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# Differential response of dwarf and tall tropical wheat cultivars to elevated ozone with and without carbon dioxide enrichment: Growth, yield and grain quality

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ABSTRACT

In past few years, atmospheric concentrations of carbon dioxide ($CO_2$) and tropospheric ozone ($O_3$) have increased due to anthropogenic activities. $CO_2$ enhances the plant growth and surface level of $O_3$ is a well-known phyto-toxic pollutant. Present study was conducted to assess the impact of elevated levels of $CO_2$ and $O_3$, singly and in combination on two wheat cultivars HUW-37 and K-9107 on their growth, yield attributes and grain quality in open top chambers (OTCs). Wheat plants under elevated $CO_2$ (EC) showed increment in growth parameters while exposure to elevated $O_3$ (EO) showed an opposite trend than EC. In elevated $CO_2 + O_3$ (ECO) exposure, elevated $CO_2$ fully protected wheat cultivars against negative effects of $O_3$. Yield parameters showed significant increase in EC followed by ECO and in EO, significant reductions in yield were noticed in both the cultivars. Protein and total free amino-acids decreased in grains of EC, ECO and EO in both the test cultivars. Total soluble sugars and starch contents in grains increased due to EC and ECO and decreased in EO, however reducing sugars showed an opposite trend in both the cultivars. The yield data obtained from the experiment showed cultivar specific response as cultivar HUW-37 proved to be sensitive as compared to K-9107 against ambient and elevated levels of $O_3$. The study also concludes that elevated $CO_2$ nullified the negative impact of elevated $O_3$ in both the test cultivars of wheat.



## 1. Introduction

Global climate change is primary environmental concern both to the scientific community and society due to its direct effect on agriculture. Carbon dioxide ($CO_2$) and tropospheric ozone ($O_3$), two important aspects of global climate change are rising continuously in the atmosphere (Ainsworth, 2008). Atmospheric concentrations of $CO_2$ have been steadily rising as a result of fossil fuel combustion and land use change, from approximately 315 ppm (parts per million) in 1959 to the current atmospheric concentration to be above 390 ppm (www.esrl.noaa.gov/gmd/ccgg/trends/global.html). Current Intergovernmental Panel on Climate Change (IPCC) projections indicates that atmospheric $CO_2$ concentrations will increase over this century, reaching 730–1020 ppm by the year 2100. In parallel with the recent trend of rising atmospheric $CO_2$, the concentration of $O_3$ has been increasing rapidly because of the anthropogenic emissions of several $O_3$-forming precursors (Kostiainen et al., 2006). $O_3$ is not only a strong phyto-toxic pollutant but also an important green-house gas (Percy et al., 2002). In industrialized countries of the northern hemisphere, 8 h tropospheric $O_3$ is estimated to have increased from approximately 10 ppb (parts per billion) prior to the industrial revolution to a current level of approximately 60 ppb during summer months, and is predicted to increase 20% more by 2050 (IPCC, 2007). In India, tropical climatic condition favors formation of tropospheric $O_3$. Studies have shown an increasing trend of $O_3$ concentrations in the past few years (Rai et al., 2011; Sarkar and Agrawal, 2010a; Tiwari et al., 2008). Sarkar and Agrawal (2010a) have reported the mean day time $O_3$ concentration to be 45.3 ppb during the year 2007–2008 and 47.3 ppb during the year 2008–2009.

Both $CO_2$ and $O_3$ have direct effects on growth and physiology of plants. $O_3$ causes foliar injury to plants, reduces photosynthesis and decreases productivity. $O_3$ at higher concentrations negatively affects crops causing biochemical and physiological alterations (Betzelberger et al., 2010; Rai et al., 2011; Sarkar et al., 2010, Singh et al., 2005), which are translated in form of changes such as reductions in growth (Biswas et al., 2008) and yield losses (Rai et al., 2010; Sarkar and Agrawal, 2010a).

In contrast, elevated atmospheric $CO_2$ improves the growth and productivity of crop plants. Atmospheric $CO_2$ is the basic source of carbon for plants and is mainly utilized in synthesizing primary metabolites via photosynthesis. Thus, its increased availability may enhance photosynthesis rate and decrease photorespiration

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thereby inducing increase in total non-structural carbohydrates (mainly starch, fructans and soluble sugars), their allocation to various organs and yield in many $C_3$ plants (Ainsworth and Long, 2005; Ainsworth et al., 2002).

Some experiments have been performed in recent years to examine the impacts of elevated $CO_2$ and $O_3$ on crop plants and have shown a variety of responses (Booker et al., 2005; Burkey et al., 2007; Craigon et al., 2002; Ishioh and Imai, 2005; Pleijel et al., 2000). Studies clearly demonstrate different directional responses of instantaneous leaf-level carbon assimilation for soybean in elevated $CO_2$ and $O_3$ (Morgan et al., 2003). The most compelling reason is that elevated concentrations of $CO_2$ lower $O_3$ flux into leaves due to decline in stomatal conductance resulting lower amount of $O_3$ absorbed by the leaf and subsequent $O_3$ injury (Cardoso-Vilhena et al., 2004). Secondly, the protective effect of elevated $CO_2$ against $O_3$ injury involves increased photosynthates availability that could be used for damage repair and detoxification processes to enable plants to maintain the growth.

Studies conducted on wheat have shown it to be most sensitive crop to $O_3$ exposure (Mills et al., 2007) and among tropical/Asian and temperate cultivars of wheat, Asian cultivars are more sensitive to $O_3$ (Emberson et al., 2009; Rai et al., 2007; Sarkar and Agrawal, 2010a; Van Dingenen et al., 2009; Wahid et al., 1995). Wheat is a $C_3$ plant and it is predicted that in near future $C_3$ plants will be more benefited in $CO_2$ enriched environment. Keeping these facts in view, the objective of the present study was (1) to assess the relative impact of $CO_2$ and $O_3$, singly and in combination, on growth and yield of tropical wheat cultivars at different developmental ages, (2) to study variations in seed quality of wheat cultivars under elevated $CO_2$ and/or $O_3$ and also (3) to assess the level of amelioration provided by elevated $CO_2$ against $O_3$.

## 2. Materials and methods

### 2.1. Study area

The field experiments were conducted during winter season between the months of December to March for two consecutive years (i.e., 2010–2011 and 2011–2012) in the same field at Botanical garden of the Banaras Hindu University, Varanasi, Uttar Pradesh (25°81′N and 83°1′E about 76 m above mean sea level) located in the eastern Gangetic plains of India. Soil of the experimental site was alluvial, pale brown and sandy loam in texture (sand 45%, silt 28% and clay 27%) with slightly alkaline pH (7.2–7.4).

During the experimental period, meteorological parameters like maximum and minimum temperature, relative humidity, total rainfall and number of sunshine hours were collected from the observatory of Indian Meteorological Division (IMD), Banaras Hindu University, Varanasi, India.

### 2.2. Raising of plants

Two cultivars of wheat (*Triticum aestivum* L.), HUW-37 (dwarf variety) and K-9107 (tall variety) were selected as test cultivars. Both the cultivars are popular and widely grown in Northern parts of India. Seeds were obtained from Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi and C. S. Azad University of Agriculture and Technology, Kanpur. The life spans of both the cultivars were 125 days from emergence to harvest. HUW-37 and K-9107 have yield potential of 50–55 qn and 45–50 qn and released in 1982 and 1996, respectively. Both the cultivars were tolerant to karnal bunt and resistant to rust and blight.

The field was prepared using standard agronomic practices. Grains were hand sown inside each open-top chamber (OTCs). Recommended dose of fertilizers (120, 80 and 40 kg $ha^{-1}$) N, P and K as urea, superphosphate and muriate of potash, respectively were added during the preparation of field. Half dose of N and full doses of P and K were given as basal dressings. Another half dose of N was supplied after 30 days of germination as top dressing. After 15 days of germination, plants were thinned to one plant every 15 cm. Regular watering was performed in each chamber to maintain similar water regime. Manual weeding was done four times during the course of experiment.

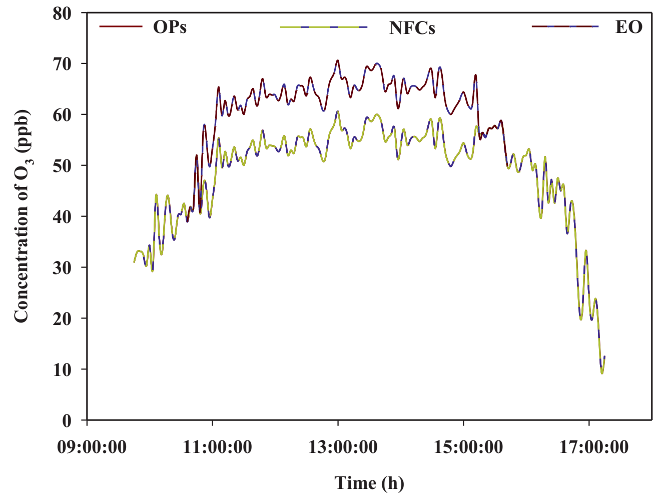


**Fig. 1.** Differential profile of $O_3$ (ppb) at a particular day (14th December, 2010) in OPs, NFCs and EO at the experimental site.

