



RESEARCH ARTICLE

Contrasting effects of elevated CO₂ and O₃ on physiology, growth and yield of rice (*Oryza sativa* L.)

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Abstract

Rising ambient levels of greenhouse gases such as carbon dioxide, with 421 ppm average concentration of tropospheric ozone with levels of 25–60 ppb, have raised concerns about food security globally. Of the food grains consumed globally, rice (*Oryza sativa* L.) is under threat due to the rapid increase in tropospheric ozone levels and the crop's relative ozone sensitivity. In South India, rice is the most consumed food grain and in this regard, this study systematically evaluated the responses of the widely cultivated, high-yielding rice cultivar ASD-16 to elevated concentrations of CO₂ (550 ± 15 ppm) and O₃ (100 ± 10 ppb), administered daily from 10:00 to 17:00 hr over 30 days spanning the critical reproductive stage. Elevated CO₂ exerted significant positive effects, enhancing net photosynthetic rates by 22, 56 and 99 % at 60, 70 and 80 days after sowing (DAS), respectively, relative to elevated O₃ exposure. Concurrently, stomatal conductance and transpiration rates exhibited significant suppression under elevated CO₂ conditions, reflecting CO₂-mediated stomatal regulation, while relative chlorophyll content mirrored photosynthetic enhancements, indicating improved physiological performance. Comprehensive assessments of plant physiological, growth and yield parameters and their interrelationships, revealed strong correlations between metabolic functions and plant productivity and showed that leaf injury percentage, a key O₃ damage metric, has strong predictive power for reductions in photosynthetic performance. These findings demonstrate the contrasting physiological and agronomic impacts of elevated CO₂ and O₃, emphasising the need to consider their interactive effects when projecting future climate change scenarios for rice production.

Keywords: abiotic stress; food security; ImageJ; open top chamber; physiology

Introduction

Rice (*Oryza sativa* L.), a principal staple crop, exhibits pronounced sensitivity to escalating climate change stressors, including drought, extreme temperatures and fluctuating environmental regimes, resulting in substantial yield reductions (1). These multifaceted pressures disrupt plant ionic homeostasis, compromising survival and productivity. Compounding this challenge, global population projections anticipate a ~25 % increase to 10 billion by 2050, while climate-induced declines have curtailed yields of major food crops by over 50 % worldwide (2, 3). Such perturbations adversely affect crop ontogeny, reproductive success and overall yield architecture, posing serious threats to global food security (4). As the preeminent cereal grain, rice contributes 21 % of global caloric intake, surpassing wheat, maize and potato in consumption in Asia, especially South Asia, with projections indicating a 3.2 % productivity decline per 1 °C temperature rise (5, 6). Developing climate-resilient cultivars thus necessitates elucidating plant stress response mechanisms capable of mitigating both biotic and abiotic adversities (7).

Since the industrial revolution, atmospheric concentrations

of CO₂ and tropospheric O₃ have risen markedly, with current CO₂ levels having reached 421 ppm in 2025 compared to the pre-industrial baselines of 280 ppm (8). Elevated CO₂ fosters beneficial growth responses by augmenting photosynthetic capacity, functioning as a vegetative growth promoter that enhances biomass accumulation and yield potential (9). Conversely, tropospheric O₃, a potent phytotoxicant formed through photochemical oxidation of precursors including carbon monoxide and methane, represents a formidable trace greenhouse gas exerting profound deleterious effects on vegetation. Documented since the 1940s, O₃'s deleterious effects stem from stomatal ingress, triggering excessive reactive oxygen species (ROS) generation within leaf apoplasts, culminating in oxidative burst and programmed cell death (10). Threshold concentrations of 4–7 ppb suffice to induce cellular damage, yet prevailing global averages of 50–80 ppb render O₃ a critical threat to sensitive crops like rice (11).

In this context, the present study investigates the comparative impacts of elevated CO₂ (eCO₂- 550 ppm) and elevated O₃ (eO₃-100 ppb) on the South Indian staple cultivar ASD-16, addressing physiological responses, growth traits and yield

attribute metrics under ambient (control), $e\text{CO}_2$ and $e\text{O}_3$ exposure. This study carries the following hypothesis in the outcomes of the study: Simulation of $e\text{CO}_2$ improves photosynthetic efficiency and reduces water loss and exposure to $e\text{O}_3$ decreases the overall yield due to altered photosynthetic activity of the cultivars. By bridging the often-overlooked continuum from plant production to consumer quality, this research fills a critical knowledge gap in climate change simulations for C_3 crops.

Materials and Methods

Experiment site and plant materials

The experiment was conducted within three open-top chambers (OTCs) situated at the TNAU wetlands, Tamil Nadu Agricultural University, Coimbatore, from 2nd December 2024 to 25th August 2025 (11°00'13.0" N latitude and 76°55'42.0" E longitude and an altitude of 430.5 m above msl). The study utilised the ASD 16 rice cultivar (parentage ADT 31/CO39), a prominent short-duration, high-yielding variety (5600 kg ha⁻¹), widely adopted in South India, characterised by a growth cycle of 110–115 days.

Three-week-old seedlings, raised in nursery conditions, were transplanted into the OTCs located at A1 block of TNAU wetlands following a factorial randomised block experimental design. The rice plants were subjected to an average ozone-weighted concentration of 100 ppb and elevated CO_2 at 550 ppm from 51–80 days after sowing (DAS), equivalent to a 30-day exposure period from 10:00 to 17:00 hr daily. Ozone was administered at a feed rate of 1.2 mg min⁻¹ in the treatment chamber (Fig. 1), yielding an accumulated AOT40 of 10 ppm hr⁻¹, while elevated CO_2 levels were precisely regulated via a SCADA-based automated system, maintaining concentrations at 550 ppm (± 15 ppm) of the target (12). Control chambers received no supplemental gas treatments, recording baseline ambient ozone at 6 ppb and CO_2 at 421 ppm.

Plant analysis

Physiological parameters

Physiological parameters were assessed through non-destructive measurements of net photosynthetic rate (A), stomatal

conductance (gs) and transpiration rate (E) using the portable LCpro-SD photosynthesis system (ADC BioScientific Ltd., UK) and five randomly selected plants ($n = 5$) from each OTC gas treatment. Measurements were standardised at leaf cuvette CO_2 concentration of 550 ppm, leaf temperature of 28 °C and photosynthetic photon flux density (PPFD) of 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Relative chlorophyll content was quantified using a handheld CCM - 200 + chlorophyll content meter (Apogee Instruments, USA), providing values proportional to actual leaf chlorophyll concentration. All assessments were conducted under optimal solar conditions between 10:00 and 14:00 hr, following 30 days of exposure to $e\text{O}_3$ and $e\text{CO}_2$ treatments in respective chambers. Readings were obtained from three distinct positions on the third fully expanded youngest leaf per plant, with mean values calculated for statistical analysis.

