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Response of rice (*Oryza sativa* L.) cultivars to elevated ozone stress

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Abstract

The current study aimed to evaluate the cultivar specific variation in rice exposed to elevated ozone. Fifteen short duration rice cultivars were exposed to 50 ppb ozone for 30 days at reproductive stage. The physiological, biochemical, growth and yield traits of all test cultivars were significantly affected in response to elevated ozone. On average, ozone stress decreased tiller number by 22.52%, number of effective tillers by 30.43%, 1000 grain weight by 0.62 % and straw weight by 23.83% over control. Spikelet sterility increased by 19.26% and linear multiregression 3D model significantly fits the spikelet sterility and photosynthetic traits with the R^2 of 0.74 under elevated ozone. Principal Component Analysis with total variance of 57.5% by first two principle components categorized 15 rice cultivars into four major groups, ie., ozone sensitive (MDU6, TRY(R)2 and ASD16), moderately ozone sensitive (ASD18, ADT43 and MDU5), moderately ozone tolerant (ADT37, ADT(R)45, TPS5, Anna(R)4, PMK(R)3 and ADT(R)48) and ozone tolerant (CO51, CO47 and ADT36).

Key words: Rice cultivars; Elevated ozone; Plant response; Plant traits relationships; Principal component analysis.

Introduction

34

35 Tropospheric ozone is the third most important contributor of greenhouse radiative forcing
36 ($0.40 \pm 0.20 \text{ Wm}^{-2}$) after CO_2 and CH_4 (IPCC, 2013). Right from the industrial revolution until
37 today, there is a continuous alarming rise in ozone forming precursors (NO_x , VOC) in the
38 atmosphere, which in turn increases the tropospheric ozone (Monks et al., 2015). According to
39 Intergovernmental Panel on Climate Change (IPCC) fifth assessment report, the developing Asian
40 and African countries are in higher food security risk due to unplanned urbanization and
41 industrialization which favours tropospheric O_3 formation (Pachauri et al., 2014). In India, the
42 ozone forming precursor, NO_x level showed an increasing trend of 0.9ppb per year from 2010 to
43 2015 (Kumari et al., 2020).

44 The observations from 2005–2010 revealed that the highest trend of ozone increase about
45 3–5.6% per decade was observed over Indo Gangetic plains, while 1.2–2% per decade was noticed
46 over southern regions of India (Lal et al. 2012). Kumari et al. (2020) reported that the annual mean
47 ozone concentration increased by 19.2% from 2010-2015 over Indo Gangetic plains. In south
48 India, the maximum ozone concentration (56 ppb) was recorded in southern Tamil Nadu (Krishna
49 Sharma and Nagaveena, 2016) and 62 ppb at higher altitude of western ghats (Udayasoorian et al.,
50 2013) during summer season. The increasing trend of tropospheric ozone concentration
51 considerably affected a variety of plant diversity including forest (Feng et al., 2019), agricultural
52 (Fischer, 2019; Shao et al., 2020) and horticultural crops (Suganthi and Udayasoorian 2016; Yang
53 et al., 2017; Singh et al., 2018).

54 Rice, an important food crop of the world is susceptible to many pollutants particularly air
55 pollutants. The tropospheric ozone, causes considerable yield loss in rice (Pandey et al., 2015,
56 2018; Singh et al., 2018). Van Dingenen et al. (2009) reported 3.7 % yield loss in rice at global
57 level. In SARRC countries, rice is cultivated almost throughout the year with two major rice
58 growing seasons (rabi and kharif) which overlap with the peak ambient ozone concentrations (Frei,
59 2015; Ziemke et al., 2019). Several studies on rice crop revealed that elevated tropospheric ozone
60 (eTO_3) causes reduced photosynthetic rate and photosynthetic pigments, oxidative stress induced
61 bronzing symptoms and altered antioxidant metabolism (Li et al., 2017; Peng et al., 2018). These
62 physiological and biochemical stress leads to reduction in growth, biomass, tiller number, spikelets
63 number and grain yield (Akhtar et al. 2010; Jing et al., 2016; Shao et al., 2020).

