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

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Abstract The plant response to elevated ozone stress reveals inter-species and intra-species disparity. Ozone-induced crop yield loss is predicted to increase in the future, posing a threat to the world economy. This study aims 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 an average, ozone stress decreased the 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% categorized 15 rice cultivars into four major groups, i.e., 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). This study indicates that the different responses of rice cultivars to elevated ozone stress through a change in plant physiology, biochemical, growth, and yield traits and the results directed to provide scientific information on plant adaptations to ozone stress and helps in efforts to search ozone tolerant gene for plant breeding.

Keywords Rice cultivars · Elevated ozone · Plant response · Principal component analysis

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Introduction

Tropospheric ozone (O_3) is the third most important contributor of greenhouse radiative forcing (+0.41 Wm^{-2}) after carbon dioxide (CO_2) and methane

(CH$_4$) (IPCC, 2013). Right from the industrial revolution until today, there is a continuous alarming rise in ozone forming precursors like oxides of nitrogen (NOx) and volatile organic compounds (VOCs) in the atmosphere, which in turn increases the tropospheric ozone (Monks et al., 2015). The ozone-forming precursor, NOx showed a strong positive trends over India (+29%), China (+26%), and the Middle East (+20%), and the negative trends over the USA (−29.4%), southern Africa (−8.2%), and Western Europe (−8.8%) per decade over a period of 2005–2014 (Miyazaki et al., 2017). According to Kumari et al. (2020), NOx level showed an increasing trend of 0.9 ppb per year from 2010 to 2015 over Indo Gangetic plains, India. This increase in NOx emissions from industries and motor vehicle raises tropospheric ozone concentration.

Since the mid-1990s, tropospheric ozone trends has increased in the northern mid-latitude regions (2–7%; 1–4 ppb) and tropics (2–14%; 1–5 ppb) with the largest increases of 8–14% (3–6 ppb) per decade in the southern Asian region (IPCC, 2021). The average ozone concentration varied from 45 to 65 ppb over Indian regions (David & Nair, 2013; Deb Roy et al., 2009). An increasing trend of ozone by 3–5.6 and 1.2–2% per decade were observed over Indo Gangetic plains and southern regions of India, respectively over the study period of 2005–2010 (Lal et al., 2012). According to Kumari et al. (2020), the annual mean ozone concentration increased by 19.2% from 2010 to 2015 over Indo Gangetic plains. Moreover, in southern regions of India, the maximum ozone concentration reached up to 56 ppb at Kanyakumari (Krishna Sharma & Nagaveena, 2016) and 62 ppb at high altitude region of Western Ghats, Ooty, Tamil Nadu (Udayasoorian et al., 2013). Hence, the current level of ozone concentration has crossed the standard permissible limit for vegetation (40 ppb) and severely affects the forest (Feng et al., 2019), agricultural (Fischer, 2019; Shao et al., 2020) and horticultural crop species (Singh et al., 2018; Yang et al., 2017).

Rice, an important food crop of the world is susceptible to many pollutants particularly air pollutants. In Asian regions, rice is cultivated almost throughout the year with two major rice growing seasons (rabi and kharif) which generally overlaps with the peak ambient ozone concentration (Frei, 2015; Ziemke et al., 2019). Several studies on rice crop revealed that elevated tropospheric ozone (eTO$_3$) reduced the photosynthetic rate, photosynthetic pigments, oxidative stress induced bronzing symptoms and altered antioxidant metabolism (Li et al., 2017; Peng et al., 2018). These physiological and biochemical stress leads to reduction in growth, biomass, tiller numbers, spikelet numbers, and grain yield (Akhtar et al., 2010; Jing et al., 2016; Shao et al., 2020).

The tropospheric ozone causes considerable yield loss in rice (Pandey et al., 2015, 2018; Singh et al., 2018). Van Dingenen et al. (2009) reported 3.7% yield loss in rice at global level. Ozone-induced damage to rice in India is estimated to be 2.1±0.8 Mt which was sufficient to feed roughly 35% 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 simulated using Weather Research and Forecasting-Chemistry (WRF-Chem) model showed 8% relative yield loss for rice crop in Indian region (Sharma et al., 2019).

Tropospheric ozone-induced yield loss estimation 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 in India and there is no information on elevated tropospheric ozone (eTO$_3$) impact on rice in southern part of India. Since rice yield loss to ozone stress is one aspect of global climate change issue, cultivar sensitivity and tolerance need to be thoroughly studied. Hence, an experimental study was conducted to explore the intra-specific variations of rice cultivars exposed to elevated ozone stress. Further, better understanding of cultivar specific variations in rice plants under ozone stress shall provide scientific information about cultivar adaptations to environmental condition. The outcome of the work would throws light on the significance of implementing ozone tolerant plant breeding programs and formulation of ozone reduction policies to protect rice productivity at global scale.

Materials and methods

Experimental site and ozone treatment

The 15 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 popularly cultivated in and around Tamil Nadu region were chosen for this study. The specific characteristics of chosen rice cultivars developed in various research stations of Tamil Nadu Agricultural University are described in Table 1. In order to maintain the elevated ozone concentration, the experimental study was carried out in Open Top Chambers (3.5×3.5 m) located in the wetlands ($11.00°$ N, $76.92°$ E) of Tamil Nadu Agricultural University (TNAU), Coimbatore, India, during January to May 2019. During the experimental period, monthly mean maximum and minimum temperature ranged between 32.7 and 17.8 °C, respectively, while the maximum and minimum relative humidity varied from 98 to 19%, respectively. Experimental soil chosen for the study has a clay loam texture with slightly alkaline pH (8.38) and an electrical conductivity (EC) of 0.32 dS m^{-1}. Furthermore, the soil had an organic carbon content of 0.53% organic carbon and 226, 12.4, and 287 kg ha^{-1} of available nitrogen (N), phosphorous (P) and potassium (K), respectively. All the crop management practices were followed as per the recommendation of crop production guide (TNAU, 2019). Seeds of 15 rice cultivars were soaked in water for 24 h to induce sprouting. The sprouted seeds were sown uniformly in the well prepared nursery bed by maintaining a thin film of water for 20 days under natural light and ambient temperature. Three-week old seedlings were transferred into two Open Top Chambers, one for control ($n = 90$) and another for ozone treatment ($n = 90$) with six replications for each cultivar ($N = 180$). The factorial completely randomized block design was followed in control and ozone chambers. Three days after transplanting, pre-emergence herbicide butachlor (1.25 kg/ha) was applied and seventh day after transplanting, gap filling was carried out to maintain equal number of seedlings in each treatment. To provide adequate nutrient supply, the fertilizer recommendation of 150:50:50 kg N:P:K ha^{-1} were supplied as four equal split. The split application of N and K were followed at basal, tillering, panicle initiation, and heading stages, while P was applied as basal alone. Submergence of 5 cm water level was maintained from seventh day after transplanting to throughout the crop period.

