

Growth, Yield and Quality of Maize under Ozone and Carbon Dioxide Interaction in North West India

Achchhelal Yadav¹, Arti Bhatia^{2*}, Sudesh Yadav¹, Archana Singh²,
Ritu Tomer², Ramesh Harit², Vinod Kumar², Bhupinder Singh²

¹Jawaharlal Nehru University, New Delhi-110067, India

²ICAR- Indian Agricultural Research Institute, New Delhi-110012, India

ABSTRACT

Maize is an important C₄ crop and how it will respond to elevated atmospheric carbon dioxide and ozone levels is not well documented. To understand how the growth and nutritional quality of maize will be affected under elevated carbon dioxide (CO₂) and tropospheric ozone (O₃) interaction, a field experiment was conducted under free air O₃ and CO₂ enrichment rings (FAOCE) growing HQPM-1 and PMH-1 maize cultivars at New Delhi, India. Each cultivar was exposed to ambient and elevated CO₂ (559 ppm) alone and along with ambient and elevated O₃ (71.8 ppb) throughout the growing period. Elevated CO₂ (EC) significantly increased the leaf area index (10.8–22.5%), chlorophyll (11.2–17.3%) and photosynthetic rate (12.1–16.5%) in the two cultivars over the ambient. O₃ exposure of 27 ppm hr (AOT40) under elevated O₃ (EO) treatment led to a significant decline in yield ($p < 0.01$) by 9.2% in HQPM-1 and 9.8% in PMH-1. Under elevated CO₂ the increase in grain yield was higher under HQPM-1 (25.4%) as compared to PMH-1 (9.04%). The protein content increased under EO (8.1–12.5%) and decreased under EC (13.4–13.6%) in the two maize cultivars due to yield dilution effect. Lysine, phosphorus and potassium content of the grain significantly decreased in both the cultivars under elevated CO₂. Carbohydrate and amylose concentrations in grains increased (9.9–15.5%) under EC and decreased (10.8–16.7%) under EO, however, no significant change in yield, protein, amylase, carbohydrate, lysine, potassium and phosphorus was observed under the interaction treatment ECO as compared to the ambient. After two years of study we could conclude that elevated CO₂ (559 ppm) was able to offset the negative effect of elevated O₃ (71 ppb) on grain yield by 11.2% in PMH-1 and by 18.8% in HQPM-1 without significantly affecting the grain quality in both the maize cultivars.

OPEN ACCESS

Received: May 23, 2020

Revised: August 23, 2020

Accepted: September 4, 2020

* Corresponding Author:

artibhatia.iari@gmail.com

Publisher:

Taiwan Association for Aerosol
Research

ISSN: 1680-8584 print

ISSN: 2071-1409 online

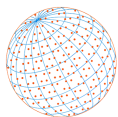
 Copyright: The Author(s).

This is an open access article distributed under the terms of the [Creative Commons Attribution License \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are cited.

Keywords: Elevated carbon dioxide (CO₂), Tropospheric ozone (O₃), Crop growth, Yield, Nutritional quality

1 INTRODUCTION

Climate change and air pollution are increasing threats to agriculture production and food security (Porter *et al.*, 2014; Tai *et al.*, 2014). Due to the incessant burning of fossil fuels and anthropogenic activities, the concentration of CO₂ has increased by 40% (400 ppm) since pre-industrial era (1750 AD) and may increase to 550 ppm by 2050 (IPCC, 2014). The greenhouse gases such as carbon dioxide (CO₂) and tropospheric ozone (O₃), also a phytotoxic air pollutant have bearings on plant growth and productivity. Atmospheric CO₂ is a source of carbon assimilation in plants and works as a carbon fertilizer, resulting in improved plant growth and production (van der Kooi *et al.*, 2016; Lopes *et al.*, 2018). However, according to literature plants that use the C₄ photosynthetic pathway are less responsive to elevated CO₂ as compared to plants that use the C₃ pathway. Maize being a C₄ plant, may thus behave differently under elevated CO₂ as compared to C₃ crops and there have been contrasting reports about the impact of elevated CO₂ in maize. Abebe *et al.* (2016) observed an increase of 53.7% in the grain yield of maize whereas, Leakey *et al.* (2006) observed reductions in photosynthesis, biomass and yield under elevated CO₂ condition. Furthermore, CO₂ enrichment leads to a decline in nutrients present in the grains such as zinc,



iron and protein content (Myers *et al.*, 2014; Broberg *et al.*, 2017). Previously it has been reported that elevated CO₂ reduced N, crude protein, P and K contents of grains (Abebe *et al.*, 2016). Recently, Reich *et al.* (2018) observed a role reversal of the C₃–C₄ concept wherein they observed that after 12 year of experimentation, the biomass increased under elevated CO₂ in C₄ plants and decreased under C₃ crops.

The concentration of ground level O₃ is increasing continuously since industrial revolution and has reached 35–40 ppb globally and it may continue to increase rapidly in the developing countries in the 21st century (Frei, 2015; Proietti *et al.*, 2016; Sicard *et al.*, 2017) According to Environmental Protection Agency if global emissions of O₃ precursors continue at present day rate then the tropospheric O₃ may reach more than 70 ppb level by 2050 (Pfister *et al.*, 2014). O₃ is produced in the troposphere due to photochemical reactions of the oxides of nitrogen (NO_x) and the volatile organic compounds (VOCs) including methane and CO₂ (Bhatia *et al.*, 2012). Several researchers reported that higher concentration of tropospheric O₃ reduced carbon storage of crops, thereby reducing plant growth and yield (Bhatia *et al.*, 2011; Zhu *et al.*, 2011; Tai *et al.*, 2014; Broberg *et al.*, 2015; Tomer *et al.*, 2015). The elevation of tropospheric O₃ in the free air significantly increases P, K and protein concentrations in the cereals (Pleijel *et al.*, 2018). However, most of the research on the effect of elevated tropospheric O₃ on photosynthesis and stomatal function has been carried out in C₃ species only (Ainsworth, 2017).

Besides the individual impacts of elevated CO₂ and O₃, the combined interaction of the elevated CO₂ and O₃ influence the crop growth and development. The enrichment of CO₂ mitigated the adverse impacts of elevated O₃ in many studies (Kumari *et al.*, 2015; Phothi *et al.*, 2016; Singh *et al.*, 2018). Torbert *et al.* (2004) in a study in grain sorghum reported that elevated CO₂ increased the total biomass production, however, the interactive influences of elevated CO₂ and O₃ have very rarely been studied in C₄ crops. Bhatia *et al.* (2014) observed a significant reduction in the growth and yield of maize under elevated O₃ in a study conducted in open top chambers. They reported that exposure of elevated (ambient+40–45 ppb) O₃ resulted in greater susceptibility of the maize plant to pest attack. Very few studies have been carried out in C₄ crops relative to C₃ crops and thus there is a generally a poorer understanding of C₄ responses to climate change (Leakey, 2009).

Maize a C₄ cereal crop is the third most important crop after rice and wheat across the world. It is being produced in 189.3 million hectares and contributes 36% (1078 Mt) to the world food production (FAO, 2016) and about 70% of it is grown in the developing nations. In India, maize is mainly grown in Kharif season (June–October) with a total production of 24.4 million tons and 2.5 ton ha⁻¹ productivity, accounting for 9% of the total food grain production (Government of India, 2017). Due to the declining ground water tables and poor soil fertility in the rice growing areas of Indo-Gangetic Plains, maize has been suggested as an alternate crop to diversify from rice for improving soil quality and reducing the global warming potential of soils in this region. However, increasing concentration of tropospheric O₃ in India, especially in the Indo-Gangetic Plains (IGP) is of major concern as it poses a threat to the quality and productivity of crops (Singh and Agrawal, 2017) and may reach 70 ppb by 2050 in South Asia (IPCC, 2014). Apart from this most of the studies on the impact of tropospheric O₃ on crops have been conducted in Europe and America (temperate regions). Very few studies have been carried out in the tropical and semi-arid regions. Keeping in view the contrasting response obtained in C₄ crops under elevated CO₂, the present study was carried out with the aim to assess the impact of elevated CO₂ and tropospheric O₃ separately as well as in combination on the growth, yield and nutritional quality of maize under free air O₃ and CO₂ enrichment.

