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RESEARCH ARTICLE

Assessment of Physiological, Biochemical and Yield Attributes of Rice Cultivars under Elevated Ozone Stress

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ABSTRACT

The present study aimed to assess the response of 15 rice cultivars exposed to elevated ozone stress. The plants were exposed to 100 ppb ozone (10:00–17:00 h) for 30 days at the reproductive stage. Elevated ozone significantly alters the physiological, biochemical, growth and yield traits of all test rice cultivars. Elevated ozone exposure significantly decreased photosynthetic rate maximally in MDU5 (42.90%) and TRY(R)2 showed maximum reduction in stomatal conductance (42.39%) and chlorophyll content (41.22%). In terms of yield traits, a decrease in tiller number, number of effective tillers, number of filled spikelets per panicle and 1000 grain weight over the control were also observed. The multivariate analysis with a total variance of 58.86% categorized the 15 rice cultivars into ozone sensitive (TRY(R) 2, ASD16, ADT(R)45 and MDU5), moderately ozone sensitive (ASD18, ADT43, Rice MDU6, ADT36 and ADT37), moderately ozone tolerant (ADT(R)48 CO47 and Rice CO51) and ozone tolerant (Rice TPS5, Anna(R)4 and PMK(R)3) under 100 ppb ozone. The present results revealed that the ozone tolerant rice cultivars would be recommended to cultivate in the regions experiencing high ozone concentration.

Keywords: *Rice cultivars; Elevated ozone; Plant traits; Principle Component Analysis.*

INTRODUCTION

Tropospheric ozone pollution is considered as an important threat to crop species and food security. In the current scenario, tropospheric ozone and precursors are increasing due to industrialization and urbanization. In southern parts of India, ozone concentrations were varied from <10 to >200 ppb. According to Pulikesi et al.(2006), ozone concentrations ranged from 30 to 69 ppb during March to October, 2005 at Chennai. The ozone concentrations were recorded as 29-67 ppb (December – July, 2009) in Anantapur (Reddy *et al.,* 2010), 19.04-50.83 ppb (summer, 2012) in Suchindrum, Tamil Nadu (Sharma et al., 2013), 16.8 – 101.2 ppb (Summer, 2016) in chennai, Tamil Nadu (Prabakaran *et al.,* 2017) and 1.11 – 292.36 ppb (pre monsoon,2016) in chennai, Tamil Nadu (Mohan and Saranya, 2019).

Some projections articulate that ozone was projected to rise at a rate of 10 ppb per decade from 2000 to 2020 (Austin and Butchart, 2003). As per the projection, a 40% increase in O3 concentration is expected in the South Asian region by the year 2050 (Tai et al., 2014). Simulations for the period 2050 project boosts in the ozone of 20 to 25 per cent and simulations through 2100 indicate that ozone may increase by 40 to 60 per cent (Hauglustaine et al., 2005). Impacts are going to be most severe over many parts of India and China in recent decades (Lal *et al.,* 2017).

Ozone incidents on the crop can be both chronic exposure (high background ozone concentration throughout the growing season) as well as acute ozone stress when concentrations exceed approximately 100 parts per billion (ppb), which can lead to the hypersensitive response and stimulation of cell death (Osborne, 2016). The global threat to food security due to ground level O3 pollution and its interaction with climate change scenario has been well documented by many researchers (Sarkar et al., 2015, Van Dingenen *et al.*, 2009; Avnery et al., 27 2013).

Rice is known to be as susceptible to tropospheric ozone pollution as other major crop species like soybean and wheat (Biswas et al., 2008; Sinha et al., 2015; Daripa et al., 2016), particularly cultivars from the Asian region (Shi et al., 2009; Wang et al., 2012; Ramya et al., 2020). During the 1990's, there were five global hot spots where three months mean tropospheric ozone reached 60–70 ppb and

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two of the hot spots were in most of the rice growing regions of China and India (Emberson et al., 2009). The global crop yield loss due to tropospheric ozone was estimated to be worth $14–26 billion for the year 2000, about 40% of which may be occurring in China and India (Van Dingenen et al., 2009). 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 the 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). Hence, the present investigation is concentrated on assessing the response physiological, biochemical and yield traits of rice cultivars under elevated ozone stress.

