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Oxalic acid enhances wheat (*Triticum aestivum* L.) resilience to combined abiotic stresses through integrated physiological and rhizospheric microbial modulation

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ABSTRACT

Soil contamination and abiotic stress have become serious global problem due to rapid development of social economy. Oxalic acid (OA), an important organic acid and fertilizer component, has been found effective in enhancing plant tolerance against various abiotic stresses. For this purpose, we have designed the current experiment to explore the contribution of OA in mediating growth and eco-physiology by alleviating abiotic stresses, in wheat (*Triticum aestivum* L.). Seedlings of *T. aestivum* were subjected to the different abiotic stresses including drought, salinity, heat, and cold stress, and were supplemented with exogenous OA at 5 mM. Results from the present study revealed that the abiotic stresses induced a substantial decrease in shoot length, root length, number of leaves, leaf area, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid content, net photosynthesis, stomatal conductance, transpiration rate, soluble sugar, reducing sugar, non-reducing sugar contents, calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), and phosphorus (P) contents, microbial diversity, richness, and evenness in *T. aestivum* plants. In contrast, abiotic stresses in the soil significantly ($P < 0.05$) increased phenolic content, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), health risk indices, bioaccumulation factors. Although, the activities of enzymatic antioxidants such as superoxide dismutase, peroxidase, catalase, ascorbate peroxidase in the *T. aestivum* plants and non-enzymatic such as phenolic, flavonoid, ascorbic acid, and anthocyanin contents were increased with the exposure of abiotic stresses. The application of OA significantly improved photosynthetic efficiency, microbial diversity, richness, and evenness, while reducing health risk indices, bioaccumulation factors, MDA, and H_2O_2 contents under stress conditions. Proteomic and transcriptomic profiling further supported the regulatory role of OA in modulating stress-responsive signaling pathways and enhancing stress tolerance in *T. aestivum* plants. Increased antioxidant enzyme activities in OA-treated plants appeared to play a crucial role in scavenging stress-induced reactive oxygen species. Research findings, therefore, suggested that OA application can ameliorate abiotic stresses toxicity in *T. aestivum* seedlings and resulted in improved plant growth and composition under abiotic stresses.

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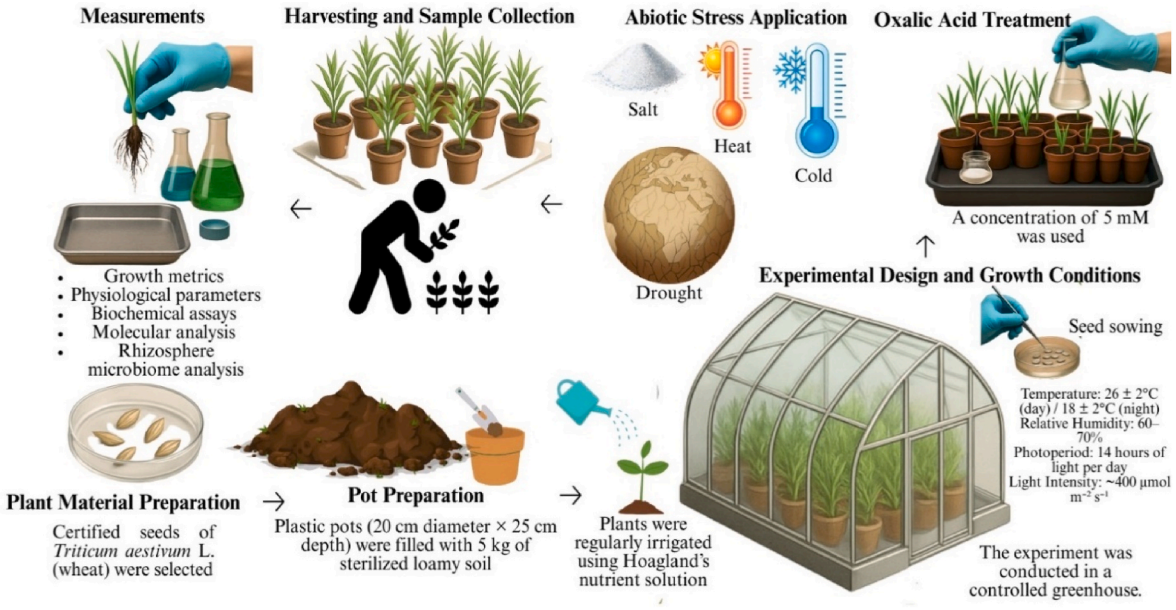

Fig. 1. Schematic overview of the experimental methodology. Wheat (*Triticum aestivum* L. cv. Sakha 93) plants were exposed to individual and combined abiotic stresses (salinity, drought, heat, and cold) with or without oxalic acid (OA; 5 mM) foliar application under controlled greenhouse conditions. Stress treatments were maintained for two months, and plants were harvested 60 days after sowing for morphological, physiological, biochemical, molecular, and rhizosphere microbiome analyses.

1. Introduction

Global agriculture is increasingly challenged by abiotic stresses such as drought, salinity, heat, metal and cold, which have intensified due to climate change and anthropogenic disturbances (Kopecká et al., 2023; Alwutayd et al., 2024). These stresses not only reduce crop yield and quality but also impair plant growth, disrupt nutrient uptake, limit photosynthetic efficiency, and trigger oxidative damage (Ullah et al., 2025). Among major food crops, wheat (*Triticum aestivum* L.), a globally cultivated staple providing approximately 20% of human caloric intake, is particularly sensitive to such environmental stressors (Sharma and Sharma, 2025). It is thus an essential goal of global food security to ensure that wheat can withstand a joint stress environment.

Mitigation strategies to overcome abiotic stress are urgently required, as there is an ever-growing concern regarding the excessive use of fertilizers and pesticides (Rahim et al., 2025; Al-Huqail et al., 2023). Oxalic acid (OA) is a plant-based and soil-based dicarboxylic acid that has gained more and more attention over the past few years owing to its stress-regulating properties (Li et al., 2022). Nevertheless, the biologically beneficial impact of OA on plants has been reported in recent literature despite its earlier association with the pathogenic fungi, including increased antioxidant activity of enzymes, increased nutrient uptake, and modified plant hormone signaling pathways (Chen et al., 2024). It should be mentioned that oxalic acid is reported to mitigate the adverse effects of abiotic stress through improved redox homeostasis, osmolytes accumulation, and enzyme system and non-enzyme defenses in plants (Gupta et al., 2024). In addition to its internal actions, OA is also one of the main root exudates that affect the shaping of the rhizosphere microbiome (Deng et al., 2024). OA has the potential to modify microbial community structure and microbial community functionality through pH control, metal chelation and selective microbial recruitment, potentially leading to improved plant-microbial interactions that are essential to nutrient cycling, pathogen suppression and stress tolerance (Xiang et al., 2020). OA also plays an important role in recruiting beneficial microbial consortia such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF).(Anandakumar et al., 2024).

During recent years, certain abiotic stresses have received considerable attention due to their effects on plant morphology and physiology owing to increasing environmental exposure, which is also likely to have a negative impact on vegetative crops, including *T. aestivum*. Previously, many studies have been conducted on different plant species using various growth regulators or chelators in different soils. However, there are very few studies which have been conducted on the effect of OA on *T. aestivum* under different abiotic stresses, including drought, salinity, heat, and cold stress. Therefore, we aimed to design this study, which will increase our knowledge about the effects of different abiotic stresses on plant growth and biomass, photosynthetic pigments, gas-exchange characteristics, antioxidant machinery (enzymatic and non-enzymatic antioxidants), rhizosphere microbial diversity, health risk indices, bioaccumulation factors, and stress-responsive signaling pathways under the application of OA. The results from the present study provide a new insight that the use of OA under different abiotic stresses may be beneficial and can improve plant yield under abiotic stress conditions.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted in the greenhouse of the Department of Biotechnology, College of Science, Taif University, Taif, Makkah Province, Saudi Arabia. Pots were placed under a glass house environment where they received natural sunlight, day/night humidity (60/70%), day/night temperature (24/12 °C), respectively, and 14-h photoperiod with a light intensity of approximately 400 µmol m^{-2} s^{-1}. Healthy and mature seeds of wheat (*Triticum aestivum* L. cv. Sakha 93) were collected from the Gene-Bank of King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia. The cultivar Sakha 93 was selected due to its wide adaptation, stable growth performance, and its frequent use in abiotic stress-related physiological studies. Before seed sowing, the seeds were carefully washed and sterilized in 0.1 % $HgCl_2$ solution for 1 min and then washed thrice with distilled water. The soil used for this experiment was collected from experimental station of Taif University, was air-dried and passed through a 2-mm sieve, and its properties were as follow: pH-6.9, EC-0.9 dS cm^{-1}, organic