2 MATERIALS

2.1 Site Description

The experiment was carried out in free air O₃ and CO₂ enrichment (FAOCE) rings at the farm of Indian Agricultural Research Institute (IARI), New Delhi, India, during the summer season of 2016 and 2017. The site is located at (28°37'N latitudes, 77°12'E longitudes at mean sea level of 228.6 meters in an overlap of monsoon-influenced humid subtropical and semi-arid climate with average annual precipitation of 797 mm. The weather parameters recorded at the site are given in Table 1. The experimental site had a typical Ustochrept soil, having sandy loam texture. The soil

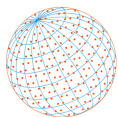


Table 1. Weather parameters during the crop growing seasons 2016 and 2017.

Month	Rainfall (mm)	Average monthly temperature (°C)		Mean temperature (°C)	Sunshine (hour)	Relative humidity (%)	Monthly average O ₃ (ppb)
		Maximum	Minimum				
July, 2016	539.4	38.8	24.4	31.6	80.6	82	27.5
August, 2016	387.5	32.6	24.2	28.4	55.8	83	26.9
September, 2016	174	34.3	22.5	28.4	189.0	72	31.1
October, 2016	37.2	33.9	16.2	25.0	161.2	63	29.2
July, 2017	170.5	34.1	26.2	30.2	145.7	78	30.4
August, 2017	127.1	33.8	25.9	29.8	127.1	82	27.1
September, 2017	198	33.6	23.7	28.7	186.0	77	31.7
October, 2017	0.0	34.0	17.1	25.6	192.2	68	30.6

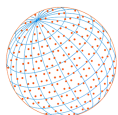
had a total organic carbon, P, K and total N content of 0.45%, 25.5 kg ha⁻¹, 249 kg ha⁻¹ and 188 kg ha⁻¹, respectively.

2.2 Treatment and Experiment Design

Two maize (*Zea mays* L) cultivars HQPM-1 (high quality protein) and PMH-1 (normal) were sown, in free air ozone and carbon dioxide enrichment (FAOCE hereafter) rings having an area of 19.6 m² (5.0-meter diameter) each. Four treatments were taken for the study: ambient CO₂ and O₃ (Amb), elevated CO₂ and ambient O₃ (EC), elevated O₃ and ambient CO₂ (EO) and elevated CO₂ and elevated O₃ (ECO). Each FAOCE ring was divided in two halves (9.8 m²) and each cultivar was sown in one half of the ring. There were two replicate rings for each of the four treatments. The crops were grown during 2016 and 2017 and were exposed to elevated levels of O₃ and CO₂ during the entire crop growth period. The targeted levels were 550 ppm of elevated CO₂ and 70 ppb of tropospheric O₃. The ambient CO₂ ranged from 388 to 416 ppm and from 389 to 419 ppm during 2016 and 2017 respectively. The elevated CO₂ ranged from 505 to 623 ppm and from 508 to 613 ppm in the FAOCE rings during 2016 and 2017 respectively. The wind vane and anemometer were installed in the center of the FAOCE at 1-meter height to monitor the wind direction and speed, respectively. For achieving the targeted elevated CO₂ level (550 ppm), pure CO₂ was released from commercial grade compressed 30 kg pure CO₂ gas cylinders, fitted with a regulator (DURA, ESAB, India) through solenoid valves and PVC tubing to the base of each FAOCE plot with laser holes (0.3mm diameter) at a distance of 30 cm to spray CO₂ in the rings uniformly. Releasing of the elevated concentration of compressed CO₂ was controlled by the solenoid valves as per the wind direction and speed in the FAOCE rings. O₃ was produced by an ozone generator (Eltech Engineers, Mumbai) by converting atmospheric oxygen into O₃ using UV lamps. Air blowers were used to mix O₃ with air and the mixture of air and O₃ was controlled by the flappers using solenoid valves. Air from the center of each ring was monitored for the concentrations of CO₂ by using a CO₂ analyzer (NDIR, Topak USA) and an ozone analyzer (2B Technologies) was used for monitoring O₃ levels. Microprocessors were used to perform control, logging and operation of CO₂ and O₃ concentrations through digital input and output modules as per wind direction and velocity on real time basis. The exposure of elevated CO₂ and tropospheric O₃ was given during day time (10.00 am to 17.00 pm) when the plant photosynthesis was taking place to save on costs as maintaining elevated CO₂ was very costly. The crop was exposed to elevated tropospheric O₃ for 7 hours as has been most commonly used practice in studies worldwide (Ashmore, 2005). Maize cultivars were sown on June, 24 and June, 25 in 2016 and 2017, respectively. Planting was done at 20 cm plant to plant and 50 cm row to row distances. The crops were fertilized with nitrogen (N) (150 kg ha⁻¹), P₂O₅, and K₂O (70, kg ha⁻¹) and zinc sulphate (Zn SO₄) (25 kg ha⁻¹). The complete doses of Zn SO₄, P₂O₅ and K₂O and 50% of the nitrogen (N) were applied at the time of crop planting. The remaining amount of nitrogen was given in two equal parts at the knee high (35 DAS) and tasseling stage (65 DAS) of crop growth.

2.3 AOT40 Calculation

The O₃ concentration during crop growth period ranged from 4 to 41.1 ppb and from 6 ppb to



38.5 ppb in the ambient air during 2016 and 2017, respectively (Fig. 1). The elevated O_3 ranged from 60 to 88 ppb and from 55 to 87 ppb in the FAOCE rings during 2016 and 2017, respectively. The average O_3 in the ambient air during crop growth period was 30.07 and 28.63 ppb whereas average elevated O_3 in the FAOCE rings was 72.75 and 71.04 ppb during 2016 and 2017. AOT40 which is the sum of hourly average values of O_3 concentration beyond 40 ppb (Mills *et al.*, 2018) was calculated from the elevated levels of O_3 in the FAOCE rings during the complete crop growth period and was 27441 ppb hr and 26292 ppb hr in 2016 and 2017 respectively.

2.4 Measurement of Plant Growth and Yield Parameters

Leaf area index (LAI) was measured by plant canopy analyzer (LAI-2200 C) at tasseling and silking stages of crop growth. The photosynthetic rates and stomatal conductance were also measured at tasseling and at silking stages on the third leaf of the plant from the top around 11.00 am by using portable photosynthesis system (Model No. LI-6400 XT; LI-COR, USA). For evaluating yield attributes of crops, maize plants from 1 m² area were harvested at maturity from each replicate. The cob length (cm), cob diameter (cm), grains number (cob⁻¹), grain weight (g cob⁻¹), biological yield (g m⁻²), grain yield (g m⁻²) and 100 grain weight (g) were determined as per standard procedures. Yield data was adjusted to 10% moisture content after drying (65°C) in an oven. The ratio of grain yield and biological yield multiplied by 100 was expressed as harvest percent.

2.5 Plant and Grain Sample Analysis

Chlorophyll content of leaves of both the cultivars was determined at tasseling as well as silking using procedure described by Wellburn (1994). Nutritional quality parameters such as potassium (K) and phosphorus (P) were determined as per method described by Jakson (1973). The crude protein content was calculated by multiplying the conversion coefficient 5.95 with the total N concentration (Juliano, 1993) which was determined by the Kjeldahl method. The carbohydrate concentration of the grains was determined by the method given by Sadasivam and Minickam (1992). The lysine content of grains was estimated as per procedure described by Tsai *et al.* (1972). The amylose content was determined by procedure described by Hu *et al.* (2010).

2.6 Statistical Analysis

Using the 9.3 SAS statistical software, growth, yield, yield components and quality attributes of maize were analyzed. Split plot design was used to conduct the experiment. Two-way analysis of variance (ANOVA) was used to analyze the impact of cultivar and the four treatments. The statistically significance of data was compared based on their 95% confidence level ($p < 0.05$) (Gomez and Gomez, 1984).

