. The absorbance of the supernatant was measured at 532 nm and was then corrected for non-specific absorbance at 600 nm. The amount of MDA was calculated by means of the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol of MDA g^{-1} FW.

2.5 Measurement of Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide was tested according to the method described by (Sekmen *et al.*, 2014). To determine H_2O_2, 0.5 g leaf tissue was homogenized at 4^{o}C with 3 mL of 50 mM K-phosphate buffer (pH 6.5) and centrifuged at 11,500 *g* for 15 min. The supernatant (3 mL) was collected to mix with 1 mL of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v), before centrifuged at 11,500 *g* for 15 min at room temperature. Spectrophotometrically the optical density of the supernatant was measured at 410 nm to determine the H_2O_2 content (Є = 0.28 µM^{-1} cm^{-1}) and converted as ìmoles g^{-1} FW.

Measurement of the superoxide radical ($O^{2\bullet-}$)

Superoxide radical was determined following the method of (Wu *et al.*, 2012) with some modifications. Accurately, 3 mL of 65 mmol L^{-1} phosphate buffer (pH 7.8) was used to homogenize leaves (0.3 g) on an ice bath which were subsequently centrifuged for 10 minutes at 4°C and 5,000 *g*. The supernatants (0.75 mL) were combined with 0.07 mL of 10 mmol L^{-1} hydroxylamine chlorhydrate and 0.675 mL of 65 mmol L^{-1} phosphate buffer (pH 7.8) before being stored at room temperature. After 20 minutes, 0.375 mL of sulfanilamide (17 mmol L^{-1}) and 0.375 mL of naphthylamine (7 mmol L^{-1}) were added, and kept for another 20 minutes at 25°C before being combined with 2.25 mL ether. The $O_2^{\bullet-}$ concentration was calculated using a standard curve of $NaNO_2$, and the absorbance was measured at 530 nm.

Measurement of methylglyoxal (MG)

Leaf tissue (0.3 g) was extracted using 3 mL of perchloric acid (0.5 M). The mixture was then centrifuged for 10 minutes at 11,000 *g* at 4°C after being incubated

Table 1: Interaction effect of genotypes and drought duration on Chl (a+b) content of sesame genotypes.

Genotype	Drought duration	Chl (a+b) (mg g^{-1} FW)	Proline (µmoles g^{-1} FW)
BD 6971	30 DAS (Control)	0.58	1.76
	45 DAS	0.55	2.16
	60 DAS	0.54	3.83
	75 DAS	0.52	5.29
	90 DAS	0.51	6.06
BD 6964	30 DAS (Control)	0.52	1.67
	45 DAS	0.52	2.26
	60 DAS	0.51	3.59
	75 DAS	0.49	4.36
	90 DAS	0.47	5.29
BD 6990	30 DAS (Control)	0.53	1.55
	45 DAS	0.51	1.82
	60 DAS	0.48	2.57
	75 DAS	0.47	3.90
	90 DAS	0.45	4.85
BD 7014	30 DAS (Control)	0.51	1.30
	45 DAS	0.50	1.33
	60 DAS	0.47	2.41
	75 DAS	0.43	3.10
	90 DAS	0.43	4.07
BD 7004	30 DAS (Control)	0.52	1.47
	45 DAS	0.50	1.10
	60 DAS	0.47	1.38
	75 DAS	0.43	2.29
	90 DAS	0.41	3.86
BD 6993	30 DAS (Control)	0.51	1.48
	45 DAS	0.48	1.82
	60 DAS	0.46	1.88
	75 DAS	0.41	2.02
	90 DAS	0.39	3.46
BD 6979	30 DAS (Control)	0.49	1.53
	45 DAS	0.47	1.46
	60 DAS	0.45	1.42
	75 DAS	0.39	2.16
	90 DAS	0.56	3.52
BD 6985	30 DAS (Control)	0.48	1.37
	45 DAS	0.45	1.71
	60 DAS	0.42	1.84
	75 DAS	0.39	2.63
	90 DAS	0.37	2.91
BD 6982	30 DAS (Control)	0.45	0.93
	45 DAS	0.42	0.99
	60 DAS	0.40	1.20
	75 DAS	0.37	2.58
	90 DAS	0.34	2.97
BD 6980	30 DAS (Control)	0.43	0.70
	45 DAS	0.40	0.78
	60 DAS	0.35	0.97
	75 DAS	0.32	2.04
	90 DAS	0.31	2.32
LSD (0.05)		**0.052**	
CV (%)		**5.26**	