Foliar injury interpretation

Rice cultivars exposed to $e\text{O}_3$ conditions exhibited characteristic foliar injury symptoms, including necrosis, brown spotting, chlorosis and bronzing, which collectively imparted a pale discolouration to the leaves. Foliar damage was quantified using established criteria for leaf bronzing and injury percentage as described previously (13, 14). For a comprehensive assessment of lesion characteristics, quantitative parameters-including lesion count, total injured area, mean lesion size and percentage of affected leaf area-were determined through image analysis employing ImageJ software (15).

Plant growth traits and yield parameters

At crop maturity, growth and yield attributes were recorded from five randomly selected plants ($n = 5$) per rice cultivar from each gas treatment OTC, encompassing plant growth traits such as: root length, plant height, panicle length and yield parameters such as: tiller number per plant, effective tiller number per plant, grain filling percentage and 1000 grain weight. To ensure measurement precision, five additional randomly selected plants per treatment were preserved intact throughout the experiment, avoiding prior destructive sampling for intermediate analyses.

Statistical analysis

All statistical analyses were conducted using Origin 2025 software. Two-way analyses of variance (ANOVA) were employed to evaluate the significance of treatment (control, $e\text{CO}_2$ and $e\text{O}_3$) at 10-day

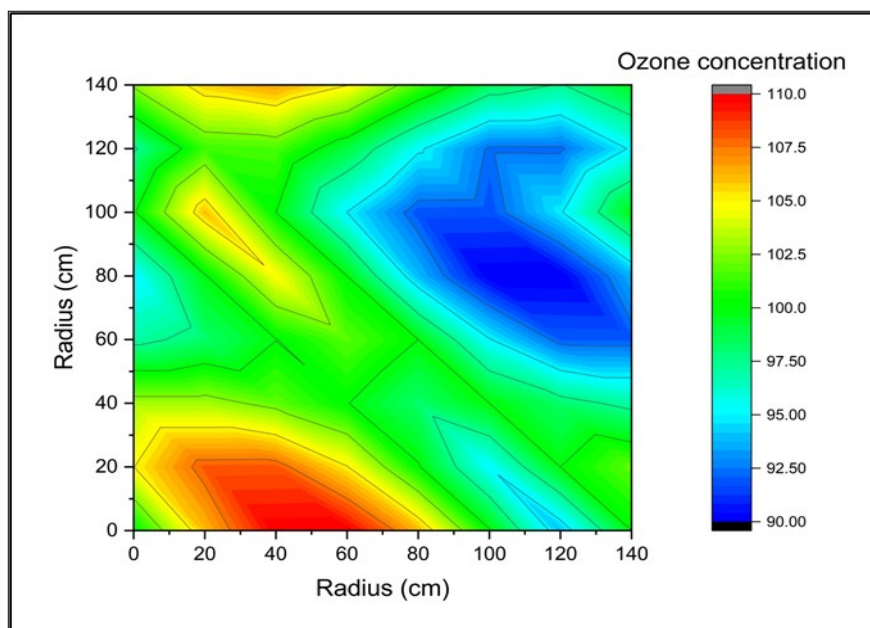


Fig. 1. Contour graph presenting varying $e\text{O}_3$ concentration in OTC; measurements were taken at 20 cm above the canopy height.

intervals (60, 70 and 80 DAS) on physiological, growth and yield parameters, with statistical significance determined at $p \leq 0.05$. Post-hoc comparisons of treatment means were performed using the Tukey-Kramer honestly significant difference (HSD) test to discern specific pairwise differences among treatments. Multivariate ordination was performed using principal component analysis (PCA) to assess correlations among physiological parameters under different gas treatments. Surface plots were used to visualise the interrelationships among selected physiological and yield traits across treatments. All graphical representations were generated using GraphPad Prism (version 10.4.2) to ensure publication-quality visualisation of statistical outcomes.

Results

Visible foliage injury

The physiological markers of stress are often indicated visually on both adaxial and abaxial sides of leaves. Elevated ozone-induced visual markers of stress are linked to the formation of leaf necrosis, chlorosis and bronzing over the leaf surface, quantified using leaf injury percentage and leaf bronzing score and digital injury analysis.

Leaf injury percentage

Leaf injury was quantified with visible symptoms on a 0–100 % scale across adaxial and abaxial surfaces. Under eO_3 (100 ppb, 30 days during flowering), ASD 16 exhibited pronounced foliar injury across all five replicates, in the form of bronzing, rusting and necrotic lesions. Mean leaf injury was 60.2 ± 3.3 % (range: 56–65 %), with individual replicate values of 56, 59, 60, 65 and 61 % (Table 1). No visible injury symptoms were observed under eCO_2 and ambient control conditions.

Leaf bronzing score

Leaf bronzing was quantified on a standardised scale of 1–10. Visible bronzing symptoms were consistently observed across replicates, with scores of 6, 7, 8, 6 and 6 for all the five plants, respectively, yielding a mean leaf bronzing score (LBZ) of 6.6 (Table 1). Bronzing patterns corroborated the percentage-based injury assessments, confirming uniform oxidative damage expression. No bronzing occurred under eCO_2 and control conditions. Conversely, plants exposed to eCO_2 and ambient control conditions exhibited no

Table 1. Foliar injury-based scoring under eO_3 exposure

Plant number	Leaf injury percentage (LIP)	Leaf bronzing score (LBZ)
1	56	6
2	59	7
3	60	8
4	65	6
5	61	6

visible bronzing symptoms, demonstrating the protective effects of eCO_2 . Quantified leaf bronzing scores are presented in Table 1.