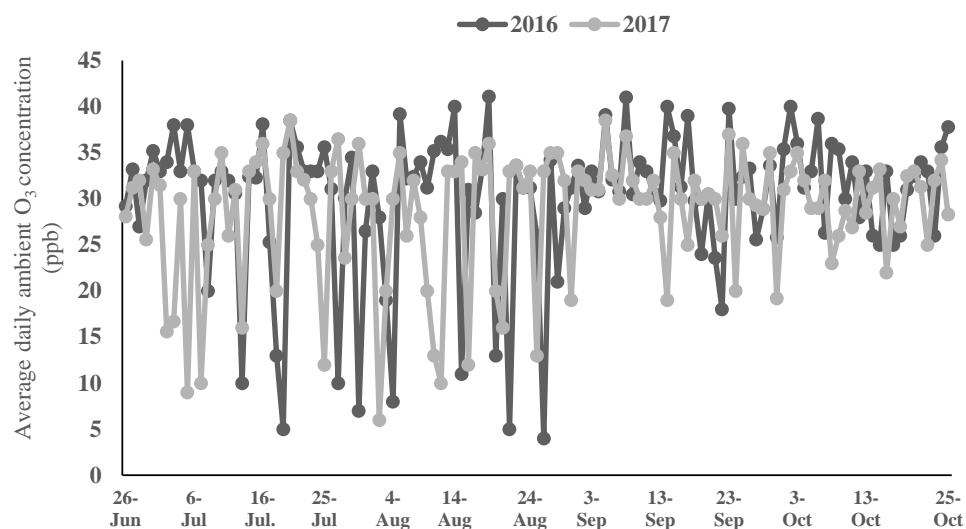
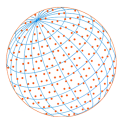


Fig. 1. The concentrations of daily average ambient O_3 during 2016 and 2017.



3 RESULTS

3.1 Impact of Elevated CO₂ and O₃ on Photosynthetic Rate, Stomatal Conductance, Chlorophyll Content and Leaf Area Index

The mean photosynthetic rates (Pn) ranged from 33–34.8 $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under EO and from 41.8–44.4 $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under EC across the crop growth stages and cultivars. It increased significantly by 12.1–16.6% ($p < 0.05$) in HQPM-1 and by 12.4–15.5% ($p < 0.05$) in PMH-1 under EC treatment across the crop growth stages in the two years (average data) (Table 2). However, increased tropospheric O₃ (EO) significantly reduced the Pn of HQPM-1 (7.8–9.4%) and PMH-1 (6.7–15.4%) ($p < 0.05$) (Table 2) at both crop stages as compared to the ambient. The Pn in the interactive treatment ECO increased by 11.9–12.4% for HQPM-1 and by 10.9–18.5% for PMH-1 (across the stages) over elevated O₃ alone and was found to be at par with the ambient.

The stomatal conductance (*gs*) significantly decreased ($p < 0.01$) in both the cultivars under EC, EO and ECO as compared to the ambient (Table 2). The lowest *gs* was observed in EC treatment at tasseling stage in PMH-1 cultivar.

Similar to the Pn the total chlorophyll content of leaves increased under EC and reduced under EO as compared to the ambient in both the cultivars (Table 2). In the ECO treatment, elevated CO₂ negated the effect of O₃ on the chlorophyll content in HQPM-1 (9.8–11%) and PMH-1 (4.8–10.4%) over elevated O₃ alone.

Significant difference in LAI was observed between the cultivars. Increased concentration of EO led to a higher decline in LAI of HQPM-1 (13.2–16.2%) as compared to PMH-1 (7.5–8.5%) at tasseling and silking stages. Under ECO, a higher increase in LAI was observed for HQPM-1 (12.1–16.1%) as compared to PMH-1 (5.4–8.5 % in PMH-1) over EO across the years and stages.

3.2 Impact of Elevated CO₂ and O₃ on Yield Components

The cob length (cm) and grains (cob^{-1}) reduced in both cultivars under EO. Elevated CO₂ significantly ($p < 0.01$) increased the cob length as well as grains (cob^{-1}) in both crop cultivars as compared to the ambient (Amb). Under the interactive treatment (ECO) averaged cob length increased by 9.4% for HQPM-1 and by 5.8% for PMH-1 over EO alone and was found to be at par with the Amb (Table 3). Average grain weight (cob^{-1}) under EC significantly increased in HQPM-1 (22.1%) and in PMH-1 (5.32%) (Table 3). While, EO reduced the grain weight (cob^{-1}) by 5.8% in HQPM-1 and by 11.3% in PMH-1 as compared to the Amb (Table 3). Under the interactive treatment ECO grain weight (cob^{-1}) increased by 12.2% in HQPM-1 as compared to EO alone, whereas there was no significant difference in PMH-1. The 100 grain weights of HQPM-1 and PMH1 were not-significantly different under ECO treatment as compared to the ambient during both the years.

Grain yield of HQPM-1 increased significantly in the first (33.3%, $p < 0.01$) and the second year (17.4%, $p < 0.01$) under EC as compared to the Amb. Similarly, grain yield of PMH-1 responded positively to the increased CO₂ during the first (10.5%) ($p < 0.01$) and the second year (7.5%) (Table 3). Under the interactive treatment ECO, averaged grain yield increased by 18.8% in HQPM-1 and by 11.2% in PMH-1 as compared to the elevated O₃ alone.

3.3 Impact of Elevated CO₂ and O₃ on Quality of the Grains

The mean of the two years' data revealed that the elevated CO₂ concentration in free air declined the crude protein by 8.1% in HQPM-1 and by 12.5% in PMH-1. On the contrary, the enrichment of O₃ increased the crude protein significantly ($p < 0.05$) by 13.5% in HQPM-1 and by 13.4% in PMH-1 as compared to the ambient (Fig. 2(a)). The EC treatment diminished the positive influence of elevated O₃ on protein concentration and it decreased by 8.2 and 8.8% for HQPM-1 and PMH-1, respectively under the ECO treatment as compared to EO alone. The protein contents in both the crop cultivars under the interactive treatment (ECO) were at par with the Amb (Fig. 2(a)).

Carbohydrate content of grains increased in HQPM-1 (4.6%) and PMH-1 (15.5%) under the EC, but responded negatively to EO in HQPM-1 (15.3%) and PMH-1 (16.6%) as compared to the ambient treatment (Fig. 2(b)). No significant change was obtained in the carbohydrate content

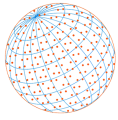


Table 2. Plant growth parameters at tasseling and silking stages of maize under the different treatments (Average of 2016 and 2017).

Cultivar	Treatment	Photosynthetic rate $\mu\text{mole (CO}_2\text{) m}^{-2}\text{ s}^{-1}$		Stomatal conductance mole (H ₂ O) m ⁻² s ⁻¹		Leaf area index		Chlorophyll (mg g ⁻¹ FW)	
		Tasseling	Silking	Tasseling	Silking	Tasseling	Silking	Tasseling	Silking
HQPM-1	Amb	36.8 ± 1.42 ^{*b}	37.1 ± 1.54 ^b	0.42 ± 0.02 ^a	0.45 ± 0.016 ^a	3.8 ± 0.18 ^b	3.7 ± 0.09 ^b	1.6 ± 0.013 ^b	1.56 ± 0.012 ^b
	EC	42.9 ± 1.1 ^a	41.6 ± 1.2 ^a	0.35 ± 0.01 ^b	0.38 ± 0.014 ^b	4.3 ± 0.15 ^a	4.1 ± 0.06 ^a	1.78 ± 0.008 ^a	1.83 ± 0.013 ^a
	EO	33.9 ± 1.3 ^c	33.6 ± 1.31 ^c	0.38 ± 0.01 ^b	0.40 ± 0.017 ^b	3.3 ± 0.25 ^c	3.1 ± 0.11 ^c	1.43 ± 0.013 ^c	1.45 ± 0.01 ^c
	ECO	38.1 ± 1.2 ^b	37.6 ± 0.99 ^b	0.36 ± 0.01 ^b	0.37 ± 0.008 ^c	3.7 ± 0.12 ^b	3.6 ± 0.07 ^b	1.57 ± 0.021 ^b	1.61 ± 0.027 ^b
PMH-1	Amb	39.5 ± 0.77 ^b	37.3 ± 0.98 ^b	0.43 ± 0.02 ^a	0.43 ± 0.012 ^a	4.0 ± 0.16 ^b	3.8 ± 0.11 ^b	1.56 ± 0.025 ^b	1.52 ± 0.016 ^b
	EC	44.4 ± 0.63 ^a	43.1 ± 0.92 ^a	0.34 ± 0.01 ^b	0.36 ± 0.014 ^b	4.6 ± 0.17 ^a	4.5 ± 0.13 ^a	1.71 ± 0.017 ^a	1.68 ± 0.018 ^a
	EO	33.4 ± 0.59 ^c	34.8 ± 1.11 ^c	0.38 ± 0.01 ^b	0.40 ± 0.015 ^b	3.7 ± 0.18 ^c	3.5 ± 0.09 ^b	1.47 ± 0.01 ^c	1.44 ± 0.011 ^c
	ECO	39.5 ± 0.60 ^a	38.6 ± 1.01 ^b	0.36 ± 0.01 ^b	0.38 ± 0.013 ^b	3.9 ± 0.13 ^b	3.8 ± 0.11 ^b	1.54 ± 0.01 ^b	1.59 ± 0.019 ^b

* SE (n = 4), Different lowercase subscripts within a variety show significant difference between the treatments according to Duncan's test.