### 2.3. Elevated $CO_2$ and $O_3$ treatment

The experimental set up consisting of 24 OTCs were established at the site by following the design of Bell and Ashmore (1986). The OTCs were cylindrical with 1.5 m diameter and 1.8 m height including the frustum. There were three chambers in replicate for each treatment, so a total of 12 chambers per cultivar. All the OTCs were equipped with non-filtered high speed blower with three air volume changes per minute. Our experimental design was a factorial design with two levels of $CO_2$ (i.e., ambient and elevated) and two levels of $O_3$ (i.e., ambient and elevated). Treatment consists of: (i) non-filtered chambers (NFCs) receiving ambient $CO_2$ and ambient $O_3$ as control; (ii) NFCs with elevated $CO_2$ (EC); (iii) NFCs with elevated $O_3$ (EO); and (iv) NFCs with elevated $CO_2 + O_3$ (ECO). Intergovernmental Panel on Climate Change (IPCC, 2007) projections indicate that atmospheric $CO_2$ concentrations will reach to 730–1020 ppm by the year 2100. In the Northern Hemisphere, mean surface $O_3$ is projected to increase by 10–30 ppb by 2100 (Prather et al., 2003). The experimental dose of $CO_2$ and $O_3$ was selected in view of the above projections. Plants were exposed to elevated $CO_2$ (700 ppm) and elevated $O_3$ (ambient + 10 ppb, i.e., nearly 25% of ambient) from seed germination to the maturity from 11.00 to 15.00 h. The treatment period was selected on the basis that $O_3$ showed its daily peak value between 11.00 and 15.00 h (Fig. 1). Elevated $CO_2$ treatment was given with $CO_2$ cylinders having regulated gas flow and elevated $O_3$ by ozone generators (Model Systrocom, India) that contain UV lamps causing oxygen break down and consequent $O_3$ formation. Open plots (OPs) were also kept to assess any chamber effect. Microclimatic measurements were performed within and outside the OTCs at canopy height at regular intervals. It was observed that temperature and relative humidity were 0.1–0.3 °C and 4–5% higher inside the OTCs as compared to OPs. The light intensity in the OTCs was 95% of the ambient levels as compared to OPs, so, OTCs provided the near natural environment just like OPs.

*2.4. Air quality monitoring and AOT 40 calculation*

Eight hourly continuous $CO_2$ and $O_3$ monitoring was done in OTCs and OPs at the experimental site using automatic $CO_2$ (Model LI-820, LI-COR Inc., USA) and $O_3$ (Model APOA 370, HORIBA Ltd., Kyoto, Japan) analyzers throughout the growth period of the crop from 9.00 to 17.00 h. Air samples were drawn through Teflon tube (0.35 cm in diameter) at canopy height inside the chamber.

AOT 40 (accumulated ozone over a threshold value of 40 ppb), an $O_3$ exposure index, was calculated by the formula given by Mauzerall and Wang (2001):

$$\mathrm{AOT\,40} = \sum_{i=1}^{n} [C_{O_3} - 40]_i$$

where $C_{O_3}$ is the mean $O_3$ concentration per hour in parts per billion (ppb), $i$ is the index, and $n$ is the number of hours with $C_{O_3} > 40$ ppb.

*2.5. Study of foliar injury*

Five plants from each setup were collected for the study of foliar injury percentage (FIP) at 40, 60 and 80 DAG. All the leaves (with or without injury symptoms) were collected (excluding totally senesced leaves) and total surface area was measured through a portable leaf area meter (Model LI 3000, LICOR Inc., Lincoln, USA) and then injured leaf area was cut with a clean scissor and area of injured parts was again calculated with the same leaf area meter. FIP was calculated according to the formula given by Rao et al. (1995).

$$\mathrm{FIP} = \frac{\text{total injured area}}{\text{total leaf area}} \times 100$$

*2.6. Plant sampling and analysis*

Plant samples were taken from each treatment after fumigation at 40, 60 and 80 DAG (days after germination) for various growth and biomass analysis. Six plants were taken randomly from each chamber. Monoliths of 10 cm × 10 cm × 20 cm size containing intact roots were carefully dug from each chamber. These were thoroughly washed by placing them on sieves of 1 mm mesh size under running tap water to remove attached soil particles. Growth parameters recorded were root and shoot lengths, number of tillers, number of leaves and leaf area per plant. Leaf area was measured using a portable leaf area meter (Model LI-3000, LI-COR, Inc., USA). For biomass determination, plant parts (root, shoot and leaf) were separated and oven dried at 80 °C to obtain constant weight. Dry weights of plant parts were taken. Growth indices such as, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR), root/shoot ratio (RSR), specific leaf area (SLA) and specific leaf weight (SLW) were calculated according to the formulae given by Hunt (1982).

*2.7. Yield attributes*

Harvesting was performed at 125 DAG. Individual as well as combined treatment effects on final yield were assessed (i.e., number of ears plant$^{-1}$, weight of ears plant$^{-1}$, number of grains plant$^{-1}$, weight of grains plant$^{-1}$, harvest index and test weight of grains). Ten plants were sampled from OTCs for each cultivar and treatment.

*2.8. Grain quality parameters*

Grain samples were oven dried and grinded in a stainless steel grinder and passed through a 2.0 mm sieve. Three replicates were taken from each treatment. For extracting sugars and starch, 50 mg powdered grain sample was boiled with 5 ml 80% ethanol (v/v) and then centrifuged. The pellets were successively washed with 80% ethanol for four times and centrifuged after each washing. Finally the pellets were washed with distilled water and centrifuged again. The supernatant collected after each washing was used for estimating soluble and reducing sugars and pellets for extracting starch. Estimation of total soluble sugar and starch was conducted by following phenol/$H_2SO_4$ colorimetric assay (Dubois et al., 1956). Reducing sugar content was determined by following the method of Somogyi–Nelson (Herbart et al., 1971). Total amino acids were estimated by the methodology given by Moore and Stein (1948). The method consists of extraction of amino acids in 80% ethanol and its colorimetric estimation using ninhydrin reagent at 570 nm wavelength. Protein content in grains was estimated by following the method as described by Lowry et al. (1951).

*2.9. Statistical analysis*

Data of growth and total biomass were subjected to multivariate ANOVA to examine the individual and combined effects of age, cultivar (Cv), $CO_2$ and $O_3$. Results of total biomass and yield of two-year experiment were subjected to multivariate ANOVA to examine the individual and combined effects of year, Cv, $CO_2$ and $O_3$. Results of seed quality parameters were analyzed through three-way ANOVA using Cv, $CO_2$ and $O_3$ as factors. Duncan's multiple range tests were performed as post hoc for various measurements after subjecting to one way ANOVA test. All the statistical tests were performed using SPSS software (SPSS Inc., version 16.0).

## 3. Results

*3.1. Meteorological parameters*

In both the experimental years, meteorological data showed similar variations. Maximum mean temperature was highest during March (33.9 °C in 2011 and 32.7 °C in 2012) and lowest during January (21.4 °C in 2011 and 21.6 °C in 2012). Minimum mean temperature also varied from 9.2 to 16.2 °C during experimental period 2010–2011 and 9.7 to 15.7 °C during 2011–2012. Maximum mean relative humidity was highest during January 2011 (88.5%) in first year and during December 2011 (95.8%) in second year of the experiment. Total monthly mean rainfall was higher in the second year (48.8 mm) than first year (2.0 mm). Number of sunshine hours ranged from 6.9 to 8.7 h during 2010–2011 and 4.8 to 8.3 h during 2011–2012.

*3.2. Air quality monitoring and AOT 40*

In NFCs, mean 8 h $CO_2$ concentration was 388.4 ppm during the first year (2010–2011) and 392.5 ppm in the second year (2011–2012). In EC and ECO, mean $CO_2$ concentration varied from 548.2 ppm during the first year (2010–2011) to 554.0 ppm during the second year (2011–2012) (Table 1). Mean $O_3$ concentration in NFCs throughout the first year (2010–2011) was 48.4 ppb with AOT 40 value of 5.1 ppm h and during second year (2011–2012), it was 52.7 ppb with AOT 40 value of 7.8 ppm h (Table 1). At first year of growth, mean $O_3$ concentration in EO and ECO was 55.2 ppb and the corresponding AOT 40 value was 8.4 ppm h (Table 1). In second year, mean 8 h $O_3$ concentrations in EO and ECO was 58.3 ppb and the corresponding AOT 40 value was 11.0 ppm h (Table 1). Similar variations were recorded during monitoring in OPs and NFCs (Fig. 1). Diurnal profiles of $CO_2$ and $O_3$ of two consecutive years of experiment have been shown in Figs. 2 and 3.

**Table 1**
Mean $CO_2$ and $O_3$ concentrations and AOT 40 in different experimental setups during experimental period 2010–2011 and 2011–2012. Values are mean ± SE.

| Experimental setup | Experimental period: 2010–2011 | | | Experimental period: 2011–2012 | | |
|---|---|---|---|---|---|---|
| | Mean $CO_2$ (ppm) | Mean $O_3$ (ppb) | AOT 40 (ppm h) | Mean $CO_2$ (ppm) | Mean $O_3$ (ppb) | AOT 40 (ppm h) |
| NFCs | 388.4 ± 37.3 | 48.4 ± 4.6 | 5.1 | 392.5 ± 36.5 | 52.7 ± 5.1 | 7.8 |
| EC | 548.2 ± 53.7 | 48.4 ± 4.6 | 5.1 | 554.0 ± 56.3 | 52.7 ± 5.1 | 7.8 |
| EO | 388.4 ± 37.3 | 55.2 ± 5.3 | 8.4 | 392.5 ± 36.5 | 58.3 ± 6.2 | 11.0 |
| ECO | 548.2 ± 53.7 | 55.2 ± 5.3 | 8.4 | 554.0 ± 56.3 | 58.3 ± 6.2 | 11.0 |

