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Research Article

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Seaweed-based biostimulants alleviate drought stress in sesame by modulating antioxidant defense and gene expression

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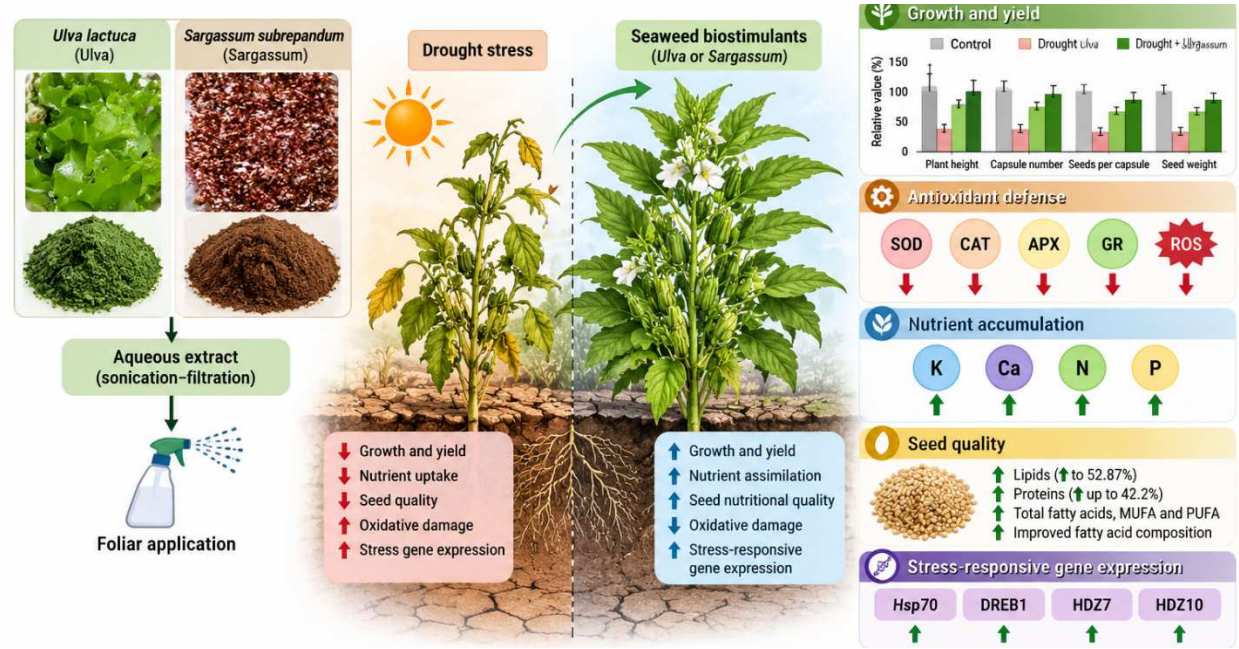
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Abstract

Drought stress significantly hampers sesame growth, yield, and oil quality. As environmentally sustainable solutions gain attention, seaweed-derived biostimulants have emerged as promising tools for improving stress tolerance, though their application in drought-stressed sesame remains underexplored. A greenhouse experiment was conducted under controlled drought and seaweed treatment conditions. Growth attributes, yield components, nutrient accumulation, antioxidant enzyme activities (SOD, CAT, APX, GR), seed biochemical composition (lipid, protein, fiber, carbohydrate), fatty acid profiles, and gene expression of *Hsp70*, *DREB1*, *HDZ7*, and *HDZ10* were analyzed to evaluate the response of sesame to drought and seaweed application. Drought stress caused significant reductions in plant height (−15%), capsule number per plant (−38%), seeds per capsule (−36.6%), and seed weight (−12.4%). Seaweed applications, particularly *S. subrepandum*, improved these yield traits, with capsule and seed numbers recovering by 17–28% under drought. Antioxidant enzyme activities increased sharply under drought (GR rose by over 3-fold), indicating oxidative stress. Seaweed treatments significantly reduced ROS-detoxifying enzyme levels, suggesting mitigation of oxidative damage. Drought also reduced key nutrients such as K (−58%) and Ca (−39%), while seaweed application restored their levels by up to 46% and 25%, respectively. Biochemical analyses revealed a 23% drop in lipid content under drought, while *U. lactuca* reversed this trend, increasing lipid levels to 52.87%. Seaweed treatments also enhanced protein content by up to 42.2% and moderated fiber and sugar fluctuations. Fatty acid profiling showed substantial drought-induced declines in total fatty acids (TFA), MUFA, and PUFA, which were partially restored by both seaweed extracts, with *S. subrepandum* better preserving PUFA content. At the molecular level, drought stress upregulated key stress-responsive genes (*DREB1* up to 39-fold), and seaweed application further enhanced expression, especially in *S. subrepandum*-treated plants (up to 43-fold), suggesting a transcriptional priming effect. The study

provides novel, integrative evidence that foliar-applied seaweed extracts particularly *S. subrepandum* effectively alleviate drought-induced damage in sesame by enhancing growth, nutrient assimilation, antioxidant balance, seed biochemical quality, lipid metabolism, and stress-responsive gene expression. These findings underscore the potential of marine algae as natural, sustainable biostimulants for improving drought tolerance and nutritional quality in oilseed crops.

Keywords: Seaweed biostimulants, *Ulva lactuca*, *Sargassum subrepandum*, antioxidant enzymes, fatty acid profile, gene expression, sustainable agriculture, climate resilience



Introduction

Drought stress is one of the most severe abiotic factors constraining global agricultural productivity, accounting for substantial yield losses in many staple and high-value crops. In water-limited environments, drought impairs plant performance by disrupting cellular homeostasis, reducing stomatal conductance, altering nutrient acquisition, and generating oxidative stress due to the accumulation of reactive oxygen species (ROS) (Aqaei et al. 2020, Hussain et al. 2021). These physiological imbalances adversely affect photosynthesis, biomass accumulation, reproductive development, and the nutritional composition of seeds. Sesame (*Sesamum indicum* L.), an oilseed crop valued for its high-quality oil and protein, exhibits moderate drought tolerance but is vulnerable to water deficit during germination and flowering (Gholinezhad and

Darvishzadeh 2021). Thus, enhancing sesame's resilience through sustainable and eco-friendly strategies is vital for stabilizing yield in arid and semi-arid regions.

In recent years, plant biostimulants derived from natural sources have gained attention as promising tools to enhance plant tolerance to abiotic stress without posing environmental risks (Hasanuzzaman et al. 2021a, Johnson et al. 2024). Among these, marine macroalgae (seaweeds) have emerged as potent biostimulants due to their rich composition of bioactive metabolites such as oligosaccharides, betaines, phytohormones (auxins, cytokinins, gibberellins), polyphenols, and essential micronutrients (Ali et al. 2021, Sujeeth et al. 2022). When applied as foliar sprays, seaweed extracts rapidly penetrate the leaf surface, triggering local and systemic responses that enhance the plant's physiological and molecular defense mechanisms against drought (Deolu-Ajayi et al. 2022, Hasanuzzaman et al. 2021a).

The mechanisms by which seaweed biostimulants mitigate drought stress are multifaceted. One major pathway involves the enhancement of the antioxidant defense system. Seaweed treatments upregulate ROS-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), which collectively prevent oxidative damage to lipids, proteins, and nucleic acids (Begum et al. 2021, Borella et al. 2023). Additionally, seaweed-derived compounds improve osmotic adjustment by stimulating the accumulation of osmolytes like proline, glycine betaine, and soluble sugars, thus maintaining cell turgor and stomatal function under water-limited conditions (Mughunth et al. 2024, Saddhe et al. 2021).

Furthermore, foliar-applied seaweed extracts enhance nutrient uptake efficiency particularly for key elements like potassium (K), calcium (Ca), and magnesium (Mg) which are essential for enzyme activation, membrane stability, and stress signaling (Aremu et al. 2022, Bhattacharya and Bhattacharya 2021, Deolu-Ajayi et al. 2022). These extracts also contain chelating agents and polysaccharides that may improve ion bioavailability and root absorption (Maghraby et al. 2022).

At the molecular level, seaweed treatments modulate the expression of stress-responsive genes involved in drought adaptation (Rasul et al. 2021). Genes such as *Hsp70* (heat shock proteins), *DREB1* (dehydration-responsive element-binding factor), and *HDZ7/HDZ10* (homeodomain-leucine zipper transcription factors) are known to regulate protective functions such as protein folding, gene regulation, and cellular development under dehydration (Wang et al. 2022). Studies suggest that seaweed biostimulants may enhance the expression of these genes by activating ABA-

dependent and independent signaling pathways, thereby priming plants for more efficient stress response (Borella et al. 2023, Rasul et al. 2021, Sujeeth et al. 2022).

In addition to physiological and molecular responses, seaweed extracts influence seed biochemical composition, including crude protein, fiber, lipid, and carbohydrate content (Battershill 2024). They also play a role in preserving oil quality under stress by stabilizing fatty acid biosynthesis, particularly unsaturated fatty acids such as linoleic and oleic acids (Wang et al. 2013). These improvements not only support crop productivity but also maintain the nutritional and commercial value of the final product.

Despite the well-documented benefits of seaweed-derived biostimulants in various crops, there remains a significant gap in our understanding of their application in *U. lactuca* and *S. indicum* L., particularly under drought stress conditions. Sesame, while moderately drought-tolerant, is highly susceptible to water deficits during critical growth stages, leading to severe reductions in yield, oil quality, and seed nutritional composition. Moreover, most existing studies have focused on model or cereal crops, with limited mechanistic insights into how seaweed biostimulants influence stress-responsive pathways in oilseed species like sesame. This study is therefore novel in its comprehensive, multi-tiered approach to evaluating the drought-mitigating potential of foliar-applied aqueous extracts from two distinct marine macroalgae *U. lactuca* (green algae) and *S. subrepandum* (brown algae).

The importance of this work lies in its contribution to climate-resilient agriculture by demonstrating a biologically based, eco-friendly strategy that enhances sesame performance under water-limited conditions. In light of increasing global water scarcity and the need to reduce reliance on synthetic agrochemicals, the use of marine biostimulants represents a sustainable alternative for boosting plant resilience while maintaining nutritional quality and productivity. Accordingly, the primary aim of this study was to investigate the effectiveness of *U. lactuca* and *S. subrepandum* extracts as foliar biostimulants for mitigating drought-induced damage in sesame. The specific objectives were to: (i) assess their impact on plant growth and reproductive yield traits; (ii) evaluate their influence on mineral nutrient accumulation under stress; (iii) quantify changes in key antioxidant enzyme activities (SOD, CAT, APX, GR); (iv) analyze modifications in seed biochemical composition (lipid, protein, fiber, carbohydrate); (v) profile fatty acid content under drought and biostimulant treatments; and (vi) determine expression patterns of major

drought-responsive genes (*Hsp70*, *DREB1*, *HDZ7*, *HDZ10*). By integrating physiological, biochemical, and molecular analyses, this study offers novel insights into the underlying mechanisms of seaweed-mediated drought tolerance and supports the strategic development of environmentally sound biostimulant technologies for sustainable sesame production in arid and semi-arid regions.

Materials and Methods

Plant Material and Seaweed Extract Preparation

Sesame (*Sesamum indicum* L.) seeds were obtained from the Ministry of Environment, Water and Agriculture Saudi Arabia. Fresh seaweed samples of *U. lactuca* and *S. subrepandum* were collected from the Red Sea in Jeddah, Saudi Arabia and transported to the laboratory in ice boxes to maintain their freshness. Selected physico-chemical characteristics of the seaweed samples, including proximate composition and mineral content are presented in Table 1.

Upon arrival, the seaweed samples were thoroughly washed several times with distilled water to remove adhering sand particles and residual salinity. The samples were taxonomically identified based on morphological and ecological characteristics, following the protocols outlined by Ghosh and Ghosh, at the National Institute for Oceanography and Fisheries (NIOF), Alexandria, Egypt (Ghosh and Ghosh 2023).

The cleaned seaweed samples were air-dried for approximately 10 hours at room temperature. Once dried, the samples were finely ground using an electric blender and sieved through an 80-mesh screen to obtain a uniform powder. Aqueous extracts were prepared by suspending 20 g of powdered *U. lactuca* or *S. subrepandum* in 1 L of distilled water (2% w/v). The suspensions were incubated overnight at 60°C in a shaking water bath to enhance the extraction of bioactive compounds. Following incubation, the mixtures were centrifuged at 40,000 rpm for 20 minutes. The resulting supernatants were collected and stored at 4°C for subsequent use in foliar applications (Figure 1).