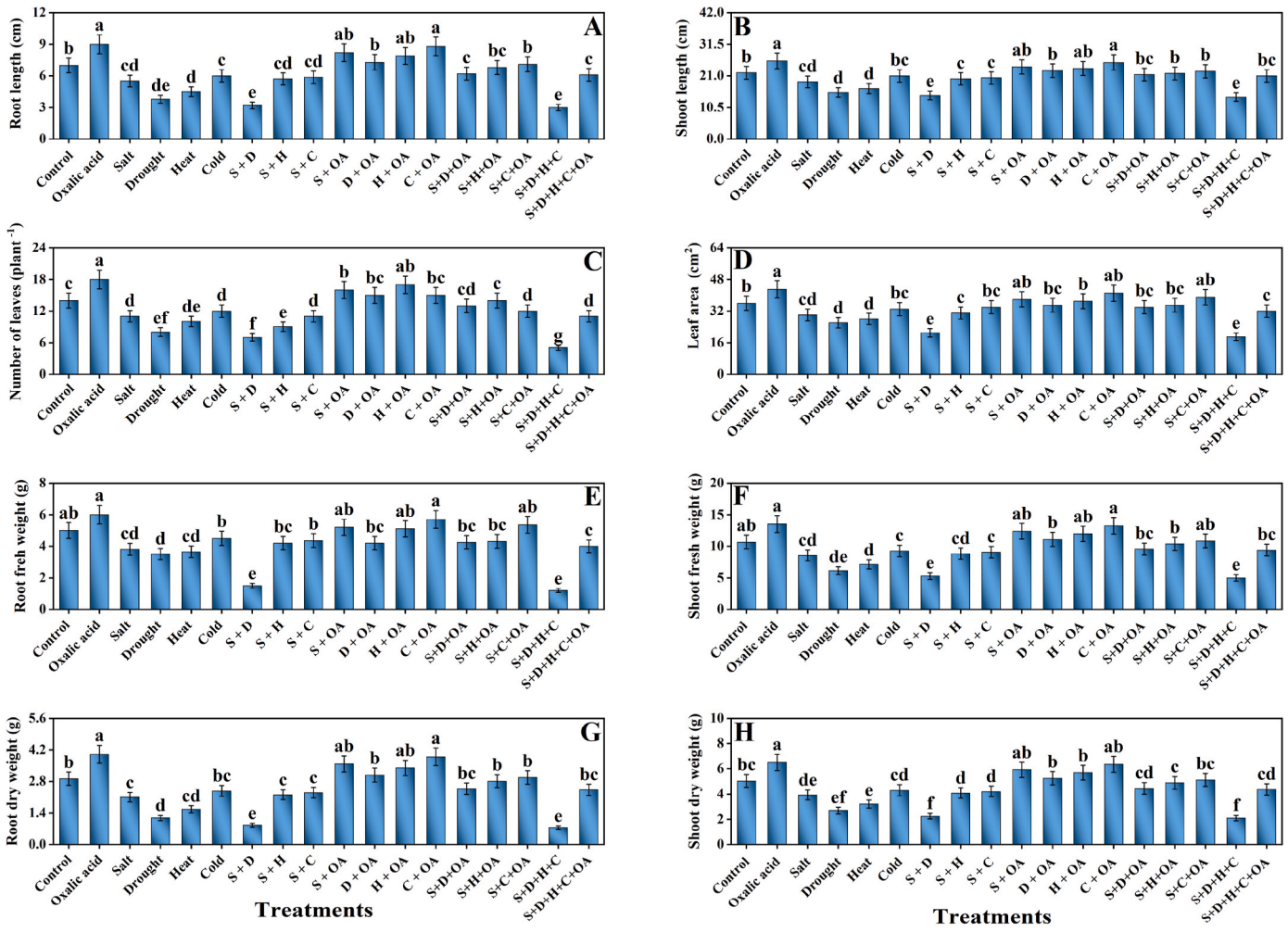

Fig. 2. Effect of oxalic acid (OA) on morphological and biomass attributes of *Triticum aestivum* L. grown under different abiotic stresses including salinity (S), drought (D), heat (H), and cold (C) stress. (A) Root length, (B) Shoot length, (C) Number of leaves plant^{-1}, (D) Leaf area, (E) Root fresh weight, (F) Shoot fresh weight, (G) Root dry weight, and (H) Shoot dry weight. Data are presented as mean ± standard deviation (SD) of five replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P < 0.05$ according to Tukey's honestly significant difference (HSD) test.

matter-17 g kg^{-1}, EK-21 mg kg^{-1}, TP-0.17 g kg^{-1} and TN-16 g kg^{-1}. Small pots (15 cm height × 20 cm width) were used in this study, each containing 5 kg of uncontaminated soil. The pot dimensions and soil volume were maintained uniformly across all treatments to ensure consistent root development, soil moisture retention, and stress exposure conditions throughout the experimental period. Before starting the pot experiment, different abiotic stresses, including drought, salinity, heat, and cold stress, were artificially imposed. Sodium chloride (NaCl) was added to irrigation water to reach an EC of 8 dS m^{-1} for salt stress. Salt concentration was increased gradually over three days to avoid osmotic shock. Drought stress was applied by withholding water until the soil moisture content reached 30% of field capacity, which was checked gravimetrically every 24 h. Heat stress was imposed by maintaining plants at 42 ± 1 °C for 8 h each day (10:00–18:00), with the temperature kept at 20 °C during the night. Cold stress was introduced by exposing plants to 4 ± 1 °C for 8 h daily (10:00–18:00) in a separate controlled chamber, while the nighttime temperature was maintained at 20 °C. All pots underwent two cycles of water saturation and air drying before the initiation of stress treatments. All stress treatments were maintained continuously for two months during the experimental period. Oxalic acid (OA) was applied to the *T. aestivum* plants 14 days after seed sowing at the seedling stage. Oxalic acid was prepared as an aqueous solution at a concentration of 5 mM and applied as a foliar spray to the *T. aestivum* seedlings (Sadak and Orabi, 2015). Foliar application of OA was performed twice per week, while control plants were sprayed with deionized water. Foliar application was selected to ensure rapid absorption and direct physiological responses under abiotic stress conditions. The pots used in this study were rotated regularly to minimize environmental variation among treatments. The total duration of the experimental treatments was two months under controlled conditions. All pots were placed in completely randomized design (CRD) with 18 treatments, having one plant in each pot with five replicates of each treatment. The treatments included control (unstressed), individual abiotic stresses (salt, drought, heat, cold), their combinations, and oxalic acid (OA) treatments either alone or in combination with stress. The treatment groups were as follows: Control (no stress, no OA), Oxalic acid only (OA), Salt stress (S), Drought stress (D), Heat stress (H), Cold stress (C), Combined stresses: S + D, S + H, S + C, D + OA, H + OA, C + OA, S + D + OA, S + H + OA, S + C + OA, S + D + H + C, S + D + H + C + OA. The plants were collected 60 days after sowing seeds for subsequent analysis. Analytical-grade chemicals were used throughout this study, all of which were acquired from Saudi Basic Industries Corporation, Saudi Arabia. The complete methodology, including the step-by-step experimental setup and procedures, is illustrated in Fig. 1 to provide a clear overview of the experimental process.

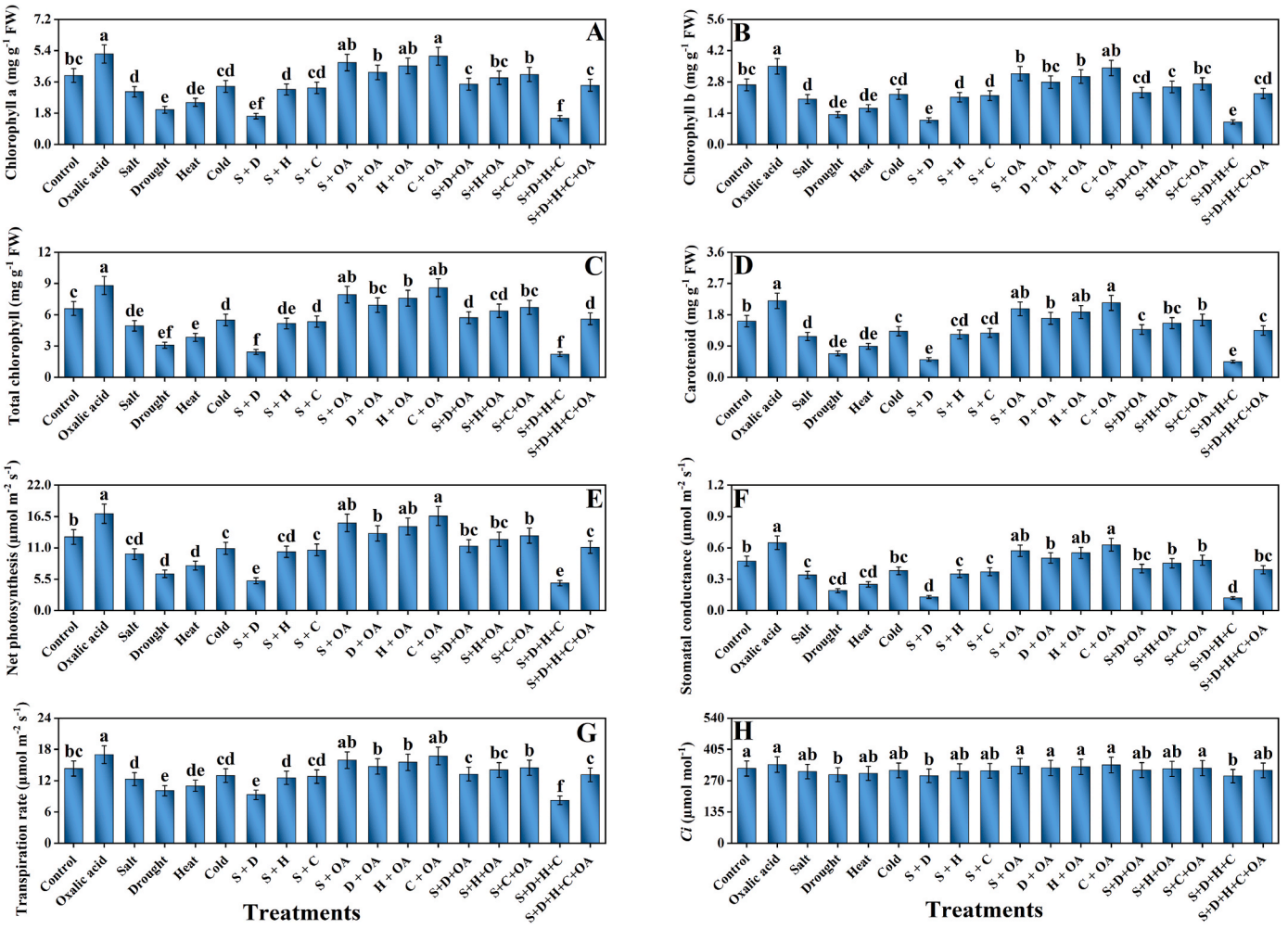

Fig. 3. Effect of oxalic acid (OA) on photosynthetic pigments and gas exchange attributes of *Triticum aestivum* L. under different abiotic stresses including salinity (S), drought (D), heat (H), and cold (C) stress. (A) Net photosynthetic rate (Pn), (B) Stomatal conductance (Gs), (C) Transpiration rate (Ts), and (D) Intercellular CO_2 concentration (Ci). Data are presented as mean ± standard deviation (SD) of five replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P < 0.05$ according to Tukey's honestly significant difference (HSD) test. Control = untreated plants; OA = oxalic acid.

2.2. Sampling and tissue collection

After 2 months, the remaining four plants were uprooted and washed gently with the help of distilled water to eliminate the aerial dust and deposition. Functional leaf in each treatment was picked at a rapid growth stage during 09:00 and 10:30. The sampled leaves were washed with distilled water, immediately placed in liquid nitrogen, and stored in a freezer at −80 °C for further analysis. All of the harvested plants were divided into two parts (i.e., roots and shoots) to study different physio-biochemical traits. Leaves from each treatment group were picked for chlorophyll, carotenoid, oxidative stress, and antioxidants analysis. Leaf area was measured using a digital leaf area meter (Model LI-3100C, LI-COR Biosciences, USA, while the number of leaves per plant was recorded manually at the time of harvest. Root and shoot lengths were measured straightway after the harvesting using a measuring scale and digital weighting balance to measure fresh biomass.

