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India

*CORRESPONDENCE

Shashi Bhushan Agrawal
✉ sbagrawal56@gmail.com
Krishna Kumar Choudhary
✉ kkc@bhu.ac.in

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Biochar modulates morphophysiological traits and yield stability in *Phaseolus vulgaris* L. via biochemical adjustments under tropospheric Ozone stress

Vineet Upadhyay¹, Krishna Kumar Choudhary^{1*},
Madhoolika Agrawal² and Shashi Bhushan Agrawal^{2*}

¹Department of Botany, MMV, Banaras Hindu University, Varanasi, India, ²Laboratory of Air Pollution and Global Climate Change, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

Introduction: Tropospheric ozone (O₃) is a major abiotic environmental stressor in the Indo-Gangetic plains of India due to more conducive conditions for O₃ formation, such as emissions of large amounts of O₃ precursors (VOCs, NOx), prolonged sunshine hours, and high temperatures, causing significant losses in crop productivity. Among various mitigation approaches, biochar has emerged as a viable and economical soil amendment with multifaceted benefits in improving plant resilience against abiotic stressors.

Methods: This field study investigates the efficacy of biochar amendment in mitigating O₃-induced phytotoxicity in two popularly grown cultivars (HUR-137 and PDR-14) of Kidney beans (*Phaseolus vulgaris* L.). Biochar was applied at 2.5% and 5% (w/w) under ambient and elevated O₃ exposures. Key markers of plant growth, development, biochemical and yield were assessed, supported by histochemical and chlorophyll fluorescence parameters to determine physiological and antioxidative responses.

Results and discussion: Biochar fertilizations (2.5 and 5% w/w) significantly ameliorated O₃-induced growth and total biomass reductions. Improvements in floral metrics were noted in biochar-treated test plants. Histochemical analyses revealed reduced oxidative damage, followed by enhanced stimulation of antioxidative defense observed in biochar amendments as reflected by biochemical assays. Plant pigments and chlorophyll fluorescence parameters reflected improvements in biochar treatments. Biochar supplementation protected yield traits under O₃ stress, improving pod formation, seed filling, and productivity in both the test cultivars. Under O₃ stress, unamended plants of PDR-14 (EOB0) displayed more pronounced results with 44.7% reduction in GWP (17.46 g plant⁻¹, 95% Confidence Interval, 15.32–19.60), relative to ambient O₃ conditions (31.58 g plant⁻¹, 95% CI, 27.78–35.38). The application of 5% biochar (EOB5) ameliorated O₃-induced yield reduction (23.06 g plant⁻¹, 95% CI, 20.60–25.53), depicting 32.1% improvements compared to EOB0. Overall findings suggest that biochar application is a potential agronomic strategy to maintain productivity in *Phaseolus vulgaris* (L.) cultivars in areas experiencing high concentrations of O₃.

KEYWORDS

agroecosystem, biochar, *Phaseolus vulgaris* L., tropospheric O₃, yield optimization

1 Introduction

Ozone (O₃) is a potent phytotoxic gas that devastatingly impacts plant growth, physiological functions, and crop productivity (Gupta et al., 2023). With hotspots in rapidly industrializing parts of Asia, Latin America, and Africa, global background O₃ concentrations have surged by 20–50% since the pre-industrial era (Sicard et al., 2021). Climate change outcomes intensify O₃ formation, as higher temperatures speed up photochemical reactions, while altered precipitation patterns prolong O₃ persistence in the atmosphere (Upadhyay et al., 2024). Ozone levels are noted to show an increase of 1.8 ± 1.3 ppb per decade with further projections to increase of 10–20% by 2050 due to current emission patterns (Christiansen et al., 2022; Ghosh et al., 2021; Solomon, 2007), gravely jeopardizing the sustainability of agriculture. Damages chloroplast ultrastructure and blocks photosynthetic electron transport, increases chlorosis, disrupts plant nutrient uptake, translocation of assimilates and respiration. ROS (Reactive Oxygen Species) generated in plants due to O₃ causes oxidative damage to chloroplast ultrastructure and thus inhibiting photosynthetic electron transport. This oxidative stress further induces chlorosis, impairs nutrient uptake, disrupts assimilate translocation, and perturbs cellular respiration, collectively compromising plant growth and productivity (Nowroz et al. 2024; Ramya et al., 2023). Sensitive crop species exhibit cellular damage, impaired photosynthesis, accelerated senescence, and reduced yield due to oxidative damage generated by O₃ (Feng et al., 2022). On average, O₃ pollution lowers staple crop yields by 5–15% worldwide, with losses of over 30% in high-O₃ places (Feng et al., 2022). O₃-induced yield losses for *Phaseolus vulgaris* L. (Kidney beans), a vital source of protein worldwide (Kimothi and Dhaliwal, 2023), threaten nutritional security, especially in low-income nations where beans constitute a dietary staple (Caregnato et al., 2013). An estimated \$14–26 billion is lost economically each year as a result of O₃ damage to agriculture (Qasim et al., 2024). These losses worsen rural poverty and food insecurity in impoverished countries when farmers face the unavailability of chemical protectants or cultivars resistant to O₃ (Qasim et al., 2024).

Scientific studies into antiozonants and mitigation of O₃ damage have led to several agronomic tools, including chemical protectants, agronomic adjustments (altered planting dates & irrigation), and breeding O₃-tolerant crop varieties (Poornima et al., 2023). However, several constraints, such as low scalability, sporadic application, minimal nutrient enrichment, and large inputs, cause limitations on their applicability as a sustainable agronomic tool. Biochar is a C-rich amorphous product of the thermal transformation of biomass in an oxygen-limited environment (Upadhyay et al., 2024). Its application in agriculture for yield optimization has historical roots, dating back to pre-Columbian Amazonian civilizations (Bezerra et al., 2019). These engineered soils were rich in biochar, organic waste, and pottery fragments and displayed remarkable fertility and resilience toward drought and nutrient leaching, two major abiotic stressors in tropical ecosystems (Bhattacharyya et al., 2024). Similar methods have been documented in traditional Chinese agriculture and Japan, where these methods were employed to improve soil fertility and nutrient content (Chen et al., 2019). These early methods, which shielded crops from salt, metal toxicity, and moisture fluctuations, demonstrate an intuitive understanding of the relationships between soil, plants, and the environment. Systematic investigation into biochar's potential was

initiated by the scientific community in the 20th century, initially through studies on soil fertility and carbon sequestration. With the growing recognition of climate change impacts, researchers have increasingly focused on biochar's role in mitigating abiotic stresses, framing it within relevant challenges such as soil salinization, drought stress, heavy metal contamination, and ground-level O₃ pollution.

Biochar's high porosity, water, and nutrient retention properties help sustain crop resilience against abiotic stressors. Biochar improves nutrient retention in the soil via high cation exchange capacity (CEC) and functional groups capable of binding essential nutrients (Upadhyay et al., 2024). Better nutrient availability supports metabolic repair and maintenance under oxidative stress (Ghosh et al., 2021). Mainly applied as a soil conditioner, biochar has been reported to boost the antioxidative defense of crop plants against drought, salinity, and O₃ (Rathinapriya et al., 2025). Ghosh et al. (2021) reported that with 5% biochar amendment, a decrease in MDA content by 24.4% and boost in total chlorophyll content by 22%, increased activities of antioxidative enzymes and maintained the yield significantly, relative to unamended O₃-stressed wheat cultivar HD 2967. Under salinity stress, biochar fertilization was noted to improve leaf area (11%), root length (11.1%) and total chlorophyll content (20%) in wheat under 2% biochar amendment (Kanwal et al., 2018). A boost in antioxidative enzyme production and nutrient bioavailability has been linked to augmentation of redox homeostasis in plants (Rathinapriya et al., 2025). In addition, biochar has also been reported to modulate microbial activity and population, leading to enhanced rhizosphere health and plant resilience against abiotic stress (Bolan et al., 2024). Increased activities of microbial populations were noted to improve soil water holding capacity, Nitrogen (N) use efficiency and reduce oxidative stress in leaves of cotton plant (Ren et al., 2025). High CEC and nutrient content of biochar allow greater phyto-availability of nutrients to the crop plants, augmenting growth and productivity (Upadhyay et al., 2024). While studies have noted the efficacy of biochar fertilization against O₃ in wheat and mungbean (Ghosh et al., 2021; Upadhyay et al., 2025), studies into the impacts on *Phaseolus vulgaris* remain unexplored. In view of continuous rising tropospheric O₃ concentrations, the scarcity of field data in developing countries creates substantial uncertainty in estimating economic yield losses (Ramya et al., 2023). Therefore, assessment of O₃ sensitivity of site-specific cultivars is paramount under the current conditions.

This study ventures into finding biochemical applicability of biochar as a soil amendment against O₃ stress and differential sensitivity of two commonly grown kidney beans (*Phaseolus vulgaris* L.) cultivars. The objective of present study is to investigate the possible modulatory role of biochar amendments under field conditions on popularly grown *Phaseolus vulgaris* (L.) cultivars under tropospheric O₃ stress through integrative assessment of morpho-physiological responses, biomass allocation, redox homeostasis and yield. The study hypothesizes that biochar amendment to soil will result in maintenance of yield and nitrogen metabolism in both cultivars via induction of antioxidative defense, lowering O₃-generated injuries, and maintenance of redox homeostasis. Moreover, it is posited that biochar supplementation will favorably modulate biomass partitioning, promoting sustained growth and reproductive yield. Biochar is also predicted to benefit more for O₃-sensitive cultivar, thus reducing the performance gap between sensitive and tolerant cultivars, supporting targeted use in O₃-polluted regions.

2 Materials and methodology

2.1 Experimental site, setup, plant materials, and treatments

The experimental site was established within the botanical garden of the Department of Botany, Banaras Hindu University, Varanasi (25.267°N and 82.99°E), situated in the Gangetic plains, India. A field experiment was conducted over 4 months from November 2024 to February 2025. The experimental site featured sandy clay loam soil with a texture composition of 45% sand, 28% silt, and 27% clay, exhibiting a close to neutral pH (7.2). For the field study, two widely cultivated kidney bean cultivars from northern India, HUR-137 and PDR-14 (Uday), were selected. Both the cultivars were sown on the first week of November, 2024 and harvested at 110–120 days after germination (DAG) on the last week of February 2025. HUR-137 was red-seeded while PDR-14 had red variegated seeds. Seeds of HUR-137 were procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University (BHU). The study utilized open-top chambers (OTCs) following the design specifications of Bell and Ashmore (1986). Each OTC measured 1.5 × 1.8 m (diameter × height) and was lined with a 0.25-mm-thick polyethylene sheet. The chambers maintained near-natural environmental conditions, with 95% ambient light transmittance, a warmer temperature by 0.1–0.3 °C, and a relative humidity elevation of 2–4%, based on standardized performance reported for these open-top chambers in previous studies (Mishra et al., 2013; Mishra and Agrawal, 2015).

The experiment comprised six treatments for each cultivar: ambient O₃ without biochar (AO), ambient O₃ with 2.5% biochar (AB2.5), ambient O₃ with 5% biochar (AB5), elevated O₃ without biochar (EOB0), elevated O₃ with 2.5% biochar (EOB2.5) and elevated O₃ with 5% biochar (EOB5). Each OTC was treated as one biological replicate, and three OTC were allocated for each treatment per cultivar, resulting in 36 chambers overall for all treatments (Supplementary Figure 1). At the experimental location, ozone generators (Paraday, India) coupled with high-speed air blowers were used to deliver elevated O₃ treatments (EOB0, EOB2.5, and EOB5). The ozone generators guaranteed a homogeneous O₃-air mixture and uniform distribution within the OTCs. The blowers operated at a flow rate of three air changes per minute. To simulate projected tropospheric ozone levels, which are anticipated to increase by 20–25% by 2050, on the basis of SRES A2 scenario (Solomon, 2007), an elevated O₃ concentration of ambient + 20 ppb was maintained daily for 4 h (11:00–15:00) from germination through seed maturity.

Biochar used in this study was procured from Greenfields Eco Solutions Pvt. Ltd. (Rajasthan, India) and produced from *Prosopis juliflora* wood biomass under pyrolytic conditions of 400–500 °C. The biochar had an average pore diameter of 2.1 nm, specific surface area of 211.5 m² g⁻¹, moisture content of 1.5–2.2%, ash content of 1.4–1.9% (w/w), volatile matter of 38–45 g kg⁻¹, and fixed carbon (residual matter) of 31–36 g kg⁻¹. The measured pH, electrical conductivity (EC), and CEC were 7.9–8.2, 1.4–1.5 dS m⁻¹, and 16–18 cmol kg⁻¹, respectively. Elemental composition included organic carbon (715–725 g kg⁻¹), total nitrogen (1.6–1.9 g kg⁻¹), C: N ratio (382–446), total phosphorus (1.9–2.1 g kg⁻¹), total potassium (24–26 g kg⁻¹), calcium (11–13 g kg⁻¹), and magnesium (0.45–0.51 g kg⁻¹), with a calorific value of 7.8–7.9 kcal g⁻¹. Biochar was administered on a weight-to-weight (w/w) basis at concentrations of

2.5 and 5% for the respective treatments. Biochar concentrations were based on our earlier research (Upadhyay et al., 2025) as well as previous studies (Ghosh et al., 2021) which established that the benefits of biochar application for yield protection saturated at 5% w/w. To calculate appropriate biochar quantities, soils in the OTCs were excavated up to a depth of 25 cm before amendment. Afterwards, the amended soil was reintroduced into the OTCs and prepared for sowing. Biochar was incorporated into the soil 10 days before sowing to facilitate proper soil conditioning. For sowing seeds, each OTC had four rows that were uniformly spaced. Post one week of germination, plants were thinned to facilitate a 15 cm gap between adjacent plants. Manual weeding was performed weekly to minimize competition between plants and harboring of pests and diseases. Plants were irrigated with equal volumes of water at fixed intervals to ensure optimum soil moisture. As kidney bean has higher requirements of N and P than other legumes, the recommended dosage of N at 120 kg/ha, P at 60 kg/ha, and K at 60 kg/ha as per the recommendation of Directorate of Pulses Development, Bhopal, India, during the preparation of the field. A basal dressing provided whole doses of P, K, along with half the dosage of N, while the remaining half dosage of N was supplied as top dressing.

2.2 Plant sampling

Three randomly selected plants were taken from each chamber for all assessments. Floral parameters were analyzed at reproductive DAG (80 DAG), while morphological, growth, and biochemical parameters were assessed at both vegetative (40 DAG) and reproductive (80 DAG) stages. Physiological parameters of test cultivars, including minimal fluorescence (F_0), variable fluorescence (F_v), maximal fluorescence (F_m), and the quantum yield of photosystem II (F_v/F_m), were measured at a 20-day interval till maturation of yield (100 days). For root assessment, soil monoliths (10 cm × 10 cm × 20 cm) were randomly excavated from each open-top chamber (OTC). These monoliths were thoroughly cleaned under running tap water to rinse off soil particles.