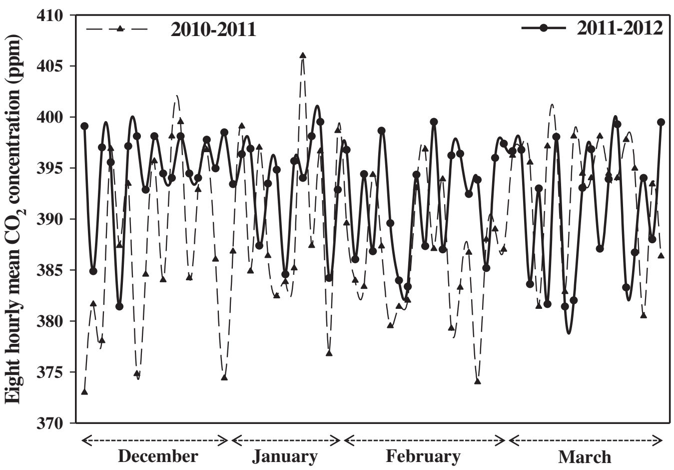


**Fig. 2.** Diurnal profile of $CO_2$ (ppm) of two consecutive years at the experimental site (8 h daily mean).

### 3.3. Foliar injury

Foliar injury in terms of interveinal chlorosis and chlorotic stippling was first observed in elevated $O_3$ exposure on adaxial surface of the leaves (Fig. 4). Initially, foliar injury was not much distinct, but at later sampling ages, it became prominent in both the cultivars. Under EO exposure, higher FIP was noticed in Cv HUW-37 than K-9107 at 60 and 80 DAG (Fig. 5). Plants grown in NFCs also showed some injury in leaves. For Cv HUW-37, FIP was 16 and 18.2% and for Cv K-9107, it was 11 and 15%, respectively at 60 and 80 DAG in NFCs (Fig. 5). In EC treatment, no foliar injury was observed throughout the growth period (Fig. 4). Foliar injury was reduced in combined treatment (ECO) due to $CO_2$ enrichment. In ECO, FIP was 13.5 and

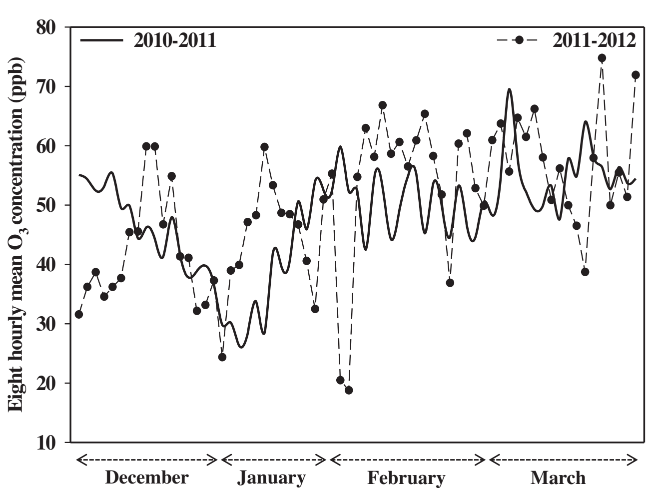


**Fig. 3.** Variations in mean concentrations (ppb) of $O_3$ for two consecutive years at the experimental site during growth period of wheat (8 h daily mean).

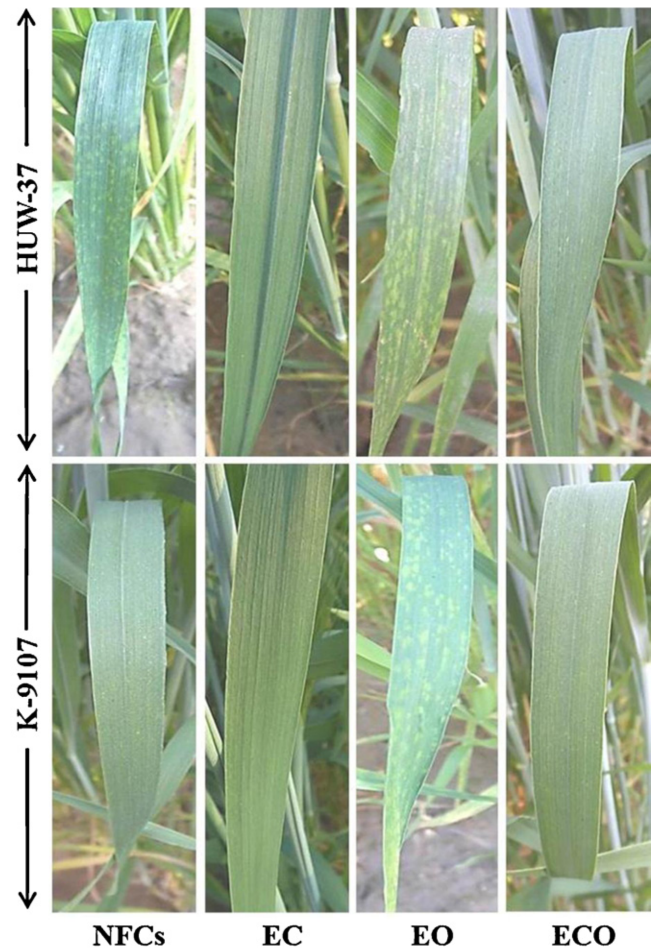


**Fig. 4.** Injury profile in leaves of wheat (*Triticum aestivum* L.) in different experimental setups at 80 DAG (days after germination).

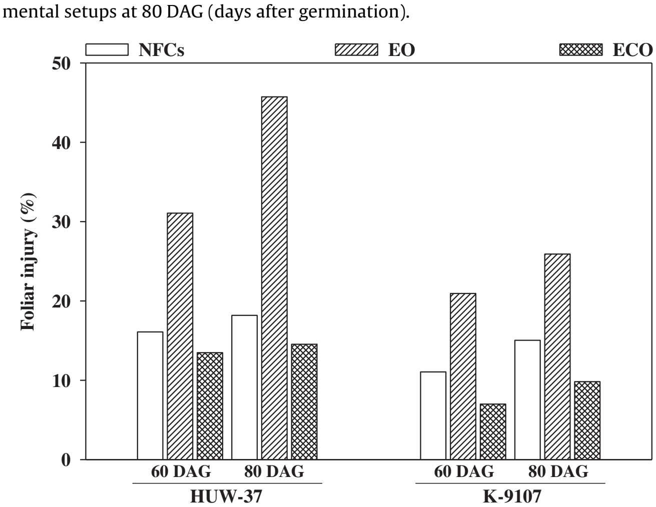


**Fig. 5.** Foliar injury percentage in wheat leaves in NFCs, EO and ECO at 60 and 80 DAG (year: 2010–2011).

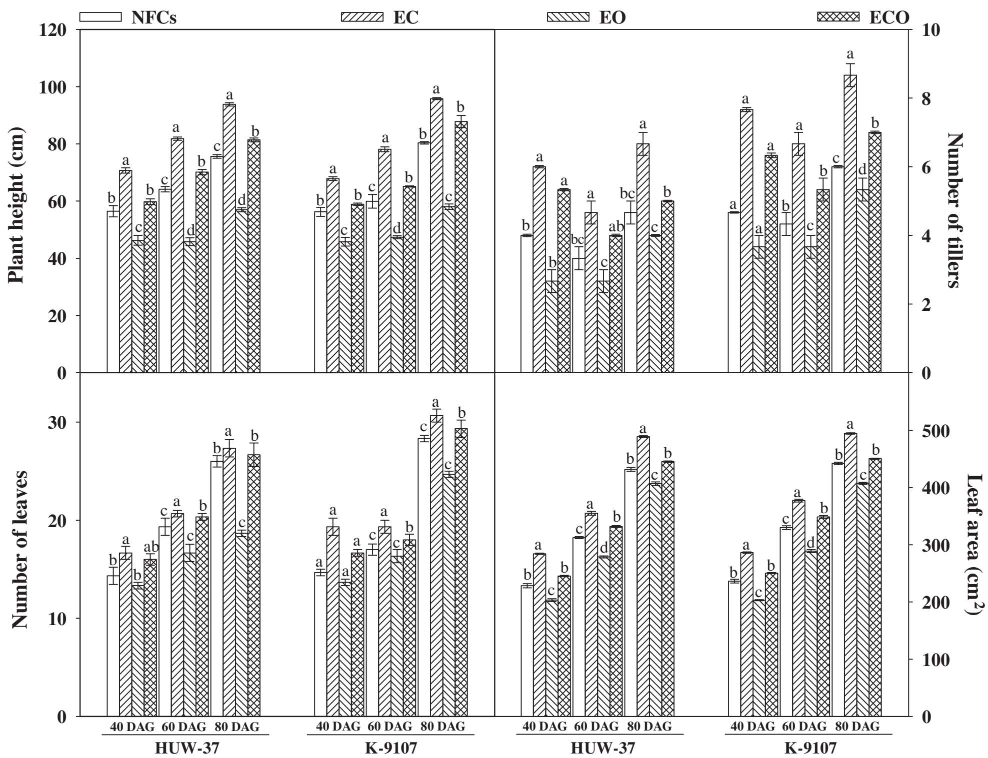


**Fig. 6.** Morphological parameters of wheat plants at 40, 60 and 80 DAG (days after germination) in NFCs, EC, EO and ECO (year: 2010–2011). Different letters on bars indicate significant differences among treatments at $p < 0.05$ according to Duncan's test.

14.5% for HUW-37 and 7.0 and 9.8% for K-9107, respectively at 60 and 80 DAG (Fig. 5).

### *3.4. Morphological parameters*

Plant height decreased significantly under elevated $O_3$ (EO) at 40, 60 and 80 DAG as compared to NFCs. Reductions were 28.5% in HUW-37 and 21% in K-9107 at 60 DAG (Fig. 6). Significant increase in plant height was observed under elevated $CO_2$ (EC) and elevated $CO_2 + O_3$ (ECO) in both the cultivars at 40, 60 and 80 DAG as compared to NFCs. At 60 DAG, plant height increased by 27.5 and 30.3% in EC and by 9.3 and 8.7% in ECO, respectively in HUW-37 and K-9107 (Fig. 6). Results of multivariate ANOVA test showed that variation in plant height was significant due to age, $CO_2$, $O_3$, and their interactions except Cv, Cv × $CO_2$, Cv × $O_3$, age × Cv × $CO_2$, age × Cv × $O_3$, and Cv × $CO_2$ × $O_3$ (Table 2).

