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Impacts of elevated ozone and CO_2 on growth and yield of double zero mustard (*Brassica juncea*)

GAYATHRI JAWAHARJOTHI[1], D K SHARMA[1], BOOMIRAJ KOVILPILLAI[2], ARTI BHATIA[1], SUDHIR KUMAR[1], MANJUNATH PRASAD[1], SACHIN SURESH SUROSHE[1], RANJEET RANJAN KUMAR[1], VIJAY DUNNA[1] and SOORA NARESH KUMAR[1]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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ABSTRACT

An experiment was conducted during 2020–21 and 2021–22 under FACE (Free air concentration enrichment) by growing PDZM 31 (Pusa Double Zero Mustard 31) genotype under different treatments (elevated ozone, carbon dioxide, their interaction, and ambient). The aim of the study was to assess the impact of elevated ozone and carbon dioxide interaction on the growth and yield of Indian mustard [*Brassica juncea* (L.) Czern.]. Growth characteristics (Crop growth rate, Absolute growth rate, Specific leaf area, Specific leaf weight, Dry weight of Biomass, Plant height, and no. of leaves) and yield attributes were negatively impacted under elevated ozone during different growth stages of Indian mustard followed by an increased growth under elevated CO_2. In seed yield under interaction treatment, the elevated CO_2 ameliorates the negative effects of elevated ozone by about 3.85% and 4.27% in both years.

Keywords: Dry weight biomass, Elevated ozone, Elevated carbon dioxide, Growth rate, Yield

Tropospheric ozone (O_3), a secondary air pollutant, a short-lived greenhouse gas (GHG) increased in concentration due to human activities in the past few decades (Pachauri *et al.* 2014). The concentration of tropospheric ozone is projected to increase by about 20–25% by 2050 (Chan and Yao 2008, Jaggard *et al.* 2010) and may reach 60–70 ppb by 2100 (IPCC 2014) across the globe. The atmospheric CO_2 concentrations (420 ppm) have increased by at least 35% and are forecasted to reach 540–970 ppm by the year 2100 (Solomon *et al.* 2007).

In India, mustard [*Brassica juncea* (L.) Czern.] is mainly grown in the northern (Rajasthan, Haryana, Madhya Pradesh, Uttar Pradesh, Gujarat etc.) and eastern parts (West Bengal, Jharkhand, Assam etc.) of the country which ranked 3rd position globally after Canada and China sharing about 11% of the global rapeseed-mustard production and 24.7 and 29.4% in terms of area and production (2018–19). The elevated ozone in crops harms the photosynthetic machinery and other gas exchange parameters such as stomatal conductance, intercellular CO_2 concentrations, water use efficiency and transpiration rate (Daripa *et al.* 2016) resulting in damaged cells in leaf tissues. To study the impact of elevated ozone and carbon dioxide interaction on the growth and yield characteristics an experiment was designed to evaluate the impact of tropospheric O_3 and CO_2 enrichment on Double Zero Mustard.

MATERIALS AND METHODS

A field experiment was conducted at the research field of ICAR-Indian Agricultural Research Institute, New Delhi by growing PDZM 31 variety during winter (*rabi*) season of 2020–21 and 2021–22. The soil type of the experimental site is Typic Haplustept which belongs to the group of Indo-Gangetic alluvium non-calcareous and slightly alkaline in reaction (Kumar *et al.* 2021).

The study was carried out under FAOE (free air ozone enrichment), FACE (free air carbon dioxide enrichment), and FAOCE (free air ozone and carbon dioxide enrichment) in circular plots and ambient open field conditions. The horizontal perforated tubing played a vital role in discharging O_3 (~65 ppb) and CO_2 (~550 ppm) above the soil surface and the rings were 6 m in diameter. The exposure begins from the date of germination till the maturity stage.

Growth characteristics and seed yield:

Crop growth rate ($g/cm^2/day$)

$$CGR = \frac{1}{\text{Ground area}} \times \frac{W2-W1}{t2-t1}$$

[1]ICAR-Indian Agricultural Research Institute, New Delhi; [2]ACRC, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. *Corresponding author email: dineshpusa69@gmail.com

Absolute growth rate (g/day)

$$AGR=\frac{W2-W1}{t2-t1}$$

where W2, W1- Dry weight of plants (g) at times t1 and t2 (day); t1, t2- Time interval (15 days)

Specific leaf area (cm^2/g)

$$SLA=\frac{\text{Leaf area } (cm^2)}{\text{Leaf weight (g)}}$$

Specific leaf weight (g/cm^2)

$$SLW=\frac{\text{Leaf weight (g)}}{\text{Leaf area } (cm^2)}$$

where Leaf area, measured by Leaf area meter (cm^2) (LI-3100 AREA METER); Plant height (cm), no. of leaves; Dry weight of biomass (g/plant), during 45 DAS, 60 DAS, 75 DAS and 90 DAS.

