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Elevated ozone and two modern wheat cultivars: An assessment of dose dependent sensitivity with respect to growth, reproductive and yield parameters

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ABSTRACT

Tropospheric ozone (O_3), throughout the globe, has become a potential risk for agriculture. The present investigation was performed with two cultivars of Indian wheat (Sonalika and HUW 510) against ambient and elevated levels of O_3 by using open top chambers (OTCs). Both the cultivars showed the negative impact of O_3 on various growth, reproductive and yield parameters but the response among cultivars was quite distinct. Cultivar HUW 510 showed higher O_3 damage in its vegetative parts (shoot and root height, leaf number, leaf area, etc.) than Sonalika, whereas the response of reproductive structures (pollen viability and viable florets per plant) was *vice versa*. Yield response to stress (YRS) analysis revealed that degree of damage in both cultivars was more severe under elevated concentrations of O_3 over ambient. The overall results of the present study showed that in future, O_3 would be a threat for wheat production but differential response among cultivars might help researchers to find out a suitable variety for an area experiencing higher concentration of O_3.

1. Introduction

Increasing food crisis during the present time has become a major point of concern throughout the world for mankind. In combat with the emerging multidimensional risks, agriculture is losing its potential yield rapidly (Barun, 2007). Over the past few years tropospheric ozone (O_3) has been recognized as a major threat to agriculture at global level. Diverse integrated and individual studies performed in different countries have confirmed this concern (Krupa et al., 2001; Mills et al., 2007; Booker et al., 2009).

Modern wheat is the second largest produced cash crop with a net production of more than 620 million metric tons year^{-1} (FAOSTAT, 2007). Nearly two third of world population depends on this crop for their primary diet. India is the second largest wheat producing country of the world with an annual productivity of nearly 72 million metric tons. In India, wheat is the primary staple food and serves as the main dietary supplement. Wahid (2006) found stunted growth and enhanced rate of leaf senescence with significant reductions in different morphological, physiological and yield parameters in three wheat cultivars due to ambient level of O_3 at Lahore, Pakistan. Rai et al. (2007) also observed a yield loss of 21% in wheat due to ambient O_3 pollution at a sub urban area of Varanasi, India. Ollerenshaw and Lyons (1999) observed a major decrease in relative growth rate (RGR), above ground biomass and different yield parameters in wheat with at elevated O_3 concentrations of 75 and 81 ppb in England. The results of Biswas et al. (2008) have clearly concluded that O_3 sensitivity was governed by genetic characters in modern wheat and supported the earlier findings of Pleijel et al. (2006) obtained with two old and modern cultivars of Swedish wheat under elevated levels of O_3.

Reproductive parts and different phases of reproductive cycle are found to be very susceptible to O_3 stress (Black et al., 2000). According to Mulchi et al. (1986), O_3 exposure for 5 days during anthesis period, at 4 h mean concentration of 123 ppb, caused larger adverse effects on the grain yield and quality of six winter wheat cultivars. Schoene et al. (2004) reported that under closed chamber exposure with ambient (65 ppb, 8 h) and elevated (110 ppb, 4 h) concentrations, O_3 negatively affected the development of pollen grains in perennial ryegrass (*Lolium perenne* L.). In another study, Ollerenshaw and Lyons (1999) have noticed that consecutive O_3 fumigations of 81 ppb (7 h day^{-1}) for 27 days and 75 ppb (6 h day^{-1}) for 16 days increased the infertile florets per spikelet by 9.2% in wheat. An examination of more than 53 studies carried out between 1980 and 2007 through a meta-analytical approach reveals that elevated levels of O_3 (an average of 72 ppb) have decreased grain yield by 29% and above ground biomass by 18% in modern wheat varieties (Feng et al., 2008).

With the current trends of emission in primary pollutants, tropospheric O_3 is predicted to rise globally by 20–25% between 2015 and 2050, and 40–60% by 2100 (Meehl et al., 2007) suggesting, O_3 to become a bigger problem than the present. According to Singh et al. (2007), the global wheat production must continue to increase at least 2% annually until 2020 to meet the future

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demand. This objective could be achieved only if the newly developed cultivars of wheat show genotypic variability having tolerance against unavoidable stresses, like elevated levels of surface O_3 and other pollutants. At present, different individual and integrated studies are concentrating mainly on the performance of the modern crop cultivars under elevated levels of pollutants and their dose–response relationship. Depending on these studies, different models are proposed to predict the future yield of these crops but all of them have certain limitations. Some existing models have overestimated crop productivity against increasing O_3 pollution (Long et al., 2005). Moreover, these models suffer from region biasness due to unavailability of data on crop yield and O_3 concentrations throughout the world. Kaliakatsou et al. (2010) also reported high discrimination between the predicted and estimated yield loss in winter wheat against O_3, by studying a large number of standardized trial plots from different sites over a 13-year period in England.

Keeping in view, the present investigation has been undertaken with the following objectives: (i) to assess the response of two high yielding wheat cultivars under elevated levels of O_3, (ii) to develop a dose–response relationship between grain yield of wheat and O_3 in Indian context, (iii) to observe growth phase specific O_3 sensitivity, and (iv) to evaluate the cultivar specific response. According to Mills et al. (2007), wheat is an O_3 sensitive crop as it demonstrates 5% yield reduction against O_3 at 3 months AOT40 of 3 ppm h (at 95% confidence interval, range: 2.3–4.4 ppm h) in European countries. Although, the depiction might not be adequate enough for tropical and sub-tropical wheat cultivars because normally the background levels of O_3 in tropical countries are higher than temperate countries. We hypothesized that being tropical in origin; Indian wheat cultivars might show better tolerance against O_3 than the temperate cultivars. Further the cultivars allocate more photosynthate towards reproductive than vegetative parts during grain filling stage will able to maintain higher yield under elevated O_3 stress.

2. Materials and methods

2.1. Experimental site

The experiment was performed for two consecutive years (i.e. 2007–2008 and 2008–2009) in the same field at Agricultural Research Farm, Banaras Hindu University, Varanasi, India (25°14′N, 82°03′E). The place is 76.1 m above mean sea level. The soil of the study site has a sandy loam characteristic with pH 7.2. No major air pollutant sources are in the vicinity of the selected experimental site.

2.2. Plant material

Two high yielding, dwarf cultivars of winter wheat, *Triticum aestivum* L. cv 'Sonalika' and cv 'HUW 510' with medium life span were selected for the present experiment. Both the cultivars are highly recommended and widely grown in Northern parts of India. Sonalika, also known as RR21, was the first variety that played important role in 'Green Revolution' of India with a huge success. It is immensely popular due to its high yield, shorter maturity and double dwarf nature. HUW 510, a relatively younger one, is also very popular for its high yield, double dwarf and disease resistance nature.