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. The ozone concentration inside the chambers was monitored using ambient ozone monitor (G09-O$_3$-3121). 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 the target level of 50 ppb O$_3$ (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

The ozone exposed plants depicting injury symptoms on leaves like necrotic dark brown spots, chlorotic, and pale color of leaves and they were evaluated based on certain criteria. After 30 days of ozone exposure, the whole plant with all leaves of each cultivar and treatment were observed for evaluating injury symptoms. A non-destructive visible scoring method was followed by assigning a leaf injury percentage (LIP) from 0 to 100 (Chaudhary & Agrawal, 2013) and leaf bronzing score (LBS) from 0–10 (Ueda et al., 2015) to all rice cultivars. The criteria for evaluating leaf visible symptom are given in Table 2.

Physiological traits

A non-destructive physiological measurement was performed on three randomly selected rice plants ($n = 3$) within each cultivar and treatment ($N = 90$). A portable photosynthesis system (ADC BioScientific LCpro-SD System, UK) was utilized to quantify photosynthetic rate (A) and stomatal conductance (gs) while chlorophyll content meter (CCM-200+, USA) was used to assess chlorophyll content (Chl). After 30 days of ozone exposure, measurement was taken at three different points of third youngest fully expanded leaf of each cultivar in treatment and the average of three points were calculated.

Biochemical traits

The destructive biochemical measurements were performed on three randomly chosen rice plants ($n = 3$) of each cultivar and treatment ($N = 90$).

Table 1 Characteristics of 15 short-duration rice cultivars

Rice cultivars	Parentage	Special characters	Grain type	Duration (days)	Tamil Nadu rice breeding station
ADT 36	Triveni×IR 20	Resistant to blast and brown plant hopper	Medium	110	Tamil Nadu Rice Research Institute, Aduthurai
ADT 37	BG 280–12×PTB 33	Resistant to many pest and diseases	Short bold	105	
ADT 43	IR 50×White ponni	Resistant to green leaf hopper	Medium slender	110	
ADT (R) 45	IR 50×ADT 37	Resistant to gall midge and moderately resistant to brown plant hopper	Medium slender	110	
ADT (R) 48	IET 11,412×IR 64	Resistant to green leaf hopper, gall midge and stem borer	Long slender	94–98	
Anna (R) 4	Pantdhan 10×IET 9911	Drought tolerant	Long slender	100–105	Agricultural Research Station, Paramakudi
ASD 16	ADT 31×CO 39	Resistant to blast	Short bold	110–115	Rice Research Station, Ambasamudram
ASD 18	ADT 31×IR50	Resistant to brown plant hopper	Medium slender	105	
CO 47	IR 50×CO 43	Resistant to blast	Medium slender	110–115	Directorate of Plant Breeding and Genetics, Coimbatore
MDU 5	*O. glaberrima*×Pokkali	Resistant to drought, suitable for dry and transplanted condition	Medium slender	95–100	Agricultural College &Research Institute, Madurai
PMK (R) 3	UPLRI 7×CO 43	Drought tolerance, semi-dwarf, non-lodging and non-shattering	Long bold	105–110	Agricultural Research Station, Paramakudi
Rice MDU 6	MDU 5×ACM 96,136	High yielding, superior cooking quality	Long slender	110–115	Agricultural College &Research Institute, Madurai
Rice TPS 5	ASD16×ADT37	High yielding, moderately resistant to stem borer, leaf folder, gall midge and brown plant hopper, good cooking quality	Short bold	118	Agricultural Research Institute, Tirupathisaram
Rice CO 51	ADT 43×RR 272–1745	High yielding semi-dwarf rice variety, moderately resistant to blast, brown plant hopper and green leaf hopper	Medium slender	105–110	Directorate of Plant Breeding and Genetics, Coimbatore
TRY (R) 2	IET 6238×IR 36	Saline/alkaline tolerant	Long slender	115–120	Agricultural College &Research Institute, Trichy

After 30 days of ozone exposure, third youngest fully expanded leaf of selected plants were collected and frozen at –80 °C until further analysis of malondialdehyde (Heath & Packer, 1968), proline (Bates et al., 1973), and ascorbic acid (Keller & Schwager, 1977).

Table 2 Criteria for evaluating ozone-induced leaf visible symptoms

Rating	Criteria	Ozone injury percentage (%)	Leaf bronzing score
None	No ozone-induced visible injury in any of the rice leaves	0	0
Very slight	Slight ozone injury on few rice leaves	0–10	1
		11–20	2
Slight	Easily visible ozone injury on few rice leaves	21–30	3
		31–40	4
Moderate	Moderate to severe ozone injury on many rice leaves	41–50	5
		51–60	6
Severe	Severe ozone injury on many rice leaves	61–70	7
		71–80	8
Very severe	Whole rice plant severely damaged	81–90	9
		91–100	10

Growth and yield traits

All the growth and yield traits of rice cultivars, i.e., 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. Three selected plants ($n = 3$) of each cultivar and treatment ($N = 90$) which were not destructed earlier for any of the analysis were chosen for observation to obtain precise yield results. Grains were separated from the panicles manually and then the whole plant was dried at 80 °C for 72 h to measure the straw weight. Grains were soaked in water and numbers of floating and sunken spikelets were counted manually to determine filled and unfilled spikelets. Twenty randomly chosen filled grains were weighed and the value was multiplied by 50 to obtain the thousand grain weight. Spikelet sterility (SS) was calculated as the number of sterile spikelets relative to the total number of spikelets.