Leaf injury prevalence

To assess the temporal progression of ozone-induced foliar injury, third leaves from the rice cultivar ASD 16 were collected at 60, 70 and 80 DAS during the panicle initiation stage under one month of eO_3 exposure. Leaf samples were meticulously washed, dried and subjected to quantitative injury analysis using ImageJ software,

enabling precise measurement of lesion count, injury area, average lesion size and percentage leaf area affected. Progressive intensification of foliar damage was evident with prolonged ozone exposure. From 60–70 DAS, the foliar injury count increased by 122 %, the injury area expanded by 29 %, average lesion size grew by 50 % and the percentage of affected leaf area escalated from 0.98–2.7 %. This trend intensified further from 70–80 DAS, with injury count rising by 21 %, increasing total injury area from 1.098–2.205 cm^2 , while lesion size was at 0.003 (Table 2). These

Table 2. Leaf injury prevalence under eO_3 exposure

Components	60 DAS	70 DAS	80 DAS
Injury count	358	798	965
Injury area (cm^2)	0.851	1.098	2.205
Average size of lesion	0.002	0.003	0.003
Leaf area covered by injury (%)	0.98	2.73	3.47

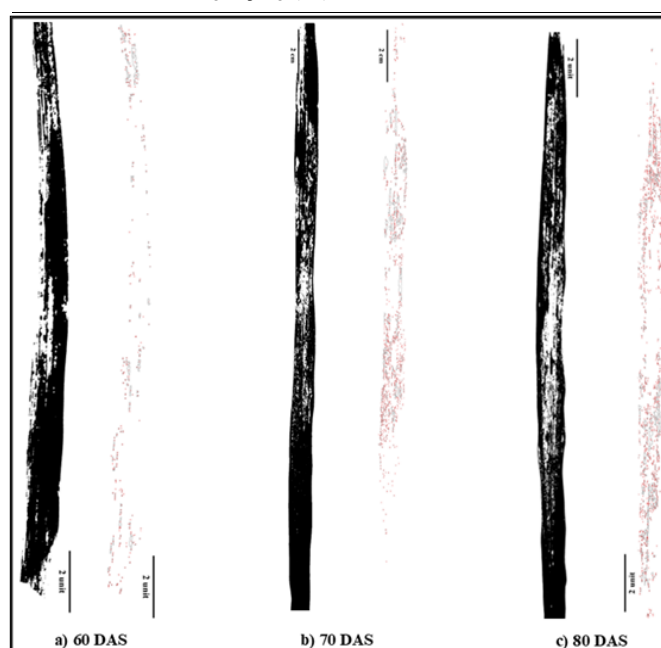


Fig. 2. Development of foliar injury under eO_3 from 60–80 DAS (days after sowing).

quantitative metrics highlight the phytotoxic impact of eO_3 on rice foliage during reproductive development. Representative images are presented in Fig. 2.

Physiological parameters

Photosynthetic rate (A)

Photosynthetic rates of the cultivar were measured at 60, 70 and 80 days after sowing (DAS) across treatments. Under eCO_2 , A increased by 17 % at 60 DAS, 28 % at 70 DAS and 27 % at 80 DAS relative to ambient controls. Progressive reduction of A under eO_3 by 4 % at 60 DAS, 18 % at 70 DAS and 36 % at 80 DAS was reported when compared to control condition cultivars. Variations among eCO_2 reported improved A rate ($\mu mol CO_2 m^{-2} s^{-1}$) by 22.5 (22 %) at 60 DAS, 31.3 (56 %) higher at 70 DAS and 29.2 (99 %) higher at 80 DAS (Table 3) relative to cultivars grown under eO_3 exposure. Temporal variations in photosynthetic rates across treatments are shown in Fig. 3.

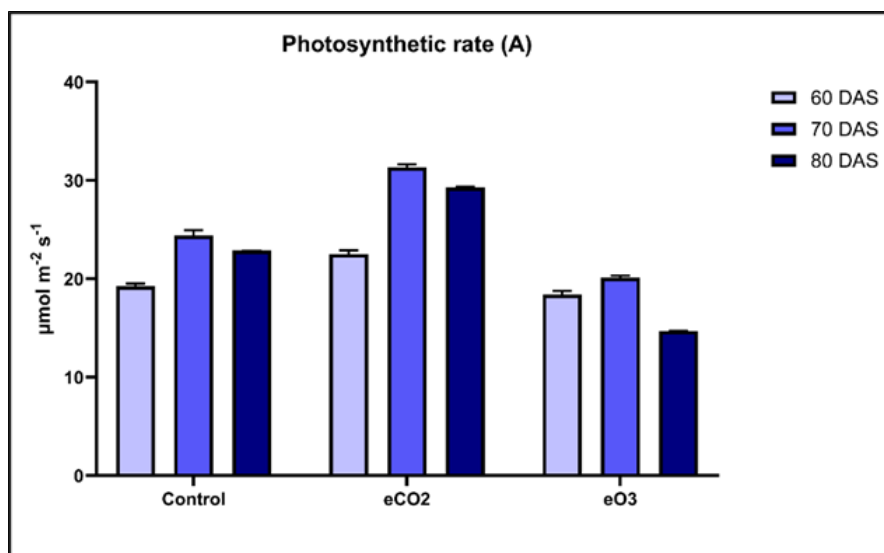
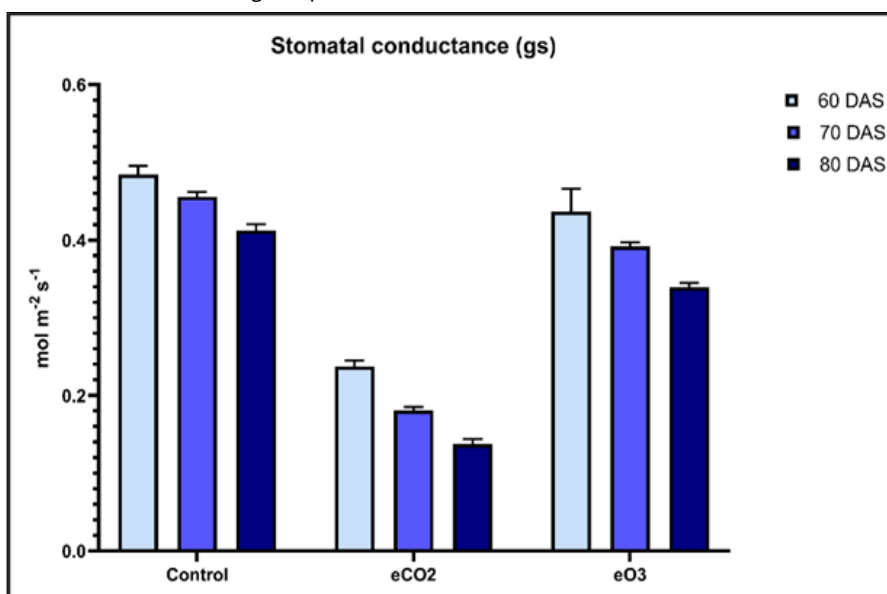
Stomatal conductance (gs)

Stomatal conductance under eCO_2 declined progressively with plant ontogeny, registering 52 % lower values at 60 DAS, 60 % lower