2.3. Wheat cultivation

Seeds of winter wheat were hand sown in all experimental setups in December 2007 and December 2008. Fertilizers (120 kg ha^{-1} N, 60 kg ha^{-1} P and 40 kg ha^{-1} K as urea, single super phosphate and muriate of potash, respectively) were added as per recommendation during preparation of the experimental field. Half dose of N and full doses of P and K were given as basal dressing and another half dose of N was given as top dressing. After emergence of seedlings, a distance of 1 plant every 15 cm was maintained. Weeding was done manually as per requirements. Field was irrigated properly at definite intervals to maintain soil moisture properly in all the treatment plots.

2.4. Meteorological parameters

Different meteorological parameters, like maximum (T_{max}) and minimum temperature (T_{min}), relative humidity (RH), total rainfall and sunshine hours of the experimental site were recorded periodically.

2.5. Ozone treatment

In first year, two different levels of O_3 treatments were provided in open top chambers (OTCs) ($n = 3$) for each cultivar. Experimental OTCs were divided as: charcoal filtered air (FCs), non-filtered air (NFCs), non-filtered air with additional lower (+10 ppb) O_3 (NFCLOs) and non-filtered air with additional higher (+20 ppb) O_3 (NFCHOs). Actually, with the help of these four treatments, plants were exposed to a series of four different O_3 concentrations: (i) nearly no O_3, (ii) ambient O_3, (iii) ambient + 10 ppb O_3, and (iv) ambient + 20 ppb O_3. Open plots (OPs, $n = 3$) were also used to monitor the effects of chamber enclosures. The treatments were distributed in a complete randomized manner. Ozone fumigation in respective OTCs was done with the help of O_3 generators (Model Systrocom, India) with daily O_3 fumigation at the peak O_3 period (10:00–15:00 h) of local time.

The OTCs were 1.5 m in diameter and 1.8 m in height, including a flexible but solid build iron frame with a cover of transparent polypropylene sheet. Each of the OTCs was attached to a high speed blower for a continuous air supply at three changes per minute. Ozone generators were attached to the respective blowers, for proper mixing of O_3 with the air entering inside the chamber. The design of the chambers was described in detail by Tiwari and Agrawal (2006). It was observed that temperature and relative humidity were higher by 0.1–0.2 °C and 2–4%, respectively in OTCs, as compared to OPs. The light intensity within the chambers was 95% of the ambient level as noticed in OPs.

2.6. Ozone monitoring

Ozone monitoring was done on a 12 h day^{-1} basis (6:00–18:00 h) at the experimental site. Air samples at canopy height were drawn through a 15 m long inert Teflon tube (0.35 cm in diameter) from each of the experimental setups for a definite time. Ozone concentrations were monitored by using UV absorption photometric O_3 analyzer (Model APOA 370, HORIBA Ltd, Japan).

2.7. AOT40 (accumulated ozone over a threshold concentration of 40 ppb) value

Exposure index for O_3, i.e. AOT40 was calculated by using the following formula given by Mauzerall and Wang (2001):

$$\text{AOT40} = \sum_{i=1}^{n} [C_{O_3} - 40]_i$$

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.

2.8. Growth parameters

To determine various growth and biomass parameters, three monoliths (10 cm × 10 cm × 20 cm) containing intact roots were carefully collected at random from each chamber and also from open plots at 25, 50 and 75 days after germination (DAG). These were thoroughly washed with tap water to remove all the soil and unwanted particles from the roots and other parts of the plants.

Growth parameters studied were root and shoot length, leaf area, number of healthy leaves, standing dead (senesced leaves) and tillers. Leaf area was measured using portable leaf area meter (Model LI-3000, LI-COR Inc., USA). Respective plant parts were separated and oven dried at 80 °C till constant weight was achieved for determination of total biomass. Different growth indices like relative growth rate (RGR), net assimilation rate (NAR), net primary productivity (NPP) and absolute growth rate (AGR) were calculated to study the biomass allocation within the plant parts by using the formulae provided by Hunt (1982).

2.9. Reproductive parameters

Fertile florets per plant and pollen viability were assessed as reproductive measurements. Ten plants in each replicate of experimental setup were tagged for this purpose from the beginning of the flowering. For counting fertile florets, the tagged spikelets were periodically evaluated until seed setting. For pollen viability, 'ready to dehisce' or fully mature anthers were collected between 06:00 and 07:00 h and immediately; the pollen grains were stained with 2% aceto-carmine in presence of a proper osmotic stabilizer and sealed with paraffin on a clean glass slide. Each slide was scored for five observations. Fifty observations for each spikelet and a total of 10 spikelets were observed from each replicate of every experimental setup. The florets were randomly collected from each spikelet.

2.10. Yield parameters

Plants (only the above ground parts) were harvested at maturity. Ten plants from each replicate were sampled for assessing different yield parameters like above ground biomass, weight of grains per plant and number of grains per plant. Harvest index (HI) was calculated as the ratio of economic yield (weight of grains per plant) and total above ground biomass. Yield response to stress (YRS) was calculated with a formula adopted from Yu et al. (2006) as follows:

$$\text{YRS}\ (\%) = \frac{Y_{\text{treatment}} - Y_{\text{control}}}{Y_{\text{control}}} \times 100,$$

where $Y_{\text{treatment}}$ and Y_{control} are the grain yields of wheat at treatment sets and control sets (control set was taken in two ways: (i) against filtered chambers (FCs) and (ii) against open plots (OPs)).

2.11. Statistical analysis

Results were subjected to one-way and two-way ANOVA for assessing the significance of quantitative changes in various parameters due to different treatments and cultivars. Duncan's multiple range test was performed as post hoc on parameters subjected to ANOVA (only if the ANOVA was significant). All the statistical tests were performed using SPSS software (SPSS Inc., version 16.0).

2.12. Derivation and analysis of dose–response functions

The correlations between grain yield and relative yield (Mills et al., 2007) of both the cultivars (Sonalika and HUW 510) and AOT40 were determined through linear regression using SPSS software (SPSS Inc., version 16.0).