MATERIAL AND METHODS

Fifteen short duration rice cultivars i.e., i) Rice CO 51, ii) CO 47, iii) ADT 36, iv) ADT 37, v) ADT 43, vi) ADT(R) 45, vii) ADT(R) 48, viii) ASD 16, ix) ASD 18, x) MDU 5, xi) MDU 6, xii) Rice TPS 5, xiii) TRY 2, xiv) Rice Anna 4 and xv) Rice PMK(R) 3, which are widely cultivated by farmers around Tamil Nadu region (Table 1) were taken for assessing the impact of elevated tropospheric ozone (100 ppb from 10:00 to 17:00 h) exposure for 30 days from 51 DAS to 80 DAS. The exposure index for ozone concentration, i.e., AOT 40 (accumulated hourly ozone concentration above 40 ppb), was calculated by the formula explained by Mauzerall and Wang (2001),

$$AOT40 = \sum_{i=1}^{n} O_3 - 40$$

where, O_3 denotes the mean ozone values per hour (ppb) which is above 40 ppb, i is the index, and n indicates the number of hours.

The physiological parameters i.e., photosynthetic rate, stomatal conductance and chlorophyll content, the biochemical parameters i.e., proline and malondialdehyde, ascorbic acid content, the growth parameters i.e., root length, shoot length, tiller number and the yield parameters i.e., panicle length, number of effective tillers per plant, number of spikelets per panicle, number of filled spikelets per panicle, 1000 grain weight and straw weight per plant were taken for analysis.

Photosynthetic rate and stomatal conductance were quantified using a portable photosynthesis system (ADC BioScientific LCpro-SD System, UK) and chlorophyll content was measured using a chlorophyll content meter (CCM-200+, USA). The malondialdehyde content (MDA) was estimated by following the protocol of Heath and Pacber (1968) and expressed as μmol g^{-1} fresh weight. The proline content was carried out according to the method of Bates *et al*. (1973) and expressed as μmol g^{-1} fresh weight. Ascorbic acid content (AsA) was measured by using 2, 6 dichlorophenol indophenol (DCPIP) reduction method (Keller and Schwager, 1977) and expressed as mg g^{-1} fresh weight. All the physiological and biochemical traits were observed after 30 days of 100 ppb ozone exposure. All the growth and yield traits were measured at the crop maturity stage.

Statistical analysis

The relative plant physiological, biochemical and yield traits were calculated using the following formula, Relative plant trait = (ozone/control)×100. All the plant traits were displayed as relative plant traits. All the statistical analyses were performed using the SPSS tool (SPSS Inc., version 16.0.0). P-value less than 0.05 ($P < 0.05$) considered as significant. The multivariate analysis (Principal component analysis) was performed using R software (Version 3.5.1) and OriginPro 2019 (version 9.6.5) was used to plot the graphs.

RESULTS AND DISCUSSION

Ozone exposure

A report by number of researchers indicated that AOT40 for rice growing season at various sites of India exceeding the threshold level. The AOT40 values of 11.2 ppm.h at Varanasi for the period July to September, 2011 were showed significant yield loss in rice cultivars (Sarkar *et al.,* 2015). Similarly, at Mohali AOT40 recorded as 19.8 and 12.6 ppm.h from June to Septembe r, 2012 and 2013, respectively registered growth and yield loss in rice cultivars (Sinha *et al*., 2015). Kumari *et al*., (2020) reported that AOT40 of 1.9 and 2.4 ppm.h were recorded from July to September, 2010 and 2015, respectively. In present study, the calculated AOT40 was recorded as 10.3 ppm.h showed significant reduction in physiological, growth and yield traits.

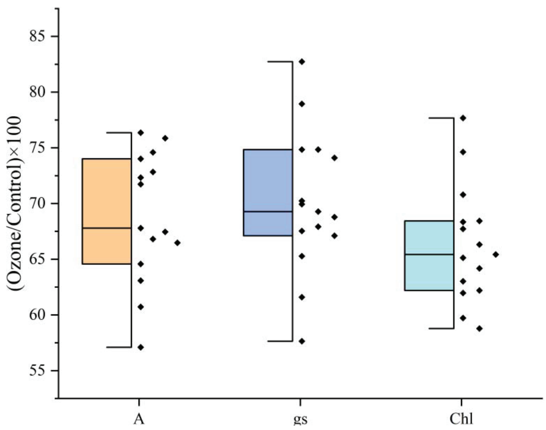

Figure 1. Relative physiological traits of 15 rice cultivars exposed to 100 ppb elevated ozone. (A=Photosynthetic rate, gs=Stomatal conductance, Chl=Chlorophyll content)