Table 3. Physiological parameters under gas exposures from 50 to 80 DAS

	Control			Elevated CO ₂			Elevated O ₃		
	60 DAS	70 DAS	80 DAS	60 DAS	70 DAS	80 DAS	60 DAS	70 DAS	80 DAS
A	19.2 ± 0.13 ^f	24.4 ± 0.24 ^c	22.8 ± 0.0 ^d	22.5 ± 0.17 ^d	31.3 ± 0.13 ^a	29.2 ± 0.03 ^b	18.3 ± 0.16 ^g	20.1 ± 0.11 ^e	14.6 ± 0.0 ^h
gs	0.48 ± 0.00 ^a	0.45 ± 0.0 ^b	0.41 ± 0.0 ^{cd}	0.23 ± 0.0 ^f	0.18 ± 0.0 ^g	0.13 ± 0.0 ^h	0.43 ± 0.01 ^{bc}	0.39 ± 0.00 ^d	0.33 ± 0.00 ^e
Chl	34.2 ± 0.15 ^f	37.2 ± 0.00 ^d	39.1 ± 0.02 ^c	36.12 ± 0.21 ^e	40.6 ± 0.00 ^b	42.6 ± 0.00 ^a	24.5 ± 0.15 ^g	23.4 ± 0.0 ^h	18.5 ± 0.0 ⁱ
E	5.5 ± 0.08 ^a	5.1 ± 0.02 ^b	5 ± 0.00 ^{bc}	3.2 ± 0.03 ^e	2.5 ± 0.16 ^f	1.4 ± 0.11 ^g	5.1 ± 0.02 ^b	4.7 ± 0.04 ^c	3.9 ± 0.06 ^d

A: Photosynthetic rate; gs: Stomatal conductance; Chl: Chlorophyll content; E: Transpiration rate. Different letters indicate statistically significant differences ($p \leq 0.05$).

**Fig. 3.** Photosynthetic rate of cultivar ASD 16 under gas exposure.**Fig. 4.** Stomatal conductance of cultivar ASD 16 under gas exposure.

at 70 DAS and 68 % lower at 80 DAS compared to ambient control conditions (Fig. 4). This temporal attenuation reflects CO₂-induced stomatal closure, a well-characterised physiological adaptation to eCO₂ concentrations. Appropriately, eO₃ exposure resulted in substantially higher gs relative to eCO₂ treatments across all sampling dates, though gs remained consistently below ambient control levels. Specifically, gs under eO₃ was 0.43, 0.39 and 0.33 H₂O m² s⁻¹, exhibiting an increase by 87, 116 and 153 % at 60, 70 and 80 DAS compared to corresponding eCO₂ values (Table 3). These divergent stomatal responses underscore the antagonistic physiological interactions between elevated CO₂ and O₃ on rice gas exchange dynamics.

Chlorophyll content

Chlorophyll pigmentation serves as a fundamental indicator of photosynthetic capacity and overall plant health, with higher chlorophyll content manifesting as enhanced leaf colouration (16). Under eCO₂ exposure, chlorophyll content increased progressively: 5 % at 60 DAS, 9 % at 70 DAS and 8 % at 80 DAS relative to ambient controls, reflecting the carbon fertilisation effect and improved photosynthetic performance (Table 3). Conversely, eO₃ exposure drastically suppressed chlorophyll accumulation (CCI) and reductions of 24.5 (-32 %) at 60 DAS, 23.4 (-42 %) at 70 DAS and 18.5 (-57 %) at 80 DAS compared to eCO₂ (Fig. 5). Notably, chlorophyll

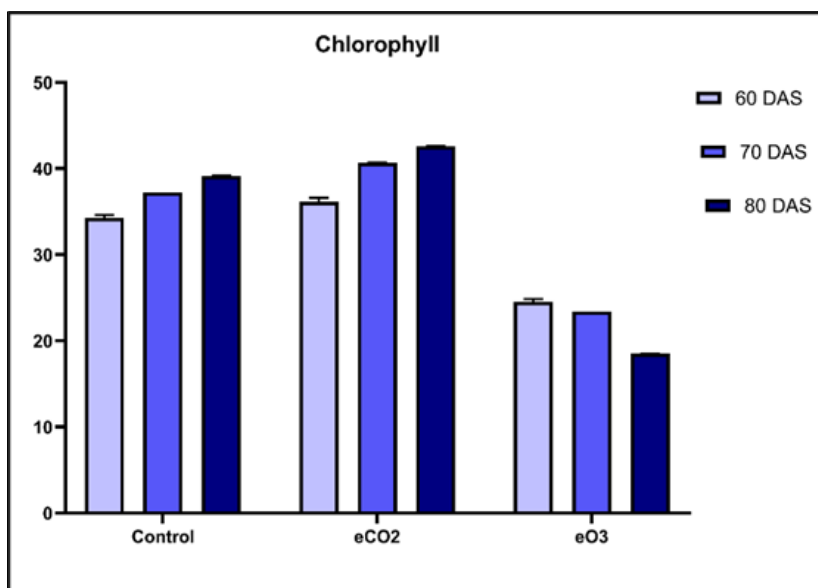


Fig. 5. Chlorophyll levels of cultivar ASD 16 under gas exposure.

content increased cumulatively with developmental stage under both eCO₂ and ambient control conditions, whereas progression under eO₃ exhibited progressive decline, reflecting the temporal intensification of ozone-induced phytotoxicity.

Transpiration rate (E)

Leaf transpiration rate (E), as a primary indicator of water loss from foliar surfaces, is directly regulated by stomatal conductance. Cultivars under eCO₂ exposure substantially suppressed transpiration, with values (mmol m⁻² s⁻¹) and reductions of 3.2 (-42 %) at 60 DAS, 2.5 (-51 %) at 70 DAS and 1.4 (-72 %) at 80 DAS compared to controls, reflecting stomatal closure mediated conservation of water resources. Conversely, eO₃ maintained transpiration rates significantly higher than eCO₂ conditions throughout reproductive development: 59 % higher at 60 DAS, 88 % at 70 DAS and 178 % at 80 DAS, as represented in Fig. 6 and Table 3.

Growth attributes

Root length

Root length, a key indicator of belowground resource acquisition capacity, was measured for rice cultivar ASD 16 across experimental treatments at harvest. Under ambient control conditions, plants

exhibited a mean root length of 11.2 cm, establishing the baseline for comparative morphological analysis (Table 4). A significant enhancement of root length was observed under eCO₂, with mean root length reaching 15.2 cm, a 36 % increase relative to controls. This substantial promotion of root growth reflects enhanced carbon assimilation and preferential allocation of photoassimilates to belowground biomass under eCO₂ exposure conditions. Conversely, eO₃ exposure markedly suppressed root development, resulting in a mean root length of 8.9 cm, representing a 21 % reduction compared to ambient controls. The diminished root elongation under eO₃ reflects phytotoxic effects of tropospheric ozone on root meristematic activity and cellular expansion, consistent with oxidative disruption of auxin-mediated growth processes.