Number of tillers, number of leaves and leaf area showed similar trend as plant height in both the cultivars. In EO exposure, number of leaves decreased significantly by 28.2% in HUW-37 at 80 DAG as compared to NFCs (Fig. 6). Reductions in number of leaves in K-9107 were insignificant at all the ages of sampling (Fig. 6). Significant increase in number of leaves were observed by 16.2 and 32% in EC and by 11.6 and 13.6% in ECO in HUW-37 and K-9107, respectively at 60 DAG as compared to NFCs (Fig. 6). Number of tillers, number of leaves and leaf area showed significant variations due to all individual factors and their interactions except $CO_2$ × $O_3$, age × $CO_2$, Cv × $CO_2$, age × Cv × $CO_2$, age × Cv × $O_3$ (Table 2).

### *3.5. Total biomass and growth indices*

Total biomass, in both the experimental years, showed significant increase in EC and ECO and declining trends in EO in both the cultivars. During first year, reductions of 49.2% in HUW-37 and 43.8% in K-9107 were recorded in EO at 80 DAG compared to NFCs (Fig. 7). Total biomass increased by 16.6 and 19.8% in EC and 7 and 11% in ECO in HUW-37 and K-9107, respectively at 80 DAG as compared to NFCs (Fig. 7). Results of multivariate ANOVA analysis revealed that variations recorded in total biomass were significant due to age, Cv, $CO_2$, $O_3$ and their interactions except Cv × $O_3$, age × Cv × $O_3$, Cv × $CO_2$ × $O_3$, age × Cv × $CO_2$ × $O_3$ (Table 2). In the second year, similar trend was noticed at 80 DAG. Multivariate ANOVA also showed that in both years, total biomass accumulation varied significantly mainly due to Cv, $CO_2$, $O_3$ and their interaction, however, no significant variations were observed in total biomass year wise (Table 3).

Effects on RGR (relative growth rate) were not significant in both the cultivars at 40–60 DAG and 60–80 DAG. In EO exposure, significant reductions in NAR (net assimilation rate) were observed by 35.8 and 29.8% in HUW-37 and K-9107, respectively at 40–60 DAG as compared to NFCs (Fig. 8). NAR increased by 24% in HUW-37 and 30% in K-9107 in EC at 40–60 DAG as compared to NFCs. Under ECO, a significant increase by 13.6% was observed only in HUW-37 (Fig. 8).

LAR (leaf area ratio) increased significantly by 48.7 and 35.1% in EO and decreased in EC by 16.8 and 18.6% in HUW-37 and K-9107, respectively at 60 DAG as compared to NFCs (Fig. 9). In ECO treatment, the changes in LAR were insignificant in both the cultivars at 60 DAG. In EO exposure, SLW (specific leaf weight) reduced by 30% in HUW-37 and 22% in K-9107 at 60 DAG as compared to NFCs (Fig. 9). Significant increase in SLW was recorded in both the cultivars under EC treatment at 60 DAG as compared to NFCs. In EO treatment, SLA (specific leaf area) increased by 44 and 29% in HUW-37 and K-9107, respectively at 60 DAG; however, changes due to other treatments were not significant as compared to NFCs (Fig. 9). LWR (leaf weight ratio) significantly increased by 42.8 and 27.6% in EC and ECO in HUW-37 at 60 DAG (Fig. 9). Exposure to EO did not cause any significant changes in LWR in both the cultivars at all the ages of sampling. Significant increments in root/shoot ratio (RSR) were observed with values 26.3 and 57% in EC and 21.2 and

**Table 2**

*F* ratios and significance levels obtained from multivariate ANOVA test performed for selected growth parameters, total biomass and growth indices of wheat plants treated with $CO_2$ and $O_3$, individually and in combination.[†]

| Parameters | A | Cv | $CO_2$ | $O_3$ | $CO_2 \times O_3$ | A × Cv | A × $CO_2$ | A × $O_3$ | Cv × $CO_2$ | Cv × $O_3$ | A × Cv × $CO_2$ | A × Cv × $O_3$ | A × $CO_2 \times O_3$ | Cv × $CO_2 \times O_3$ | A × Cv × $CO_2 \times O_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant height | 628.47$^{***}$ | 0.000$^{ns}$ | 1345$^{***}$ | 665.03$^{***}$ | 25.36$^{***}$ | 13.51$^{***}$ | 28.67$^{***}$ | 9.26$^{***}$ | 0.44$^{ns}$ | 2.08$^{ns}$ | 0.98$^{ns}$ | 0.71$^{ns}$ | 8.40$^{**}$ | 0.96$^{ns}$ | 3.43$^{*}$ |
| Number of tillers | 15.05$^{***}$ | 41.30$^{***}$ | 78.36$^{***}$ | 10.72$^{**}$ | 0.13$^{ns}$ | 0.41$^{ns}$ | 2.88$^{ns}$ | 4.76$^{*}$ | 0.02$^{ns}$ | 0.01$^{ns}$ | 1.64$^{ns}$ | 2.17$^{ns}$ | 0.17$^{ns}$ | 0.721$^{ns}$ | 3.94$^{*}$ |
| Number of leaves | 31.20$^{***}$ | 8.93$^{**}$ | 23.79$^{***}$ | 11.52$^{**}$ | 1.52$^{ns}$ | 2.82$^{ns}$ | 2.43$^{ns}$ | 1.18$^{ns}$ | 0.08$^{ns}$ | 0.005$^{ns}$ | 0.27$^{ns}$ | 0.24$^{ns}$ | 0.44$^{ns}$ | 1.419$^{ns}$ | 0.47$^{ns}$ |
| Leaf area | 6103$^{***}$ | 45.01$^{***}$ | 992.46$^{***}$ | 550.16$^{***}$ | 0.03$^{ns}$ | 13.41$^{***}$ | 0.26$^{ns}$ | 0.49$^{ns}$ | 0.17$^{ns}$ | 6.53$^{*}$ | 0.12$^{ns}$ | 1.70$^{ns}$ | 12.44$^{***}$ | 5.067$^{*}$ | 0.29$^{ns}$ |
| Total biomass | 2714$^{***}$ | 61.55$^{***}$ | 727.19$^{***}$ | 353.16$^{***}$ | 77.33$^{***}$ | 22.69$^{***}$ | 88.68$^{***}$ | 61.62$^{***}$ | 7.49$^{**}$ | 3.46$^{ns}$ | 4.11$^{*}$ | 0.87$^{ns}$ | 47.93$^{***}$ | 0.58$^{ns}$ | 1.63$^{ns}$ |
| RGR | 17.69$^{***}$ | 0.03$^{ns}$ | 11.95$^{**}$ | 4.59$^{*}$ | 0.000$^{ns}$ | 1.67$^{ns}$ | 0.11$^{ns}$ | 0.15$^{ns}$ | 0.20$^{ns}$ | 0.11$^{ns}$ | 0.008$^{ns}$ | 0.39$^{ns}$ | 0.001$^{ns}$ | 0.08$^{ns}$ | 0.38$^{ns}$ |
| NAR | 36.98$^{***}$ | 0.49$^{ns}$ | 116.13$^{***}$ | 64.10$^{***}$ | 7.75$^{**}$ | 0.34$^{ns}$ | 6.22$^{*}$ | 6.09$^{*}$ | 0.11$^{ns}$ | 0.53$^{ns}$ | 0.000$^{ns}$ | 0.79$^{ns}$ | 0.49$^{ns}$ | 1.76$^{ns}$ | 5.04$^{*}$ |
| SLA | 35.72$^{***}$ | 1.10$^{ns}$ | 14.29$^{**}$ | 10.78$^{**}$ | 5.87$^{*}$ | 1.49$^{ns}$ | 8.16$^{**}$ | 7.49$^{*}$ | 0.26$^{ns}$ | 0.22$^{ns}$ | 0.72$^{ns}$ | 0.38$^{ns}$ | 4.46$^{*}$ | 0.16$^{ns}$ | 0.24$^{ns}$ |
| SLW | 748.81$^{***}$ | 1.39$^{ns}$ | 146.92$^{***}$ | 60.10$^{***}$ | 2.07$^{ns}$ | 2.65$^{ns}$ | 14.09$^{**}$ | 0.41$^{ns}$ | 8.19$^{**}$ | 1.40$^{ns}$ | 5.50$^{*}$ | 0.29$^{ns}$ | 0.07$^{ns}$ | 0.000$^{ns}$ | 0.52$^{ns}$ |
| LAR | 505.88$^{***}$ | 5.19$^{*}$ | 737.57$^{***}$ | 504.477$^{***}$ | 236.09$^{***}$ | 2.60$^{ns}$ | 0.44$^{ns}$ | 1.23$^{ns}$ | 0.27$^{ns}$ | 1.02$^{ns}$ | 4.97$^{*}$ | 1.79$^{ns}$ | 4.74$^{*}$ | 0.79$^{ns}$ | 18.58$^{***}$ |
| LWR | 717.82$^{***}$ | 0.93$^{ns}$ | 5.65$^{*}$ | 0.41$^{ns}$ | 0.32$^{ns}$ | 0.81$^{ns}$ | 0.06$^{ns}$ | 1.38$^{ns}$ | 4.39$^{*}$ | 0.41$^{ns}$ | 3.51$^{ns}$ | 0.39$^{ns}$ | 0.47$^{ns}$ | 0.006$^{ns}$ | 1.00$^{ns}$ |
| RSR | 223.96$^{***}$ | 30.93$^{***}$ | 111.25$^{***}$ | 40.38$^{***}$ | 0.73$^{ns}$ | 56.86$^{***}$ | 12.49$^{**}$ | 2.87$^{ns}$ | 8.05$^{**}$ | 3.76$^{ns}$ | 2.27$^{ns}$ | 3.42$^{ns}$ | 1.73$^{ns}$ | 0.01$^{ns}$ | 5.52$^{*}$ |

A: age; Cv: cultivar.