2.3 O₃ concentration monitoring and associated meteorological metrics

O₃ concentrations were consistently measured over 8 h (9:00–17:00) throughout the cropping period (November 2024–February 2025), using an ultraviolet (UV) absorption photometric O₃ analyser (Model APOA-370, HORIBA Ltd., Japan). A 0.35 cm diameter Teflon tube was inserted into the OTCs to monitor O₃ levels. Between November 2024 and February 2025, the average maximum and minimum temperatures were 27.2 °C and 14.9 °C, respectively (Figure 1). The highest mean temperature was recorded in November, and the lowest during January. Sunshine hours, precipitation, and relative humidity and variations therein are illustrated in Figure 2. Mean O₃ concentrations during the OTCs for the 8-h monitoring period were 72.56 ppb for the elevated conditions and 51.48 ppb for the ambient conditions. The total monthly mean AOT40 (accumulated O₃ exposure over threshold of phytotoxic 40 ppb) values under ambient O₃ conditions were 128.5 ppbh for November, 236.5 ppbh for

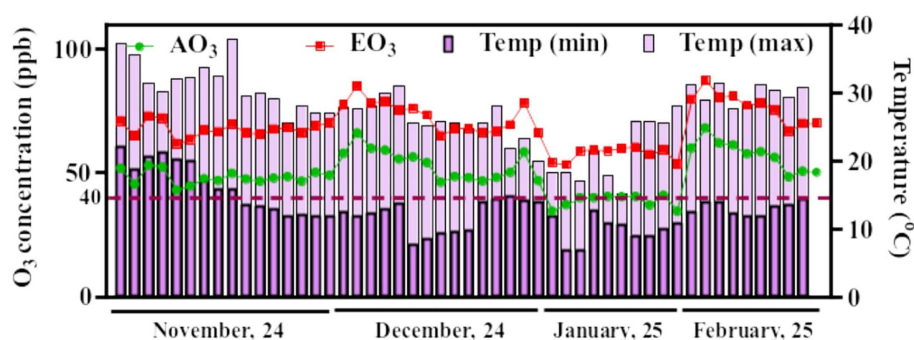


FIGURE 1

Temporal changes in 8-h average ambient (AO₃) and elevated (EO₃) O₃ levels (ppb), and temperature changes throughout the experiment.

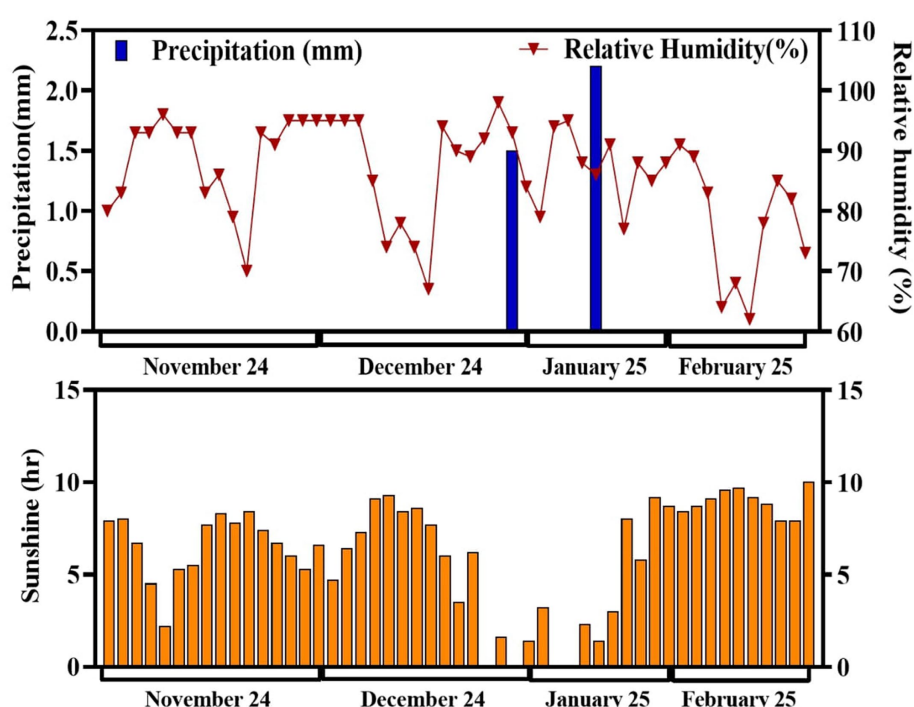


FIGURE 2

Variations in precipitation (mm), relative humidity (%), and sunshine (hr) during November 2024–February 2025.

December, 128.5 ppbh for January, and 269.27 ppbh for February. The corresponding concentrations for elevated O₃ treatments were 477.8, 577.7, 393.57, and 616.4 ppbh for the same months, respectively.

2.4 Morphometric measurements

Root length (RL), root weight (RW), shoot length (SL), shoot weight (SW), plant height (PH), leaf weight (LW), leaf area (LA), FIP (Foliar Injury Percent), number of leaves plant⁻¹ (NoL), root-shoot ratio of weight (RTST) and total biomass (TB) was measured. Floral parameters included the number of flowers plant⁻¹, the petal area. Quantification of plant biomass was done after oven-drying at 80 °C,

until constant weight was achieved; subsequently, gravimetric measurements were conducted employing a weighing balance (Sartorius BSA223S). LA was measured using a portable leaf area meter (Model Li-3100, Li-COR, Inc., USA). Calculations of foliar injury percent (FIP) was done according to Upadhyay et al. (2025).

$$\text{FIP} = (\text{Total leaf area injured} / \text{Total leaf area}) * 100$$

2.5 Photosynthetic pigments and chlorophyll fluorescence measurements

Chlorophyll and carotenoid content were quantified employing the methodology of Takshak and Agrawal (2015). Chlorophyll fluorescence parameters were measured using a PAM

(Pulse-Amplitude-Modulation) chlorophyll fluorometer (FluorPen, Photon system Instruments Ltd., Czech Republic), under field conditions between 9:00 and 10:00 a.m. Before measurements, to ensure complete relaxation of the PSII reaction centres, dark-adaptation of leaves was performed using leaf clips on the adaxial surface for 10 min. The following fluorescence metrics were measured: minimal fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence ($F_v = F_m - F_0$), and the maximum quantum yield of PSII photochemistry (F_v/F_m). These measurements were utilized to evaluate the effects of ozone (O_3) stress on the photosynthetic performance of the studied plant species.

2.6 *In-situ* ROS detection via histochemical assay and microscopic visualization

ROS, specifically Superoxide anion and hydrogen peroxide, were histochemically localized at the 80 DAG using the third fully expanded leaf. Leaf samples were collected from three randomly selected plants for each treatment. Superoxide and hydrogen peroxide radical accumulation were visualized as per the procedure detailed by Yadav et al. (2019). Nitro blue tetrazolium, serving as a chromogenic substrate, forms insoluble blue formazan deposits after being reduced by O_2^- , while H_2O_2 was localized using 3,3'-diaminobenzidine (DAB), which forms reddish-brown precipitate in the presence of endogenous peroxides. Stained leaf samples were examined under a Dewinter optical microscope employing 4X and digitally captured for further qualitative assessment.

2.7 Biochemical parameters

Fully expanded third leaves were randomly collected from test plants at phenological stages for biochemical assays. Solute leakage was quantified employing a conductivity meter (Model 306, Systronics, India) following Dijak and Ormrod (1982). Lipid peroxidation was estimated as malondialdehyde (MDA) concentration (Heath and Packer, 1968). Total phenolic content was measured spectrophotometrically as per Bray and Thorpe (1954), while ascorbic acid levels were evaluated following Keller and Schwager (1977). Enzymatic antioxidants—ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) were extracted and assayed according to Nakano and Asada (1987), Aebi (1984), Fridovich (1974), respectively. Glutathione reductase (GR) activity was determined using the method of Anderson (1996). Total soluble protein content was estimated via the Bradford assay (Bradford, 1976). Hydrogen peroxide (HPS) and superoxide (SOS) radical scavenging activities were assessed following Takshak and Agrawal (2014, 2015).

Choudhary et al. (2013) provided detailed instructions for measuring the activities of nitrate reductase (NR) and nitrite reductase (NiR).

2.8 Yield attributes

Yield metrics were assessed at harvest stages of test cultivars. Dried pods were collected from the plants after ripening of pods. Pod length, number of pods plant^{-1} , seed weight plant^{-1} in terms of grain weight plant^{-1} (GWP), seed weight pod^{-1} , test weight of 100 seeds,

number of seeds plant^{-1} , number of seeds pod^{-1} were measured to analyse impact of individual and combined application of O_3 and biochar on test plants.

2.9 Statistical analyses

All assessed parameters were normalized and homogenized, with their adherence to normality and homogeneity of variance verified using Shapiro–Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) was utilized to examine the fluorescence, biochemical, and yield data. Statistically significant differences between treatment groups were assessed using Tukey's *post hoc* test, with results denoted by lowercase letters above the corresponding bar graphs and scattered plot for each treatment. Redundancy Analysis (RDA) was performed to evaluate biochar and O_3 treatments on the studied parameters. Before RDA, the parameters were subjected to Box-Cox transformations. In addition, the Euclidean similarity and dissimilarity indices were used to analyse similarities in the performance of different individual and combined treatments of O_3 and biochar. A Partial Least Squares (PLS) test was employed to find out relationships between different treatments (explanatory variables) and the plant's physiological and biochemical response (response variables). The response variables were selected based upon RDA analysis and post PLS analysis, and a two-block PLS biplot was plotted to visualize relationships between treatments and response variables for each cultivar. For Partial Least Squares (PLS) and Redundancy Analysis (RDA), Past 4.03 performed automatic cross-validation using the leave-one-out (jackknife) approach. In this procedure, each data point is sequentially excluded, and the analysis is recalculated using the remaining dataset. This method allows for robust estimation of model parameters and reduces potential bias in the results. SPSS was used for Tukey's *post hoc* test (SPSS Inc. version 21.0, IBM Corp, Armonk, NY). GraphPad Prism (version 8.0.2) was used to generate the graphs, and RDA, Euclidean similarity index calculation, and PLS were carried out in Past 4.03.

3 Results

3.1 Growth morphometrics

Exposure to O_3 stress significantly reduced NoL (number of leaves) in both cultivars, especially at 80 Days after germination (DAG) (HUR-137:18%; PDR-14: 22%) (Figure 3). Biochar application had no restorative effect under elevated O_3 ; however, biochar at high doses improved NoL under ambient conditions (HUR-137:19%; PDR-14: 31%). LA showed maximum reductions in PDR-14 in comparison to HUR-137 at both developmental stages. Interestingly, PDR-14 displayed less reduction in LA at 80 DAG than at 40 DAG, corresponding to lesser foliar reductions (AO 769.81 cm^2 , 95% Confidence Interval, i.e., CI: 750.72–788.89; EOB0 533.03 cm^2 , 95% CI: 484.55–581.52 for PDR-14 at 80 DAG) (Figure 3).

Biochar amendment demonstrated dose-dependent improvement in LA at ambient conditions in both cultivars, nonetheless, significant improvements under high O_3 were noted only at 5% biochar supplementation. Progression of developmental stages in test cultivars was marked with an increase in foliar injury (Figure 3). EOB0 of

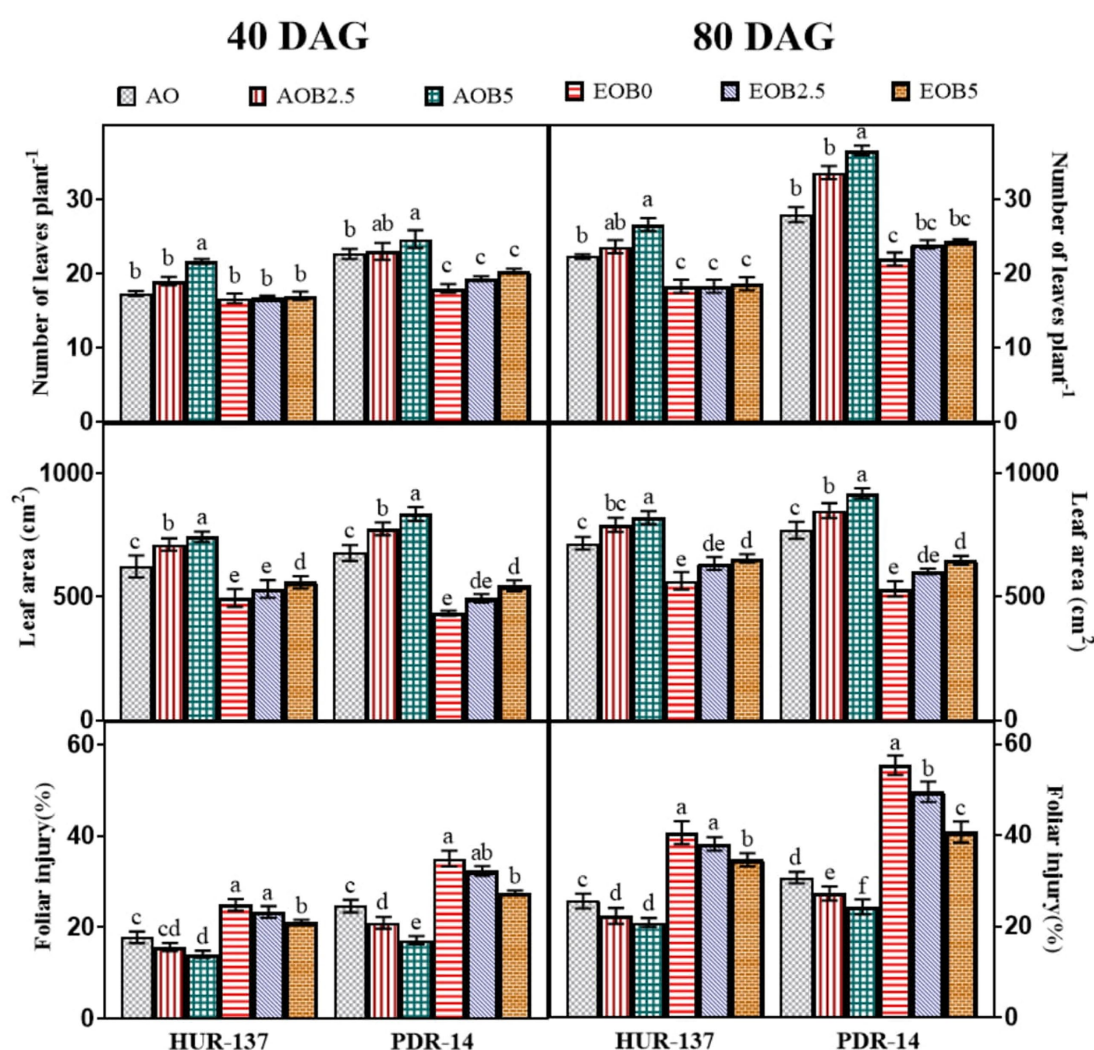


FIGURE 3

Foliar parameters of the test cultivars under individual and combined treatments of O_3 and biochar at vegetative and reproductive stages (Mean $\pm$ S.E.). Bars showing different letters indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$ $n = 5$. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar, EOB0 Elevated O_3 without biochar, EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar.