for 15 minutes on ice. Plant extracts were decolored by adding charcoal (10 mg mL^{-1}), allowed to stand for 15 minutes at room temperature, and centrifuged for 10 minutes at 11,000 *g* to get a colored supernatant. Before being used in the MG test, the supernatant was neutralized by centrifuging it again for ten minutes at 11,000 *g*. It was then allowed to rest at room temperature with a saturated potassium carbonate solution for 15 minutes. In accordance with Rohman *et al*., (2016), neutralized supernatant was utilized for MG estimation. Freshly, 500 mM aqueous solution of N-acetyl-L-cysteine was made. The reaction was conducted in sodium dihydrogen phosphate buffer (100 mM) at 25°C. The buffer's pH was adjusted to 7.0 using 10 M NaOH. Firstly, sodium dihydrogen phosphate buffer was added to a volume of 980 µL together with the MG solutions of 5, 10, 15, 20, and 25 µL, corresponding to 0.5, 2, and 5 mM. The spectrophotometer was then set to zero. About 20 µL of N-acetyl-L-cysteine solution (final concentration up to 10 mM) was added to initiate the reaction. The formation of N-α-acetyl-S-(1-hydroxy-2-oxo-prop-1-yl) cysteine was noted for 10 min at 288 nm wavelength.

Statistical analysis

Statistical analysis of the data was carried out using the computer-based statistical package STATISTIX 10 and STAR 2.0.1 software. Means were compared by employing the Least Significant Difference (LSD) Test at 5% significance level of probability.

Results

Chlorophyll content

Photosynthetic pigment contents decreased sharply with increasing the duration of drought stress at all the genotypes (Table 1). The Chlorophyll (a+b) content was significantly reduced by drought stress (Table 1). Among ten sesame genotypes, BD 6964 and BD 6971 showed minimum reduction (9.61 and 12.06%, respectively) under extreme drought stress (90DAS) as against the maximum (27.90 and 24.49%) in BD 6980 and BD 6979, respectively compared to their corresponding control at the end of the stress period. However, the reduction percentage was medium in other genotypes.

Proline content

Proline accumulation did not show any significant differences at early drought (45 DAS) conditions in BD 6980 and BD 6982 genotypes, however, a significant increase in proline accumulation was found in BD 6984 and BD 6971 genotypes (Table 2). Irrespective of genotype, proline content was also increased with increasing the duration of drought. Compared to the other genotypes, maximum (6.06 µmoles g^{-1} FW) proline content was found in BD 6971 as against the minimum (2.32 µmoles g^{-1} FW) in BD 6980 at 90 DAS of drought.

Superoxide radical anion ($O_2^{\bullet-}$)

Superoxide radical anion production rate varied significantly among the genotypes, duration of drought, and their interaction (Table 2). At the prolonged drought stage (90 DAS), the lowest value (10.44) was recorded from BD 6971, followed by BD 6964 (11.82) as against the highest value (29.89) from BD 6980 genotype. It was more remarkable that $O_2^{\bullet-}$ production increased sharply under prolonged drought stress (90 DAS) in the BD 6980 genotype compared to the gradual increase in the BD 6971 genotype.

