Indian Journal of Experimental Biology
Vol. 57, September 2019, pp. 690-699

Identification of drought tolerant *Sesamum* genotypes using biochemical markers

Tapaswini Hota[1], Chinmaya Pradhan[2] & Gyana R Rout[1]*

[1]Department of Agril. Biotechnology, College of Agriculture, OUAT, Bhubaneswar-3, Odisha, India
[2]Post Graduate Department of Botany, Utkal University, Bhubaneswar-4, Odisha, India

Received 04 December 2017; *revised 11 December 2018*

Sesamum indicum L. (Pedaliaceae) is an economically important oil seed crop and used for food, nutraceutical, pharmaceutical industries. Drought stress is considered as the most important abiotic stress limiting to crop production. In this context, the present study was to illustrate the biochemical markers of *Sesamum* genotypes to identify the drought tolerance. The irrigation was made in three treatments [T-1 Control (100% FC), T-II (50% FC) and T-III (25% FC)]. Growth rate, fresh and dry weight were recorded in all treatments. The biochemical analysis showed that the total pigment content was more in 'ENT-78-301' and minimum in 'SI-205' at T-III (25% FC). The total carbohydrates, proline and polyphenol content were also increased at treatment-III in all the genotypes after 30 days of water stress. Enzyme activities are variable in different treatments under water stress. The super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities differentiate the genotypes reflected to drought stress. The enzyme activity was higher at T-III (25 % FC) in all the genotypes. However, the maximum was observed in genotype 'Prachi' and minimum in 'SI-1025'. On the basis of biochemical and enzymatic analysis, the genotypes 'SI-1025', 'SI-205', 'SI-2138-2' and 'KM-13' are more tolerant to drought and 'ENT-78-301', 'Prachi', 'Kanaka','S-0140', 'SI-1926' and 'IS-607-1-84' are the susceptible to drought. The results of this study are useful for *Sesamum* breeding programme.

Keywords: Abiotic stress, Benne, Drought stress, Oil seed, Sesame

Sesamum indicum L. (Pedaliaceae) is one of the oldest and important oil seed crops. Sesame is widely used in food, nutraceutical and pharmaceutical industry in many countries because of its high oil, protein and antioxidant contents[1]. It is usually cultivated in arid and semi-arid regions of the world for its quality edible oil and it is very responsive to the changing environmental conditions[2]. Growth of plant was adversely affected with increasing drought level[3-4]. Drought stress is a common problem seriously influencing grain production and quality. Severe water stress may result in the arrest of photosynthesis and disturbance of metabolism[5]. Drought stress leads to increase accumulation of reactive oxygen species (ROS) in plants and causes to damage the various cellular mechanisms, such as enzyme inhibition, protein degradation, membrane lipid peroxidation and damage of nucleic acid, which ultimately culminate in cell death. In different crops, physiological responses to drought vary with the genotype[6-7]. The different irrigation intervals cause in stunted growth and decreased seed yield of sesame through effect on photosynthesis process in addition to the yield, oil and protein contents[8]. However, to respond the changing environmental and growing conditions, plant behaviour can change the biosynthesis of bioactive compounds when subjected to abiotic constraints instead of yield parameters[9]. Drought is a wide spread problem mostly in arid and semiarid regions that seriously influencing crop production[10].

Drought tolerance consists of plant ability to growth under water deficit conditions[11]. Understanding the response of plant to dry environments has great importance and also a fundamental part of producing stress tolerant crops. Increasing plant resistance to drought stress would be the most economical approach to improve agricultural productivity and reduce agricultural use of fresh water resources. In this context, the present investigation, we tried to assess the influence of drought stress on growth, physiological and biochemical changes in *Sesamum indicum* L.

Materials and Methods

Plant material and different levels of irrigation

The genetically pure seed material of 33 *Sesamum* genotypes representing different geographical locations in India was collected from the germplasm centre of

*Correspondence:
E-mail: grrout@rediffmail.com

AICRP in *Sesamum* at Odisha University of Agriculture & Technology, Bhubaneswar, India**.** Out of them, 10 genotypes ('SI-2138-2', 'S-0140', 'Prachi', 'Kanaka', 'SI-1025', 'SI-205', 'KM-13', 'IS-607-1-84', 'SI-1926' and 'ENT-78-301') were selected for this study on the basis of high yield. A factorial experiment in a completely randomized design with three replications was performed in a greenhouse of the Department of Agricultural Biotechnology, Orissa university of Agriculture and Technology, Bhubaneswar. After surface sterilization the seeds were sown into plastic pots (20×30 cm) filled with 5 kg soil mixture (soil, sand and cow-dung in the ratio 1:1:1). Experimental soil was a sandy-clay loam texture with pH 8.0. Experiments were conducted in a green house with 65% relative humidity. All the pots were irrigated up to 30 days after sowing and then applied to various levels of irrigation to maintain the field capacity (FC). Various levels of irrigation are follows i.e. T-1 Control (100% FC), T-II (50% FC) and T-III (25% FC). Gravimetric method was employed to determine the field capacity of soil[12]. For drought treatment one group of 30 days old plant was maintained under optimum irrigation (Field Capacity), and the other groups were subjected to 50% FC and 25% FC. Drought treatment continued for 30 days, and leaves were collected on 7^{th}, 15^{th}, 21^{th} and 30^{th} day intervals and frozen in liquid N_2 immediately and stored at –20°C for analysis. Drought stress in change of chlorophyll content, proline, proteins, polyphenol and total carbohydrates contents, antioxidant enzymes (POD, SOD, GPX) were analyzed. Ten seedlings from each treatment were sampled randomly at 7^{th}, 15^{th} , 21^{th} and 30^{th} days after drought treatment. Data on root and shoot length, fresh and dry weight of shoots and root were determined. All the plant samples were dried at 55°C for 2 days in hot air oven to a constant weight as dry weight was determined.

Estimation of chlorophyll

Sesamum leaves of both control and treated were washed with distilled water. One gram of leaves, chopped into small pieces grinded thoroughly using sterilized sand and homogenate with of 80% (v/v) acetone. The homogenate was transferred to a 50 mL falcon tube and centrifuged at 8000 rpm for 20 min. To remove residue as pellet, supernatant green colour was collected and volume of extracted chlorophyll was measured. Absorbance was recorded at 663 nm (chlorophyll-a), 645 nm (chlorophyll-b), 470 nm (carotenoid) under a UV-Vis Spectrophotometer (Perkinmer, Germany).The amount of chlorophyll was estimated according to Vernon[13]. This process was repeated for all the treated plant samples. The amount of chlorophylls and carotenoid were calculated using the formula in µg/mL fresh weight basis.

Determination of total carbohydrate

Five hundred milligrams of fresh leaves were taken into a tube and hydrolyzed with 5 mL 2.5N HCl for 3 h by the hot water bath. Further, the test tubes were cooled at room temperature and the sample was neutralized by adding solid sodium carbonate till the effervescence ceases. The volume was made to 100 mL and centrifuged at 5000 rpm for 15 min. The supernatant was collected and 0.1 mL aliquots from each sample were taken for analysis. The volume was made up to 1.0 mL in all test tubes by adding distilled water. Four milliliter of anthrone reagent was added to each tube and heated for 8 min in boiling water bath and cool down rapidly. Then, the measurement was taken at 630 nm.