Table 3. Yield and yield attributes under different treatments.

Cultivar	Treatment	Cob length (cm)		Number of grains (cob ⁻¹)		Grain weight (g)		Grain yield (g m ⁻²)		Biological yield (g m ⁻²)		100 Grain weight (g)	
		2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
HQPM-1	Amb	12.3 ± 0.18 ^{*b}	12.9 ± 0.15 ^b	222 ± 5.5 ^b	237 ± 6.8 ^b	58.1 ± 1.2 ^b	61.2 ± 1.3 ^b	420 ± 15.81 ^b	460 ± 18.25 ^b	1380 ± 30.82 ^b	1140 ± 28.39 ^b	23.7 ± 0.24 ^b	24.3 ± 0.23 ^a
	EC	14.1 ± 0.17 ^a	13.7 ± 0.11 ^a	270 ± 4.5 ^a	261 ± 11.6 ^a	71.0 ± 1.1 ^a	74.7 ± 2.1 ^a	560 ± 15.11 ^a	540 ± 14.93 ^a	1510 ± 41.43 ^a	1460 ± 25.21 ^a	26.1 ± 0.39 ^a	25.4 ± 0.39 ^a
	EO	11.4 ± 0.11 ^c	11.9 ± 0.16 ^c	207 ± 3.6 ^c	231 ± 8.6 ^b	53.6 ± 1.5 ^c	58.8 ± 2.8 ^b	380 ± 18.25 ^c	420 ± 15.81 ^b	1280 ± 35.35 ^c	1060 ± 37.63 ^c	22.6 ± 0.30 ^c	22.8 ± 0.72 ^b
	ECO	12.5 ± 0.24 ^b	13.0 ± 0.17 ^b	239 ± 8.0 ^b	255 ± 6.7 ^b	64.5 ± 2.7 ^c	68.7 ± 0.8 ^b	460 ± 19.14 ^b	490 ± 23.80 ^c	1360 ± 28.57 ^b	1280 ± 43.77 ^b	24.1 ± 0.42 ^b	24.8 ± 0.65 ^a
PMH-1	Amb	16.7 ± 0.17 ^b	16.6 ± 0.15 ^b	283 ± 8.9 ^b	246 ± 7.0 ^b	79.9 ± .93 ^b	77.1 ± 1.15 ^b	570 ± 14.71 ^b	530 ± 19.31 ^b	1480 ± 50.82 ^b	1440 ± 35.59 ^b	25.4 ± 0.58 ^b	25.5 ± 0.65 ^b
	EC	18.4 ± 0.23 ^a	18.0 ± 0.16 ^a	298 ± 10.4 ^a	283 ± 7.2 ^a	85.3 ± 1.1 ^a	80.1 ± .89 ^a	630 ± 22.81 ^a	570 ± 19.57 ^a	1730 ± 56.12 ^a	1690 ± 43.27 ^a	26.9 ± 0.80 ^a	26.4 ± 0.84 ^a
	EO	15.5 ± 0.36 ^c	16.1 ± 0.06 ^b	239 ± 8.8 ^c	206 ± 12.1 ^c	70.0 ± 0.57 ^b	69.1 ± 0.99 ^c	510 ± 18.87 ^c	480 ± 19.73 ^c	1350 ± 53.90 ^b	1340 ± 46.00 ^c	25.0 ± 0.98 ^b	25.1 ± 0.40 ^b
	ECO	16.4 ± 0.18 ^b	17.1 ± 0.21 ^b	272 ± 7.5 ^b	264 ± 7.2 ^b	77.2 ± .81 ^b	75.9 ± 0.83 ^b	560 ± 14.93 ^b	540 ± 11.08 ^b	1500 ± 35.82 ^b	1470 ± 34.15 ^b	26.3 ± 0.71 ^b	25.2 ± 0.97 ^b

* SE (n = 4), Different lower case subscripts within a variety show significant difference between the treatments in the same year according to Duncan's test.

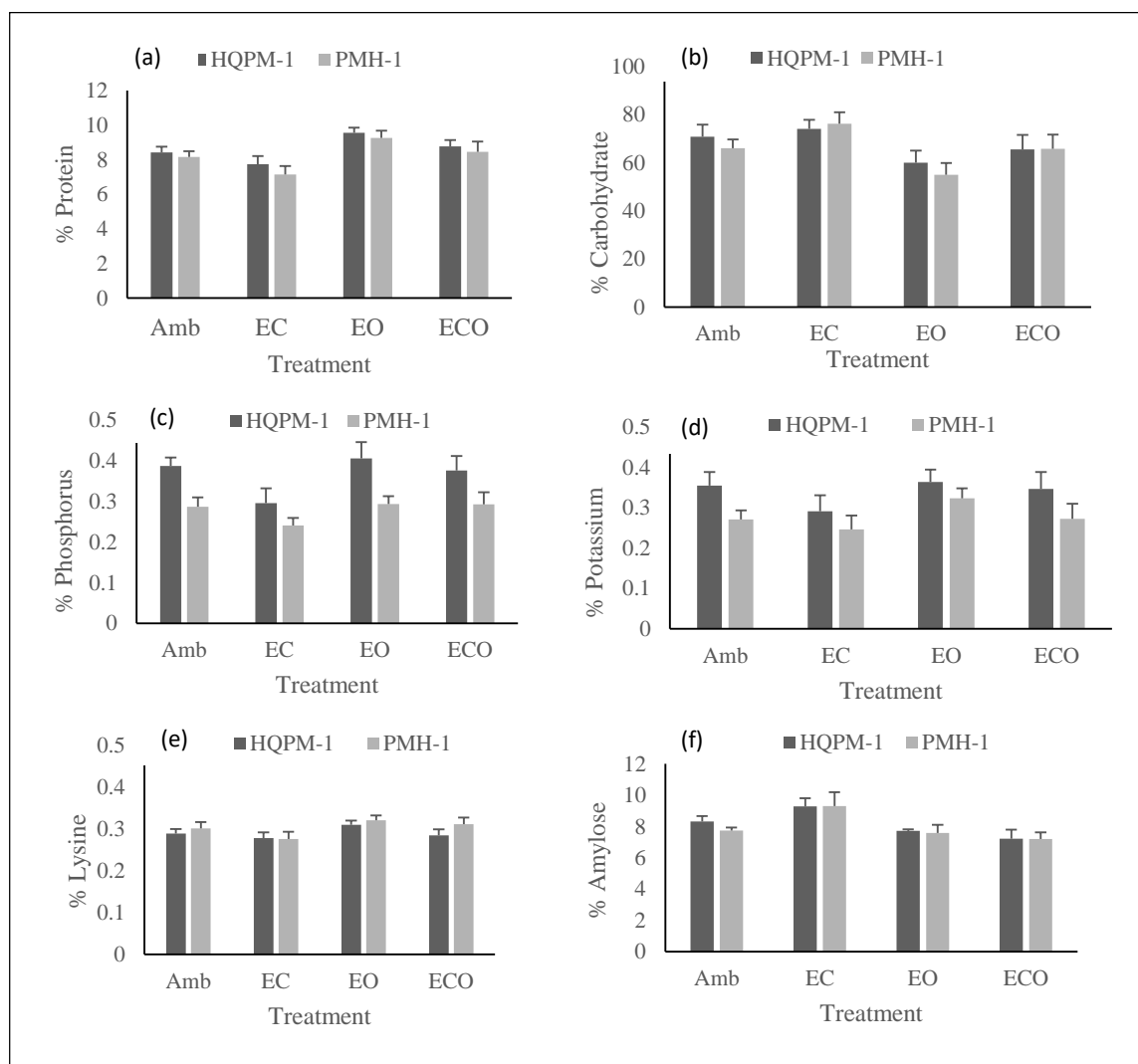
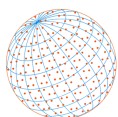


