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Impact of Elevated Ozone on Growth, Yield and Nutritional Quality of Two Wheat Species in Northern India

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ABSTRACT

Sensitivity to tropospheric ozone is highly variable in cultivars of different plant species. Wheat, an important cereal crop, has been found to be sensitive to elevated ozone levels leading to differences in grain yields. The objective of this study was to compare the effects of elevated tropospheric ozone on growth, yield and nutritional quality of two species of wheat, *Triticum aestivum* (PBW 343) and *Triticum durum* (HD 2936), which are tropical wheat cultivars commonly grown in northern India. Experiments were conducted growing winter wheat (*rabi* season) under elevated tropospheric ozone in northern India for two years in open-top chambers (OTCs) under charcoal-filtered air (CF), non-filtered air (NF), open air (OA) and elevated ozone (EO) concentration (NF + 25–35 ppb O_3). There were different species responses to EO, with the modern *aestivum* wheat cultivar being more sensitive than *durum* wheat. The declines in all growth and yield parameters were greater in *T. aestivum* than *T. durum* in both the years. On average there was a 7% greater reduction in the photosynthetic rate and stomatal conductance in *T. aestivum* as compared to *T. durum* under EO at the flowering stage, and a 6% more reduction in leaf chlorophyll was observed on *T. aestivum* as compared to *T. durum*. Exposure to elevated O_3 caused a decrease in the number of leaves and leaf area index, rubisco enzyme activity and chlorophyll in both the species. More reductions in grain yield were observed in *T. aestivum* (15 and 19%) as compared to *T. durum* (9 and 13%) under EO in the two years, respectively. Filtration of air significantly increased all growth and yield parameters in both species of wheat.

***Keywords*:** Tropospheric ozone; *Triticum aestivum L.*; *Triticum durum L.*; Grain yield.

INTRODUCTION

O_3 is one of the most damaging tropospheric air pollutants affecting plant growth and productivity (Leisner and Ainsworth, 2012). Concentrations of tropospheric O_3 have increased considerably since the Industrial Revolution, especially during the past 60 years, and are likely to continue to increase by 10–30 parts per billion (ppb) by 2100 if current high emission rates continue in the Northern Hemisphere (The Royal Society, 2008). The IPCC Fourth Assessment Report projects an increase in tropospheric O_3 across the globe of 20–25% by 2050 (Jaggard *et al.*, 2010). Due to its strong oxidative property, ozone damages crops by reducing photosynthesis and other important physiological functions, resulting in weaker plants, inferior crop quality, and decreased yields (Fuhrer, 2009; Singh *et al.*, 2013). Ozone enters into the underside of the leaf of plants through their stomata and most likely reacts with molecules in the cell wall that end up triggering production of ROS molecules, which damage the cells, inhibiting photosynthesis, accelerating leaf senescence, reducing plant growth and impairing yield attributes (Bhatia *et al.*, 2012).

Impacts of tropospheric O_3 on crop plants are reported from many developing countries, which are rapidly industrializing like China (Wang *et al.*, 2007, 2008), India (Bhatia *et al.*, 2011) and Pakistan (Wahid, 2006; Rai *et al.*, 2007; Wahid *et al.*, 2012). O_3 impairs plant metabolism leading to yield reduction in agricultural crops and its influence is dependent on dose, genetic background and the developmental phase of plants and varies between species and cultivars (Fiscus, 2005; Rai *et al.*, 2010). Cereals are highly sensitive and have shown decreased yields with increasing O_3 levels (Singh *et al.*, 2010).Wheat, one of the crucially important crops in the global food supply, has been found to be particularly sensitive to O_3, yields decreasing with increasing O_3 (Mills *et al.*, 2007; Feng *et al.*, 2008, 2010). Wheat (*Triticum sps.*) is the second most important food crop in India after rice both in terms of area and production, contributing substantially to the national food security (Mughal *et al.*, 2011). Globally, India is the second largest

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wheat producing country, averaging an annual production of 85.93 million tonnes (MOA, 2013) and accounts for nearly 12 per cent of world wheat production. There have been some reports that ground level ozone is having tremendous impact on yield of cereals especially wheat due to tropospheric ozone (Ambasht and Agrawal, 2003; Wilkinson *et al.*, 2012). Wheat has been identified as especially sensitive to O_3 due to likely co-occurrence of peak levels of O_3 during growing season of wheat (Avnery *et al.*, 2011). Wheat is grown during the months of November to April in Northern and central India. 95% of the cultivated wheat is of the *aestivum* (hexaploid) type used for the preparation of bread and other baked products and the remaining 5% is *durum* (tetraploid) wheat, which is used essentially for making pasta and macaroni (Patnaik and Khurana, 2003). With urbanization the demand for durum wheat is on the rise due to higher fast food consumption in India. Picchi *et al.* (2010) studied the potential risk of O_3 damage for modern wheat cultivars; however, there are not many studies on the impact of elevated ozone levels on growth and productivity of *T. durum* species of wheat especially in the tropical region. Thus the present study was carried out to assess the impact of elevated ozone on growth, yield and nutritional quality of two wheat species grown in Northern India.

METHODS

Experimental Site and Design

A field experiment was conducted growing wheat cultivars (*T. aestivum* and *T. durum* variety PBW 343 and HD 2936 during *rabi* season (winter) from December to April in 2008–2009 and 2009–2010 at the experimental farm of the Indian Agricultural Research Institute, New Delhi, India. The site is located in the Indo-Gangetic alluvial tract at 28°40′N and 77°12′E, at an altitude of 228 m above mean sea level. The climate of the region is subtropical, semi-arid and January is the coldest month of the year with a minimum temperature ranging from 5 to 7°C. The mean maximum and minimum temperatures from December to April are 36.6 and 22.6°C, respectively. Average rainfall of this area is 72 cm annually, approximately 80% of which occurs during the kharif (monsoon) season. The alluvial soil of experimental site was sandy loam texture (46% sand, 33% silt and 21% clay) (Typic Ustochrept) with bulk density of 1.42 g/cm^3, pH (1:2 soil:water) of 8.61, electrical conductivity of 0.158 dS/m, cation exchange capacity of 7.3 C mol (p+)/kg; and organic carbon, total N, Olsen P, and ammonium acetate extractable K contents of 0.32%, 156.8 kg/ha, 13.83 kg/ha, and 171.56 kg/ha, respectively. The experimental soil was under rice-wheat rotation for the past two years.