After harvest, from each replication, 4 plants were chosen and the total mean of treatments for no. of siliquae/plant was counted, in no. of seeds/silique, 25 siliquae were randomly selected from each replication and mean values were obtained. For test weight (g/1000 seeds), from each replication 4 sets of randomly drawn seeds were counted by using seed counter. Harvest index (%) and seed yield was quantified as:

$$HI = \frac{\text{Economic yield } (gm^{-2})}{\text{Biological yield } (gm^{-2})} \times 100$$

Statistical analysis: Analysis of variance (ANOVA) was done using a CRD to check whether the treatment differences were statistically significant using Web Agri Stat Package 2.0 (WASP 2.0). Multivariate analysis was performed using JMP Pro 16 statistical software from SAS.

RESULTS AND DISCUSSION

Growth parameters: The crop growth rate exhibited significant differences among the stages in both seasons (Table 1). The highest CGR in all the seasons was reflected in elevated CO_2 treatment and a decline in CGR was observed under elevated ozone treatments (0.3 g/cm^2/day). The difference in loss of CGR in eO_3 atop in control was about 17–38% in different stages of Indian mustard. At 60–75 DAS the highest decline in eO_3 was observed as ~38%. At the same time, the elevated CO_2 compensated for the effect of elevated O_3 by about 25.7% at 60–75 DAS. Under all the elevated treatments (Table 1), the AGR showed a significant difference and a decline of 17 to 34.75% was observed throughout both seasons. The highest AGR in both seasons under elevated CO_2 was found to be 1.34 g/day (2020–21) and 1.22 g/day (2021–22) during 45–60

Table 1 Effects of elevated O_3 and CO_2 on crop growth rate and absolute growth rate of Indian mustard

Treatment	Crop growth rate (g/cm^2/day)			Absolute growth rate (g/day)		
	45–60 DAS	60–75 DAS	75–90 DAS	45–60 DAS	60–75 DAS	75–90 DAS
			2020–21			
eO_3	0.30 ± 0.02^c	0.07 ± 0.00^d	0.09 ± 0.01^c	0.90 ± 0.07^c	0.20 ± 0.03^c	0.23 ± 0.04^b
eCO_2	0.45 ± 0.02^a	0.13 ± 0.01^a	0.14 ± 0.00^a	1.34 ± 0.07^a	0.44 ± 0.00^a	0.46 ± 0.06^a
eO_3*eCO_2	0.33 ± 0.01^{bc}	0.08 ± 0.00^c	0.09 ± 0.01^{bc}	0.98 ± 0.04^{bc}	0.25 ± 0.02^{bc}	0.28 ± 0.03^b
Ambient	0.36 ± 0.02^b	0.11 ± 0.00^b	0.11 ± 0.01^{ab}	1.08 ± 0.06^b	0.31 ± 0.02^b	0.34 ± 0.05^{ab}
SEm±	0.020	0.004	0.006	0.059	0.018	0.042
CD (P≤0.05)	0.061	0.013	0.023	0.183	0.064	0.135
Per cent change (eO_3 over control)	-17.04	-37.78	-23.33	-17.04	-34.75	-32.06
Per cent change (eCO_2 over eO_3)	32.78	48.36	35.78	32.78	54.29	48.72
Compensation effect (%) eCO_2 over eO_3	8.84	24.19	6.58	8.84	24.19	20.27
			2021–22			
eO_3	0.28 ± 0.02^c	0.07 ± 0.00^c	0.08 ± 0.00^d	0.84 ± 0.06^c	0.28 ± 0.02^b	0.24 ± 0.03^c
eCO_2	0.41 ± 0.01^a	0.12 ± 0.01^a	0.14 ± 0.01^a	1.22 ± 0.04^a	0.37 ± 0.03^a	0.47 ± 0.02^a
eO_3*eCO_2	0.30 ± 0.01^{bc}	0.09 ± 0.01^{bc}	0.10 ± 0.00^c	0.91 ± 0.04^{bc}	0.30 ± 0.01^b	0.28 ± 0.02^c
Ambient	0.35 ± 0.02^b	0.10 ± 0.01^{ab}	0.12 ± 0.01^b	1.04 ± 0.05^b	0.34 ± 0.01^{ab}	0.36 ± 0.01^b
SEm±	0.016	0.010	0.005	0.047	0.017	0.019
CD (P≤0.05)	0.048	0.032	0.018	0.145	0.059	0.065
Per cent change (eO_3 over control)	-19.35	-32.04	-33.35	-19.35	-15.84	-33.35
Per cent change (eCO_2 over eO_3)	31.39	43.81	43.97	31.39	23.63	48.76
Compensation effect (%) eCO_2 over eO_3	8.37	27.21	23.19	8.37	6.81	16.93