$^{*}$ Level of significance, $p < 0.05$.

$^{**}$ Level of significance, $p < 0.01$.

$^{***}$ Level of significance, $p < 0.001$.

$^{ns}$ Level of significance, not significant.

[†] Data used from first year experiment (2010–2011).

**Table 3**

Results of multivariate ANOVA showing *F*-ratios and level of significance for total biomass and yield parameters of wheat cultivars for two consecutive years treated with elevated $CO_2$, elevated $O_3$ and elevated $CO_2 + O_3$.

| Parameters | Year | Cv | $CO_2$ | $O_3$ | $CO_2 \times O_3$ | Y × Cv | Y × $CO_2$ | Y × $O_3$ | Cv × $CO_2$ | Cv × $O_3$ | Y × Cv × $CO_2$ | Y × Cv × $O_3$ | Y × $CO_2 \times O_3$ | Cv × $CO_2 \times O_3$ | Y × Cv × $CO_2 \times O_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TB | 0.61$^{ns}$ | 55.30$^{***}$ | 2094$^{***}$ | 1024$^{***}$ | 114.55$^{***}$ | 0.13$^{ns}$ | 0.22$^{ns}$ | 0.37 $^{ns}$ | 1.83$^{ns}$ | 0.14$^{ns}$ | 0.35$^{ns}$ | 0.31$^{ns}$ | 0.001$^{ns}$ | 17.97$^{***}$ | 0.67$^{ns}$ |
| NOEPP | 0.14$^{ns}$ | 45.43 $^{***}$ | 104.54$^{***}$ | 32.26$^{***}$ | 2.53$^{ns}$ | 0.14$^{ns}$ | 0.14$^{ns}$ | 0.14$^{ns}$ | 0.006$^{ns}$ | 2.07$^{ns}$ | 0.14$^{ns}$ | 0.14$^{ns}$ | 0.14$^{ns}$ | 2.07$^{ns}$ | 0.14$^{ns}$ |
| WOEPP | 0.23$^{ns}$ | 40.71$^{***}$ | 414.29$^{***}$ | 48.63$^{***}$ | 3.30$^{ns}$ | 0.17$^{ns}$ | 0.32$^{ns}$ | 0.41$^{ns}$ | 69.99$^{***}$ | 1.2$^{ns}$ | 0.83$^{ns}$ | 0.19$^{ns}$ | 0.26$^{ns}$ | 0.21$^{ns}$ | 0.77$^{ns}$ |
| NOGPP | 0.47$^{ns}$ | 296.60$^{***}$ | 194.90$^{***}$ | 59.62$^{***}$ | 0.69$^{ns}$ | 0.57$^{ns}$ | 0.06$^{ns}$ | 0.63$^{ns}$ | 4.87$^{*}$ | 0.93$^{ns}$ | 0.03$^{ns}$ | 0.52$^{ns}$ | 0.02$^{ns}$ | 2.42$^{ns}$ | 0.04$^{ns}$ |
| WOGPP | 0.24$^{ns}$ | 215.74$^{***}$ | 1957$^{***}$ | 259.72$^{***}$ | 18.53$^{***}$ | 0.43$^{ns}$ | 0.39$^{ns}$ | 0.39$^{ns}$ | 1.74$^{ns}$ | 12.13$^{**}$ | 0.26$^{ns}$ | 0.34$^{ns}$ | 0.73$^{ns}$ | 37.47$^{***}$ | 0.56$^{ns}$ |
| HI | 0.03$^{ns}$ | 73.53$^{***}$ | 20.82$^{***}$ | 9.59$^{**}$ | 0.72$^{ns}$ | 0.002$^{ns}$ | 0.20$^{ns}$ | 0.15$^{ns}$ | 0.49$^{ns}$ | 0.38$^{ns}$ | 0.31$^{ns}$ | 0.002$^{ns}$ | 0.01$^{ns}$ | 7.62$^{*}$ | 0.49$^{ns}$ |
| TW | 0.19$^{ns}$ | 32.54$^{***}$ | 147.58$^{***}$ | 142.49$^{***}$ | 6.97$^{**}$ | 0.29$^{ns}$ | 0.13$^{ns}$ | 0.45$^{ns}$ | 0.40$^{ns}$ | 4.46$^{*}$ | 0.23$^{ns}$ | 0.58$^{ns}$ | 0.64$^{ns}$ | 2.18$^{ns}$ | 0.72$^{ns}$ |

TB: total biomass; NOEPP: number of ears plant$^{-1}$; WOEPP: weight of ears plant$^{-1}$; NOGPP: number of grains plant$^{-1}$; WOGPP: weight of grains plant$^{-1}$; HI: harvest index; TW: test weight; Y: year; Cv: cultivar.

$^{*}$ Level of significance, $p < 0.05$.

$^{**}$ Level of significance, $p < 0.01$.

$^{***}$ Level of significance, $p < 0.001$.

$^{ns}$ Level of significance, not significant.

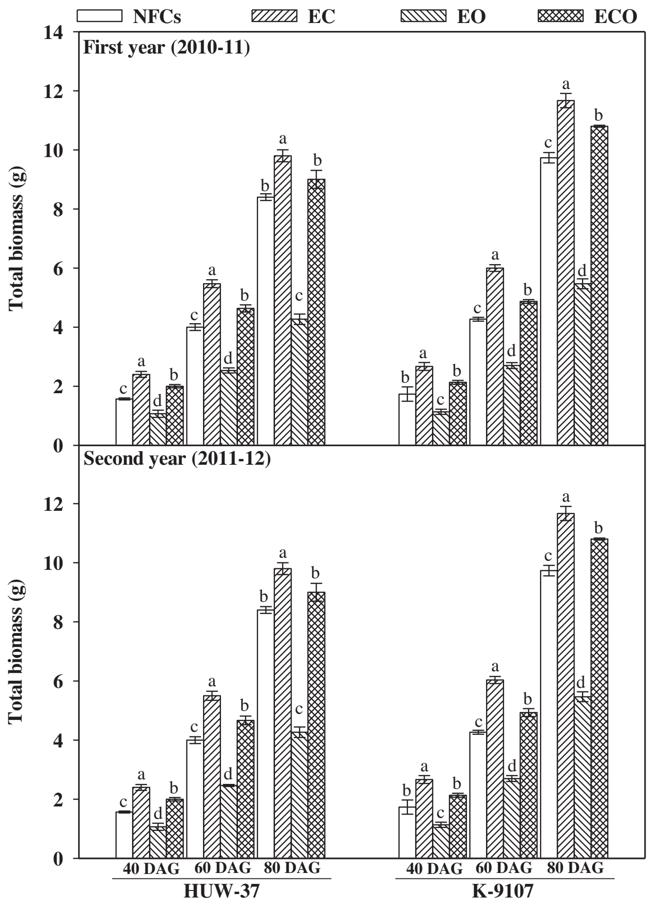


**Fig. 7.** Effect of $CO_2$, $O_3$ and $CO_2 + O_3$ on total biomass of two cultivars of wheat at different stages of growth for two consecutive years of experiment. Values are mean ± SE. Bars showing different letters indicate significant differences among treatments according to Duncan's test at $p < 0.05$.

18.3% in ECO, while in EO, reductions were recorded at 60 DAG as compared to NFCs (Fig. 9).

Results of multivariate ANOVA analysis showed that RGR, NAR, SLA, SLW, LAR, LWR and RSR varied significantly due to age, $CO_2$, $O_3$ and their interactions except Cv × $O_3$, age × Cv × $O_3$ and Cv × $CO_2$ × $O_3$ (Table 2).

### 3.6. Yield attributes

Yield parameters, in both the experimental years, increased in EC and ECO and reduced in EO as compared to NFCs. Number and weight of ears plant$^{-1}$ increased by 29.7 and 64.7% in HUW-37, while in K-9107, significant increase in weight of ears plant$^{-1}$ was recorded in EC. Significant increments in number and weight of grains plant$^{-1}$ were 28 and 46% in HUW-37 and 36 and 54.6% in K-9107, respectively in EC as compared to NFCs. Harvest index (HI) increased significantly by 12.5% in K-9107 as compared to NFCs. Significant increments in test weight (wt. of 1000 grains) were noticed to be 11.8 and 14.4% in HUW-37 and K-9107, respectively as compared to NFCs (Fig. 10).

Number of ears plant$^{-1}$ reduced significantly by 25.5 and 20.6% in HUW-37 and K-9107 and higher reductions were observed in HUW-37 under EO exposure. Weight of ears plant$^{-1}$ decreased significantly by 31.1% in K-9107. Number and weight of grains plant$^{-1}$ decreased by 21.1 and 39% in HUW-37 and 18.3 and 12.4% in K-9107, respectively. During second year, weight of grains plant$^{-1}$ reduced by 40.8% in HUW-37 and 14% in K-9107. Significant reduction in test weight was 17.3% in HUW-37 and 13.5% in K-9107 as compared to NFCs. Under EO exposure, change in HI was not significant in both the cultivars (Fig. 10).

In ECO, significant increase in number and weight of ears plant$^{-1}$ was observed by 10.6 and 55.6% in HUW-37 only. Number and weight of grains plant$^{-1}$ increased by 12.8 and 34.8% in HUW-37 and 19 and 37.5% in K-9107, respectively as compared to NFCs. Changes in HI and test weight were not found significant in both the cultivars as compared to NFCs (Fig. 10).