Ozone induced damage to rice in India is estimated to be 2.1 ± 0.8 Mt which was sufficient to feed roughly 35 per cent of population in India (Ghude et al., 2014). Furthermore, in India annual loss of 0.3–6.7 million ton (0.3–6.3%) for rice crop is estimated based on the accumulated ozone over a threshold of 40 ppb (AOT40) and mean ozone for 7 h during the day (M7) (Lal et al., 2017). The crop economic loss is simulated using WRF-Chem model showed 8% relative yield loss for rice crop (Sharma et al., 2019).

Tropospheric ozone induced yield loss is important for countries like India with increased population and urbanization, which needs to be addressed urgently to maintain the food security. Only very few studies has been reported so far and to our knowledge there is no studies on eTO₃ impact on rice in southern part of India. Hence an experimental study was done to explore the response of rice genotypes to eTO₃.

Materials and Methods

Experimental site and ozone treatment

The fifteen short duration rice cultivars viz. ADT36, ADT37, ADT43, ADT(R)45, ADT(R)48, Anna(R)4, ASD16, ASD18, CO47, MDU5, PMK(R)3, Rice MDU6, Rice TPS5, Rice CO51 and TRY(R)2, which are cultivated in and around Tamil Nadu region were chosen for the present study. In order to maintain the elevated ozone concentration, the experimental study was carried out in Open Top Chambers located in the wetland (11.00° N, 76.92° E), Tamil Nadu Agricultural University, Coimbatore, India. The experimental soil were characterized as per the standard procedures and all the package of practices were followed as per the recommendation of TNAU (2019). During the experimental period, monthly mean maximum and minimum temperature reached to 32.7 and 17.8°C, respectively and maximum and minimum relative humidity attained to 98 and 19%, respectively. Experimental soil chosen for the study has a clay loam in texture and slightly alkaline pH (8.38) with an EC of 0.32 dS m⁻¹. Characteristics of soil were 0.53% organic carbon and 226, 12.4 and 287 kg ha⁻¹ of available N, P and K, respectively. The factorial experiment in completely randomized block design was followed in control and ozone chambers. For each rice cultivar, three replications were maintained for control and ozone treatment (N=90).

Open Top Chambers (control and ozone treatment) with a diameter of 3.5 m and height of 3.5 m was used for the study. Ozone generator (A4G, Faraday, India) was used for ozone production and the ozone emission was set at a distance of 30 cm above the plant canopy and the ozone concentrations inside the chamber were monitored using ambient ozone monitor (G09-O₃-3121). In addition the monthly mean maximum and minimum temperature and relative humidity was also recorded. The plants were exposed to ozone fumigation for 30 days (10.00 h-17.00 h) from 51 days after sowing to 80 days after sowing (reproductive stage). The daily average ozone concentration in ozone treatment chamber ranged from 46 to 56 ppb, to achieve 50 ppb O₃ (AOT40= 2.1 ppm.h) (Table S1, Supplementary Information). In control chamber, plants were grown equivalently without ozone and the concentration was <10 ppb.

Leaf visible symptom

After 30 days of ozone exposure at reproductive stage, whole plant with all leaves of each cultivar was taken for examination. The ozone induced damage was quantified by assigning a leaf injury percentage (LIP) from 0-100 (Chaudhary and Agrawal, 2013) and leaf bronzing score (LBS) from 0-10 (Ueda et al., 2015).

Physiological traits

A portable photosynthesis system (ADC BioScientific LCpro-SD System, UK) was utilized to quantify photosynthetic rate (A) and stomatal conductance (gs) and chlorophyll content meter (CCM-200+, USA) was used to assess chlorophyll content (Chl). After thirty days of ozone exposure, measurements were taken at three different points of third youngest fully expanded leaves and averages of three points were calculated.

Biochemical traits

Fresh fully expanded leaves were collected and pooled after 30 days of ozone exposure, for the analyses of malondialdehyde (Heath and Packer, 1968), proline (Bates et al., 1973) and ascorbic acid content (Keller and Schwager, 1977).

Growth and yield traits

The root length (RL), shoot length (SL), panicle length (PL), number of tillers (NTP), number of effective tillers per plant (NETP), number of spikelets per panicle (NSPi), number of filled spikelets per panicle (NFSPi), thousand grain weight (1000 GW) and straw weight (SWP) were measured at crop maturity stage for each treatment. Grains were soaked in water and number of floating and sunken spikelets was counted manually to determine filled and unfilled spikelets. Spikelet sterility (SS) was calculated as the number of sterile spikelets relative to the total number of spikelets.

