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Effect of elevated ozone and carbon dioxide on growth and yield of rice (*Oryza sativa*)

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ABSTRACT

An experiment was conducted by growing Pusa 44 rice (*Oryza sativa* L.) variety under different treatments: Ambient, Elevated O_3 (EO_3: 60 ± 10 ppb), Elevated CO_2 (ECO_2: 550 ± 10 ppm) and Elevated CO_2 and O_3 (550 ± 10 ppm CO_2 and 60 ± 10 ppb EO_3) in free air carbon dioxide and ozone enrichment facility (FACE-O_3) with the objective to assess the impact of elevated tropospheric ozone (O_3) and carbon dioxide (CO_2) interaction on growth and yield of rice. The crop was exposed to elevated levels of gases from transplanting to maturity. EO_3 was found to be having a negative impact on all the growth parameters at vegetative, anthesis and maturity stages. ECO_2 was found to be having a compensatory effect over EO_3 for the different growth parameters. On an average the compensatory effect of ECO_2 over EO_3 on yield was about 40% and negative impact of elevated O_3 over control was around 26% in both the years.

Keywords: Elevated CO_2, Elevated O_3, Growth stage, Rice, Yield

Increasing ozone (O_3) and carbon dioxide (CO_2) concentration in the atmosphere are of major concern (Wang *et al.* 2017) due to its direct effect on agriculture. Ground level O_3 concentrations are increasing at the rate of 1-2% per year (Wang and Frie 2011) and may reach 60-70 ppb by 2100 (IPCC 2014), whereas atmospheric CO_2 is currently 415 ppm and is expected to reach 700 ppm by 2100 (NOAA 2019). The most rapid increase in O_3 is occurring in south Asia and may result in crop production losses (Tiwari and Agrawal 2018).

Rice (*Oryza sativa* L.) is the widely grown crop globally, occupying about 161.8 Mha area, of which 10.5 Mha lies in the rice-wheat system of the Indo-Gangetic Plains (Gupta *et al.* 2016). Ozone is known to impact stomatal conductivity (g_s), net CO_2 assimilation and carboxylation efficiency (Singh *et al.* 2013). Elevated O_3 causes physiological changes in crops, which are translated into morphological changes and reduces plant growth (Bhatia *et al*. 2013). Decline in photosynthetic rate (P_N) in the plants exposed to O_3 stress is associated with damage to photosynthetic machinery (Daripa *et al*. 2016), biophysical parameters such as g_s and internal CO_2 also play important roles in determining the photosynthetic yield and sensitivity of O_3 exposed plants. Under CO_2 rich and warm atmosphere, plants may show a tendency to reduce g_s, thus, indirectly alleviate the O_3 stress in plants (Yadav *et al*. 2019). Thus the objective of this study was to quantify the effect of elevated O_3 and CO_2 interaction on growth and yield of rice.

MATERIALS AND METHODS

Experimental site and soil: A field experiment was conducted at the farm of ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi by growing Pusa 44 rice genotype in *kharif* (July−Mid November) during 2016–17. The soil at the experimental site belongs to the major group of Indo-Gangetic alluvium (Typic Haplustept), non-calcareous and slightly alkaline in reaction. The climatic variables and soil properties of the experimental site were as in Bhatia *et al*. (2021).

Treatments and crop management: This experiment was carried out in under free air Carbon Dioxide and Ozone enrichment (FACE-O_3) in four 6-m diameter circular plots with perforated horizontal tubings, with one ambient plot (ACO_2 + AO_3) and three plots each receiving elevated CO_2 (ECO_2+ AO_3), elevated O_3 (EO_3 + ACO_2) and both ECO_2 and EO_3. CO_2 and O_3 were released through horizontal perforated tubing's above the soil surface at canopy level to attain the target CO_2 (550 ppm) and O_3 (60 ppb) concentration. The O_3 concentration was measured using an O_3 concentration analyzer (2B technologies) and CO_2 analyser (Fuji).