Proline content

Five hundred milligram of leaf samples were taken from each treatment and homogenized in 3% (w/v) sulphosalycylic acid and filtered through filter paper. Mixture was heated at 100°C for 1 h in a hot water bath after addition of ninhydrin and glacial acetic acid. Reaction was stopped by putting in ice bath. The mixture was extracted with toluene and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and expressed as µg proline per g fresh weight basis[14].

Estimation of protein

The total soluble protein content was determined according to the method of Lowry *et al*.[15]. One gram fresh leaves of *Sesamum* were homogenized in ice cold trichloroacetic acid (TCA) buffer and incubated overnight at 4°C. Then, the sample was centrifuged at 8000 rpm for 20 min. Supernatant was discarded and pellet was washed with acetone to remove the pigments. The sample was centrifuged at 10000 rpm for 10 min and washed with 80% alcohol to remove phenolic compounds. Further, centrifuged at 5000 rpm for 10 min and the pellet was suspended in known volume of extraction buffer and kept in boiling water bath for 2 min. Aliquot (100 µL) was taken and volume was made up to 1.0 mL. Five milliliter of reagent-C (50 mL of 2% (w/v) sodium carbonate

dissolved in 0.1N NaOH + 1.0 mL of 0.5 % (w/v) copper sulphate dissolved in 1% sodium potassium tatarate) was added, mixed well and kept for 10 min. Further, 0.5 mL of Folin-Ciocalteau reagent was added, mixed well and incubated in the dark for 30 min at room temperature. After blue colour developed, the OD was taken at 660 nm. The protein content was calculated against the standard graph.

Estimation of total polyphenols

Total polyphenol content was determined by Folin-Ciocalteu method[16]. Five hundred milligram of fresh leaf sample of both control and treated were grinded with a pmotor and pestle in 10-time volume of 80% (v/v) ethanol. The homogenate was centrifuged at 10000 rpm for 20 min and the supernatant was collected. The residue was re-extracted with 5 times the volume of 80% (v/v) ethanol and the supernatant was collected. The supernatant was evaporated to dryness. Then, the residue was dissolved in a known volume of distilled water. Different aliquots were pipetted out into test tubes. Volume was making up to 3 mL with water and added 0.5 mL (v/v) of Folin-Ciocalteau reagent. After 3 min, 2mL of 20% (w/v) of Na_2CO_3 solution was added in each tube. Further, the sample tubes were kept in a boiling water bath for 1min. The absorbance was recorded at 650 nm against the blank. A standard curve was prepared using different concentration of catechol.

Enzyme assays

Five hundred milligrams of leaf tissues were grinded by using a mortar and pestle with 5 mL extraction buffer containing 50 mM potassium phosphate buffer pH 7.6 and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15,000g for 15 min and the supernatant fraction used to assay for the various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C. The superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT)[17]. The reaction solution consisted of 50 µL of the enzyme extract, 50 mM phosphate buffer (pH 7.8), 0.1 µM EDTA, 13 mM methionine, 75 µM nitroblue tetrazolium and 2 µM riboflavin in a total volume of 1.5 mL. After adding riboflavin, the test tubes were shaken manually and placed under fluorescent light with two 20W lamps. After 15 min, the sample tubes were taken back from light to stop the reaction. Reduction in NBT was estimated by reading the absorbance of the reaction mixture at 560 nm, and one unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT reduction and the results were expressed as Unit/mg.protein .

Peroxidase (POD) (EC 1.11.1.7) activity was determined with guaiacol as reducing substrate in a reaction mixture containing 0.1M potassium-phosphate buffer (pH-6), 20 mM guaiacol, and 30 mM H_2O_2. The oxidation of guaiacol was assessed by recording the absorbance increase at 470 nm at 25°C. The enzyme unit was express as the absorbance change at 470 nm, per min, under the above conditions.

Glutathione peroxidase (GPX) was analysed by the method of Wendel[18]. One mg of NADPH, 9.2 mL of 1 mM sodium azide solution (in 50 mM sodium phosphate buffer with 0.4 mM EDTA), 0.1 mL of glutathione reductase enzyme solution (100 U/mL), and 0.05 mL of glutathione reduced (GSH) were added and mixed by inversion. Then, 3 mL of the mixture in 0.05 mL of enzyme extract was added. It was vortexes and incubated for 5 min at room temperature. After the incubation, 0.05 mL of H_2O_2 was mixed immediately and measure at 340 nm after every 30 s over a period of 5 min. GPX activity was calculated from the change in optical density per min in the maximum linear rate range using a molar extinction coefficient for NADPH of 6.22×103/µmol and assuming 2 mol of GSH formed for each mole of NADPH consumed. One unit activity was defined as 1 µmol NADPH oxidized per minute.

Statistical analysis

Each data point represents the mean of three samples ± SE. Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test, and the significance level of P <0.05 was employed

Results and Discussion

Water shortage is considered to be one of the most adverse abiotic stress factor influencing plant growth and their physiological and biochemical response in crop production. Significant effects of water regimes on sesame growth parameters were found after sowing the sample using 100% FC (control) (Treatment-I) as compared with treatments 50% FC (Treatment-II) and 25% FC (Treatment-III) water regime. The growth was decreased by decreasing irrigation water quantity. The results showed that both shoot and root fresh weight decreased in all genotypes

but more prominent in one genotype 'SI-1926'. Moreover, dry weight of shoot and root was reduced in the treatment of 50%, and 25% of water regime respectively comparing with 100 % FC (Tables 1). Genotypes like 'SI-1025, SI-205', 'SI-2138-2'' and 'KM-13' showed higher dry weight of shoot and root after 30 days in presence of water stress (25% FC) and therefore categorized as drought tolerant. These results are in agreement with the findings of Mensah *et al.*[8]. The increase of dry weight could be due to the generation of hypoxia and stomatal conductance under drought conditions.

Photosynthetic pigments are major drivers of plants photosynthetic capacity due to their crucial role in both absorption (chlorophyll) and dissipation (carotenoids) light energy. The chlorophyll content was decreased with increase of drought stress but in some cases chlorophyll-b increased at lower water regime (25% FC) and then again decreased with increasing the stress. The lowest total chlorophyll content was recorded after 30 days of stress treatment i.e 1047.3 and 977.9 µg/g fresh weight basis in genotype 'SI-2138-2' and 'S-0140' at 25% water regime, respectively (Tables 2). The highest carotenoid content was observed in 'Prachi' in treatment–III (765.9 µg/g fresh weight) after 15 days and 632.7 µg/g fresh weight basis) after 30 days of stress treatment. Our results are in agreement with the findings of other researchers[19,20]. The adverse effect of water stress on chlorophyll content has previously been shown in different plant species[21]. Kamanga *et al.*[21] reported that the increased electrolyte leakage in reduction of chlorophyll content. A decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess

Table 1 — Fresh and dry weight (gm) of ten genotypes of *Sesamum indicum* after 15 and 30 days of water stress