2.3. Measurement of chlorophyll and carotenoids content and gas exchange traits

Leaves were collected for the determination of chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95 % acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll content was calculated by the standard method of (Arnon, 1949)

Net photosynthesis (*Pn*), leaf stomatal conductance (*Gs*), transpiration rate (*Ts*), and intercellular carbon dioxide concentration (*Ci*) were measured from four different plants in each treatment group. Measurements were conducted between 11:30 and 13:30 on days with a clear sky. Rates of leaf *Pn*, *Gs*, *Ts*, and *Ci* were measured with a LI-COR gas-exchange system (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) with a red-blue LED light source on the leaf chamber. In the LI-COR cuvette, CO_2 concentration was set as 380 μmol mol^{-1} and LED light intensity was set at 1000 μmol m^{-2} s^{-1}, which was the average saturation intensity for photosynthesis in *T. aestivum*.

2.4. Determination of oxidative stress markers

Lipid peroxidation was estimated by determining malondialdehyde (MDA) levels in leaf tissues. For this, 0.1 g of frozen leaf sample was homogenized in a pre-chilled mortar at 4 °C. The homogenate was centrifuged at 10,000×g for 15 min, and the resulting supernatant was subjected to heating at 100 °C for 15–30 min, followed by immediate cooling. Absorbance was measured by using a microplate spectrophotometer (xMark™; Bio-Rad, USA) to calculate MDA content. Hydrogen peroxide (H_2O_2) content was quantified by mixing 3 mL of the tissue extract with 0.1% titanium sulfate dissolved in 20% sulfuric acid. After

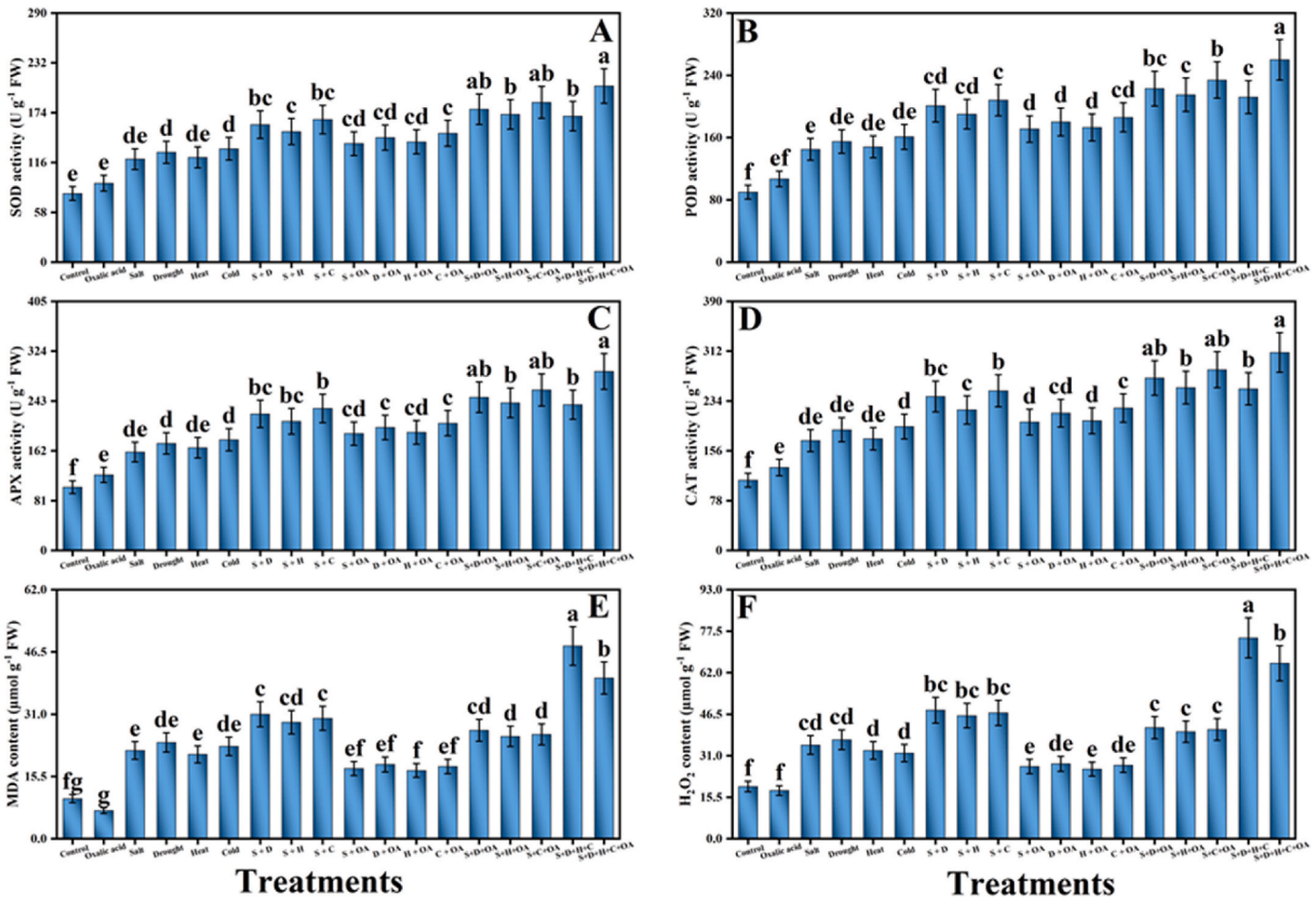

Fig. 4. Effect of oxalic acid (OA) on antioxidant enzyme activities and oxidative stress biomarkers in *Triticum aestivum* L. under different abiotic stresses including salinity (S), drought (D), heat (H), and cold (C) stress. (A) Superoxide dismutase (SOD) activity, (B) Peroxidase (POD) activity, (C) Ascorbate peroxidase (APX) activity, (D) Catalase (CAT) activity, (E) Malondialdehyde (MDA) content, and (F) Hydrogen peroxide (H_2O_2) content. Data are presented as mean ± standard deviation (SD) of five replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P < 0.05$ according to Tukey's honestly significant difference (HSD) test. Control = untreated plants; OA = oxalic acid.

centrifugation, the absorbance was measured at 410 nm (Heath and Packer, 1968; Jana and Choudhuri, 1981).

2.5. Determination of antioxidants and nutrients content

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenized in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0), including 0.5 mmol EDTA and 0.15 mol NaCl. The homogenate was centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant was used for measurement of superoxide dismutase (SOD) and peroxidase (POD) activities. SOD activity was assayed in 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro blue tetrazolium, 1.17 mM riboflavin, 10 mM methionine, and 100 μL enzyme extract. Finally, the sample was measured by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad). Enzyme activity was measured by using a method by (Chen and Pan, 1996).

POD activity in the leaves was estimated by using the method of (Sakharov and Ardila, 1999) by using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1 % H_2O_2, and 0.1 mL of 4 % guaiacol solution was prepared. Increases in the absorbance at 470 nm because of guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme.

Catalase (CAT) activity was analyzed according to (Aebi, 1984). The assay mixture (3.0 mL) was comprised of 100 μL enzyme extract, 100 μL H_2O_2 (300 mM), and 2.8 mL 50 mM phosphate buffer with 2 mM EDTA (pH 7.0). The CAT activity was measured from the decline in absorbance at 240 nm as a result of H_2O_2 loss ($\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$).

Ascorbate peroxidase (APX) activity was measured according to (Nakano and Asada, 1981). The mixture containing 100 μL enzyme extract, 100 μL ascorbate (7.5 mM), 100 μL H_2O_2 (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring APX activity. The oxidation pattern of ascorbate was estimated from the variations in wavelength at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$).

2.6. Gene expression analysis of antioxidant enzymes

Quantitative real-time PCR (RT-qPCR) assay was applied to investigate the expression levels of 4 stress-related genes, including Fe-SOD, POD, CAT and APX. Total RNA was extracted from leaf tissue samples using RNeasy Plant Mini kits (Qiagen, Manchester, UK). Contaminating DNA was then removed and first-strand cDNAs were prepared using Reverse Transcription kits (Qiagen, Manchester, UK). RT-qPCR analysis was conducted as reported in the protocol of QuantiTect SYBR Green PCR kit (Qiagen, Manchester, UK). Reaction volume and PCR amplification conditions were adjusted as mentioned by (El-Esawi et al., 2020)