Statistical analysis

All the statistical analyses were performed using the SPSS statistical package (SPSS Inc., version 16.0.0). One-way ANOVA (Analysis of variance) was used to test the effect of ozone on physiological, biochemical, growth and yield traits of 15 rice cultivars. Two-way ANOVA was used to test the treatment; cultivar and their interaction effect of various plant traits and Tukey-Kramer method was used to identify difference among treatment means. The ozone induced percentage reduction of yield traits over control were estimated by the following formula, $100 - [(ozone/control) \times 100]$. Shapiro-Wilk test was used to determine the normality of the data and linear multiregression analysis was used to fit the 3D model of the plant traits (Urban et al., 2017). The regression equation used in the 3D model was described in Table 1. The degree of correlation between leaf injury percentage, photosynthetic and yield traits were determined based on Pearson's correlation coefficient. P values less than 0.05 ($P < 0.05$) considered as significant. Principal component analysis (PCA) was performed in R software (Version 3.5.1) using all observed physiological, biochemical, growth and yield traits. SigmaPlot 14 and OriginPro 2019 (Version 9.6.5) were used to plot the graphs.

Result and discussion

Tropospheric ozone induced loss in rice production is still unknown in southern parts of India. It is important to generate data with reference to tropospheric ozone induced impact on rice cultivars that mostly growing in this region. In the present study, an average ozone concentration

of 50 ppb was fixed to mimic current ozone level over southern India with popularly growing short duration rice cultivars.

Leaf visible symptom

The leaf bronzing score and leaf injury percentage were worked out based on bronzing injury symptom which varied from 3 to 6 and 23.3 to 51.7%, respectively. Among the cultivars, MDU6 and TRY(R)2 showed high leaf injury percentage (51.7%) and leaf bronzing score of 6, while less leaf injury percentage (23.3%) and leaf bronzing score (3) were noticed in Anna(R)4 and PMK(R)3 (Table 2). The ozone-induced leaf injury symptom in present study may be due to entry of O₃ into the plant system via gas exchange during photosynthesis and breakdown of ozone into reactive oxygen species (ROS) in the apoplast which might have caused cell death and development of necrotic symptoms (Baier et al., 2005; Kangasjarvi et al., 2005). Few cultivars, Anna(R) 4 and PMK(R) 3 showed less injury symptoms might be related to enhanced antioxidant system compared to MDU6 and TRY(R)2. This was also correlated with higher lipid peroxidation. This result is consistent with the study by Wang et al. (2014) who reported that lesser bronzing symptoms were associated with improved antioxidant system in ozone tolerant rice and also presence of quantitative trait loci OzT9, which is responsible for leaf bronzing formation under ozone stress.

Physiological response

Reduction in stomatal conductance observed between 8.62% (Anna(R)4) and 29.31% (TRY(R)2) (Fig. 1). On average across all test cultivars, 21.35% reduction in stomatal conductance under elevated ozone stress would be related to controlling gas influx in leaf mesophyll region and closure of stomata with response to ozone stress (Fiscus et al., 2005). This stomatal closure also related with the production of ROS under ozone stress controls the activity of guard cell ion channels and protein kinase activity in stomata, resulting in the reduced stomatal conductance (Vainonen and Kangasjarvi, 2015). Similar results found by Pang et al. (2009) who reported that rice cultivars, Shanyou63 and Wuyunjing3 were significantly decreased stomatal conductance upto 36.7% under elevated ozone which were mediated via stomatal closure with response to elevated ozone stress.

The stomatal response to elevated ozone alters the photosynthetic capacity of the rice cultivars (Chen et al., 2011, Pandey et al., 2018). In photosynthetic rate, highest percent reduction was observed in ASD16 (26.78 %) and lowest in PMK(R)3 (11.11%) (Fig. 1). The reduction in stomatal conductance by ozone was proportional to decline in photosynthetic rate by 19.23% in current study was related to stomatal limitation in all rice cultivars. These stomatal limitations directly inhibited the photosynthetic CO₂ fixation in the plant system. It is also correlated with the report of Masutomi et al. (2019) who confirmed the fact that elevated ozone altered the linear relationship between stomatal conductance and net photosynthetic rate. Consistently, the observation by Akhtar et al. (2010) confirmed a significant reduction in photosynthetic rate with the maximum of 66.3% in Bangladeshi rice cultivars under 100 ppb ozone were not only attributed to damage of photosynthetic enzyme but also ozone induced stomatal closure.