Genotypes	Treatments	Fresh weight (Mean ± SE)*				Dry weight (Mean±SE)*			
		Shoot (15days)	Root (15 days)	Shoot (30days)	Root (30 days)	Shoot (15days)	Root (15 days)	Shoot (30days)	Root (30 days)
SI-2138-2	T-I (100% FC)	23.8±1.08^{n}	1.66±0.05^{j}	30.5±1.7^{i}	5.24±0.03^{e}	5.33±0.6^{o}	0.36±0.07^{d}	6.57±0.3^{e}	0.97±0.04^{f}
	T-II (50% FC)	15.2±1.12^{i}	0.87±0.02^{f}	12.6±1.2^{e}	2.11±0.07^{b}	2.68±0.8^{j}	0.20±0.03^{b}	3.86±0.2^{c}	0.75±0.08^{e}
	T-III (25% FC)	8.5±2.14^{d}	0.64±0.08^{c}	4.58±1.8^{a}	0.87±0.04^{a}	1.02±0.9^{c}	0.09±0.09^{a}	1.06±0.4^{a}	0.23±0.03^{b}
S-0140	T-I (100% FC)	25.1±1.17^{o}	1.96±0.07^{m}	37.9±1.3^{l}	7.99±0.06^{f}	5.85±0.5^{p}	0.37±0.07^{d}	4.21±0.3^{d}	2.88±0.07^{i}
	T-II (50% FC)	12.8±1.18^{g}	1.03±0.03^{g}	16.9±1.5^{g}	2.86±0.02^{c}	3.18±0.7^{l}	0.21±0.08^{b}	2.99±0.2^{c}	1.45±0.05^{h}
	T-III (25% FC)	10.3±1.29^{f}	0.86±0.08^{f}	0.96±0.9^{a}	1.53±0.07^{b}	1.63±0.3^{e}	0.15±0.08^{a}	0.84±0.6^{a}	0.84±0.07^{e}
Prachi	T-I (100% FC)	22.4±1.12^{m}	1.98±0.02^{m}	38.8±1.7^{l}	6.98±0.04^{f}	4.95±0.5^{n}	0.49±0.06^{e}	3.18±0.4^{c}	0.98±0.06^{f}
	T-II (50% FC)	18.4±1.27^{k}	1.04±0.03^{g}	15.5±1.9^{f}	3.22±0.08^{c}	2.34±0.8^{i}	0.23±0.08^{b}	2.03±0.7^{b}	0.75±0.04^{e}
	T-III (25% FC)	13.3±1.24^{g}	0.65±0.08^{c}	4.22±0.4^{b}	1.33±0.03^{b}	1.76±0.9^{f}	0.15±0.03^{b}	1.10±0.6^{a}	0.12±0.09^{a}
Kanaka	T-I (100% FC)	26.4±1.18^{o}	2.14±0.02^{m}	30.3±1.2^{i}	4.10±0.06^{d}	4.33±0.6^{m}	0.48±0.07^{e}	3.08±0.3^{c}	0.67±0.02^{d}
	T-II (50% FC)	19.5±1.17^{l}	1.31±0.09^{h}	16.8±1.5^{g}	2.42±0.05^{b}	2.16±0.4^{h}	0.25±0.05^{b}	1.36±0.6^{a}	0.34±0.07^{c}
	T-III (25% FC)	12.5±1.24^{g}	0.67±0.08^{d}	6.77±1.4^{c}	0.87±0.09^{a}	1.35±0.7^{d}	0.12±0.02^{a}	1.68±0.9^{b}	0.15±0.04^{a}
SI-1025	T-I (100% FC)	17.6±1.28^{j}	1.87±0.07^{l}	32.6±1.1^{j}	6.97±0.02^{a}	2.97±0.1^{k}	0.32±0.08^{c}	2.06±0.1^{b}	0.63±0.06^{d}
	T-II (50% FC)	9.32±2.19^{e}	1.12±0.04^{g}	15.1±1.7^{f}	3.71±0.08^{d}	2.03±0.6^{g}	0.21±0.04^{b}	1.11±0.2^{a}	0.41±0.05^{c}
	T-III (25% FC)	6.35±2.48^{b}	0.76±0.06^{e}	7.42±1.9^{c}	1.44±0.04^{b}	1.22±0.2^{d}	0.10±0.06^{a}	0.64±0.8^{a}	0.12±0.08^{a}
SI-205	T-I (100% FC)	15.1±1.19^{i}	1.56±0.06^{i}	22.5±1.1^{h}	3.99±0.07^{d}	2.03±0.8^{g}	0.32±0.02^{c}	2.88±0.2^{c}	0.78±0.02^{e}
	T-II (50% FC)	7.83±2.12^{d}	0.93±0.09^{f}	14.8±1.2^{f}	1.45±0.06^{b}	1.13±0.9^{c}	0.21±0.05^{b}	1.35±0.8^{a}	0.52±0.07^{d}
	T-III (25% FC)	4.66±2.47^{a}	0.55±0.08^{b}	7.24±1.8^{c}	0.75±0.05^{a}	0.76±1.3^{b}	0.11±0.08^{a}	0.69±0.9^{a}	0.19±0.06^{b}
KM-13	T-I (100% FC)	17.2±1.14^{j}	1.30±0.02^{h}	55.3±1.0^{m}	6.99±0.04^{f}	2.32±0.7^{i}	0.37±0.03^{d}	2.86±0.1^{c}	0.87±0.04^{e}
	T-II (50% FC)	10.4±1.28^{f}	0.89±0.09^{f}	23.1±1.2^{h}	3.56±0.03^{d}	1.21±0.9^{d}	0.18±0.09^{b}	1.63±0.9^{b}	0.62±0.02^{d}
	T-III (25% FC)	6.77±2.16^{d}	0.53±0.06^{b}	10.5±1.4^{d}	1.64±0.02^{b}	0.74±1.3^{b}	0.11±0.04^{a}	0.87±0.4^{a}	0.21±0.08^{b}
IS-607-1-84	T-I (100% FC)	12.7±1.21^{g}	0.99±0.05^{g}	15.6±1.9^{f}	4.88±0.08^{e}	2.03±0.2^{g}	0.22±0.07^{b}	1.76±0.6^{b}	0.73±0.09^{e}
	T-II (50% FC)	7.49±2.16^{d}	0.53±0.03^{b}	7.93±1.8^{c}	2.41±0.06^{b}	1.21±0.1^{d}	0.10±0.08^{a}	0.96±0.8^{a}	0.43±0.07^{c}
	T-III (25% FC)	5.23±2.16^{b}	0.32±0.02^{a}	4.24±0.7^{b}	1.67±0.07^{b}	0.72±0.3^{b}	0.08±0.09^{a}	0.42±0.3^{a}	0.22±0.05^{b}
SI-1926	T-I (100% FC)	19.3±1.18^{l}	2.33±0.03^{n}	34.3±1.3^{k}	4.85±0.04^{e}	0.86±1.1^{c}	0.43±0.06^{d}	1.97±0.7^{b}	0.41±0.03^{c}
	T-II (50% FC)	10.6±1.15^{f}	1.57±0.09^{i}	17.7±1.8^{g}	1.37±0.03^{a}	0.73±0.3^{b}	0.34±0.07^{c}	1.05±0.2^{b}	0.25±0.02^{b}
	T-III (25% FC)	6.89±2.14^{c}	0.89±0.07^{f}	9.34±1.5^{d}	0.99±0.08^{a}	0.54±1.6^{a}	0.14±0.09^{a}	0.46±0.4^{a}	0.11±0.07^{a}
ENT-78-301	T-I (100% FC)	14.3±1.17^{h}	2.56±0.08^{o}	18.3±1.6^{g}	1.97±0.05^{b}	1.48±0.5^{e}	0.17±0.03^{b}	1.21±0.7^{a}	0.57±0.04^{d}
	T-II (50% FC)	8.75±2.18^{e}	1.29±0.04^{h}	9.45±1.2^{d}	1.02±0.07^{a}	0.98±1.2^{c}	0.10±0.06^{a}	0.97±0.2^{a}	0.47±0.08^{d}
	T-III (25% FC)	6.85±2.17^{c}	0.95±0.05^{f}	5.13±1.1^{b}	0.52±0.04^{a}	0.54±0.6^{a}	0.08±0.09^{a}	0.56±0.8^{a}	0.18±0.05^{b}