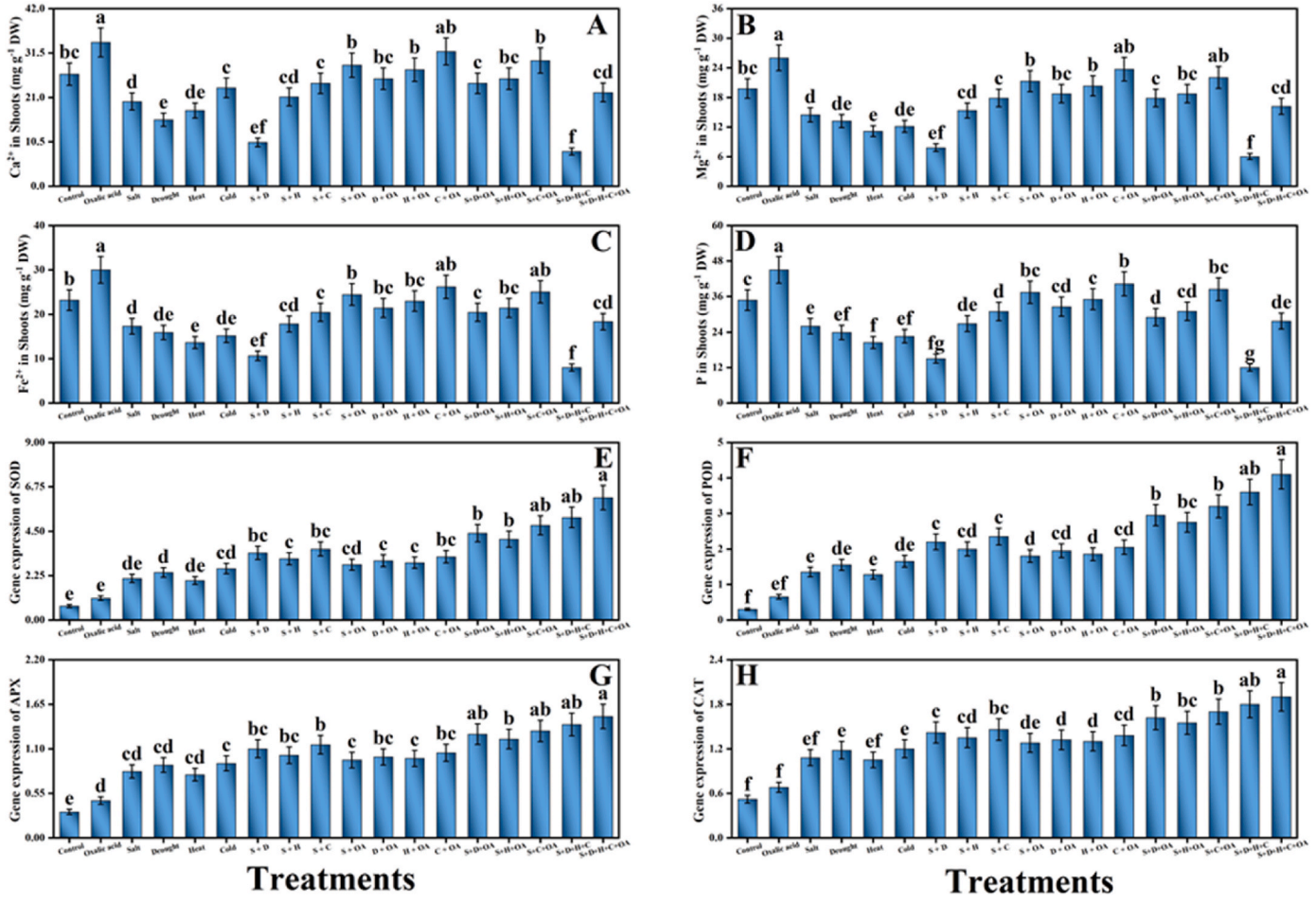

Fig. 5. Effect of oxalic acid (OA) on mineral nutrient contents and antioxidant-related gene expression in *Triticum aestivum* L. under different abiotic stresses including salinity (S), drought (D), heat (H), and cold (C) stress. (A) Calcium (Ca^{2+}) content, (B) Magnesium (Mg^{2+}) content, (C) Iron (Fe^{2+}) content, (D) Phosphorus (P) content, (E) Relative expression of SOD gene, (F) Relative expression of POD gene, (G) Relative expression of APX gene, and (H) Relative expression of CAT gene. Data are presented as mean ± standard deviation (SD) of five replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P < 0.05$ according to Tukey's honestly significant difference (HSD) test. Control = untreated plants; OA = oxalic acid.

2.7. Determination of non-enzymatic antioxidants

Plant ethanol extracts were prepared for the determination of nonenzymatic antioxidants and some key osmolytes. For this purpose, 50 mg of dry plant material was homogenized with 10 mL ethanol (80 %) and filtered through Whatman No. 41 filter paper. The residue was reextracted with ethanol, and the 2 extracts were pooled together to a final volume of 20 mL. The determination of flavonoids (Pękal and Pyrzynska, 2014), phenolics (Bray and Thorpe, 1954), anthocyanin (Lewis et al., 1998), and total sugars (Dubois et al., 1956) was performed from the extracts.

2.8. Mineral nutrient analysis

Essential nutrients such as calcium (Ca^{2+})' magnesium (Mg^{2+}), iron (Fe^{2+}), and phosphorus (P) analysis, plant shoots were washed twice in redistilled water, dipped in 20 mM EDTA for 3 s and then, again washed with deionized water twice for the removal of adsorbed metal on the plant surface. The washed samples were then oven dried for 24 h at 105 °C. The dried roots and shoots were digested using the wet digestion method in HNO_3: $HClO_4$ (7:3 V/V) until clear samples were obtained. Each sample was filtered and diluted with redistilled water up to 50 mL. The shoot contents of Ca^{2+}, Mg^{2+}, Fe^{2+}, and P were analyzed using the Atomic Absorption Spectrophotometer (AAS) model Agilent 240FS-AA.

2.9. Assessment of proline content

To measure proline concentrations, 0.5 g of shoot tissues were ground in sulfosalicylic acid and then centrifuged, and the supernatant was collected from each sample. The proline concentration in each sample was measured (Bates et al., 1973). Specifically, the supernatant from each sample was reacted with acid ninhydrin, and the resulting colorimetric reaction was measured to determine the proline concentration by "UV-1700 pharmaSpec spectrophotometer". The ProDH "proline dehydrogenase", P5CR "pyrroline-5-carboxylate reductase", and P5C "pyrroline-5-carboxylate" were measured using kits provided by Jiangsu Meibiao Biological Technology Co., Ltd. Enzyme activities were accurately measured using these reagent kits, which include all chemicals and related instructions by "UV-1700 pharmaSpec spectrophotometer".

2.10. Determination of ASA-GSH cycle and cell wall remodeling component

Glutathione (GSH), glutathione disulfide (GSSH), DHA (dehydroascorbic acid), and ascorbic acid (AsA) were determined in fresh leaves (Hodges et al., 2001) and were extracted by homogenizing 0.2 g of leaves in TCA and then collecting the supernatant by centrifugation. GSH concentration was measured in a solution including phosphate buffer, supernatant, and DTNB reagent (PBS, pH 7.0). The amount of

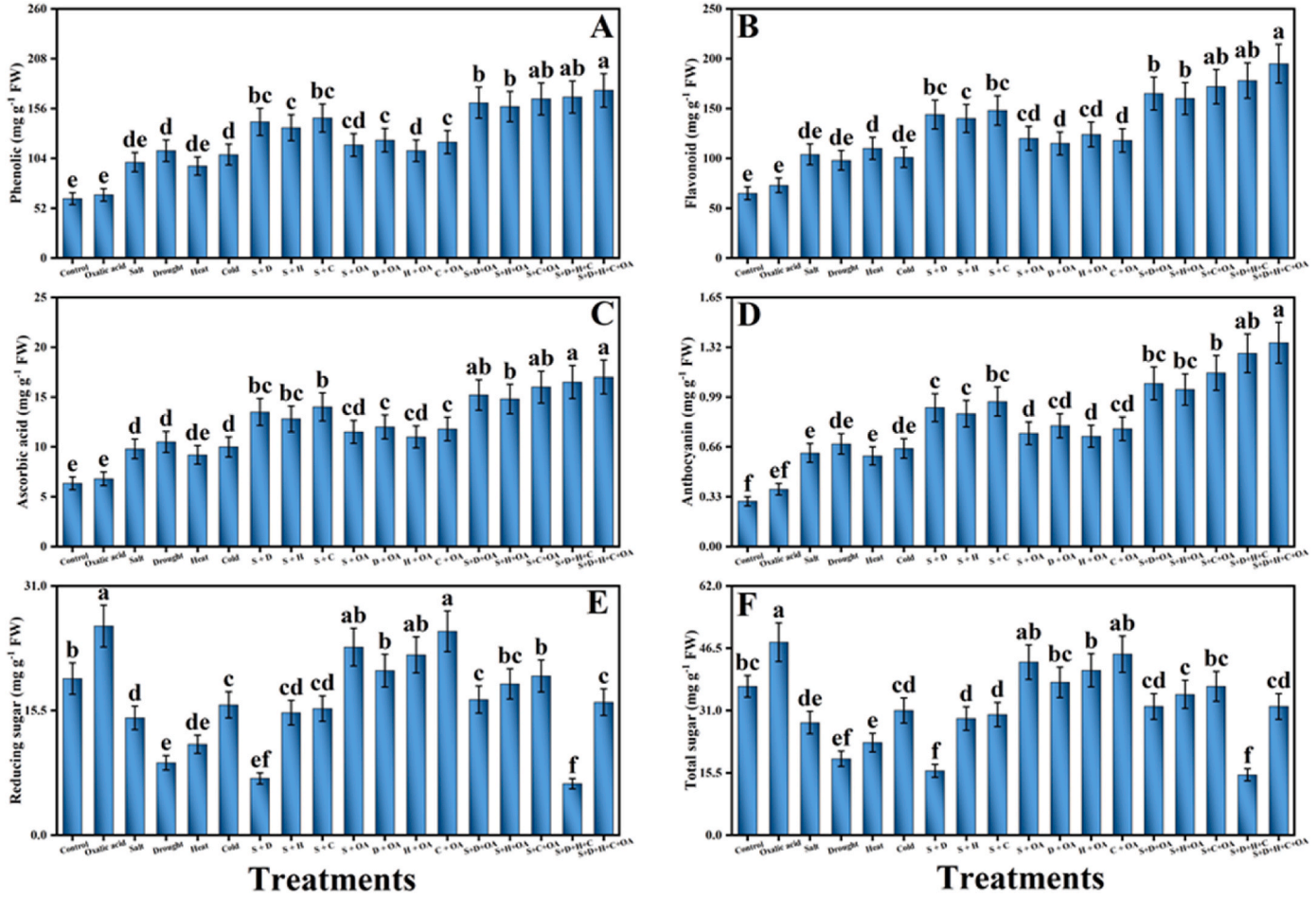

Fig. 6. Effect of oxalic acid (OA) on non-enzymatic antioxidants and sugar contents in *Triticum aestivum* L. under different abiotic stresses including salinity (S), drought (D), heat (H), and cold (C) stress. (A) Phenolic content, (B) Flavonoid content, (C) Ascorbic acid content, (D) Anthocyanin content, (E) Reducing sugar content, and (F) Total sugar content. Data are presented as mean ± standard deviation (SD) of five replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P < 0.05$ according to Tukey's honestly significant difference (HSD) test. Control = untreated plants; OA = oxalic acid.

GSH was determined by a spectrophotometer. To measure the AsA content, NaH_2PO_4 solution, enzyme extract, distilled water, and 10 % TCA were mixed to determine the concentration of AsA in the samples. After a 30-s incubation period, $FeCl_3$ solution, H_3PO_4, and 2,2′ -dipyridine were added to the reaction mixture. The $FeCl_3$ and 2,2′ -dipyridine reacted with the AsA to produce a red-colored complex that can be measured spectrophotometrically at 525 nm. The amount of AsA present in the sample was calculated.