Reduction in chlorophyll content varied between 17.04% (Anna(R)4) and 35.08% (Rice MDU6) in present study (Fig. 1) ultimately decreased photosynthetic pigments, especially chlorophyll located in thylakoids which was 27.19% reduction in present study might results from lipid peroxidation in PSII reaction center undoubtedly affects the light harvesting efficiency of all test rice cultivars (Ueda et al., 2015; Jing et al., 2016). The report of Li et al. (2017) correlated with current result that both SY63 and Bt-SY63 rice cultivars reduced chlorophyll content under elevated ozone stress was associated with degenerated chloroplasts which mediates changes in carbon assimilation cycle. This observed physiological disorder under elevated ozone stress causes photosynthetic instability which results in alteration of sub-cellular, cellular, plant organ and whole plant level.

Thirty days of ozone exposure at reproductive stages significantly reduced all physiological traits. A significant treatment and cultivar interaction effect were noticed in stomatal conductance and chlorophyll content; while there was no interaction effect observed in photosynthetic rate.

Biochemical response

Oxidative stress leads to damage in membrane lipids of plant system measured by malondialdehyde concentration (Ueda et al., 2013; Li et al., 2017) which used as important indicating parameter for assessing ozone stress by measuring lipid peroxidation. The percentage increment of MDA content was highest in MDU6 (191.62%) and lowest in CO51 (50.00%) in present study (Fig. 2). MDA content increased by 121.43% over control indicating altered reactive

oxygen species metabolism in ozone exposed plants. Higher the concentration of reactive oxygen species generated in ozone exposed plant leads to membrane damage and cell death (Frei, 2015). Correspondingly, Ashrafuzzaman et al. (2017) reported that a rice variety, Nipponbare significantly increased MDA content under elevated ozone confirmed continuous accumulation of ozone inside the leaves consequently generate ROS mediated lipid peroxidation.

A non-enzymatic antioxidant, proline acts as scavenger of singlet oxygen and hydroxyl radicals in response to environmental stress (Rejeb et al., 2014). In current study, increment in proline content varied between 50.15% (PMK(R)3) to 145.15% (MDU6) (Fig. 2); proline accumulation increased by 98.38% over control might be correlated with participation of proline in ROS scavenging mechanism in plant tissues that would be beneficial for its tolerance to environmental stresses (Gill and Tuteja, 2010). The current result coincide with the observation of Kibria et al. (2017) and Nahar et al. (2018) who reported that rice cultivars accumulate free proline under biotic and abiotic stresses. Similarly, Upadhayaya et al. (2007) reported that increased proline concentration in rice cultivars under hydrogen peroxide treatment results from defense response of plant system to oxidative stress.

Ascorbic acid, a low molecular weight antioxidant showed defense against ROS by detoxification mechanism (Kao, 2015). Reduction in ascorbic acid content observed between 10.19% (ADT37) to 28.75% (ASD18) (Fig. 2). A decreasing AsA content by 20.99% was observed in present study might be due to continues ozone exposure gradually depletes AsA pool of plant system by O₃ derived ROS. This inability of AsA regeneration in ozone exposed plant suggested that increasing plant's susceptibility towards ozone stress. In the same way, Wang et al. (2013) reported that AsA content in rice cultivar, Shanyou63 decreased by 22.75% under 250 ppb ozone was associated with accumulation of O₃ derived ROS degraded AsA scavenging system which leads to increasing sensitivity of the plants when it encounters ozone stress. On contrary, Ashrafuzzaman et al. (2018) observed no changes in AsA content even at 108 ppb ozone in few rice cultivars (BINA11, BR28, NB and L81). Furthermore, the relationship between AsA and O₃ is remain in debate (Bellini and De Tullio, 2019).

A significant increment in the level of malondialdehyde and proline content was noticed in all rice cultivars; while ascorbic acid content was decreased. In all biochemical traits, significant cultivar and treatment effect was observed. An interaction effect was significant in

malondialdehyde and proline content; whereas ascorbic acid content showed not significant interaction effect.