3. Results

3.1. Meteorological parameters

Climate data, in both the seasons, did not vary significantly. Maximum temperature was highest during March (34.1 °C at 2008 and 38.1 °C at 2009) and lowest during January (22.6 °C at 2008 and 23.6 °C at 2009). Minimum temperature also varied from 9.0 to 17.5 °C during 2007–2008 and 10.0–21.5 °C during experimental period 2008–2009. Maximum relative humidity was highest during December 2007 (80.5%) at first year and during January, 2009 (82.5%) at second year of experiment. Total rainfall was higher in first year (28.4 mm) than second year (5.6 mm). Sunshine hours varied from 6.2 to 8.4 h during 2007–2008 and 6.8 to 9.2 h during 2008–2009.

3.2. O_3 monitoring and AOT40

In filtered chambers (FCs), air filtration with activated charcoal filters resulted in reduction of O_3 concentrations by 88% compared to non-filtered chambers (NFCs) and open plots (OPs). Day time ambient O_3 concentration throughout the first year (2007–2008) was 45.3 ppb in average with a range of 4.0–125 ppb. During second year (2008–2009), O_3 was 47.3 ppb in average with a range of 2.0–137 ppb (Table 1). In both the growing years, O_3 concentrations were higher during the reproductive phase than the vegetative phase of wheat. During first year, day time ambient O_3 concentration increased from 41.8 ppb during vegetative phase to 54.6 ppb during reproductive phase. At first year of growth, mean day time O_3 concentrations were 4.7 ppb in FCs, 45.3 ppb in OPs and NFCs, 50.4 ppb in NFCLOs and 55.6 ppb in NFCHOs and the corresponding AOT40 values were 0 ppm h in FCs, 7.9 ppm h in OPs and NFCs, 10.4 ppm h in NFCLOs and 13.1 ppm h in NFCHOs, respectively (Table 1). In second year, mean day time O_3 concentrations were 4.9 ppb in FCs, 47.3 ppb in OPs and NFCs and 56.9 ppb in NFCHOs and the corresponding AOT40 values were 0 ppm h in FCs, 8.7 ppm h in OPs and NFCs and 14.4 ppm h in NFCHOs, respectively (Table 1).

Table 1
Mean O_3 concentrations and AOT40 in different experimental setups during experimental period 2007–2008 and 2008–2009.

Experimental set	Experimental period: 2007–2008		Experimental period: 2008–2009	
	Mean O_3 concentration (ppb)	AOT40 (ppm h)	Mean O_3 concentration (ppb)	AOT40 (ppm h)
FCs	4.7	0	4.9	0
OPs	45.3	7.9	47.3	8.7
NFCs	45.3	7.9	47.3	8.7
NFCLOs	50.4	10.4	–	–
NFCHOs	55.6	13.1	56.9	14.4

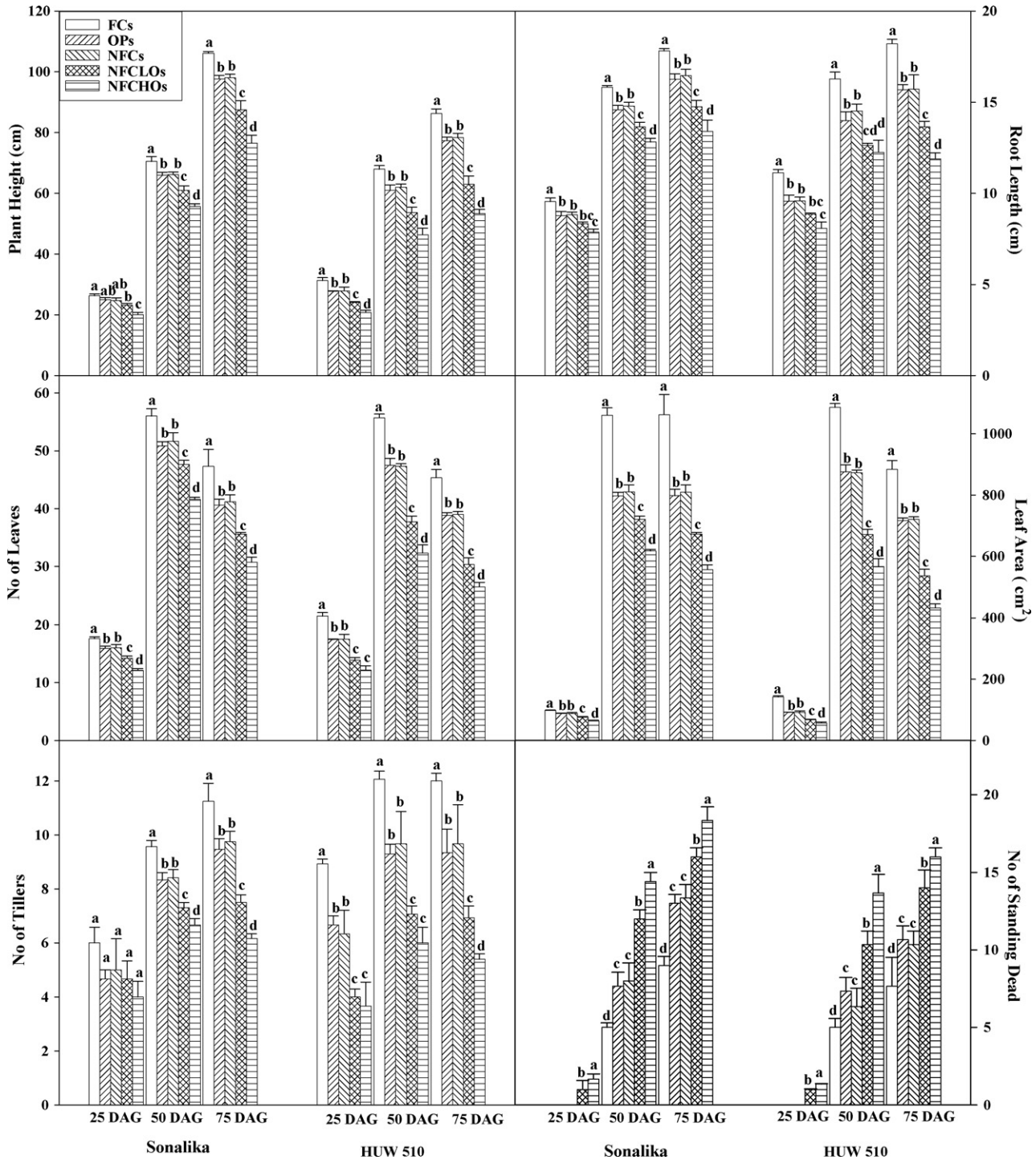

Fig. 1. Effect of O_3 on plant height, root length, number of leaves, leaf area, number of tillers and number of standing dead of two wheat cultivars at different stages of growth. Values are mean ± SE. Bars showing different letters indicate significant differences according to Duncan's test at $p < 0.05$.