Hydrogen peroxide (H_2O_2) content

Hydrogen peroxide content was significantly influenced by sesame genotypes and the duration of drought conditions (Table 2). Under control conditions, H_2O_2 contents of sesame genotypes ranged from 5.87 to 9.20 µmole g^{-1} FW, whereas, under prolonged (90 DAS) drought, it ranged from 9.39 to 32.06 µmole g^{-1} FW. Moreover, the increment was lower at 90 DAS drought in BD 6971 (60.07%), BD 6964 (70.44%), and BD 7004 (82.81), as against a higher increment at BD 6982 (228.60%) and BD 6980 (257.40%).

Table 2: Interaction effect of genotypes and drought duration on MDA, $O_2^{\bullet-}$ -, H_2O_2, MG and proline content of sesame genotypes.

Genotype	Drought	A	B	C	D
BD 6971	30 DAS (Control)	27.32	6.45	5.87	4.70
	45 DAS	30.39	7.42	6.82	5.17
	60 DAS	31.79	8.16	8.34	5.77
	75 DAS	33.10	8.74	8.51	6.59
	90 DAS	34.73	10.44	9.39	9.04
BD 6964	30 DAS (Control)	28.51	5.41	6.28	4.70
	45 DAS	31.52	7.71	7.02	5.19
	60 DAS	32.82	9.07	8.60	7.15
	75 DAS	34.22	9.68	9.75	8.13
	90 DAS	35.70	11.82	10.70	10.17
BD 6990	30 DAS (Control)	32.37	7.44	8.09	4.97
	45 DAS	36.66	9.36	10.17	5.83
	60 DAS	41.92	11.65	11.19	8.76
	75 DAS	48.15	11.76	14.68	9.86
	90 DAS	50.71	15.54	15.72	12.54
BD 7014	30 DAS (Control)	31.95	8.12	8.56	5.20
	45 DAS	36.38	10.42	9.86	5.75
	60 DAS	39.76	11.47	12.62	8.79
	75 DAS	49.66	13.36	14.19	11.16
	90 DAS	51.95	14.46	16.42	13.65
BD 7004	30 DAS (Control)	32.01	8.17	9.20	5.92
	45 DAS	37.74	9.54	11.26	6.44
	60 DAS	44.37	11.16	11.64	9.74
	75 DAS	55.04	15.19	15.27	12.07
	90 DAS	61.24	18.39	16.80	15.94
BD 6993	30 DAS (Control)	33.56	7.03	9.00	5.53
	45 DAS	41.52	10.76	10.64	6.67
	60 DAS	48.07	11.76	11.59	11.77
	75 DAS	58.35	17.45	17.10	14.90
	90 DAS	66.15	19.39	20.03	18.43
BD 6979	30 DAS (Control)	35.73	9.16	8.69	6.39
	45 DAS	43.89	10.49	10.48	6.87
	60 DAS	49.97	13.36	13.24	12.12
	75 DAS	61.36	15.43	15.41	14.50
	90 DAS	69.70	21.20	19.52	17.77
BD 6985	30 DAS (Control)	36.28	8.55	8.62	6.62
	45 DAS	42.32	11.11	12.43	7.67
	60 DAS	53.98	14.37	14.02	11.71
	75 DAS	65.08	17.13	17.78	13.32
	90 DAS	73.00	23.05	23.03	18.37
BD 6982	30 DAS (Control)	39.15	9.87	8.86	6.73
	45 DAS	46.43	13.08	13.71	8.56
	60 DAS	54.98	16.73	16.32	13.10
	75 DAS	68.83	21.73	23.02	19.07
	90 DAS	78.47	27.48	29.11	24.84
BD 6980	30 DAS (Control)	40.91	9.61	8.97	6.78
	45 DAS	50.72	12.41	12.77	9.50
	60 DAS	58.57	15.70	15.73	13.80
	75 DAS	74.42	20.74	25.43	21.35
	90 DAS	82.55	29.89	32.06	25.75
LSD (0.05)		2.32	1.76	2.59	1.98
CV (%)		3.06	7.40	8.12	9.64

A: MDA (nmol g^{-1} FW); **B:** $O_2^{\bullet-}$ (nmol g^{-1} FW min^{-1});
C: H_2O_2 (µmoles g^{-1} FW); **D:** MG (µmoles ml^{-1})