Treatments and Crop Management

The experiment was carried out growing wheat in open top chambers (OTCs) of 3 meter diameter and 2.5 meter height consisting of a circular aluminum frame covered with transparent film. The experiment was carried out with four treatments arranged in randomized block design with three replications. The treatments were: charcoal filtered air (CF), elevated ozone (EO), non-filtered control (NF) and open air (OA) plots (Table 1). The OTCs were fitted with an inert PVC pipe of 10 cm diameter (adjustable height) with many small holes which released either charcoal filtered air (CF), non-filtered air (NF) or elevated ozone along with non-filtered air (EO) at the crop canopy level. Air was blown into the OTCs through a fan that provided uniform air speeds. The ventilation rates were kept at 3 air changes per minute to keep the leaf boundary layer resistances down and the chamber temperature close to ambient. Charcoal filters were used to adsorb ozone from ambient air reduced by around 80–85% blown inside the CF treatment. In NF treatment only air was blown in the open top chambers. The NF treatment was the open top chamber control. In the EO treatment 25–35 ppb of additional ozone was maintained over the non-filtered air levels. O_3 was applied for 7 h/d from 09.30–16.30 h in the elevated O_3 chambers. Additional O_3 was generated from oxygen with the help of reaction with UV radiation < 200 nm using ozone generators (Systocom, Varanasi, India). Air was sampled from the middle of each OTC at the crop canopy level and fed to an O_3 analyzer (Model APOA-370, Horiba, Germany) for measuring the ozone concentrations daily using a cross flow modulated ultraviolet absorption method. The light intensity inside and outside the OTCs was measured using a portable light meter (Metravi 1332), temperature with a constantan-copper thermocouple and relative humidity was measured using a digital humidity sensor at 10.00 and 16.00 h daily. Cumulative ozone exposure above 40 ppb during daylight hours at different growth stages was characterized by the AOT40 index (Fuhrer *et al.*, 1997) and is listed in Table 1.

Experimental Practices

Two most popular wheat cultivars of northern India *T. aestivum* (variety PBW 343) and *T. durum* (variety, HD 2936) were grown in open top chambers for the study. The seeds were sown at a recommended spacing of 20 × 15 (row × row). Nitrogen (120 kg/N/ha) was applied in 3 splits, 50% as basal and 25% each at top dressing while all P (60 kg/P/ha) and K (40 kg/K/ha) and zinc sulphate (20 kg/ha) was applied basally. A week after sowing of crops, gap filling was done to maintain uniform plant population. Irrigation was given at crown root initiation (CRI), tillering, flowering, milking and dough stages respectively. Weed management was carried out as per standard procedures. At every harvesting, three plants were taken for growth and biochemical measurements. At the final harvest, the yield of one meter square area was recorded.

Three destructive plant harvestings were performed during the crop growth period at 34, 69 and 105 DAS (Days after Sowing) in Ist year and 35, 71 and 108 DAS in IInd year respectively at tillering, flowering and maturing stages for measurement of plant height, root dry weight, shoot dry weight and number of leaves and tillers per plant.

Growth and Physiology

Single-leaf net photosynthetic rates (P_N), and stomatal conductance (g_s), were measured with portable photosynthesis systems (LI-6400-40 Portable Photosynthesis System). Leaf area index (LAI) was calculated using leaf area meter (Model

Table 1. Treatments and mean ozone concentrations in open top chambers (OTCs) during 2008–09 and 2009–10 wheat season.

Treatment	Ozone concentration (ppb)	Seasonal mean O_3 (ppb)		AOT 40 (ppmV h)	
		I[st] Year	II[nd] Year	I[st] Year	II[nd] Year
Open air (OA)	Ambient O_3	31.8 ± 1.9	36.1 ± 2.1	2.56 ± 0.3	3.16 ± 0.2
Non-filtered air (NF)	5–10% less than ambient	30.9 ± 1.4	34.9 ± 1.9	2.05 ± 0.1	2.39 ± 0.3
Charcoal filtered air (CF)	85–90% less than ambient	5.25 ± 0.8	7.69 ± 1.3	0	0
Elevated ozone (EO)	Ambient + 25–35 ppbO_3	59.2 ± 2.5	65.35 ± 3.7	16.62 ± 2.2	17.96 ± 3.4

LI-3100, LI-COR, Inc., USA). Total chlorophyll content of the flag leaf was determined as per method described by Barnes *et al.* (1992). Total chlorophyll, photosynthetic rates and stomatal conductance were measured at tillering and flowering and Leaf Area Index (LAI) was measured at tillering, flowering and maturing stages in both the years. The rubisco enzyme activity was also measured at tillering stage (43 DAS) in both the years as per method described by Kobza and Seemann (1988).

Final Harvest

Final harvesting of the crop was carried out at123 DAS and 135 DAS in the I[st] year and II[nd] year respectively, when the crop had a golden yellow colour, by measuring various yield parameters of the straw and ears, viz., average number of tillers, average number of spikes, average number of grains/spike, grain yield, total biomass and 1000 grain weight. The grains were separated from the straw and straw weights for wheat in both cultivars viz. *T. aestivum* and *T. durum* and dry weight was got by oven drying at 65°C reaching a constant weight.

Grain Quality Parameters

Total Soluble sugar, starch and protein content were analyzed in the wheat grain. The total soluble sugar of grain was analyzed as per the procedure described by Mc. Cready *et al.* (1950). The starch content of grain was analyzed by using Bradford Dye Binding method as per the procedure described by Bradford, (1976). Protein content was calculated from percentages of total nitrogen by multiplication by conversion factor of 5.7 for wheat grains by macro Kjeldahl method (Jackson, 1973).

Statistical Analysis

Data of growth, yield and quality parameters were subjected to two ways ANOVA to examine the individual and combined effects of species, treatments and their interaction. Duncan's multiple range tests were performed for various measurements after subjecting to one way ANOVA test. All the statistical tests were performed using Statistical package for agricultural research (SPAR, version 2.0).