Statistical analysis

All the statistical analysis was 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 method was used to identify difference among treatment means. The ozone-induced percentage reduction of yield traits over control was estimated by the following formula, $100 - [(\text{ozone}/\text{control}) \times 100]$. 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 is described in Table 3. 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 ($P < 0.05$) is 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 on rice production is still unknown in southern parts of India and hence it is important to generate data with reference to tropospheric ozone-induced impact on rice cultivars. In this study, an average ozone concentration of 50 ppb

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

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

was fixed to mimic current ozone level over southern India with popularly growing 15 short-duration rice cultivars.

Leaf visible symptom

The leaf bronzing score and leaf injury percentage were given 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 4). The ozone-induced leaf injury symptom in this study may be due to entry of O_3 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 the development of necrotic symptoms (Baier et al., 2005). Few cultivars, Anna(R) 4 and PMK(R) 3 showed less injury symptoms which might be related to enhanced antioxidant system compared to MDU6 and TRY(R)2. This result is consistent with the study by Wang et al. (2014) who reported that lesser bronzing symptoms were associated with improved antioxidant system like lipid peroxidation in ozone tolerant rice and also presence of quantitative trait loci OzT9, which is responsible for leaf bronzing formation under ozone stress.

Physiological response

A reduction in stomatal conductance was observed between 8.62% (Anna(R)4) and 29.31% (TRY(R)2)

Table 4 Leaf injury percentage (LIP) and leaf bronzing score (LBS) on rice cultivars exposed to elevated ozone stress

Cultivars	Leaf injury percentage	Leaf bronzing score
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

(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 is also related with the production of reactive oxygen species under ozone stress, which controls the activity of guard cell ion channels and protein kinase activity in stomata, resulting in the reduced stomatal conductance (Vainonen & Kangasjarvi, 2015). Similar results were found by Pang et al. (2009) who reported that rice cultivars, Shanyou63 and Wuyunjing3 experienced a significant decreased in stomatal conductance up to 36.70% 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 percentage 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 the decline in photosynthetic rate by 19.23% which was related to the stomatal limitation in all rice cultivars that inhibited the photosynthetic CO_2 fixation in the plant system. It is also correlated with the report of Masutomi et al.
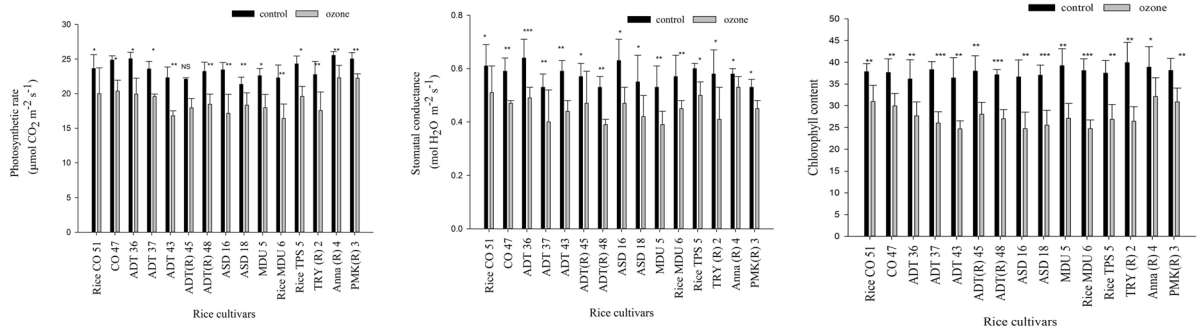
Fig. 1 Physiological traits of rice cultivars exposed to elevated ozone stress. Bars indicate ± 1 SEM, (sample size, $N = 90$). Asterisk denotes significant difference between control and ozone treatment within the cultivar.* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, and NS = not significant

(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 which was not only attributed to damage of photosynthetic enzyme but also due to ozone-induced stomatal closure.

As a consequence of lipid peroxidation in PSII reaction center undoubtedly affecting the light harvesting efficiency of all test cultivars (Jing et al., 2016; Ueda et al., 2015), this would result in the reduction of chlorophyll content between 17.04% (Anna(R)4) and 35.08% (Rice MDU6) (Fig. 1). Moreover, on an average across 15 rice cultivars, chlorophyll content reduced by 27.19% was associated with decreased photosynthetic pigments especially chlorophyll located in the thylakoids. Similar findings were

reported by Li et al. (2017) wherein both SY63 and Bt-SY63 rice cultivars showed a reduction in chlorophyll content under elevated ozone stress that was associated with degenerated chloroplasts which mediates the changes in carbon assimilation cycle. This observed physiological disorder under elevated ozone stress causes photosynthetic instability which results in alteration at 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
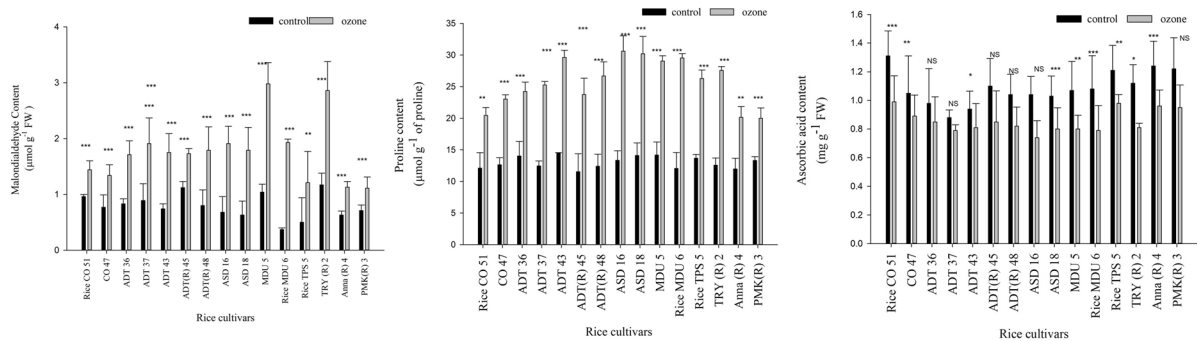
Fig. 2 Biochemical traits of rice cultivars exposed to elevated ozone stress. Bars indicate ± 1 SEM, (sample size, $N = 90$). Asterisk denotes significant difference between control and ozone treatment within the cultivar.* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, and NS = not significant
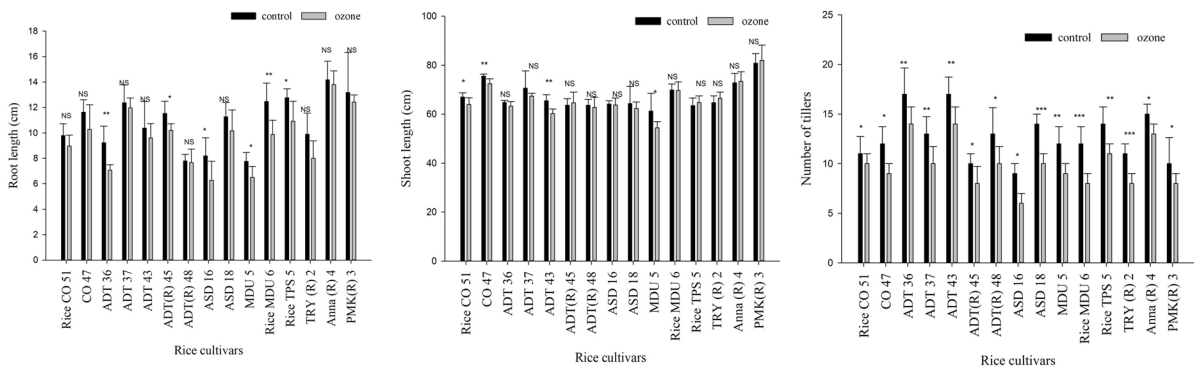
Fig. 3 Growth traits of rice cultivars exposed to elevated ozone stress. Bars indicate ±1 SEM, (sample size, $N=90$). Asterisk denotes significant difference between control and ozone treatment within the cultivar. $* \leq 0.05$, $** \leq 0.01$, $*** \leq 0.001$ and NS = not significant