Healthy sesame seeds were surface sterilized by immersion in 3% (v/v) hydrogen peroxide (H_2O_2) for 20 minutes and then thoroughly rinsed with sterile distilled water. Prior to sowing, the physical and chemical properties of the soil were analyzed and are presented in Table 1. Ten seeds were planted in each plastic pot (40 × 35 cm) containing a mixture of clay soil and compost in a 10:1

ratio. The compost fertilizer provided essential macronutrients, containing 4% ammonium nitrate (N), 3% ammonium phosphate (P), and 3% potassium chloride (K). Pots were placed in a controlled greenhouse environment maintained at 20 ± 2 °C during the day, 20 ± 1 °C at night, with 60–75% relative humidity and a 12-hour photoperiod. Pots were irrigated with tap water every two days, and seed germination was observed within 5–7 days.

Thirty days after sowing, seedlings were thinned to five plants per pot to ensure uniform plant density. Following the thinning stage, plants were assigned to different treatments. Control plants (normal irrigation, WW) continued to receive tap water every three days. Drought stress (DS) was imposed by withholding water to maintain 50% soil moisture, beginning from day 7 to day 21 after sowing. For bio-fertilizer treatments, plants were sprayed with either *Ulva lactuca* or *Sargassum subrepandum* aqueous extracts. Foliar applications involved spraying 20 mL of 2% (20 g/L) seaweed extract per plant every three days, starting from day 10 to day 26 after sowing. *Ulva lactuca* treatments used 25 mL of extract per pot, while *S. subrepandum* treatments used 50 mL per pot. Additional groups combined drought stress with seaweed applications: drought-stressed plants received either *U. lactuca* extract (DS + U) or *S. subrepandum* extract (DS + S) following the same foliar application schedule. The experiment was conducted in a completely randomized design with three replicates per treatment to ensure robust and reliable comparisons.

Soil physico-chemical characterization

A representative soil sample was collected and analyzed to determine its physico-chemical and mechanical properties. The soil had a pH of 7.54 and an electrical conductivity (EC) of 1329 ± 13.24 µS/cm, indicating moderate salinity levels. The concentrations of major cations were as follows: magnesium (Mg^{2+}) 17.8 ± 1.6 meq/L, calcium (Ca^{2+}) 124.6 ± 3.45 meq/L, sodium (Na^{+}) 163 ± 4.22 meq/L, and potassium (K^{+}) 16 ± 0.74 meq/L. Carbonate (CO_3^{2-}) and bicarbonate (HCO_3^{-}) levels were 0.005 ± 0.001 meq/L and 59.2 ± 2.51 meq/L, respectively. The soil organic matter (O.M.) content was relatively low at 0.06 ± 0.002%, and the available nitrogen content was 64.9 ± 3.29 ppm, suggesting a need for nutrient supplementation to support plant growth (Table 2).

Growth and Yield Parameters

Morphological Measurements

Yield Measurements

The growth and yield attributes of sesame plants were evaluated at physiological maturity to assess the effects of drought stress and foliar application of seaweed extracts. Plant height was measured from the base to the tip of the main stem using a meter scale on ten randomly selected plants per treatment. The number of capsules per plant was counted manually from the same plants to estimate reproductive performance. To determine the number of seeds per capsule, ten capsules were randomly selected from each replicate, and the seeds were manually counted. Seed development was further assessed by measuring the weight of 1000 air-dried seeds using a precision balance, expressed in grams. Above-ground biomass and grain yield were obtained by harvesting five representative plants per treatment; the samples were oven-dried at 70 °C until constant weight, and grain yield was recorded after threshing. These measurements followed standard protocols described by Anjum et al. (2011), Boureima et al. (2012), Farooq et al. (2009), and Ali et al. (2013), and provided a comprehensive evaluation of the morphological and reproductive responses of sesame to drought and seaweed-based biostimulants.

Photosynthetic pigment determination

Fresh, fully expanded leaves were collected from each experimental pot at the end of the drought-treatment period. Leaves of the same developmental stage were sampled at a consistent time of day. Approximately 0.20 g of fresh leaf tissue was homogenized under dim light in 10 mL of 80% (v/v) acetone. The homogenate was centrifuged at 4,000 × g for 15 min at 4 °C. The absorbance of the resulting supernatant was measured at 663.2, 646.8, and 470 nm using a UV–visible spectrophotometer, with 80% acetone as the blank. Chlorophyll a, chlorophyll b, and total carotenoid concentrations were calculated using the equations of Lichtenthaler and Wellburn (Lichtenthaler and Wellburn 1983).

Pigment concentrations in the extract (μg mL^{-1}) were calculated as follows:

Chlorophyll a = $12.25\ A_{663.2} - 2.79\ A_{646.8}$

Chlorophyll b = $21.50\ A_{646.8} - 5.10\ A_{663.2}$

Total carotenoids = [$1000\ A_{470} - 1.82$ (chlorophyll a) − 85.02 (chlorophyll b)] / 198

Pigment contents were expressed on a fresh-weight basis (mg g^{-1} FW) using:

Pigment content (mg g^{-1} FW) = [C × V] / [1000 × W]

Here, C is the pigment concentration in the extract (μg mL^{-1}), V is the final extract volume (mL), and W is the fresh leaf mass (g). A dilution factor was applied when relevant. The values were calculated separately for each independent pot replicate and are reported as mean ± SE. Statistical comparisons should follow the same statistical model and post-hoc test used for the other measured traits.

Relative water content (**RWC**)

RWC was determined at 21 days after sowing (DAS), at the end of the stated drought period, using the relative-turgidity method of Barrs and Weatherley (1962). One healthy, fully expanded leaf was sampled from one representative plant in each pot (three independent pots per treatment) (Barrs and Weatherley 1962). Immediately after excision, three 10-mm-diameter discs were punched from the interveinal area of each leaf, avoiding the midrib, and weighed together to obtain fresh mass (FW). The discs were floated on distilled water in covered Petri dishes for 4 h in darkness at 4 °C. After blotting surface water gently with lint-free tissue, the discs were weighed to obtain turgid mass (TW). They were then oven-dried at 70 °C for 48 h and reweighed; drying and weighing were repeated until the mass was constant to obtain dry mass (DW). RWC was calculated for each pot as:

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

Here, FW is fresh mass immediately after sampling, TW is mass after full rehydration, and DW is oven-dry mass. The pot was treated as the experimental unit; leaf discs from the same pot were pooled for one RWC value.

Leaf gas exchange

Net photosynthetic rate (A), stomatal conductance to water vapour (g_s), and transpiration rate (E) were measured at 21 DAS between 09:00 and 11:00 h using a portable infrared gas analyser (LI-6400XT; LI-COR Biosciences, Lincoln, Nebraska, USA), following the stable-reading approach described by Evans and Santiago (Evans and Santiago 2014). One healthy, fully expanded, sun-exposed leaf was measured on one representative plant in each pot (n = 3 pots per treatment). Measurements were made on the same day and in randomized treatment order. The analyser was set to a reference CO_2 concentration of 400 μmol mol^{-1}, photosynthetic photon flux density of

1,000 µmol photons m^{-2} s^{-1}, leaf-chamber temperature of 22 °C, and flow rate of 500 µmol s^{-1}. Chamber vapour-pressure deficit was maintained at approximately 1.2 kPa. After the displayed gas-exchange values stabilized, three consecutive readings were recorded at approximately 30-s intervals and averaged to give one value per pot. A, g_s, and E were expressed as µmol CO_2 m^{-2} s^{-1}, mol H_2O m^{-2} s^{-1}, and mmol H_2O m^{-2} s^{-1}, respectively. This protocol is consistent with gas-exchange traits used in sesame drought studies (Baghery et al. 2023).

Lipid peroxidation

Lipid peroxidation was estimated as thiobarbituric acid-reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalents, following Heath and Packer (Heath and Packer 1968). Fresh leaf tissue (0.25 g) was homogenized in 5 mL of ice-cold 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000 × g for 10 min at 4 °C. An aliquot of 1 mL supernatant was mixed with 4 mL of 0.5% (w/v) thiobarbituric acid (TBA) prepared in 20% (w/v) TCA. A reagent blank containing extraction medium instead of sample was prepared in parallel. Samples were heated at 95 °C for 30 min, rapidly cooled on ice, and centrifuged to clarify. Absorbance was read at 532 nm and corrected for nonspecific turbidity at 600 nm. MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and reported as nmol MDA equivalents g^{-1} FW, accounting for extraction and reaction volumes.

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) was quantified colorimetrically using potassium iodide (KI), based on Velikova et al. (Velikova et al. 2000). Fresh leaf tissue (0.5 g) was homogenized on ice in 5 mL of 0.1% (w/v) TCA and centrifuged at 12,000 × g for 15 min at 4 °C. The reaction mixture contained 0.5 mL supernatant, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0), and 1 mL of 1 M KI. Absorbance was measured at 390 nm against a reagent blank. H_2O_2 concentration was determined from a standard curve prepared with known H_2O_2 concentrations and expressed as µmol H_2O_2 g^{-1} FW.

Electrolyte leakage

Relative electrolyte leakage was determined using a conductivity-based membrane injury assay adapted from Blum (Blum 2009). Fresh leaves were rinsed thoroughly with deionized water to remove surface electrolytes, blotted dry, and cut into small segments. A 0.20 g sample was placed in a tube with 10 mL deionized water and incubated at 25 °C with gentle shaking for 24 h. Initial conductivity (C_1) of the bathing solution was measured using a calibrated conductivity meter. The

tubes were then heated in boiling water for 20 min, cooled to 25 °C, and the final conductivity (C_2) was recorded. Electrolyte leakage was calculated as (C_1/C_2) × 100 and reported as a percentage. A water-only blank was measured and used to correct conductivities where needed.

Free proline

Free proline was determined using the acid-ninhydrin method of Bates et al. (Bates et al. 1973). Fresh leaf tissue (0.5 g) was homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid and filtered. Two milliliters of filtrate were mixed with 2 mL glacial acetic acid and 2 mL acid-ninhydrin reagent. The reaction mixture was heated at 100 °C for 1 h, cooled rapidly in an ice bath, and the chromophore was extracted into 4 mL toluene by vigorous mixing. The absorbance of the toluene phase was measured at 520 nm against a toluene blank. Proline concentration was calculated from an L-proline standard curve and expressed as μmol proline g^{-1} FW.

Total soluble sugars

Total soluble sugars were measured by the phenol–sulfuric acid method (DuBois et al. 2002). Fresh leaf tissue (0.20 g) was extracted in 10 mL of 80% (v/v) ethanol at 80 °C for 30 min, cooled, and centrifuged at 8,000 × g for 10 min. The extraction was repeated once, and the supernatants were combined and brought to a known final volume with 80% ethanol. An aliquot of 0.2 mL extract was mixed with 0.2 mL of 5% (w/v) phenol; 1 mL concentrated sulfuric acid was added rapidly, and the mixture was allowed to stand for 10 min before mixing and incubating for 20 min at room temperature. Absorbance was read at 490 nm. Total soluble sugar concentration was calculated against a glucose standard curve and expressed as mg glucose equivalents g^{-1} FW.

Antioxidant Enzyme Assays

Enzyme Extraction

Fresh plant tissue (0.5 g) was homogenized in 5 mL of ice-cold 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 15,000 × g for 20 minutes at 4 °C, and the supernatant was used for enzyme activity assays (Nakano and Asada 1981).

Superoxide Dismutase (SOD, EC 1.15.1.1)

SOD activity was measured according to the method of Giannopolitis and Ries (1977) by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT). The reaction

mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA, and 100 µL of enzyme extract. The reaction was initiated by illuminating the samples for 10 minutes under fluorescent light. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of NBT reduction (Giannopolitis and Ries 1977).

Catalase (CAT, EC 1.11.1.6)

CAT activity was assayed following the decomposition of H_2O_2 at 240 nm according to the method of Aebi (1984). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2, and 100 µL of enzyme extract. The decrease in absorbance was recorded for 1 minute, and CAT activity was expressed as µmol H_2O_2 decomposed per minute per mg protein using an extinction coefficient of 39.4 mM^{-1} cm^{-1} (Aebi 1984).

Ascorbate Peroxidase (APX, EC 1.11.1.11)

APX activity was determined by monitoring the decrease in absorbance at 290 nm due to ascorbate oxidation (Nakano and Asada, 1981). The 1 mL reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2, 0.1 mM EDTA, and 100 µL enzyme extract. The extinction coefficient of 2.8 mM^{-1} cm^{-1} was used to calculate enzyme activity, which was expressed as µmol ascorbate oxidized per minute per mg protein (Nakano and Asada 1981).