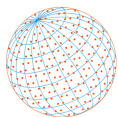
Fig. 2. (a) Protein (b) carbohydrate (c) phosphorus (d) potassium (e) lysine and (f) amylose content in maize grains under the different treatments (Average of 2016 and 2017).

in maize grains under ECO in both the cultivars as compared to the Amb. Phosphorus (P) (16.2–23.6%) and potassium (K) (8.9–17.3%) concentrations significantly decreased ($p < 0.01$) under elevated CO₂ treatment in both the cultivars as compared to the Amb (Table 4(c)). However, P (2.3–4.9%) and K (2.5–19.3%) increased under elevated tropospheric O₃. The interactive environment under ECO did not lead to any change in the P and K concentrations of grains as compared to the ambient (Figs. 2(c) and 2(d)). The amylose content also varied similar to the carbohydrate content of grains in the different treatments (Fig. 2(f)).

Lysine is an important α amino acid that is needed for the biosynthesis of proteins. It ranged under from 0.30 to 0.32% under elevated O₃ (EO) and from 0.27 to 0.28% under elevated CO₂ (EC). Lysine contents of maize grains decreased under EC by 3.7–8.4% and increased by 6.5–7.3% under EO over the ambient treatment in both HQPM-1 and PMH-1 maize cultivars (Fig. 2(e)).

3.4 Varietal Response to Yield and Quality under Different Treatments

During the first year AOT 40 of 27741 ppb hr of O₃ exposure led to decline in grain yield in HQPM-1 by 9.5%, and by 15.7% in PMH-1, while during the second year AOT exposure of 26292 ppb hr, decreased the grain yield of HQPM-1 by 8.7% and by 3.8% in PMH-1. On an average the exposure of AOT40 of 27016-ppbhr O₃ led to the yield loss of 9.2% in HQPM1 and 9.8% in PMH1 during the two years of the study. Under EC there was higher increase in grain yield of HQPM-1 as compared to PMH-1. Under ECO the grain yield of HQPM-1 increased by (6.5–9.5%) over ambient



but in case of PMH-1 it was not significantly different than ambient during the two years. There was higher increase in biological yield under EC in HQPM-1 (11.4–28%) as compared to PMH-1 (15.9–17.3%) ($p < 0.05$) during the two years as compared to the Amb. The increase in grain number cob^{-1} under elevated CO_2 was higher for HQPM-1 (10.1–21.6%) as compared to PMH-1 (5.3–15%) ($p < 0.05$) over Amb. Under the interactive treatment ECO grain weight cob^{-1} increased by 12.2% in HQPM-1 as compared to EO alone, whereas there was no significant difference in PMH-1. No significant difference in protein, carbohydrate, P and K content was observed under ECO treatment as compared to Amb in any of the crop cultivars (Fig. 2).

4 DISCUSSIONS

Present study was carried out under free air field condition to study the responses of two varieties of maize, grown under elevated levels of tropospheric O_3 and CO_2 individually as well as in combination. Due to low humidity, high light intensity, and high temperature in the Indo-Gangetic Plains (IGP) there are high concentrations of tropospheric O_3 along with atmospheric CO_2 (IPCC, 2014; Mills *et al.*, 2018) in this region. Leakey *et al.* (2009) reported that C_4 crops under elevated CO_2 did not increase the photosynthetic rate directly and only stimulated the uptake of carbon during drought or at midday when short-term water stress developed under high atmospheric vapour pressure deficits and temperatures in temperate climatic conditions. However, our results and also those of Abebe *et al.* (2016) and Pingale *et al.* (2017) confirmed that the elevated CO_2 increased Pn of maize grown under tropical semi-arid environment. Higher daytime temperatures in tropical conditions, may result in higher vapour pressure deficit resulting in short term water stress, thereby increasing the CO_2 uptake even in C_4 maize.

EO significantly reduced the photosynthetic rate in both the crop cultivars and that might be due to entering of O_3 molecules through stomatal pores during normal gas exchange process that created the reactive oxygen species (ROS), which may lead to programmed cell death (McGrath *et al.*, 2015; Yendrek *et al.*, 2017). No significant change in photosynthetic rate was observed under the interactive treatment (ECO) over the ambient (Amb), which could be due to reduced penetration of higher concentration of CO_2 and tropospheric O_3 through the stomata (Table 4(a)), because elevated CO_2 and O_3 both prohibit the opening of stomatal pores of the leaves, that minimize O_3 flux in plant apoplast (Oikawa and Ainsworth, 2016). EC and EO conditions significantly reduced the g_s in both crop varieties, and it might be attributed to reduced stomatal density and less opening of the stomatal pores (Xu *et al.*, 2016; Singh *et al.*, 2018). In previous research it was documented that g_s reduced under CO_2 condition in C_4 crops (Harley and Baldocchi, 1995; De Souza *et al.*, 2015; Hoshika *et al.*, 2015). However, O_3 induced damage may be less for C_4 species, given their intrinsically lower stomatal conductance, as well as for plants under rising CO_2 (McGrath *et al.*, 2015).

Leaf area index and chlorophyll content of leaves were also significantly impacted under the different treatments however, the cultivar treatment interaction was not significantly different at both the crop stages (Table 4(a)). Adishesha *et al.* (2017) reported that the chlorophyll content of maize increased under elevated CO_2 environment and reduced under the tropospheric O_3 concentration (Bhatia *et al.*, 2014). Deterioration in chlorophyll content of leaves under EO might be because of damage to the chloroplast due to change in membrane permeability (Zhu *et al.*, 2011). EC (550 ppm) and EO (70 ppb) treatment significantly ($p < 0.01$) impacted the crop yield parameters such as cob length, grains (cob^{-1}), biological yield and grain yield (Table 4(b)). The cultivar * treatment interaction did not significantly impact crop yield components except cob length and 100 grain weights (Table 4(b)). Higher grain number (cob^{-1}) and cob length (cm) of HQPM-1 by 11.5% and 9.7%, respectively in the first year as compared to the second year resulted in higher grain yield in the first year. Improvement in the biological yield and grain yield of cultivars under EC condition was due to the increased photosynthetic rate (12.1–16.6%), cob length (9.3–10.4%), number of grains (cob^{-1}) (10.2–15.8%) and test weight. In previous studies it was reported that cob length, grains (cob^{-1}), biological yield and 100 grain weights increased significantly under the EC and vice-versa in the EO condition (Singh *et al.*, 2014; Ainsworth, 2017). Basu *et al.* (2014) assessed the responses of 23 maize simulation models and concluded that the doubling of CO_2 from 360 to 720 ppm would increase grain yield by 7.5% on an average across the sites.

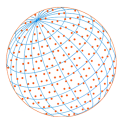


Table 4. (a) F-ratio and level of significance for plant growth parameters at tasseling and silking. (b) F –ratio and level of significance for maize yield and yield attributes. (c) F –ratio and level of significance for maize quality attributes.

(a)

Parameter	Tasseling			Silking		
	Cultivar	Treatment	Cultivar × Treat	Cultivar	Treatment	Cultivar × Treat
Photosynthetic rate	0.003ns	2.79*	0.77ns	0.03ns	44.56**	0.28ns
Stomatal conductance	9.85*	30.95**	2.01ns	8.11*	26.76**	1.76ns
LAI	10.25*	21.20**	1.91ns	13.33*	31.77**	2.22ns
Chlorophyll	0.20ns	52.11**	0.29ns	0.34ns	48.9**	0.29ns

* significant at 0.05, ** significant at 0.01 level, and ns = non-significant.