[*15 replicates/treatment; repeated twice. Irrigation given every 2-day interval. Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level]

Table 2 — Pigment content of ten genotypes of *Sesamum indicum* after 15 and 30 days of water stress

Genotypes	Treatments	Pigment content (Mean ± SE)* after 15 days (µg/g fresh weight basis)				Pigment content (Mean ± SE)* after 30 days (µg/g fresh weight basis)			
		Chl-a	Chl-b	Chl (a+b)	Carotenoid	Chl-a	Chl-b	Chl (a+b)	Carotenoid
SI-2138-2	T-I (100% FC)	1327.6±11.5^{d}	413.6±12.5^{c}	1741.2±10.5^{f}	598.2±10.2^{b,c}	1234.2±10.6^{f}	326.7±11.3^{a}	1560.9±10.2^{f}	428.5±11.4^{b}
	T-II (50% FC)	1204.6±10.8^{c}	467.3±13.2^{c}	1671.9±12.3^{e}	563.5±11.4^{b}	892.8±11.2^{b}	330.5±12.7^{a}	1223.3±11.4^{d}	436.2±12.5^{b}
	T-III (25% FC)	1023.7±12.4^{b}	312.8±11.6^{b}	1336.5±11.8^{b}	522.8±13.6^{b}	734.6±12.8^{a}	312.7±13.6^{a}	1047.3±12.7^{b}	418.5±14.2^{b}
S-0140	T-I (100% FC)	1138.4±13.2^{c}	432.7±10.5^{c}	1571.1±10.2^{d}	545.7±14.7^{b}	1036.5±11.4^{d}	432.7±10.6^{b}	1469.2±11.5^{e}	534.8±13.9^{c}
	T-II (50% FC)	1029.6±11.3^{b}	412.6±11.2^{c}	1442.2±11.2^{c}	542.4±12.4^{b}	869.4±13.9^{b}	383.2±12.8^{b}	1252.6±10.3^{d}	487.6±14.2^{b}
	T-III (25% FC)	988.2±10.4^{a}	387.4±10.3^{b}	1375.6±12.6^{b}	431.9±15.2^{a}	652.3±14.3^{a}	325.6±11.2^{a}	977.9±14.2^{a}	427.5±11.7^{b}
Prachi	T-I (100% FC)	1837.2±12.5^{i}	723.5±11.4^{f}	2560.7±13.5^{l}	834.3±11.6^{e}	1589.3±12.6^{i}	654.8±10.4^{d}	2244.1±10.6^{k}	752.8±10.6^{f}
	T-II (50% FC)	1769.4±11.2^{h}	826.1±12.4^{g}	2595.5±14.2^{l}	838.2±12.4^{e}	1238.5±10.4^{f}	610.3±12.4^{d}	1848.8±11.4^{h}	715.5±11.6^{f}
	T-III (25% FC)	1276.3±13.5^{d}	721.8±13.9^{f}	1998.1±11.7^{h}	765.9±13.5^{d}	932.6±13.2^{c}	549.7±14.2^{c}	1482.3±12.3^{e}	632.7±12.4^{e}
Kanaka	T-I (100% FC)	1682.5±12.5^{g}	521.0±14.2^{d}	2203.5±10.6^{j}	632.6±14.2^{c}	1762.8±11.5^{j}	514.7±11.9^{c}	2277.5±11.7^{k}	625.9±13.2^{e}
	T-II (50% FC)	1573.9±11.8^{f}	520.3±116^{d}	2094.2±11.3^{i}	618.3±13.6^{c}	1417.3±12.4^{h}	502.5±12.4^{c}	1919.8±12.9^{i}	605.0±11.8^{e}
	T-III (25% FC)	1186.3±10.2^{c}	419.6±12.3^{c}	1505.9±12.8^{d}	613.7±14.3^{c}	1015.5±12.8^{d}	491.7±13.7^{b}	1507.2±13.2^{f}	589.7±14.2^{d}
SI-1025	T-I (100% FC)	1253.9±11.4^{d}	378.5±10.4^{b}	1632.4±13.9^{e}	482.6±15.2^{a}	1183.4±10.2^{e}	413.6±11.8^{b}	1597.0±12.6^{f}	520.5±12.6^{c}
	T-II (50% FC)	1187.3±12.5^{c}	343.7±12.8^{b}	1531.0±11.7^{d}	454.8±13.6^{a}	953.7±13.7^{c}	485.2±14.3^{b}	1428.9±11.2^{e}	484.7±13.2^{b}
	T-III (25% FC)	1032.4±10.8^{b}	202.5±11.5^{a}	1234.9±12.4^{a}	412.6±11.4^{a}	825.8±14.9^{b}	364.7±11.4^{a}	1190.5±13.2^{c}	467.1±15.7^{b}
SI-205	T-I (100% FC)	1272.8±11.5^{d}	592.3±13.4^{d}	1865.1±10.5^{g}	695.3±10.2^{c}	1316.3±11.6^{g}	437.2±12.2^{b}	1753.5±10.5^{g}	542.6±12.3^{d}
	T-II (50% FC)	1133.6±11.8^{c}	574.5±11.8^{d}	1708.1±11.8^{f}	683.5±12.5^{c}	1034.9±12.9^{d}	414.8±14.3b	1449.7±11.8^{e}	513.9±13.2^{d}
	T-III (25% FC)	976.3±13.5^{a}	325.8±12.5^{b}	1291.1±10.4^{b}	547.6±14.2^{b}	852.1±14.5^{b}	365.9±15.2^{b}	1218.0±12.6^{d}	468.5±14.7^{b}
KM-13	T-I (100% FC)	1643.7±14.2^{g}	411.4±10.7^{c}	2055.1±12.5^{h}	521.4±13.6^{b}	1467.2±13.5^{h}	415.7±13.6^{b}	1882.9±10.3^{h}	519.4±11.3^{c}
	T-II (50% FC)	1428.3±11.7^{e}	403.7±11.6^{c}	1832.0±11.4^{g}	510.8±12.9^{b}	1028.3±11.4^{d}	383.2±14.8^{b}	1411.5±12.7^{e}	486.3±12.4^{b}
	T-III (25% FC)	1105.6±12.8^{c}	378.3±12.9^{b}	1483.9±10.6^{d}	482.5±15.4^{a}	845.7±14.4^{b}	348.1±15.7^{a}	1193.8±11.6^{c}	449.4±10.5^{b}
IS-607	T-I (100% FC)	2420.6±13.4^{m}	387.9±13.4^{b}	2808.5±13.5^{m}	494.6±13.7^{a}	2167.2±10,3^{l}	307.9±13.9^{a}	2465.1±12.9^{l}	469.3±11.8^{b}
	T-II (50% FC)	2365.8±11.6^{l}	399.3±12.6^{b}	2765.1±11.3^{m}	499.5±11.4^{a}	1932.8±11.2^{k}	321.7±14.2^{a}	2254.5±10.2^{k}	342.5±14.2^{a}
	T-III 25% FC)	2013.7±10.3^{k}	245.8±11.8^{a}	2259.5±10.2^{j}	456.4±12.5^{a}	1578.3±11.8^{i}	305.2±13.2^{a}	1883.5±11.6^{h}	410.6±13.9^{b}
SI-1926	T-I (100% FC)	1665.8±11.4^{g}	476.5±12.5^{c}	2142.3±11.4^{i}	579.6±15.2^{b}	1526.9±12.3^{i}	452.8±11.7^{b}	1979.7±12.5^{i}	564.4±12.6^{c}
	T-II (50% FC)	1432.5±12.7^{e}	453.8±14.2^{c}	1886.3±12.8^{g}	566.8±16.3^{b}	1201.6±10.6^{f}	405.6±12.3^{b}	1607.0±11.9^{f}	512.8±11.7^{c}
	T-III (25% FC)	1189.3±11.9^{c}	403.6±13.5^{c}	1592.9 ±13.5^{e}	510.3±15.7^{b}	956.2±14.3^{c}	375.9±13.6^{a}	1332.1±12.3^{d}	478.6±10.5^{b}
ENT-78-301	T-I (100% FC)	2450.5±10.5^{m}	678.4±12.7^{e}	3128.9±11.5^{o}	756.9±14.9^{d}	2167.2±10.5^{l}	421.8±11.5^{b}	2589.0±11.6^{m}	730.5±10.3^{f}
	T-II (50% FC)	2286.4±10.7^{k}	694.5±13.2^{e}	2980.9± 3.7^{n}	760.5±12.4^{d}	1943.8±11.4^{k}	583.7±12.9^{c}	2527.5±12.5^{m}	679.5±11.3^{e}
	T-III (25% FC)	1983.5±11.2^{j}	454.9±11.7^{c}	2438.4±12.9^{e}	753.8±10.3^{d}	1762.9±12.3^{j}	362.3±10.4^{a}	2125.2±12.8^{j}	673.5±13.8^{e}