3.3. Morphological parameters

Plant height decreased significantly in plants of OPs, NFCs, NFCLOs and NFCHOs as compared to FCs at 25, 50 and 75 DAG at both the years of growth. During first year, the reductions were 7.9, 7.6, 17.5 and 38.7% in Sonalika and 10.4, 9.3, 27 and 61.8% in HUW 510 at 75 DAG in OPs, NFCs, NFCLOs and NFCHOs, respectively as compared to FCs (Fig. 1). Three-way ANOVA showed significant variations in plant height due to treatment, age and cultivar and their interaction (Table 2). Root length also reduced significantly in both the cultivars in O_3 exposed plants at 50 and 75 DAG (Fig. 1). Root length also varied significantly due to treatment, cultivar and interaction between them (Table 2). Total number of leaves and leaf area were also affected by O_3 exposure in both the cultivars at all the samplings dates. In Sonalika, total number of leaves reduced by 12.9, 24.7 and 54% and leaf area by 23.8, 36.7 and 48% at 75 DAG in NFCs, NFCLOs and NFCHOs, respectively as compared to FCs (Fig. 1). HUW 510 also followed the same trend, but the percentage of reduction was more. Three-way ANOVA also showed significant variations in number of leaves and leaf area due to treatment, age and cultivar

Table 2
Results of three-way ANOVA showing *F*-values and level of significance for plant height, root length, number of tillers, number of leaves, leaf area, number of standing dead, total biomass, AGR, NPP, NAR, and RGR of two cultivars of *Triticum aestivum* L. at different concentrations of O_3.

Parameter	Cultivar	Age	Treatment	Cultivar × age	Cultivar × treatment	Age × treatment	Cultivar × age × treatment
Plant height	2963.23***	192.43***	195.69***	138.27***	4.05*	25.20***	0.201^{NS}
Root length	733.99***	0.199^{NS}	142.72***	11.33***	7.05**	7.06***	0.179^{NS}
No. of tillers	68.38***	3.944^{NS}	63.61***	1.741^{NS}	5.50**	1.45^{NS}	0.427^{NS}
No. of leaves	1865.48***	41.25***	232.88***	25.42***	9.82***	14.53***	1.379^{NS}
Leaf Area	3233.56***	28.57***	347.92***	32.31***	2.75*	59.34***	2.067^{NS}
No. of standing dead	519.84***	11.31*	78.05***	3.46*	0.52^{NS}	11.37***	0.202^{NS}
Total biomass	2267.53***	11.45**	687.37***	6.68**	0.88^{NS}	208.64***	0.954^{NS}
AGR	208.26***	138.67***	55.99***	78.89***	0.43^{NS}	1.3^{NS}	0.685^{NS}
NPP	1320.32***	13.62**	765.37***	13.56***	1.16^{NS}	131.18***	0.901^{NS}
RGR	1084.85***	0.615^{NS}	44.65***	19.90***	0.253^{NS}	9.81***	0.851^{NS}
NAR	1121.25***	0.220^{NS}	52.27***	9.16***	0.95^{NS}	2.94*	0.967^{NS}

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

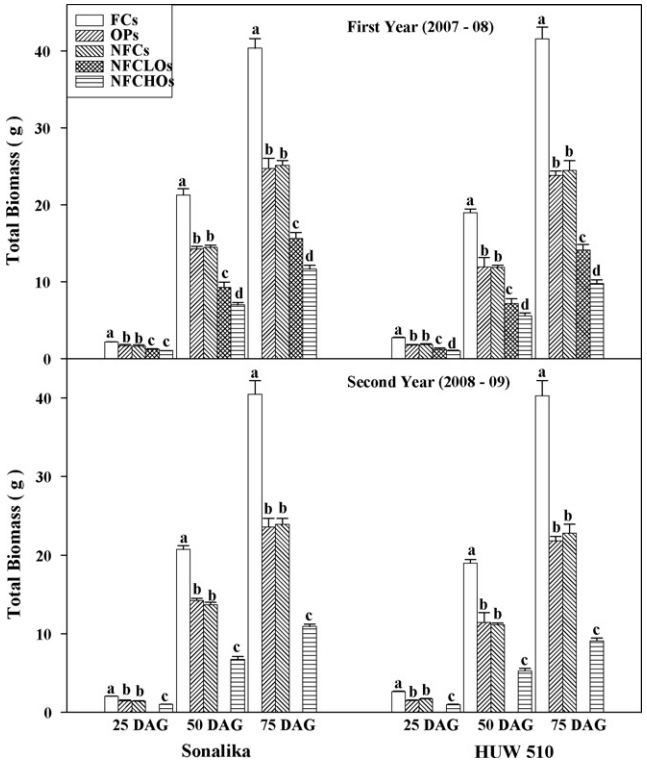

Fig. 2. Effect of O_3 on total biomass of two cultivars of wheat at different stages of growth for two consecutive years of experiment. Values are mean ± SE. Bars showing different letters indicate significant differences according to Duncan's test at $p < 0.05$.

and also their interactions (Table 2). Number of tillers also showed the same trend of reduction in O_3 exposed plants for both the cultivars at all the ages (Fig. 1). Total numbers of standing dead (fully senesced leaves) were significantly higher in O_3 exposed plants at 25, 50 and 75 DAG in both the cultivars (Fig. 1). Increments of standing dead by 48, 77.7 and 103.7% in Sonalika and 38.7, 82.6 and 106.7% in HUW 510 were observed in NFCs, NFCLOs and NFCHOs, respectively as compared to FCs at 75 DAG. Total numbers of standing dead varied significantly due to treatment, age and cultivar and also due to their interactions (Table 2).

3.4. Total biomass and growth indices

Total biomass accumulation, in both the experimental years, showed significant declining trends under O_3 exposure for both the cultivars. During first year, at 75 DAG, reductions of 37.1, 61.3 and 71.1% in Sonalika and 41, 66 and 76% in HUW 510 were observed at NFCs, NFCLOs and NFCHOs, respectively, as compared to FCs (Fig. 2). Three-way ANOVA showed that total biomass accumulation varied significantly due to individual effects of treatment, age and cultivar and interactive effects of treatment × age and cultivar × age (Table 2). In second year, the trend was almost similar for total biomass and at 75 DAG, 41 and 73% reductions in Sonalika and 43 and 77% in HUW 510 were observed in NFCs and NFCHOs, respectively, as compared to FCs (Fig. 2). Three-way ANOVA also showed that in both the years, total biomass accumulation varied significantly mainly due to individual effect of treatment and cultivar and also due to interactive effect of treatment × cultivar (Table 3).