(b)

Parameter	Year	Cultivar	Treatment	Year × Cultivar	Year × Treat.	Cultivar × Treat.	Year × Cultivar × Treat.
Cob length	47.28**	7.46**	105.73**	0.217ns	0.62 ^{ns}	5.68*	1.12 ^{ns}
Grains (cob ⁻¹)	35.19**	1.61 ^{ns}	44.03**	18.23*	3.86*	1.12 ^{ns}	2.71 ^{ns}
Grain weight (cob ⁻¹)	72.99**	17.12**	292.88**	13.06*	8.27*	3.86*	10.13**
Grain yield	13.27*	0.35 ^{ns}	71.91**	3.93*	2.74 ^{ns}	1.30 ^{ns}	0.247 ^{ns}
Biological yield	20.10*	32.46**	112.01**	28.34*	3.41*	0.73 ^{ns}	0.031 ^{ns}
100 grain weight	8.49*	5.15*	23.1**	9.95*	0.96 ^{ns}	4.71*	2.95*

* significant at 0.05, ** significant at 0.01 level, and ns = non-significant.

(c)

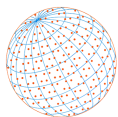
Element	Cultivar	Treatment	Cultivar × Treat.
Protein	9.22*	11.72*	2.44 ^{ns}
Carbohydrate	11.03*	23.45*	2.11 ^{ns}
Phosphorus	66.86**	16.71**	1.65 ^{ns}
Potassium	47.18**	17.34**	2.23 ^{ns}
Lysine	1.22 ^{ns}	17.61*	1.99 ^{ns}
Amylose	0.84 ^{ns}	22.46**	0.53 ^{ns}

* significant at 0.05, ** significant at 0.01 level, and ns = non-significant.

The fumigation of elevated O₃ throughout the plant growth, reduced photosynthetic rate, grain weight (cob⁻¹), cob length, grains (cob⁻¹) and 100 grain weight of crop cultivars and led to reduced grain yield in the EO treatment. It may be attributed to the entry of O₃ molecules in plants through leaf stomata and its quick reaction with the molecules of the intercellular space and production of reactive oxygen species (ROS) in the apoplast (Ainsworth, 2017). The produced ROS in apoplast trigger metabolic defense mechanism and causing programme cell death that promote leaf senescence, resulting in diversion of resources during growth and seed production and ultimately influences the crop yield. Feng *et al.* (2015) and Leisner and Ainsworth (2012) observed that the elevated tropospheric O₃ damages the crop during the reproductive stage of growth due to increased abiotic stress sensitivity and high demand of resources for seed development. In our study the physiological maturity was reached earlier by 4 days under the EO treatment in both the crop cultivars, and it was delayed by five days in the elevated CO₂ treatment as compared to the ambient and this may have contributed to the decrease or increase in maize yield under EO and EC respectively. Similar to our findings Broberg *et al.* (2015) and Pleijel *et al.* (2015) pointed out that the senescence promoted the negative effects of tropospheric O₃ on the duration of grain filling, resulting in lower grain mass of wheat and reduced yield.

An increase in grain yield of maize was observed under the interactive treatment ECO over the ambient, implying that 559 ppm of elevated CO₂ was able to offset the negative impact of elevated O₃ (71 ppb) on the grain yield. The mitigative effect of EC under the combined interactive treatment may be attributed to the reduced stomatal conductance and declined stomatal density which reduced the influx of O₃ into the plant leaves (Gray *et al.*, 2000; Ainsworth *et al.*, 2008).

Besides, impacts on growth and grain yield, elevated carbon dioxide (EC) and elevated tropospheric (EO) also impacted quality of grains. In this study phosphorus (P), potassium (K),



protein and lysine reduced under EC and increased under EO (Table 4(c)). However, carbohydrate and amylose contents of grains increased under EC but reduced under EO treatment in both the maize varieties. In our study the average photosynthetic rates (12.1–16.6%) of crop cultivars under EC was more compared to the Amb, which might have caused improved carbohydrate accumulation in the plants, resulting in dilution of P, K, protein and lysine concentrations under EC treatment and vice versa under EO. No significant interaction could be observed between the cultivars and the treatments (Table 4(c)). It has been reported in other crops that the concentration of P, K and crude protein of the grains reduced significantly under the elevated CO₂ and increased under elevated O₃ (Hogy *et al.*, 2009; Zhang *et al.*, 2013; Zhou *et al.*, 2015; Zhu *et al.*, 2018). Mishra *et al.* (2013) noticed a negative correlation between grain yield and grain protein content due to the growth dilution effect.

The average decline in yield over the two years under elevated O₃ in both the cultivars was similar, however they responded differently to elevated CO₂. The increase in grain yield was more in HQPM1 as compared to PMH-1 under EC treatment. Thus, under the interaction ECO the offsetting of grain yield as compared to decline in yield due to EO was much higher in case of HQPM-1. The increase in grain yield was higher in HQPM-1 under EC due to higher increase in 100 grain weight. The differences obtained in the grain weights (cob⁻¹) of crop cultivars under various treatment was due to the number of grains (cob⁻¹) and 100 grain weight (g).

5 CONCLUSIONS

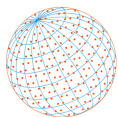
In our study the presence of elevated CO₂ increased the carbon uptake and yield of maize, but the increased carbohydrates in the grain led to a decline in the nutrient content (P, K & crude protein) due to the dilution effect and lowered the grain quality. Just the opposite was observed under elevated O₃. The elevated CO₂ mitigated the adverse impacts of elevated O₃ on the maize growth and yield under the interaction treatment. After two years of study we could conclude that elevated CO₂ (559 ppm) was able to offset the negative effect of elevated O₃ (71 ppb) on growth parameters (4.8–18.3%) and yield (11.2–18.8%) without significantly affecting the grain quality in both the maize cultivars. This amelioration was primarily due to a reduction in stomatal conductance, as a consequence of CO₂ elevation. The yield and the grain nutrient content of maize grown under the interaction of elevated CO₂ and O₃ (ECO) was observed to be at par with the ambient. Therefore, the yield and nutrient content of maize would be the least affected under the future climatic scenario of increasing atmospheric CO₂ and O₃ concentrations in tropical environments.

ACKNOWLEDGEMENTS

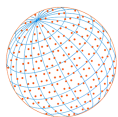
Authors thank the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi and Director, ICAR- Indian Agricultural Research Institute, New Delhi for providing the facilities to execute the experimental work. Authors acknowledge the funding support provided by the NICRA project, ICAR, GOI for carrying out this research. Authors also extend their sincere thanks to Dr. S.D. Singh, Dr. Bidisha Chakrabarti, Mr. Raghuvver and Miss Abhilasha for help and guidance during the experiment.

REFERENCES

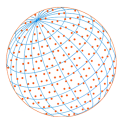
- Abebe, A., Pathak, H., Singh, S.D., Bhatia, A., Harit, R., Kumar, V. (2016). Growth, yield and quality of maize with elevated atmospheric carbon dioxide and temperature in North West India. *Agric. Ecosyst. Environ.* 218, 66–72. <https://doi.org/10.1016/j.agee.2015.11.014>
- Adishesha, K., Janagoudar, B.S., Amaregouda, A. (2017). Response of maize (*Zea mays* L.) genotypes to elevated carbon dioxide and temperature regimes. *Int. J. Chem. Stud.* 5, 2448–2256. <https://www.chemjournal.com/archives/2017/vol5issue5/PartAG/5-5-421-234.pdf>
- Ainsworth, E.A. (2008). Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. *Global Change Biol.* 14, 1642–