Glutathione Reductase (GR, EC 1.6.4.2)

GR activity was measured based on the method of Foyer and Halliwell (1976) by following the oxidation of NADPH at 340 nm. The 1 mL reaction mixture contained 100 mM phosphate buffer (pH 7.8), 0.5 mM oxidized glutathione (GSSG), 0.1 mM NADPH, and 100 µL of enzyme extract. The decrease in absorbance was monitored for 1 minute, and activity was expressed as µmol NADPH oxidized per minute per mg protein using an extinction coefficient of 6.22 mM^{-1} cm^{-1} (Foyer and Halliwell 1976).

Crude lipid content

Crude lipid content was determined using the Soxhlet extraction method, following the standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005). Approximately 2–3 g of finely ground, oven-dried sesame seed or leaf sample was weighed into a

cellulose extraction thimble and placed in a Soxhlet apparatus. Hexane was used as the extraction solvent, and the extraction was carried out for 6 hours at a consistent boiling temperature. After extraction, the solvent was evaporated using a rotary evaporator, and the lipid residue was dried in an oven at 60 °C to a constant weight. The crude lipid content was calculated gravimetrically and expressed as a percentage of dry weight. This method provides a reliable estimation of total lipids and allows for comparison of lipid accumulation among treatments under different stress conditions.

Crude fiber content

Crude fiber content was determined using the conventional acid-alkali digestion method as described by the Association of Official Analytical Chemists (AOAC, 2005). Approximately 2 g of oven-dried and finely ground sesame plant material was accurately weighed and subjected to sequential digestion with 1.25% sulfuric acid (H_2SO_4) followed by 1.25% sodium hydroxide (NaOH) under controlled heating. After digestion, the residue was filtered, washed thoroughly with hot distilled water, and dried in an oven at 105 °C to constant weight. The dried residue was then incinerated in a muffle furnace at 550 °C for 4 hours to remove all organic matter. Crude fiber content was calculated as the difference in weight before and after ashing and expressed as a percentage of dry weight. This method effectively estimates the indigestible fibrous fraction of plant tissues, such as cellulose and lignin, which is important for assessing the nutritional quality of sesame under stress and treatment conditions.

Carbohydrate content

Total carbohydrate content was determined by the phenol-sulfuric acid method as described by Dubois et al. (1956), which is suitable for estimating both simple and complex carbohydrates. Briefly, 100 mg of finely ground, oven-dried sesame tissue was hydrolyzed with distilled water and centrifuged, and 1 mL of the supernatant was used for the assay (DuBois et al. 1956). To this, 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid (H_2SO_4) were added rapidly. The mixture was vortexed and allowed to stand for 30 minutes at room temperature to develop a yellow-orange color (DuBois et al. 1956). Absorbance was then measured at 490 nm using a UV–Vis spectrophotometer. Glucose was used to prepare the standard calibration curve, and total carbohydrate content was expressed as mg glucose equivalents per gram of dry weight. This

colorimetric assay provides a reliable and sensitive measure of carbohydrate accumulation in plant tissues under different environmental conditions.

Fatty acid profile

Fatty acid composition of sesame seed oil was analyzed using gas chromatography (GC) following methyl esterification of lipid extracts, according to the method of Christie (1982). Approximately 1 g of finely ground seed sample was subjected to Soxhlet extraction using hexane to obtain crude oil. The extracted oil was then converted to fatty acid methyl esters (FAMEs) by transesterification using 2% sulfuric acid in methanol and heated at 60 °C for 2 hours. After cooling, the FAMEs were extracted with hexane, washed with distilled water to remove impurities, and dried over anhydrous sodium sulfate. The prepared FAMEs were injected into a gas chromatograph equipped with a flame ionization detector (GC-FID) and a capillary column (HP-88, 100 m × 0.25 mm × 0.2 µm). The oven temperature was programmed to increase from 140 °C to 240 °C at a rate of 4 °C/min. Fatty acids were identified by comparing their retention times with those of known FAME standards. The results were expressed as percentages of total identified fatty acids, and individual components were classified as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and total fatty

Mineral nutrient content

The macro- and micronutrient content of sesame leaves was determined to evaluate the impact of drought stress and seaweed extract treatments on nutrient uptake and accumulation. Fresh leaf samples were collected at the flowering stage, washed thoroughly with deionized water, oven-dried at 70 °C to a constant weight, and finely ground. Approximately 0.5 g of the powdered sample was subjected to wet digestion using a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 3:1 ratio (v/v) following the protocol described by Jackson (Jackson 1973). After complete digestion, the solution was filtered and diluted with deionized water to a final known volume. Nitrogen (N) content was determined using the micro-Kjeldahl method (Bremner 1996), while phosphorus (P) was analyzed colorimetrically using the vanadomolybdate yellow method with absorbance measured at 420 nm. Potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) contents were quantified using a flame photometer (Jenway PFP7) or atomic absorption spectrophotometer (AAS) depending on the required sensitivity and element. The results were expressed as percentage or mg/g of dry weight (DW). These standardized

procedures allowed for accurate comparison of nutrient concentrations across treatments and elucidated the beneficial role of seaweed-based biostimulants in enhancing nutrient retention under drought conditions.

Moisture and Ash Content

Moisture and ash contents were determined using standard gravimetric procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005). For moisture content, approximately 5 g of fresh sesame tissue was weighed into a pre-weighed crucible and dried in a hot air oven at 105 °C until a constant weight was achieved. The loss in weight was recorded as the moisture content and expressed as a percentage of the fresh sample weight. For ash content, a separate dried sample was placed in a muffle furnace and incinerated at 550 °C for 4–6 hours until a white or gray ash was obtained. The residue was then cooled in a desiccator and weighed. Ash content was calculated as the percentage of the original dry weight, representing the total mineral residue present in the sample. These methods are essential for evaluating the basic proximate composition and nutritional quality of plant materials.

acids (TFA).

Gene expression analysis

Gene expression analysis was conducted using quantitative real-time PCR (qRT-PCR) to evaluate the transcriptional responses of selected stress-related genes (Hsp70, DREB1, HDZ7, and HDZ10) in sesame plants. Total RNA was extracted from fresh leaf tissue using the TRIzol reagent (Invitrogen, USA) following the manufacturer's protocol. RNA quality and concentration were assessed using a NanoDrop spectrophotometer and 1.2% agarose gel electrophoresis. First-strand cDNA synthesis was performed using 1 µg of total RNA with a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). qRT-PCR was carried out on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA) using SYBR Green Master Mix (Takara, Japan). Gene-specific primers were designed using Primer3 software and validated for efficiency. Each 20 µL reaction contained 10 µL of SYBR Green mix, 0.5 µM of each primer, 2 µL of diluted cDNA, and nuclease-free water. The PCR cycling conditions were: initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. Melt curve analysis was performed to verify specificity. The sesame Actin gene was used as the internal reference.

Relative expression levels were calculated using the 2^–ΔΔCt method (Livak and Schmittgen 2001), and data were presented as fold change compared to the control group.

Statistical Analysis

All experimental data were statistically analyzed using one-way analysis of variance (ANOVA) to evaluate the effects of drought stress and seaweed extract treatments on various morphological, physiological, biochemical, and molecular traits of sesame. When the ANOVA indicated significant differences ($p < 0.05$), treatment means were compared using the Least Significant Difference (LSD) test at the 5% probability level. Results were expressed as mean ± standard error (SE) based on at least three biological replicates. For gene expression data, $\log_2$-transformed fold changes were used to satisfy assumptions of normality and homoscedasticity. All analyses were performed using IBM SPSS Statistics (version 25.0). Different letters or asterisks were used in figures and tables to indicate statistically significant differences among treatments based on the LSD test.

Results

Pigment concentrations

Pigment concentrations differed numerically among treatments in the supplied workbook (Figure 2). Compared with the well-watered control, drought treatment had lower chlorophyll *a* (0.70 vs. 1.22 mg g^{-1} FW), chlorophyll *b* (0.24 vs. 0.43 mg g^{-1} FW), and total carotenoids (0.11 vs. 0.19 mg g^{-1} FW). Under drought, the corresponding values were higher in plants treated with *U. lactuca* (0.93, 0.33, and 0.15 mg g^{-1} FW) and *S. subrepandum* (1.02, 0.36, and 0.16 mg g^{-1} FW). Among drought-treated groups, *S. subrepandum* had the highest numerical values for all three pigments.

Gas exchange

Drought treatment was associated with lower net photosynthetic rate (Pn), stomatal conductance (gs), and transpiration rate (E) than the well-watered control (Figure 3). Pn decreased from 20.6 to 8.3 µmol CO_2 m^{-2} s^{-1}, gs from 0.36 to 0.12 mol H_2O m^{-2} s^{-1}, and E from 4.2 to 1.4 mmol H_2O m^{-2} s^{-1}. Under drought, plants treated with *U. lactuca* had Pn, gs, and E values of 13.6 µmol CO_2 m^{-2} s^{-1}, 0.22 mol H_2O m^{-2} s^{-1}, and 2.7 mmol H_2O m^{-2} s^{-1}, respectively. Corresponding values for

S. subrepandum-treated plants were 15.1 µmol CO_2 m^{-2} s^{-1}, 0.25 mol H_2O m^{-2} s^{-1}, and 3.0 mmol H_2O m^{-2} s^{-1}. Among the drought-treated groups, the *S. subrepandum* treatment had the highest numerical values for all three parameters.

Oxidative damage and osmotic adjustment

Compared with well-watered plants, drought-treated plants showed higher MDA (30.0 vs. 12.0 nmol g^{-1} FW), H_2O_2 (1.50 vs. 0.45 µmol g^{-1} FW), and electrolyte leakage (44.0% vs. 18.0%). Proline concentration also increased under drought, from 1.10 to 4.50 µmol g^{-1} FW. Under drought, both seaweed treatments were associated with lower values for these variables than drought alone. Among the drought-treated groups, *S. subrepandum* treatment had the lowest values for MDA (18.5 nmol g^{-1} FW), H_2O_2 (0.78 µmol g^{-1} FW), electrolyte leakage (26.5%), and proline (2.75 µmol g^{-1} FW) (Figure 4).

Soluble sugars increased from 17.5 mg g^{-1} FW in the well-watered control to 26.0 mg g^{-1} FW under drought. The drought-plus-*U. lactuca* and drought-plus-*S. subrepandum* treatments had illustrative values of 29.0 and 31.5 mg g^{-1} FW, respectively (Figure 4).

Antioxidant Enzyme Activities

Drought stress had a pronounced impact on the antioxidant defense system of sesame plants, as reflected in the activities of key enzymes: SOD, CAT, APX, and GR (Figure5). Under drought conditions (Dr), the activity of SOD increased markedly to 48.65 U/mg protein compared to 33.47 U/mg in the control plants. This 45% elevation suggests a heightened oxidative environment, prompting the plant to boost its primary reactive oxygen species (ROS)-scavenging enzyme. However, when *Ulva lactuca* and *Sargassum subrepandum* were applied under drought stress (DrU and DrS), SOD levels declined to 40.87 U/mg and 41.46 U/mg, respectively (Figure 5A). These reductions imply that the seaweed extracts alleviated oxidative stress, reducing the enzymatic burden.

A similar trend was observed for CAT, which detoxifies hydrogen peroxide. CAT activity more than doubled in drought-stressed plants (7.92 U/mg) compared to the control (3.74 U/mg), confirming the presence of elevated H_2O_2. The DrU and DrS treatments lowered CAT activity to

4.31 U/mg and 4.41 U/mg, respectively, approaching normal levels and suggesting reduced oxidative load due to seaweed intervention (Figure 5B). Ascorbate peroxidase, another critical enzyme in the ROS-scavenging pathway, increased from 18.22 U/mg in the control group to 29.39 U/mg under drought conditions. Seaweed-treated drought-stressed plants showed moderated APX levels: 21.50 U/mg (DrU) and 25.73 U/mg (DrS). Notably, DrS resulted in higher APX activity than DrU, indicating that *S. subrepandum* was more effective in maintaining oxidative detoxification (Figure 5C).

The most striking changes were observed in GR activity. Drought stress led to a dramatic increase in GR, from 57.47 U/mg in the control to 182.74 U/mg under drought a more than threefold elevation. While seaweed treatments did not restore GR to control levels, they significantly reduced its activity to 125.50 U/mg (DrU) and 114.57 U/mg (DrS), highlighting their protective role against excessive oxidative stress (Figure 5D).

Growth and Yield Attributes

Drought stress had a significant adverse effect on the morphological and reproductive traits of sesame plants, including capsule production, seed development, and overall biomass accumulation. As shown in Figure 6, the number of capsules per plant was reduced from 47 in control plants to 29 under drought stress (Dr), reflecting a substantial 38% decline. However, seaweed applications mitigated this reduction, with CP values of 31.33 for *Ulva lactuca* (DrU) and 34 for *S. subrepandum* (DrS), indicating partial recovery of reproductive output under drought conditions (Figure 6A).