Growth response

The ozone induced reduction in biomass and yield has been well documented for a wide range of crop species (Shi et al., 2009; Zheng et al., 2013; Lal et al., 2017). In terms of growth traits, the cultivar ASD16 showed maximum reduction (23.58%) in root length while minimum reduction was observed in ADT(R)48 (1.71%). The maximum reduction in shoot length was noticed in MDU5 (11.25%) while slight increment was observed in TRY(R)2 (2.88%) and number of tillers showed maximum reduction in MDU6 (33.33%) and minimum in CO51 (9.09%) (Fig. 3). In present study, on average, elevated ozone stress decreased growth traits namely root length by 11.99%, shoot length by 2.11% and tiller number by 22.52% might be attributed to loss of photosynthetic capacity directly affects the foliar carbon assimilation rate, which inhibit the growth and development of rice cultivars (Wang et al., 2012; Jing et al., 2016). Similar results have been noticed in *indica*, *japonica* and bangladeshi rice cultivars were reduced plant height and tillering at elevated ozone stress indicating accumulative ozone damages in leaves inhibited the plant growth and development (Frei et al., 2008; Akhtar et al., 2010; Shao et al., 2020).

A significant cultivar and treatment effect were noticed in root length, shoot length and number of tillers. An interaction effect was significant in shoot length and number of tillers, while root length showed no interaction effect (Table 3).

Yield response

All yield parameters were significantly influenced by elevated ozone treatment. In present study, a significant maximum reduction in number of effective tillers were noticed in MDU6 (50.00%) and minimum in Anna(R)4 (15.38%). Panicle length showed maximum reduction in ASD18 (21.05%) while slight increment was observed in TRY(R)2 (0.22%) (Fig. 4). On an average, decrease in number of effective tillers by 30.43% and panicle length by 11.69% in current study was primarily caused by the inhibition of tillering formation and reduced plant height which leads to reduction in effective tillers and panicle size. Similar results were observed in previous

study by Shao et al. (2020) who reported that the smaller panicle size was coincided with smaller plant and reduced tiller numbers inhibited the effective tillers under ozone stress.

Cultivar ADT43 depicted maximum reduction (38.52%) and ADT(R)45 showed minimum reduction (13.56%) in number of spikelets per panicle. The maximum reduction in number of filled spikelets per panicle were noticed in ADT43 (50.99%) and minimum in Anna(R)4 (22.87%) (Fig. 4). Decrease in yield traits ie., number of spikelets per panicle by 23.32% and number of filled spikelets per panicle by 35.27% were observed in present study had confirmed the negative effects of ozone on yield. This indicated that assimilates allocation to panicles were significantly reduced due to more assimilates were utilized for plant respiration and for regulating antioxidant metabolism when plant encounters ozone stress. Similarly, the spikelet number and filled spikelets per panicle were reduced in ozone sensitive Nipponbare rice variety under 100 ppb ozone were associated with imbalance in assimilates allocation (Wang et al., 2014). Further, a gene APO1 located at the end of Chromosome 6 were affected due to ozone stress which were responsible for panicle branch number may involved in yield loss (Terao et al., 2010).

On average, the percentage of spikelets sterility per panicle increased by 19.26% (Fig. 5) revealed that weaken fertilization efficiency and reduction in availability of carbohydrate might limited grain filling process are the major reason for decreased grain yield to a larger extent as reported by Lin et al. (2014) and Jing et al. (2016).

The maximum reduction in 1000 grain weight were noticed in ADT37 (4.01%) while slight increment were observed in ASD18 (1.05%), ADT(R)45 (1.61%) and ADT36 (1.74%) (Fig. 4). A decrease in 1000 grain weight by 0.62% in present study was coinciding with O₃ induced reduction in net photosynthesis leads to decrease in cumulative carbon gain. Correspondingly, ozone derived reduction in individual grain mass was reported in bangladeshi rice varieties (Ashrafuzzaman et al., 2017), transgenic Bt-SY63 (Li et al., 2017) and modern *indica* and *japonica* rice cultivars (Shao et al., 2020) under elevated ozone stress were proven the negative impact of ozone on pollen fertilization efficiency and weaken sink strength due to limited CO₂ assimilation rate.