DAS. AGR decreased exponentially in all the treatments at all other stages. The compensation effect of elevated CO_2 over elevated O_3 was about 8.6%. The crop growth rates are an indication of the production efficiency of crops under different treatments and the CO_2 treatments imply carbon gain that increases the net photosynthetic rate (Mishra *et al.* 2013). The crop growth rate under eCO_2 is mainly due to an increase in crops' net assimilation rate, resulting in the rise of dry-weight biomass (Watanabe *et al.* 2010). The specific leaf area (SLA) (Fig 1a and 1b) and specific leaf weight

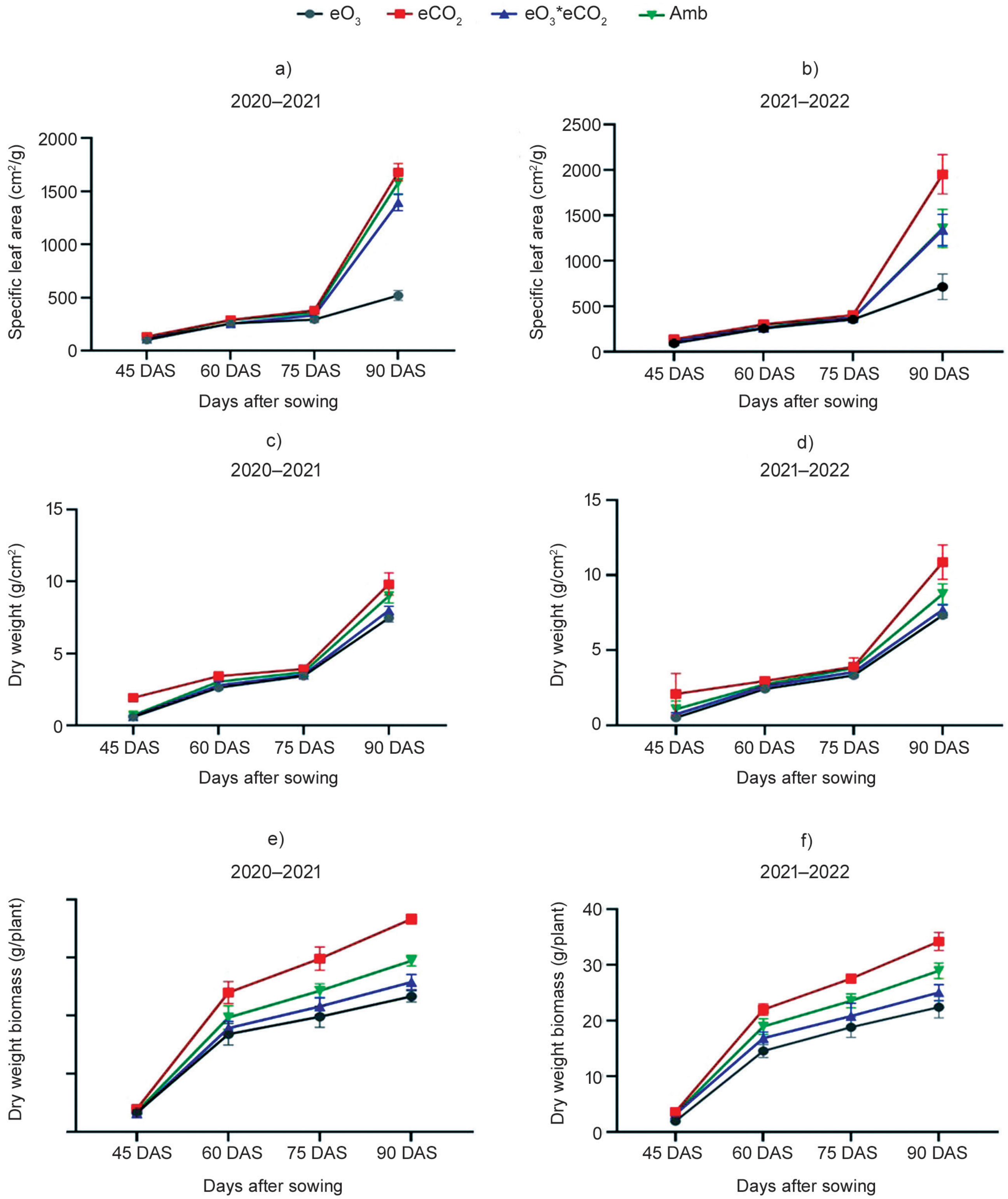

Fig 1 Specific leaf area (SLA), Specific leaf weight (SLW) and Dry weight biomass of Indian mustard plants exposed under different treatments.

(SLW) (Fig 1c and 1d) exhibited similar results. However, at peak flowering stage, i.e. at 75 DAS the differences in these traits were found to be non-significant. In the vegetative stage, at 45 DAS both SLA and SLW recorded the highest range in elevated CO_2, followed by control treatment and exponential increase of the SLA and SLW was observed in 60, 75 and 90 DAS. Among the treatment effects, in SLA, the eO_3 showed a significant decrease in the range of 102.27 cm^2/g at 45 DAS (1st season) and 92.96 cm^2/g (2nd season) to 521.14 