Similarly, the number of seeds per capsule followed a comparable pattern. While control plants had 47.33 seeds per capsule, drought stress significantly reduced this to 30. DrU and DrS treatments improved CS to 41.33 and 38, respectively, demonstrating the protective role of seaweed biostimulants in maintaining reproductive capacity under stress (Figure 6B).

Seed weight was also negatively impacted by drought. Control plants exhibited an average seed weight of 4.28 g, while drought-stressed plants dropped to 3.75 g. Treatment with *U. lactuca* slightly improved WS to 4.06 g, while *S. subrepandum* resulted in 3.90 g, suggesting limited but beneficial effects of seaweed extracts on seed development under stress conditions (Figure 6C).

Plant height, another key morphological parameter, was markedly reduced under drought stress. Control plants reached an average height of 71.23 cm, whereas drought stress limited growth to 60.59 cm. Application of *U. lactuca* (DrU) and *S. subrepandum* (DrS) under drought stress resulted in improved plant heights of 66.52 cm and 64.31 cm, respectively. Notably, *U. lactuca* performed slightly better in promoting vegetative growth, while *S. subrepandum* was more effective in enhancing reproductive traits such as capsule and seed numbers (Figure 6D).

Mineral Nutrient Content

Drought stress had a clear detrimental effect on the macro- and micronutrient content of sesame plants, as shown in Figure 7. The concentration of N in drought-stressed plants (Dr) was 1.18%, slightly higher than the control (1.12%) but significantly lower in drought + seaweed treatments, with 0.92% (DrU) and 0.88% (DrS). Interestingly, foliar application of seaweeds alone led to increased N content in non-stressed plants, reaching 1.89% in *Ulva lactuca* and 1.85% in *S. subrepandum* treatments, indicating improved nitrogen assimilation under optimal conditions (Figure 7A).

Phosphorus content was notably reduced under drought stress, decreasing from 0.28% in the control to 0.15% in the Dr group. Seaweed treatments under drought restored P levels slightly to 0.19% (DrU) and 0.16% (DrS), yet they remained below control levels (Figure 7B). Similar trends were observed for K, where drought stress led to a substantial decline from 54.57 mg/g DW (control) to 22.66 mg/g DW. Application of *U. lactuca* and *S. subrepandum* under drought raised K levels to 33.11 mg/g DW and 28.37 mg/g DW, respectively, showing moderate recovery of K uptake (Figure 7C).

Magnesium content dropped from 1.60 mg/g in the control to 0.87 mg/g in drought-stressed plants. Seaweed supplementation improved Mg accumulation to 1.29 mg/g (DrU) and 1.21 mg/g (DrS), nearing the values observed under normal conditions (Figure 7D). For Ca, drought stress caused a marked reduction from 41.83 mg/g in the control to 25.56 mg/g, while seaweed-treated plants under drought showed improved Ca levels of 31.77 mg/g (DrU) and 28.78 mg/g (DrS) (Figure 7E).

Sodium content was also impacted by drought, decreasing from 16.21 mg/g in the control to 12.77 mg/g in drought-stressed plants. Treatment with *U. lactuca* (DrU) and *S. subrepandum* (DrS) improved Na content to 13.75 mg/g and 14.90 mg/g, respectively. Notably, all macronutrients were better retained in seaweed-treated plants under drought, with *S. subrepandum* showing slightly stronger effects on Mg, Ca, and Na uptake compared to *U. Lactuca* (Figure 7H).

Biochemical Composition

The biochemical profile of sesame seeds was notably affected by drought stress and modified by seaweed treatments, as shown in Figure 8. Drought stress (Dr) significantly reduced crude lipid content to 38.7%, compared to 50.3% in control plants. However, application of *U. lactuca* (DrU) restored crude lipid levels to 52.87%, surpassing even the non-stressed treatments, while *S. subrepandum* (DrS) yielded a more modest recovery to 39.44%, still above drought-alone levels (Figure 8A).

Protein content followed an increasing trend under stress and supplementation. Drought alone surprisingly increased protein to 36.45%, up from 22.67% in the control. Seaweed application further boosted this response, with *S. subrepandum* (DrS) achieving the highest value at 42.20%, followed closely by *U. lactuca* (DrU) at 41.13%, suggesting enhanced nitrogen metabolism or stress-related protein synthesis under combined treatment conditions (Figure 8B).

Crude fiber content declined under drought stress, dropping to 3.63% from 4.26% in the control. Treatment with *U. lactuca* improved Crude fiber to 3.92%, while *S. subrepandum* treatment resulted in 3.80%, reflecting a moderate restorative effect of seaweeds on dietary fiber retention (Figure 8C). Interestingly, carbohydrate content increased under drought, reaching 16.8%, in contrast to 13.96% in control plants. Seaweed applications slightly reduced this accumulation, with 15.69% (DrU) and 15.27% (DrS), indicating partial normalization of sugar metabolism (Figure 8D).

Moisture and Ash Content

Drought stress significantly affected the proximate composition of sesame plants, particularly moisture and ash content, as shown in Figure 9. The moisture content in control plants was 18.47%,

which declined drastically to 8.27% under drought stress (Dr), representing more than a 55% reduction. Application of seaweed extracts under drought conditions partially restored moisture levels to 12.90% with *U. lactuca* (DrU) and 10.24% with *S. subrepandum* (DrS), indicating an improvement in water retention capacity. Despite not fully returning to control levels, the observed increase suggests that seaweed treatments enhance the physiological water status of sesame under stress (Figure 9A).

Unexpectedly, ash content slightly increased under drought stress, rising from 4.66% in the control to 5.30% in Dr plants. This increase may be attributed to a relative concentration effect due to reduced moisture. Seaweed applications under drought resulted in marginally lower ash levels 5.15% (DrU) and 5.20% (DrS) compared to the drought-only group, suggesting stabilization of mineral content. Interestingly, ash content under seaweed treatments in both stress and non-stress conditions remained relatively stable, supporting their role in modulating mineral homeostasis (Figure 9B).

Fatty Acid Profile

Drought stress significantly altered the lipid composition of sesame seeds, with marked reductions in TFA, MUFA, and PUFA, as shown in Figure 10. Under well-watered control conditions, the TFA content was 49.67%, which dropped sharply to 28.58% under drought stress (Dr). Seaweed supplementation helped mitigate this decline: *U. lactuca* (DrU) increased TFA to 38.38%, while *S. subrepandum* (DrS) also improved it to 33.90%, indicating partial restoration of oil content (Figure 10A).

Saturated fatty acids showed a slight decline under drought, from 11.25% in control plants to 9.74% in drought-stressed ones. With seaweed treatment, SFA levels rose to 10.50% (DrU) and 10.37% (DrS), approaching normal levels, while remaining within a desirable nutritional range (Figure 10B).

The MUFA content declined from 18.78% in control plants to 14.86% under drought. Seaweed applications modestly increased MUFA levels to 16.71% (DrU) and 17.24% (DrS), suggesting an improved balance of fatty acid types. Notably, the MUFA content in DrS-treated plants was closer to that of the control group, indicating better recovery of oil quality (Figure 10C).

PUFA levels, essential for nutritional value, were also severely affected by drought, decreasing from 23.42% in the control to 17.64% under drought stress. Both seaweed treatments helped recover PUFA content, with *U. lactuca* (DrU) reaching 21.44% and *S. subrepandum* (DrS) 20.52%. These values highlight the potential of seaweed biostimulants in restoring unsaturated fatty acid profiles under abiotic stress (Figure 10D).

Gene Expression Analysis

Gene expression profiling revealed significant transcriptional responses in sesame plants subjected to drought stress and seaweed treatments. As shown in Table 3 and Figures 11 and 12, the expression levels of four key genes Hsp70, DREB1, HDZ7, and HDZ10 were substantially upregulated in response to drought stress and further modulated by the application of *U. lactuca* and *S. subrepandum*.

Under well-watered conditions (control), the expression levels of all genes remained low and stable, ranging between 0.96 and 0.99. Drought stress (Dr) triggered a dramatic increase in gene expression, with Hsp70 rising to 28.64-fold, DREB1 to 39.27-fold, HDZ7 to 18.52-fold, and HDZ10 to 34.11-fold, indicating a strong activation of stress-responsive pathways.

Application of seaweed extracts alone under non-stress conditions resulted in mild upregulation of these genes. *U. lactuca* induced moderate expression increases for Hsp70 (2.94), DREB1 (2.46), HDZ7 (1.97), and HDZ10 (3.49). *S. subrepandum* elicited slightly stronger responses, with expression levels of 4.67, 3.88, 3.17, and 2.29 for the respective genes.

Remarkably, the combination of drought stress with seaweed treatments further amplified gene expression beyond drought stress alone. The highest levels were observed under drought + *S. subrepandum* (DrS), with Hsp70 at 33.56, DREB1 at 43.08, HDZ7 at 26.73, and HDZ10 at 36.15. Similarly, drought + *U. lactuca* (DrU) significantly enhanced gene expression to 31.50 (Hsp70), 41.28 (DREB1), 23.41 (HDZ7), and 38.26 (HDZ10), demonstrating the efficacy of both seaweed extracts in boosting drought-induced molecular responses.

Discussion

This study shows that drought affected sesame across several levels of organization, including antioxidant-enzyme activity, growth and reproductive traits, leaf mineral composition, seed quality, fatty-acid profile, and stress-responsive gene expression. Foliar applications of *Ulva lactuca* and *Sargassum subrepandum* modified many of these responses, although their relative effects depended on the trait. The findings support a multi-trait response to seaweed treatment, rather than a uniform advantage of one extract across all measured outcomes. Seaweed-based biostimulants have been reported to influence stress signaling, redox regulation, nutrient acquisition, metabolism, and gene expression, although their effects depend on the crop, extract composition, and application conditions (Deolu-Ajayi et al. 2022, Sujeeth et al. 2022).

Drought increased the activities of SOD, CAT, APX, and GR. SOD activity rose from 33.47 to 48.65 U mg^{-1} protein, CAT from 3.74 to 7.92 U mg^{-1}, APX from 18.22 to 29.39 U mg^{-1}, and GR from 57.47 to 182.74 U mg^{-1}. These increases are consistent with greater demand for enzymatic antioxidant defenses during water deficit. SOD converts superoxide radicals to H_2O_2, which is subsequently removed by enzymes including CAT and APX. GR supports cellular redox balance by maintaining the reduced glutathione pool. Seaweed application moderated several enzyme activities, but responses differed among enzymes: *U. lactuca* resulted in lower SOD and APX activities than *S. subrepandum*, whereas *S. subrepandum* resulted in lower GR activity. Thus, neither extract showed uniformly greater effects across the antioxidant system. Lower enzyme activity in seaweed-treated plants could reflect lower demand for ROS detoxification, but enzyme activity alone cannot establish that oxidative damage declined. That interpretation requires direct measurements of ROS and cellular injury, such as H_2O_2, MDA, or electrolyte leakage (Hasanuzzaman et al. 2021b, Liu et al. 2022).

Drought reduced plant height, capsule number, seeds per capsule, and seed weight, indicating effects on both vegetative growth and reproductive development. The responses to the extracts were trait-specific. Under drought, *U. lactuca* produced greater plant height, seeds per capsule, and seed weight than *S. subrepandum*, whereas *S. subrepandum* produced more capsules per plant. These differences may reflect the sensitivity of growth and reproductive processes to water limitation and the different ways in which the two extracts influence plant development. Reduced turgor and cell expansion, altered carbon assimilation, and impaired reproductive development are plausible mechanisms. However, the manuscript does not report verified measurements of leaf

water status or gas exchange; therefore, these pathways should be presented as possible explanations rather than as mechanisms directly demonstrated in this experiment. Seaweed extracts can modify crop responses through several interacting physiological processes, but the magnitude and direction of effects are context-dependent (Deolu-Ajayi et al. 2022, Sujeeth et al. 2022).

The nutrient responses were also element-specific. Drought reduced leaf P, K, Mg, Ca, and Na concentrations, while leaf N increased slightly from 1.12% in the control to 1.18% under drought. Under drought, *U. lactuca* resulted in greater P, K, Mg, and Ca concentrations than *S. subrepandum*, whereas *S. subrepandum* resulted in greater Na concentration. Reduced soil-water movement can constrain nutrient transport to roots, while drought can alter root activity and nutrient distribution within the plant. K is involved in osmotic regulation and stomatal function, Ca participates in membrane stability and stress signaling, and Mg is required for chlorophyll structure and numerous enzyme reactions. These functions provide a physiological context for the observed mineral patterns, but leaf concentrations do not directly establish root uptake or ion transport. The results should therefore be described as changes in leaf nutrient content, not as direct evidence of increased nutrient uptake or assimilation (Bhattacharya and Bhattacharya 2021, Weisany et al. 2021).