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Lipid peroxidation (Malondialdehyde)

Malondialdehyde (MDA) content varied significantly among genotypes, drought duration, and their interactions (Table 2). Irrespective of genotypes, MDA content gradually increased with the increasing duration of drought stress. The MDA content was higher in BD 6980 (40.91, 50.72, 58.57, 74.42, and 82.55 at 30, 45, 60, 75, and 90 DAS, respectively) followed by BD 6982 (39.15, 46.43, 54.98, 68.83 and 78.47 at 30, 45, 60, 75 and 90 DAS, respectively), in comparison to lower MDA content in BD 6971 (27.32, 30.39, 31.79, 33.10 and 34.73 at 30, 45, 60, 75 and 90 DAS, respectively) and BD 6964 (28.51, 31.52, 32.82, 34.22 and 35.70 30, 45, 60, 75 and 90 DAS, respectively). The rest of the genotypes showed moderate performance in MDA content. At the control condition, MDA content was minimal for all genotypes, which was significantly increased under drought at different days after sowing. However, the increment was higher in BD 6980, which was 23.98, 43.18, 81.92, and 101.79% at 45, 60, 75, and 90 DAS, respectively, compared to their control. Conversely, BD 6971 showed 11.23, 16.36, 21.15, and 27.12% increases at 45, 60, 75, and 90 DAS, respectively, which was at par with BD 6964 (10.55, 15.11, 20.02, 25.21% at 45, 60, 75 and 90 DAS, respectively).

Methylglyoxal (MG) content

Methylglyoxal content was increased significantly with the increase in the duration of drought in all the genotypes (Table 2). The higher (6.78) accumulation of MG was recorded in BD 6980 genotypes and lower (4.70) in BD 6971 and BD 6964 genotypes under drought stress. Under prolonged drought (90 DAS), MG content increased by 92.34 and 116.40% in BD 6971 and BD 6964, whereas, 269.20 and 279.80% in BD 6982 and BD 6980 genotypes, respectively compared to their respective control.

Discussion

Drought stress stands as a significant impediment to crop production. The alterations in biochemical attributes serve as the primary determinants of the impact of stress on plants (Alvi *et al*., 2022). An evident impact of drought stress on diverse biochemical attributes of sesame genotypes was noted, as these parameters were notably influenced by the duration of drought. Photosynthetic pigments play a crucial role for plants by capturing sunlight essential for photosynthesis. Any decrease in these pigments directly diminishes the photosynthesis rate in plants (Chavoushi *et al*., 2020). The concentration of photosynthetic pigments (chlorophyll a, chlorophyll b, and total chlorophyll) was negatively impacted by both genotype variations and the duration of drought. Photo inhibition and the photo-destruction of pigments may have contributed to such alterations. Damage caused by active oxygen species to chloroplasts is the primary cause of the reduction in chlorophyll under drought stress (Kusvuran & Dasgan, 2017). Moreover, (Kamanga *et al*., 2018) also reported that the increased electrolyte leakage is the main cause of the reduction of chlorophyll content. The alterations in chlorophyll content also resulted in lipid peroxidation, leading to the destruction of chlorophyll and the transformation of leaf color from green to yellow. Moreover, the decrease or depletion of chlorophyll content during drought conditions may result from pigment photo-oxidation (Nahakpam, 2017), loss of chloroplast membranes, distortion of lamellar vesicles, excessive swelling, slower synthesis, and/or accelerated breakdown or dissociation of chlorophyll molecules (Kadkhodaie *et al*., 2014).