cm^2/g (1st season) and 714.45 cm^2/g (2nd season) at 90 DAS. A significant per cent difference of elevated CO_2 over eO_3 was observed between 23.73 to 68.97% and 31.87 to 63.40%. In SLW, the eCO_2 exhibited a range of 32.46 to 23.99% at 45 DAS and 69.10 to 75.35% at 90 DAS. Contrary results of SLA and SLW were obtained by Saha *et al.* (2015) in the open-top chambered conditions of chickpea and wheat. The increased SLA and SLW are due to the increased leaf area and leaf weight under CO_2 conditions.

The dry matter production (biomass g/m^2) (Fig 1e and 1f) was significantly lowered by 3.4 to 20.84% during 2020–21 and 20.11 to 41.03% during 2021–22 in the ozone treatments in comparison with ambient conditions. An increase of dry matter accumulation was found in the range of 15 to 36.37% during 2020–21 and in the range of 31.4 to 46% during 2021–22 in elevated CO_2 over elevated ozone. Significant higher plant height (Table 2) was observed for plants growing in elevated CO_2 in comparison to those in ambient, O_3, and CO_2 interaction and O_3 treatments. Reduced plant height was found in elevated O_3 treatment followed by O_3*CO_2 treatments. The no. of leaves did not vary significantly between treatments

Table 2 Effects of elevated O_3 and CO_2 on plant height and no. of leaves of Indian mustard