Seed composition changed under drought. Crude lipid and fiber contents declined, whereas protein and carbohydrate contents increased. The increase in protein concentration could reflect altered seed composition or allocation, but the measurements do not distinguish these explanations. The extracts also produced different responses: *U. lactuca* increased crude lipid content to 52.87%, above the control value of 50.30%, whereas *S. subrepandandum* produced the highest protein content among the drought-treated groups. Drought also reduced seed moisture content, which was partly increased by both seaweed treatments. Seed moisture is a compositional measurement and should not be interpreted as a direct measure of plant water status. Taken together, these results indicate that the extracts influenced seed composition, with the direction of response varying among constituents.

Drought reduced total fatty acids, MUFA, and PUFA. Both extracts partly moderated these decreases, but their effects again differed by measure: *U. lactuca* resulted in higher total fatty-acid and PUFA values under drought, while *S. subrepandum* resulted in a slightly higher MUFA value.

Changes in fatty-acid composition could involve altered lipid synthesis, desaturation, or oxidative turnover, but these processes were not measured. The data therefore demonstrate changes in the seed fatty-acid profile without establishing the biochemical pathway responsible for them. Previous work has also reported drought-associated changes in sesame fatty-acid composition (Gholinezhad and Darvishzadeh 2021).

Drought strongly increased expression of *Hsp70*, *DREB1*, *HDZ7*, and *HDZ10*, and seaweed application further modified their expression. Under drought, *S. subrepandum* produced the highest reported values for *Hsp70*, *DREB1*, and *HDZ7*, whereas *U. lactuca* produced the highest *HDZ10* value. HSP70 proteins are associated with protein folding and cellular proteostasis, while DREB transcription factors regulate dehydration-responsive gene networks. These expression patterns are consistent with treatment-related modulation of stress-responsive transcription. However, gene-expression changes alone do not demonstrate that these genes caused the observed growth or seed-quality responses. In addition, because seaweed sprays overlapped with the drought-treatment period, these results support enhanced or modified stress-responsive expression but do not establish a priming mechanism. Testing priming would require a design that separates pre-treatment from subsequent stress exposure and evaluates the timing of the response (Hussain et al. 2021, Sujeeth et al. 2022, Wang et al. 2022).

Concurrent drought-associated declines in chlorophyll content, relative water content, photosynthetic rate, stomatal conductance, and transpiration would indicate impaired leaf hydration and photosynthetic function. Increases in MDA, H_2O_2, and electrolyte leakage alongside these declines would be consistent with greater oxidative injury and membrane disruption. Higher proline and soluble sugar concentrations could reflect osmotic adjustment, although sugar accumulation may also result from altered metabolism or reduced utilization. Together, these measurements could help connect leaf-level stress responses with growth and seed outcomes. The results indicate that both seaweed extracts modified drought responses in sesame, but their relative effects varied among traits. *U. lactuca* showed stronger responses for several vegetative and seed-quality measures, whereas *S. subrepandandum* resulted in more capsules per plant, higher protein content, and greater expression of some stress-responsive genes. These patterns do not establish one extract as uniformly superior. In addition, the mechanisms discussed here are plausible

interpretations of the measured responses; the study did not directly test causal links among gene expression, antioxidant activity, mineral status, and seed performance.

Conclusion

Drought altered sesame growth and reproductive traits, leaf mineral composition, antioxidant-enzyme activity, seed composition, fatty-acid profile, and stress-responsive gene expression. Foliar application of *U. lactuca* and *S. subrepandandum* partly modified these responses, with effects that differed among traits. *U. lactuca* showed stronger responses for plant height, seeds per capsule, seed weight, crude lipid, and several fatty-acid measures. *S. subrepandandum* produced more capsules per plant, the highest protein content among drought-treated plants, and higher expression of several—but not all—target genes. These findings indicate distinct response profiles for the two extracts under the tested greenhouse conditions. Field experiments and validated measurements of water status, gas exchange, pigments, and oxidative damage are needed to test the proposed physiological mechanisms and determine the agronomic value of the treatments.

Ethical Approval: Ethical approval was not required for this work

Conflicts of Interest: Authors declare no conflict of interest.

Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contribution

Aisha A.M. Alayafi: Conceptualization, methodology, investigation, formal analysis, data curation, visualization, and writing—original draft, review and editing**.**

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812



(A)



(B)

Figure 1: Air-dried seaweed samples *Ulva Lactuca* (A) *and Sargassum subrepandum* (B)

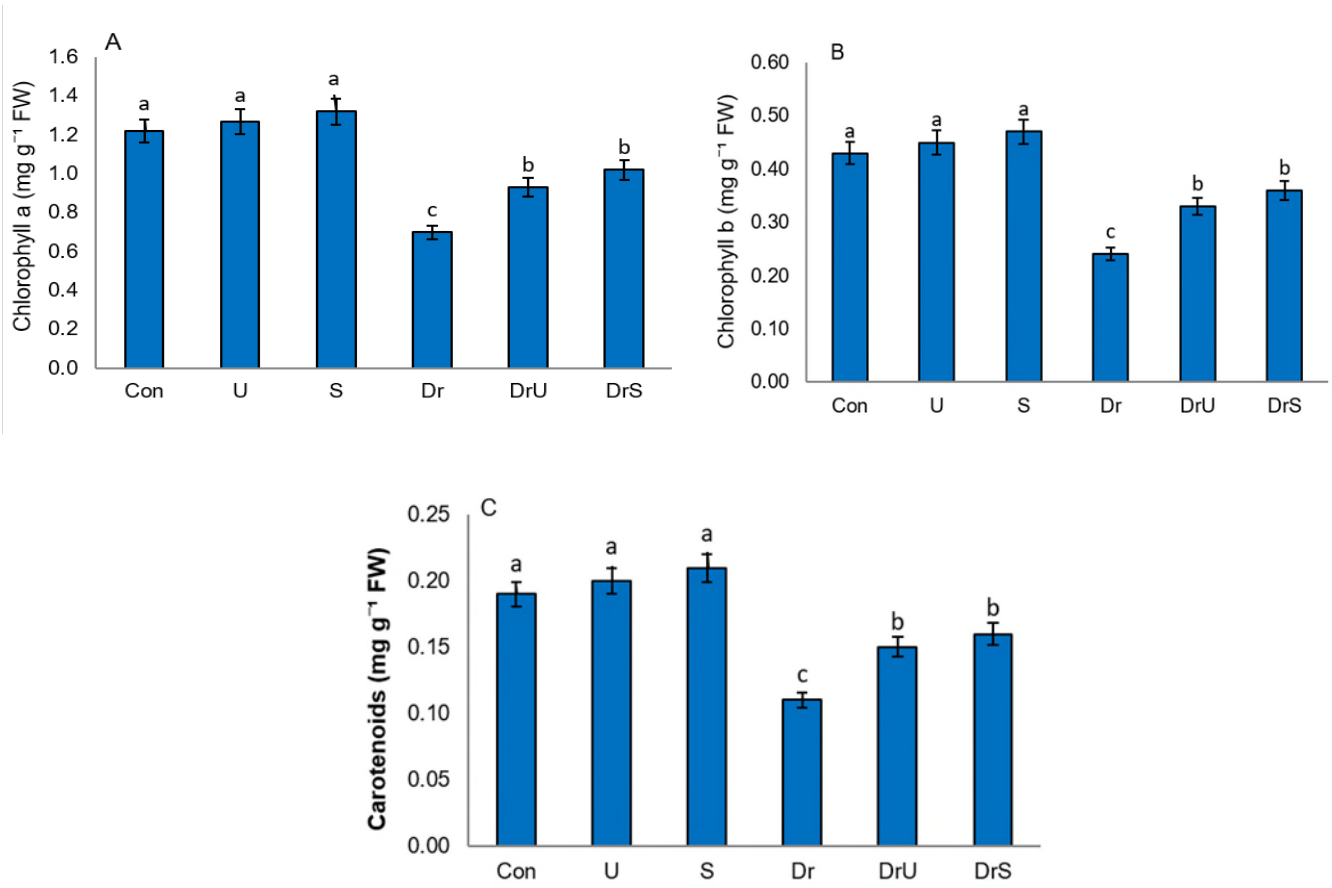


Figure 2. Leaf pigment concentrations in sesame plants under different treatments: (A) chlorophyll *a*, (B) chlorophyll *b*, and (C) total carotenoids. Treatments were well-watered control (Con), *Ulva lactuca* (U), *Stoechospermum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard error (SE) of three replicates. Different letters indicate significant differences among treatments according to the LSD test at $P \leq 0.05$.

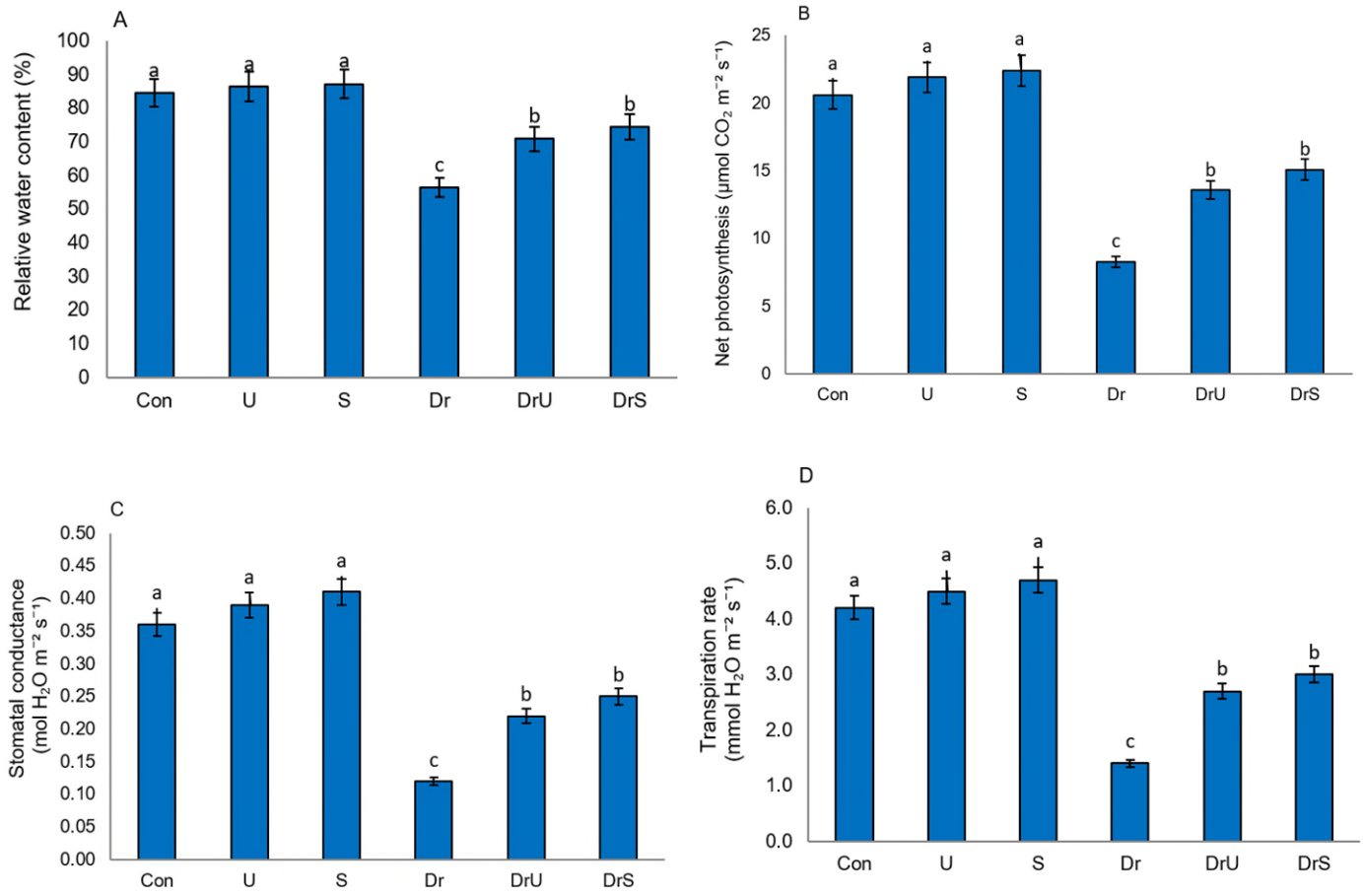


Figure 3. Relative water content and gas-exchange responses of sesame plants under different treatments: (A) relative water content (RWC), (B) net photosynthetic rate (Pn), (C) stomatal conductance (gs), and (D) transpiration rate (E). Treatments were well-watered control (Con), *Ulva lactuca* (U), *Stoechospermum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard error (SE) of three replicates. Different letters indicate significant differences among treatments according to the LSD test at $P \leq 0.05$.