[*3 replicates/treatment; repeated twice. Irrigation given every 2-day interval. Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level]

energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments. The present results also revealed that there was change of osmotic adjustments (proline and total soluble carbohydrates) in all irrigation regimes. High level maintenance of water in all treatments (water deficit), prevents accumulation of osmotic adjustments, like proline and total soluble sugars (Fig. 1 A and B). The accumulation of osmolyte compounds in the cells, as a result of water stress was often associated with a possible mechanism to tolerate the harmful effect of water shortage. The utilization of sugars as an osmotic solute in expanded leaves of sunflower was studied[22]. Hassanzadeh *et al*.[23] observed that decrease in chlorophyll-a but increase in chlorophyll-b content under drought stress in seasame due to the water deficit under drought stress by producing reactive oxygen species (ROS), such as O_2 and H_2O_2. The changes of chlorophyll content was also lead to lipid peroxidation and consequently chlorophyll destruction due to the changing green color of the leaf into yellow, the reflectance of the incident radiation was increased.

Proline act as a signaling molecule to modulate mitochondrial functions and influence cell proliferation or cell death and trigger specific gene expression, which can be need for plant recovery from stress. Furthermore, it also contributes to stabilizing sub-cellular structures, buffering cellular redox potential and scavenging free radicals under stress conditions[24,25]. In the present study, drought significantly led to an increase in proline content in sesame as compared to the control plant during vegetative and flowering stages, which could be due to the putative role of drought in increasing the production of proline precursor's. These results were in agreement with the findings of Ghorbanli *et al*.[26]. They have reported that the leaf proline increased significantly under mild and severe drought stress in comparison with control in tomato. Proline accumulation was the first response of plants exposed to drought stress in order to reduce injury to cells. It also acts as an electron receptor

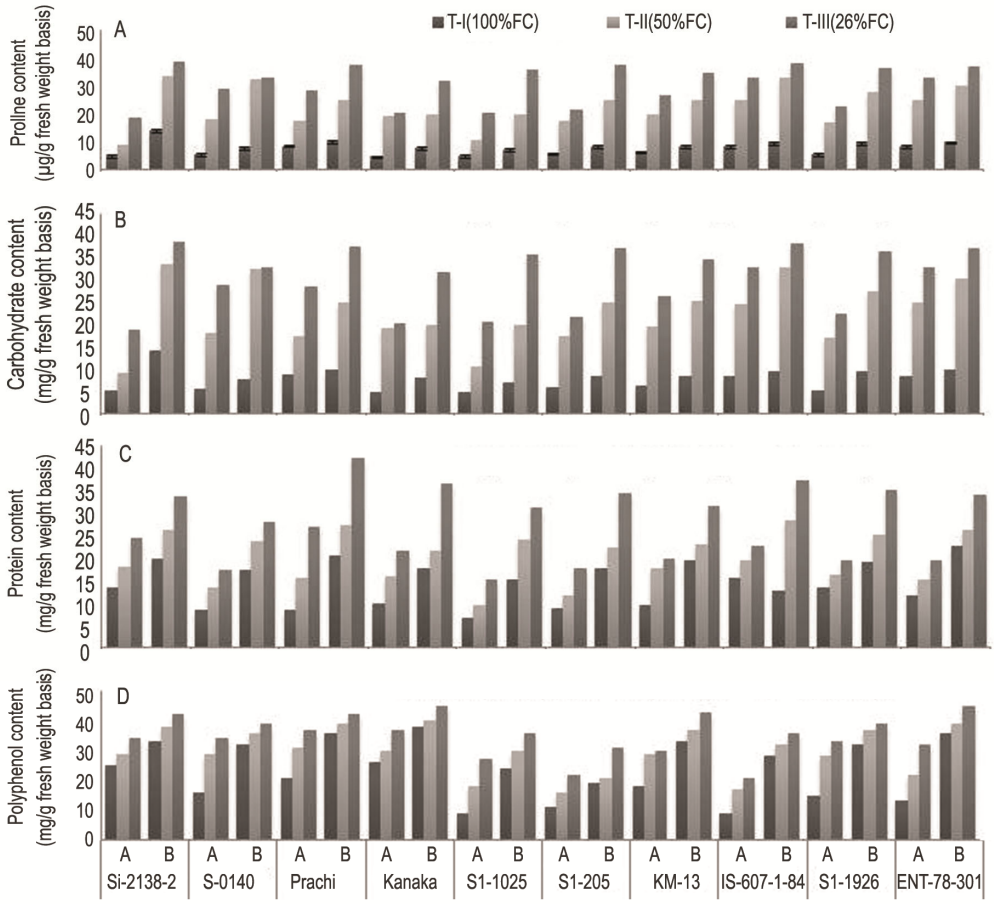


Fig. 1 A-D — Proline, carbohydrate, polyphenol and protein content of 10 genotypes of *Sesamum indicum* under water stress after (A) 15 days; and (B) 30 days of growth.