HUR-137 showed 40.6% Foliar Injury Percent (FIP) at 80 DAG compared to 25.6% FIP at AO, while at 40 DAG EOB0 displayed 24.9% FIP, relative to 17.85 under AO. Whereas EOB0 for PDR-14 suffered 55% FIP at the reproductive stage, with an increase from 38% at 40 DAG. EOB5 exhibited maximal reductions in FIP for PDR-14 (28%, $p < 0.05$) compared to HUR-137 (13% reduction in FIP compared to EOB0, with mean value of 21.04%, $p < 0.05$). Visible O_3 -induced foliar injuries emerged on leaves of test plants within 10–15 days of O_3 exposure. Initially manifesting as chlorotic stipples on older mature leaves and progressed into necrotic patches, with greater severity in biochar non-amended plants (Supplementary Figure 2).

Ozone triggered significant reductions in Root Length (RL) of both cultivars, demonstrating greater decline at the reproductive phase. HUR-137 (23.8%) displayed lesser reductions compared to PDR-14 (33%) for RL, nevertheless, biochar at higher doses exhibited abatement of RL reductions, maximally in PDR-14 (16%). Improved RL was observed for AOB5 of PDR-14 with mean value of 13.07 cm ($p < 0.05$) from 9.13 cm of AO representing a 43%

increase. In HUR-137, RL improved from 8.83 cm ($p < 0.05$) under AO to 11.98 cm in AOB5, reflecting an improvement of 35.6% (Figure 4). Significant enhancements for plant height were observed under ambient conditions for 5% biochar supplementation, with a greater increase noticed for PDR-14 (40 DAG: 31%). Despite significant declines in plant height for EOB0 (27% for PDR-14; 21% for HUR-137), biochar treatment showed improvements with dose-dependent amelioration, more prominent in PDR-14. Both test cultivars suffered greater losses in total biomass at 80 DAG (HUR-137: 32%; PDR-14: 39%) compared to 40 DAG (HUR-137: 26%; PDR-14: 27%). Root weight suffered greater declines for EOB0 in PDR-14 (80 DAG: 51%) when compared to HUR-137 (80 DAG: 42%). Biochar in EOB5 displayed maximal improvements for PDR-14 in root weight (34%), shoot weight (16%), leaf weight (33%) and total biomass (27%) at 80 DAG (Figure 5). Compared to elevated O_3 , biochar supplementation exhibited greater boost in biomass metrics under ambient O_3 levels. Diminished allocation of biomass toward below-ground biomass was evident in reduced

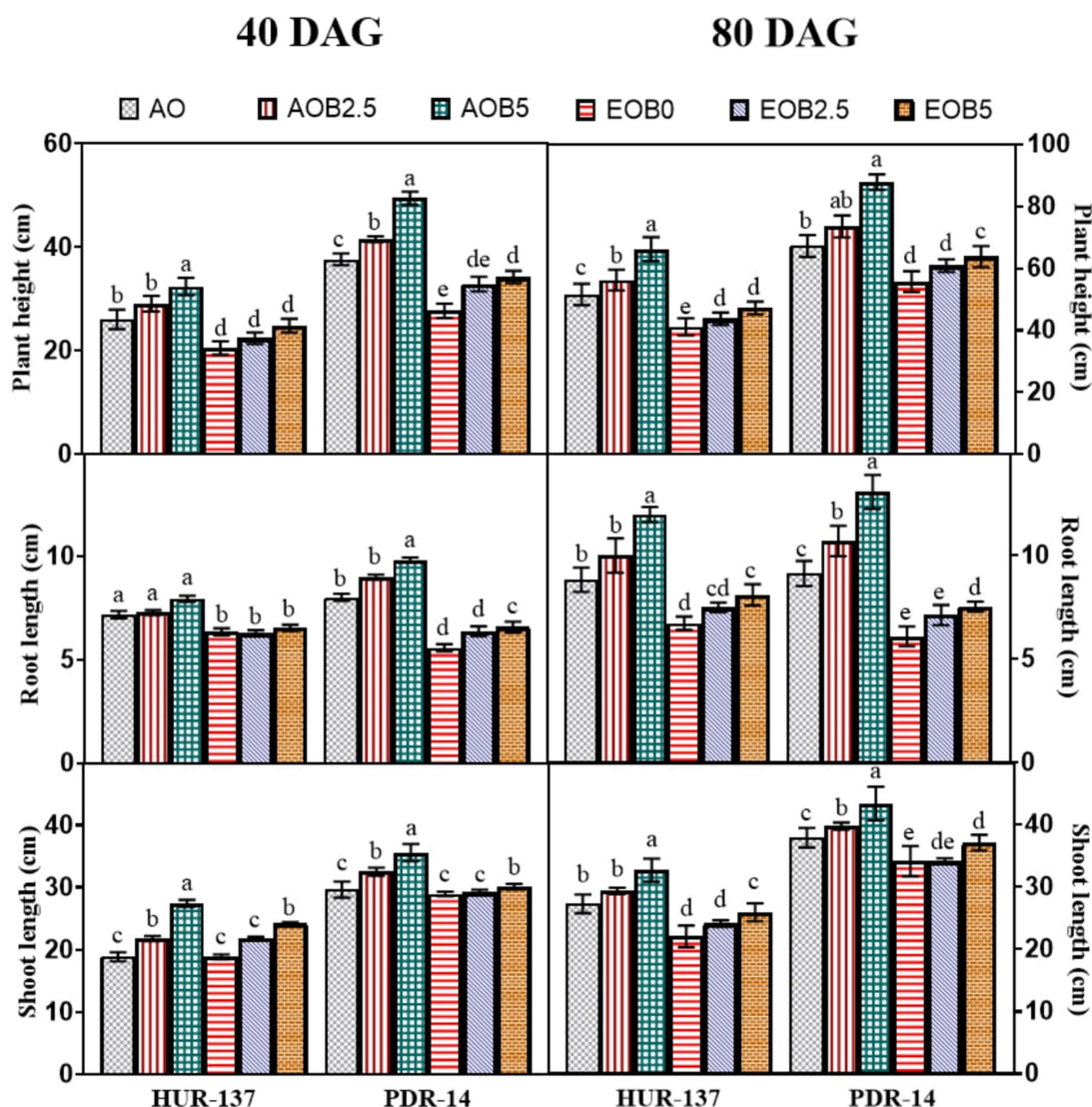


FIGURE 4

Effect of ambient (AO) and elevated O_3 on Root length, shoot length, and plant height of HUR-137 and PDR-14 cultivars under various treatments at 40 and 80 DAG (Mean $\pm$ S.E.). Bars showing different letters indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$; $n = 5$. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar; EOB0 Elevated O_3 without biochar; EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar.

RTST at both stages under a high O_3 environment. Reductions in RTST were more pronounced in PDR-14 (21% at 80 DAG), and biochar amendment improved by 16% at 5% concentration (w/w).

Biochar treatments did not display ameliorative impacts under high O_3 environments in HUR-137, nonetheless, EOB5 showed an increase in the number of flowers plant⁻¹ in PDR-14. Under ambient conditions dose-dependent increment in measured floral metrics was noticed for PDR-14, whereas HUR-137 exhibited improvements under 5% biochar treatments (Figure 6).

3.2 Solute leakage rate, MDA content, total phenolics, and protein content

Malondialdehyde (MDA) is produced by the lipid membrane of plants under stress in response to ROS (Reactive oxygen species),

reflecting the degree of membrane damage. Relative to AO, EOB0 test plants at 80 DAG displayed significant elevations in MDA content of 26.59% in HUR-137 and 23% in PDR-14, respectively (Figure 7). Transition of plants into the reproductive stage was marked with a significant increase in lipid peroxidation and permeability of the cell membrane. Escalation of solute leakage in EOB0 test plants was noted in both cultivars, although to a higher degree in PDR-14 (80 DAG: 29%) compared to HUR-137 (80 DAG: 26%). SOS activities presented overwhelming increments in ozonated plants (EOB0), with PDR-14 displaying the sharpest boost (80 DAG: 1.45 times compared to AO) compared to HUR-137 (80 DAG: 1.25 times compared to AO). Biochar at 5% displayed substantially greater ameliorative results on solute leakage, MDA contents, and total phenolics for both cultivars. EOB5 test plants noted 15% reduction in solute leakage and a drop of 10.8% in MDA content for PDR-14 at reproductive stage (Figure 7). AOB5 treatments showed significant reductions in total phenolics content in

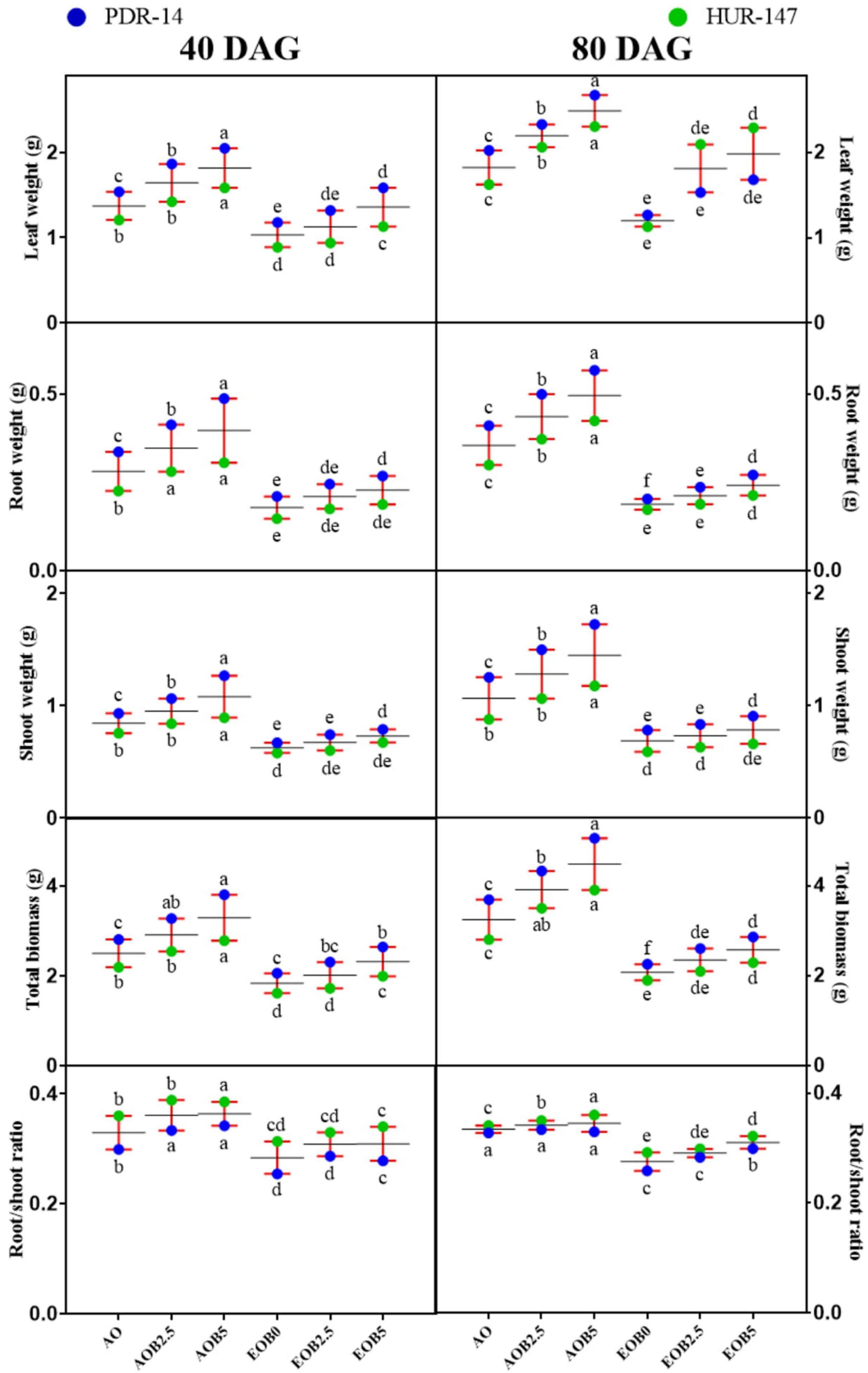


FIGURE 5
Total biomass, root-shoot ratio, and dry weight of various plant parts of HUR-137 and PDR-14 under treatments AO, AOB2.5, AOB5, EOB0, EOB2.5, EOB5 at 40 and 80 DAG (Mean $\pm$ SE). Different letters indicate significant differences between the treatments for each growth stage according to Duncan's multiple range test.
(Continued)

FIGURE 5 (Continued)

Tukey's test at $p < 0.05$; $n = 5$. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar, EOB0 Elevated O_3 without biochar, EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar.

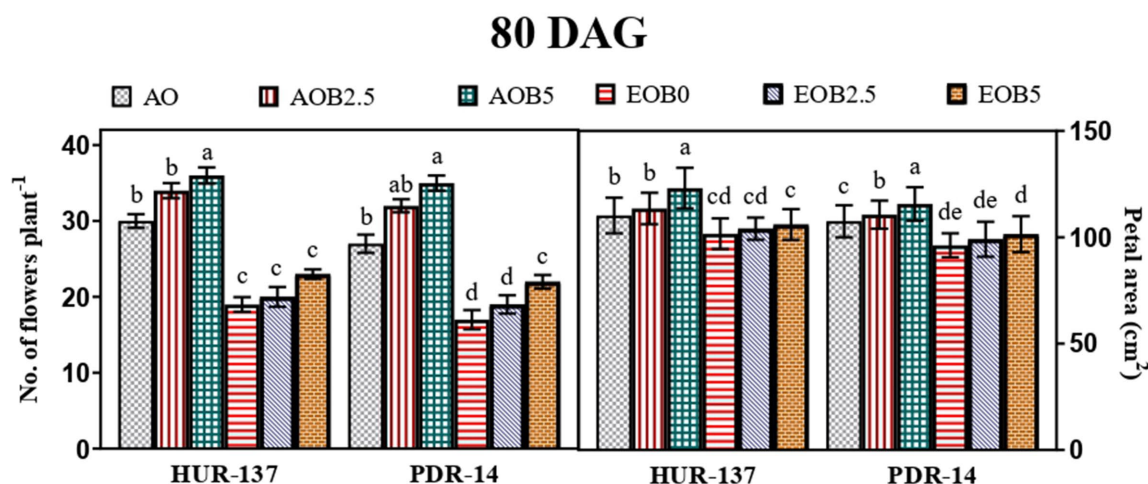


FIGURE 6

Floral parameters of the test cultivars under individual and combined treatments of O_3 and biochar at vegetative and reproductive stages (Mean $\pm$ S.E.). Bars showing different letters indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$; $n = 5$. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar, EOB0 Elevated O_3 without biochar, EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar.

both the test cultivars at both the stages (Figure 7). Biochar at 5% concentration significantly reduced total phenolics in the test cultivars at both the sampling stages under ambient and elevated O_3 levels. Protein content in PDR-14 at 40 DAG exhibited maximum reductions under a high O_3 environment (29%) compared to a 19% drop in HUR-137, relative to AO. Higher doses of biochar (EOB5) consistently maintained protein content in O_3 -stressed plants.