Proline accumulation is an important physiological index for plant response to drought stress as well as to other types of stress to reduce the injury of the cell (Khan *et al*., 2019). It acts as a signaling molecule to modulate mitochondrial functions influence cell proliferation or cell death and trigger specific gene expression. In this study, drought notably resulted in an elevation of proline content (Table 2), likely attributed to the potential effect of drought in enhancing the production of proline precursors. These findings align with those of (Hota *et al*., 2019), who observed heightened proline accumulation in all sesame genotypes under stress conditions. Furthermore, they noted that the activation of pyrroline-5-carboxylate synthetase (an enzyme involved in proline biosynthesis) and the inhibition of proline dehydrogenase (an enzyme involved in proline degradation) occurred more rapidly under severe drought conditions. Elevated proline content in plants experiencing water stress has been documented across various species. It serves as a regulatory or signaling molecule, triggering multiple responses integral to the adaptation process. (Peymaninia *et al*., 2012) investigated both drought tolerant and sensitive genotypes in Sesamum indicum. They observed a notable rise in proline levels in the leaves of both tolerant and sensitive genotypes. (Ghorbanli *et al*., 2013) also reported that the leaf proline increased significantly under mild and severe drought stress in comparison with control in tomato.

Lipid peroxidation, defined as the oxidative degradation of polyunsaturated lipids is a well-known index for determining the extent of oxidative stress because increased MDA content is highly correlated with oxidative damages induced by various abiotic stresses including drought (Morales & Munné-Bosch, 2019). As per (Zu *et al*., 2017), cultivars exhibiting greater drought tolerance demonstrate lower MDA content under stress conditions. This study also confirmed a time-dependent enhancement of MDA levels under drought stress (Table 2). The heightened production of reactive oxygen species (ROS) in drought-stressed tissues leads to increased lipid peroxidation, limiting the antioxidant defense system's capacity to counteract the ROS generated by stress. The overproduction of oxygen species (ROS) induced by abiotic stress, along with subsequent lipid peroxidation, has been evidenced in various crops including sesame (Ullah *et al*., 2017; W. Wei *et al*., 2013; Zheng *et al*., 2017).

In addition to MDA, both O_2•- and H_2O_2 are harmful substances detrimental to the cell, and their excessive accumulation indicates oxidative stress (Hasanuzzaman & Fujita, 2011). Our experiment revealed elevated levels of both O_2•- and H_2O_2 under drought stress (Table 2), consistent with findings from several prior studies (Rani *et al*., 2018; Rohman *et al*., 2016). The restricted ability to scavenge O_2•- and H_2O_2 under abiotic stress conditions could account for this observation.

MG is unavoidably generated (either through enzymatic processes or spontaneously) as a byproduct of fatty acid, protein, and glucose metabolism. Nevertheless, its overproduction is observed in plants when exposed to various abiotic stresses. Moreover, beyond its direct cytotoxic impact on cellular components, MG is recognized as an inherent mediator of O2•- production(Saito *et al*., 2011), thereby exacerbating oxidative damage. This study documented a notable rise in MG content in drought-stressed plants (Table 2), suggesting that drought-induced oxidative damage is augmented by the production of MG alongside ROS. This present investigation follows the result of (Rohman *et al*., 2016). Reports have also documented an increase in glyoxalase activities under drought conditions in rapeseed (Hasanuzzaman & Fujita, 2011). Considering biochemical characteristics (viz. chlorophyll, proline, $O_2^{\bullet-}$, H_2O_2, MDA, and MG content), BD 6971 and BD 6964 were identified as relatively tolerant, BD 6982 and BD 6980 were classified as relatively susceptible genotypes.

Conclusion

Drought stress significantly altered the biochemical responses of studied genotypes. According to the biochemical reactions, certain genotypes demonstrated superior performance and were categorized as drought-tolerant (e.g., BD 6964 and BD 6971), whereas others, such as BD 6980 and BD 6982, were deemed drought-sensitive due to their significant reduction in biochemical parameters under drought conditions. Moreover, the genotypes (BD 6990 and BD 7014) were considered moderately tolerant as their biochemical parameters were slightly affected under drought conditions. The potential of sesame genotypes like BD 6964 and BD 6971 makes them suitable candidates for inclusion in future breeding programs aimed at developing drought-tolerant varieties. Their cultivation in regions with limited water resources could expand the cultivated area and enhance production efficiency in Bangladesh.

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