Cell wall isolation was done as reported by (Yang et al., 2011). Shoots (4 g) were placed in a mortar and ground with liquid nitrogen. The homogenized samples were transferred to centrifuge tubes and 75 % ethanol was added and incubated at 25 °C. The samples were centrifuged. The bottom sediment was further homogenized in 10 mL of each of acetone, chloroform, and methanol (v:v = 1:1) for 30 min each, with shaking at room temperature. The homogenate was centrifuged. The remaining cell wall components were lyophilized until dry sediment was obtained. The lyophilized cell wall components were analyzed for biochemical assays. Subsequently, the separation of the hemicellulose fraction was carried out. Approximately 3 mg CW was mixed with water in an Eppendorf tube. The mixture was boiled for 1 h using a heating block or hot plate set at 100 °C and centrifuged. The above procedure was repeated for duplicate samples. After 12 h, the precipitate was extracted twice with 1 mL of KOH (24 %, w/v) at room temperature. After each extraction, centrifugation was done. The hemicellulose concentration was measured at 540 nm absorbance. Pectin Assay Kit was used to detect pectin. Pectinesterase Assay Kit was used to detect PME activity. The Cellulose Assay Kit was used to detect cellulose concentrations using kits provided by Jiangsu Meibiao Biological Technology Co., Ltd. Enzyme activities were accurately measured using these reagent kits, which include all chemicals and related instructions. DM was calculated using the formula: demethylation degree = 100 - DM, where DM is the degree of methylation.

2.11. Rhizosphere microbiome, health risk assessment, and molecular analysis

Rhizosphere soil samples were collected at the harvesting stage by carefully uprooting *T. aestivum* plants from each treatment. Loosely attached soil particles were gently removed by shaking, while the soil tightly adhering to the root surface was considered rhizosphere soil. Soil samples were collected from a depth of 0–15 cm, and five biological replicates were obtained for each treatment. Each biological replicate was further analyzed using three technical replicates for microbial and molecular analyses. Total genomic DNA was isolated from rhizosphere soil samples using a commercial soil DNA isolation kit according to the manufacturer's instructions. The V3–V4 hypervariable regions of bacterial 16S rRNA genes were amplified using region-specific primers and subjected to high-throughput sequencing using the Illumina MiSeq platform to determine bacterial community richness, diversity, and evenness following the general methodology of (Henckel et al., 1999). For health risk assessment, the Health Risk Index (HRI), Daily Intake of Metals (DIM), and Bioaccumulation Factor (BAF) were calculated using

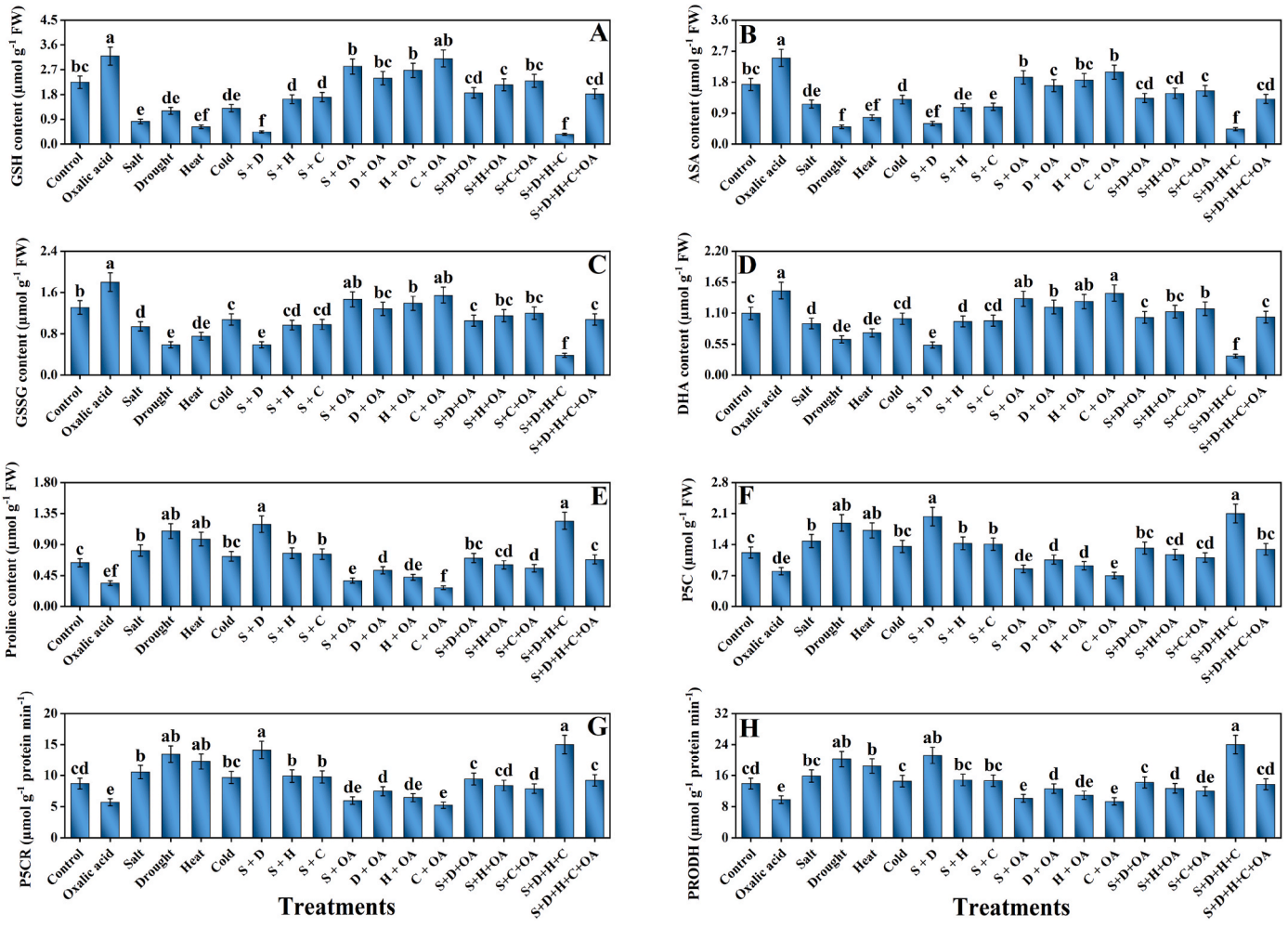

Fig. 7. Effect of oxalic acid (OA) on glutathione–ascorbate cycle components and proline metabolism in *Triticum aestivum* L. under different abiotic stresses including salinity (S), drought (D), heat (H), and cold (C) stress. (A) Glutathione (GSH) content, (B) Ascorbic acid (AsA) content, (C) Glutathione disulfide (GSSG) content, (D) Dehydroascorbic acid (DHA) content, (E) Proline content, (F) Pyrroline-5-carboxylate (P5C) content, (G) Pyrroline-5-carboxylate reductase (P5CR) activity, and (H) Pyrroline-5-carboxylate dehydrogenase (PRODH) activity. Data are presented as mean ± standard deviation (SD) of five replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P < 0.05$ according to Tukey's honestly significant difference (HSD) test. Control = untreated plants; OA = oxalic acid.

established equations based on the measured elemental concentrations in shoot tissues, average dietary intake, and reference body weight. Elemental concentrations were quantified from acid-digested shoot samples using atomic absorption spectrophotometry (AAS) following the method described by (Sanaei et al., 2021). For proteomic analysis, total proteins were extracted from frozen *T. aestivum* leaf tissues using the phenol extraction method and quantified using the Bradford assay. Protein profiling was performed through SDS-PAGE, and selected protein bands were analyzed using mass spectrometry to identify stress-associated proteins according to the method of (Bhushan et al., 2007). Total RNA was isolated from *T. aestivum* leaf tissues using TRIzol reagent, and RNA purity and integrity were evaluated using a NanoDrop spectrophotometer. Quantitative real-time PCR (qRT-PCR) was performed to determine the expression levels of differentially expressed genes (DEGs) and stress-responsive genes following the protocol of (Sun et al., 2019).

2.12. Statistical analysis

All data were analyzed using Statistix 8.1 software. Normality of the data was assessed prior to analysis. The experiment was conducted in a completely randomized design (CRD) with five biological replicates per treatment. Data were subjected to two-way analysis of variance (ANOVA) to evaluate the effects of abiotic stresses (drought, salinity, heat, and cold) and OA treatment, as well as their interaction. Mean comparisons were performed using Tukey's honestly significant difference (HSD) test at $P < 0.05$. Graphical representations were prepared using Origin software (OriginLab, USA).

3. Results

3.1. Plant morphological photosynthetic, and gas exchange attributes

In the present study, various growth parameters, photosynthetic pigments, and gas exchange attributes in wheat (*Triticum aestivum* L.) under different abiotic stresses with the application of oxalic acid (OA) were measured. Growth and biomass of *T. aestivum* are presented in Fig. 2, while gas exchange attributes are presented in Fig. 3. According to the results, abiotic stresses caused significant toxicity in *T. aestivum* and decreased root length, root fresh weight, shoot length, shoot fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoids, net photosynthesis, stomatal conductance, transpiration rate, and intercellular CO_2 concentration. However, the application of OA significantly increased these parameters compared to untreated plants under both abiotic-stressed and non-stressed conditions.