3.3 Detection of ROS through histochemical localization

Histochemical localization of $O_2^{\cdot-}$ and H_2O_2 showed increased concentrations of free radicals under O_3 fumigation (Figures 8a,b). PDR-14 exhibited high staining intensity for both DAB and NBT, reflecting higher production of $O_2^{\cdot-}$ and H_2O_2 . Both concentrations of biochar under ambient conditions reduced free radicals more significantly. Nonetheless, notable reductions in free radicals were noted at higher dosage (5% w/w) of biochar for elevated O_3 treatments. Biochar fertilization showed significantly lesser localization in PDR-14 for both $O_2^{\cdot-}$ and H_2O_2 , compared to HUR-137.

3.4 Photosynthetic pigments, anthocyanin and chlorophyll fluorescence measurements

Examination of physiological impacts of biochar and O_3 exposure, both individually and in combination reveals a divergent response in test cultivars. Relative to AO, total chlorophyll content for EOB0

reduced by 30.2% in PDR-14 and 24.9% in HUR-137 (80 DAG) (Figure 9). Temporal patterns of total chlorophyll content exhibited further reductions with progression of the plant's developmental phase. Significant reductions were detected in the chlorophyll a/b ratio in both cultivars, with maximum reductions in PDR-14. Biochar amendment showed considerable minimization of reduction in chlorophyll content and a/b ratio, with EOB5 of PDR-14 registering maximum improvement in chlorophyll a/b (31% at 40 DAG). For total chlorophyll content, EOB5 plants noted improvements ranging from 25.8% (HUR-137) to 32.8% (PDR-14) compared to EOB0, at 80 DAG. Biochar displayed greater amelioration at the vegetative stage of plants, with significant benefits observed at a 5% rate of application. 5% biochar fertilized plants had 32.65% boost at ambient O_3 levels for both cultivars; however, under elevated O_3 conditions PDR-14 registered greater enhancements in a/b ratio (31%) compared to HUR-137 (18.4%). Biochar partially ameliorated the drop in Fv/Fm values for both cultivars, indicating improved photosynthetic performance of plants. EOB2.5 treatments noted significant improvements compared to EOB0 (Table 1). Nonetheless, an increase in the dose of biochar supplementation resulted in no considerable amelioratory impact (Figure 9).

3.5 Enzymatic antioxidants, ROS scavenging activity

Increased activities of antioxidative enzymes were noted in both cultivars in response to both biochar amendments and O_3 , underscoring convergent responses of treatments. Both biochar and O_3 showed greater increments for PDR-14 compared to

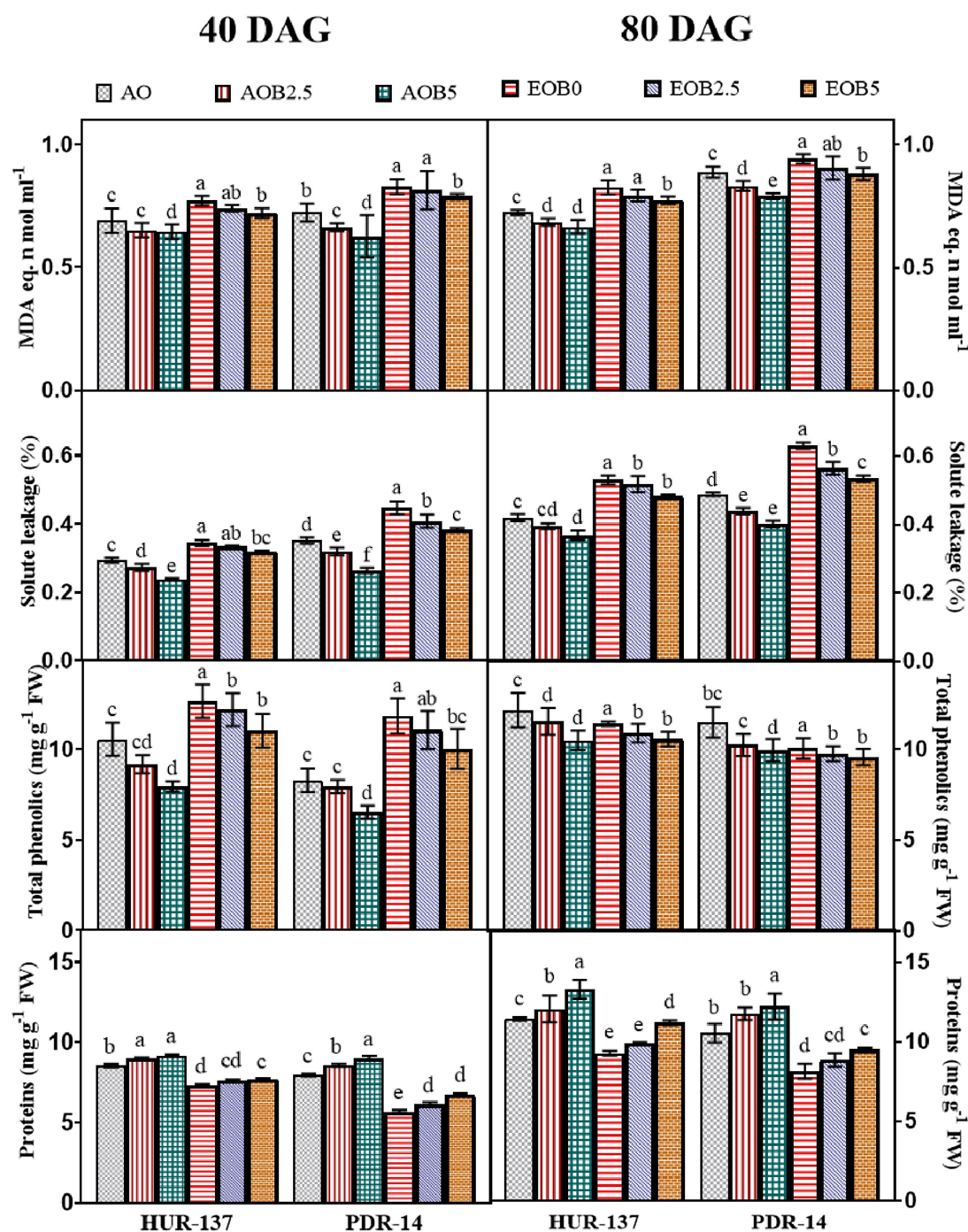


FIGURE 7

Effect of individual and combined doses of O_3 and biochar on MDA content, solute leakage (%), total phenolics, and protein contents in the two test cultivars at 40 and 80 DAG (Mean $\pm$ S.E). Bars showing different letters indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$; $n = 5$. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar; EOB0 Elevated O_3 without biochar; EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar. MDA, Malondialdehyde.

HUR-137 (Figure 10). SOD (Superoxide dismutase) increased 1.3 times for EOB0 plants of PDR-14 and 1.14 times for HUR-137 at 80 DAG, while biochar application at 5% further boosted enzyme activities for PDR-14 (1.44 times) and HUR-137 (1.2 times) in comparison to AO. Activities for APX and GR followed the same trend of synergistic impact of biochar and O_3 fumigation. Induction of plant's antioxidative defense was further manifested in terms of

increased activity of catalase, with the highest increment under AOB5 treatments for PDR-14. At 40 DAG, O_3 fumigation noted a 22% increase while biochar further increased activities (EOB2.5: 32%; EOB5: 47.8%) relative to AO. In EOB0, both HPS and SOS showed greater increments for PDR-14 in comparison to HUR-137, especially at 80 DAG (HPS: 21%; SOS: 25%). AOB5 treatments showed increased HPS by 1.52 times and SOS by 1.4 times in HUR-137, and

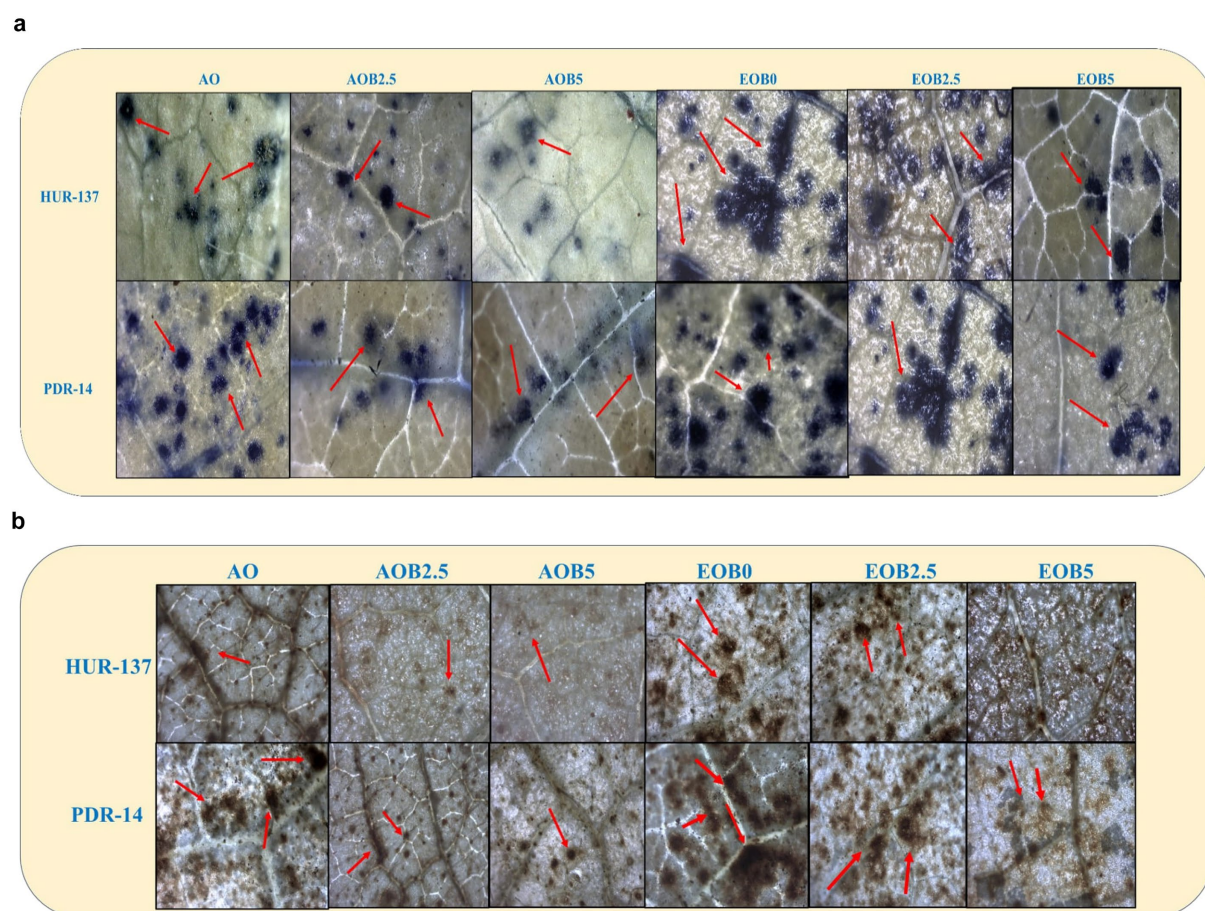


FIGURE 8

(A) Histochemical localization of superoxide radicals in leaves of test plants of various treatments using Nitroblue tetrazolium (NBT) staining. Dark blue formazan deposits indicate sites of superoxide ($O_2^{\cdot -}$) accumulation. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar; EOB0 Elevated O_3 without biochar; EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar. (B) Histochemical localization of hydrogen peroxide in leaves using 3,3'-diaminobenzidine (DAB) staining. Brown polymerization products denote H_2O_2 accumulation. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar; EOB0 Elevated O_3 without biochar; EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar.

for the PDR-14, the increases were 1.54 times and 1.5 times, respectively, compared to AO (Figure 10).

3.6 Enzymes of nitrogen assimilation

Activities of key enzymes in the nitrogen assimilation pathway (NR and NiR) were significantly and antagonistically impacted by biochar and O_3 treatments. Plants exposed to O_3 displayed a general decline in enzyme activity, in comparison to AO. HUR-137 exhibited higher NR and NiR activities than PDR-14. However, PDR-14 suffered greater decline under O_3 exposure for both NR (33.7%) and NiR (30.6%) activity, especially at the vegetative stage (Figure 11). Biochar application, particularly at 5% concentration, partially mitigated the drop in enzyme activities. Increasing the concentration of biochar fertilization resulted in a further improvement of enzyme activities. This was more prominent in PDR-14 under ambient conditions, where, compared to AOB2.5 (10%), AOB5 (31%) had greater enhancements in NR activity at 40 DAG.