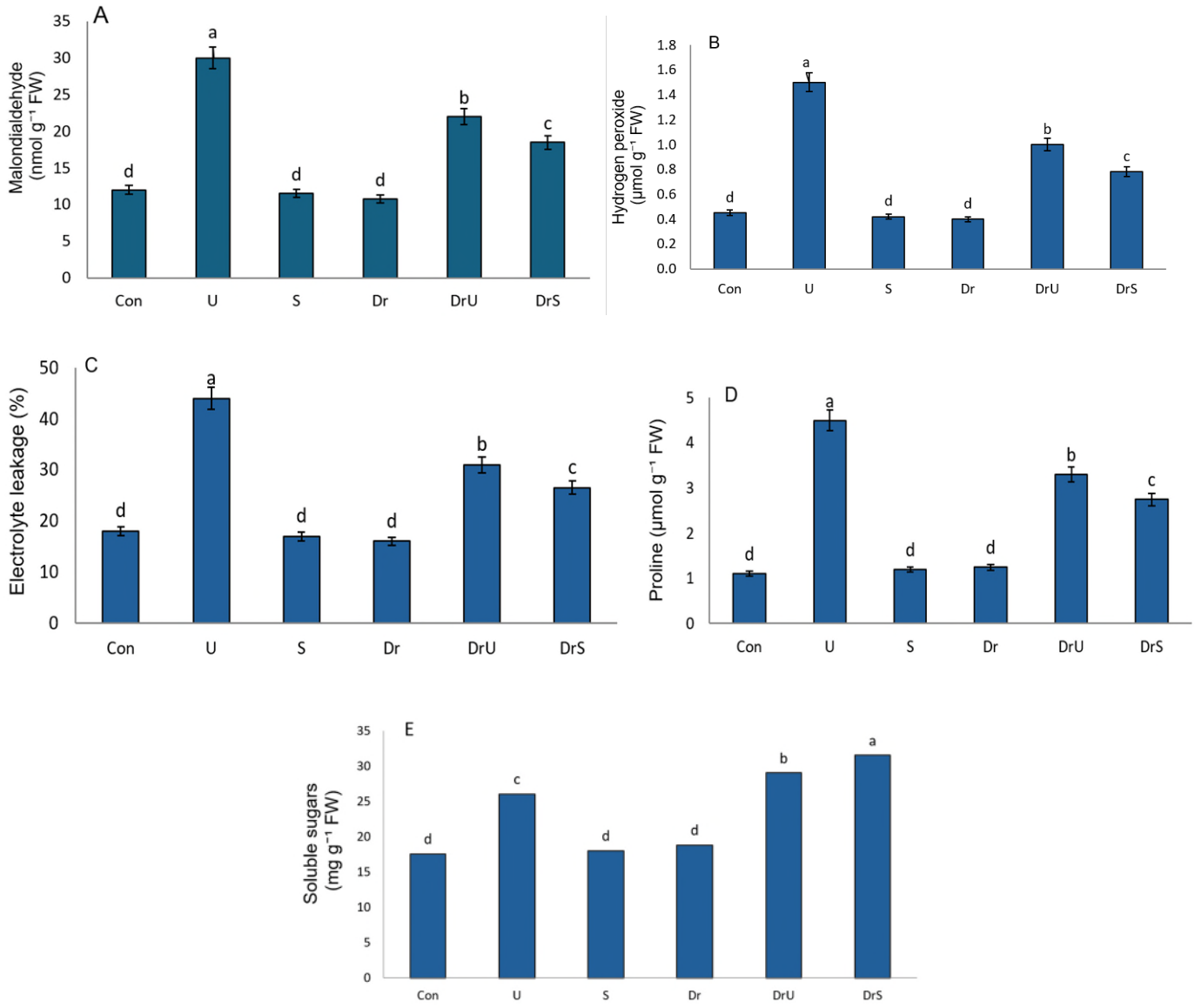


Figure 4. Oxidative damage and osmotic adjustment responses of sesame plants under different treatments: (A) malondialdehyde (MDA) content, (B) hydrogen peroxide (H_2O_2) content, (C) electrolyte leakage, (D) proline content, and (E) soluble sugar content. Treatments included the well-watered control (Con), *Ulva lactuca* (U), *Stoechospermum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard error (SE) of three replicates. Different letters indicate significant differences among treatments according to the LSD test at $P \leq 0.05$.

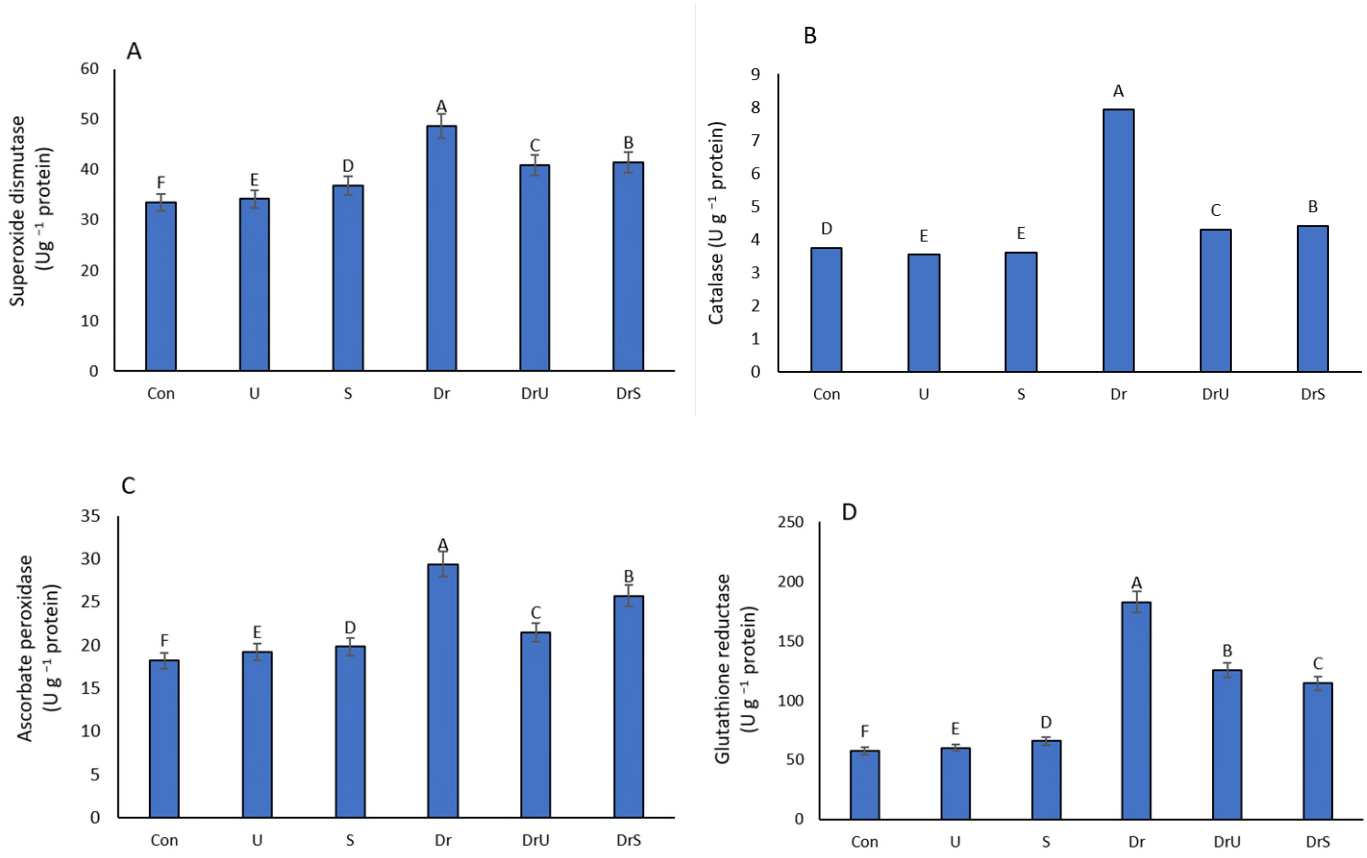


Figure 5. Activities of antioxidant enzymes in sesame plants under different treatments: (A) superoxide dismutase (SOD), (B) catalase (CAT), (C) ascorbate peroxidase (APX), and (D) glutathione reductase (GR). Treatments include Control (Con), *Ulva lactuca* (U), *Sargassum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard deviation of three replicates. Statistical differences were assessed using the Least Significant Difference (LSD) test at the 5% probability level. Different letters indicate significant differences among treatments.

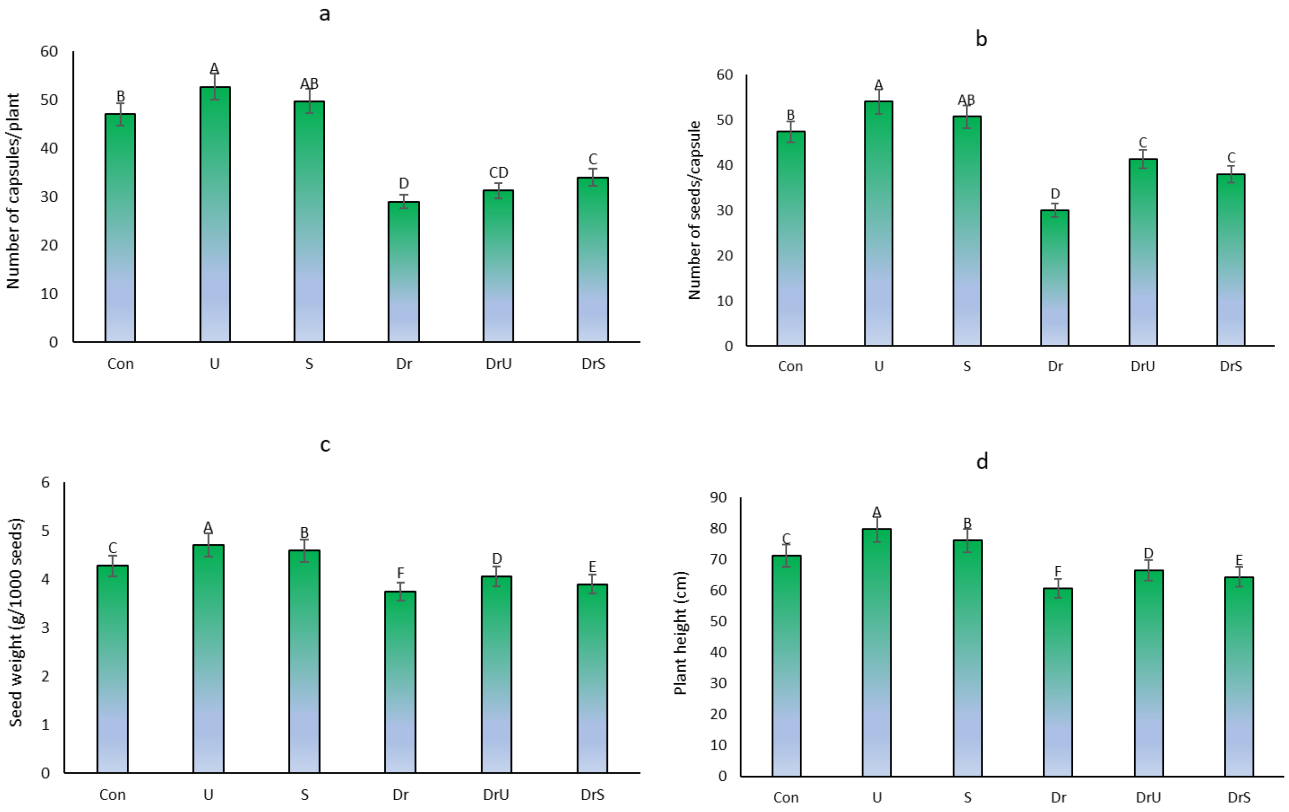


Figure 6. Morphological and reproductive characteristics of sesame plants under different treatments: (A) number of capsules per plant, (B) number of seeds per capsule, (C) seed weight, and (D) plant height. Treatments include Control (Con), *Ulva lactuca* (U), *Sargassum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard deviation of three replicates. Statistical significance was determined using the LSD test at a 5% probability level; means sharing the same letter are not significantly different.