Transplanting of seedlings of rice sown on 20 June was carried out in 5 replicate crates (size 0.24 m^2) on 12 July in 2016 and 16 July in 2017. EO_3 and ECO_2 exposure in

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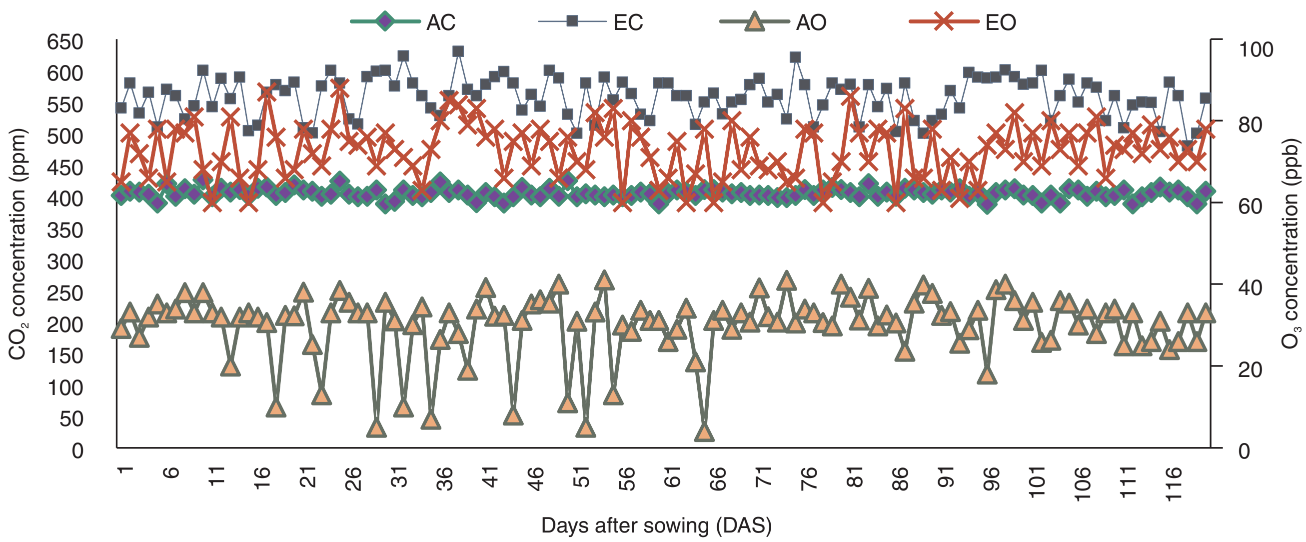

Fig 1 Daily average concentrations of ambient CO_2 (AC), elevated CO_2 (EC), ambient O_3 (AO) and elevated O_3 (EO) during rice growth period in 2016 and 2017.

rings began on 19 July 2016 and 23 July 2017 and ended on 29 October, 2016 and 1 November 2017, respectively. Urea at the rate of 12 g/m^2 was added in three splits of 6, 3 and 3 g/m^2 at 0, 35 and 70 days after transplanting (DAT). The rice soil was saturated with water till 85 DAT. Weeds, pests and diseases were controlled as required.

Plant sampling and analysis: Plant samples were collected for recording of biomass of different plant parts at three different growth stages, viz. active tillering, anthesis and maturity stage. For taking the dry weight of different plant parts, they were kept in a pre-heated hot air oven 65°C for 72 hr till a constant dry weight was achieved.

Data analysis: Statistical analysis of the data was done using SPSS 23.0 (SPSS Inc., Chicago, USA) for windows. Analysis of variance (ANOVA) was done to test whether the differences were statistically significant using completely randomized design.

RESULTS AND DISCUSSION

Concentration of CO_2 and O_3 in the rings: The season-long daytime average CO_2 concentration in the ambient and in the elevated plots was 404.6 ± 4.6 ppm and 557.8 ± 11.2 ppm, respectively. The target O_3 concentration in FACE-O_3 was higher (62.6 ± 5.5 ppb) than the ambient (30.1 ± 4.3 ppb) (Fig 1).