concentration (Li et al., 2017; Ueda et al., 2013) which is used as an important 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%) (Fig. 2). MDA content increased by 121.43% over control indicating an altered reactive oxygen species metabolism in ozone exposed plants. Consequently, higher concentration of reactive oxygen species generated in ozone exposed plant leads to membrane damage and cell death (Frei, 2015). Ashrafuzzaman et al. (2017) also reported that Nipponbare rice variety exhibited an increase in MDA content under elevated ozone, thereby validating a continuous accumulation of reactive oxygen species mediated lipid peroxidation.

A non-enzymatic antioxidant, proline acts as a scavenger of singlet oxygen and hydroxyl radicals in response to environmental stress (Rejeb et al., 2014). In this study, a hike in proline content varied between

50.15% (PMK(R)3) to 145.15% (MDU6) (Fig. 2) over control might be correlated with participation of proline in reactive oxygen species scavenging mechanism in plant tissues that would be beneficial for its tolerance to environmental stress (Gill & Tuteja, 2010). This result coincides 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, Upadhyaya 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 reactive oxygen species (ROS) by detoxification mechanism (Kao, 2015). Reduction in ascorbic acid content was observed between 10.19% (ADT37) and 28.75% (ASD18) (Fig. 2). A decrease in ascorbic acid content by 20.99% was observed in this study, which might be due to continuous ozone exposure gradually depleting

Table 5 ANOVA results of plant growth traits of 15 rice cultivars exposed to elevated ozone stress

ANOVA results (P values)	Root length (cm)	Shoot length (cm)	Number of tillers per plant
Cultivar	<0.001	<0.001	<0.001
Treatment	<0.001	<0.001	<0.001
Cultivar × treatment	0.185	0.005	<0.001
Treatment means			
Control	10.83	67.50	12.51
Ozone	9.58	66.12	8.51
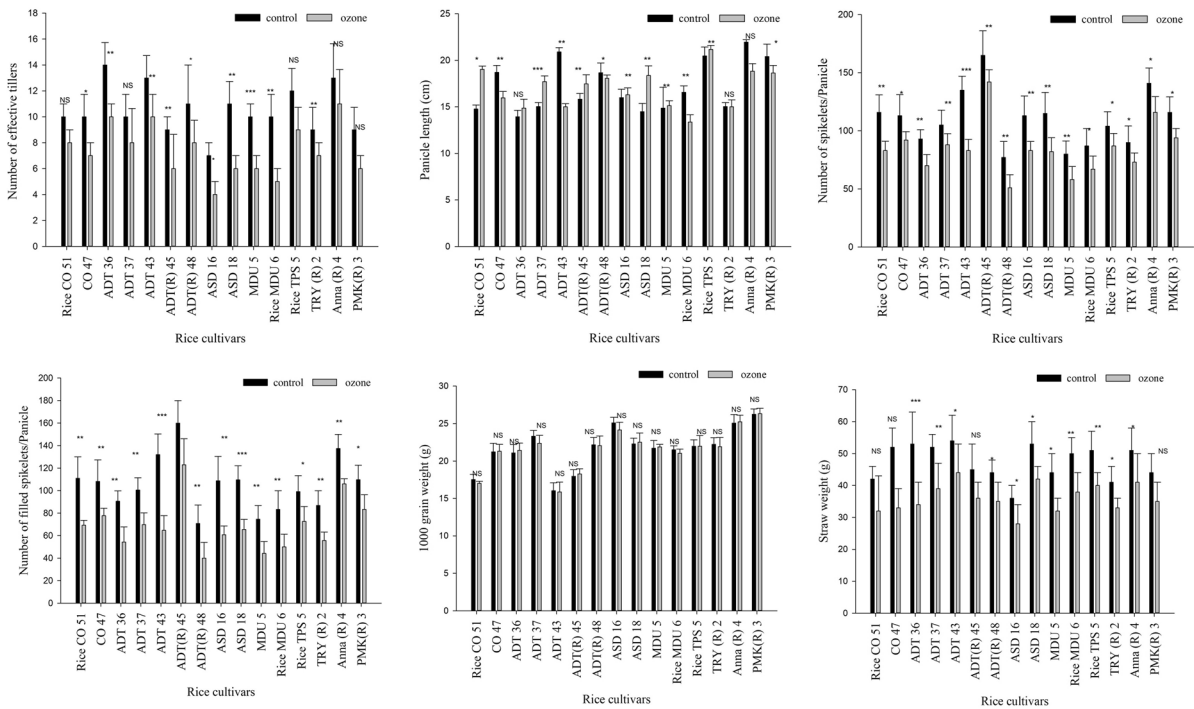
Fig. 4 Yield traits of rice cultivars exposed to elevated ozone stress. Bars indicate ± 1 SEM (sample size, N=90). Asterisk denotes significant difference between control and ozone treatment within the cultivar.* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, and NS = not significant