3.7 Yield

Several key yield parameters were evaluated to comprehend the impact of O_3 and biochar on test cultivars. O_3 negatively impacted reproductive development and yield fixation by generating a substantial reduction across all parameters ($p < 0.05$). Reductions in yield components were more pronounced for PDR-14 than HUR-137. In PDR-14, pod no. $plant^{-1}$ (39.5%), pod length (20%), seeds pod^{-1} (19.5%), and seeds $plant^{-1}$ (44.8%) declined, whereas in HUR-137, reductions were 26.7, 21, 11, and 31.9%, respectively. Biochar effectively amended the reduction on yield parameters (Figure 12). Pod number $plant^{-1}$ improved under both ambient and elevated O_3 levels, for biochar treatments in both cultivars. Higher doses of biochar exhibited significantly greater impact in PDR-14 compared to HUR-137. In HUR-137, biochar did not significantly affect pod length under elevated O_3 conditions, while in PDR-14, 5% biochar improved pod length. Biochar had no impact on seed pod^{-1} for both the cultivars, while seed $plant^{-1}$, noted significant improvements under biochar. O_3 stress also impacted seed quality and

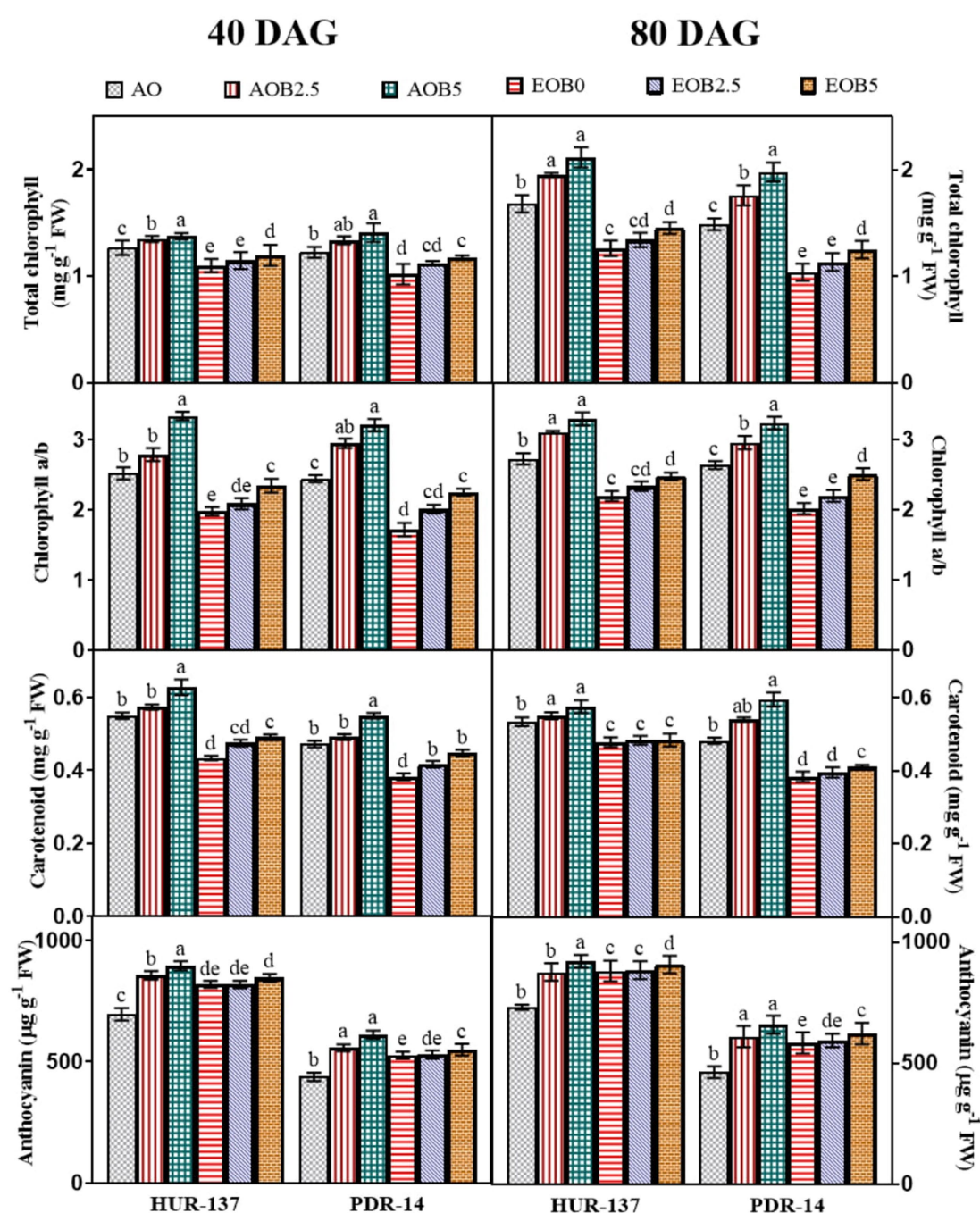


FIGURE 9

Plant pigments (Total chlorophyll, Chlorophyll a/b, carotenoid, and anthocyanin content) at 40 and 80 DAG stages in different treatments (AO, AOB2.5, AOB5, EOB0, EOB2.5, EOB5) of HUR-137 and PDR-14 cultivars (Mean $\pm$ S.E). Bars showing different letters indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$; $n = 5$. Treatment: AO Ambient O₃ without biochar; AOB2.5 Ambient O₃ with 2.5% biochar; AOB5 Ambient O₃ with 5% biochar; EOB0 Elevated O₃ without biochar; EOB2.5 Elevated O₃ with 2.5% biochar; EOB5 Elevated O₃ with 5% biochar.

prompted shriveling of seeds. HUR-137 observed a 38% boost in the production of shriveled seeds while PDR-14 recorded a 49% (Figures 13a,b). Biochar at 2.5% showed 21% (ambient O₃) and 36% (elevated O₃) drop in shriveled seeds generation in HUR-137. 5% biochar demonstrated greater efficacy in the reduction of shriveled seeds under high O₃ levels in both HUR-137 (12.5%) and PDR 14 (17%).

EOB0 plants of HUR-137 displayed a 35.8% reduction in GWP (30.38 g plant⁻¹) and a 44.7% decline in PDR-14 (17.46 g plant⁻¹) compared with respective AO test plants of HUR-137 (47.43 g plant⁻¹) and PDR-14 (31.58 g plant⁻¹). Biochar amendments noted a more amelioratory impact on PDR-14 with higher values under ambient conditions. For PDR-14, AOB5 (47.18 g) displayed a 49.5% improvement relative to AO, while EOB5 partially restored GWP to

TABLE 1 Various chlorophyll fluorescence parameters (*Fo*, *Fm*, *Fv*, *Fv/Fm*) under individual and combined doses of O₃ and biochar of Hur-137 and PDR-14 at both the sampling stages (Mean ± S.E).

Parameter	Sampling stages	PDR-14						HUR-137					
		AO	AOB2.5	AOB5	EOB0	EOB2.5	EOB5	AO	AOB2.5	AOB5	EOB0	EOB2.5	EOB5
<i>Fo</i>	20 DAG	9021.6 ± 102 ^b	5,925 ± 592 ^c	5152.2 ± 113 ^c	15,233 ± 129.2 ^a	14922.32 ± 310 ^a	13,786 ± 198.2 ^a	6258.33 ± 197.3 ^b	5693.3 ± 186 ^b	4459.3 ± 352.2 ^c	9463.52 ± 287.3 ^a	8413.5 ± 319.5 ^a	7981.35 ± 219.36 ^a
	40 DAG	10143.3 ± 114 ^c	8625.3 ± 686 ^d	7702.56 ± 120.8 ^c	16,277 ± 169.5 ^a	15279.67 ± 328.6 ^a	12,683 ± 96.7 ^b	7241.6 ± 26 ^d	6,220 ± 239.2 ^{de}	5,710 ± 229 ^c	11,387 ± 258 ^a	9,233 ± 302.2 ^b	8975.7 ± 274.8 ^c
	60 DAG	12,209 ± 343 ^d	9,597 ± 261 ^c	8,896 ± 254 ^c	17,740 ± 284 ^a	15,789 ± 378 ^b	14,131 ± 357 ^{bc}	8,236 ± 231 ^c	7,727 ± 139 ^c	6,784 ± 75.6 ^d	14,359 ± 275 ^a	12,176 ± 209 ^b	10,667 ± 300 ^b
	80 DAG	13,900 ± 393 ^{bc}	10429.3 ± 169 ^c	9111.2 ± 312 ^d	18,639 ± 323.2 ^a	18,154 ± 311.2 ^a	16100.3 ± 325 ^b	9,103 ± 198.3 ^c	8,741 ± 124.3 ^{cd}	8,014 ± 89.3 ^d	13,214 ± 237.3 ^a	11,359 ± 178.3 ^b	10596.3 ± 269.7 ^{bc}
	100 DAG	16325.3 ± 269 ^{bc}	14,781 ± 241 ^c	13,987 ± 31.3 ^d	19963.1 ± 311 ^a	16,832 ± 298 ^b	15762.4 ± 235 ^b	9752.7 ± 215 ^c	9,628 ± 139.1 ^c	8,957 ± 128.9 ^{cd}	12,896 ± 218.3 ^a	11968.3 ± 289.3 ^{ab}	11003.8 ± 279.6 ^b
<i>Fm</i>	20 DAG	55987.2 ± 456.3 ^b	60365.62 ± 297.9 ^a	70,963 ± 2951.8 ^a	39986.3 ± 3218.23 ^{cd}	41,187 ± 298.65 ^{cd}	43983.8 ± 683.7 ^d	52898.8 ± 2,815 ^{ab}	56,864 ± 2149.3 ^a	57,863 ± 3368.36 ^a	46,568 ± 658.9 ^{de}	48,963 ± 783.9 ^{de}	48999.8 ± 328.2 ^d
	40 DAG	53,681 ± 669.6 ^b	62817.7 ± 2787.5 ^a	63150.3 ± 3337 ^a	43100.7 ± 3657.6 ^d	44,173 ± 1562.9 ^{cd}	45,186 ± 545.5 ^c	53,734 ± 2315 ^{ab}	54,401 ± 3859 ^{ab}	56,656 ± 1633 ^a	45,183 ± 578 ^d	45,880 ± 670 ^{cd}	47,670 ± 1051 ^{bc}
	60 DAG	46,995 ± 1063.2 ^b	52,238 ± 892.9 ^a	54,927 ± 3019.2 ^a	41357.3 ± 2985.9 ^c	42956.5 ± 1786.3 ^{de}	45,954 ± 429.3 ^d	45249.8 ± 2935.3 ^b	47359.2 ± 2,937 ^{ab}	47,996 ± 583.5 ^a	33652.9 ± 698.3 ^c	35897.3 ± 529.3 ^{de}	38,158 ± 387 ^{cd}
	80 DAG	40,417 ± 3055 ^b	43,105 ± 1892 ^a	44,554 ± 3986 ^a	35,667 ± 3,490 ^d	37,247 ± 2758 ^{cd}	37,968 ± 1305 ^c	40,623 ± 2737 ^c	42,253 ± 3757 ^{bc}	44,146 ± 500 ^a	33,302 ± 12790 ^{de}	36,881 ± 12,211 ^d	39,749 ± 3160 ^d
	100 DAG	40,128 ± 2065.9 ^b	41,974 ± 985.7 ^{ab}	43,023 ± 2652.3 ^a	30,159 ± 3149.2 ^c	31,198 ± 359.7 ^{cd}	31,876 ± 863.7 ^d	42,029 ± 1659.8 ^b	42,398 ± 259.9 ^b	46863.7 ± 483.6 ^a	33269.3 ± 875.9 ^{de}	34932.9 ± 387 ^{cd}	35983.8 ± 683 ^{cd}
<i>Fv</i>	20 DAG	42986.3 ± 965 ^b	48569.3 ± 659 ^b	59,875 ± 2637 ^a	28754.2 ± 2014 ^d	30693.8 ± 1,053 ^{cd}	32698.2 ± 365 ^c	42158.6 ± 2103 ^b	46,657 ± 3011 ^b	48,621 ± 1011 ^a	36,038 ± 364 ^c	37,207 ± 394 ^c	37,983 ± 2013 ^c
	40 DAG	42312.7 ± 1032 ^c	51,728 ± 2801 ^b	55,067 ± 2484 ^a	31,157 ± 1543 ^c	33,033 ± 1068 ^{de}	36,617 ± 1122.7 ^d	44,161 ± 1591 ^b	45,514 ± 2965 ^{ab}	49,346 ± 1459 ^a	32,411 ± 289 ^d	33,350 ± 676 ^d	35,028 ± 556 ^c
	60 DAG	37895.3 ± 1268.3 ^c	42193.2 ± 956 ^b	45893.2 ± 639 ^a	26982.3 ± 658 ^d	28298.3 ± 3,621 ^d	31,863 ± 593 ^c	37,419 ± 721 ^b	37,891 ± 982 ^{ab}	40,444 ± 436 ^a	24,359 ± 286 ^{de}	25,543 ± 1,011 ^{de}	28,936 ± 219 ^c
	80 DAG	30,387 ± 2666 ^{bc}	34,527 ± 3,184 ^a	37,362 ± 435 ^{ab}	23,429 ± 714 ^d	25,615 ± 2215 ^c	26,819 ± 2177 ^a	33,315 ± 2126 ^b	34,326 ± 2385 ^b	37,787 ± 565 ^a	26,567 ± 2014 ^{de}	28,615 ± 7900 ^{de}	29,819 ± 2177 ^d
	100 DAG	28945.3 ± 1,036 ^b	32579.1 ± 2096 ^a	34891.3 ± 758 ^a	18,529 ± 1625 ^d	19,025 ± 3,627 ^d	22365.9 ± 875 ^c	31258.9 ± 1,019 ^b	33,456 ± 2,604 ^b	36,982 ± 2,657 ^a	24,353 ± 2083 ^{de}	26,057 ± 2024 ^d	28,967 ± 2,280 ^c
<i>Fv/Fm</i>	20 DAG	0.775 ± 0.007 ^b	0.825 ± 0.021 ^b	0.865 ± 0.007 ^a	0.721 ± 0.061 ^c	0.724 ± 0.003 ^c	0.784 ± 0.021 ^c	0.795 ± 0.01 ^b	0.821 ± 0.061 ^b	0.834 ± 0.007 ^a	0.731 ± 0.005 ^d	0.762 ± 0.003 ^{cd}	0.761 ± 0.01 ^{cd}
	40 DAG	0.808 ± 0.021 ^b	0.833 ± 0.003 ^b	0.869 ± 0.005 ^a	0.740 ± 0.01 ^{cd}	0.753 ± 0.021 ^d	0.816 ± 0.005 ^c	0.826 ± 0.007 ^b	0.850 ± 0.003 ^b	0.867 ± 0.061 ^a	0.724 ± 0.01 ^c	0.739 ± 0.021 ^c	0.753 ± 0.061 ^c
	60 DAG	0.815 ± 0.003 ^b	0.857 ± 0.01 ^a	0.886 ± 0.007 ^a	0.652 ± 0.021 ^d	0.687 ± 0.01 ^{cd}	0.747 ± 0.06 ¹	0.848 ± 0.003 ^c	0.867 ± 0.007 ^b	0.886 ± 0.021 ^a	0.714 ± 0.061 ^c	0.726 ± 0.01 ^c	0.759 ± 0.005 ^d
	80 DAG	0.774 ± 0.021 ^b	0.822 ± 0.003 ^a	0.848 ± 0.01 ^a	0.657 ± 0.007 ^{de}	0.681 ± 0.061 ^{de}	0.787 ± 0.005 ^c	0.807 ± 0.01 ^b	0.832 ± 0.003 ^{ab}	0.852 ± 0.021 ^a	0.691 ± 0.005 ^d	0.708 ± 0.021 ^d	0.727 ± 0.01 ^c
	100 DAG	0.717 ± 0.01 ^b	0.796 ± 0.061 ^a	0.813 ± 0.005 ^a	0.608 ± 0.01 ^c	0.642 ± 0.021 ^{de}	0.718 ± 0.003 ^{cd}	0.751 ± 0.061 ^b	0.799 ± 0.01 ^{ab}	0.812 ± 0.005 ^a	0.702 ± 0.021 ^{de}	0.726 ± 0.003 ^d	0.734 ± 0.01 ^c

Bars showing different letters indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$; $n = 5$. Treatment: AO Ambient O₃ without biochar; AOB2.5 Ambient O₃ with 2.5% biochar; AOB5 Ambient O₃ with 5% biochar, EOB0 Elevated O₃ without biochar, EOB2.5 Elevated O₃ with 2.5% biochar; EOB5 Elevated O₃ with 5% biochar. *Fo* minimum fluorescence yield; *Fm* maximum fluorescence yield; *Fv* variable fluorescence; *Fv/Fm* Maximum Quantum Yield of Photosystem II.