Leaf area: Total leaf area ranged from 1263.17 cm^2/hill in ACO_2+EO_3 at tillering to 3467.88 cm^2/hill in ECO_2+AO_3 at maturity stage in 2016 and 1546.92 cm/hill in ECO_2+EO_3 during tillering to 3544.77 cm^2/hill in ECO_2+AO_3 during maturity stage in 2017 (Fig 2). Negative impact of EO_3 on leaf area was most pronounced at tillering followed by anthesis and maturity stages. Elevated CO_2 has been found to increase the leaf area (Kimball *et al.* 2016) and EO_3 has been found to decrease the leaf area (Yadav *et al.* 2020). Leaf area is a basis of measuring productivity during the growth and development under different environmental condition.

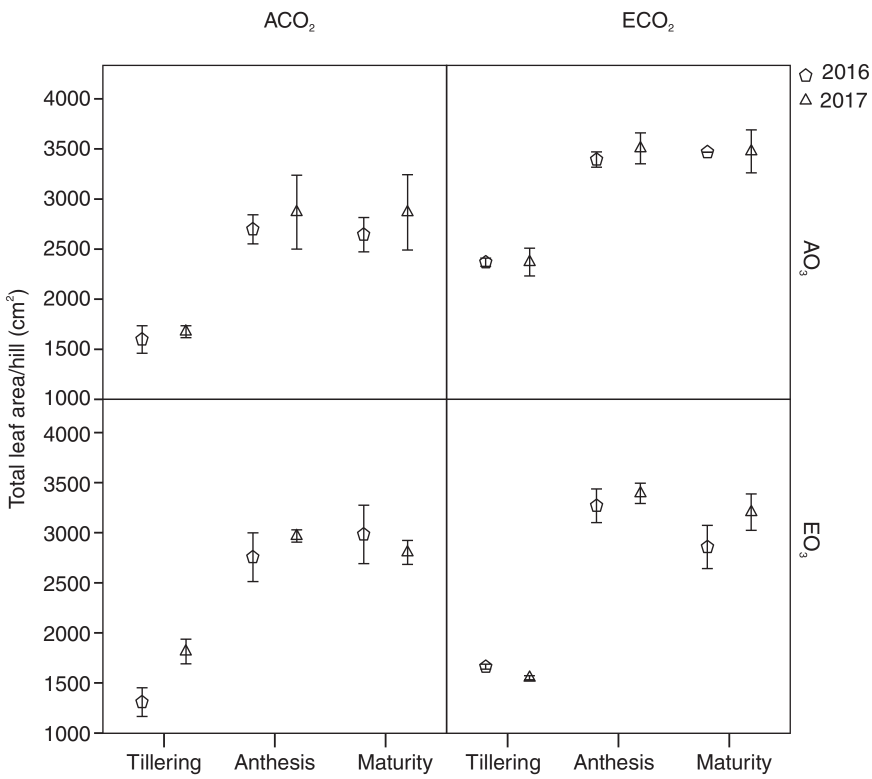

Fig 2 Effect of different treatments of CO_2 and O_3 on total leaf area/hill of rice at tillering, anthesis and maturity stages during the year 2016 and 2017. ACO_2; Ambient CO_2, ECO_2; Elevated CO_2, AO_3; Ambient O_3, EO_3; Elevated O_3.

Table 1 Effect of different treatments of CO_2 and O_3 on growth parameters of rice during the years 2016 and 2017