Absolute growth rate (AGR) was affected significantly due to O_3 exposure in both the cultivars. During 50–75 DAG, it reduced by 10.4, 25.6 and 41.4% in Sonalika and 11, 49 and 70% in HUW 510 at NFCs, NFCLOs and NFCHOs, respectively as compared to FCs (Fig. 3). Three-way ANOVA showed that AGR varied significantly due to treatment, age and cultivar (Table 2). Net primary productivity (NPP) decreased by 38, 61.3 and 71% in Sonalika and 41, 66 and 76.5% in HUW 510 at NFCs, NFCLOs and NFCHOs, respectively as compared to FCs during 50–75 DAG (Fig. 3). Both RGR and NAR followed the same trend against O_3 exposure (Fig. 3). Three-way ANOVA showed that these growth indices varied significantly due to treatment and cultivar (Table 2).

3.5. Reproductive parameters

Total number of viable pollens and viable florets per plant affected significantly due to O_3 exposure. Number of viable pollens reduced by 21, 33 and 41% in Sonalika and 18, 28 and 35% in

Table 3
Results of three-way ANOVA showing level of significance for total biomass, yield, HI, pollen viability and viable florets of both the cultivars of *T. aestivum* L. at different concentrations of O_3 for two consecutive years.

Parameter	Year	Cultivar	Treatment	Year × cultivar	Year × treatment	Cultivars × treatment	Year × cultivar × treatment
Total biomass	NS	*	***	NS	NS	***	NS
Yield	NS	**	***	NS	NS	***	NS
HI	NS	NS	***	NS	NS	**	NS
Pollen viability	NS	**	***	NS	NS	**	NS
Viable florets	NS	*	***	NS	NS	***	NS

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

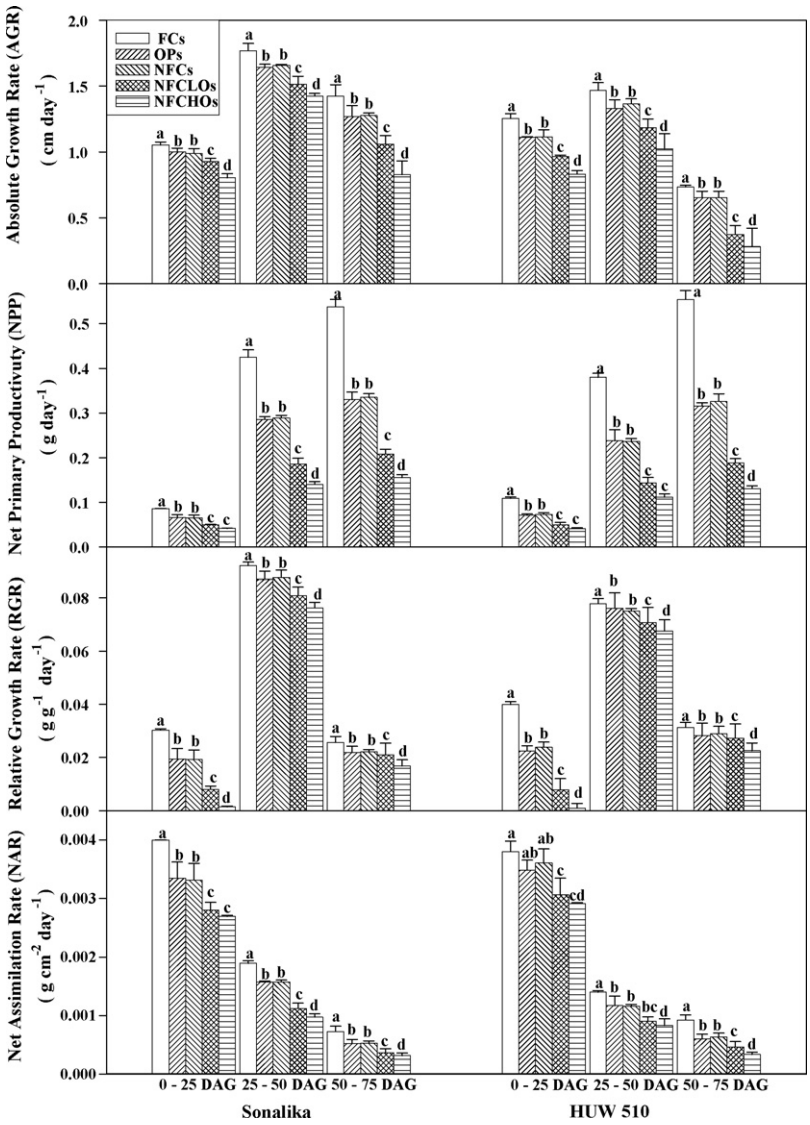

Fig. 3. Effect of O_3 on AGR, NPP, RGR and NAR of two cultivars of wheat at different stages of growth. Values are mean ± SE. Bars showing different letters indicate significant differences according to Duncan's test at $p < 0.05$.

HUW 510 at NFCs, NFCLOs and NFCHOs, respectively as compared to FCs. Viable florets per plant also reduced by 22, 35.4 and 36% in Sonalika and 16, 26.4 and 30% in HUW 510 at NFCs, NFCLOs and NFCHOs, respectively as compared to FCs (Fig. 4). Two-way ANOVA showed that both the parameters varied significantly due to treatment and cultivar (Fig. 4). Response of viable pollens and viable florets per plant did not vary significantly between both the years of growth (Table 3).

3.6. Yield

Above ground biomass was significantly decreased under O_3 stress. Reductions of 7, 16.7 and 22% in Sonalika and 8.4, 18.5 and 25% in HUW 510 were observed at NFCs, NFCLOs and NFCHOs, respectively as compared to FCs (Fig. 4). Two-way ANOVA showed that above ground biomass varied significantly due to O_3 treatment and cultivar (Fig. 4). Total number of grains per plant decreased by 18, 26.5 and 33% in Sonalika and 12, 25 and 28% in HUW 510 at NFCs, NFCLOs and NFCHOs, respectively as compared to FCs (Fig. 4). Weight of grains ($g\,m^{-2}$) was also reduced significantly in O_3 exposed plants. Reductions of 11, 25 and 38.5% in Sonalika and 20, 37 and 46% in HUW 510 were observed at NFCs, NFCLOs and NFCHOs, respectively as compared to FCs (Fig. 4). Harvest index (HI) also followed a same trend of reduction in both the cultivars under O_3 treatments. Two-way ANOVA showed that both weight of grains ($g\,m^{-2}$) and HI varied significantly due to O_3 treatment only (Fig. 4).