the ascorbic acid pool of plant system by O₃-derived ROS. This inability of ascorbic acid regeneration in ozone exposed plant suggests the plant's susceptibility towards ozone stress. In the same way, Wang et al. (2013) reported that ascorbic acid content in rice cultivar, Shanyou63 decreased by 22.75% under 250 ppb ozone which was associated with the accumulation of O₃-derived ROS degraded ascorbic acid scavenging system thereby leading to increased sensitivity of the plants when it encounters ozone stress. On contrary, Ashrafuzzaman et al. (2018) observed no changes in ascorbic acid content even at 108 ppb ozone in few rice cultivars (BINA11, BR28, NB, and L81). Furthermore, the relationship between ascorbic acid and O₃ remains unclear (Bellini & De Tullio, 2019).

A significant increment in the level of malondialdehyde and proline content was noticed in all rice cultivars; while ascorbic acid content 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 no significant variation.

Growth response

The ozone-induced reduction in biomass and yield has been well documented for a wide range of crop species (Lal et al., 2017; Shi et al., 2009; Zheng et al., 2013). 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 this study, on an average, elevated ozone stress decreased the growth traits namely root length by 11.99%, shoot length by 2.11% and tiller numbers by 22.52%, which might be attributed to loss of photosynthetic capacity directly affecting the foliar carbon assimilation rate, which in turn inhibits the growth and development of rice cultivars (Jing et al., 2016; Wang et al., 2012). Similar results have been noticed in

Fig. 5 Box plots for spike-
let sterility of 15 rice cul-
tivars exposed to elevated
ozone stress
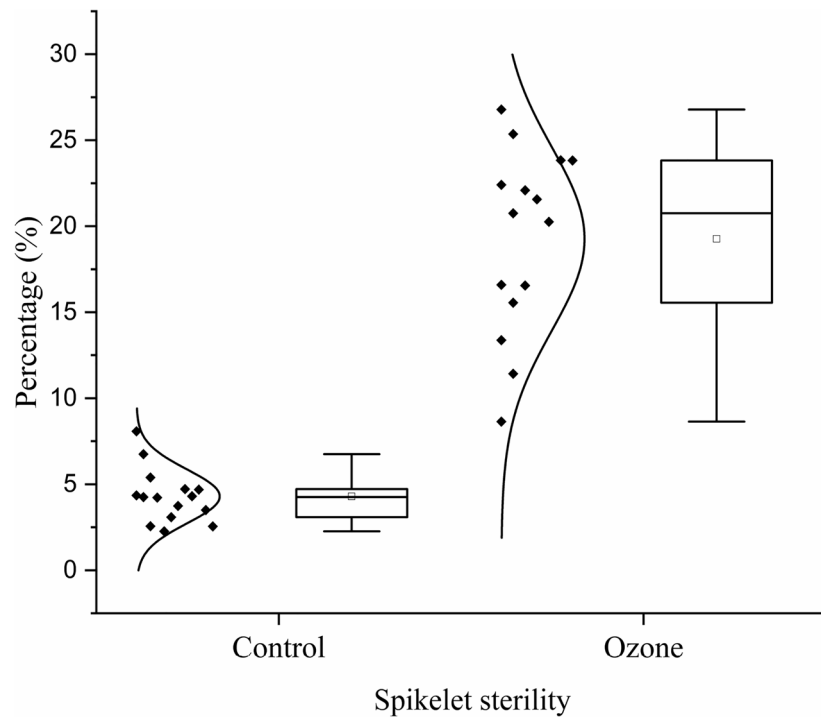
indica, *japonica*, and Bangladeshi rice cultivars
with reduced plant height and tillering at elevated
ozone stress indicating ozone damages in leaves
thereby preventing the plant growth and develop-
ment (Akhtar et al., 2010; Frei et al., 2008; Shao
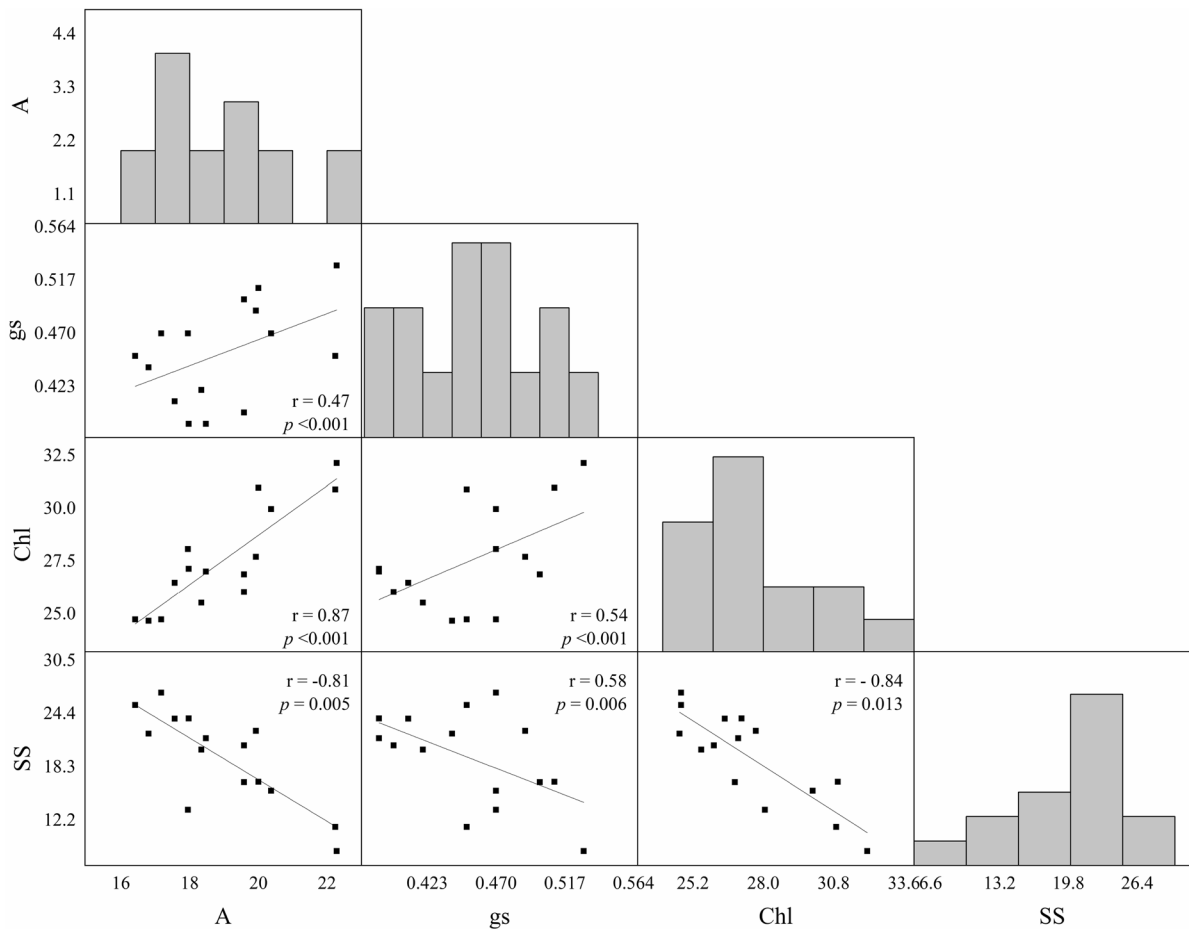et al., 2020).