RESULTS AND DISCUSSION

Variations were observed in O_3 concentration during the crop growth period. Mean hourly ozone concentrations in the CF chambers did not exceed 10 ppb as the charcoal filters removed 85–90% of ambient ozone from the OTCs. The average daytime O_3 concentration in non-filtered air during the growing season was 30.9 ± 1.4 and 34.9 ± 1.9 ppb in I[st] year and II[nd] year respectively. The mean 7-h ozone concentration in the EO treatment was 59.2 ± 2.5 and 65.35 ± 3.7 ppb in the two years, respectively (Table 1). Levels of sun light on the crop canopy were, on an average, reduced by 12% inside the OTC, whereas mean air temperature inside the chamber was 1.06 ± 0.2°C higher than that on outside the chambers (Table 2). Relative humidity was higher inside the chambers and average mean difference of relative humidity was 4.6 and 2.9% in I[st] year and II[nd] year, respectively (Table 2). Higher concentrations of ozone were observed during the second year as compared to the I[st] year. During the experiment variations were observed in O_3 concentration during the crop growth period. Higher daily ambient O_3 concentration (7-hr mean) was observed during the flowering and post-flowering period i.e., February to March (40–67 ppb) as compared to the vegetative phase i.e., December and January (18–35 ppb). The average O_3 concentrations during grain filling period were higher during the second year (49 ppb) as compared to first year (42 ppb) of the study. Highest daily O_3 concentration during the crop growth period was observed during the month of April in both the years of the study (65–73 ppb) (Fig. 1). The AOT 40 under EO was higher in the second year of the study (Table 1). The AOT 40 under EO at the different growth stages of sowing to tillering, tillering to flowering and flowering to maturity varied from 4.35, 4.52 and 7.76 ppmv h in 2009 to 4.28, 5.04 and 8.65 ppmv h in 2010 respectively.

Effect on Growth

Stomatal Conductance (g_s) and Photosynthetic Rate (P_N)

Previous research has shown that higher O_3 sensitivity of modern day cultivars of wheat is due to greater stomatal conductance (g_s) leading to higher O_3 flux (Pleijel *et al.*, 2006). In our study, at tillering the difference observed in g_s among the species were not significant, while at flowering stage a clear difference was observed in g_s between the species (Fig. 2(a)). At flowering, reduction in g_s was more in *T. durum* than *T. aestivum*. An average decrease of 12% was observed in *T. durum* whereas it was 19% in *T. aestivum* under EO. This decrease in g_s was probably due to stomatal closure which is caused by an increase in internal CO_2 concentration resulting from O_3-induced inhibition of carbon assimilation by chloroplasts (Heath and Taylor, 1997).

Partial closure of stomata in response to high O_3 concentration was responsible for decreased photosynthetic rate (P_N) of the plants. A significant lowering in P_N was obtained in the two wheat species under EO at the tillering stage (results not shown). As the plant growth progressed

Table 2. Mean monthly microclimatic conditions inside and outside the open top chambers (OTCs) during 2008–2009 and 2009–2010 wheat season.

Months	Temperature °C			Light Intensity (Klx)			Relative humidity (%)		
	Outside	Inside	Difference	Outside	Inside	Difference	Outside	Inside	Difference
Dec-09	23.3	24.2	+0.9	57.9	53.8	–4.1	61.9	66.8	+4.9
Jan-09	17.6	18.7	+1.1	49.8	48.3	–1.5	58.3	63.6	+5.3
Feb-09	22.9	23.8	+0.9	52.1	49.2	–2.9	63.8	68.6	+4.8
Mar-09	34.8	35.8	+1.0	61.3	56.7	–4.6	66.3	71	+4.7
Apr-09	34.4	35.3	+0.9	59.8	56.6	–3.2	68.3	71.5	+3.2
Dec-10	23.2	24.3	+1.1	55.6	52.1	–3.5	63.2	66.3	+3.1
Jan-10	17.3	18.5	+1.2	52.1	50.3	–1.8	54.6	57.4	+2.8
Feb-10	22.6	23.7	+1.1	54.7	52.6	–2.1	67.8	70.5	+2.7
Mar-10	34.5	35.7	+1.2	61.9	58.7	–3.2	67.9	71.5	+3.6
Apr-10	35.2	35.8	+1.2	60.7	57.6	–3.1	68.3	70.7	+2.4

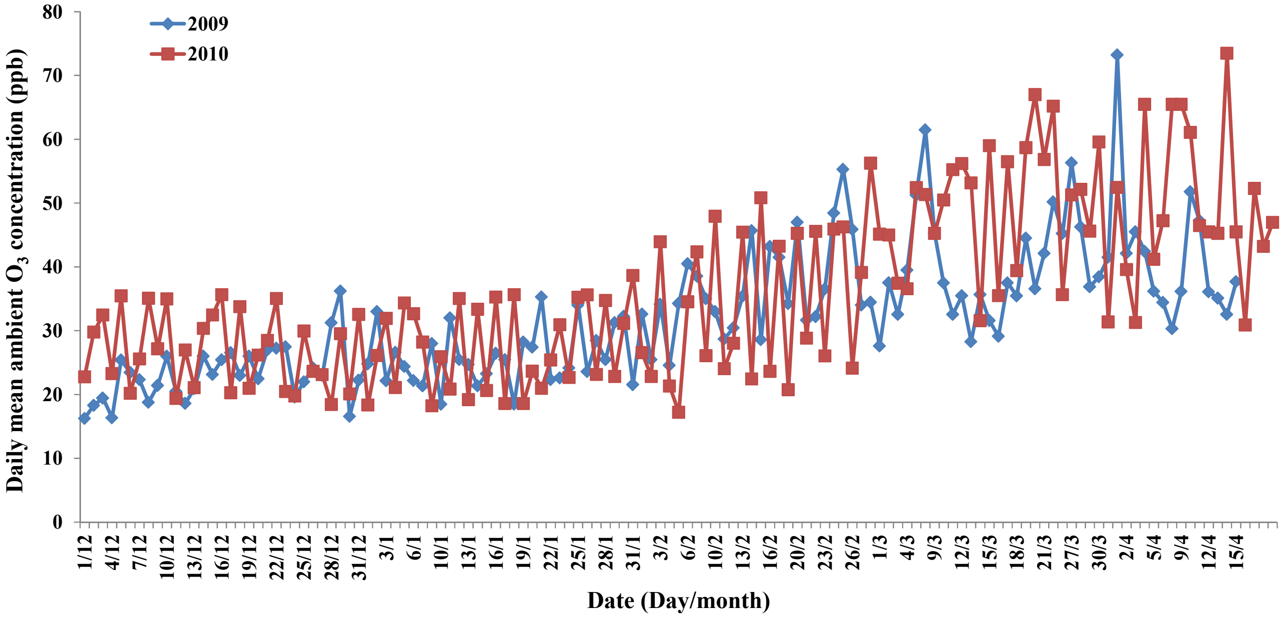

Fig. 1. Mean daily ambient ozone concentrations during crop growth period in 2009 and 2010.

more reduction in P_N was observed at flowering stage. The P_N was less in *T. aestivum* (15.70 and 17.02 µmol/m^2/s) as compared to *T. durum* (26.29 and 20.95 µmol/m^2/s) under EO in both the years (Fig. 2(b)). On an average there was 7% more reduction in P_N in *T. aestivum* as compared to *T. durum* under EO. Cao *et al.* (2009) also observed a significant decrease of photosynthetic rate in wheat cultivars grown under O_3 treatment. After entering through stomata, O_3 is converted to reactive oxygen species (ROS) causing membrane damage, resulting in injuries and destroying photosynthetic pigments (Noormets *et al.*, 2010; Rai *et al.*, 2010) and ultimately reduction in photosynthetic rate. In our study the P_N of *T. durum* was significantly higher than *T. aestivum* in CF at all growth stages.