Treatment	Plant height (cm)				No. of leaves			
	45 DAS	60 DAS	75 DAS	90 DAS	45 DAS	60 DAS	75 DAS	90 DAS
2020–21								
eO_3	15.68 ± 0.13^d	46.53 ± 0.99^d	124.3 ± 1.48^d	165.13 ± 0.74^d	5.5 ± 0.65^d	11 ± 0.41	12.75 ± 0.48	14.5 ± 1.85
eCO_2	20.53 ± 0.21^a	64.98 ± 1.21^a	155.13 ± 1.39^a	184.5 ± 0.89^a	8.25 ± 0.25^a	16.25 ± 2.59	17 ± 1.08	21.75 ± 1.31
eO_3*eCO_2	16.9 ± 0.52^c	51.5 ± 0.42^c	133.95 ± 1.18^c	168.25 ± 0.51^c	6.75 ± 0.48^c	12.5 ± 0.65	15.25 ± 0.85	16.75 ± 0.75
Ambient	19.1 ± 0.41^b	60.15 ± 0.75^d	143.78 ± 1.03^b	174.93 ± 0.38^d	7.5 ± 0.29^b	15 ± 1.68	16.25 ± 3.42	20.5 ± 3.10
SEm±	0.316	0.842	1.270	0.629	0.416	1.333	1.459	1.752
CD (P≤ 0.05)	1.085	2.747	3.951	2.032	0.737	NS	NS	NS
Per cent change (eO_3 over control)	-17.93	-22.65	-13.55	-5.60	-26.67	-26.67	-21.54	-29.27
Per cent change (eCO_2 over eO_3)	23.63	28.40	19.87	10.50	33.33	32.31	25	33.33
Compensation effect (%) eCO_2 over eO_3	7.81	10.69	7.76	1.89	22.73	13.64	19.61	15.52
2021–22								
eO_3	16.18 ± 0.35^b	48.28 ± 1.58^d	116.55 ± 1.99^d	153.88 ± 1.34^c	5.75 ± 0.25^b	10.75 ± 1.44	12.25 ± 1.03	12.75 ±2.84
eCO_2	20.78 ± 0.72^a	72.48 ± 2.34^a	150.13 ± 2.20^a	175 ± 0.93^a	7.75 ± 0.63^a	15.75 ± 2.78	16.25 ± 1.49	20.5 ± 2.36
eO_3*eCO_2	17.15 ± 0.53^b	54 ± 1.53^c	126.45 ± 1.93^c	156.75 ± 0.63^c	6.5 ± 0.5^{ab}	11.5 ± 0.87	14.75 ± 1.03	16 ± 1.41
Ambient	19.6 ± 0.70^a	63.9 ± 0.63^b	133.53 ± 1.55^b	167.43 ± 2.35^b	7.25 ± 0.25^a	13.5 ± 2.25	15.25 ± 3.90	20 ± 3.42
SEm±	0.575	1.521	1.918	1.313	0.407	1.834	1.864	2.508
CD (P≤0.05)	1.829	5.046	5.957	4.513	1.353	NS	NS	NS
Per cent change (eO_3 over control)	-17.47	-24.45	-12.71	-8.09	-20.69	-20.37	-19.67	-36.25
Per cent change (eCO_2 over eO_3)	22.14	33.39	22.36	12.07	25.81	31.75	24.62	37.80
Compensation effect (%) eCO_2 over eO_3	6.03	11.86	8.49	1.87	13.04	6.98	20.41	25.49

in both seasons. The no. of leaves decreased by 21.54 to 29.27% in 1st season and -19.67 to 36.25% in 2nd season respectively at elevated O_3 treatments of Indian mustard compared to the ambient conditions (Table 2). Generally, under elevated ozone treatments, the shoot and root biomass significantly decreased similar to plant height and dry matter production (Bhatia *et al.* 2011, Kumar *et al.* 2021) which alters the photosynthetic partitioning. Simultaneously in the interaction treatments, a compensatory effect of CO_2 over O_3 was observed in the dry weight of biomass, plant height, morphological and growth characteristics (Mishra *et al.* 2013). Though the leaf numbers were non-significant, somehow the observations under CO_2 suggested that plants retain the photosynthates for O_3 repair and restore the leaf cell division, formation, and expansion (Singh *et al.* 2009). Under elevated CO_2, the decreased stomatal aperture might decline the O_3 entry in the leaves creating higher availability of substrates that detoxify the negative effects of O_3 by encouraging in repair process (Mishra *et al.* 2013).

Yield attributes: The no. of siliquae/plant, no. of seeds/siliqua and harvest index were found to be higher under eCO_2 and the least values were observed in the eO_3 conditions in both seasons (Table 3). Similarly, the test weight (g/1000 seeds) was observed highest in elevated CO_2 and under ambient conditions. A reduction of seed yield in Indian mustard was about 14.91 and 4.27% in two seasons which was quantified under O_3 treatments compared to ambient conditions. The reduction in growth characteristics is directly proportional to yield attributes. The O_3 effect on the seed yield of Indian mustard was altered by about 3.85% (2020–21) and 27.25% (2021–22) by the elevated CO_2 which implied the compensation effect. Similar results of lowered seed yield were obtained by Singh *et al.* (2009) and Bhatia *et al.* (2011) in *Brassica campestris* var. Kranti, 5–10% in maize and soybean (McGrath *et al.* 2015), garlic (Gayathri *et al.* 2019), rice (Kumar *et al.* 2021), maize (Yadav *et al.* 2021) and wheat (Hansen *et al.* 2019), while CO_2 enrichment escalated the crop yields by 9–14% in maize and soybean (McGrath and Lobell 2011), 11% of chickpea (Lamichaney *et al.* 2021) and 37.15% rice (Kumar *et al.* 2021). The CO_2 treatments ameliorate the damage of photosynthesis caused by elevated O_3, thus enhancing the yield and compensating the ozone phytotoxicity (Kobayakawa and Imai 2011, Bhatia *et al.* 2021, Kumar *et al.* 2021).