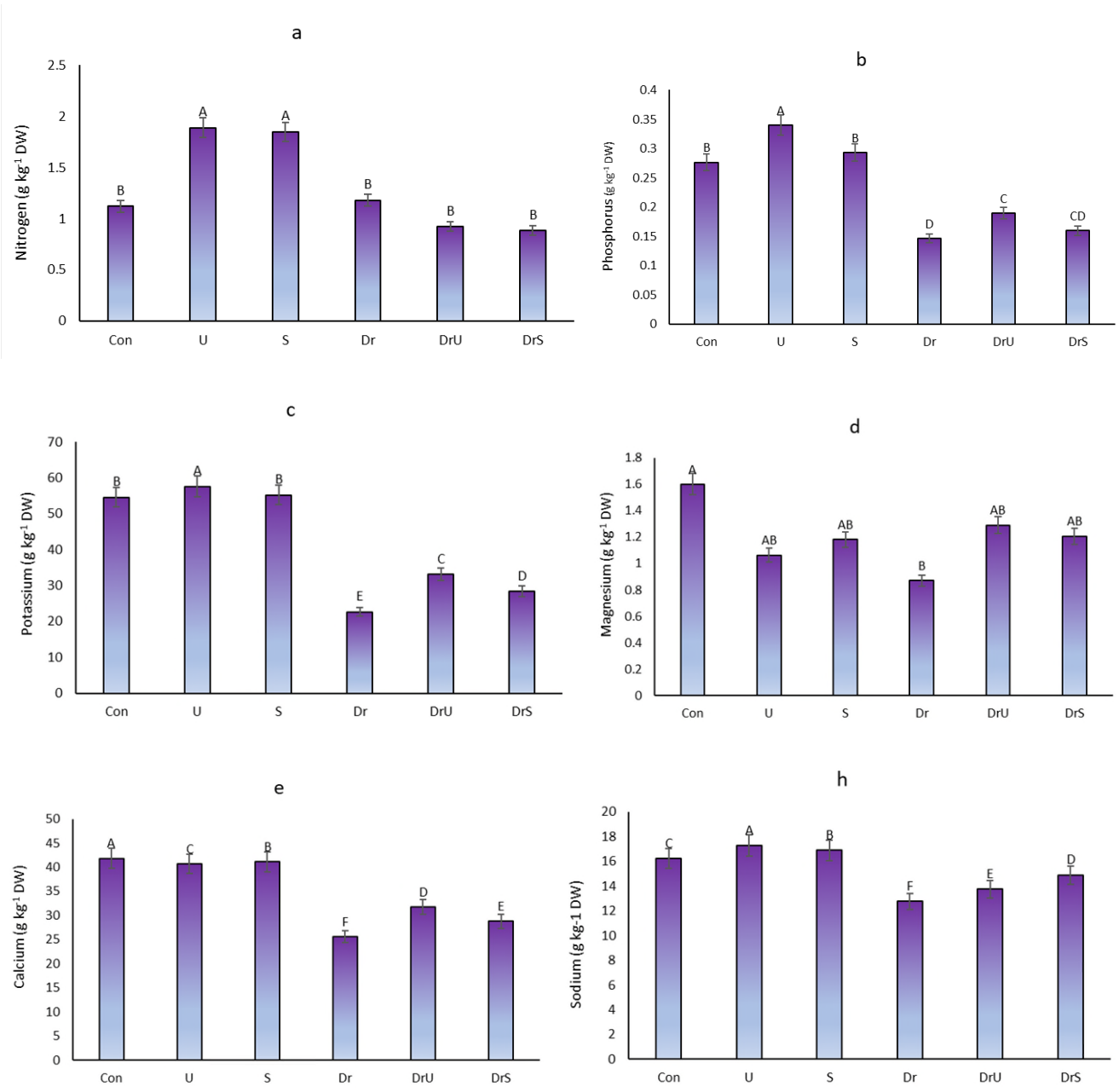


Figure 7. Mineral nutrient content in sesame plants under different treatments: (A) nitrogen, (B) phosphorus, (C) potassium, (D) magnesium, (E) calcium, and (F) sodium. Treatments include Control (Con), *Ulva lactuca* (U), *Sargassum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard deviation of three replicates. Statistical significance was determined using the LSD test at the 5% probability level; means sharing the same letter are not significantly different.

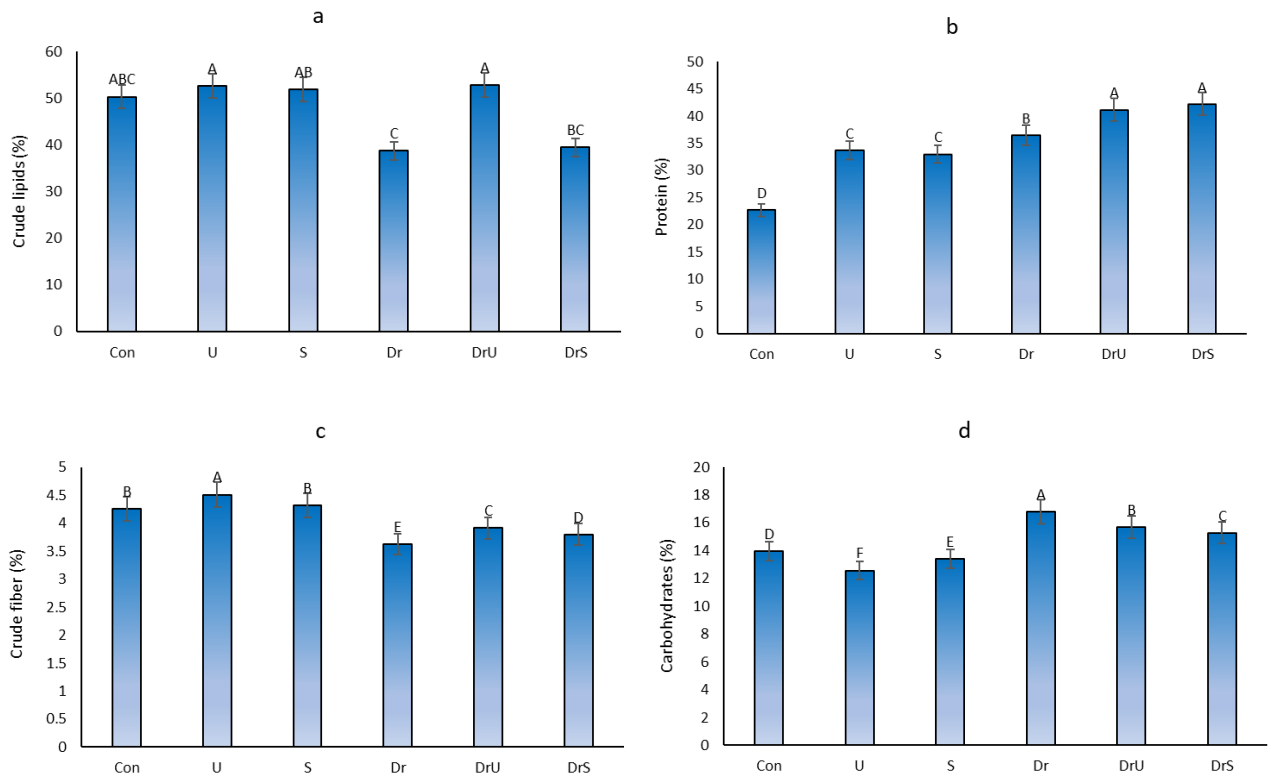


Figure 8. Biochemical composition of sesame plants under different treatments: (A) crude lipids, (B) protein content, (C) crude fiber, and (D) carbohydrate content. Treatments include Control (Con), *Ulva lactuca* (U), *Sargassum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard deviation of three replicates. Statistical significance was determined using the LSD test at the 5% probability level; means sharing the same letter are not significantly different.

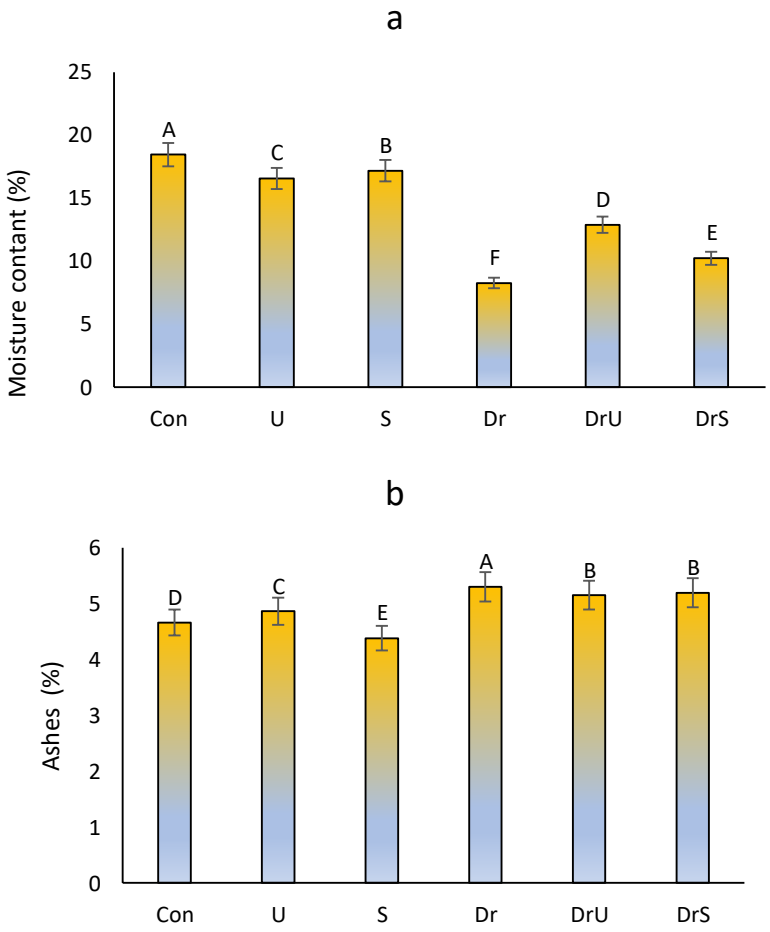


Figure 9. Proximate composition of sesame plants under different treatments: (A) moisture content and (B) ash content. Treatments include Control (Con), *Ulva lactuca* (U), *Sargassum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard deviation of three replicates. Statistical significance was determined using the LSD test at the 5% probability level; means sharing the same letter are not significantly different.

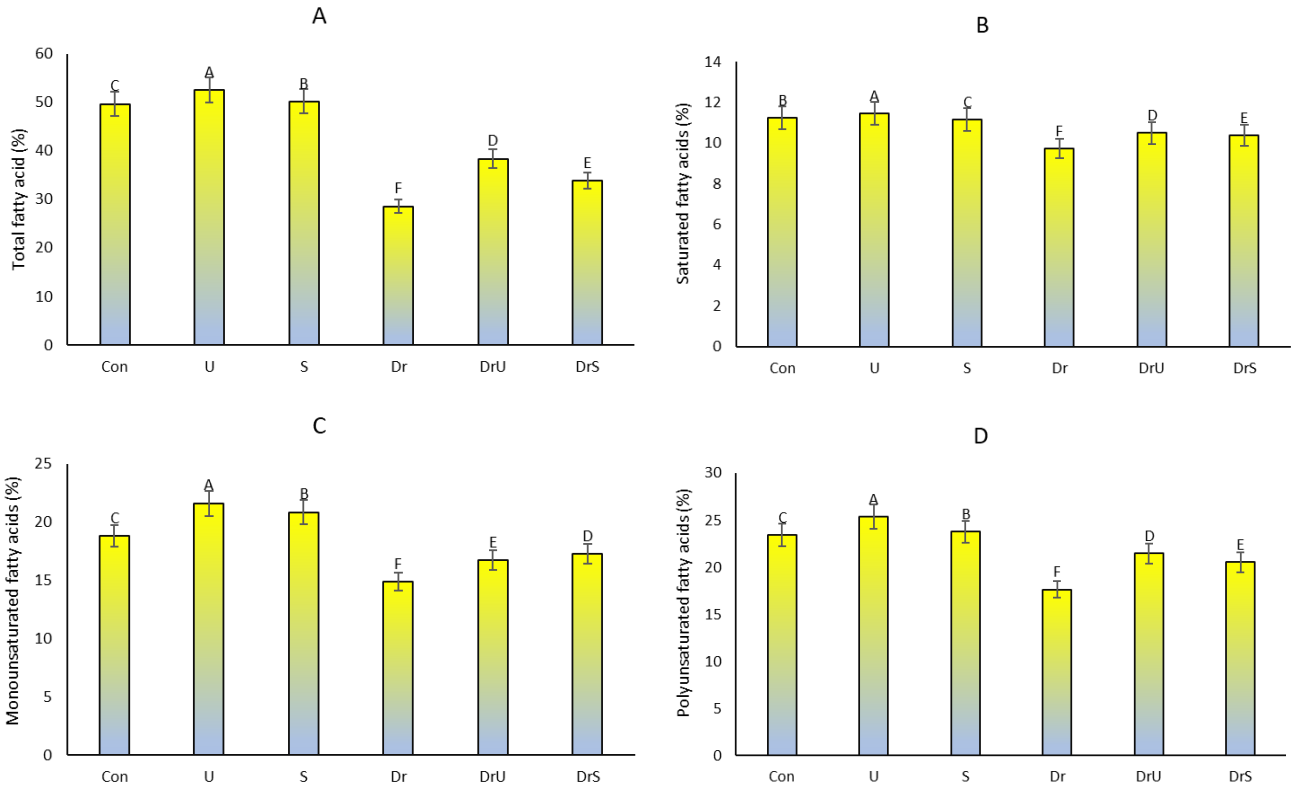


Figure 10. Fatty acid composition of sesame seeds under different treatments: (A) total fatty acids, (B) saturated fatty acids (SFA), (C) monounsaturated fatty acids (MUFA), and (D) polyunsaturated fatty acids (PUFA). Treatments include Control (Con), *Ulva lactuca* (U), *Sargassum subrepandum* (S), drought stress (Dr), drought + *U. lactuca* (DrU), and drought + *S. subrepandum* (DrS). Data are presented as means ± standard deviation of three replicates. Statistical significance was determined using the LSD test at the 5% probability level; means sharing the same letter are not significantly different.