Plant Height and above Ground Biomass

Wheat is an O_3 sensitive crop (Mishra *et al.*, 2013) and in our study we observed a differential response in the two species under different levels of ozone. At tillering stage there were significant differences observed in plant height between the two species of wheat, however no significant differences were observed among the different treatments at this stage. At flowering stage under EO, more decrease was observed in *T. aestivum* (16–18%) as compared to *T. durum* (11–13%) in the two years of the study (Table 3(a)). The plant growth was visibly very healthy in CF treatment in both the wheat species. There significant differences in plant height at flowering stage under the two species of wheat (Table 3(b)). Rai *et al.* (2007) observed reduced shoot length in wheat under non filtered air as compared to filtered air which had higher ozone levels. Feng *et al.*, (2008) while carrying out a meta-analysis of wheat responses to elevated ozone observed similar reduction (5%) in stem height of wheat under elevated ozone levels. No significant reductions in plant dry weight were observed between the two species however significant differences were observed under the O_3 treatments. In *T. aestivum*, average plant dry weight reduced by 19% while it reduced by 13% in T. *durum* under EO (Table 3(a)). At flowering, the plant dry weight was significantly reduced in the EO treatment

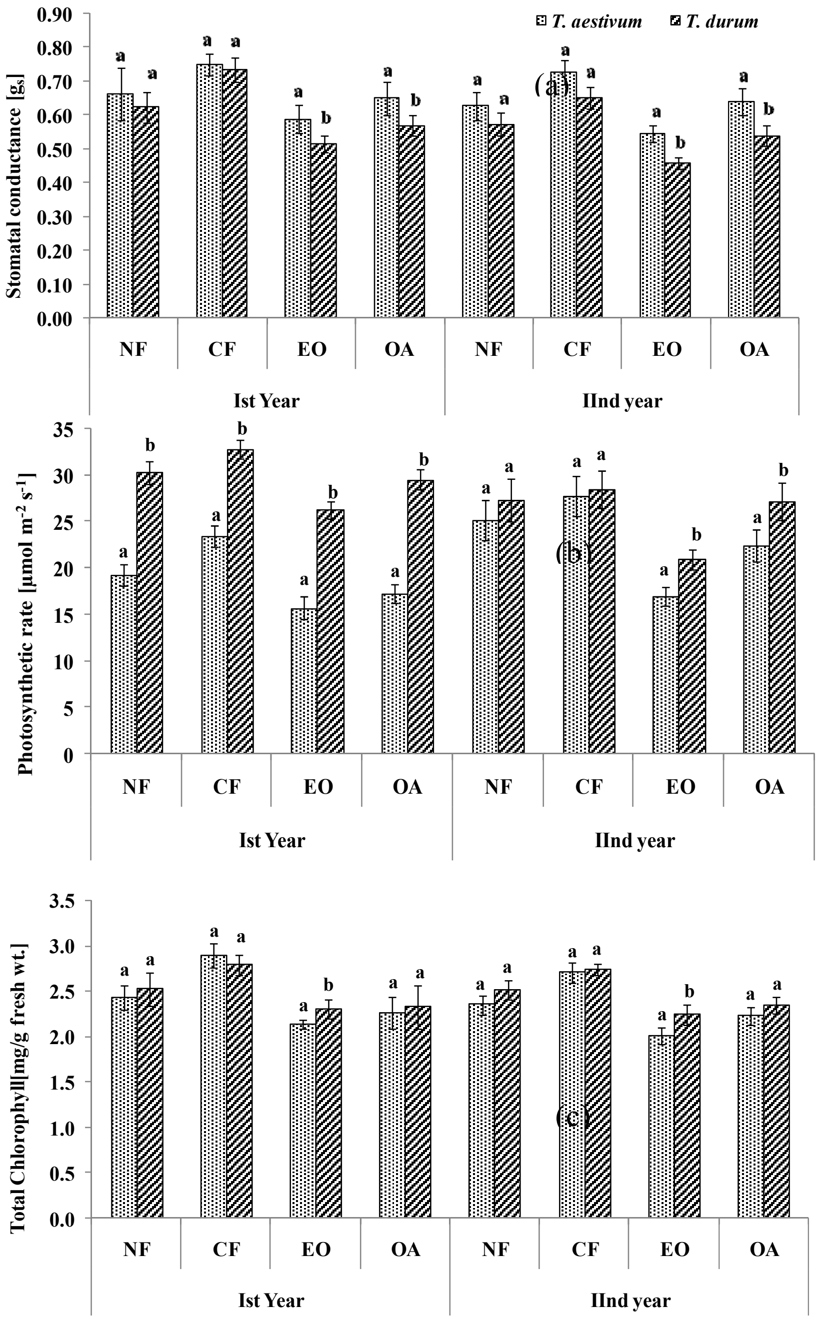

Fig. 2. Impact of elevated ozone on (a) stomatal conductance, (b) photosynthetic rate and (c) total chlorophyll in wheat varieties at flowering.

possibly due in part to reductions in plant height which were significant. The presence of elevated levels of O_3 impacted the plant growth by reducing the g_s which inhibited the photosynthesis, leading to a reduction in dry matter accumulation. As photosynthesis and C-fixation decreased more in *T. aestivum*, this led to lower biomass production in *T. aestivum* as compared to *T. durum*. Accelerating leaf senescence, reducing plant growth and impaired yield attributes were also obtained by Sarkar *et al.* (2010) and Wahid (2006) under higher ozone levels in wheat. However, no significant differences in plant dry weight under CF at flowering was observed among the species.