preventing photosystems injuries in dealing with ROS function. Accumulation of proline facilitates the synthesis of soluble substances in closing stomata[26]. The proline accumulation increased in all *Sesamum* genotypes when plant subjected to stress condition. In genotype 'SI-2138-2' and 'IS-607-1-84' showed highest proline accumulation recorded (38.1-38.5 µg/g fresh weight basis) at Treatment-III (25% FC) after 30 days of stress application. Moreover, they also reported that the activation of pyrroline-5-carboxylate synthetase (an enzyme from proline biosynthesis) as well as inhibition of proline dehydrogenase (an enzyme from proline degradation), were both faster in high drought than in slow drought. High proline content in plants under water stress was observed in many species and act as a regulatory or signaling molecule to activate multiple responses that are part of the adaptation process[27]. Proline contents were increased linked with stomata close and photosynthesis inhibition under drought stress. Such responses are dominantly mediated by plant hormone, i.e. abscisic acid (ABA). Arish *et al.*[28] examined drought tolerance and sensitive genotypes of *Sesamum indicum* subjected to water stress. Water stress caused a significant increase in proline levels in leaves of both tolerant and sensitive genotypes. The leaf of tolerant genotype maintained higher relative water content, photosynthetic activity and proline levels under water stress than that of drought sensitive. Vendruscolo *et al.*[29] observed that proline was involved in tolerance mechanisms against oxidative stress and this was the main strategy of plant to avoid detrimental effects of water stress.

Soluble protein is an important indicator of reversible and irreversible changes in plant metabolism, was known to a wide variety of phytohormones[30]. The present study revealed that total soluble protein content increased in under drought stress sesame plants as compared with control. In genotypes 'Prachi' and 'IS-607-1-84' having higher protein content (42.5 and

37.4 mg/g fresh weight basis) in 25% FC after 30 days of drought stress (Fig. 1C). These results were in agreement with the findings of Anosheh *et al*.[31]. They stated that total soluble protein content increased in response to drought stress in wheat plants. The synthesis and accumulation of protein was studied in two *Zea mays* species and remarkable changes were noticed in 78 out of 413 proteins. These evidences indicated that a kind of relationship existed between protein accumulation and plant physiological resistance against drought stress[32]. They also observed that water stress reduced the synthesis of protein in sesame leaf and suggested that water stress causes the generation of ROS. The ROS brings about inhibition of protein synthesis or causes protein denaturation. The increase in total soluble protein level was a natural occurring under drought stress to increase plant adaptation to water deficit conditions. To avoid damage of endogenous components, antioxidant defense proteins and metabolites are essential to maintain cellular functions. The majority of proteins belongs to dehydrin-like proteins and abundantly induced during embryo maturation of many higher plants as well as in water stressed seedlings[25]. There was a gradual increase in polyphenols content with the increase of drought stress. The genotype like 'Kanaka; and 'ENT-78-301' showed the highest value of polyphenol (45.0 mg/100 g fresh weight basis) in 25% FC in 30 days drought stress treatment (Fig. 1D).

Water stress is inevitably associated with increased oxidative stress due to enhanced accumulation of ROS, particularly $O2^-$ and H_2O_2 in chloroplasts, mitochondria, and peroxisomes. As a result, the induction of antioxidant enzyme activities is a general adaptation strategy which plants use to overcome oxidative stresses[33]. The ability of plants to overcome oxidative stress partly relies on the induction of superoxide dismutase activity and subsequently on the upregulation of other downstream antioxidant enzymes[34]. The present study showed that the antioxidant enzyme activity was increased under drought stress in all the genotypes. The SOD activity significantly enhanced in plants exposed to water stress with different field capacity (Fig. 2A). A gradual increase was observed in SOD activities

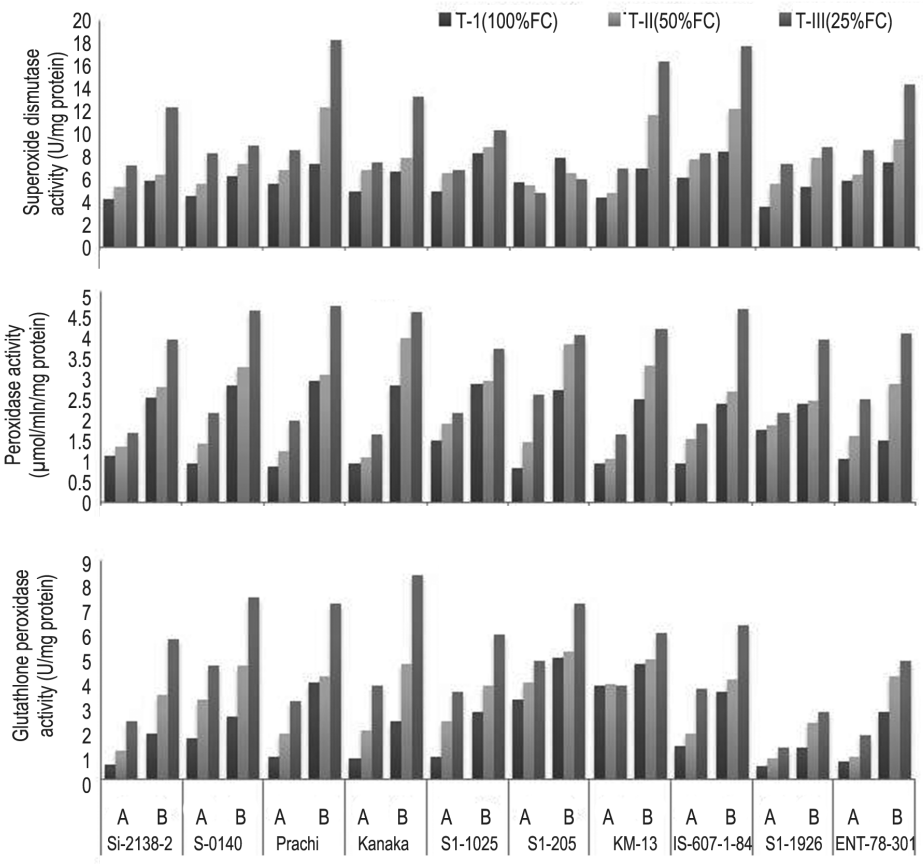


Fig. 2 A-C — Superoxide dismutase, peroxidase and glutathione peroxidase activity of 10 genotypes of *Sesamum indicum* under water stress after (A) 15 days; and (B) 30 days of growth.

under T-II (50% FC) and T-III (25% FC) in comparison with the T-I (100% FC). The interaction effect of cultivar × drought treatments was highly significant for SOD. The maximum increase in the SOD activities was observed in the genotypes 'Prachi' and 'IS-607-1-84' (3 fold and 2 fold, respectively) in 30 days water stress, while the minimum increase was indicated in the genotype 'SI-205' compared to the treatment-I. Among the genotype, 'SI-205' showed a decrease in SOD activity. In 'SI-1025', no much difference is found in 50% FC (Treatment-II) and 25% FC in 15, 21 and 30 days after stress. Our results are consistent with other studies reporting the increased SOD activity in response to drought stress[21,35]. The present study indicates a significant increase in POD activity in sesame plants under drought stress (Fig. 2B). Some previous studies, as parallel with our results, reported the increased POD activity under drought stress conditions in various plant species[21,36]. The maximum increase in the peroxidase activities was observed in the genotypes 'Prachi' and 'ENT-78-301' after 30 days water stress, while the minimum increase was indicated in the genotype SI-205 compared to the control.