23.06 g plant⁻¹, reflecting a 32.1% increase compared to EOB0 test plants.

3.8 Redundancy analysis, Euclidean similarity and dissimilarity index, and biplot visualization of PLS

RDA was conducted to find out variations in analysed parameters with different treatments under O₃ and biochar, separately for each cultivar. The plot diagram displayed that in HUR-137 (Supplementary Figure 3), biochar treatments under ambient O₃ conditions strongly align with total chlorophyll, nitrate reductase and nitrite reductase, grain weight plant⁻¹, and other growth-related parameters. Treatments under ambient O₃ (AO, AOB2.5, AOB5) are clustered on the left, indicating similar influence. EOB0 shows strong associations with MDA and Solute leakage, indicating significant cellular damage, in addition to being positioned on the far right, showing the strongest deviation and high stress impact. EOB2.5 and EOB5 display alignment with antioxidative enzyme and deviate to the left from EOB0, meaning increased amelioration with an increase in biochar concentration. RDA biplot of PDR-14 (Supplementary Figure 4) suggests similar trends, with EOB2.5 and EOB5 implying partial to strong recovery on analysed metrics. EOB0 shows strong associations with MDA content, solute leakage, and foliar injury. Ambient treatments with biochar additions note the strongest associations with plant pigments, nitrogen metabolism, and antioxidative defense. PLS biplots reveal grouping and patterns between treatments, shedding light on the influence of treatments on traits. Treatments showed a gradient of EOB0 to EOB2.5 to EOB5 to AO to AOB2.5 to AOB5 in both the test cultivars, suggesting a progression from highly stressed (EOB0) to the most optimum conditions (AOB5). AOB5 and EOB5 both shifted away from their respective unamended treatment groups (AO and EOB0, respectively), reflecting a directional effect of biochar (Supplementary Figures 5, 6). Further similarities and dissimilarities between treatment groups were analysed based on Euclidean index (Figure 14). The conversions of indices into heat map demonstrate that biochar fertilization had the most pronounced impacts on PDR-14, as biochar treatments (AOB5 and EOB5) show the largest dissimilarities with unamended treatment groups. For HUR-137, O₃ influenced most traits compared to biochar, as ozonation of plants increased dissimilarity values more than biochar additions.

4 Discussion

Understanding the intricacies of O₃-induced perturbations in plant's biochemical and physiological aspects is critical before embarking upon the search for anti-ozonants or ameliorative agents. The present investigation unveils an intriguing narrative concerning the potential of biochar to ameliorate the detrimental impacts of elevated O₃ in *Phaseolus vulgaris* (L.), revealing nuanced responses dependent upon both cultivar and biochar concentration.

4.1 Oxidative stress, redox homeostasis, and membrane integrity

Stomatal pores on leaves facilitate O₃ entrance into the plant, followed by generation of reactive oxygen species (ROS) in the leaf apoplast pathway, such as O₂⁻ and H₂O₂ (Wedow et al., 2021). Production of ROS was reflected in histochemical localization, in which EOB0 (elevated O₃ without biochar) showed maximum dark spots of DAB (3,3'-Diaminobenzidine) and blue spots for O₂⁻. Intense staining under O₃ exposure indicates rapid production of ROS, especially in leaf mesophyll tissues. Further corroboration of cellular redox disruption was evidenced by increased MDA (malondialdehyde) content and solute leakage. MDA content and solute leakage rate are key indicators of cellular redox imbalance and their elevated levels signify increased lipid peroxidation, compromised cell membrane fluidity, structure, and functionality (Nowroz et al. 2024). These O₃-generated disruptions were further elucidated in pictorial ordination of RDA (Redundancy analysis) (Supplementary Figures 3, 4), showing a strong association of MDA and solute leakage with ozonated treatments for both cultivars. As plants progressed into reproductive stage MDA content and solute leakage increased across cultivars. This is primarily because at reproductive phase increased metabolic load and shifting of photosynthetic assimilates toward flowering and seed development intensifies lipid peroxidation (Monson et al., 2022.)

Application of biochar conferred partial but significant amelioration of phytotoxic effects, with a concentration-dependent trend observed across most parameters. Reductions in MDA content and solute leakage rate in plants demonstrate a partial restoration of membrane integrity and stabilization. Microscopic evaluation of ROS via histochemical assay endorsed this biochar-driven ameliorative impact, as lowered staining intensity under biochar amendments suggests redox stabilization. HUR-137 showed lesser ROS accumulation, MDA content, and solute leakage rates than PDR-14, reflecting inherent tolerance for oxidative stress. Phenolic compounds are essential secondary metabolites in plant stress responses, acting as antioxidants and signaling molecules (Saini et al., 2024). Our study noted an increased production of phenolics under O₃, suggesting a hike in cellular demands for antioxidants and signaling molecules due to increased cellular redox imbalance. Biochar amendments resulted in a significant reduction in total phenolics, attributable to lowered oxidative burden due to increased activities of antioxidative enzymes and free radical scavenging. Similar observations were confirmed by Wang et al. (2014) in root secretions of *Malus hupehensis* Rehd., suggesting absorptive capacity of biochar to alleviate phenolics concentrations.

The enzymatic antioxidative defense system plays a critical role in redox homeostasis and protection of cellular components from oxidative disruptions (Gupta et al., 2023). In the current study, O₃ stress significantly elicited the activities of key enzymatic antioxidants such as APX (ascorbate peroxidase), GR (glutathione reductase), SOD (superoxide dismutase) and CAT (catalase), in both *Phaseolus vulgaris* (L.) cultivars. The elevated responses of antioxidative enzymes underscore a targeted defense system aimed at detoxification of ROS-mediated stress. However, the magnitude of induction varied between cultivars, with PDR-14 generally exhibiting a stronger percent increase for EOB0 in antioxidative

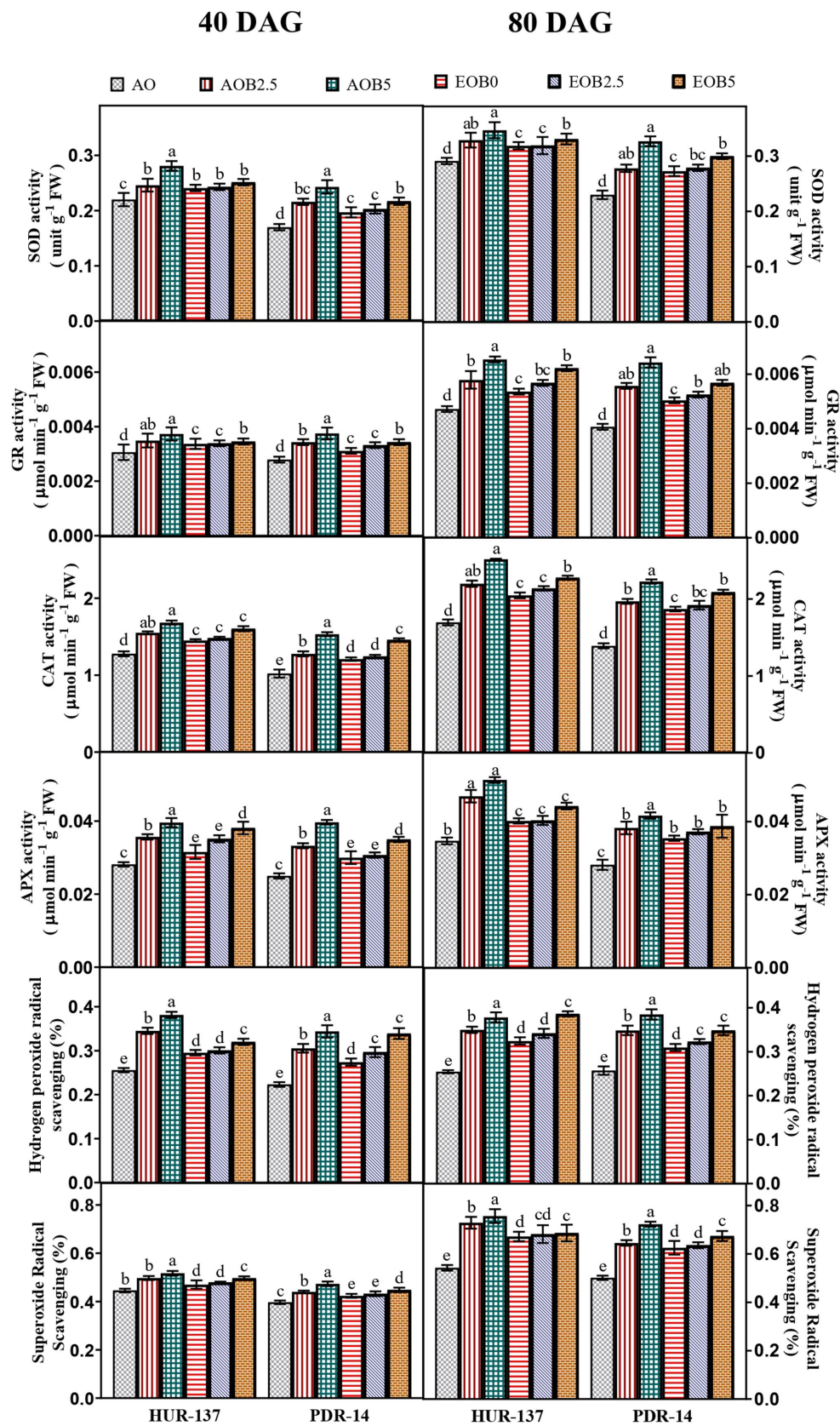


FIGURE 10 Depiction of various enzymatic antioxidant activity and free radical scavenging (Hydrogen peroxide scavenging and Superoxide scavenging) in different treatments (AO, AOB2.5, AOB5, EOB0, EOB2.5, EOB5) of both the test cultivars at both sampling stages (Mean $\pm$ S.E). Bars showing different letters (Continued)

FIGURE 10 (Continued)

indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$; $n = 5$. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar; EOB0 Elevated O_3 without biochar; EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar. SOD Superoxide dismutase; CAT Catalase; APX Ascorbate peroxidase; GR, Glutathione reductase.

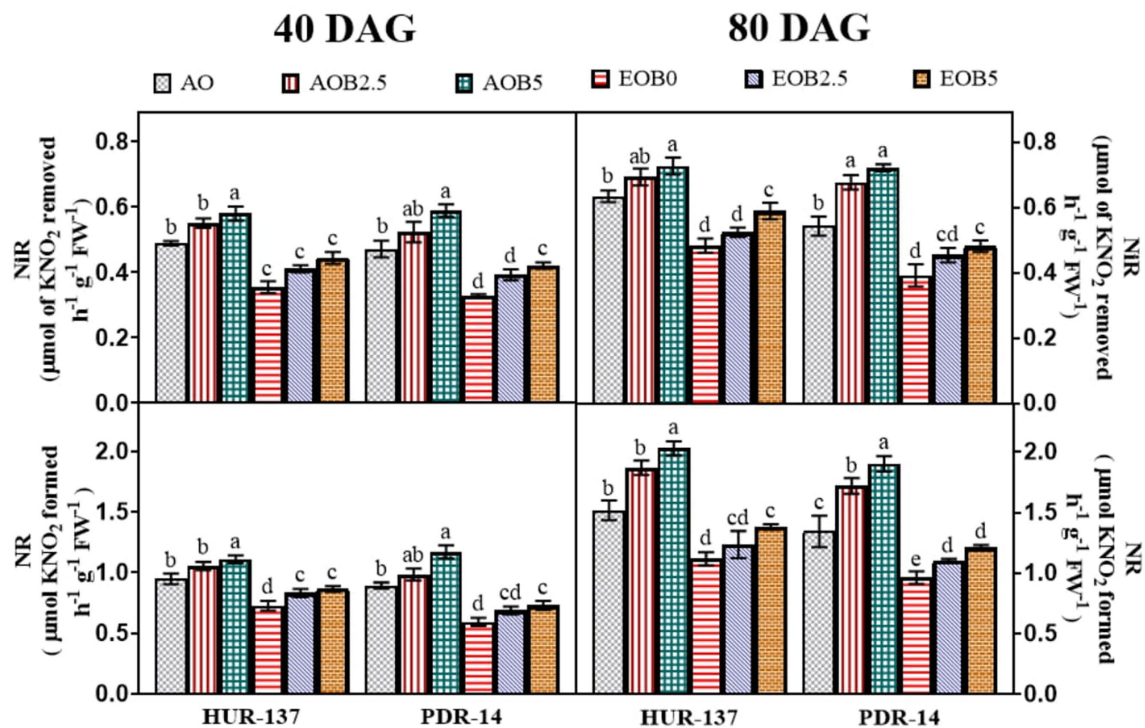


FIGURE 11

Effect of individual and combined doses of O_3 and biochar on NR and NiR activities of HUR-137 and PDR-14 cultivars at both the sampling stages (Mean $\pm$ SE). Bars showing different letters indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$; $n = 5$. Treatment: AO Ambient O_3 without biochar; AOB2.5 Ambient O_3 with 2.5% biochar; AOB5 Ambient O_3 with 5% biochar; EOB0 Elevated O_3 without biochar; EOB2.5 Elevated O_3 with 2.5% biochar; EOB5 Elevated O_3 with 5% biochar. NR, Nitrate reductase; NiR, Nitrite reductase.

enzyme production relative to AO, indicating a possible lower baseline level of enzyme activities under ambient conditions. Despite a higher percent increase under elevated O_3 for PDR-14, HUR-137 showed higher levels of enzyme activities, suggesting a better redox homeostasis mechanism in HUR-137. In line with these observations, higher levels of free radical scavenging were also noted for HUR-137. These findings align with the work of Caregnato et al. (2013), who demonstrated that higher endogenous levels of antioxidative enzyme activity in *Phaseolus vulgaris* (L) cultivars confer greater protection toward foliage and growth. Biochar fertilization further amplified the antioxidative enzymatic response, especially under EOB5 treatments. A dose-dependent increase in antioxidative enzyme activity was observed in both the test cultivars, demonstrating a proportional increment in correction capacity of biochar concerning redox imbalance. While biochar fertilization at ambient O_3 levels resulted in the highest increase in ROS scavenging activities, combined treatments of biochar and elevated O_3 noted partial mitigation of ROS stress and augmentation of membrane stability. SOD is a critical first-line defense enzyme in the antioxidative pathway, and it enables

dismutation of O_2 . Elevated levels of SOD indicated better conversion of superoxide ions into hydrogen peroxide and high CAT, APX and GR activities implied efficient hydrogen peroxide detoxification and recycling of glutathione as well as, indicating adaptive responses of plants under stress and further strengthening of the antioxidative defense system under biochar supplementations (Gupta et al., 2023). The synergistic effect of biochar on the modulation of redox homeostasis further explains the observed reductions in oxidative cytotoxicity in terms of MDA content and solute leakage rate.