Leaf Area Index

Leaf area index (LAI) is expressed as total leaf area of the crop per unit ground area occupied by the crop. Significant differences were observed in LAI under the two species in the different O_3 treatments at flowering (Table 3(b)). The decrease in LAI was more in *T. aestivum* species than in *T. durum*. During Ist year, the LAI decreased from 3.50 in *T. aestivum* and 3.40 in *T. durum* in CF to 2.60 in *T. aestivum* and 3.0 in *T. durum* under EO at flowering stage. In IInd year it decreased by 10% in *T. aestivum* and 6.3% in *T. durum* under EO as compared to NF at flowering. The decrease in LAI was due to a decrease in number of leaves

Table 3(a). Impact of ozone treatments on growth parameters at flowering stage in wheat.

Parameters	Treatments							
	NF	CF	EO	OA	NF	CF	EO	OA
	T. aestivum				*T. durum*			
I[st] year								
Plant height (cm)	61.5 ± 3.0[a]	66.5 ± 5.6[a]	51.5 ± 0.6[b]	58.5 ± 2.8[a]	56.7 ± 1.2[b]	62.6 ± 2.3[a]	49.5 ± 1.0[c]	55.9 ± 3.1[b]
Plant dry weight (g)	15.5 ± 1.3[b]	17.8 ± 0.4[a]	12.6 ± 0.5[c]	13.9 ± 1.2[b]	14.5 ± 1.5[b]	16.5 ± 2.9[a]	12.7 ± 0.4[c]	13.6 ± 1.3[b]
Leaf area index	3.3 ± 0.07[b]	3.5 ± .08[a]	2.6 ± .05[c]	3.2 ± .04a[b]	3.2 ± .07[b]	3.4 ± 0.03[a]	3.0 ± 0.2[c]	3.1 ± 0.04[b]
Tillers/hill	12.3 ± 0.3[b]	13.7 ± 0.2[a]	10.3 ± 0.3[c]	12.0 ± 0.3[b]	12.0 ± 0.5[b]	14.3 ± 1.3[a]	11.7 ± 0.1[c]	12.0 ± 0.2[b]
Leaves/plant	29 ± 1.06[b]	33 ± 1.61[a]	22.7 ± 0.2[c]	27 ± 1.42[b]	26 ± 0.73[b]	30.3 ± 2.6[a]	22 ± 0.15[c]	25 ± 0.97[b]
II[nd] year								
Plant height (cm)	57.9 ± 1.2[a]	61.6 ± 3.5[a]	47.6 ± 0.1[c]	53.3 ± 1.9[b]	54.7 ± 0.6[b]	57.4 ± 2.1[a]	47.1 ± 0.6[c]	52.9 ± 1.4[b]
Plant dry weight (g)	14.0 ± 1.0[a]	16.4 ± 2.1[a]	11.1 ± 0.2[b]	13.8 ± 1.1[a]	13.8 ± 0.6[b]	15.9 ± 1.2[a]	11.9 ± 0.2[c]	13.5 ± 0.2[b]
Leaf area index	3.1 ± 0.02[b]	3.3 ± 0.04[a]	2.8 ± 0.02[c]	3.1 ± 0.04[b]	3.2 ± 0.03[b]	3.3 ± 0.05[a]	3.0 ± .02[c]	3.1 ± 0.08[b]
Tillers/hill	11.7 ± 0.4[b]	12.7 ± 0.2[a]	10.1 ± 0.1[c]	11.4 ± 0.2[b]	12.3 ± 0.1[b]	13.7 ± 0.3[a]	10.5 ± 0.1[c]	12.0 ± 0.2[b]
Leaves/plant	28 ± 1.2[b]	31 ± 1.1[a]	23 ± 0.3[c]	28 ± 1.3[b]	26 ± 1.2[b]	30 ± 0.2[a]	23 ± 0.9[c]	25 ± 1.3[b]

* Different letters within a row indicate significant differences among treatments at $p < 0.05$ by Duncan's test.

per plant under EO (Table 3(a)). The reduction in numbers of leaves was more in *T. aestivum* as compared to *T. durum* at flowering and maturity in EO. In our study there was significant reduction in number of leaves per plant between the two species and the treatments, however, no significant differences was observed in the species × treatment interaction (Table 3(b)). Under EO while senescence is accelerated, formation of new leaves may be slowed by decreased supply of assimilates and translocation (Ewert and Pleijel, 1999; Grantz and Farrar, 1999; Morgan *et al.*, 2003). Leaf senescence in our study was characterised by a general yellowing, which was more pronounced at the leaf tip in the beginning and then it spread downwards along the leaf. In EO treatment, the leaves colour changed from green to yellow. Symptoms of ozone-induced premature senescence of basal leaves became evident during the latter part of February in the EO treatment. Senescence rate was higher in *T. aestivum* as compared to *T. durum*. Plants under EO treatment were accompanied by black or brown darkening of the affected area after 65 DAS and it was more visible in *T. aestivum* than *T. durum* (Fig. 3). Visibly the numbers of dark spots were more in *T. aestivum* as compared to *T. durum* species of wheat. Feng *et al.* (2010) also observed accelerated senescence of flag leaves in wheat under higher O_3 concentrations. Kharel and Amgain, (2010) observed that elevated O_3 reduced the number of leaves per plant, leaf area and LAI in crop plants. Dermody *et al.* (2006) reported that lowering in LAI was primarily due to accelerated senescence under elevated ozone in soybean.

Total Chlorophyll and Rubisco Enzyme Activity

Total chlorophyll content of leave was also more affected in *T. aestivum* as compared to *T. durum* in both the years. At tillering stage decrease in chlorophyll was not significant between the two species. At flowering total chlorophyll significantly decreased under EO in both the species (Fig. 2(c)). In *T. aestivum*, the decrease was 6% more than in *T. durum*. Under CF also the two species showed different chlorophyll levels. A 17% increase in chlorophyll was observed in *T. aestivum* and 10% in *T. durum* in CF over NF in both the years.

Ozone induced ROS can damage chloroplasts by changing membrane permeability, thereby causing chlorophyll destruction. Thus, in our study the reduction in chlorophyll was due to higher ozone induced ROS in EO. More reduction in chlorophyll in *T. aestivum* as compared to *T. durum* under exposure to O_3 was probably due to higher O_3 uptake (less decrease in g_s in *aestivum* as compared to *durum*) and a greater loss of photosynthetic activity (more reduction in photosynthetic rate in *aestivum* as compared to *durum*). Zhu *et al.* (2011) growing winter wheat under elevated O_3 in FACE-ozone system observed corpus adiposum inside chloroplast broken down, resulting in a significant decrease of the chlorophyll content. Pleijel *et al.* (2006) reported significant reduction of chlorophyll in the flag leaf in different cultivars of wheat after exposure to O_3 above 50 ppb for 1 month, which shortened the grain filling period and resulted in lower yields under elevated O_3. It has been reported that the decline in photosynthetic capacity induced

Table 3(b). F-ratio and level of significance for growth parameters at flowering stage in wheat.