Plant height

At physiological maturity (80 DAS), rice cultivar ASD 16 attained a height of 94 cm under ambient control conditions. Gas enrichment treatments produced differential morphological responses. Simulation of eCO₂ modestly enhanced shoot height by 2 cm (to 96 cm), reflecting increased photosynthetic carbon fixation and enhanced biomass accumulation. Contrarily, eO₃ exposure

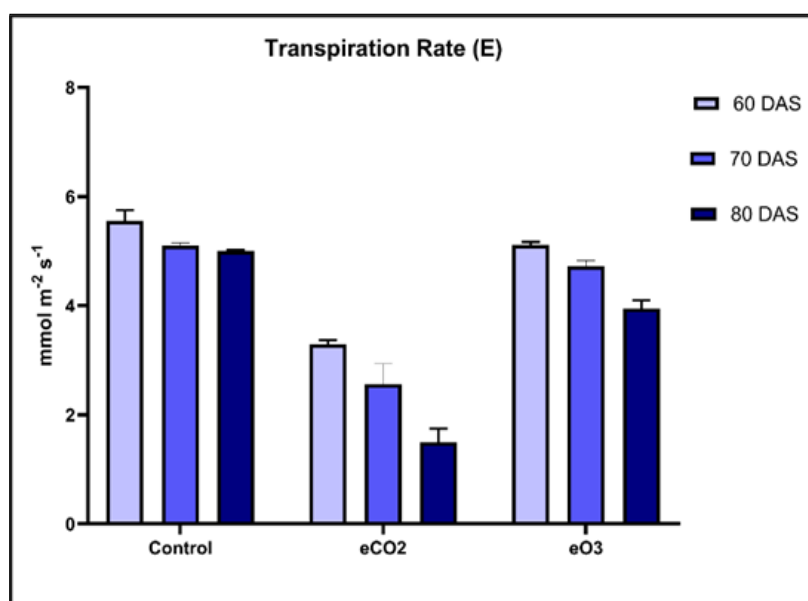


Fig. 6. Transpiration rate of cultivar ASD 16 under gas exposure.

Table 4. Growth attributes by cultivar under gas simulation

Treatments	Root length (cm)	Plant height	Panicle length
Control	11.2 ± 0.5	94 ± 0.5	22.7 ± 0.0
Elevated CO ₂	15.2 ± 0.2	96 ± 1.2	23.5 ± 0.1
Elevated O ₃	8.9 ± 0.3	81.3 ± 0.6	15.41 ± 0.1

markedly suppressed plant height, reducing stem length by 13 cm relative to controls (final height: 81 cm), representing a 13.8 % reduction. Relative to eCO₂ conditions, the O₃-exposed plants were further stunted by 15 cm, as reported in Table 4.

Yield parameters

Number of tillers and effective tillers

Yield parameters under eCO₂ showed significant improvements, such as enhanced tillering capacity in rice cultivar ASD 16, increasing total tiller number from 7 tillers per plant under ambient controls to 9 tillers per plant (a 28.6 % increase). Of these 9 tillers, 8 proved effective (88.9 % effectiveness), indicating that eCO₂ enrichment not only promotes tiller initiation but also maintains high spikelet fertility and grain-filling potential per tiller. Under ambient conditions, 6 out of 7 tillers were effective (85.7 %), representing the baseline fertility profile in well-managed growth conditions as illustrated in Fig. 7. Conversely, eO₃ exposure markedly suppressed both tiller formation and reproductive competence. Ozone-stressed plants produced only 5 total tillers per plant (a 28.6 % reduction), yet only 2 of these tillers proved effective in grain production (40 % effectiveness), representing a 58 % decrease in the number of productive tillers compared to ambient controls.

Grain filling of rice grains

Grain-filling status was assessed using the standard water flotation method, wherein filled grains sink to the bottom of the water column due to higher density, while unfilled (immature or chalky) grains remain floating. Under eCO₂ conditions, the rice cultivar produced predominantly filled grains at a ratio of 92 filled: 8 unfilled (92 % grain-filling percentage), reflecting enhanced photosynthetic carbon assimilation and improved source-sink carbon translocation to developing grains. Under ambient control conditions, the ratio was 82:18 (82 % filled), establishing a realistic baseline for normally managed rice. Conversely, eO₃ exposure dramatically reduced grain-filling capacity, with only 61 filled:39 unfilled grains (61 % filling percentage), representing a 21 % point

decline relative to ambient controls and a 31 % point deterioration compared to eCO₂-grown plants, as illustrated in Fig. 7.

1000-grain weight

Rice grain weight, assessed via 1000-grain weight as a rapid quality and yield component measure, serves as a critical indicator of grain development and harvest quality. Under ambient control conditions, the cultivar produced 1000 grains weighing 21.4 g. Enhancement of grain weight under eCO₂ exposure increased the 1000-grain weight by 9 % to approximately 23.3 g, reflecting improved photosynthetic carbon assimilation and enhanced grain-filling capacity (17) and conversely decreased to 14.2 g under eO₃ exposure as illustrated in Fig. 7.

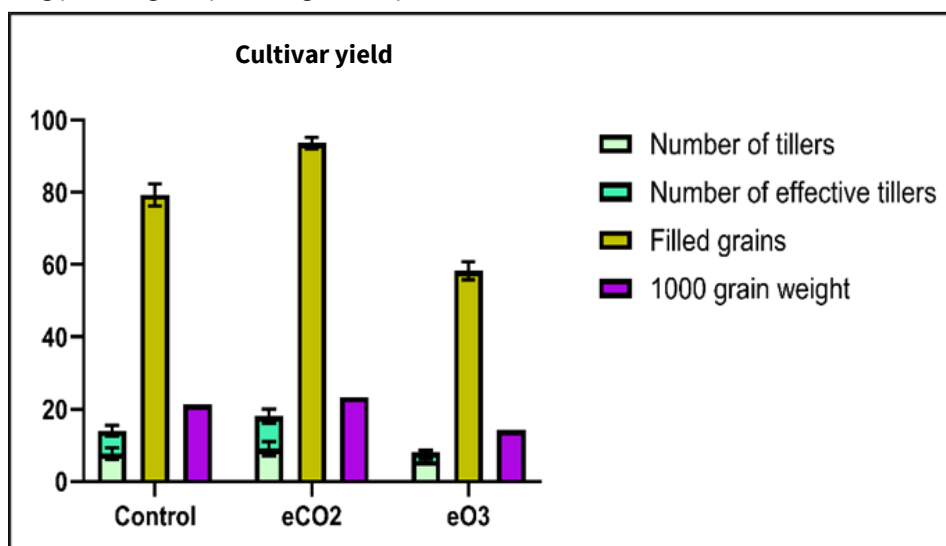
Discussion

Visible foliage injury

Leaf injury and leaf bronzing under eO₃ are indicators of disruptions in hormonal regulation and carbohydrate metabolism, leading to compromised membrane integrity and impaired plant function and premature senescence. These symptoms arise from excessive accumulation of ROS such as O₂^{•-} (superoxide anions), •OH (hydroxyl radicals) and H₂O₂ (hydrogen peroxide) in the mesophyll and apoplastic regions of the leaves, resulting in visible rusting, necrotic lesions and leaf bronzing (18–21). Leaf bronzing indicates loss of chlorophyll pigments, manifesting as bronze discolouration, in contrast to the characteristic green colouration of healthy leaf tissue (22, 23). Conversely, plants grown under eCO₂ and ambient conditions exhibited no visible signs of foliar injury. Elevated CO₂ levels are known to mitigate oxidative stress by modulating physiological parameters, including stomatal conductance and photosynthetic efficiency, hence maintaining chlorophyll pigments (24, 25).