Physiological traits

Rice cultivars exposed to elevated ozone exhibited a significant reduction in photosynthetic rate, stomatal conductance and chlorophyll content. At elevated ozone treatment, the photosynthetic rate was significantly reduced maximally in MDU5 (Control: 24.44 and O3: 13.95 µmol CO_2 $m^{-2}s^{-1}$) and minimum reduction were observed in Anna(R)4 (Control: 26.10 and O3: 19.93 µmol CO_2 m^{-2} s^{-1}). Similarly, ozone exposure caused a marked reduction in stomatal conductance were maximum in TRY(R)2 (Control: 0.48 and O_3: 0.28 mol H_2O $m^{-2}s^{-1}$) and minimum reduction in Anna(R)4 (Control: 0.56 and O_3: 0.46 mol H_2O $m^{-2}s^{-1}$). The maximum reduction in chlorophyll content were noticed in TRY(R)2 (Control: 34.93 and O_3: 20.53) and minimum in Anna(R)4 (Control: 37.93 and O_3: 29.47) at 30 days of ozone exposure.

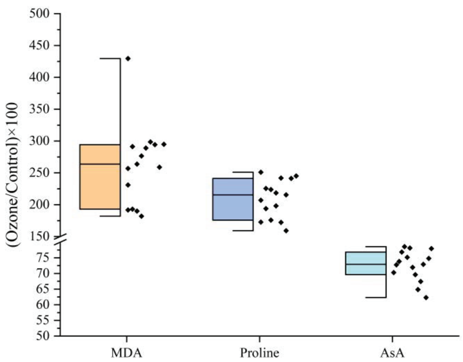

Figure 2. Relative biochemical traits of 15 rice cultivars exposed to 100 ppb elevated ozone. (MDA= Malondialdehyde content, Proline= Proline content, AsA=Ascorbic acid content)

The relative physiological traits were varied among the photosynthetic rate, stomatal conductance and chlorophyll content. The boxplot of relative physiological traits of 15 rice cultivars are depicted in Figure 1. The maximum and minimum relative physiological traits were recorded 76.36 and 57.09% for photosynthetic rate, 82.73 and 57.63% for stomatal conductance and 77.68 and 58.77% for chlorophyll content. The results showed that the deviation between rice cultivars were less for photosynthetic rate and chlorophyll content compared to stomatal conductance. The mean values for relative plant traits were recorded as 67.79, 69.27 and 65.42% for photosynthetic rate, stomatal conductance and chlorophyll content, respectively. In terms of relative photosynthetic rate, stomatal conductance and chlorophyll content, 75 percentile of rice cultivars lies between 73.42, 74.46 and 68.39%, respectively and 25 percentile of rice cultivars located between 65.52, 67.31 and 62.60%, respectively. These variations in relative plant traits were related to the genotypic difference among test rice cultivars. Furthermore, deviations in relative plant traits showed differences in tolerance levels of various rice cultivars under elevated ozone stress. The decrease in stomatal conductance was also observed by Chen et al. (2011) in rice genotypes of SL46 and NB under 5 days of ozone stress. Moreover, plants exposed to elevated ozone on a daily basis continuously decrease stomatal conductance, which negatively influences the photosynthetic rate (Fiscus et al. 2005). Similar to the current report, Akhtar et al. (2010) observed that 100 ppb of ozone exposure significantly decreased the photosynthetic rate of BR11, BR14, BR28, and BR29 (Bangladeshi rice cultivars). Consistently decrease in chlorophyll content in the present study might be due to degeneration of chloroplast under elevated ozone stress, which ultimately alters the carbon assimilation capacity of the plant species (Jing et al., 2016).

Biochemical traits

Malondialdehyde and proline contents in all test rice cultivars showed significant increment under elevated ozone condition, whereas decrease in ascorbic acid content was noticed. The thirty days of ozone exposure increased MDA content maximally in Rice MDU6 (Control: 0.61 and O_3: 3.21 µmol g^{-1} FW) and least in PMK(R)3 (Control: 0.81 and O_3: 1.47 µmol g^{-1} FW).