Parameters	Species	Treatment	Interaction (Species × Treatment)
		Ist year	
Plant height (cm)	34.45***	103.23***	1.24^{ns}
Plant dry weight (g)	1.43^{ns}	13.09***	0.37^{ns}
Leaf area index	1.29^{ns}	153***	32.3***
Tillers/hill	4.83*	7.20**	3.36*
Leaves/plant	10.99**	34.02***	0.73^{ns}
		IInd year	
Plant height (cm)	7.68*	48.25***	1.63^{ns}
Plant dry weight (g)	0.03^{ns}	25.94**	0.67^{ns}
Leaf area index	0.09^{ns}	104.51***	13.44**
Tillers/hill	5.01*	3.62*	4.08*
Leaves/plant	7.89*	37.16***	1.56^{ns}

Level of significance: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ns: not significant.

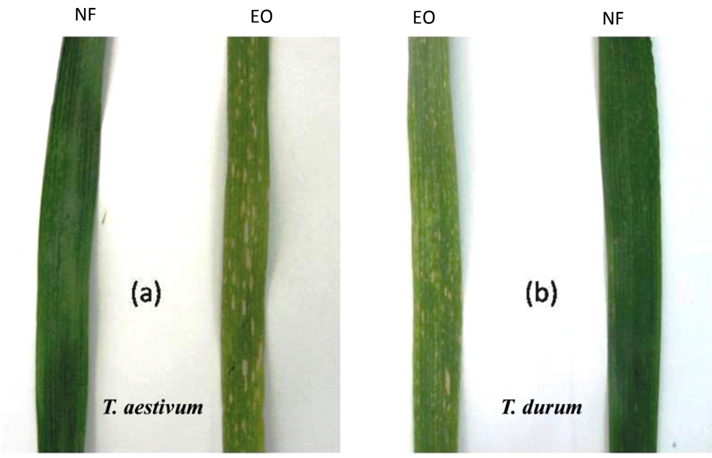

Fig. 3. Injury symptoms on plant leaf under elevated ozone in (a) *T. aestivum* (b) *T. durum.*

by O_3 is caused primarily by a decrease in the maximum *in vivo* rate of Rubisco carboxylation due to a reduction in the activity and/or quantity of Rubisco (Long and Naidu, 2002; Biswas and Jiang, 2011). The decrease in ribulose bisphosphate carboxylase/oxygenase (Rubisco) activity was significantly different between the two species of wheat (Fig. 4) and might have been a reason for differential response in our study between the two species of wheat. It reduced by 19% in *T. aestivum* and by 13% in *T. durum* under EO as compared to NF. Rubisco is the key protein involved in net CO_2 fixation during photosynthesis (Miziorka and Lorimer, 1983).

Effect on Yield Parameters

The results of final harvest of crop showed reduction in yield parameters for both the varieties under EO, though changes were more significant in case of *T. aestivum* species. In contrast, the filtration of air (CF) significantly increased the major yield components, in both the species. There was significant decline in average no. of spikelet/spike, average no. of grains/spike and 1000 grain weight (Table 4) under different treatments (Table 4(a)) however, only significant difference in average no. of grains/spike was observed among the species and in species × treatment interaction (Table 4(b)). As EO affected more the *T. aestivum* species (growth and physiological parameters described earlier) so that plants of *T. aestivum* were more affected under EO as compared to the *T. durum* species. In *T. aestivum*, the average number of spikelet/spike reduced by 8% and in *T. durum*, it decreased by 5% under EO (Table 4(a)). The effect on average grains/spike was largely due to an increase in percentage sterility in EO treatment. Percentage sterility increased by 8% *T. aestivum* and by 5% in *T. durum* under EO. Accelerated senescence observed under EO (result discussed earlier) shortened the grain filling duration and there by reduced average no. of grains/spike and ultimately to lower grain yield under elevated O_3 (Gelang *et al.*, 2000).

From our results it was observed that *T. aestivum* was more affected by EO as compared to *T. durum* and this ultimately resulted in a greater decline in grain yield of wheat in *T. aestivum.* Significant differences were obtained between *T. aestivum* and *T. durum* in grain yield under EO in both the years (Table 4(b)). The grain yield decreased by 17% in *T. aestivum* and by 11% in *T. durum*. Grain yield ranged from 345 g/m^2 to 538 g/m^2 in the different treatments (Table 4(a)). Under low O_3 levels of CF treatment the grain yield increased by 19% *T. aestivum* and by 16% in *T. durum* over NF. Significant impact of EO was also observed

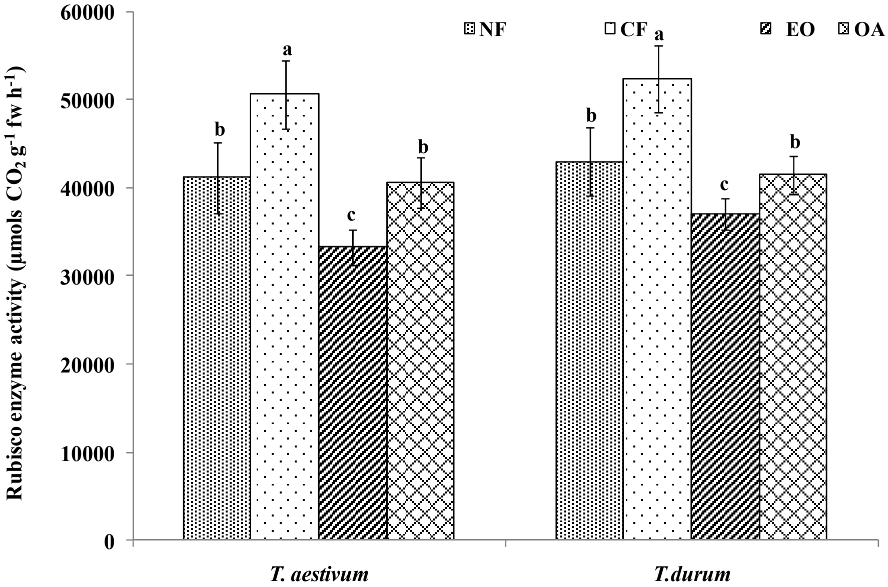

Fig. 4. Impact of different treatments on rubisco enzyme activity at tillering.

on 1000 grain weight, however no significant differences were obtained among the two wheat species (Table 4(b)). Feng *et al.* (2009) in a meta analysis of impacts of elevated O_3 on wheat obtained results indicating decreased grain yield due to decreased 1000 grain weight, grains/spike, spikes/plant, relative to ambient air. This was due to higher translocation of carbohydrates to sink, leading to higher grain filling and grain weight (Wahid *et al.*, 2006) in filtered air. Picchi *et al.* (2010) studied the potential risk of O_3 damage for modern wheat cultivars and found *durum* wheat to be more sensitive showing more severe symptoms than common *aestivum* wheat. However, in our study we observed that *T. aestivum* was more sensitive than *T. durum* with respect many yield parameters under EO.