Glutathione peroxidases (GPXs, EC 1.11.1.9) are a diverse group of isozymes having generous substrate spectrum and serve as antioxidant enzymes. They occur in plant cells in the cytosol, chloroplasts, mitochondria, and the endoplasmic reticulum, and catalyze the detoxification of H_2O_2 and lipid hydroperoxides with GSH as reductor, and thus protect biomolecules from oxidative damage. It is now known that plant glutathione peroxidases exhibit substrate specificity and can use both GSH and thioredoxins (Trxs) as reductions[37]. In this experiment, GPXs activity increased with increasing water deficit. Similar results were found in *Glycine max*[38], Sunflower[39] and *Beta vulgaris*[40]. The maximum increase in the GPX activities was observed in the genotypes 'Prachi' and 'Kanaka' (2- and 3- fold, respectively) in 30 days water stress, while the minimum increase was indicated in the genotype KM-13 compared to other treatment (Fig. 2C). Among the genotype, 'SI-1926' showed a decrease in GPX activity. Masoumi *et al.*[38] reported a positive and significant correlation between GPX activity and seed yield under optimal irrigation (0.99) conditions, mild water deficit stress (0.74), and high water deficit stress (0.95). Tohidi-Moghaddam *et al.*[41] reported that plants under water deficit stress showed a significant increase in SOD, CAT and GPX activities of canola leaves compared with control plants. Pourtaghi *et al.*[39] demonstrated that water deficit significantly increased the activity of GPXs in sunflower plants compared with fully irrigated control plants. Moreover, the relationship between seed yield and GPXs activity in fully irrigated (0.78) and moderately water stressed (0.91) plants was both positive and significant. GPXs can be used as a marker of drought tolerance in selecting tolerant genotypes under moderate and extreme water deficiency conditions. The expression of many GPX is enhanced in response to abiotic and biotic stresses, including salinity, heavy metal toxicity and infection with bacterial or viral pathogens[42]. On comparison, it was observed that the genotypes like 'SI-1025', 'SI-205', 'SI-2138-2' and 'KM-13' were tolerant to drought. Antioxidant enzymes activity has a beneficial function on plant tolerance in drought stress. It was observed that the tolerant genotypes having higher growth and yield than non-tolerant under drought stress (Table 3). Hatzig *et al.*[43] reported that water stress has a positive transgenerational effect on seedling vigour, vegetative and reproductive growth due to an altered reservoir of seed storage metabolites and inter-generational stress memory, formed by stress-induced changes in the epigenome of the seedlings. Drought stress has substantially affected the seed yields and seed quality. Seed filling was influenced by various metabolic processes occurring in

Table 3 — Comparison of growth and yield of drought tolerant and non-tolerant genotypes of *Sesamum*

Types of genotype	Genotypes	Plant height (cm)	Stem height to the first capsule (cm)	Days to maturity	Days to 50% flowering	No. of fruiting branches	No. of capsule per plant	No. of seeds per capsule	No. of seeds per plant	1000 seeds weight in (g)
Tolerant	SI-1025	115.4	46.23 ±1.7	85	40	4	58	48	2784	3.63
	SI-205	123.7	59.52 ±1.3	92	39	7	65	45	2925	2.78
	SI-2138-2	143.5	48.31 ±1.0	91	44	3	63	50	3150	2.52
	KM-13	138.7	52.47 ±1.5	85	39	4	60	48	2880	3.29
Non-Tolerant	S-0140	113.6	35.23 ±1.9	86	42	3	56	46	2576	3.26
	Prachi	112.8	34.85 ±1.2	84	40	3	52	50	2600	3.65
	Kanaka	99.6	32.59 ±1.6	82	38	3	55	44	2420	3.57
	IS-607-1-84	98.7	58.37 ±1.2	85	43	4	43	42	1806	3.32
	SI-1926	108.5	59.43 ±1.4	92	42	5	48	44	2112	2.79
	ENT-78-301	102..4	54.13 ±1.0	85	45	4	36	48	1728	2.43

the leaves, especially production and translocation of photoassimilates, importing precursors for biosynthesis of seed reserves, minerals and other functional constituents[44].

Conclusion

Drought is greater challenge that decreases agricultural production substantially all over world. It induces several common physiological responses in plants, such as water relation and photosynthetic ability. The biochemical analysis showed that the photosynthetic pigments are major drivers of plant photosynthetic capacity. Other biochemical traits i.e. carbohydrates, proline and polyphenol content were increased at 25% FC in all *Sesamum* growth after 30 days of water stress. Water stress is a major source of oxidative stress increasing ROS production and lipid peroxidation and production of variable antioxidant enzyme. The enzyme activity has a beneficial function in plant tolerance in water stress. On the basis of biochemical and enzymatic analysis, the genotypes 'SI-1025', 'SI-205', 'SI-2138-2' and 'KM-13' are more tolerant to drought. This study should be useful for *Sesamum* improvement programme.

Acknowledgement

The authors acknowledge the Department of Science & Technology, Govt. of India for providing FIST grant, and also AICRP Sesamum, OUAT, Bhubaneswar for providing seed materials for experiment.

Conflict of Interest

Authors have no conflict of interest to declare.

References

1 Morris JB, Food, industrial, nutraceutical, and pharmaceutical uses of sesame genetic resources. In: *Proceedings of Trends in New Crops and New Uses*, (Eds, Janick J & Whipkey A, ASHS Press, Alexandria, USA), 10 (2002) 153.

2 Eskandari H, Zehtab-Salmasi S, Ghassemi-Golezani K & Gharineh MH, Effects of water limitation on grain and oil yields of sesame cultivars. *J Food Agricul Environ*, 7 (2009) 339.

3 Bahrami H, Razmjoo J & Ostadi Jafari A, Effect of drought stress on germination and seedling growth of sesame cultivars (*Sesamum indicum* L.). *Int J Agri Sci*, 2 (2012) 423.

4 Hassanzadeh M, Ebadi A, Panahyan-e-Kivi M, Eshghi AG, Jamaati-e-somarin SH, Saeidi M & Zabihi-e-Mahmoodabad R, Evaluation of drought stress on relative water content and chlorophyll content of Sesame (*Sesamum indicum* L.) genotypes at early flowering stage. *Res J Environ Sci* 3 (2009a) 345.

5 Jaleel CA, Manivannan P, Lakshmanan GMA, Gomathinayagam M, & Panneerselvam R, Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. *Colloids Surf Biointerfaces*, 61 (2008) 298.

6 Bacelar EA, Moutinho-Pereira JM, Goncalves BC, Ferreira HF & Correia CM, Changes in growth, gas exchange, xylem hydraulic properties and water 7use efficiency of three olive cultivars under contrasting water availability regimes. *Environ Exp Bot*, 60 (2007) 183.

7 Centritto M, Lauteri M, Monteverdi MC & Serraj R, Leaf gas exchange, carbon isotope discrimination and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. *J Exp Bot*, 60 (2009) 2325.

8 Mensah, JK, Obadoni BO, Eruotor PG & Onome-Irieguna F, Simulated flooding and drought effects on germination, growth and yield parameters of sesame (*Sesamum indicum* L.). *Afr J Biotech*, 5 (2006) 1249.