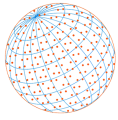
1650. <https://doi.org/10.1111/j.1365-2486.2008.01594.x>
- Ainsworth, E.A. (2017). Understanding and improving global crop responses to ozone. *Plant J.* 90, 886–897. <https://doi.org/10.1111/tpj.13298>
- Ashmore, M.R. (2005). Assessing the future global impacts of ozone on vegetation. *Plant Cell Environ.* 28, 949–964. <https://doi.org/10.1111/j.1365-3040.2005.01341.x>
- Basu, S., Brisson, N., Durand, D.L., Boote, K., Lizaso, J., Jones, J.W., Rosenzweig, C., Ruane, A.C., Adam, M., Baron, C., Baso, B., Biernath, C., Boogaard, H., Conijn, S., Corbeels, M., Deryng, D., De Sanctis, G., Gayler, S., Grassini, P., ... Waha, K. (2014). How do various maize crop models vary their responses to climate change factors? *Global Change Biol.* 20, 2301–2320. <https://doi.org/10.1111/gcb.12520>
- Bhatia, A., Ghosh, A., Kumar, V., Tomer, R., Singh, S.D., Pathak, H. (2011). Effect of elevated tropospheric ozone on methane and nitrous oxide emission from rice soil in North India. *Agric. Ecosyst. Environ.* 144, 21–28. <https://doi.org/10.1016/j.agee.2011.07.003>
- Bhatia, A., Tomer, R., Kumar, V., Singh, S.D., Pathak, H. (2012). Impact of tropospheric ozone on crop growth and productivity - A review. *J. Sci. Ind. Res.* 71, 97–112. <http://nopr.niscair.res.in/handle/123456789/13486>
- Bhatia, A., Kumar, V., Kumar, Amit. and Singh, S.D. (2014). Effect of elevated ozone and carbon dioxide interaction on growth and yield of maize. *Maydica* 58, 291–98. <https://journals-crea.4science.it/index.php/maydica/article/view/957/817>
- Broberg, M.C., Feng, Z., Xin, Y., Pleijel, H. (2015). Ozone effects on wheat grain quality – A summary. *Environ. Pollut.* 197, 203–213. <https://doi.org/10.1016/j.envpol.2014.12.009>
- Broberg, M.C., Uddling, J.M.G., Peijel, H. (2017). Fertilizer efficiency in wheat is reduced by ozone pollution. *Sci. Total Environ.* 607–608, 876–880. <https://doi.org/10.1016/j.scitotenv.2017.07.069>
- De Souza, A.P., Cocuron, J.C., Garcia, A.C., Alonso, A.P., Buckeridge, M.S. (2015). Changes in whole-plant metabolism during the grain-filling stage in sorghum grown under elevated CO₂ and drought. *Plant Physiol.* 169, 1755–1765. <https://doi.org/10.1104/pp.15.01054>
- European Environment Agency (EEA) (2000). <http://glossary.eea.europa.eu/EEAGlossary>
- Feng, Z., Hu, E., Wang, X., Jiang, L., Liu, X. (2015). Ground-level O₃ pollution and its impacts on food crops in China: A review. *Environ. Pollut.* 199, 42–48. <https://doi.org/10.1016/j.envpol.2015.01.016>
- Food and Agriculture Organization (FAO) (2016). Agricultural data. Food and Agriculture Organization of The United Nations, Rome, <http://faostat.fao.org/>
- Frei, M. (2015). Breeding of ozone resistant rice: Relevance, approaches and challenges. *Environ. Pollut.* 197, 144–155. <https://doi.org/10.1016/j.envpol.2014.12.011>
- Gomez, K.A., Gomez A. (1984). *Statistical Procedure for Agricultural Research* 2nd ed. Wiley New York, USA, p. 704.
- Government of India (2017). Chapter 7: Agriculture and Food Management. in *Economic Survey 2016-17, Economic Survey - Volume II*, Ministry of Finance, Government of India. https://www.indiabudget.gov.in/budget2017-2018/es2016-17/echap07_vol2.pdf
- Gray, J.F., Holroyd, G.H., Van Der Lee, F.M., Ahrami, A.R., Sijmons, P.C., Woodward, F.I., Schuch, W., Hetherington, AM. (2000). The III C signaling pathway links CO₂ perception to stomatal development. *Nature* 408, 713–716. <https://doi.org/10.1038/35047071>
- Harley, P.C., Baldocchi, D.D. (1995). Scaling carbon dioxide and water vapour exchange from leaf to canopy in a deciduous forest. I. Leaf model parametrization. *Plant Cell Environ.* 18, 1146–1156. <https://doi.org/10.1111/j.1365-3040.1995.tb00625.x>
- Hogy, P., Weiser, H., Kohler, P., Schwadorf, K., Breuer, J., Muntiferig, R., Fangmeir, A. (2009). Effect of elevated CO₂ on grain yield and quality of wheat: Results from 3-year free air CO₂ experiment. *Plant Biol.* 11, 60–69. <https://doi.org/10.1111/j.1438-8677.2009.00230.x>
- Hoshika, Y., Katata, G., Deushi, M., Watanabe, M., Koike, T., Paoletti, E. (2015). Ozone-induced stomatal sluggishness changes carbon and water balance of temperate deciduous forests. *Sci. Rep.* 5, 9871. <https://doi.org/10.1038/srep09871>
- Hu, G., Burton, C., Yang, C. (2010). Efficient measurement of amylose content in cereal grains. *J. Cereal Sci.* 51, 35–40. <https://doi.org/10.1016/j.jcs.2009.08.007>
- IPCC (2014). *Climate Change. 2014. Impacts, adaptation and vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.



- <https://www.ipcc.ch/report/ar5/wg2/>
- Jackson, M.L. (1973). Soil chemical analysis. Prentice. Hall of India, New Delhi, pp. 219–221.
- Juliano, B.O. (1993). Rice in human nutrition, FAO Food and Nutrition Series, No. 26, International Rice Research Institute, Los Baños, Philippines.
- Kumari, S., Agrawal, M., Singh, A. (2015). Effects of ambient and elevated CO₂ and ozone on physiological characteristics, antioxidative defense system and metabolites of potato in relation to ozone flux. *Environ. Exp. Bot.* 109, 276–287. <https://doi.org/10.1016/j.envexpbot.2014.06.015>
- Leakey, A.D.B., Uribeharrea, M., Ainsworth, E.A., Naidu, S.L., Rogers, A., Ort, D.R., Long S.P. (2006). Photosynthesis productivity and yield of maize are not affected by open -air -elevation of CO₂ concentration in the absence of drought. *Plant Physiol.* 140, 779–790. <https://doi.org/10.1104/pp.105.073957>
- Leakey, A.D.B. (2009). Rising atmospheric carbon dioxide concentration and the future of C₄ crops for food and fuel. *Proc. R. Soc. London, Ser. B* 276, 2333–2343. <https://doi.org/10.1098/rspb.2008.1517>
- Leisner, C.P., Ainsworth, E.A. (2012). Quantifying the effects of ozone on plant reproductive growth and development. *Global Change Biol.* 18, 606–616. <https://doi.org/10.1111/j.1365-2486.2011.02535.x>
- Lopes, A., Ferreira, A.B., Pantoja, P.O., Parolin, P., Premade, M.T.F. (2018). Combined effect of elevated CO₂ level and temperature on germination and initial growth of *Montrichardia arborescens* (L.) Schott (Araceae): A microcosm experiment. *Hydrobiol.* 814, 19–30. <https://doi.org/10.1007/s10750-015-2598-1>
- McGrath, J.M., Betzelberger, A.M., Wang, S., Shook, E., Zhu, X.G., Long, S.P., Ainsworth, E.A. (2015). An analysis of ozone damage to historical maize and soybean yields in the United States. *Proc. Natl. Acad. Sci. U.S.A.* 112, 14390–14395. <https://doi.org/10.1073/pnas.1509777112>
- Mills, G., Sharps, K., Simpson, D., Pleijel, H., Broberg, M., Uddling, J., Jaramillo, F., Davies, W.J., Agrawal, M., Agrawal, S.B., Ainsworth, E.A., Buker, P., Dentener, F., Emberson, L., Feng, Z., Harmens, H., Hayes, F., Kobayashi, K., Paoletti, E., Van den Berg, M., Van, D.R. (2018). Ozone pollution will compromise efforts to increase global wheat production. *Global Change Biol.* 24, 3560–3574. <https://doi.org/10.1111/gcb.14157>
- Mishra, A.K., Rai, R., Agrawal, S.B. (2013). Differential response of dwarf and tall tropical wheat cultivars to elevated ozone with and without carbon dioxide enrichment: Growth, yield and rain quality. *Field Crops Res.* 145, 21–32. <https://doi.org/10.1016/j.fcr.2013.02.007>
- Myers, S.S., Zanobetti, A., Kloog, I., Huybers, P., Leakey, A.D.B., Bloom, A.J., Carlisle, E., Dietterich, L.H., Fitzgerald, G., Hasegawa, T., Holbrook, N.M., Nelson, R.L., Ottman, M.J., Raboy, V., Sakai, H., Sartor, K.A., Schwartz, J., Seneweera, S., Tausz, M., Usui, Y. (2014). Increasing CO₂ threatens human nutrition. *Nature* 510, 139–142. <https://doi.org/10.1038/nature13179>
- Oikawa, S., Ainsworth, E.A. (2016). Changes in leaf area, nitrogen content and canopy photosynthesis in soybean exposed to an ozone concentration gradient. *Environ Pollut.* 215, 347–355. <https://doi.org/10.1016/j.envpol.2016.05.005>
- Pfister, G.G., Walters, S., Lamarque, J.F., Fast, J., Barth, M.C., Wong, J., Done, J., Holland, G., Bruyère, C.L. (2014). Projections of future summertime ozone over the US. *J. Geophys. Res.* 119, 5559–5582. <https://doi.org/10.1002/2013JD020932>
- Phothi, R., Umponstira, C., Sarin, C.S.W., Nabheerong, N. (2016). Combining effects of ozone and carbon dioxide application on photosynthesis of Thai jasmine rice (*Oryza sativa* L.) cultivar KhaoDawk Mali 105. *Aust. J. Crop Sci.* 10, 591–597. <https://doi.org/10.21475/ajcs.2016.10.04.p7595x>
- Pingale, B.N., Singh, S.D., Yadav, A. (2017). Potential impacts of increasing atmospheric carbon dioxide on yield and plant growth of rice (*Oryza sativa*) and maize (*Zea mays*) crops. *J. Agric Sci.* 87, 1041–44. <http://epubs.icar.org.in/ejournal/index.php/IJAgS/article/view/73235>
- Pleijel, H., Høgy, P. (2015). CO₂ dose response functions for wheat grains, protein and mineral yield based on FACE and open top chamber experiments. *Environ. Pollut.* 198, 70–77. <https://doi.org/10.1016/j.envpol.2014.12.030>
- Pleijel, H., Broberg, M.C., Uddling, J., Mills, G. (2018). Current surface ozone concentrations significantly decrease wheat growth yield and quality. *Sci Total Environ.* 613–614, 687–692.