Straw yield was significantly affected among the two wheat species which was largely due to reduced level of tillering under EO in *T. aestivum*. It decreased by 10.5% in *T. aestivum* and, and by 4.5% in *T. durum* under EO (Table 3(a)). Significant reduction was also observed in the average number of productive tillers among the two species (Table 4(a)). Harvest index ranged from 0.40 to 0.42 in Ist year and 0.38 to 0.43 in IInd year among the different treatments in both the years, however no significant species × treatment interaction was observed in HI (Table 4(b)). Decline in HI under EO showed that there was less biomass portioning towards grain as compared to non-filtered air. All the yield related parameters showed more decline in the IInd year of the study which might be due to higher ambient O_3 levels between flowering to maturity stage (Fig. 1).

Effect of Ozone Treatments on Grain Quality

In addition to reductions in grain yield, there was also an impact of O_3 levels on nutritional quality of the two wheat species (Fig. 5). The sugar content decreased by 10 and 15% in *T. aestivum* and by 8 and 9% in *T. durum* in the two years of the study and the differences were significant (Fig. 5(a)). The decline in starch content however, was significant among the species only in the first year of the study. Ozone reduces photosynthesis, leading to lower translocation of carbon to the grain resulting in the reduced sugar and starch in the grain (Bhatia *et al.*, 2012). Mishra *et al.* (2013) observed similar decrease in soluble sugar and protein content in grain in tall and dwarf wheat cultivars under EO. In our study there was a decline in grain protein in both the species however no significant difference was observed. The decrease in grain protein content was due to growth dilution effect which occurs due to dilution of accumulation of more non-nitrogenous compounds in the grain. Evans (1993) and Mishra *et al.* (2013) observed a negative correlation between grain yield (GY) and grain protein concentration (GPC) due to growth dilution effect. The decrease observed in carbohydrate (sugar and starch) were more in *T. aestivum* than *T. durum* showing that the more primitive wheat (*T. durum*) showed less decline than the modern species (*T. aestivum*) under EO. No significant differences were observed in nutritional quality among the two species in the CF (Fig. 5(b)).

CONCLUSIONS

Comparison of responses of wheat species (*T. aestivum* and *T. durum*) with plants grown in different environments with filtered, non-filtered air, and elevated O_3 showed that the current ambient levels of O_3 may be significantly affecting the growth, yield and nutritional quality of wheat and it may vary from species to species. Elevated ozone caused more decline in physiological and growth parameters, of *T. aestivum* as compared to *T. durum* which ultimately resulted in significant reduction in yield and nutritional quality, however more studies are required to be carried out evaluating different wheat cultivars of the two wheat species. Effect of tropospheric O_3 on both growth and yield of an important cereal crop like wheat needs attention to maintain the productivity of agricultural systems in future climate change scenarios.

Table 4(a). Impact of ozone treatments on yield parameters at final harvest.

Parameters	Treatments							
	NF	CF	EO	OA	NF	CF	EO	OA
	T. aestivum				*T. durum*			
Ist year								
Av. No. productive tillers	10.8 ± 0.1^{b}	12.0 ± 0.2^{a}	9.5 ± 0.04^{c}	10.5 ± 0.2^{b}	11.0 ± 0.1^{b}	12.2 ± 0.3^{a}	10.0 ± 0.1^{c}	11.0 ± 0.1^{b}
Spikelet/spike	20.6 ± 0.4^{b}	21.4 ± 0.9^{a}	18.6 ± 0.6^{c}	19.5 ± 0.4^{b}	20.6 ± 0.3^{b}	21.1 ± 0.8^{a}	19.4 ± 0.0^{c}	19.5 ± 0.5^{b}
Av. No. grains/spike	52.3 ± 0.5^{b}	56.3 ± 0.5^{a}	48.5 ± 0.1^{c}	50.1 ± 0.2^{b}	50.6 ± 0.4^{b}	54.0 ± 0.1^{a}	49.6 ± 0.2^{c}	49.3 ± 0.3^{b}
1000 grain wt. (g)	42.8 ± 0.5^{b}	45.3 ± 1.1^{a}	37.6 ± 0.1^{c}	40.3 ± 0.3^{b}	42.3 ± 0.8^{b}	44.5 ± 1.3^{a}	38.3 ± 0.0^{c}	38.6 ± 0.9^{b}
Straw yield (g/m^2)	634 ± 5.1^{b}	740 ± 9.3^{a}	558 ± 2.3^{c}	618 ± 4.2^{b}	632 ± 5.0^{b}	762 ± 9.1^{a}	610 ± 3.1^{c}	627 ± 5.0^{b}
Harvest Index	0.41 ± 0.01^{b}	0.41 ± 0.0^{a}	0.40 ± 0.01^{bc}	0.40 ± 0.01^{b}	0.42 ± 0.01^{b}	0.41 ± 0.01^{a}	0.41 ± 0.01^{c}	0.41 ± 0.01^{b}
Grain yield (g/m^2)	439 ± 5.3^{b}	527 ± 7.8^{a}	369 ± 8.5^{c}	421 ± 7.5^{b}	459 ± 5.6^{b}	538 ± 8.1^{a}	409 ± 8.2^{c}	441 ± 8.6^{b}
IInd year								
Av. No. productive tillers	10.9 ± 0.1^{b}	11.8 ± 0.2^{a}	9.8 ± 0.01^{c}	11.0 ± 0.03^{b}	11.1 ± 0.1^{b}	12.1 ± 0.2^{a}	10.3 ± 0.06^{c}	11.0 ± 0.1^{b}
Spikelet/spike	17.6 ± 0.27^{b}	18.2 ± 0.33^{a}	16.5 ± 0.04^{c}	17.1 ± 0.23^{b}	18.2 ± 0.36^{b}	19.2 ± 1.11^{a}	17.4 ± 0.05^{c}	17.1 ± 0.15^{b}
Av. No. grains/spike	44.8 ± 0.88^{b}	48.1 ± 0.45^{a}	40.8 ± 0.17^{c}	44.2 ± 0.37^{b}	46.5 ± 0.35^{b}	48.3 ± 0.21^{a}	43 ± 0.10^{c}	45.4 ± 0.28^{b}
1000 grain wt. (g)	42.1 ± 0.43^{b}	43.5 ± 2.17^{a}	37.1 ± 0.10^{c}	40.9 ± 0.34^{b}	40.4 ± 0.51^{b}	43.3 ± 1.63^{a}	37.7 ± 0.14^{c}	39.8 ± 0.26^{b}
Straw yield (g/m^2)	631 ± 4.2^{b}	658 ± 8.1^{a}	564 ± 2.6^{c}	622 ± 3.4^{b}	636 ± 5.7^{b}	678 ± 9.3^{a}	605 ± 2.2^{c}	621 ± 4.1^{b}
Harvest Index	0.40 ± 0.01^{a}	0.42 ± 0.01^{a}	0.38 ± 0.01^{a}	0.40 ± 0.00^{a}	0.41 ± 0.01^{b}	0.43 ± 0.00^{a}	0.38 ± 0.01^{c}	0.41 ± 0.01^{b}
Grain yield (g/m^2)	423 ± 5.1^{b}	499 ± 8.0^{a}	345 ± 7.5^{a}	404 ± 7.9^{b}	446 ± 8.6^{b}	517 ± 6.3^{a}	396 ± 10.8^{c}	430 ± 8.7^{b}