3.7. Analysis of dose–response functions

Dose–response analysis demonstrated that O_3 showed significant negative effects at higher concentrations. YRS analysis and linear regression analysis between grain yield and AOT40 pointed out that even slight increase in the concentrations of O_3 over ambient might cause drastic damage (Figs. 5 and 6). Relative yield analysis with AOT40 ($y = 1.02 - 0.03x$, $r^2 = 0.88$) showed yield loss of 5.4% at a 3 months AOT40 of 3 ppm h (95% confidence interval, range: 0–14.4 ppm h) (Fig. 7).

4. Discussion

Present experiment performed with OTCs showed significant negative effects of O_3 on two cultivars of wheat grown at a rural site of Varanasi, India. The microclimatic conditions varied to a small extent between the chambers and open plots. The increase

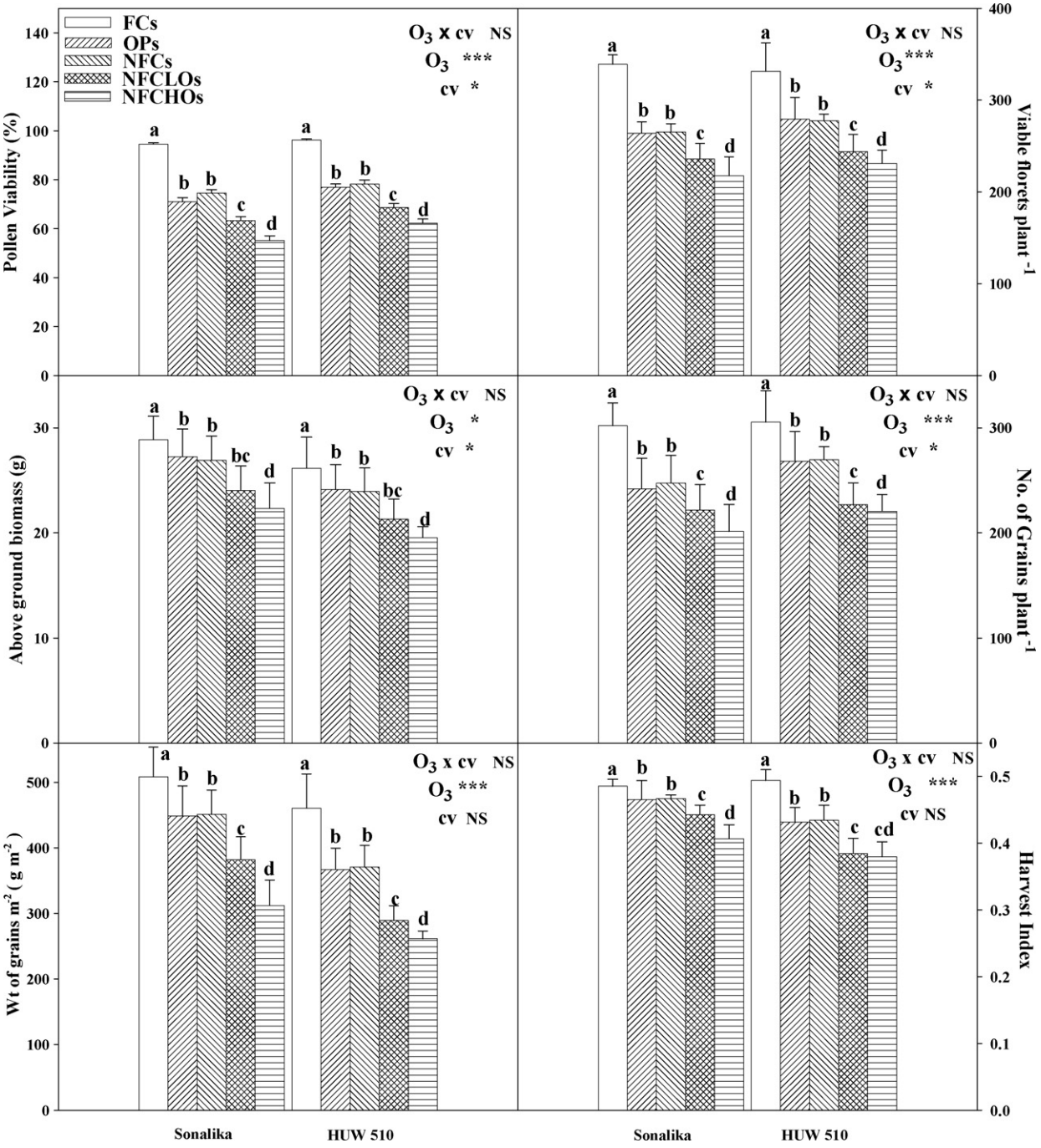

Fig. 4. Effect of O_3 on various reproductive and yield parameters of two cultivars of wheat at different stages of growth. Values are mean ± SE. Bars showing different letters indicate significant differences according to Duncan's test at $p < 0.05$. Results of two-way ANOVA shown as $O_3 \times$ cv, O_3 and cv. Level of significance $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ and NS: not significant.

in temperature (0.1–0.2 °C) within chambers as compared to OPs during the present study was similar to that reported earlier by Rai et al. (2007) and Sarkar and Agrawal (2010), and much less than the reports of Wahid (2006). The other parameters like relative humidity and light intensity were also similar as reported by Rai et al. (2007) and Sarkar and Agrawal (2010). Minor changes in microclimatic parameters showed the efficacy of chambers for providing an almost natural environment as open plots to the test plants. Musselman et al. (2006) have already concluded that O_3 uptake by plants inside the chambers was likely to be similar to the field conditions. Results of the present study also showed non-significant variations between the responses of plants grown under OPs and NFCs.