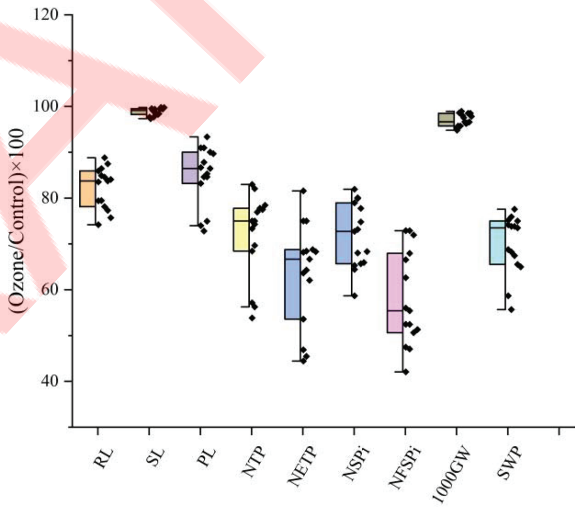

Figure 3. Relative growth and yield traits of 15 rice cultivars exposed to 100 ppb elevated ozone. (RL=Root length, SL=Shoot length, PL=Panicle length, NTP=Number of tillers, NETP=Number of effectivetillersper plant, NSPi=Numberofspikelets per panicle, NFSPi=Number of filled spikelets per panicle, 1000 GW=Thousand grain weight, SWP=Straw weight)

The relative biochemical traits of 15 rice cultivars were depicted in Figure 2. The maximum and minimum relative biochemical traits were recorded 429.52 and 181.69% for photosynthetic rate, 251.05 and 159.19% for stomatal conductance and 78.54 and 62.28% for chlorophyll content. The mean values for relative biochemical traits were higher for malondialdehyde (263.79%) and proline content (215.49%); whereas ascorbic acid content showed a less mean value of 72.89%.

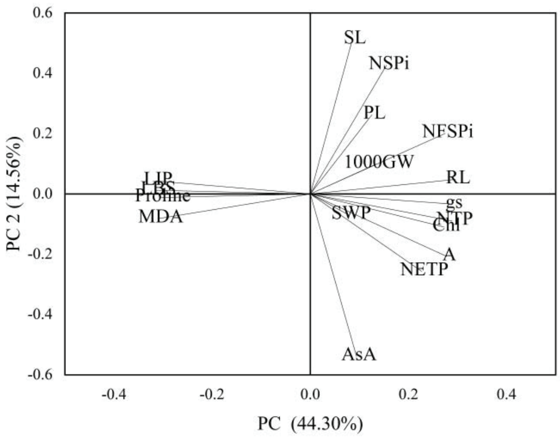

Figure 4. Principal component analysis describing plant traits of 15 rice cultivars exposed to 100 ppb elevated ozone.

Higher in malondialdehyde and proline content were mainly due to a significant increase in malondialdehyde and proline content under elevated ozone exposure, while ascorbic acid content showed a decrease in the trend under elevated ozone stress. Moreover, in all biochemical traits, maximum number of rice cultivars were spread up to 75 percentile and the deviation was very higher for malondialdehyde and proline content compared to ascorbic acid content.

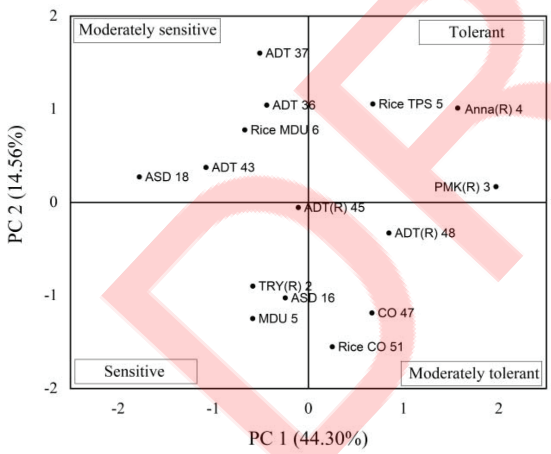

Figure 5. Principal component analysis describing classification of 15 rice cultivars exposed to 100 ppb elevated ozone.