The multivariate analysis (Fig 2) carried out between the growth parameters at the vegetative stage and yield characteristics revealed that the growth and yield parameters are positively correlated to each other whereas CGR and AGR were very highly and positively correlated to each other as well as with seed number/siliqua (82%). Likewise, SLA was highly positively correlated with harvest index (r=0.84), test weight (r=0.80) and seed no./siliqua (r=0.82).

Table 3 Effects of elevated O_3 and CO_2 on no. of siliquae/plant, no. of seeds/siliqua, test weight, harvest index and seed yield of Indian mustard

Treatment	No. of siliquae/plant	No. of seeds/siliqua	Test weight (g/1000 seeds)	Harvest Index (%)	Seed yield (kg/m^2)
	After harvest				
2020–21					
eO_3	94.25 ± 2.50^d	32.5 ± 1.03^d	4.01 ± 0.11^c	38.67 ± 0.65^d	0.176 ± 0.01^c
eCO_2	125.5 ± 2.22^a	45.75 ± 0.48^a	4.92 ± 0.07^a	45.69 ± 0.55^a	0.233 ± 0.03^a
eO_3*eCO_2	109.25 ± 0.48^c	35.75 ± 0.48^c	4.33 ± 0.04^b	40.55 ± 0.32^c	0.182 ± 0.01^{bc}
Ambient	118 ± 1.08^b	42.5 ± 0.65^b	4.74 ± 0.06^a	43.66 ± 0.49^b	0.206 ± 0.01^{ab}
SEm±	1.568	0.661	0.069	0.501	0.011
CD ($P\leq 0.05$)	5.457	2.156	0.227	1.588	0.029
Per cent change (eO_3 over control)	-25.20	-30.77	-18.18	-12.90	-14.91
Per cent change eCO_2 over eO_3	24.90	28.96	18.33	15.36	24.52
Compensation effect (%) eCO_2 over eO_3	13.73	9.09	7.21	4.64	3.85
2021–22					
eO_3	85 ± 4.06^b	31.75 ± 0.48^d	3.91 ± 0.06^c	39.92 ± 0.84^c	0.176 ± 0.01^b
eCO_2	117.5 ± 3.40^a	43.75 ± 0.63^a	4.87 ± 0.11^a	46.94 ± 0.48^a	0.241 ± 0.00^a
eO_3*eCO_2	105.5 ± 5.30^a	35.5 ± 0.65^c	4.23 ± 0.06^b	41.05 ± 0.59^c	0.183 ± 0.01^b
Ambient	114.25 ± 3.90^a	41 ± 0.58^b	4.64 ± 0.12^a	43.26 ± 0.77^b	0.232 ± 0.01^a
SEm±	4.167	0.583	0.089	0.670	0.007
CD ($P\leq 0.05$)	13.019	1.807	0.287	2.111	0.022
Per cent change (eO_3 over control)	-34.41	-29.13	-18.64	-8.36	-24.35
Per cent change (eCO_2 over eO_3)	27.66	27.43	19.55	14.95	27.25
Compensation effect (%) eCO_2 over eO_3	19.43	10.56	7.38	2.76	4.27