4.2 Modulation of photosynthetic performance and biochemical pathways under O_3 stress and biochar amendment

Chlorophyll fluorescence measurements serve as a proxy for assessment of the functional status of photosystem II (PSII) (Basara and Gorzelany, 2025). Ozone exposure generated a decline across key fluorescence matrices. F_0 assesses fluorescence emitted when all

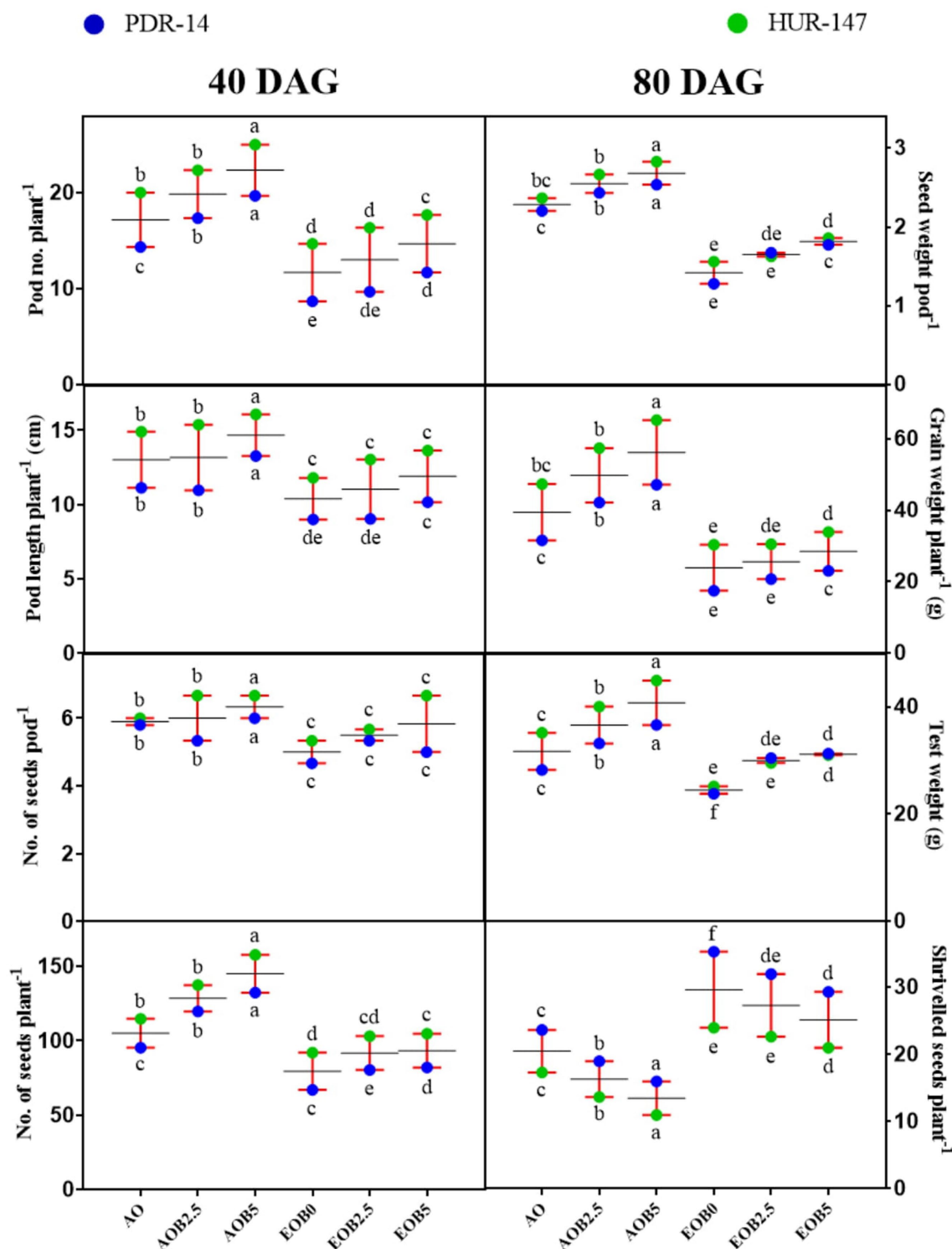


FIGURE 12

Yield characteristics of HUR-137 and PDR-14 cultivars under various treatments (AO, AOB2.5, AOB5, EOB0, EOB2.5 and EOB5). Values are mean $\pm$ Standard Error ($n = 15$). Different letters indicate significant differences between the treatments for each growth stage according to Tukey's test at $p < 0.05$. Treatment: AO Ambient O₃ without biochar; AOB2.5 Ambient O₃ with 2.5% biochar; AOB5 Ambient O₃ with 5% biochar; EOB0 Elevated O₃ without biochar; EOB2.5 Elevated O₃ with 2.5% biochar; EOB5 Elevated O₃ with 5% biochar.

reaction centres of PSII are open, and thus increased F_0 characterizes damage and photoinhibition to PSII. Environmental stressors, including O₃, are well established to decrease variable fluorescence F_v

and maximum fluorescence F_m , due to disruption of thylakoids and also photo-inhibition (Basara and Gorzelany, 2025). Reduction in maximum quantum yield of PSII underscores reduced light energy

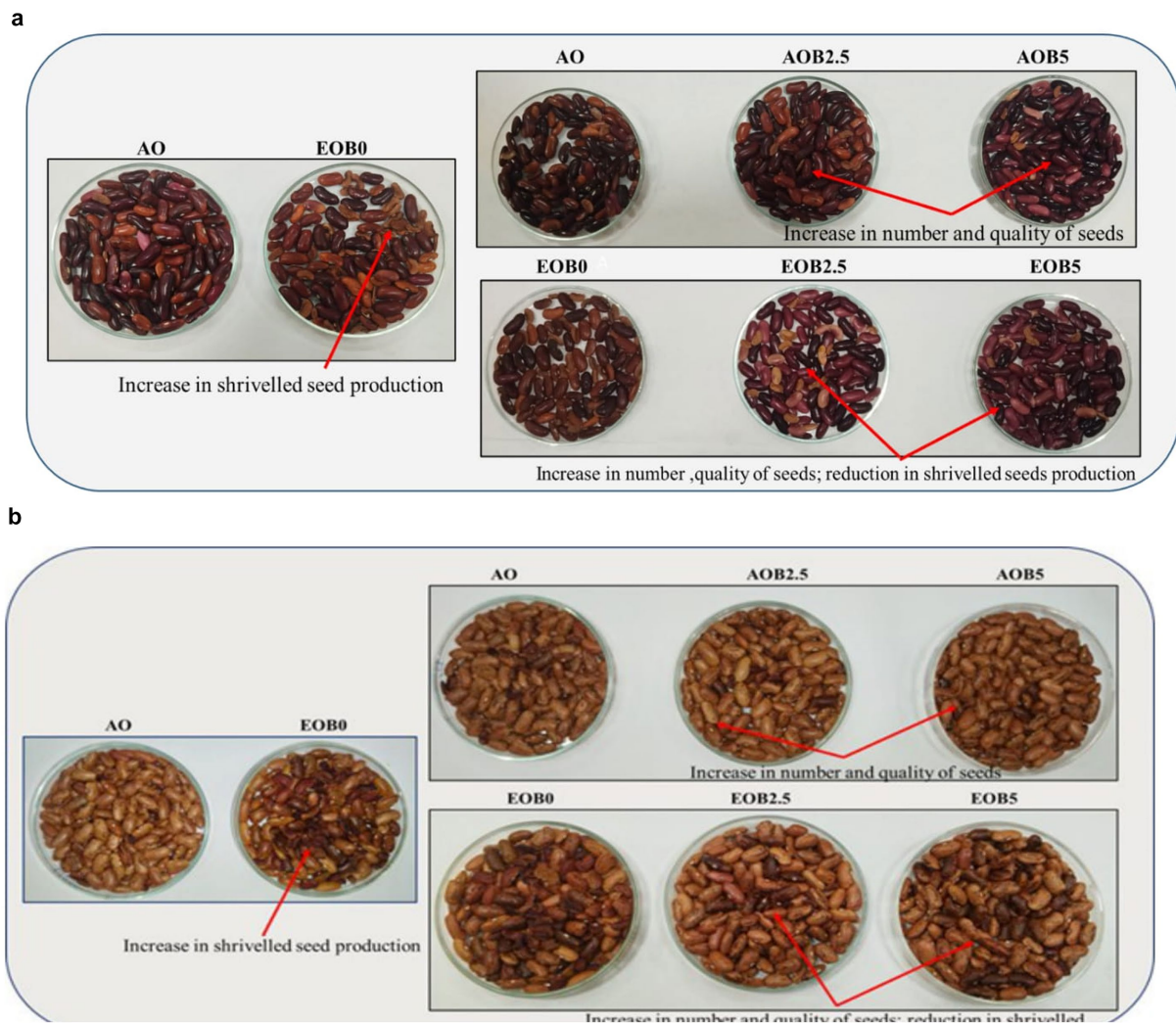


FIGURE 13

(a) Comparison of yield of HUR-137 of Rajma (*Phaseolus vulgaris* L.) plants under various treatments (AO, AOB2.5, AOB5, EOB0, EOB2.5, and EOB5). Treatment: AO Ambient O₃ without biochar; AOB2.5 Ambient O₃ with 2.5% biochar; AOB5 Ambient O₃ with 5% biochar; EOB0 Elevated O₃ without biochar; EOB2.5 Elevated O₃ with 2.5% biochar; EOB5 Elevated O₃ with 5% biochar. (b) Comparison of yield of PDR-14 of Rajma (*Phaseolus vulgaris* L.) plants under various treatments (AO, AOB2.5, AOB5, EOB0, EOB2.5 and EOB5). Treatment: AO Ambient O₃ without biochar; AOB2.5 Ambient O₃ with 2.5% biochar; AOB5 Ambient O₃ with 5% biochar; EOB0 Elevated O₃ without biochar; EOB2.5 Elevated O₃ with 2.5% biochar; EOB5 Elevated O₃ with 5% biochar.

conversion efficiency of PSII under O₃. Increased ROS has been noted by Bhattacharjee (2019) and Guo et al. (2024) to cause oxidative damage to cell organelles and impact photochemical efficiency. HUR-137 also presents higher F_v/F_m values under O₃ stress, implying superior photochemical efficiency of PSII and tolerance to cellular oxidative burst. The partial restoration of photochemical efficiency and improved efficiency of PSII under biochar can be attributed to detoxification of ROS and reduction of cellular oxidative stress. These observations were confirmed in *Vicia faba* (L.) grown under drought stress (Abd El-Mageed et al., 2021), *Medicago ciliaris* under water-deficient conditions (Gharred et al., 2022) and peanut under a 2-year field experiment (Wang et al., 2021), with increased F_v/F_m under biochar supplementations ascribed to improved water status and Na⁺, K⁺ contents in soil and plants.

O₃ stress is recorded to accelerate proteolysis and protein oxidation, likely due to oxidative destruction of cellular components

and obstruction of nitrogen assimilation pathways (Wu T. et al., 2023). Such observations are also echoed in our study with declines in activities of NR and NiR in both the test cultivars. Protein content can serve as a key marker of nitrogen assimilation in plants (Dell'Aversana et al., 2021). NR catalyses the conversion of NO₃⁻ into NO₂⁻ and NiR catalyses NO₂⁻ to NH₄⁺; therefore, a drastic decline in their activity hampers amino acid metabolism, representing oxidative damage to enzymatic proteins and metabolic reallocation toward stress response to the detriment of growth and metabolism. Interestingly, biochar supplementation lowered the O₃-generated suppression of NR and NiR activities, with EOB5 offering better protection. HUR-137 showed higher NR and NiR activities under both ambient and elevated O₃ levels, suggesting stronger metabolic resilience and efficient nitrogen metabolism pathways. The et al. (2021) emphasized that maintenance of NR and NiR activities is critical for the sustenance of amino acid and protein biosynthesis.

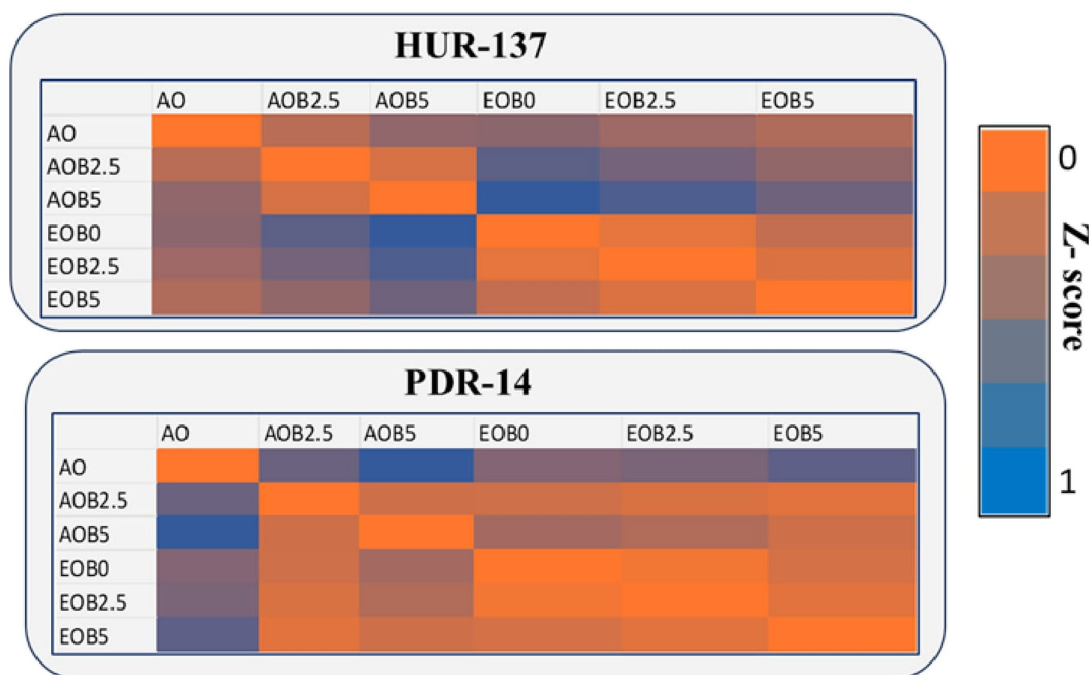


FIGURE 14

Heat map generated based on Euclidean similarity and dissimilarity index. Higher shades of red denote values closer to 0 (lower distance) and indicate greater similarity between treatments, whereas bluer shades signify higher values and distances (closer to 1), highlighting dissimilarity between treatments. (Treatment: AO Ambient O₃ without biochar; AOB2.5 Ambient O₃ with 2.5% biochar; AOB5 Ambient O₃ with 5% biochar; EOB0 Elevated O₃ without biochar, 60,825 Elevated O₃ with 2.5% biochar; EOB5 Elevated O₃ with 5% biochar).