In current study, the cultivar, CO47 showed maximum reduction (35.90%), while minimum reduction were observed in ADT43 (18.52%) (Fig. 4); on average, straw weight decreased by 23.83% across all test rice cultivars was attributed to reduction in net photosynthetic rate and modification in phloem loading and/or translocation (Ainsworth, 2008; Frei et al., 2008; Akhtar et al., 2010). Similarly, total dry weight was considerably reduced in Nipponbare and *Indica* rice

cultivars under elevated ozone were correlated with reduced photosynthetic carbon assimilation rate (Chen et al., 2011; Peng et al., 2018).

A significant cultivar and treatment effect were noticed in number of effective tillers, number of spikelets and filled spikelets per panicle, percentage of spikelets sterility per panicle, 1000 grain weight and straw weight. All observed yield traits showed a significant interaction effect except for 1000 grain weight and straw weight (Table 3).

Plant traits relationships

The positive relationship between photosynthetic rate and chlorophyll content were existed ($r = 0.87^{***}$) while photosynthetic rate and chlorophyll content were significantly negative correlation with spikelet sterility ($r = -0.81^{***}$ and $r = -0.84^{**}$) (Fig. 6). A regression analysis used in 3D model clearly depicted the relationship of photosynthetic traits with spikelet sterility i.e., decrease in photosynthetic rate and chlorophyll content increased spikelet sterility ($R^2 = 0.74^{***}$) (Fig. 7). This significant negative correlation between plant physiological traits and spikelet sterility might be due to sink limitations to the plant economic parts. Further, altered carbon assimilation rate would limiting grain yield rather than storage and phloem loading (Ueda et al., 2013). This negative correlation suggests that the plants were exposed to ozone during heading and flowering hence the grain yield was affected. Correspondingly, leaf injury percentage showed positive correlation with spikelet sterility ($r = 0.85^{***}$) while weaker association observed with straw weight (Fig. 8). This results confirmed the alteration in source-sink regulation and alteration in assimilate partitioning (Crous et al., 2006). Similarly, Ueda et al. (2015) showed weak relation between injury percentage and biomass production.

Principal component analysis (PCA) has been used to identify ozone responsive parameters in European beech (*Fagus sylvatica*) (Löw et al., 2012) and wheat cultivars (Fatima et al., 2019). In present study, principal component analysis was performed to categorize the principal components of plant physiological, biochemical, growth and yield traits of 15 rice cultivars that best explain the response to ozone stress to identify ozone tolerant cultivars. The first two principal components (PC1 and PC2) accounted for 46.1 % and 11.4 % among rice cultivars, respectively with cumulative of 57.5% and clustered all physiological traits and most of the yield traits as the best descriptors followed by growth and biochemical traits (Fig. 9). PCA analysis revealed that the

strong correlation between physiological and yield traits (cluster together) more than with biochemical parameters using PC1 and PC2.

The first principal component (PC1) correspond to higher values for all physiological, growth and yield traits except for straw weight, but lesser loadings for leaf visible symptoms and biochemical traits except for ascorbic acid content. The second principal component (PC2) explained higher values for leaf visible symptoms, biochemical traits (proline and MDA), growth (shoot length) and all the yield traits except for 1000 GW, and lesser loading for all physiological traits, biochemical trait (AsA), growth (RL and NTP) and yield traits (1000 GW). This multivariate analysis allows the identification of ozone responsive variables that are best described to ozone tolerance.

Cultivars showing highest values for observed physiological, biochemical, growth and yield traits for PC1 and PC2 located in the positive quadrant were considered as ozone tolerant cultivars. In contrast, cultivars showing the low values for PC1 and PC2 fall in negative quadrant were considered as ozone sensitive cultivars. Cultivars with moderate values for PC1 and PC2 fall in positive and negative quadrant respectively were considered as moderately ozone tolerant cultivars while PC1 and PC2 with negative and positive quadrant, respectively were considered as moderately ozone sensitive cultivars. Hence, the 15 rice cultivars were categorized into four major groups indicating ozone tolerant (Rice CO51, CO47 and ADT36), moderately ozone tolerant (ADT37, ADT(R)45, Rice TPS5, Anna(R)4, PMK(R)3 and ADT(R)48), moderately ozone sensitive (ASD18, ADT43 and MDU5) and ozone sensitive (Rice MDU6, TRY(R)2, ASD16) cultivars (Fig. 10). Similar to the present investigation, Mazid et al. (2013) clustered 41 different rice genotypes for screening bacterial blight resistance genotypes using PCA. Kakar et al. (2019) also clustered 74 rice genotypes for identifying salt tolerance using principal component analysis for accuracy and reliability.