The present experiment has been designed to develop a complete gradient of O_3 exposure for wheat plants. Some of the earlier studies have reported that the ambient day time O_3 concentrations, in Varanasi, India, varied from 36.4 to 48 ppb during December 2004–March 2005 (Rai et al., 2007) and 35.3–54.2 ppb during December 2006 to March 2007 (Singh and Agrawal, 2009). Tiwari et al. (2005) also reported that the ambient O_3 concentrations were lower than 40 ppb during December 2002 and January 2003, but it often exceeded 40 ppb levels for several hours during February 2003 and March 2003. So, the mean ambient day time O_3 concentration for the complete wheat season was around 40 ppb. Results of present study also showed that the mean day time O_3 concentration was 45.3 ppb during the first year and 47.3 ppb during

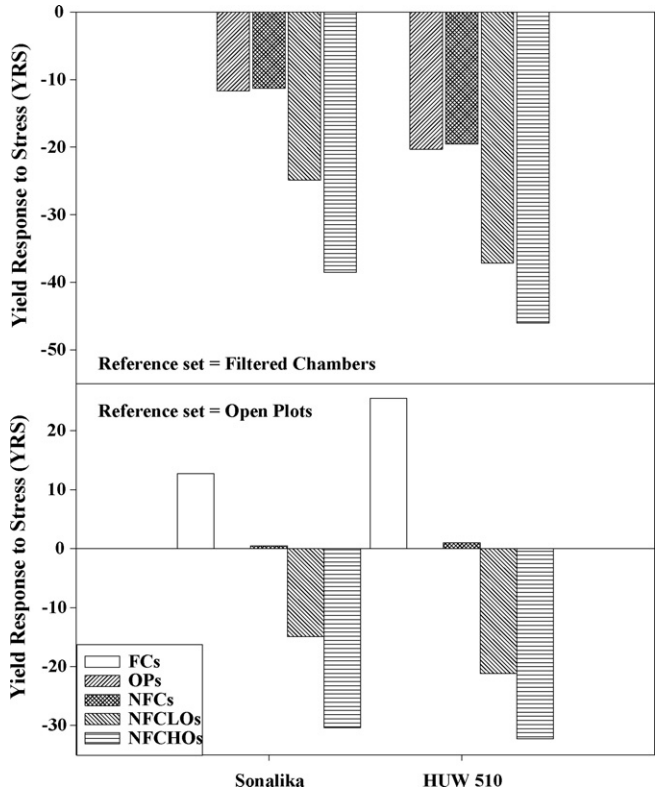

Fig. 5. Yield response to stress (YRS %) at different experimental sets from filtered chambers and open plots. YRS (%) = $((Y_{treatment} - Y_{contol})/Y_{control}) \times 100$, where $Y_{treatment}$ and $Y_{control}$ are the grain yields of wheat at treatment sets and control sets (control set has been taken in two ways: first one against filtered chambers (FCs) and second one against open plots (OPs), respectively.

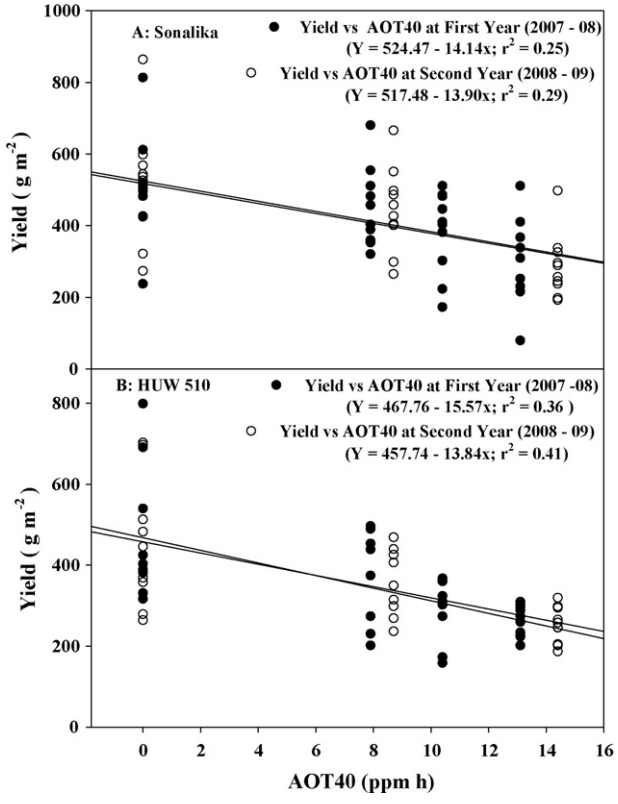

Fig. 6. Correlation coefficients and regression equations between individual yield responses and cumulative AOT40 of two wheat cultivars for two consecutive years.

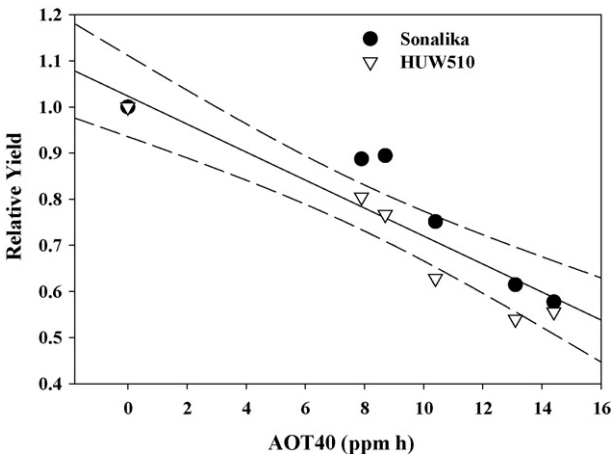

Fig. 7. Correlation coefficients and regression equations between relative yield responses and cumulative AOT40 of two wheat cultivars for two consecutive year.

the second year of experiment (Table 1). So, 10 ppb (nearly 25% over ambient) and 20 ppb (nearly 50% over ambient) elevated O_3 doses over ambient were selected for exposure studies. The mean day time O_3 concentrations and AOT40 values reflected that the amount of O_3 was the only factor which varied between different experimental sets and formed an O_3 dose dependent gradient (Table 1). Recently, Mittal et al. (2007) showed that Indo-Gangetic plain situated in the north-east of India had very high AOT40 as well as higher concentrations of O_3 as compared to other parts of India.

Different morphological parameters were negatively affected in wheat plants due to O_3 exposure. Wahid (2006) reported 10–20% reduction in shoot length and 8–20% reduction in root length of three different cultivars of wheat in Lahore, Pakistan at ambient O_3 concentration. Singh and Agrawal (2009) also reported reductions of 12.8–17.3% in shoot height and 6.3–9.2% in root height in their study made with EDU in five cultivars of wheat at a rural area of Varanasi, India. Results of the present study also followed the same trend though the negative effects were of higher magnitude with higher concentrations of O_3 (Fig. 1). Total number of healthy leaves and leaf area are the two most important parameters for the survival and performance of any healthy plant. Results showed that both of these parameters were drastically affected by O_3 exposure (Fig. 1). Wahid (2006) reported 7–18% decrease in flag leaf area in wheat plants. Singh and Agrawal (2009) also found a significant loss in total number of leaves and leaf area in wheat plants under ambient O_3 pollution. Miller et al. (1999) reported that O_3 could induce several senescence associated genes (SAGs) in *Arabidopsis* plants resulted in early senescence. In the present study, an increasing trend in total number of standing dead under elevated concentrations of O_3, could be a result of activation of senescence associated genes (SAGs) under stress conditions (Fig. 1). Pleijel et al. (1998) also found early senescence of leaves in wheat plants under O_3 pollution. Both the test cultivars were affected under O_3 exposure but the performance of Sonalika was better than HUW 510 with respect to morphological responses (Fig. 1).