Results of multivariate ANOVA analysis showed that in both years, yield components varied significantly due to individual and combined treatment of $CO_2$ and $O_3$. WOGPP (weight of grains plant$^{-1}$) showed significant variations due to Cv, $CO_2$, $O_3$ and their interaction (Table 3).

### 3.7. Grain quality parameters

Protein content in grains (GP) decreased significantly by 8.9 and 5.4% in EC and 18.8 and 15.3% in EO in HUW-37 and K-9107, respectively as compared to NFCs. In ECO, the variations were not significant in both the cultivars (Table 4). Total free amino acids (TFAA) showed significant reductions by 10.4 and 6.9% in HUW-37 and K-9107, respectively in EC. Under EO exposure, TFAA decreased significantly by 20.2 and 17.4% in HUW-37 and K-9107, respectively as compared to NFCs. The changes observed in ECO were insignificant in both the cultivars (Table 4).

Total soluble sugars (TSS) increased significantly by 18.6 and 28.9% in EC and by 9.7 and 13.9% in ECO in HUW-37 and K-9107, respectively as compared to NFCs (Table 4). In HUW-37, starch content (SC) significantly increased by 19.7 and 8.3% and in K-9107, increments were 26.6 and 10.2% in treatment EC and ECO, respectively. Reductions in TSS and SC were observed in HUW-37 by 17.4 and 24.1% and 16.2 and 12.1% in K-9107, respectively in EO as compared to NFCs (Table 4). Reducing sugars (RS) reduced significantly by 23.1% in HUW-37 and 22.3% in K-9107 in EC. Increments recorded in EO were 9.6 and 7.4% in HUW-37 and K-9107, respectively as compared to NFCs (Table 4). Under ECO treatment, a significant increase by 3.9% was noticed only in K-9107 (Table 4).

Results of three-way ANOVA revealed that protein content, TFAA, TSS, RS and SC in grains varied significantly due to all the individual factors and their interactions except Cv × $O_3$ (Table 4).

## 4. Discussion

The results of the present study clearly indicate the adverse effects of elevated $O_3$ on two experimental wheat varieties. The negative effect was, however, more prominent in HUW-37 than in K-9107. Ozone is well-known to generate reactive oxygen species (ROS), which may affect the structure and function of the biological membranes (Gillespie et al., 2011). Prolonged exposure of crop plants to elevated $O_3$ led to development of injury symptoms such as interveinal yellowing or chlorotic stippling (Picchi et al., 2010; Sarkar and Agrawal, 2010b). Under EO, cultivars performed differentially at 80 DAG, showing interveinal chlorosis in Cv HUW-37 and chlorotic stippling in K-9107. In elevated $CO_2$ (EC) and elevated $CO_2 + O_3$ (ECO), elevated levels of $CO_2$ might have provided protection against ambient and elevated $O_3$ by reducing stomatal conductance and hence decreasing $O_3$ uptake by plants (Kumari et al., 2013).

At 60 DAG, plants treated with EC showed higher increment in plant height, number of tillers, number of leaves and leaf area in Cv K-9107 than HUW-37. This result may attribute to improve dry matter production and thus it causes the share increase of photosynthetic material toward shoot growth. Gent (1995) reported 20% increase in biomass and canopy photosynthesis in tall as compared to semi-dwarf isolines of wheat early in development. The growth

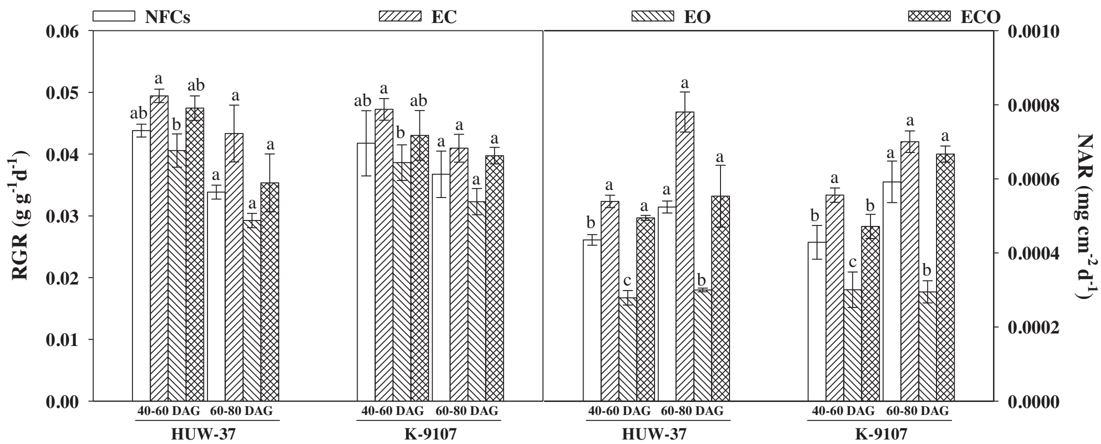


**Fig. 8.** Relative growth rate (RGR) and net assimilation rate (NAR) of wheat plants at 40–60 and 60–80 DAG in NFCs, EC, EO and ECO (year: 2010–2011). Bars showing different letters indicate significant differences among treatments at $p < 0.05$ according to Duncan's test.

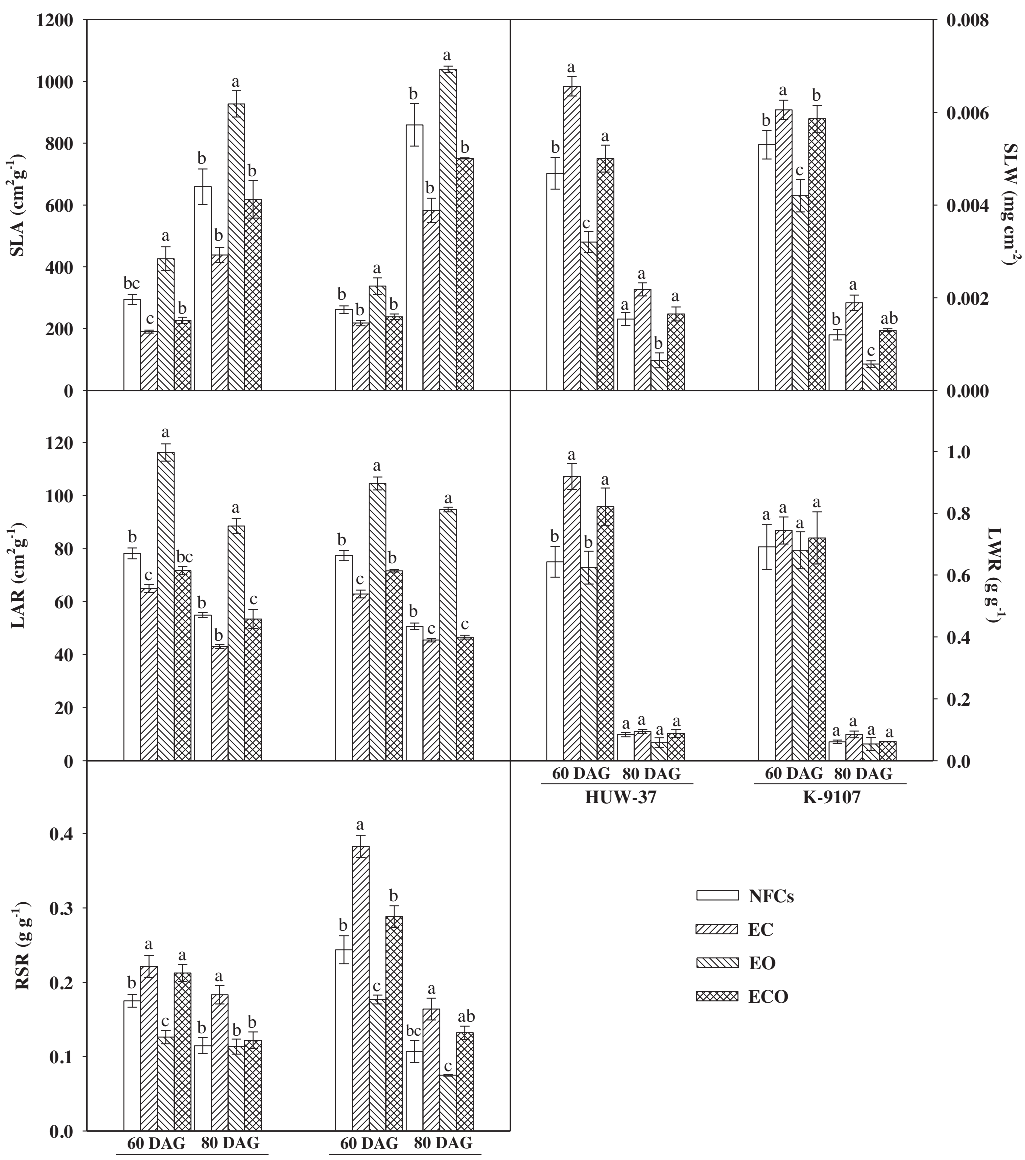


**Fig. 9.** Growth indices of wheat plants at 60 and 80 DAG in NFCs, EC, EO and ECO (year: 2010–2011). Different letters on bars indicate significant differences among treatments at $p < 0.05$ according to Duncan's test.