Treatment	DW (leaves) (g/hill)			DW (Culms) (g/hill)			DW (roots) (g/hill)			Plant height (cm)			No. of tillers/hill		
	T	A	M	T	A	M	T	A	M	T	A	M	T	A	M
2016															
ACO_2+AO_3	12.65	15.67	19.94	11.90	10.44	13.17	30.15	29.93	28.66	84.00	102.50	100.00	27.50	33.50	34.00
ACO_2+EO_3	8.65	15.69	20.76	8.25	10.56	16.65	8.95	15.61	21.06	83.50	96.50	103.00	23.50	28.50	28.50
ECO_2+AO_3	13.15	23.61	30.01	12.25	17.41	26.76	19.95	48.38	37.42	94.00	107.50	100.50	22.00	30.00	28.00
ECO_2+EO_3	9.25	14.71	20.21	8.55	15.99	21.61	21.00	23.01	29.03	89.50	108.50	117.50	22.50	33.00	34.50
SE (d)	1.33	3.40	3.78	2.06	2.01	2.18	5.02	7.28	5.29	2.90	3.12	4.07	1.50	2.72	1.81
LSD (P=0.05)	2.90	2.10	NS	4.50	4.39	4.76	10.94	15.88	11.53	6.32	6.80	8.87	3.26	5.93	3.95
CO_2	**	**	NS	**	**	**	**	**	NS	**	**	**	NS	**	NS
O_3	**	**	NS	NS	NS	**	**	**	**	**	**	**	**	**	**
$CO_2\times O_3$	NS	NS	NS	**	**	**	NS	NS	NS	**	NS	**	**	**	**
Impact of EO_3 (%) over Control	-46.2	0.1	3.9	-44.2	1.1	20.9	-236.9	-91.7	-36.1	-0.6	-6.2	2.9	-17.0	-17.5	-19.3
Compensation effect of ECO_3 (%) over EO_3	6.9	-6.2	-2.6	3.6	51.4	29.8	134.6	47.4	37.8	7.2	12.4	14.1	-4.3	15.8	21.1
2017															
ACO_2+AO_3	11.05	17.18	25.00	8.70	11.00	12.44	22.35	21.00	31.46	82.00	103.50	107.00	27.00	28.50	32.50
ACO_2+EO_3	9.90	13.05	20.39	8.25	11.96	17.34	4.60	16.81	20.65	79.50	101.50	104.00	25.00	30.50	30.00
ECO_2+AO_3	14.60	22.72	29.51	15.60	16.83	25.85	20.75	51.96	35.19	91.50	108.00	102.00	25.00	31.50	28.50
ECO_2+EO_3	7.65	16.35	19.51	8.45	15.89	21.42	10.40	26.96	30.35	86.00	106.50	107.50	25.00	30.50	33.00
SE (d)	0.56	0.85	1.41	0.50	0.57	1.32	1.07	1.45	1.50	2.08	2.38	2.47	1.36	1.59	1.67
LSD (P=0.05)	1.23	1.85	3.08	1.10	1.25	2.88	2.34	3.16	3.27	4.53	5.20	5.39	2.98	3.47	3.64
CO_2	**	**	NS	**	NS	**	**	**	NS	**	**	NS	**	**	NS
O_3	**	**	NS	**	**	NS	**	**	**	**	**	**	NS	**	NS
$CO_2\times O_3$	NS	**	**	**	**	NS	**	**	**	NS	NS	**	**	NS	**
Impact of EO_3 (%) over Control	-11.6	-31.6	-22.6	-5.5	8.0	28.3	-385.9	-24.9	-52.3	-3.1	-2.0	-2.9	-8.0	6.6	-8.3
Compensation effect of ECO_2 (%) over EO_3	-22.7	25.3	-4.3	2.4	32.9	23.5	126.1	60.4	47.0	8.2	4.9	3.4	0.0	0.0	10.0

Values are means. NS, not significant; ** $P < 0.05$. n=5. T; Tillering stage, A; Anthesis stage, M; Maturity stage, ACO_2+AO_3; Ambient CO_2+Ambient O_3, ACO_2+EO_3; Ambient CO_2+Elevated O_3, ECO_2+AO_3; Elevated CO_2+Ambient O_3, ECO_2+EO_3; Elevated CO_2+Elevated O_3.

Plant height: Plant height ranged from 79.5 cm in ACO_2+EO_3 at tillering to 117 cm in ECO_2+EO_3 at maturity in the two years (Table 1 and 2). Plant height of varieties is generally genetically specified but many studies on plant height showed that the increase of O_3 concentration generally reduces the plant height of rice.

Number of tillers: The tillers/hill increased from active tillering to anthesis and then decreased at maturity stage under different treatments (Table 1 a, b). The average tillers/hill in ECO_2 rings was always higher when compared with other treatments. Reduced tiller number under O_3 stress has been also reported in other studies (Tomer *et al*. 2015). Juan *et al.* (2021) reported significant increase in number of tillers under ECO_2 condition as compared to ambient due to redistribution of nitrogen to promote tillering.