An increase in malondialdehyde concentration under elevated ozone exposure in the present study was and this may be associated with an increase in lipid peroxidation and alterations in reactive oxygen species scavenging mechanism (Li *et al.,* 2017). Ozone accumulation under 100 ppb elevated ozone stress degenerated the ozone scavenging capacity of the leaves leads to an increase in malondialdehyde concentration and production of proline content in all test rice cultivars (Loreto, 2001). Similarly, Li *et al.* (2017) observed that MDA content increased in rice cultivars of Bt-SY63 and SY63 of about 30.6 and 23.7%, respectively, in elevated ozone-treated plants. Furthermore, the production of proline in ozone-treated plants observed in the present study might be associated with the production of proline under abiotic stress conditions as an adaptive mechanism (Yang *et al.,* 2012). Similarly, Upadhyaya *et al*. (2007) reported that a significant increase in proline content were observed in rice leaves exposed to 0.2 millimoles of hydrogen peroxide. Fumigation with SO_2, NH_3, and NO_2 increased proline accumulation in rice cultivars of GR3 and TKM9 (Anbazhagan *et al.,* 1988). Furthermore, a decrease in ascorbic acid content in rice leaves expose to elevated ozone is linked with poor non-enzymatic defense mechanism and AsA pool depleted gradually under continuous ozone exposure (Rai and Agrawal, 2008). Similarly, Frei *et al*. (2012) reported that rice ND6172 had decreased AsA contents by 20–30% compared to Nipponbare under 100 ppb ozone. Wang et al. (2013) observed that ozone sensitive rice cultivar Shanyou63 decreased AsA content by 22.75% than ozone tolerant Wuxiangjing14 exposed to elevated ozone (>250 nmol mol^{-1}).

Growth and yield traits

The magnitude of decline in growth and yield traits i.e., root length, shoot length, number of tillers and 1000 grain weight were observed in all the rice cultivars due to elevated ozone exposure. The maximum reduction in tiller number were noticed in MDU5 (Control: 13.0 and O_3: 7.0) and showed minimum reduction for Anna(R)4 (Control: 15.7 and O_3: 13.0). The maximum reduction in 1000 grain weight was noticed in ASD16 (Control: 25.1 and O_3: 23.8 g) while minimum reduction was observed in ADT43 (Control: 15.7 and O_3: 15.5 g).

The relative growth and yield traits were described in Figure 3. The mean relative root, shoot and panicle length were recorded to be 83.74, 99.13 and 86.46%, respectively. In terms of the number of tillers per plant, number of effective tillers per plant and number of spikelets per panicle were showed mean of 75.00, 66.66 and 72.73%, respectively. In the current study, a decrease in root length, shoot length, panicle length and panicle number were attributed to a decrease in foliar carbon assimilation rate and it would have inhibited the growth and development of rice cultivars (Wang et al., 2013). Similarly, indica, japonica and bangladeshi rice cultivars also showed a decease in plant height and tiller number under elevated ozone treatment (Akhtar et al., 2010). Also, Shao et al. (2020) reported that the smaller panicle length was corresponded with a smaller plant and reduced tiller numbers which inhibited the effective tillers under ozone stress.

Similarly, the number of filled spikelets per panicle, 1000 grain weight and straw weight per plant were registered mean values of 55.42, 96.65 and 73.50%, respectively. Among all growth and yield traits, most of the rice cultivars lie above 25 percentile for panicle length, the number of tillers per plant and number of effective tillers per plant. The deviation in relative plant traits was very high for number of effective tillers per plant and number of filled spikelets per panicle, whereas very low for shoot length and 1000 grain weight.

Table 1. Parentage and characteristics of 15 rice cultivars

Rice cultivars	Parentage	Agronomic characteristics of cultivars
ADT 36	Triveni x IR 20	Resistant to BPH and Blast
ADT 37	BG 280-12 x PTB 33	Resistant to many pest and diseases
ADT 43	IR 50 x White ponni	Resistant to Green Leaf Hopper
ADT (R) 45	IR 50 x ADT 37	Resistant to Gall midge
ADT (R) 48	IET 11412 x IR 64	Resistant to Green Leaf Hopper, Stem borer and Gall midge
Anna (R) 4	Pantdhan 10 x IET 9911	Drought tolerant
ASD 16	ADT 31 x CO 39	Resistant to Blast
ASD 18	ADT 31 x IR50	Resistant to BPH
CO 47	IR 50 x CO 43	Resistant to Blast
MDU 5	O.glaberrima x Pokkali	Resistant to Drought
PMK (R) 3	UPLRI 7 x CO 43	Drought tolerance,
Rice CO 51	ADT 43 x RR 272 – 1745	High yielding semi dwarf rice variety, Moderately resistant to Brown Plant Hopper (BPH) and Green Leaf hopper
Rice MDU 6	MDU 5 x ACM 96136	High yielding
Rice TPS 5	ASD16 x ADT37	High yielding, Moderately resistant to stem borer and gall midge
TRY (R) 2	IET 6238 x IR 36	Saline/Alkaline tolerant