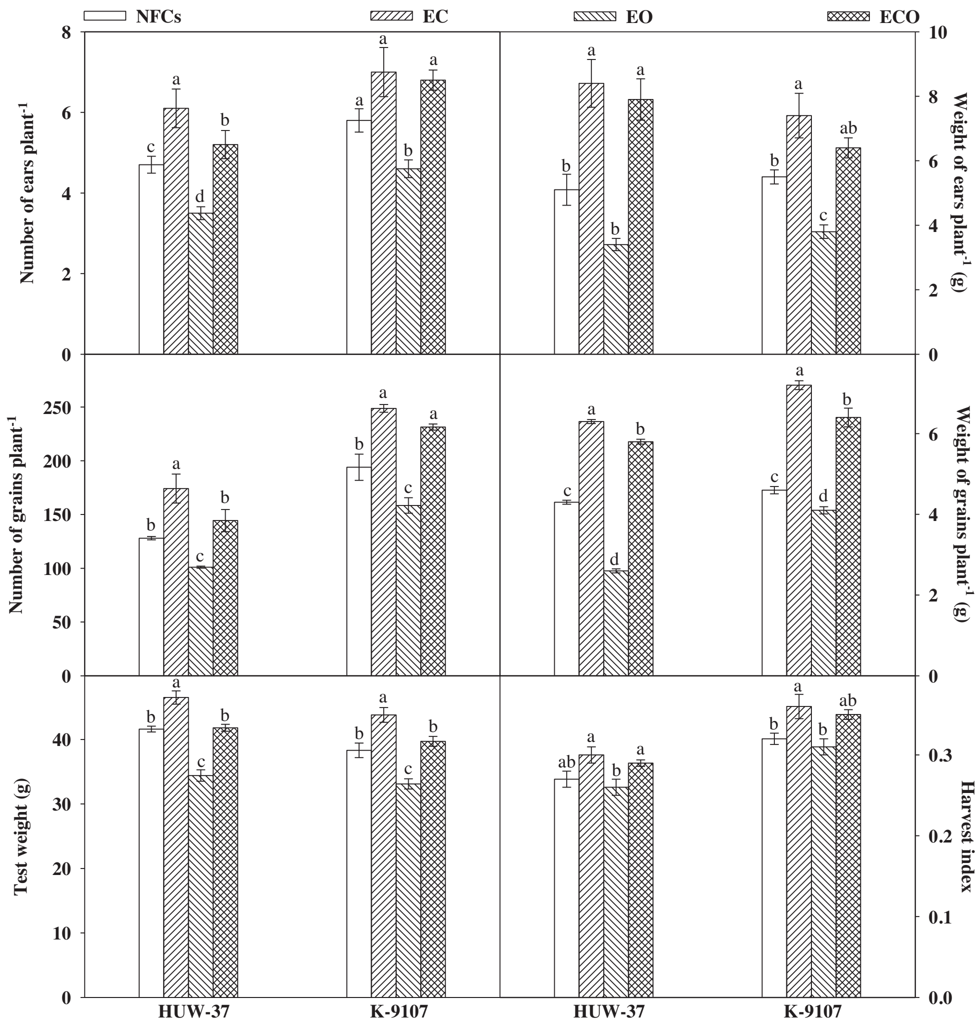


**Fig. 10.** Yield attributes of wheat plants in NFCs, EC, EO and ECO (year: 2010–2011). Values are mean ± SE. Bars showing different letters indicate significant differences among treatments according to Duncan's test at $p < 0.05$.

**Table 4**
Grain quality parameters† of wheat cultivars in non-filtered chambers (NFCs), elevated $CO_2$ (EC), elevated $O_3$ (EO) and elevated $CO_2 + O_3$ (ECO) (values are mean ± SE).

| Cultivars | Treatment | GP | TFAA | TSS | RS | SC |
|---|---|---|---|---|---|---|
| HUW-37 | NFCs | $28.8 \pm 0.23^{a}$ | $0.72 \pm 0.003^{a}$ | $91.4 \pm 1.02^{c}$ | $28.3 \pm 0.37^{b}$ | $102.5 \pm 1.66^{c}$ |
| | EC | $26.2 \pm 0.19^{b}$ | $0.64 \pm 0.004^{b}$ | $108.4 \pm 1.36^{a}$ | $21.7 \pm 0.20^{c}$ | $122.8 \pm 1.01^{a}$ |
| | EO | $23.3 \pm 0.28^{c}$ | $0.57 \pm 0.004^{c}$ | $75.4 \pm 1.35^{d}$ | $31.0 \pm 0.30^{a}$ | $77.7 \pm 1.35^{d}$ |
| | ECO | $28.5 \pm 0.45^{a}$ | $0.71 \pm 0.003^{a}$ | $100.3 \pm 0.82^{b}$ | $29.0 \pm 0.47^{b}$ | $111.1 \pm 1.21^{b}$ |
| K-9107 | NFCs | $33.9 \pm 0.13^{a}$ | $0.68 \pm 0.003^{a}$ | $95.2 \pm 1.12^{c}$ | $26.7 \pm 0.34^{c}$ | $107.4 \pm 1.40^{c}$ |
| | EC | $32.1 \pm 0.08^{b}$ | $0.63 \pm 0.007^{b}$ | $122.9 \pm 1.21^{a}$ | $20.7 \pm 0.17^{d}$ | $136.2 \pm 1.54^{a}$ |
| | EO | $28.7 \pm 0.10^{c}$ | $0.56 \pm 0.003^{c}$ | $79.8 \pm 0.86^{d}$ | $28.7 \pm 0.27^{a}$ | $94.3 \pm 1.52^{d}$ |
| | ECO | $33.7 \pm 0.39^{a}$ | $0.67 \pm 0.003^{a}$ | $108.5 \pm 0.97^{b}$ | $27.7 \pm 0.14^{b}$ | $118.2 \pm 1.52^{b}$ |

*F* ratios and significance levels from three-way ANOVA test

| | GP | TFAA | TSS | RS | SC |
|---|---|---|---|---|---|
| Cv | $804.67^{***}$ | $65.67^{***}$ | $97.53^{***}$ | $52.67^{***}$ | $110.03^{***}$ |
| $CO_2$ | $57.09^{***}$ | $85.77^{***}$ | $974.48^{***}$ | $322.66^{***}$ | $703.10^{***}$ |
| $O_3$ | $81.57^{***}$ | $175.53^{***}$ | $293.16^{***}$ | $490.00^{***}$ | $282.34^{***}$ |
| $CO_2 \times O_3$ | $374.83^{***}$ | $830.21^{***}$ | $8.00^{*}$ | $124.51^{***}$ | $4.16^{ns}$ |
| Cv × $CO_2$ | $0.47^{ns}$ | $0.01^{ns}$ | $21.30^{***}$ | $3.63^{ns}$ | $0.03^{ns}$ |
| Cv × $O_3$ | $0.40^{ns}$ | $0.06^{ns}$ | $3.37^{ns}$ | $1.39^{ns}$ | $1.84^{ns}$ |
| Cv × $CO_2 \times O_3$ | $1.55^{ns}$ | $20.26^{***}$ | $4.55^{*}$ | $0.29^{ns}$ | $19.96^{***}$ |

Different letters within a group of column indicate significant differences among treatments at $p < 0.05$ according to Duncan's test.
GP: grain protein; TFAA: total free amino acids; TSS: total soluble sugars; RS: reducing sugars; SC: starch content; Cv: cultivar.
* Level of significance, $p < 0.05$.
** Level of significance, $p < 0.01$.
*** Level of significance, $p < 0.001$.
ns Level of significance, not significant.
† Data used from first year experiment (2010–2011).

enhancement under EC exposure might have resulted from higher carbohydrate flux (Mulchi et al., 1992). Lawlor and Mitchell (1991) have shown that the extra carbon due to elevated $CO_2$ concentrations in plant leaves increased the number of tillers and branches which prop up the leaves and resulting increment in leaf area. In the present study, elevated $CO_2$ has increased the sink growth as revealed by growth and proliferation of existing sinks (i.e., number of tillers). Increments in growth parameters exposed to ECO were similar to those observed under EC exposure. In present experiment, growth parameters reduced under EO exposure in both the test cultivars and reduction was more in HUW-37 than K-9107. Under EO treatment, reduction in plant height revealed less dry mass fraction in the stem, which may be related to the greater dry mass partitioned to the ear in the dwarf variety (HUW-37) compared to the taller variety (K-9107). Number of leaves and leaf area are two important parameters for plant survival and growth. Both the parameters are strongly affected by elevated $O_3$ suggesting increased senescence of leaves leading to reduction in leaf area. Sarkar and Agrawal (2010a) have reported significant reductions in growth parameters of wheat plants under ambient + 10 ppb and ambient + 20 ppb of $O_3$.

In the present study, total biomass was higher in EC and ECO, however in EO, total biomass decreased in both the cultivars. At 80 DAG, in both the years, Cv K-9107 showed higher total biomass than HUW-37 under EC which may be attributed to higher assimilation and accumulation of photosynthates due to higher number of leaves and leaf area. In ECO and EO treatments, total biomass showed similar response in both the cultivars. Under ECO, elevated $CO_2$ helped the plants to accrue more biomass to conquer the negative effects of $O_3$ which might have resulted due to higher rate of photosynthesis, hence Cv K-9107 showed higher total biomass than HUW-37. Pearson et al. (1995) have reported reduction in stomatal aperture under elevated $CO_2$ environment. Under $CO_2$ enrichment, the reduced stomatal aperture may decrease the entry of $O_3$ inside the leaves leading to increased availability of substrates which may detoxify the negative effects of $O_3$ by prompting the repair process. Decrease in total biomass under EO exposure can be directly correlated to the $O_3$ induced negative changes in morphological and growth characteristics. Sarkar and Agrawal (2010a) have shown reductions in total biomass of wheat plants under elevated concentrations of $O_3$. Rai et al. (2007) and Agrawal et al. (2005) also reported significant reduction in biomass of wheat and mung bean plants under ambient $O_3$.