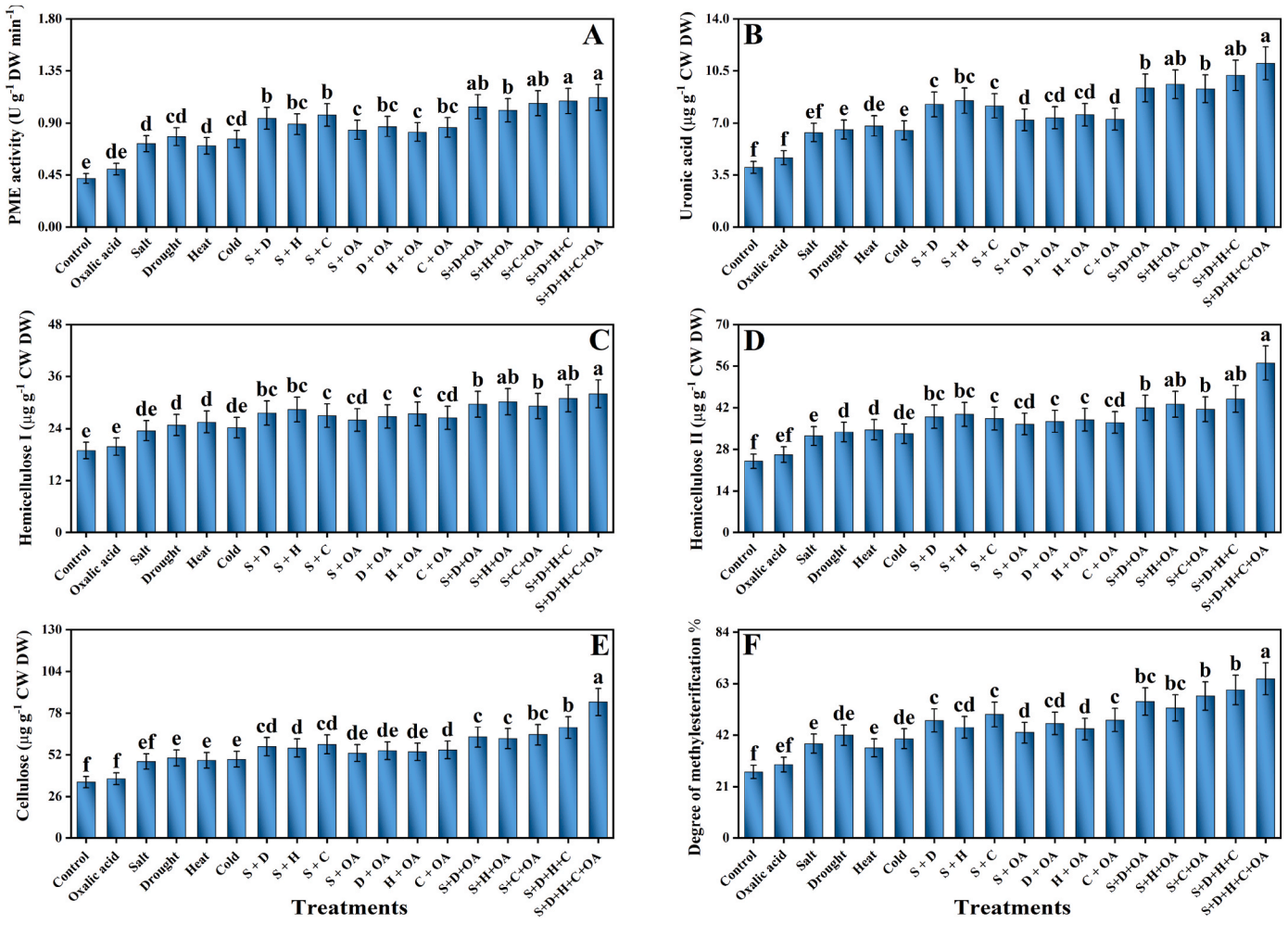

Fig. 8. Effect of oxalic acid (OA) on cell wall remodeling parameters in *Triticum aestivum* L. under different abiotic stresses including salinity (S), drought (D), heat (H), and cold (C) stress. (A) Pectin methylesterase (PME) activity, (B) Uronic acid content, (C) Hemicellulose I content, (D) Hemicellulose II content, (E) Cellulose content, and (F) Degree of methylesterification. Data are presented as mean ± standard deviation (SD) of five replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P < 0.05$ according to Tukey's honestly significant difference (HSD) test. Control = untreated plants; OA = oxalic acid.

3.2. Antioxidant defense system, stress biomarkers, and gene expression analysis

In the present study, oxidative stress biomarkers, i.e., malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), were measured from the leaves of *T. aestivum* as presented in Fig. (4E-F). According to the results, Abiotic stresses caused a significant increase in MDA and H_2O_2 contents compared to the control. However, the application of OA decreased these contents in the leaves of *T. aestivum*. Enzymatic antioxidants, i.e., superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), and catalase (CAT), as well as non-enzymatic compounds, i.e., phenolics, anthocyanin, ascorbic acid, and flavonoids, along with their related gene expression (SOD, POD, CAT, and APX), were also measured from the leaves of *T. aestivum*. The results for enzymatic antioxidants are presented in Fig. 4, gene expression in Fig. 5, and non-enzymatic compounds in Fig. 6. According to the results, abiotic stresses significantly increased the activity of enzymatic antioxidants and their gene expression, as well as the accumulation of non-enzymatic compounds compared to the control. The application of OA further enhanced these parameters under both abiotic-stressed and non-stressed conditions.

3.3. Mineral nutrient contents, osmolyte accumulation, and Glutathione–Ascorbate cycle analysis

In the present study, total sugar and reducing sugar contents were determined in *T. aestivum* under different abiotic stresses and the results are presented in Fig. 6, whereas proline metabolism and the AsA–GSH cycle are illustrated in Fig. 7. The findings demonstrated that abiotic stress significantly reduced total and reducing sugar contents compared with the control treatment. However, the exogenous application of OA markedly enhanced these contents under both stressed and non-stressed conditions.

Components associated with proline metabolism, including proline, pyrroline-5-carboxylate, pyrroline-5-carboxylate reductase, and pyrroline-5-carboxylate dehydrogenase, were also analyzed in *T. aestivum* tissues (Fig. 7). The obtained results indicated that abiotic stress significantly decreased these parameters relative to the control. In contrast, OA supplementation significantly enhanced their accumulation under stress conditions. Meanwhile, under non-stressed conditions, a slight reduction was observed compared to the control treatment.

The components of the AsA–GSH cycle, including glutathione, ascorbate, glutathione disulfide, and dehydroascorbic acid, were also assessed (Fig. 7). Exposure to abiotic stress significantly decreased glutathione, ascorbate, and dehydroascorbic acid contents, whereas glutathione disulfide levels were significantly increased. Nevertheless,

Table 1
Effect of oxalic acid and abiotic stress on rhizosphere microbiome composition, health risk indicators, and molecular responses.

Treatment	Rhizosphere Microbiome Analysis - Microbial Diversity Index	Rhizosphere Microbiome Analysis - Richness	Rhizosphere Microbiome Analysis - Evenness	Health Risk Assessment - HRI	Health Risk Assessment - DIM	Health Risk Assessment - BAF	Proteomic Analysis - Stress Marker Fold Change	Transcriptomic Analysis - DEGs	Transcriptomic Analysis - Stress-Responsive Gene Expression
Control	4.43 ± 0.22 (e)	243.9 ± 12.20 (e)	0.63 ± 0.03 (a)	1.28 ± 0.06 (f)	0.034 ± 0.00 (a)	0.39 ± 0.02 (a)	1.16 ± 0.06 (d)	243 ± 12.15 (a)	4.06 ± 0.20 (b)
Oxalic acid	2.59 ± 0.13 (f)	212.3 ± 10.62 (f)	0.65 ± 0.03 (f)	0.52 ± 0.03 (f)	0.035 ± 0.00 (f)	0.49 ± 0.02 (c)	1.2 ± 0.06 (d)	139 ± 6.95 (a)	2.87 ± 0.14 (c)
Salt	3.52 ± 0.18 (f)	151.4 ± 7.57 (c)	0.86 ± 0.04 (f)	1.01 ± 0.05 (e)	0.027 ± 0.00 (c)	0.62 ± 0.03 (c)	4.35 ± 0.22 (c)	381 ± 19.05 (f)	4.29 ± 0.21 (b)
Drought	4.4 ± 0.22 (e)	188.1 ± 9.40 (c)	0.88 ± 0.04 (a)	0.63 ± 0.03 (f)	0.05 ± 0.00 (b)	1.07 ± 0.05 (a)	3.13 ± 0.16 (c)	496 ± 24.80 (e)	1.35 ± 0.07 (e)
Heat	3.55 ± 0.18 (a)	198.8 ± 9.94 (b)	0.73 ± 0.04 (f)	0.52 ± 0.03 (d)	0.008 ± 0.00 (c)	0.49 ± 0.02 (b)	1.19 ± 0.06 (e)	155 ± 7.75 (d)	1.22 ± 0.06 (e)
Cold	4.33 ± 0.22 (d)	155.0 ± 7.75 (b)	0.75 ± 0.04 (b)	0.67 ± 0.03 (c)	0.041 ± 0.00 (c)	0.46 ± 0.02 (f)	2.06 ± 0.10 (a)	488 ± 24.40 (c)	2.15 ± 0.11 (a)
S + D	3.15 ± 0.16 (d)	191.2 ± 9.56 (d)	0.7 ± 0.03 (c)	1.49 ± 0.07 (f)	0.041 ± 0.00 (e)	0.73 ± 0.04 (e)	2.98 ± 0.15 (d)	99 ± 4.95 (d)	2.71 ± 0.14 (e)
S + H	2.96 ± 0.15 (b)	158.0 ± 7.90 (a)	0.64 ± 0.03 (e)	1.24 ± 0.06 (a)	0.036 ± 0.00 (b)	1.12 ± 0.06 (a)	2.43 ± 0.12 (d)	409 ± 20.45 (b)	4.42 ± 0.22 (b)
S + C	3.38 ± 0.17 (a)	230.4 ± 11.52 (c)	0.81 ± 0.04 (c)	0.53 ± 0.03 (d)	0.043 ± 0.00 (d)	1.15 ± 0.06 (f)	4.93 ± 0.25 (e)	348 ± 17.40 (d)	4.44 ± 0.22 (b)
S + OA	4.4 ± 0.22 (b)	152.9 ± 7.65 (e)	0.76 ± 0.04 (a)	0.46 ± 0.02 (e)	0.019 ± 0.00 (e)	0.86 ± 0.04 (a)	2.21 ± 0.11 (f)	386 ± 19.30 (a)	2.4 ± 0.12 (a)
D + OA	3.2 ± 0.16 (f)	261.5 ± 13.08 (b)	0.78 ± 0.04 (c)	0.77 ± 0.04 (e)	0.045 ± 0.00 (c)	0.84 ± 0.04 (e)	2.98 ± 0.15 (a)	175 ± 8.75 (b)	1.7 ± 0.09 (f)
H + OA	3.92 ± 0.20 (a)	264.6 ± 13.23 (c)	0.65 ± 0.03 (d)	0.55 ± 0.03 (b)	0.038 ± 0.00 (d)	1.13 ± 0.06 (f)	1.47 ± 0.07 (e)	285 ± 14.25 (f)	2.76 ± 0.14 (f)
C + OA	3.99 ± 0.20 (a)	257.6 ± 12.88 (c)	0.64 ± 0.03 (a)	0.94 ± 0.05 (a)	0.047 ± 0.00 (b)	0.14 ± 0.01 (f)	3.0 ± 0.15 (f)	365 ± 18.25 (f)	4.06 ± 0.20 (a)
S + D + OA	2.69 ± 0.13 (e)	170.0 ± 8.50 (f)	0.88 ± 0.04 (e)	1.31 ± 0.07 (a)	0.027 ± 0.00 (d)	0.36 ± 0.02 (d)	4.01 ± 0.20 (f)	335 ± 16.75 (a)	2.88 ± 0.14 (a)
S + H + OA	3.56 ± 0.18 (b)	191.4 ± 9.57 (e)	0.62 ± 0.03 (b)	0.69 ± 0.03 (d)	0.014 ± 0.00 (b)	0.68 ± 0.03 (d)	3.85 ± 0.19 (c)	393 ± 19.65 (c)	3.52 ± 0.18 (a)
S + C + OA	3.11 ± 0.16 (f)	159.0 ± 7.95 (b)	0.81 ± 0.04 (c)	0.45 ± 0.02 (b)	0.026 ± 0.00 (e)	1.0 ± 0.05 (f)	1.9 ± 0.10 (b)	74 ± 3.70 (c)	3.32 ± 0.17 (e)
S + D + H + C	3.78 ± 0.19 (e)	170.8 ± 8.54 (e)	0.69 ± 0.03 (b)	1.42 ± 0.07 (d)	0.033 ± 0.00 (d)	0.11 ± 0.01 (d)	2.84 ± 0.14 (c)	231 ± 11.55 (c)	3.32 ± 0.17 (e)
S + D + H + C + OA	2.96 ± 0.15 (b)	259.6 ± 12.98 (b)	0.64 ± 0.03 (b)	0.96 ± 0.05 (c)	0.046 ± 0.00 (b)	0.42 ± 0.02 (e)	3.24 ± 0.16 (d)	461 ± 23.05 (c)	2.55 ± 0.13 (b)

OA treatment effectively ameliorated these adverse effects by enhancing glutathione, ascorbate, and dehydroascorbic acid contents while decreasing glutathione disulfide levels in OA-treated plants.