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 5).

Yield response

All yield parameters were significantly influenced by
elevated ozone treatment. In this study, a maximum
reduction in number of effective tillers were noticed
in MDU6 (50.00%) and minimum in Anna(R)4
(15.38%). Panicle length showed a maximum reduc-
tion in ASD18 (21.05%) while slight increment was
observed in TRY(R)2 (0.22%) (Fig. 4). On an aver-
age, decrease in number of effective tillers by 30.43%
and panicle length by 11.69% was primarily caused

Table 6 ANOVA results of plant yield traits of 15 rice cultivars exposed to elevated ozone stress

ANOVA results (*P* values)	Panicle length (cm)	Number of effective tillers per plant	Number of spikelets per panicle	Number of filled spikelets per panicle	Spikelet sterility (%)	1000 grain weight (g)	Straw weight per plant (g)
Cultivar	<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	0.928	<0.001
Cultivar × treatment	<0.001	<0.001	0.011	0.008	<0.001	0.819	0.283
Treatment means							
Control	17.18	9.31	109.96	100.00	9.30	21.69	47.51
Ozone	16.99	6.36	84.71	63.49	26.73	21.55	36.11

Fig. 6 Correlation and histogram of photosynthetic traits and spikelet sterility of 15 rice cultivars exposed to elevated ozone stress. (A—photosynthetic rate; gs—stomatal conductance; Chl—chlorophyll content; SS—spikelet sterility)

by the inhibition of tillering formation and reduced plant height which leads to reduction in effective tillers and panicle size. Similar results were observed by Shao et al. (2020) who reported that smaller panicle size was coincides with reduced plant height and effective tillers under ozone stress.

Cultivar ADT43 depicted a 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 was noticed in ADT43 (50.99%) and minimum in Anna(R)4 (22.87%) (Fig. 4). Decrease in yield traits, i.e., number of spikelets per panicle by 23.32% and number of filled spikelets per panicle by 35.27% had confirmed the negative effects of ozone on yield. This indicates that under ozone stress assimilate allocation to panicles

were significantly reduced due to more utilization for plant respiration and regulating antioxidant metabolism. Similar observations were reported by Wang et al. (2014) under 100 ppb ozone in ozone sensitive Nipponbare rice variety. Further, Terao et al. (2010) reported that a gene APO1 located at the end of Chromosome 6 was affected due to ozone stress.

On an average, the percentage of spikelets sterility per panicle increased by 19.26% (Fig. 5) revealed that reduction in availability of carbohydrate might have limited the grain filling process eventually decreasing the 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

Fig. 7 Relationship between photosynthetic traits and spikelet sterility of 15 rice cultivars exposed to elevated ozone stress. (A—photosynthetic rate)
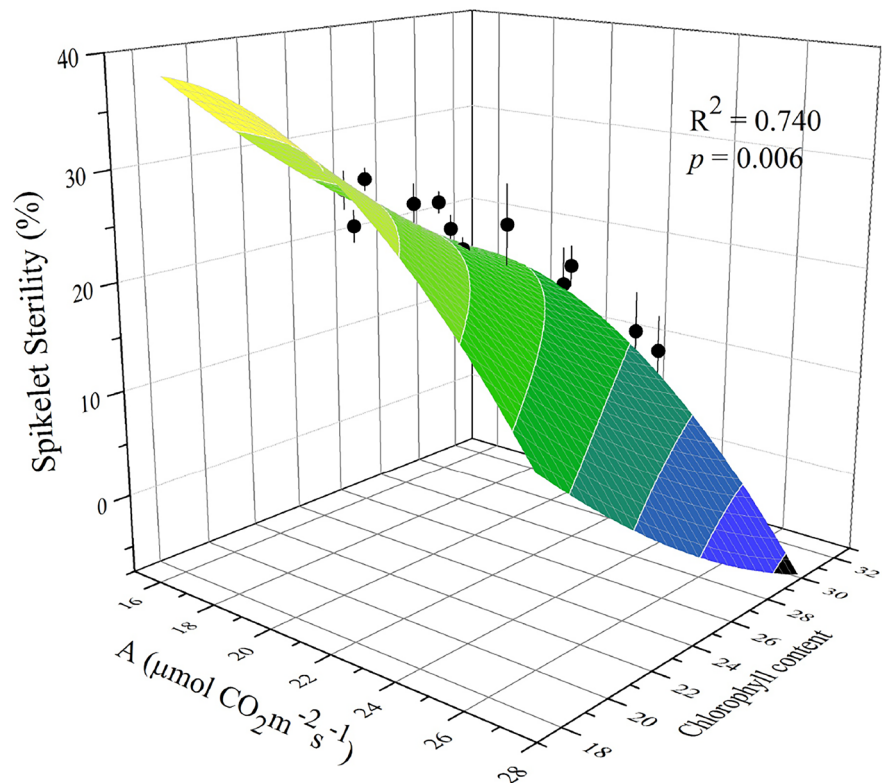

(1.61%), and ADT36 (1.74%) (Fig. 4). A decrease in 1000 grain weight by 0.62% coincides with O_3-induced reduction in net photosynthesis leading to decrease in cumulative carbon gain. Correspondingly, ozone-derived reduction in individual grain mass was reported in Bangladeshi rice varieties (Ashrafuzzaman 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 proving the negative impact of ozone on pollen fertilization efficiency and weaken sink strength due to limited CO_2 assimilation rate.