9 Golbashy M, Ebrahimi M, Khavari Khorasani S & Choukan R, Evaluation of drought tolerance of some corn (*Zea mays* L.) hybrids in Iran. *Afr J Agric Res,* 5 (2010) 2714.

10 Blum A. *Plant Breeding for Stress Environments*. CRC Press Inc., Boca Raton, Florida, USA, 1988, 233.

11 Reddy AR, Chaitanya KV & Vivekananda M, Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *Plant Physiol J*, 161 (2004)1189.

12 Johnson AI (Ed.) Methods of measuring soil moisture in the field. Ist Edition, USA, Washington, 1962, pp. 1-24.

13 Vernon, LP, Spectrophotometric Determination of Chlorophylls and Pheophytins in Plant Extracts. *Anal Chem*, 32 (1960) 1144.

14 Bates LS, Waldren RP, & Teare ID, Rapid determination of free proline for water-stress studies. *Plant & Soil*, 39 (1973) 205.

15 Lowry, OH, Rosebrough NI, Farr AL. & Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*,193 (1951) 265.

16 Singleton VL, Orthofer R, Lamuela-Raventós RM, & Lester P, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol,* 299 (1999) 152.

17 Giannopolitis CN, & Ries SK, Superoxide dismutase: I. Occurrence in higher plants. *Plant Physiol*, 59 (1977) 309.

18 Wendel A. Glutathione Peroxidases. In: *Enzymatic basis of detoxification*, (Ed. Jakoby WB. Academic Press, New York), 1980, 333.

19 Bradford KJ & Hsiao TC, Physiological responses to moderate water stress. In: *Physiological Plant Ecology: II. Water Relations and Carbon Assimilation*, (Eds. Lange, O, Nobel PS, Osmond CB & Zeigler H; Springer, Berlin, Heidelberg), 1982, 263.

20 Chartzoulakis K, Noitsakis B, & Therios I, Photosynthesis, plant growth and dry matter distribution in kiwifruit as influenced by water deficits. *Irrig Sci*, 14 (1993) 1.

21 Kamanga RM, Mbega E, & Ndakidemi P, Drought tolerance mechanisms in plants:physiological responses associated with water deficit stress in *Solanum lycopersicum*. *Adv Crop Sci Tech*, 6 (2018) 362.

22 Jones MM & Turner NC. Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. *Aus. J Plant Physiol*, 7 (1980) 193.

23 Hassanzadeh M, Asghari A, Jamaati-e-Somarin SH, Saeidi M, Zabihi-e-Mahmoodabad R, & Hokmalipour S. Effects of water deficit on drought tolerance indices of sesame (*Sesamum indicum* L.) genotypes in Moghan Region. *Res J Environ Sci*, 3 (2009b)116.

24 Ashraf M & Foolad MR, Roles of glycine, betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot*, 59 (2007) 206.

25 Rahimian Boogar A, Salehi H & Jowkar A. Exogenous nitric oxide alleviates oxidative damage in turfgrasses under drought stress. *South Afr J Bot*. 92 (2014) 78.

26 Ghorbanli M, Gafarabad M, Amirkian T & Mamaghani BA, Investigation of proline, total protein, chlorophyll, ascorbate and dehydroascorbate changes under drought stress in Akria and Mobil tomato cultivars. *Iran J Plant Physiol*, 3 (2013) 651.

27 Clifford SC, Arndt SK, Corlett JE, Joshi S, Sankhla N, Popp M & Jones HG, The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk.). *J Exp Bot,* 49 (1998) 967.

28 Peyman M, Ali E, Namvar A & Teymur KB, Water relation, solute accumulation and cell membrane injury in sesame (*Sesamum indicum* L.) cultivars subjected to water stress. *Ann Biol Res*, 3 (2012) 1833.

29 Vendruscolo EC, Schuster I, Pileggi M, Scapim CA, Molinari HB, Marur CJ & Vieira LG, Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J Plant Physiol*, 164 (2007)136.

30 Bajguz A, & Piotrowska-Niczyporuk A, Synergistic effect of auxins and brassinosteroids on the growth and regulation of metabolite content in the green alga *Chlorella vulgaris* (Trebouxiophyceae)*, Plant Physiol Biochem,* 71 (2013) 290.

31 Anosheh HP, Emam Y, Ashraf M & Foolad MR. Exogenous application of salicylic acid and chlormequat chloride alleviates negative effects of drought stress in wheat. *Adv Stud Biol*, 4 (2012) 501.

32 Fazeli F, Ghorbani M & Niknam V, Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. *Biol Plant,* 51 (2007) 98.

33 Foyer C & Noctor G, Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol Plant*, 119 (2003) 355.

34 Alscher RG, Erturk N & Heath LS, Role of superoxide dismutases (SODs) in controlling oxidative stress. *J Exp Bot*, 53 (2002) 1331.

35 Bakalova S, Nikolova A, & Wedera D. Isoenzyme profiles of peroxidase, catalase and superoxide dismutase as affected by dehydration stress and ABA during germination of wheat seeds. *J Plant Physiol*, 30 (2004) 64.

36 Das R & Uprety DC. Interactive effect of moisture stress and elevated CO_2 on the oxidative stress in *Brassica* species. *J Food Agric Environ*, 4 (2006) 298.

37 Anjum SA, Saleem MF, Cheema MA, Bilal MF & Khaliq T, An assessment to vulnerability, extent, characteristics and severity of drought hazard in Pakistan. *Pak J Sci*, 64 (1012) 138

38 Masoumi H, Darvish F, Daneshian J, Normohammadi G & Habibi D. Effects of water deficit stress on seed yield and antioxidants content in soybean (*Glycine max* L.) cultivars. *Afr J Agric Res*, 6 (2011) 1209.

39 Pourtaghi A, Darvish F, Habibi D, Nourmohammadi G & Daneshian J, Effect of irrigation water deficit on antioxidant activity and yield of some sunflower hybrids. *Aust J Crop Sci*, 5 (2011)197.

40 Sayfzadeh S & Rashidi M, Response of antioxidant enzymes activities of sugar beet to drought stress. *J Agric Biol Sci*, 6 (2011) 27.

41 Tohidi-Moghaddam HR., Shirani-Rad AR, Noormohammadi G, Habibi D, & Boojar MMA, Effect of super absorbent application on antioxidant enzyme activities in canola (*Brassica napus* L.) cultivars under water stress conditions. *Am J Agric Biol Sci*, 4 (2009) 215.

42 Avsian-Kretchmer O, Eshdat Y, Gueta-Dahan Y & BenHayyim G, Regulation of stress induced phospholipid hydroperoxide glutathione peroxidase expression in citrus. *Planta*, 209 (1999) 469.

43 Hatzig SV, Nuppenau JN, Snowdon RJ & Schieb SV, Drought stress has transgenerational effects on seeds and seedlings in winter oilseed rape (*Brassica napus* L.). *BMC Plant Biol*, 18 (2018) 297.

44 Sehgal A, Sita K, Kadambot H, Siddique M, Kumar R, Bhogireddy S, Varshney RK, Rao BH, Nair RM, Vara Prasad PV & Nayyar H, Drought or/and heat-stress effects on seed filling in food crops: impacts on functional biochemistry, seed yields, and nutritional quality. *Front Plant Sci*., 27 (2018) https://doi.org/10.3389/fpls. 2018.01705.