3.4. Cell wall remodeling and mineral nutrient contents

In this investigation, the contents of calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), and phosphorus (P) in *T. aestivum* under abiotic stresses were quantified and are presented in Fig. 5. The results indicated that abiotic toxicity significantly reduced Ca^{2+}, Mg^{2+}, Fe^{2+}, and P contents in shoots compared to the control. However, the application of OA enhanced these nutrient levels under both abiotic-stressed and non-stressed conditions.

Cellular compartment fractionation, including pectin methylesterase activity, uronic acid, hemicellulose I, hemicellulose II, and cellulose contents, was also evaluated and is presented in Fig. 8. The results showed that abiotic stresses significantly increased these parameters compared to the control. In contrast, plants grown without abiotic stress exhibited relatively lower values. However, the application of OA under abiotic stress further enhanced these cellular components.

3.5. Microbial diversity, health risk parameters, and omics-level stress responses in the rhizosphere

In the present study, microbial diversity indices, including diversity index, Chao1 richness, and evenness, along with health risk parameters such as health risk index (HRI), daily intake of metals (DIM), and bioaccumulation factor (BAF), were evaluated in *T. aestivum* grown under different abiotic stresses and are presented in Table 1. According to the obtained results, abiotic stress significantly reduced microbial attributes, including diversity index, Chao1 richness, and evenness, compared with the control treatment. In contrast, abiotic stress significantly increased health risk parameters, including HRI, DIM, and BAF, in plants grown under stress conditions. However, the application of OA significantly improved microbial diversity indices and reduced health risk parameters under abiotic stress conditions. Furthermore, the combined application of OA with abiotic stress effectively alleviated the adverse effects of abiotic toxicity in *T. aestivum* plants. Proteomic and transcriptomic responses were also analyzed under different abiotic stress conditions. Proteomic analysis revealed a significant increase in stress marker fold change under abiotic stress treatments compared with the control. Similarly, transcriptomic profiling demonstrated marked alterations in differentially expressed genes (DEGs) and stress-responsive gene expression under abiotic stress conditions. Nevertheless, OA application regulated proteomic and transcriptomic responses by reducing stress marker fold change and modulating stress-responsive gene expression, thereby enhancing stress tolerance in *T. aestivum* plants under abiotic stress conditions.

3.6. Pearson's correlation analysis

A Pearson's correlation was illustrated to depict the relationship among various morpho-physiological, biochemical, molecular, and rhizosphere parameters of *T. aestivum* grown under different abiotic

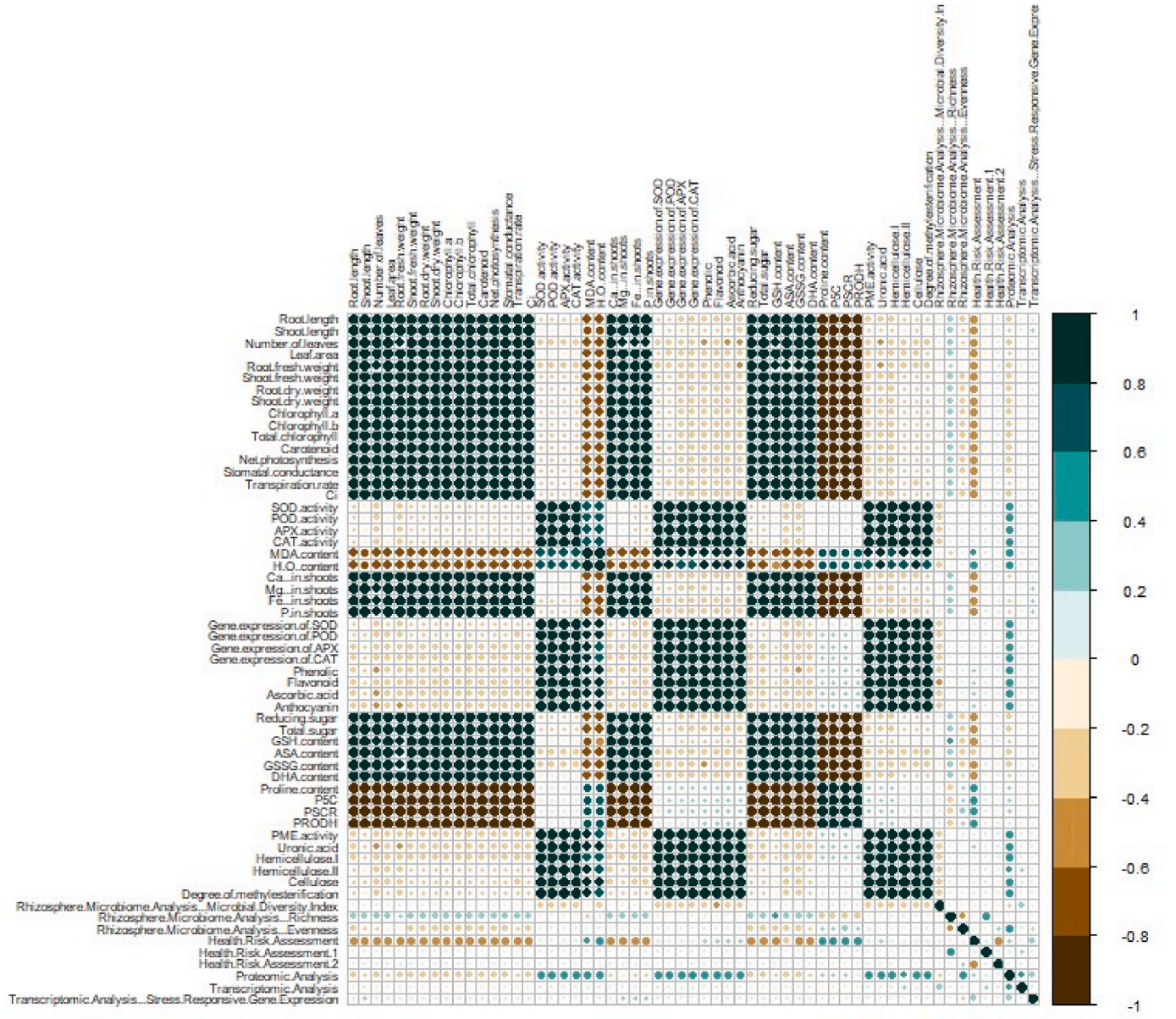

Fig. 9. Pearson correlation heatmap showing the interrelationship among agro-physiological, biochemical, gene expression, ionomic, and rhizosphere microbial traits. Strong positive correlations (green) were observed between growth, chlorophyll content, antioxidant enzyme activities, and osmolytes, while negative correlations (brown) were found between oxidative stress markers and physiological traits. Rhizosphere diversity and evenness showed significant associations with plant performance, while health risk indices negatively correlated with beneficial plant and microbial traits.

stresses with or without the application of OA (Fig. 9). H_2O_2, MDA, glutathione disulfide (GSSG), health risk index (HRI), daily intake of metals (DIM), bioaccumulation factor (BAF), pectin methylesterase (PME), uronic acid, stress marker fold change, pyrroline-5-carboxylate reductase (P5CR), and pyrroline-5-carboxylate dehydrogenase (PRODH) were positively correlated with each other, while negatively correlated with growth and physiological parameters including chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), total chlorophyll (TC), carotenoid content, root length (RL), shoot length (SL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), photosynthetic rate, stomatal conductance, transpiration rate, microbial diversity index, richness, and evenness. Similarly, antioxidant enzyme activities including SOD, POD, CAT, and APX along with their corresponding gene expression were positively correlated with growth, physiological, and rhizosphere-related parameters, indicating the beneficial role of OA in mitigating abiotic stress toxicity. This relationship demonstrated a close association among various growth, biochemical, molecular, and rhizosphere traits studied in the present experiment.