Physiological parameters

Elevated CO₂ substantially enhanced photosynthetic rates in rice cultivar, recording 17, 28 and 27 % increases at 60, 70 and 80 DAS, respectively, relative to ambient controls. This augmentation stems from improved Rubisco carboxylation efficiency, reduced photorespiration (typically 20–50 % yield loss in C₃ crops like rice) and greater electron transport capacity under assimilated carbon availability (26–28). Contrarily, eO₃ induced progressive

**Fig. 7.** Yield variations under different gas simulations.

photosynthetic inhibitions by: 4, 18 and 36 % reductions at successive stages, through ROS-mediated photodamage to PSII reaction centres and chloroplast dysfunction. Notably, $e\text{CO}_2$ exerted profound amelioration by elevating photosynthetic rate by 22, 56 and 99 % relative to $e\text{O}_3$, thereby restoring source strength and facilitating superior source-sink carbon partitioning during critical reproductive phases (29).

Stomatal conductance (g_s), which governs stomatal aperture and regulates atmospheric gas fluxes into leaf intercellular spaces, responded divergently under $e\text{CO}_2$ and $e\text{O}_3$. Under $e\text{CO}_2$ conditions, g_s exhibited pronounced reductions, as consistently documented across studies reflecting $e\text{CO}_2$ -mediated stomatal closure (30, 31). This adaptive response minimises water loss through transpiration, substantially enhancing water use efficiency, a critical advantage for crop production generally and rice specifically (8, 32). In contrast, $e\text{O}_3$ disrupted stomatal regulation through characteristic stomatal sluggishness, marked by impaired responsiveness and erratic opening-closing dynamics (33). The dynamics of stomatal closure under stress conditions are determined through abscisic acid (ABA) induced cellular signalling, followed by Ca^{2+} and ROS (34). The production and accumulation of apoplastic ROS occur in ABA-mediated stomatal closure (35). Besides ABA, Ca^{2+} participates in stomatal closure through increased concentrations of cytosolic Ca^{2+} inducing stomatal closure (36). Hence, under $e\text{O}_3$ stress, the cellular signalling promotes stomatal closure as a coping mechanism, which, however, alters the

photosynthetic processes and increases senescence (29). This dysfunction generates a physiological paradox: while cumulative ROS-induced oxidative damage and hormonal perturbations suppress photosynthetic carbon assimilation, they simultaneously trigger compensatory stomatal opening to boost carbon uptake via increased g_s as represented in Pearson correlation among physiological factors and treatments in Fig. 8 (23). Paradoxically maladaptive, this response amplifies $e\text{O}_3$ influx into mesophyll tissues, intensifying phytotoxicity. Consequently, the canonical Ball-Woodrow-Berry (A- g_s) relationship, wherein photosynthetic rate and stomatal conductance remain tightly coordinated, breaks down, culminating in depressed net photosynthesis, compromised biomass accumulation and diminished grain yield (22).

This also results in chlorophyll degradation under $e\text{O}_3$, resulting from the synergistic effects of impaired photosynthetic carbon assimilation and ozone-induced stomatal dysfunction. Despite reduced photosynthetic rates, $e\text{O}_3$ -exposed plants maintained abnormally higher g_s due to stomatal sluggishness, thereby perpetuating excess $e\text{O}_3$ flux into the intercellular spaces. This sustained $e\text{O}_3$ uptake drives excessive ROS accumulation in the chloroplast, triggering preferential degradation of chlorophyll a and b and consequent loss of leaf pigmentation and disruption of PSII quantum efficiency (ΦPSII , Fv/Fm') (37). Under $e\text{O}_3$, a higher transpiration rate, despite reduced photosynthetic capacity, reflects ozone-induced stomatal sluggishness that impairs proper stomatal closure, leading to inefficient water utilisation. Hence, increased