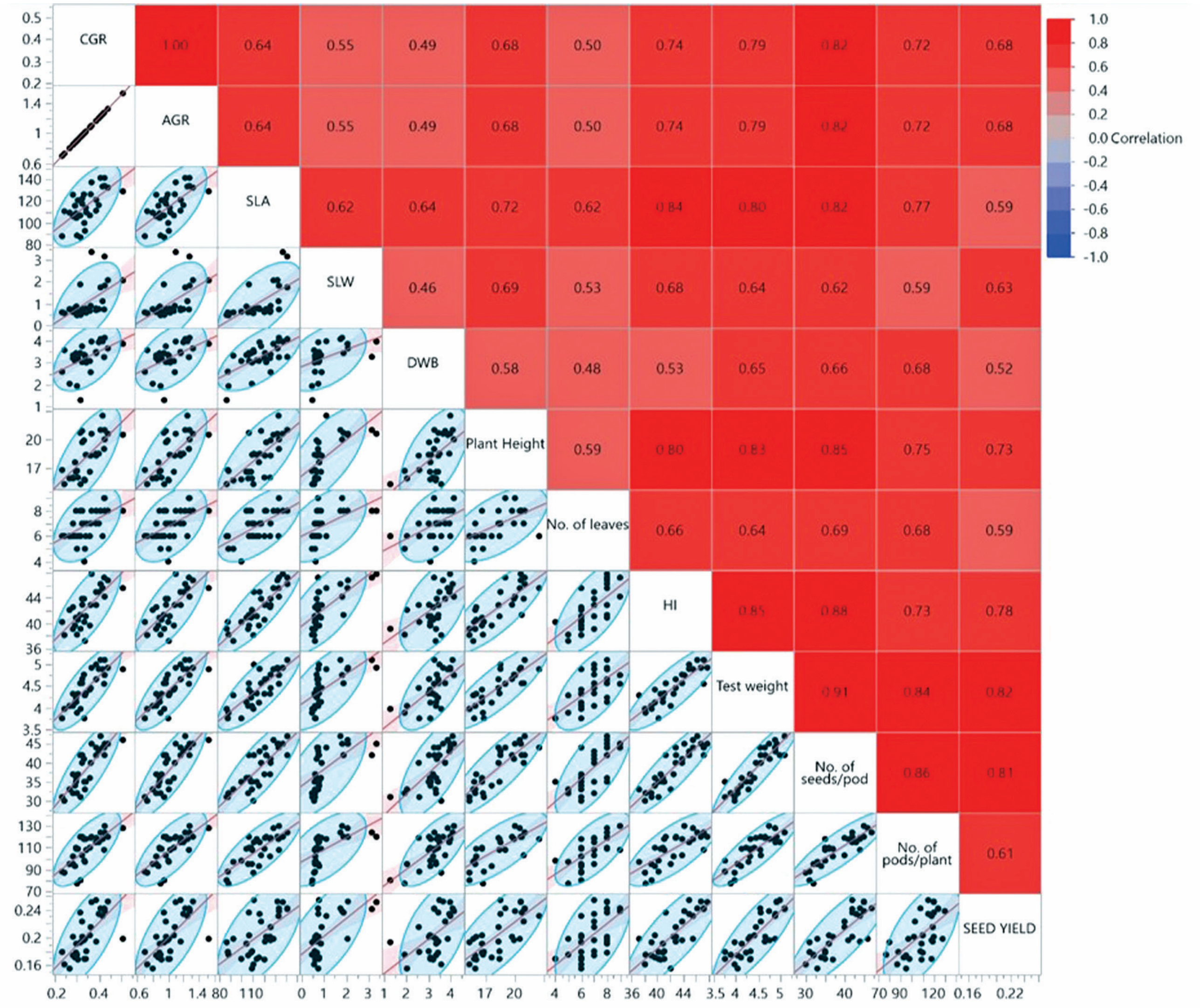

Fig 2 Multivariate analysis correlation between growth and yield parameters.

High correlation was fund between plant height, harvest index (r=0.80), test weight (r=0.83) and no. of seeds/siliqua (r=0.85). Similarly, harvest index was found to have a high positive correlation with test weight (85%) and no. of seeds/siliqua (88%). High positive correlations of test weight were observed between seed no/siliqua (r=0.91), siliquae/plant (r=0.84) and seed yield (r=0.82), likewise number of seeds was also positively correlated with siliquae/plant (r=0.86) and seed yield (r=0.81)

The economic yield decline is related to a decrease in various growth parameters (RGR, CGR, AGR, plant height, SLA, SLW, etc.) and yield attributes under O_3 exposed treatments (Singh *et al.* 2017). The tropospheric O_3 in the atmosphere enters the plants through stomatal pores and encounters apoplastic damage over antioxidants which causes cell membrane dysfunction that leads to membrane leakage and causes metabolic malfunctioning in physiological disorders such as photosynthesis, stomatal conductance and transpiration rate resulting in decreased growth rates, carbon assimilation, and yield (Bhatia *et al.* 2012, Emberson *et al.* 2018).

It is concluded that elevated ozone showed detrimental effects on mustard growth and yield. Mustard being C_3 crop, manifested a positive response with an increase of about 26% under CO_2-enriched treatment. As per the results, the rise in CO_2 levels prolonged the photosynthates activity which altered the negative effects of ozone on the growth and yield of PDZM 31. The evidence on the amelioration effects of CO_2 over O_3 was limited and its physiological role needs to be documented in future research.

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