3.7. Principal Component Analysis (PCA)

The loading plots of principal component analysis (PCA) depicted a close relationship among various morpho-physiological, biochemical, molecular, and rhizosphere parameters in *T. aestivum* under different abiotic stresses and OA application, as presented in Fig. 10. Among the entire dataset, Dim1 and Dim2 exhibited the maximum contribution and collectively occupied more than 77% of the total database. Among these, Dim1 exhibited 57.3%, whereas Dim2 exhibited 20.6% contribution from the total dataset. All studied parameters were successfully distributed in the database, clearly indicating that abiotic stress significantly affected the growth and eco-physiology of *T. aestivum*. From the obtained results, it can be concluded that H_2O_2, MDA, glutathione disulfide (GSSG), proline, pyrroline-5-carboxylate reductase (P5CR),

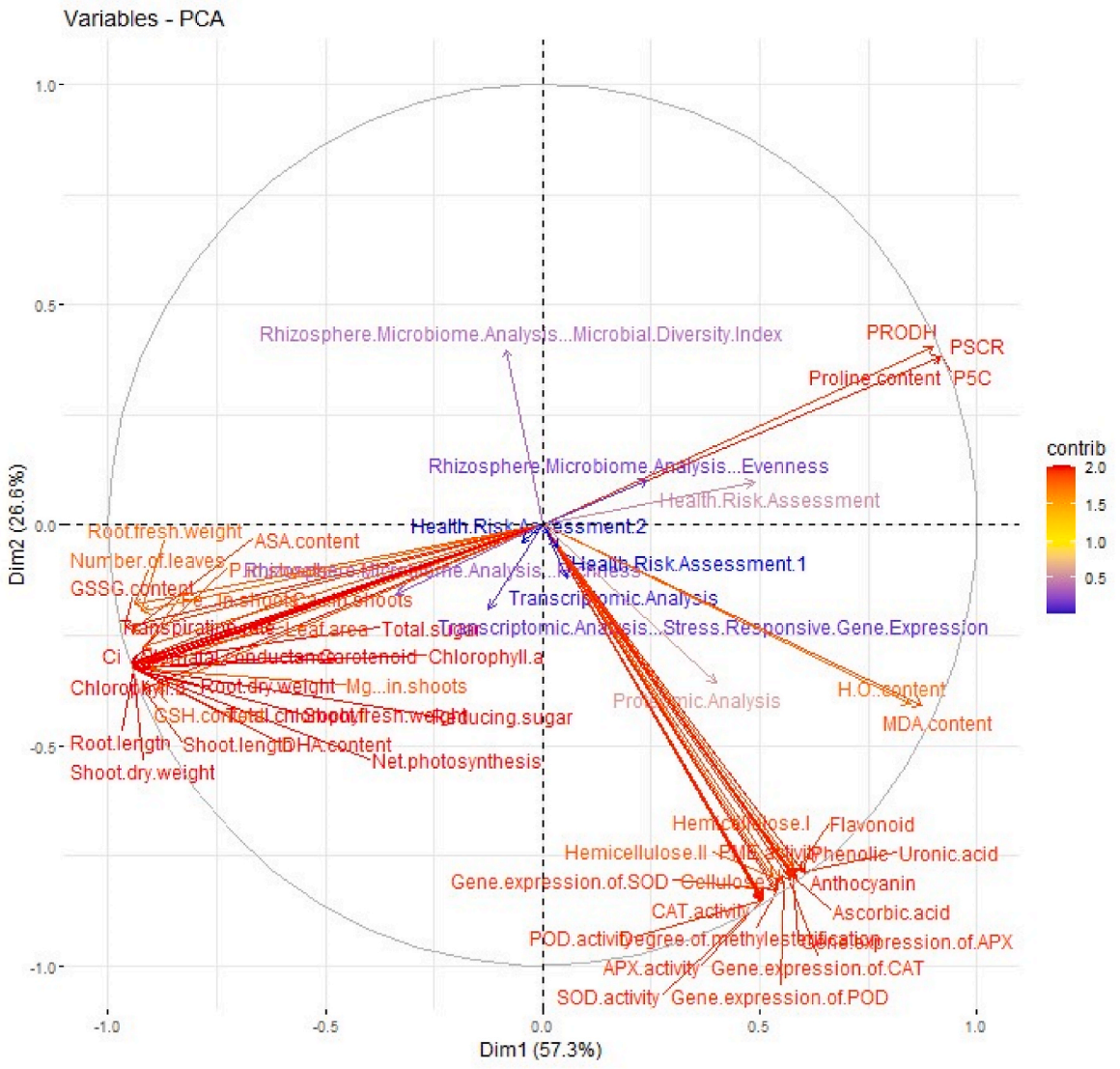

Fig. 10. Principal Component Analysis (PCA) biplot illustrating multivariate relationships among physiological, biochemical, molecular, and rhizospheric parameters in wheat under different abiotic stress treatments with or without oxalic acid (OA) application. Dim1 and Dim2 represent the first and second principal components explaining 57.3% and 20.6% of the total variance, respectively. The length and orientation of vectors indicate the contribution and correlation of variables. Strong clustering of growth, photosynthesis, and antioxidant traits with OA treatments highlights their coordinated enhancement under stress.

pyrroline-5-carboxylate dehydrogenase (PRODH), pectin methylesterase (PME), uronic acid, health risk index (HRI), daily intake of metals (DIM), bioaccumulation factor (BAF), and stress marker fold change were positively correlated in the database. Whereas root length (RL), shoot length (SL), chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), total chlorophyll (TC), carotenoid content, stomatal conductance, transpiration rate, photosynthetic rate, shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), antioxidant enzymes (SOD, POD, CAT, and APX), antioxidant-related gene expression, microbial diversity index, richness, and evenness were negatively correlated when compared with the stress-associated variables in the database.

4. Discussion

The application of oxalic acid (OA) exhibited a significant effect on mitigating the adverse impacts of different abiotic stresses, including salinity, drought, heat, and cold stress, in wheat (*Triticum aestivum* L.), as evidenced by the improvement in plant growth and biomass, photosynthetic pigments, and gas exchange parameters (Khan et al., 2023). The use of OA likely increased the ability of plants to maintain higher chlorophyll contents by preserving chloroplast membrane stability and protecting photosynthetic pigments from oxidative damage induced by abiotic stress conditions (Chen et al., 2024). The application of OA improved the net photosynthetic rate, stomatal conductance, and transpiration rate, which may be attributed to its role in enhancing carbon assimilation and maintaining stomatal regulation, thereby ensuring effective gaseous exchange under stress conditions (Khan et al., 2023). Oxalic acid, known as an important signaling and chelating compound, may have played a crucial role in alleviating abiotic stress toxicity by regulating ion homeostasis, improving nutrient availability, and reducing oxidative damage within plant tissues (Rao et al., 2025; Mumtaz et al., 2025). This protective role likely contributed to the observed reduction in oxidative stress indicators such as MDA and H_2O_2 contents, as OA enhanced ROS scavenging capacity, thereby protecting cellular components from oxidative injury (Li et al., 2022). Additionally, the increased activities of antioxidant enzymes, including SOD, POD, CAT, and APX under OA application, suggest that OA may have enhanced the expression of stress-responsive genes, thereby strengthening the antioxidant defense system against stress-induced oxidative

damage (Chen et al., 2024; Irkitbay et al., 2023).

The accumulation of reducing sugar, total sugar, phenolics, flavonoids, anthocyanins, ascorbic acid, and proline under OA application suggests enhanced carbon metabolism, osmotic adjustment, and activation of antioxidant defense pathways under abiotic stress conditions (Francini et al., 2019). Increased activities of proline metabolism-related enzymes, including P5CR and PRODH, along with enhanced GSH and AsA contents, indicate that OA maintained cellular redox homeostasis through regulation of the AsA–GSH cycle and antioxidant defense mechanisms (Ghosh et al., 2022). The reduction in oxidized antioxidant forms such as GSSG and DHA further suggests that OA maintained a more stable intracellular environment, thereby minimizing oxidative damage under stress conditions (Chauhan et al., 2022). Moreover, OA significantly improved calcium, magnesium, iron, and phosphorus contents, possibly by enhancing nutrient solubility, uptake, and ionic homeostasis in *T. aestivum* plants exposed to salinity, drought, heat, and cold stress (Panchal et al., 2021).

Oxalic acid influenced structural components of the cell wall, as evidenced by alterations in pectin methylesterase (PME) activity, hemicellulose and cellulose content, and the degree of methylesterification (DME). These changes are critical for mechanical stability and stress perception. Under stress, cell wall remodeling is an adaptive strategy that modulates porosity, ion exchange, and pathogen resistance (Tenhaken, 2015). Furthermore, OA significantly improved rhizosphere microbial diversity, richness, and evenness, while reducing health risk parameters including HRI, DIM, and BAF under abiotic stress conditions (Muhammad et al., 2024; Anyebe et al., 2025). Proteomic and transcriptomic analyses further confirmed the regulatory role of OA through enhanced stress-responsive gene expression and modulation of stress-related signaling pathways, indicating that OA acts as an important signaling and protective molecule involved in stress adaptation mechanisms (Singhal et al., 2021; Akbar et al., 2018). Although abiotic stresses frequently occur sequentially under natural field conditions, simultaneous stress combinations are also increasingly common under rapidly changing climatic environments, particularly in arid and semi-arid agricultural regions. Therefore, the present experimental design was intended to evaluate the integrated physiological, biochemical, molecular, and rhizosphere responses of *T. aestivum* under combined stress conditions, which may mimic acute environmental fluctuations occurring in agricultural systems. Collectively, these findings suggest that OA effectively mitigates abiotic stress toxicity by regulating physiological, biochemical, molecular, and rhizosphere-related processes, highlighting its potential as an eco-friendly biostimulant for sustainable agriculture under adverse environmental conditions.

5. Conclusion

The outcomes of the present study revealed that abiotic stresses, including drought, salinity, heat, and cold stress, significantly impaired plant growth, gas exchange attributes, sugar metabolism, the AsA–GSH cycle, cellular fractionation, proline metabolism, rhizosphere microbial diversity, richness, evenness, and mineral nutrient uptake in wheat (*Triticum aestivum* L.) seedlings. Furthermore, abiotic stresses enhanced oxidative stress indicators, health risk indices, and bioaccumulation factors. However, the application of oxalic acid (OA), an important organic acid and plant biostimulant, significantly alleviated abiotic stress toxicity by lowering reactive oxygen species (ROS) levels and enhancing antioxidant activities, microbial diversity, and essential nutrient uptake. This improvement was closely associated with the regulation of the AsA–GSH cycle, enhanced proline metabolism, improved cellular compartmentalization, and modulation of stress-responsive signaling pathways, which collectively contributed to maintaining redox homeostasis. Overall, the findings suggest that OA plays a crucial role in improving plant tolerance against abiotic stresses. However, the present study was conducted under controlled pot conditions, which may not fully represent complex field environments. Therefore, caution should be taken when extrapolating these results to large-scale agricultural systems. Further long-term field-based studies are required to validate the effectiveness of OA under natural conditions and to better understand its interactions with soil dynamics, rhizosphere microbial communities, and plant systems.

Consent to publish

Written consent was sought from each author to publish the manuscript.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Author contribution

Seham Sater Alhelaify: Conceptualization, supervision, methodology design, and critical revision of the manuscript. Rana M. Alshegaihi: Data collection, formal analysis, and validation of experimental results. Eman Fayad: Laboratory experiments, investigation, and preparation of initial draft. Dalal Nasser Binjawhar: Statistical analysis, data interpretation, and contribution to the results section. Mohammed O. Alshaharni: Resources, technical support, and assistance in experimental setup. Mohammed Alqurashi: Literature review, preparation of figures and tables, and manuscript editing. Ohud Muslat Alharthy: Data curation, writing—review & editing, and project administration. Willie Peijnenburg: Corresponding author, overall study design, funding acquisition, manuscript writing, revision, and final approval for submission.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

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

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