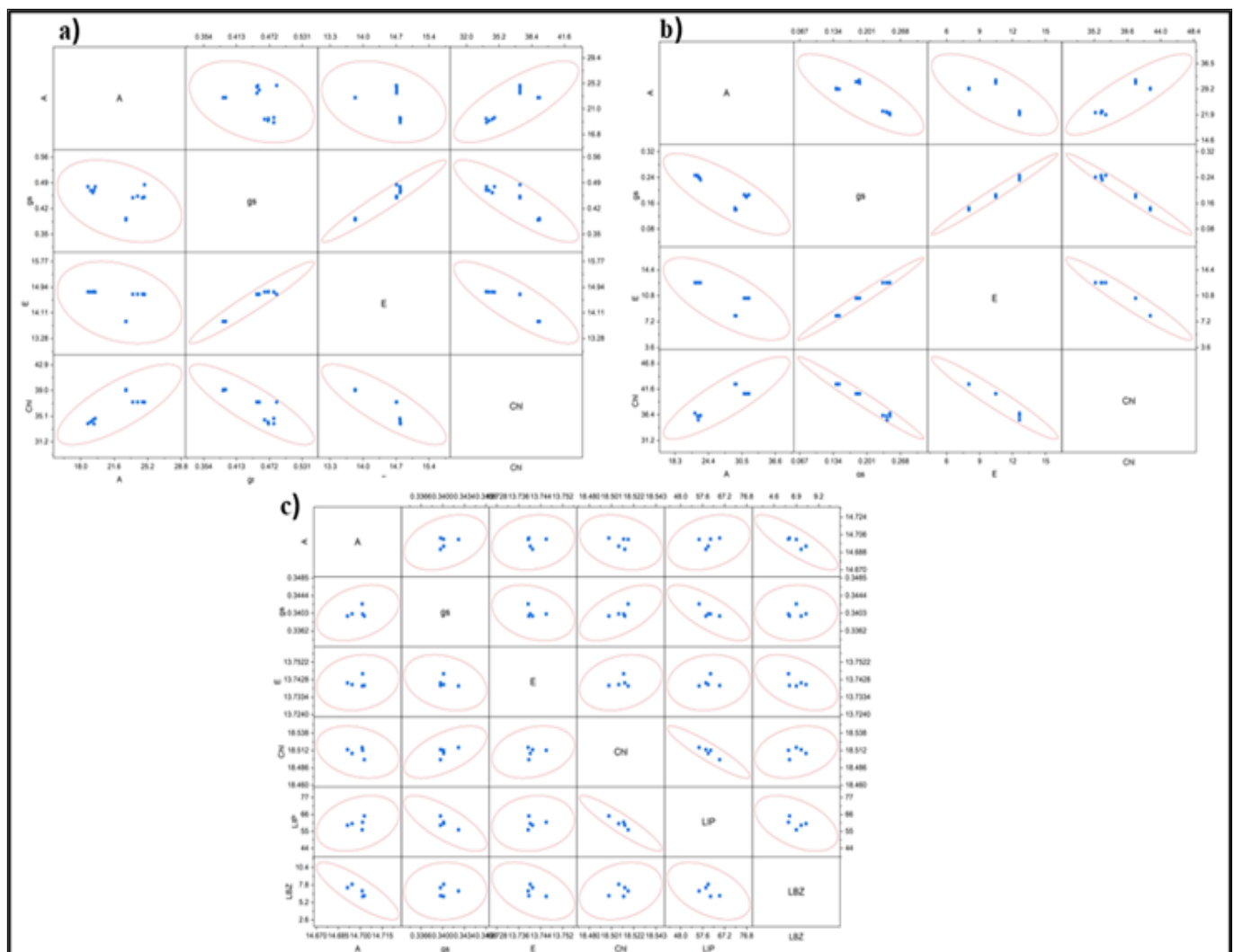


Fig. 8. Pearson correlation coefficient among physiological factors in a. Control; b. $e\text{CO}_2$ and c. $e\text{O}_3$.

transpirational rate coupled with impaired carbon assimilation dramatically reduces water use efficiency, synergistically constraining plant biomass accumulation and physiological traits (20).

Yield parameters

Yield parameters under $e\text{CO}_2$ exposure had a positive impact on all the yield components. The improvement in the photosynthetic rate and lower stomatal conductance aided in less transpiration loss, which enhanced the photosynthetic process in the cultivar. This resulted in a 28 % increase in tillers and improved production of effective tillers when compared against ambient control conditions. Elevated CO_2 enhances carbohydrate production and mobilises additional photosynthates towards the shoot (38). This remobilisation improves the carbon-to-nitrogen ratio through improved source-sink synchronisation with carbon flow associated among source and sink organs and connected transport pathways (39). Apparently, detrimental effects were observed under $e\text{O}_3$ exposure, wherein $e\text{O}_3$ severely impaired reproductive development: effective tillers aborted due to spikelet differentiation failure; grain filling collapsed (39 % unfilled grains) from pollen sterility, phloem failure and senescence; and 1000-grain weight fell 50 % (14.2 g) via defective starch deposition (19, 40–42). This occurs as $e\text{O}_3$ curtails productivity through accelerated senescence, along with reducing carbohydrate assimilate transport to reproductive sinks, hence lowering final yield (43). This yield cascade, with fewer fertile sinks, spikelet sterility and less grain weight, translates physiological phytotoxicity into harvest failure under $e\text{O}_3$.

Multivariate analysis

Principal component analysis (PCA) of the physiological parameters revealed that the first two principal components (PC1 and PC2) collectively accounted for 97.7 % of the total variance, with PC1 explaining 75.39 % (eigenvalue = 3.015) and PC2 contributing 21.81 % (eigenvalue = 0.872). The biplot illustrates the loadings of these variables as directional vectors emanating from the origin, wherein PC1 delineates a primary axis of discrimination characterised by contrasting contributions of key gas-exchange traits (Fig. 9). Transpiration rate (E) exhibited a strong positive loading (0.516), whereas stomatal conductance (gs; 0.516) and chlorophyll levels (Chl; -0.438) displayed substantial negative loadings, indicating an inverse relationship along this axis. On PC2,

Table 5. Scree plot eigenvalues from correlation matrix for physiological responses

Sl. No	Eigenvalue	Percentage of variance
1.	3.015	75.39
2.	0.872	21.81
Extracted eigenvectors		
Physiological factors	Coefficients of PC1	Coefficients of PC2
Photosynthetic rate	-0.524	0.360
Stomatal conductance	0.516	0.464
Transpiration rate	0.516	0.463
Chlorophyll levels	-0.438	0.662

all variables loaded positively, gs (0.464), E (0.463) and particularly Chl (0.662), highlighting a coordinated secondary gradient associated with pigment status and associated physiological processes independent of the primary gas-exchange axis (Table 5).

Interpreted in the context of plant physiological responses and gas simulations, the distribution of treatments in PCA reflects distinct functional strategies. Control samples clustered in the positive quadrant of both PC1 and PC2, indicating a coordinated and balanced physiological state characterised by higher gs, E and A activity. However, $e\text{CO}_2$ exposure samples were positioned predominantly along the negative side of PC1, opposite to the gas-exchange vectors, suggesting an efficient water-use strategy marked by reduced gs and E, with a relatively weak association between stomatal behaviour and photosynthetic performance.

Plants exposed to $e\text{O}_3$ were distributed toward the positive side of PC1 but negatively along PC2, indicating proximity to the gas-exchange axis but a clear separation from chlorophyll-associated variation. This positioning suggests a disrupted physiological coordination, where stomatal activity does not correspond with overall functional integrity. The negative association along PC2 further reflects reduced chlorophyll status, consistent with stress-induced impairment. Collectively, the PCA indicates that $e\text{CO}_2$ induces a regulated reduction in stomatal activity, whereas $e\text{O}_3$ exposure leads to a loss of coordination among key physiological traits, reflecting contrasting plant response strategies under these environmental conditions.