Conclusion

Decline in rice production with increasing tropospheric ozone concentration instigates researchers to select and develop ozone tolerant rice cultivars for retaining global food security. The present investigation revealed that elevated ozone significantly decreased plant photosynthetic traits, altered antioxidant metabolism, reduced tiller number and increased spikelet sterility. Ultimately, these altered plant traits led to substantial yield loss suggests all fifteen rice cultivars

are relatively susceptible to eTO₃. Moreover, a significant genetic variation in ozone sensitivity among test cultivars would provide significant information for model predication and ozone stress adaptation strategies for future rice production.

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Availability of data material

The authors declare that complete data set is provided in the results and supplementary file of this paper.

Declaration of interests

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

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Table 1. Regression equations and leaf traits used in the 3D model

Formula	$f = y_0 + (a \times x) + (b \times y) + (c \times x^2) + (d \times y^2)$
y₀	22.094
a	3.805
b	-62.594
c	-0.153
d	42.482
x	Photosynthetic rate
y	Chlorophyll content

Table 2. Leaf injury percentage (LIP) and leaf bronzing score (LBS) on 15 rice cultivars under elevated ozone stress

Cultivars	LIP	LBS
Rice CO 51	32.0	4
CO 47	28.3	3
ADT 36	33.0	3
ADT 37	38.3	5
ADT 43	48.3	5
ADT (R) 45	32.7	4
ADT (R) 48	31.7	4
ASD 16	49.3	5
ASD 18	39.7	4
MDU 5	49.0	5
Rice MDU 6	51.7	6
Rice TPS 5	25.3	3
TRY (R) 2	51.7	6
Anna (R) 4	23.3	3
PMK (R) 3	23.3	3

Table 3. ANOVA results of plant growth and yield traits of 15 rice cultivars under elevated ozone stress

ANOVA results (P values)	RL (cm)	SL (cm)	NTP	PL (cm)	NETP	NSPi	NFSPi	SS (%)	1000 GW (g)	SWP (g)
Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.928	<0.001
Cultivar ×Treatment	0.185	0.005	<0.001	<0.001	<0.001	0.011	0.008	<0.001	0.819	0.283
Treatment means										
Control	10.83	67.50	12.51	17.18	9.31	109.96	100.00	9.30	21.69	47.51
Ozone	9.58	66.12	8.51	16.99	6.36	84.71	63.49	26.73	21.55	36.11

Figure 1. Physiological traits of 15 rice cultivars under elevated ozone stress. Bars indicate ± 1 SEM, (Sample size, N=90). Asterisk denotes significant difference between control and ozone treatment within the cultivar. * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 and NS=Not Significant.

Figure 2. Biochemical traits of 15 rice cultivars under elevated ozone stress. Bars indicate ± 1 SEM, (Sample size, N=90). Asterisk denotes significant difference between control and ozone treatment within the cultivar. * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 and NS=Not Significant.

Figure 3. Growth traits of 15 rice cultivars under elevated ozone stress. Bars indicate ± 1 SEM, (Sample size, N=90). Asterisk denotes significant difference between control and ozone treatment within the cultivar. * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 and NS=Not Significant.

Figure 4. Yield traits of 15 rice cultivars under elevated ozone stress. Bars indicate ± 1 SEM (Sample size, N=90). Asterisk denotes significant difference between control and ozone treatment within the cultivar. * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 and NS=Not Significant.

Figure 5. Box plots for spikelet sterility of 15 rice cultivars under elevated ozone stress.

Figure 6. Correlation and histogram of photosynthetic traits and spikelet sterility of 15 rice cultivars under elevated ozone stress.

Figure 7. Relationship between photosynthetic traits and spikelet sterility of 15 rice cultivars under elevated ozone stress.

Figure 8. Correlation and histogram of leaf injury percentage and yield traits of 15 rice cultivars under elevated ozone stress.

Figure 9. Principal component analysis for the first two principal components (PC) scores, PC1 vs. PC2 describing the classification of ozone response plant traits for 15 rice cultivars.

Figure 10. Principal component analysis for the first two principal components (PC) scores, PC1 vs. PC2 describing the classification of 15 rice cultivars into different ozone response groups based on all the physiological, biochemical, growth and yield traits.

Supplementary Files

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