Growth indices reflect the mechanism by which $O_3$, $CO_2$ and their combined treatment affect the growth and development of test cultivars. Relative growth rate (RGR) which is a measure of production efficiency of plants showed variable response in plants exposed to different treatments. In the present study, RGR increased in EC and ECO treated plants and decreased in EO. RGR has two components NAR (net assimilation rate) and LAR (leaf area ratio) in which former is a physiological component and latter is a morphological component. Higher RGR in EC and ECO treated plants and reductions in RGR in EO treated plants may be due to variations observed in net assimilation rate (NAR). Higher NAR observed under EC and ECO may be due to carbon gain through increase in net photosynthetic rate. Lower NAR under EO treated plants suggests the adverse effect of $O_3$ on assimilation capacity. However, the changes in RGR were not significant in both the cultivars. Deepak and Agrawal (1999) also observed the similar trend in wheat cultivar HUW-234 exposed to elevated levels of $CO_2$ and $SO_2$.

LAR reflects the amount of leaf area a plant develops after per unit total plant biomass and therefore, depends on the proportion of biomass allocated to leaves relative to total plant weight (LWR) and on the amount of leaf area which a plant develops per unit leaf biomass (specific leaf area, SLA). In the present study, LAR decreased under EC and ECO treated plants and this may be attributed to increase in LWR (one of the component of LAR) suggesting more partitioning of fixed carbon for leaf growth. A reduction in LAR at elevated $CO_2$ may represent an adaptive acclimation mechanism by which the plants adjust the balance between carbon assimilation and its utilization. Larger LAR was recorded in EO exposure, suggesting major contribution of photosynthates in leaf expansion and it could be one of compensatory mechanism to maintain NAR. Increment of SLA under EO treatment denotes the decrease in leaf thickness as justified by reductions recorded in SLW and LWR. Hence, reduction in plant biomass under EO may be correlated with substantial modification in the partitioning of biomass into component plant organs. This trend is clearly evident with significant variations in RGR, NAR, SLA, SLW, LAR and LWR due to $O_3$ and as both the cultivars showed similar response, hence no variation due to $Cv \times O_3$ was observed.

In cultivar K-9107, higher RSR ratio was observed in EC treated plants than HUW-37 at 60 DAG. Increase in root: shoot ratio under EC may be attributed to greater biomass partitioning to the roots due to higher photosynthetic rate and the probable cause might be to acquire more nitrogen (N) by roots to maintain leaf N concentration and associated photosynthetic capacity. Under EO treatment, maximum reduction of RSR values was observed in HUW-37 as compared to K-9107. Ozone exposure alters the source–sink balance, decreasing assimilates partitioning to below ground organs and increased retention in the shoot portion, thus reducing the root: shoot ratio. Level of amelioration provided by ECO was higher in HUW-37 than K-9107 in relation to root: shoot ratio, but during reproductive phase, when the level of $O_3$ was high, extent of amelioration was less compared to K-9107.

The marked effect on growth parameters, biomass accumulation and its allocation to different parts is reflected in terms of economic yield. Wheat cultivars showed cultivar specific response as observed by significant variations in number and weight of grains plant$^{-1}$ due to elevated $CO_2$ and $O_3$, singly and in combination. Under EO, higher reductions in yield attributes were noticed in HUW-37 than K-9107. The main cause of reduction may be ascribed to decrease in number and weight of ears followed by reductions in weight of grains. Yield loss under $O_3$ may be attributed to reduction in photosynthetic activity and lower supply of assimilates to reproductive parts responsible for seed growth (Black et al., 2000; Fiscus et al., 2005). Reductions in wheat yield by 25 and 37% were reported by Sarkar and Agrawal (2010a) for cultivars Sonalika and HUW-510, respectively under mean EO treatment (NFCs + 10 ppb) of 50.4 ppb. In the present study, plants of EO (NFCs + 10 ppb) experienced greater $O_3$ stress compared to the plants of NFCs, but the magnitude of response was quite high in Cv HUW-37. This might show that any additional $O_3$ concentration of 10 and 20 ppb over ambient during day time will result in higher damaging effects on plants (Sarkar et al., 2010; Sarkar and Agrawal, 2010b).

AOT 40 is an exposure–response approach based on the linear decline in crop yield resulting from cumulative exposure of $O_3$ above threshold of 40 ppb during three months of growing season of crop (Fuhrer et al., 1997). According to UNECE (2008), critical level of AOT 40 for agricultural crop is 3 ppm h and 10 ppm h for forest species. In the present study, in both the years, mean concentration of elevated $O_3$ was more than 40 ppb and AOT 40 was quite high than 3 ppm h, which could be possible explanation of reductions in grain yield under elevated $O_3$. Debaje et al. (2010) have reported high AOT 40 value (7.3 ppm h) during winter season in rural part of India. During second year of experiment, reduction in yield was more which may be due to higher AOT 40 value. The results showed that HUW-37 (dwarf Cv with higher yield potential) was more $O_3$ sensitive than K-9107 (tall Cv with lower yield potential).

Stimulation in grain yield (GY) by elevated $CO_2$ may be primarily associated with increased number of grains (GN), whereas negative effect of elevated $O_3$ on GY may be more strongly associated with change in grain mass (GM) compared with GN. Our results are in agreement with earlier observations (Jablonski et al., 2002; Piikki et al., 2008). GM is considered an important quality trait in wheat (Pleijel and Uddling, 2012) which is strongly affected by elevated $O_3$ in Cv HUW-37. Under ECO treatment, elevated levels of $CO_2$ counteracted negative effects of elevated $O_3$ as proved by higher grain yield. Also, level of amelioration by elevated $CO_2$ against negative effects of $O_3$ was higher in K-9107 (less sensitive to $O_3$) than HUW-37. This result probably originated from enhanced assimilate production resulting from higher rate of photosynthesis under $CO_2$ enriched conditions (Mulholland et al., 1997) which invested the extra portion of carbon resulting in increased number of tillers and ears and consequently higher number of grains. Elevated $CO_2$ can promote the development of larger number of seeds by stimulating photosynthesis (Jablonski et al., 2002). The findings of the present study contradict the results of Heagle et al. (2000) which showed that $O_3$ sensitive wheat Cv C9904 showed greater response against elevated $CO_2$ than resistant Cv C9835.

Harvest index (HI) indicates the partitioning of dry matter between grains (economic yield) and above ground biomass (biological yield). Reduction in HI of plants under elevated levels of $O_3$ exposure revealed less dry matter partitioning toward grains as compared to NFCs. Under EC treatment, higher HI was observed in K-9107 suggesting higher rate of carbon assimilation and its translocation toward reproductive parts thus enables to develop healthy grains. No effect of elevated $CO_2$ on HI in Cv HUW-37 reveals that $CO_2$ stimulates the production of reproductive and vegetative parts of the above-ground biomass to the same extent.

Contents of protein and total free amino-acids (TFAA) in grains decreased in EC, ECO and EO in both the cultivars. Previous studies have shown a decrease in protein content under elevated $CO_2$ (Rao et al., 1995; Webber et al., 1994). Less reduction in protein content under elevated $CO_2+O_3$ suggests that $O_3$ induced oxidative damage to the protein was reduced significantly in presence of elevated $CO_2$. Under individual treatment of $CO_2$ and $O_3$, HUW-37 showed more decrease in grain protein content and in combined treatment, K-9107 showed lower reduction than HUW-37. Evans (1993) has reported a negative correlation between grain yield (GY) and grain protein concentration (GPC). This phenomenon is called 'growth dilution' which occurs due to dilution of accumulation of more non-nitrogenous compounds in the grains. The negative effect of elevated $CO_2$ on GPC may be due to growth dilution in combination with a direct, yield-independent, effect on N acquisition (Pleijel and Uddling, 2012). Similar correlation between GY and GPC was reported by Pleijel et al. (1999) in wheat.

In our study, elevated $CO_2$ and elevated $CO_2+O_3$ provoked the accumulation of non-nitrogenous compounds i.e., total soluble sugar (TSS) and starch. Total soluble sugars increased significantly due to $CO_2$ and $CO_2+O_3$ and decreased due to $O_3$ in both the cultivars. Increase in TSS was more in K-9107 which may be due to greater stimulation in photosynthesis compared to HUW-37. Starch content also showed similar response in both the cultivars as TSS. Under elevated $CO_2$, increased concentration of starch may be due to increase in photosynthetic rate (Poorter et al., 1997). The decrease in starch content under EO may be due to the hydrolysis of polysaccharides. Findings of present investigation showed decrease in reducing sugar contents (RS) in EC and increment in ECO and EO in both the cultivars. Reduction in RS was higher in HUW-37 than K-9107 under elevated $CO_2$ exposure, however, increase in RS was more in HUW-37 under EO treatment. In combined treatment of $CO_2$ and $O_3$, RS was higher in Cv K-9107. The possible explanation for this trend may be that under combined exposure, elevated levels of $CO_2$ led to stomatal closure and hence checked the entry of $O_3$ inside plant leaves, thus investing more in sugar production by counteracting the oxidative stress induced by $O_3$.

## 5. Conclusions

Growth, biomass accumulation and allocation, yield and grain quality parameters have shown differential response in both the wheat cultivars under elevated $CO_2$, elevated $O_3$ and elevated $CO_2+O_3$. Results of two-year experiment have clearly shown that $CO_2$ enrichment can prevent $O_3$ induced yield losses in tropical wheat cultivars and the degree of interaction depends on the concentrations of $CO_2$ and $O_3$ and differential sensitivity of cultivars against $O_3$. It can be concluded that Cv HUW-37 (dwarf variety with higher yield potential) was more $O_3$ sensitive than K-9107 (tall variety with lower yield potential) and level of amelioration due to elevated $CO_2$ was more in K-9107 during reproductive phase experiencing higher concentrations of $O_3$. The results of the present study can be useful to plant breeders in selecting more adapted plants in view of global climate change.

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