The cultivar, CO47 showed maximum reduction (35.90%), while minimum reduction was observed in ADT43 (18.52%) (Fig. 4). On an average, the straw weight decreased by 23.83% across all rice cultivars which was attributed to reduction in net photosynthetic rate and modification in phloem loading and/or translocation (Ainsworth, 2008; Akhtar et al., 2010; Frei et al., 2008). Similarly, total dry weight was considerably reduced in Nipponbare and *Indica* rice cultivars under elevated ozone (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 the observed yield traits showed a significant interaction effect except for 1000 grain weight and straw weight (Table 6).

Plant trait relationship

A positive relationship between photosynthetic rate and chlorophyll content existed ($r=0.87^{***}$) while both had a 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 limit the grain yield
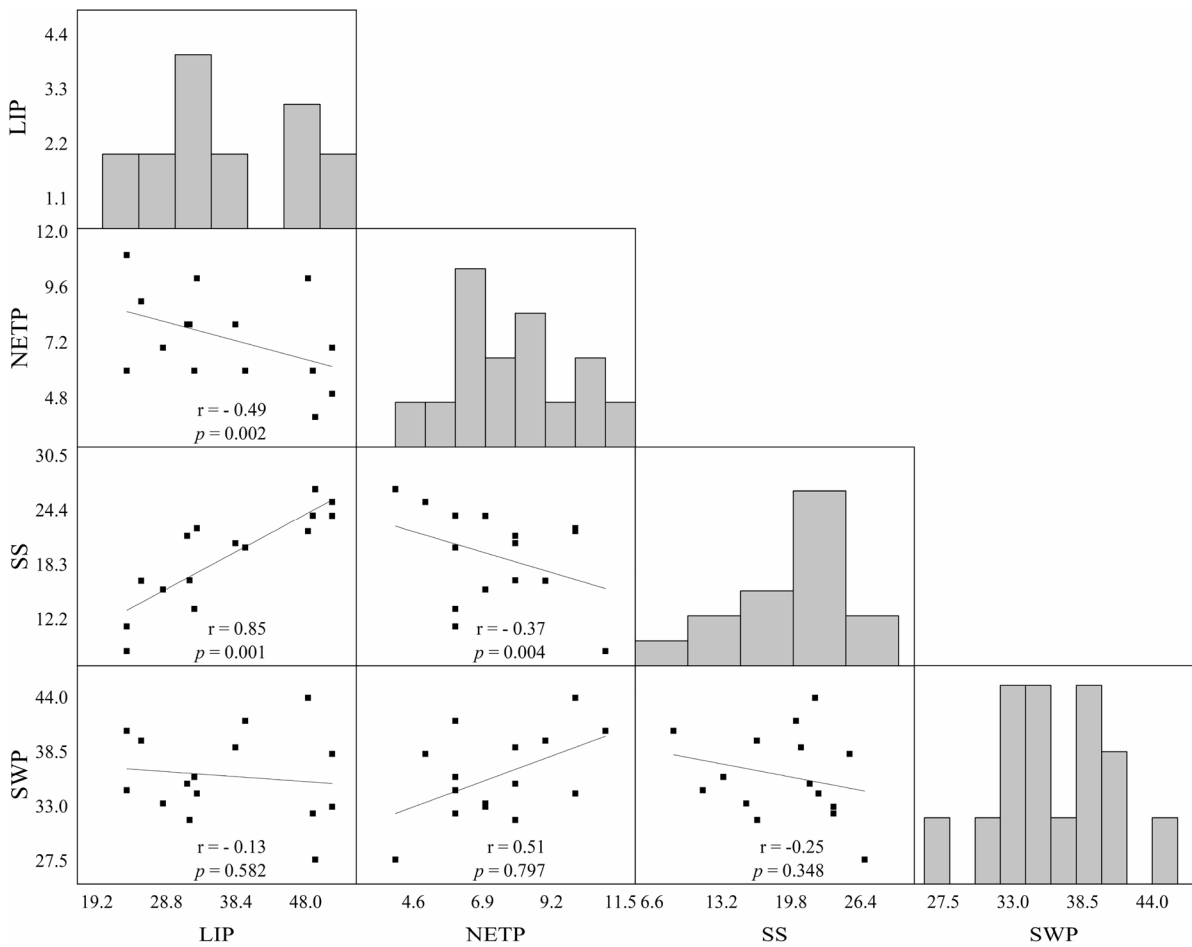
Fig. 8 Correlation and histogram of leaf injury percentage and yield traits of 15 rice cultivars exposed to elevated ozone stress. (LIP—leaf injury percentage; NETP—number of effective tillers per plant; SS—spikelet sterility; SWP—straw weight per plant)

rather than storage and phloem loading (Ueda et al., 2013). The above correlation suggests that the plants were exposed to ozone during heading and flowering stages and hence the grain yield was affected. Correspondingly, leaf injury percentage showed a positive correlation with spikelet sterility ($r = 0.85^{***}$) while weaker association was 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 a 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 this 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 a cumulative eigen value 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 a 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