These deviations in plant relative traits were related to genotypic variations among the test rice cultivars. Furthermore, these results showing a difference in ozone tolerance level under elevated ozone stress. The decrease in yield traits ie., number of spikelets per panicle, number of filled spikelets per panicle and 1000 grain weight, were attributed to an alteration in assimilates allocation to panicles. This could be due to plant utilized more assimilates for their respiration and regulating antioxidant metabolism under elevated ozone stress. Similarly, elevated levels of ozone (100 ppb) decreased the spikelet number and filled spikelets per panicle in the Nipponbare rice variety (Wang *et al*., 2014). The decrease in filled spikelets per panicle and 1000 grain weight might be due to reduced fertilization efficiency of rice cultivars under ozone stress and it would affect the carbohydrate available for the grain filling process, which ultimately leads to reduction in grain yield (Jing *et al*., 2016). Similarly, ozone-induced reduction in grain weight was observed in bangladeshi rice cultivars (Ashrafuzzaman et al., 2017), modern indica and japonica rice cultivars (Shao *et al*., 2020) under elevated ozone stress. Furthermore, reduction in straw weight was associated with decrease in photosynthetic rate, modification in phloem loading and translocation (Akhtar *et al*., 2010).

Multivariate analysis

Principal component analysis (PCA) was carried out to identify ozone tolerant and sensitive rice cultivars with respect to various plant traits under elevated ozone stress. PCA has been used to group the ozone tolerant and sensitive wheat cultivars (Fatima *et al*., 2019) and European beech (Löw *et al*., 2012). The first two principal components (PC1 and PC2) explained 44.30% and 14.56%, respectively with the cumulative eigenvalue of 58.86%. Most of the plant yield traits were clustered together and showed higher loading in first component, followed by physiological traits and biochemical traits showed lesser loading in the second component. These relative plant traits in two principal components representing the best descriptor for grouping rice cultivars (Figure 4). Further, Principle component categorized the 15 rice cultivars into four major groups indicating TRY(R) 2, ASD16, ADT(R)45 and

MDU5 were ozone sensitive, ASD18, ADT43, Rice MDU6, ADT36 and ADT37 were moderately ozone sensitive, ADT(R)48 CO47 and Rice CO51 were moderately ozone tolerant and Rice TPS5, Anna(R)4 and PMK(R)3 were ozone tolerant (Figure 5). Similarly, for accurate and reliable screening of salt-tolerant and sensitive rice cultivars (74 rice cultivars) Kakar *et al*. (2019) applied principal component analysis and Mazid *et al*. (2013) categorized 41 rice genotypes for screening of bacterial blight resistance genotypes using PCA.

CONCLUSION

The present investigation demonstrated the response of 15 rice cultivars in terms of physiological, biochemical, growth and yield traits to elevated ozone stress. The results indicated that all test rice cultivars significantly reduced the important plant traits, namely photosynthetic rate, stomatal conductance, chlorophyll content, tiller number, filled spikelets per panicle and 1000 grain weight. Moreover, studied plant traits clearly categorized the 15 rice cultivars into four different ozone sensitive (TRY(R) 2, ASD16, ADT(R)45 and MDU5), moderately ozone sensitive (ASD18, ADT43, Rice MDU6, ADT36 and ADT37), moderately ozone tolerant (ADT(R)48 CO47 and Rice CO51) and ozone tolerant (Rice TPS5, Anna(R)4 and PMK(R)3) groups. Hence, the present outcome would be useful for breeding ozone tolerant rice cultivars to overcome future climate change scenarios.

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Competing interests

The authors declare that they have no known competingfinancialinterestsorpersonalrelationships that could have appeared to influence the work reported in this paper.

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