Huang et al. (2024) corroborated the biochar-mediated boost in antioxidative defense via increased shoot N uptake and nitrogen assimilation against heat stress. Biochar amendment in the soil has been noted to increase soil NO₃ availability, leading to improved nitrogen assimilation in sugar beets (Chen et al., 2022). Increased nitrogen bioavailability has been linked to improved photosynthetic performance, *Fv/Fm* values, and chlorophyll pigments (Chen et al., 2022; Ullah et al., 2021). Our study echoed this observation, where biochar fertilization significantly lessened the O₃-led chlorophyll and carotenoids loss. Exposure to O₃ stress caused drastic reductions in total chlorophyll and carotenoid contents, with PDR-14 exhibiting a 30% reduction relative to AO, compared to a 24.4% decrease in HUR-137 at the reproductive stage. However, EOB5 plants showed significant recoveries, with total chlorophyll levels improving by 8.9% in HUR-137 and 15% in PDR-14 relative to EOB0. Such reductions were greater at reproductive stages, primarily because at reproductive stages, plants prioritize allocation of metabolic resources toward reproductive appendages and success (Monson et al., 2022). Increased stabilization of pigments and proteins under biochar can be attributed to contribute in improvements in photochemical efficiency under biochar amendment. The concomitant increase in total chlorophyll and carotenoids reflects this inference as these pigments play crucial role in light harvesting, energy transfer and photoprotection (Zhang et al., 2012).

Biochar-induced retention of chlorophyll content can be attributed to well-studied increased phytoavailability and mobility of nutrients, especially Mg²⁺ and N to the plants (Hu et al., 2023). Additionally, better protection of the thylakoid membrane and reduced oxidative stress under biochar fertilization are also significant for preserving

chlorophyll. The chlorophyll a/b ratio is particularly significant as a key marker for photosystem stoichiometry and stress tolerance. Chla/b dropped significantly under O₃ conditions while biochar fertilization resulted in partial restoration of Chla/b, with ameliorative impacts contingent upon the concentration of biochar applied. Under ambient O₃ levels, biochar led to a similar increment in Chla/b in both cultivars. A decline in Chla/b indicates decreased photosynthetic efficiency and potential alterations in light-harvesting complexes (LHCs) (Simkin et al., 2022), as PSII, which is abundant in Chlorophyll a, bears maximum photooxidative damage under O₃ stress (Vannini and Petraglia, 2024). In contrast, LHCs containing Chlorophyll b are less affected under elevated O₃ conditions (Lee et al., 2021). In addition to being an integral component of light-harvesting complexes, carotenoids perform photo-protective roles for chloroplasts by dissipating excess excitation energy via non-photochemical quenching (NPQ) (Beltrán and Wurtzel, 2024). Reductions in carotenoid content under high O₃ rendered the test plant's chloroplasts vulnerable to photo-oxidative damage.

Anthocyanins are secondary metabolites that, in addition to imparting characteristic red colour to *Phaseolus vulgaris* seeds, provide strong antioxidative properties (Choi et al., 2022). Higher amounts of anthocyanin were found in elevated O₃ conditions, suggesting an increased protective role of anthocyanin against photooxidative damage and strengthened membrane integrity. Interestingly, HUR-137 showed consistently high amounts of anthocyanins and better retention of carotenoids across all treatments, indicating an inherent robust and stronger antioxidative defense potential. Notably, production of anthocyanins increased more at 80 DAG in PDR-14 compared to 40 DAG, implying a

strategic allocation of resources toward protective roles at the reproductive stage. The percent increase of anthocyanins was higher in PDR-14, elucidating a greater allocation of metabolic resources toward defensive functions under high O₃ levels. Biochar further significantly enhanced anthocyanins and carotenoids in both the cultivars, contributing to the mitigation of redox imbalance, particularly at 5% w/w application rates. Higher anthocyanin content has been established to minimize visible foliar injury in the plants exposed to O₃ (de Rezende and Furlan, 2009). The present investigation confirms the maximum protection from foliar injury in treatments exhibiting higher levels of anthocyanin and antioxidant production.

4.3 Growth dynamics, biomass allocation, and yield stabilization

Plant foliar appendages, such as the number of leaves and leaf area, are key components of the source-sink relationships, where mainly mature leaves photosynthesize and ensure photo-assimilates availability for biomass fixation (Upadhyay et al., 2025).

Observed impairments in leaf area effectively compromised the synthesis of photo-assimilates under O₃ stress. Ozone-induced foliar injury was manifested as chlorotic and necrotic spots, leading to impairment of source capacity, which further impacted sink limitation, lowering metabolic resource availability. Such injuries were more prominent in PDR-14, under ambient and elevated O₃ levels, compared to HUR-137. Foliar damage under O₃-stress has also been noted by Upadhyay et al. (2025) in *Vigna radiata* (L.), Ansari et al. (2021) in *Sida cordifolia* (L.), Ramya et al. (2021) in rice, and Tisdale et al. (2021) in soybean. Foliar injury progressed with the developmental phases of plants, and greater injury was inflicted on mature leaves, thereby further reducing photosynthetic rates and efficiency of photosystems. This was reflected in reduced chlorophyll fluorescence and chlorophyll content of O₃-stressed plants. Better maintenance of leaf area and a lesser degree of foliar injury in HUR-137 ensured sustained photo-assimilate production and translocation toward flowering. This was reflected in less reductions in the number of flowers and petal area in HUR-137 under O₃ fumigation, while PDR-14 showed greater reductions in floral organs. Moreover, as a consequence of source restrictions, total biomass decreased under high O₃, with significant reductions observed in both root and shoot biomass.

However, root biomass declined more when compared to shoot biomass, as reflected in the decrease of the root-shoot ratio. Root-shoot ratio is a significant index for O₃ stress, illustrating prioritization of C allocation under stress (Gu et al., 2023). Greater retention of root-shoot in HUR-137 further highlighted its greater resistance to physiological and biochemical perturbations induced by high O₃ levels. Biochar application at 5% augmented photo-assimilate fixations at the source, enabling greater access for below-ground biomass. These results echoed our previous work on mungbean (Upadhyay et al., 2025), wherein biochar induced improvements of assimilates at source, leading to more allocation toward roots. Jabbarova et al. (2021) confirmed these observations on biochar-amended Okra (*Abelmoschus esculentus*) plants, noting significant enhancements across all biometrics of growth against drought stress. Plant height and root length also increased with the higher dose of biochar, implying

partial correction in biomass fixation and realignment of metabolic resources toward growth and reproduction. Such biochar-induced improvements in plant height and root length have been noted in tomato against salinity (Kul et al., 2021), *Brassica napa* (L.) under drought stress (Lalay et al., 2022), rice under water-deficit (Chen et al., 2021), and cereals under salt stress (Ababsa et al., 2023).

Ameliorative effects of biochar can be credited to an interplay of physicochemical, biochemical, and physical attributes that enhance plant's resilience to oxidative burst generated by O₃ stress. Increased activities of SOD, APX, CAT, GR and phenolics have been ascribed to improved nutrient phyto-availability and mobility (Ghosh et al., 2021). Biochar has been noted to enhance pH, Bulk Density, microbial biomass C and N, EC, and water retention capacity of salinity (Rathinapriya et al., 2025), O₃ (Ghosh et al., 2021), and drought-stressed soils (Wu T. et al., 2023). Alleviation of oxidative stress under biochar has been ascribed to improved TCA cycle, GSH metabolism, sucrose, and fatty acids synthesis in wheat (Sun et al., 2023) and *Pennisetum* (Jia et al., 2025). Jia et al. (2025) credited a reduction in ROS to improved functionality of the electron transport chain and, consequently, the respiratory activity of the plant. Biochar used in the present study had a high pH, EC and CEC of 7.9–8.2, 1.4–1.5 dS m⁻¹, and 16–18 cmol kg⁻¹, respectively, which concomitant with bioavailability of nutrients, especially Mg²⁺ and N, might have improved protein content and chlorophyll content (Abid et al., 2017). In addition, availability of nutrients in biochar such as total phosphorus (1.9–2.1 g kg⁻¹), total potassium (24–26 g kg⁻¹), calcium (11–13 g kg⁻¹), and magnesium (0.45–0.51 g kg⁻¹) have been associated with improved ionic balance and stomatal regulation, helping in maintenance of osmotic adjustments and membrane integrity under O₃ stress (Farouk and Al-Huqail, 2022). The average pore diameter (2.1 nm) and specific surface area (211.5 m² g⁻¹) in the present study enhanced soil aeration and water-holding capacity, moderating soil moisture fluctuations (Wang et al., 2021). Coupled with improved cell membrane structure and function, biochar fertilized plants showed improvements in quantum yield of PSII, suggesting less photoinhibition and dissipation of light energy into heat. Yield formation in crop plants is an outcome of the intricate interplay of source-sink relationships, C assimilation, metabolic resource allocation, and reproductive development (Cakmak and Engels, 2024). Despite the drastic loss in several flowers and petal area under O₃ stress (Figure 6), biochar fertilization alleviated O₃-induced phytotoxicity in *Phaseolus vulgaris* (L.), ensuring a greater degree of reproductive success. These observations are also validated by Liu et al. (2022) and Tadesse et al. (2025) in faba bean (*Vicia faba* L.), noting improvement in flowering, plant growth and yield formation. Biochar-treated test plants registered an increased number of pods and their length, likely due to enhancements in leaf area, chlorophyll contents, and PSII functionality, ensuring sustained metabolic resource availability.

High O₃ stress, reflected by the highest values of total AOT40 in February (Figure 1), around the time of seed-setting, adversely impacted yield formation in both cultivars of kidney beans. O₃ has been documented to alter photosynthetic assimilate partitioning, leading to poor seed filling and formation of shriveled seeds (Upadhyay et al., 2025). ROS generated in plants have been linked with increased ovule abortion and lowered success of fertilization (Hauser et al., 2025). Furthermore, increased cost of defense and

greater allocation of metabolic resources toward the antioxidative defense system reduced assimilate availability for reproductive development. These reductions were more prominent in O₃-stressed PDR-14, which registered a higher percent increase in enzymatic and non-enzymatic antioxidant activity at the reproductive stage. Nonetheless, despite more pronounced stimulation of antioxidants in PDR-14, HUR-137 recorded higher levels of antioxidative enzyme activity and free radical scavenging. Impacts on cellular redox balance and reproductive success were reflected in terms of GWP, in which HUR-137 registered lesser losses compared to PDR-14. Moreover, PDR-14 showed a large number of shriveled seeds under EOB0 treatments, echoing our previous observations in mungbean (Upadhyay et al., 2025). Impact on seed endosperm water potential and sugar content has been associated with the production of wrinkled seeds (Chugh and Sharma, 2022). Ozone has been recorded to adversely impact phloem loading and sugar transport toward the sink, contributing to impaired seed development (Polle et al., 2023).

Intriguingly, biochar concentration played a pivotal role in the efficacy of maintaining yield. Biochar concentration of 5% consistently provided superior antioxidative protection by higher stimulation of antioxidative enzymes, anthocyanin production, and minimizing cellular damage. This dose-dependent response of biochar was supported by PLS biplot (Supplementary Figures 5, 6), illustrating a positive gradation of treatments from EOB0 to AOB5, suggesting dose-dependent ameliorative impacts on kidney beans. Beneficial impact of biochar particularly at higher doses could be facilitated by augmented N uptake and assimilation pathways (Wang et al., 2021). Our findings highlight a multifactorial protective impact of biochar on *Phaseolus vulgaris* (L.) against tropospheric O₃. Differential responses of HUR-137 and PDR-14 signify the inherent greater resilience of HUR-137 against oxidative stress. Between cultivars, the greater impact of biochar on PDR-14 was deciphered by Euclidean similarity indices (Figure 14), suggesting a greater scope of yield preservation in O₃-sensitive cultivars.

Despite agro-economic significance of present study for O₃-sensitive cultivars, several critical gaps remain unaddressed and should be focused on by future research. The study focused on short-term single season responses under controlled conditions, long-term field trials across multiple seasons are required to validate the persistence and scalability of the observed effects. The molecular mechanism underlying biochar's beneficial impacts should be examined. Genome-wide association study (GWAS) to pinpoint loci imparting O₃ resilience to HUR-137 can further shed more light on the study. Metabolite profiling of kidney beans under O₃ and biochar treatments can provide deep insights into regulatory networks for oxidative stress and its alleviation by biochar. Interactions of *Phaseolus vulgaris* (L.) plants and soil, especially in the rhizosphere, should be studied to find a clear picture of biochar's impact on its growth and yield.

5 Conclusion

The conducted study conclusively demonstrates that soil amendment with biochar at 5% concentration (w/w) alleviates O₃-induced redox imbalance in *Phaseolus vulgaris* (L.), by enhancing

PSII functionality and boosting enzymatic and non-enzymatic antioxidants. Biochar maintained membrane stability by lowering lipid peroxidation and solute leakage rate. These improvements translate into realignment of photosynthetic assimilates toward reproductive parts, resulting in an increased number of flowers with petal area. HUR-137 displayed greater intrinsic tolerance to O₃-generated oxidative stress compared to PDR-14, across all measured parameters. Biochar supplementation significantly improved O₃-induced growth retardation and enhanced biomass fixation, root-shoot dynamics, thereby strengthening reproductive sink potential and maintenance of yield, with maximal efficacy observed under higher biochar concentrations. Key yield parameters including podsno. Plant⁻¹, seeds plant⁻¹, GWP noted enhancements after biochar application, suggesting partial restoration of reproductive efficiency and yield stability under O₃ stress. Seed formation and pod characters improved under biochar supplementation, suggesting yield-enhancing potentials of biochar application in O₃-polluted areas. Hence, the present study advocates for the integration of biochar as a sustainable crop management strategy in O₃-stressed agroecosystems to maintain crop productivity and enhance the resilience of agroecosystems for strengthening food security in the context of changing climatic patterns. The present findings substantiate the potential of biochar as a scalable soil amendment (5%) to mitigate O₃-yield losses particularly for sensitive cultivar (PDR-14) in highly O₃ polluted Indo-Gangetic plains of India. However, the present study evaluated short-term, single-season responses under controlled conditions, therefore, long-term field trials over multiple seasons are necessary to endorse the persistence, and scalability of the observed effects. Future researches should focus on long-term field validation, rhizosphere interactions and molecular mechanisms underlying biochar-mediated modulation of redox signaling and nitrogen metabolism.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

VU: Methodology, Investigation, Conceptualization, Visualization, Writing – original draft, Data curation. KC: Validation, Writing – review & editing, Formal analysis, Conceptualization, Visualization. MA: Writing – review & editing, Formal analysis, Visualization, Conceptualization. SA: Investigation, Conceptualization, Supervision, Writing – review & editing, Validation, Visualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2025.1634511/full#supplementary-material>

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