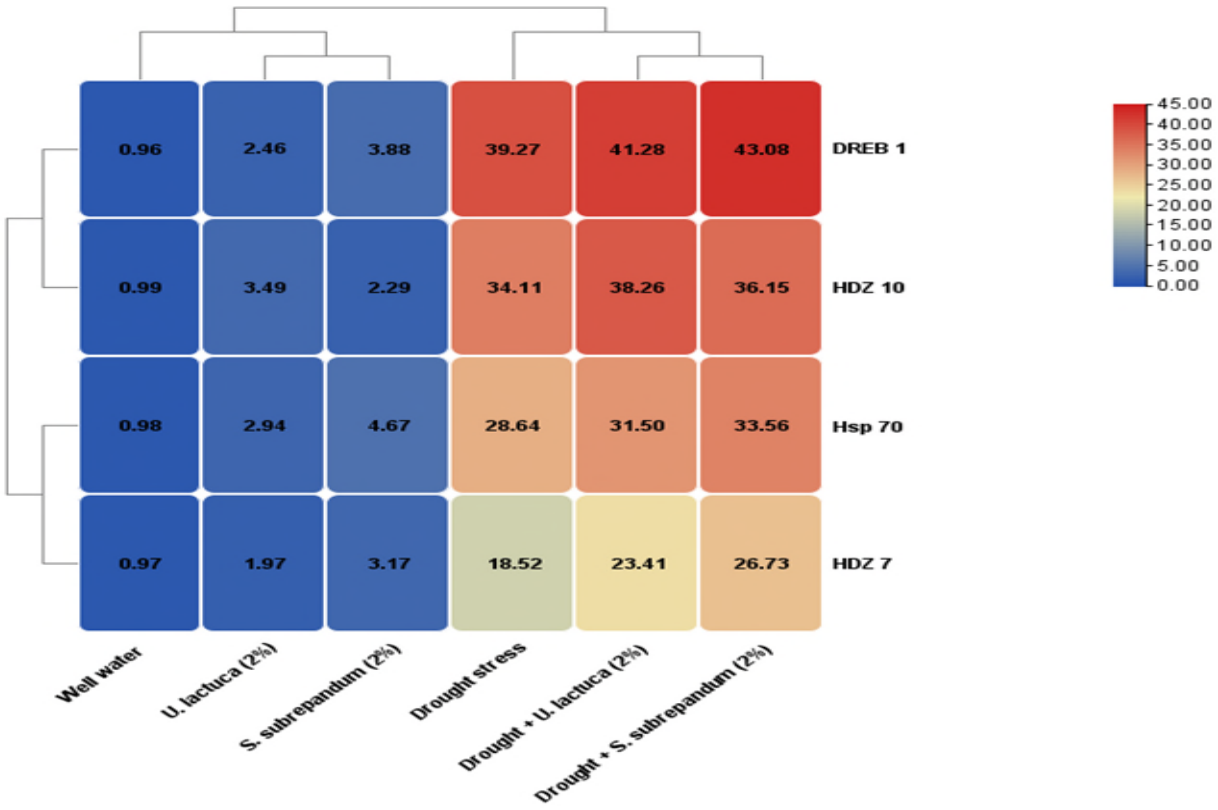
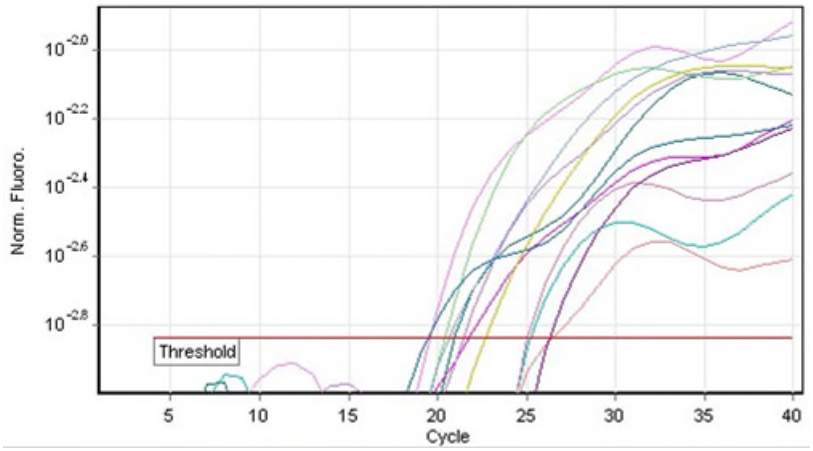
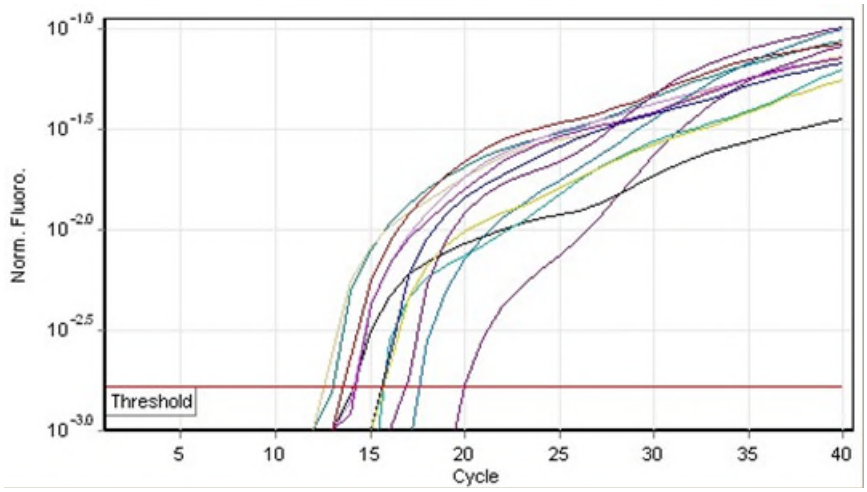


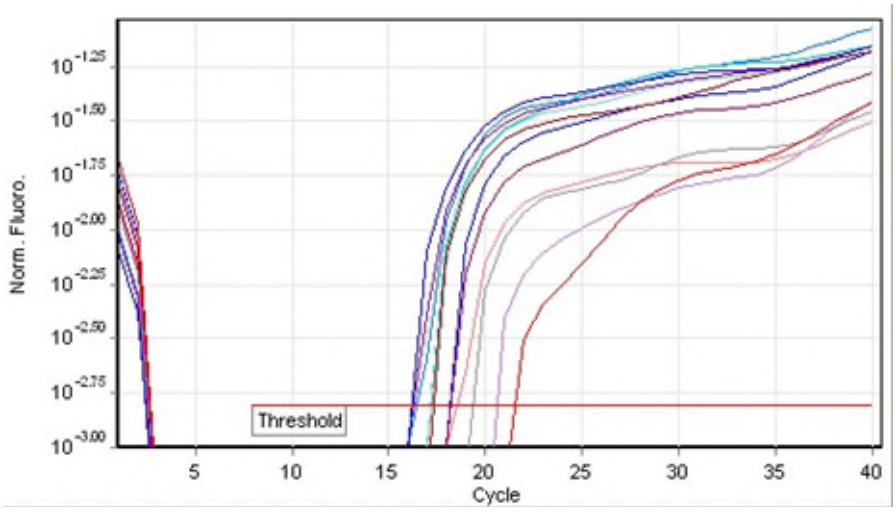
Figure 11: Heatmap showing the effects of different treatments on gene expression levels of sesame plants. Treatments include well water (Con), *Ulva lactuca* (U), *Sargassum subrepandum* (S), Drought stress (Dr), and drought combined with algae extracts (DrU, DrS). Warmer colors represent higher values, and cooler colors represent lower values across measured parameters.



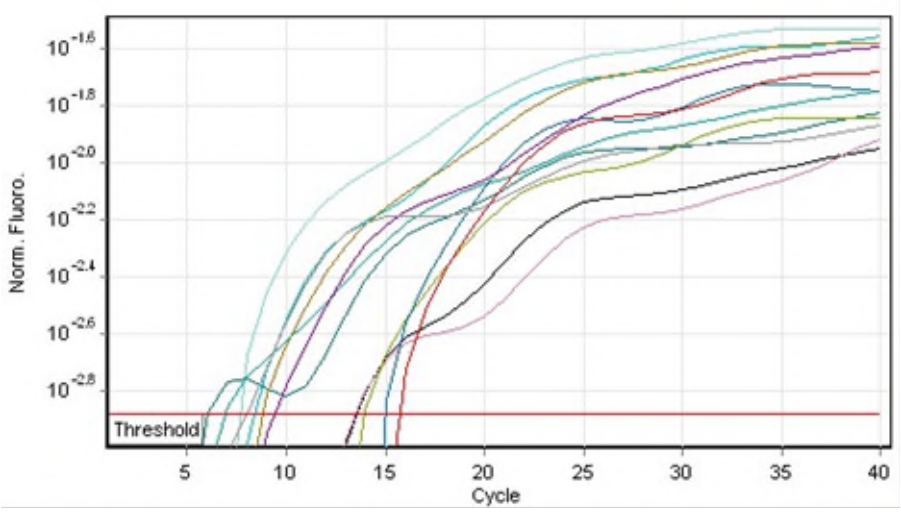
HSP 70



HDZ 7



HDZ 10



DREB 1

Figure 12: Relative gene expression levels of selected stress-responsive and growth-regulating genes in sesame plants under different treatments.

Table 1: Proximate composition and mineral content of *Ulva lactuca* and *Sargassum subrepandum*. Values are means ± standard error on a dry weight basis.

	Crude Lipids (%)	T. Protein	Crude Fiber (%)	Carbohydrates (%)	Moisture (%)	Ashes Content (%)	N (g kg^{-1} DW)	P (g kg^{-1} DW)	K (g kg^{-1} DW)	Mg (g kg^{-1} DW)	Ca (g kg^{-1} DW)	Na (g kg^{-1} DW)
Ulva lactuca	1.79±0.4	23.41±1.9	13.3±1.02	48.63±2.4	20.61±1.6	12.56±0.7	2.12±0.2	0.28±0.002	9.6±1.0	2.04±0.03	3.519±0.02	4.35±0.3
Sargassum subrepandum	2.53±0.3	4.91±0.5	31.66±2.1	30.05±2.2	65.28±3.4	16.42±1.5	1.98±0.2	0.23±0.004	18.6±1.6	1.86±0.04	26.77±1.8	13.2±1.2

Table 2: Physico-chemical properties of soil sample

pH	EC (us/cm)	Mg^{++} (mg/L)	Ca^{++} (mg/L)	Na+ (mg/L)	K+ (mg/L)	Co3- (mg/L)	HCO^{3-} (mg/L)	O.M. (%)	N (ppm)
7.54	1329±13.24	17.8±1.6	124.6±3.45	163±4.22	16±0.74	0.005±0.001	59.2±2.51	0.06±0.002	64.9±3.29

Table 3: Relative gene expression levels of selected stress-responsive and growth-regulating genes in sesame plants under different treatments.

Genes	Well water	Drought stress	*U. lactuca*	*S. subrepandum*	Drought + *U. lactuca*	Drought + *S. subrepandum*
Hsp 70	0.98	28.64	2.94	4.67	31.5	33.56
DREB 1	0.96	39.27	2.46	3.88	41.28	43.08
HDZ 7	0.97	18.52	1.97	3.17	23.41	26.73
HDZ 10	0.99	34.11	3.49	2.29	38.26	36.15