The response surface plot analysis revealed complex interactions between gs and A on grain weight and chlorophyll content across different gas simulations (Fig. 10). Under $e\text{CO}_2$, grain weight demonstrated a non-linear response pattern, increasing progressively with photosynthetic rate across the measured stomatal conductance range ($0.13\text{--}0.23\text{ H}_2\text{O m}^{-2}\text{s}^{-1}$), reaching maximum values of approximately 23.3 g per 1000 grains at higher photosynthetic rates ($29.24\text{--}31.3\text{ }\mu\text{mol CO}_2\text{ m}^{-2}\text{s}^{-1}$). Conversely, $e\text{O}_3$ stress induced substantial photoinhibition, leading to a marked decline in chlorophyll content, imperative to the oxidative stress-induced increase in leaf injury percentage. Control conditions exhibited intermediate grain weight values of 21.4 g per 1000 grains with a more stable response surface, suggesting that optimal photo-assimilation rates ($19.2\text{--}24.4\text{ }\mu\text{mol CO}_2\text{ m}^{-2}\text{s}^{-1}$) occurred at lower stomatal conductance values ($0.41\text{--}0.48\text{ H}_2\text{O m}^{-2}\text{s}^{-1}$). The differential response patterns between $e\text{CO}_2$ and $e\text{O}_3$ underline the contrasting mechanisms governing yield-related traits under oxidative stress, wherein $e\text{O}_3$ -induced impairment of the photosynthetic process substantially constrains the translocation of photosynthates to

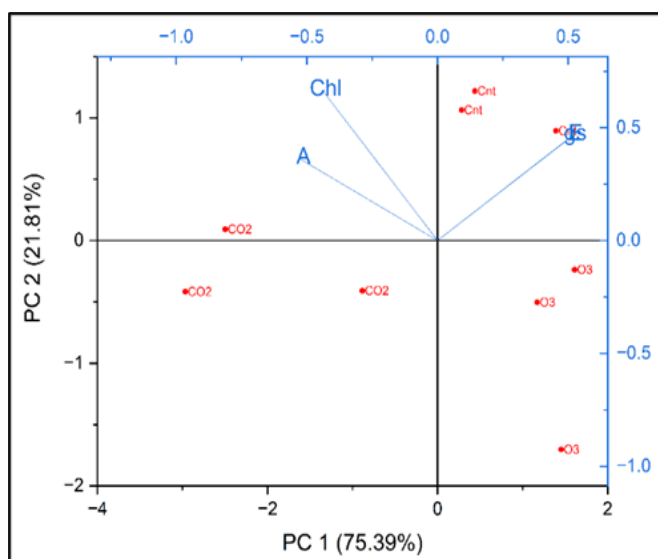


Fig. 9. PCA biplot illustrating physiological parameters across varying exposure conditions.

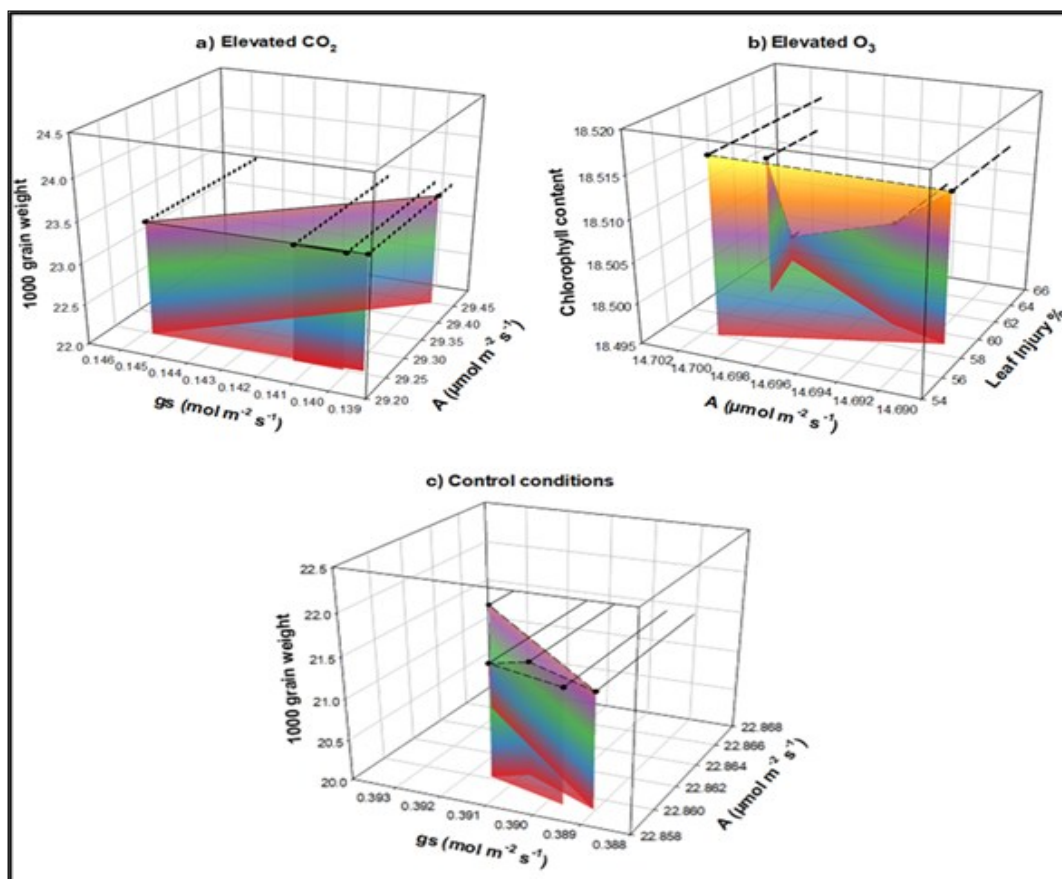


Fig. 10. Surface plot inter-relating physiological and yield parameters under: a. eCO₂; b. eO₃; c. Control conditions.

grain, thereby reducing final grain weight and quality parameters.

Conclusion

This investigation demonstrates that eCO₂ and eO₃ fluxes profoundly influence the physiological processes of rice cultivar ASD 16, resulting in significant alterations in photosynthesis, stomatal behaviour, chlorophyll status, growth attributes and yield components. The first hypothesis stands true as eCO₂ exposure exerted beneficial effects on overall plant performance, enhancing photosynthetic efficiency and resource allocation, reflecting resource use efficiency and abridged water loss through reduced transpiration rate by the cultivars. The second hypothesis supports that eO₃ imposed clear physiological constraints, particularly deterioration of chlorophyll status and disruption of coordinated gas-exchange processes, culminating in reduced growth and yield. From an applied perspective, these findings emphasise the need to prioritise traits related to stomatal regulation efficiency and O₃ tolerance in crop breeding and management strategies. Future research should focus on long-term, field-based evaluations of these individual stressors, with particular emphasis on genotype-specific variability and the identification of key physiological and yield-related traits associated with tolerance. Such efforts are essential for strengthening trait-based selection and improving the resilience and stability of rice production under future climate scenarios.

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Authors' contributions

NS contributed to writing original draft, conceptualisation and editing. AB Supervised the research and edited the manuscript. PD, SK, CK, KB and PKG contributed to conceptualisation, methodology, supervision and editing. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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