- <https://doi.org/10.1016/j.scitotenv.2017.09.111>
- Porter, J.R., Xie, L., Challinor, A.J., Cochrane, K., Howden, M., Iqbal, M.M., Lobell, D.B., Travasso, M.I. (2014). Chapter 7. Food Security and Food Production Systems. *Climate Change 2014: Impacts, Adaptation and Vulnerability. Working Group II Contribution to the IPCC 5th Assessment Report*. Geneva, Switzerland. <https://orcid.org/0000-0002-8551-6617>
- Proietti, C., Anav, A., De Marco, A., Sicard, P., Vitale M. (2016). A multi-sites analysis on the ozone effects on Gross Primary Production of European forests. *Sci Total Environ.* 556, 1–8. <https://doi.org/10.1016/j.scitotenv.2016.02.187>
- Reich, Peter. B., Hobbie, S.E., Lee, T.D., Pastore, M.A. (2018). Unexpected reversal of C₃ versus C₄ grass response to elevated CO₂ during a 20 year field experiment. *Science* 360, 317–320. <https://doi.org/10.1126/science.aas.9313>
- Sadasivam, S., Manickam, A. (1992). In *Biochemical Methods for Agricultural Sciences*, Wiley Eastern Limited, New Delhi, pp. 12–13.
- Sicard, P., Anav, A., De Marco, A., Paoletti, E. (2017). Projected global ground-level ozone impacts on vegetation under different emission and climate scenarios. *Atmos. Chem. Phys.* 17, 12177–12196. <https://doi.org/10.5194/acp-17-12177-2017>
- Singh, A.A., Agrawal, S.B., Shahi, J.P., Agrawal, M. (2014). Assessment of growth and yield losses in two *Zea mays* L. cultivars (quality protein maize and non-quality protein maize) under projected levels of ozone. *Environ. Sci. Pollut. Res.* 21, 2628–2641. <https://doi.org/10.1007/s11356-013-2188-6>
- Singh, A.A., Agrawal, S.B. (2017). Tropospheric ozone pollution in India: Effects on crop yield and product quality. *Environ. Sci. Pollut. Res.* 24, 4367–4382. <https://doi.org/10.1007/s11356-016-8178-8>
- Singh, E., Rai, R., Pandey, B., Agrawal, M. (2018). Development of resistance in two wheat cultivars against constant fumigation of ozone. *Proc. Natl. Acad. Sci., India, Sect. B* 88, 1121–1134. <https://doi.org/10.1007/s40011-017-0849-9>
- Tai, A.P.K., Val Martin, M., Heald, C.L. (2014). Threat to future global food security from climate change and ozone air pollution. *Nat. Clim. Change* 4, 817–821. <https://doi.org/10.1038/nclimate2317>
- Tomer, R., Bhatia, A., Kumar, V., Kumar, A., Singh, R., Singh, B., Singh, S.D. (2015). Impact of elevated ozone on growth, yield and nutritional quality of two wheat species in Northern India. *Aerosol Air Qual. Res.* 15, 329–340. <https://doi.org/10.4209/aaqr.2013.12.0354>
- Torbert, H.A., Prior, S.A., Rogers, H.H., Runion, G.B. (2004). Elevated atmospheric CO₂ effects on N fertilization in grain sorghum and soybean. *Field Crops Res.* 88, 57–67. <https://doi.org/10.1016/j.fcr.2003.11.011>
- Tsai, C.Y., Hansel, L.W., Nelson, O.E. (1972). Caloric method of screening maize seeds for lysine content. *Cereal Chem.* 49, 572–579.
- van der Kooi, C.J., Reich, M., Löw, M., De Kok, L.J., Tausz, M. (2016). Growth and yield stimulation under elevated CO₂ and drought: A meta-analysis on crops. *Environ. Exp. Bot.* 122, 150–157. <https://doi.org/10.1016/j.envexpbot.2015.10.004>
- Wellburn, A.R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* 144, 307–313. [https://doi.org/10.1016/S0176-1617\(11\)81192-2](https://doi.org/10.1016/S0176-1617(11)81192-2)
- Xu, Z., Jiang, Y., Jia, B., Zhou, G. (2016). Elevated –CO₂ response of stomata and its dependence on environmental factors. *Plant Cell Sci.* 7, 657–663. <https://doi.org/10.3389/fpls.2016.00657>
- Yendrek, C.R., Erice, G., Montes, C.M., Tomaz, T., Sorgini, C.A., Brown, P.J., McIntyre, L.M., Leakey, A.D., Ainsworth, E.A. (2017). Elevated ozone reduces photosynthetic carbon gain by accelerating leaf senescence of inbred and hybrid maize in a genotype-specific manner. *Plant Cell Environ.* 40, 3088–3100. <https://doi.org/10.1111/pce.13075>
- Zhang, R., Hu, H., Zhao, Z., Yang, D., Zhu, X., Guo, W., Zhu, J., Kobayashi, K. (2013). Effects of elevated ozone concentration on starch and starch synthesis enzymes of Yangmai 16 under fully open-air field conditions. *J. Integr. Agric.* 12, 2157–2163. [https://doi.org/10.1016/S2095-3119\(13\)60345-7](https://doi.org/10.1016/S2095-3119(13)60345-7)
- Zhou, X., Zhou, J., Wang, Yunxia, Peng, B., Zhu, J., Yang, L., Wang, Y. (2015). Elevated tropospheric ozone increased grain protein and amino acid content of a hybrid rice without manipulation by planting density. *J. Sci. Food Agric.* 95, 72–78. <https://doi.org/10.1002/jsfa.6684>



- Zhu, C., Kobayshi, K., Loladze, I., Zhu, J., Jiang, Q., Xu, X., Liu, G., Seneweera, S., Ebi, K.L., Drewnowski, A., Fukagawa, N.K., Ziska, L.H. (2018). Carbon dioxide (CO₂) levels this century will alter the protein, Micronutrients and vitamin content of rice grains with potential health consequences for the poorest rice-dependent countries. *Sci. Adv.* 4, 1–8. <https://doi.org/10.1126/sciadv.aag1012>
- Zhu, X., Feng, Z., Sun, T., Liu, X., Tang, H., Zhu, J., Guo, W., Kobayashi, K. (2011). Effects of elevated ozone concentration on yield of four Chinese cultivars of winter wheat under fully open-air field conditions. *Global Change Biol.* 17, 2697–2706. <https://doi.org/10.1111/j.1365-2486.2011.02400.x>