Total biomass, in both the cultivars, was also affected by O_3. Variations were not significant initially but became significant at later stages. Rai et al. (2007) and Singh and Agrawal (2009) also found similar trend in wheat under ambient concentrations of O_3. Under stressful condition, primary metabolism of plants got negatively affected and caused reductions in different growth indices like AGR, NPP, NAR and RGR (Singh and Agrawal, 2009). In the present study, same trend has been observed under elevated O_3 exposure.

Reproductive development is an important phase in the life cycle of any higher plant because any kind of anomaly might cause

significant reductions in the productivity and even prove to be a threat for its survival. O_3 proved itself as a potent inhibitor of reproductive structure development in different plant species (Black et al., 2000). Schoene et al. (2004) reported that O_3 affected the development of pollen by inhibiting starch accumulation in pollen throughout the anther in perennial ryegrass plants (*Lolium perenne* L.). Roshchina and Karnaukhov (1999), in their microspectrofluorometric study on *Philadelphus grandiflorus*, *Epiphylum hybridum* and *Plantago major*, observed that after 100 h of 150 ppm O_3 exposure (3 h day^{-1} for 5 day per week) pollen grains of these plants showed an irreversible shift in fluorescence spectra, showing loss of pollen viability. Recently, Black et al. (2010) reported direct effect of O_3 on the reproductive development of *P. major* L. population. Present results also showed the loss of pollen viability under elevated levels of O_3. Percentage of viable pollen grains reduced depending on the O_3 dose in both the wheat cultivars (Fig. 4). Viable florets per plant also affected significantly under high concentrations of O_3 (Fig. 4). Bergweilier and Manning (1999) reported that ambient O_3 can significantly inhibit flower development in spreading dogbane (*Apocynum androsaemifolium* L.) by 24% at Vermont, USA. Linear regression analysis between O_3 exposure and responses of reproductive parameters indicated that performance of HUW 510 (pollen viability: $r^2 = 0.65$; viable florets: $r^2 = 0.29$) was better than Sonalika (pollen viability: $r^2 = 0.74$; viable florets: $r^2 = 0.52$).

Present study clearly showed that, both the cultivars of wheat (Sonalika and HUW 510) showed differential responses under O_3 stress. Singh and Agrawal (2009) and Feng et al. (2010) also reported significant differences between the responses of various wheat cultivars under O_3 pollution. Each genotype (or cultivar) is unique in genetic constitution and shows exclusive response. So, this kind of differential response study, against O_3 stress, could ultimately help the crop scientists to screen a suitable cultivar for a particular area experiencing elevated levels of O_3.

For any crop, yield has always been the primary point of interest. Different studies throughout the world have shown that seed and fruit yields are commonly reduced in a wide range of crops, not only under simulated levels of O_3 but also under the prevailing ambient concentrations of O_3 (Black et al., 2000; Fuhrer and Booker, 2003; Ashmore, 2005; Singh and Agrawal, 2009). Most of the available reports on O_3–wheat interaction showed that O_3 could be a potential threat for its productivity. Feng et al. (2008), in their meta-analytic evaluation have mentioned that an average 72 ppb O_3 (which is higher than ambient) of 7–8 h, with a range of 30–200 ppb reduced the grain yield of wheat by 29%. Rai et al. (2007) from India found 21% reduction in grain yield at ambient O_3. Results of present study also showed reductions in grain yield due to O_3 under ambient and elevated levels of O_3. Harvest index (HI) reflects the partitioning of photosynthates between grains and above ground biomass. This was also negatively affected by O_3 exposure (Fig. 4). Linear regression analysis showed that the inhibitory effect of O_3 on total number of grains, above ground biomass and HI increased with increasing concentrations of O_3 (Table 4).

The whole discussion has been centered by considering FCs as reference but the manifestation of results of present could be explained more convincingly if we take OPs as reference. So, YRS were calculated for both the conditions considering FCs as reference in first case and OPs as reference in second case (Fig. 5). Results showed that the yield losses over ambient O_3 concentrations ($\equiv$40 ppb) were more drastic. This clearly indicates that in coming future, under the present trend of increase in O_3 concentrations, the yield reduction might be more than the predicted levels.

Dose–response analysis between yield and AOT40 values showed almost linear relationship. Cultivar Sonalika (first year: $r^2 = 0.24$, second year: $r^2 = 0.29$) performed better than HUW 510 (first year: $r^2 = 0.36$, second year: $r^2 = 0.41$) (Fig. 6). Depending on this dose–response analysis of yield and relative yield, Mills et al. (2007) have decisively synthesized the critical level of AOT40 of 3 ppm h for 3 months for wheat for European countries. One of the basic aims of present study was to understand the feasibility of that critical level of AOT40 on Indian wheat cultivars. Both dose–response analysis of yield and relative yield, showed that Indian wheat cultivars also followed the same critical level as that of temperate wheat. Yield loss of 5.4% has been observed at AOT40 of 3 ppm h (95% confidence interval, range: 0–14.4 ppm h) in the present investigation performed with two cultivars of wheat for two consecutive years (Fig. 7).

Table 4
Correlation coefficients and linear regression between some selected parameters and AOT40 of both the cultivars of *T. aestivum* L.

Relationship between	Correlation coefficient	Regression equation
Sonalika		
Pollen viability (%)	0.737***	$Y = 93.19 - 1.87x$
Number of viable florets per plant	0.517***	$Y = 339.92 - 11.7x$
Above ground biomass	0.122*	$Y = 29.38 - 0.60x$
Number of grains per plant	0.389***	$Y = 307.74 - 11.54x$
Harvest Index	0.461***	$Y = 0.49 - 0.01x$
HUW 510		
Pollen viability (%)	0.655***	$Y = 98.87 - 2.23x$
Number of viable florets per plant	0.292***	$Y = 333.51 - 9.71x$
Above ground biomass	0.118*	$Y = 26.63 - 0.60x$
Number of grains per plant	0.214**	$Y = 309.40 - 8.38x$
Harvest Index	0.330***	$Y = 0.50 - 0.01x$

Level of significance: $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

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