Dry matter production: EO_3 has been found to be having negative impact on root and shoot biomass. O_3 generally reduces plant growth and the amount of dry biomass (Bhatia *et al.* 2011). Under different treatments the growth parameters of dry weight (DW) of leaves/hill, DW of culms/hill and DW of roots/hill were negatively affected under EO_3. Under ECO_2+EO_3 treatment, ECO_2 was found to be having compensatory effect on EO_3 (Table 1a, b). In both the years highest compensatory effect was observed in DW of roots (134.6% in 2016 and 126.08% in 2017) at tillering. DW of roots was larger under ECO_2+AO_3 treatment at all growth stages. It has been reported that EO_3 enhances carbon allocation to reproductive parts during anthesis, compromising the growth of roots (Emberson *et al.* 2018) which was evident in this study where under EO_3 the DW of roots reduced at different growth stages. Higher O_3 levels reduce root growth by altering photosynthetic partitioning (Bhatia *et al*. 2012). Reproductive stage is considered as most O_3 sensitive however; our results suggest that higher O_3 may reduce the dry mass of the leaf, root, and whole-plant even during the tillering stage.

Yield: Grain yield of rice ranged from 359 g/m^2 in ACO_2+EO_3 to 687 g/m^2 in ECO_2+AO_3 in during the two years. Compensatory effect of ECO_2 over EO_3 on yield was above 40% (Table 2) and negative impact of EO_3 over control was above 26%. ECO_2 interactively ameliorates the inhibition of photosynthesis induced by O_3 exposure (Kobayakawa and Imai 2011, Bhatia *et al.* 2021) that results in compensation of yield. The decline in grain yield was associated with decrease in various growth parameters under elevated O_3 conditions (Singh *et al.* 2017). O_3 from the environment enters into plants generally through stomata; encounters with apoplastic antioxidants which causes plasma membrane dysfunction which leads to membrane leakage. This causes metabolic dysfunction resulting in physiological changes including decreased photosynthesis and stomatal conductance and increased respiration rates (Bhatia *et al.* 2012). These physiological changes reduce carbon assimilation, plant growth and yield (Emberson *et al.* 2018). Harvest Index was found to decrease by approximately 6% and 4% under EO_3 condition as compared to control (Table 2). A meta-analysis by Ainsworth (2008) showed that the high O_3 concentration may decrease the harvest index in rice by 5%.

Elevated O_3 reduced the dry mass of the leaf, root, and whole-plant at both the tillering and flowering stages. Elevated O_3 significantly reduced the grain yield of rice. However, elevated CO_2 countered the yield decrease. The compensatory effect of ECO_2 over EO_3 on yield was above 40%.

Table 2 Grain yield, dry biomass and harvest index of rice during the years 2016 and 2017 under different treatments of CO_2 and O_3

Treatment	2016			2017		
	Grain yield (g/m^2)	Dry biomass (g/m^2)	Harvest Index (%)	Grain yield (g/m^2)	Dry biomass (g/m^2)	Harvest Index (%)
ACO_2+AO_3	500.97	1557.22	32.17	453.33	1797.36	25.24
ACO_2+EO_3	397.22	1522.36	26.09	359.03	1651.94	21.84
ECO_2+AO_3	687.08	2224.31	30.93	631.25	2066.67	30.60
ECO_2+EO_3	670.56	2506.67	26.76	603.06	1872.64	32.29
SE (d)	20.01	51.49	1.25	14.93	72.38	1.63
LSD (P=0.05)	41.31	106.21	2.59	30.81	149.39	3.37
ANOVA results						
CO_2	**	**	**	**	**	**
O_3	**	**	**	**	**	**
$CO_2 \times O_3$	**	**	NS	**	NS	**
Impact of EO_3 (%) over Control	-26.1	-2.3	-23.3	-26.3	-8.8	-15.6
Compensation effect of ECO_2 (%) over EO_3	40.8	39.3	2.5	40.5	11.8	32.4

Values are means. NS, not significant, **P<0.05. n=5. ACO_2+AO_3; Ambient CO_2+Ambient O_3, ACO_2+EO_3; Ambient CO_2+Elevated O_3, ECO_2+AO_3; Elevated CO_2+Ambient O_3, ECO_2+EO_3; Elevated CO_2+Elevated O_3

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