Fig. 9 Principal component analysis describing the classification of ozone responsive plant traits of 15 rice cultivars. (LIP—leaf injury percentage; LBS—leaf bronzing score; A—photosynthetic rate; gs—stomatal conductance; Chl—chlorophyll content; MDA—malondialdehyde content; Proline—proline content; AsA—ascorbic acid content; RL—root length; SL—shoot length; PL—panicle length; NTP—number of tillers per plant; NETP—number of effective tillers per plant; NSPi—number of spikelets per panicle; NFSPi—Number of filled spikelets per panicle; 1000 GW—100 grain weight; SWP—straw weight per plant)
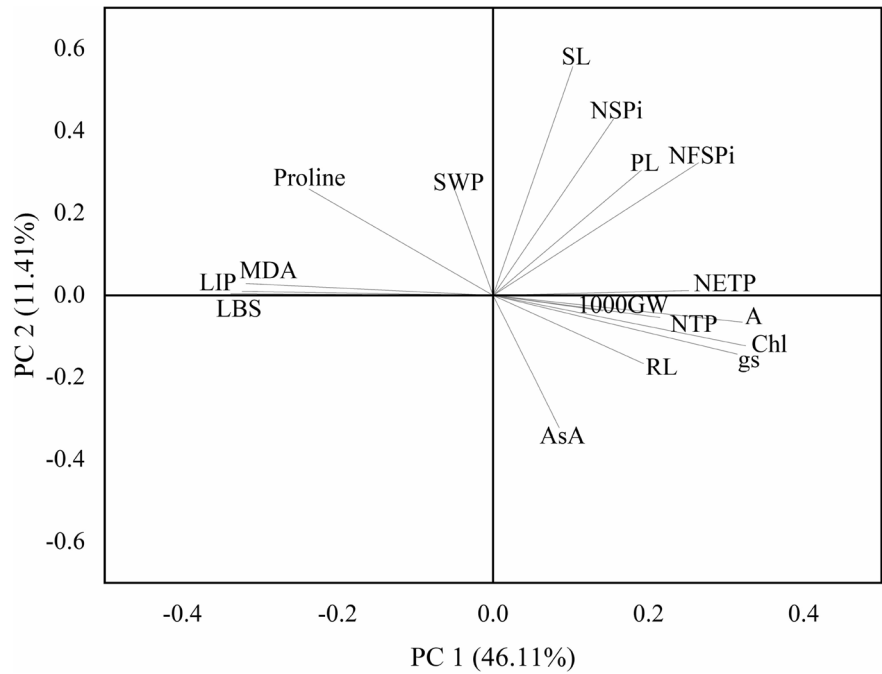
traits except for ascorbic acid content. The second principal component (PC2) explained higher values for leaf visible symptoms, biochemical traits (proline and MDA), growth (shoot length), and all the yield traits except for 1000 grain weight, and lesser loading for all physiological traits, biochemical trait (ascorbic acid), growth (root length and number of tillers per plant), and yield traits (1000 grain weight).

Fig. 10 Principal component analysis describing classification of rice cultivars
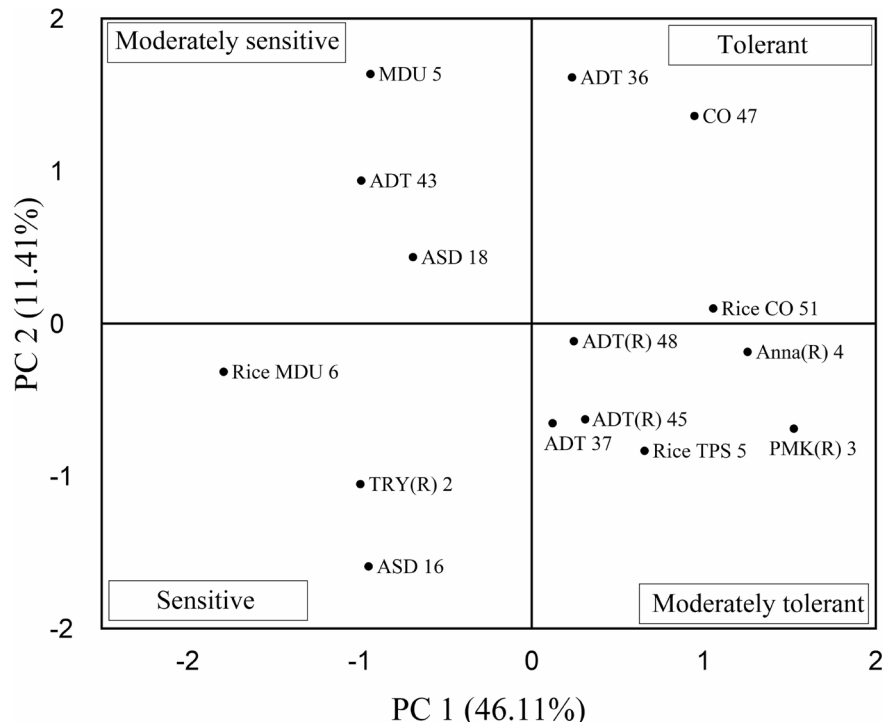
This multivariate analysis allows the identification of ozone responsive variables that are best described to ozone tolerance.

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 this 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 current investigation revealed that elevated ozone significantly decreased plant photosynthetic traits, altered antioxidant metabolism, reduced tiller numbers and increased spikelet sterility. Ultimately, these altered plant traits led to substantial yield loss suggesting that all 15 rice cultivars are relatively susceptible to elevated tropospheric ozone stress. Nevertheless, the test cultivars with different genetic diversity depicted variations in ozone tolerance under elevated ozone stress. The cultivars under ozone tolerant and moderately tolerant category namely Rice CO51, CO47, ADT36, ADT37, ADT(R)45,

Rice TPS5, Anna(R)4, PMK(R)3, and ADT(R)48 would be recommended for cultivation in the region experiencing the ozone concentration around 50 ppb level. Improved agronomic practices and nutrient management would improve crop production and reduce ozone-induced yield loss of rice cultivars grown under elevated ozone stress. Since prevalence of ozone concentration shows seasonal variation, shifting cropping season that records negligible amount of tropospheric ozone would be a possible option for cultivation of ozone sensitive rice cultivars. Moreover, the cultivar specific variations in plant traits under ozone stress could offer the prospect for developing of ozone tolerant rice cultivars through genome mapping and quantitative trait loci (QTL) based approach which would potentially improve the rice production at global scale. A significant genetic variation in ozone sensitivity and tolerance among test cultivars would perhaps provide a vital information for developing models to predict ozone-induced yield loss and adaptation strategies for sustainable rice production.

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

Declarations

Competing interests The authors declare that they have no known competing interests.

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