* Different letters within a row indicate significant differences among treatments at $p < 0.05$ by Duncan's test.

Table 4(b). F-ratio and level of significance for yield parameters at final harvest in wheat.

Parameters	Species	Treatment	Interaction (Species × Treatment)
		I st year	
Av. No. productive tillers	3.6*	3.41*	0.03ns
Spikelet/spike	0.09 ns	4.69*	0.25ns
Av. No. grains/spike	4.51*	7.40**	3.52*
1000 grain wt. (g)	0.96ns	12.63***	0.69ns
Straw yield (g/m^2)	11.44**	142.34***	3.85*
Harvest Index	0.05 ns	0.06ns	0.009ns
Grain yield (g/m^2)	53.92***	386.13***	3.77*
		II nd year	
Av. No. productive tillers	5.19*	4.44*	0.02ns
Spikelet/spike	1.02ns	1.57ns	0.13ns
Av. No. grains/spike	5.72*	6.30**	4.19*
1000 grain wt. (g)	0.60ns	9.31***	0.41ns
Straw yield (g/m^2)	3.60*	16.61***	3.32*
Harvest Index	0.23ns	0.86ns	0.05ns
Grain yield (g/m^2)	85.87***	158.35***	5.05*

Level of significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: not significant.

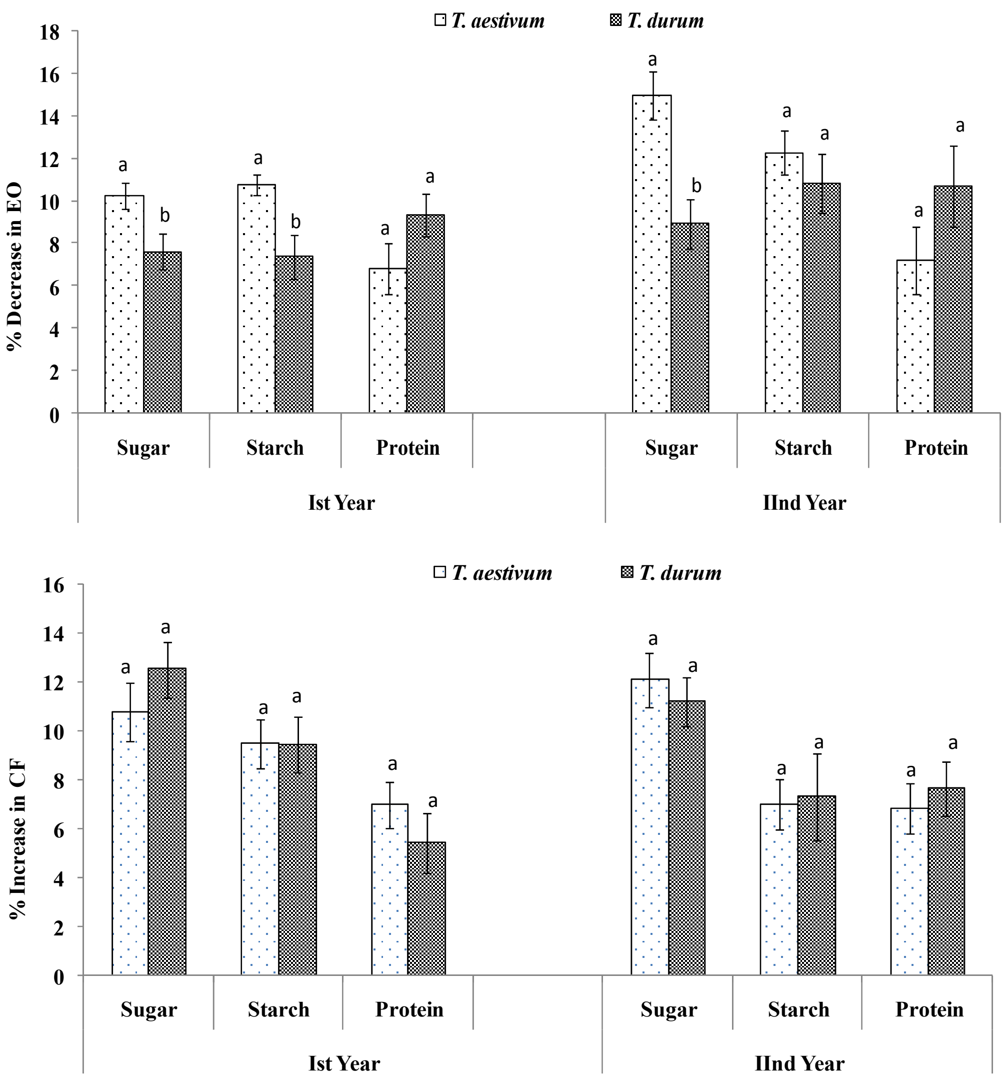

Fig. 5. Impact of different treatments on (a) decrease in grain quality parameters under elevated ozone (b) increase in grain quality parameters in charcoal filtered air.

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