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Respective and Interactive Effects of O_3 and CO_2 and Drought Stress on Photosynthesis, Stomatal Conductance, Antioxidative Ability and Yield of Wheat Plants

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ABSTRACT

Effects of O_3, Doubled CO_2 Concentration and drought stress on wheat (*Triticum aestivum L.*) plants were studied in open-top chambers (OTC). Under doubled CO_2 concentration, grain yield and biomass increased, the SOD activity, and carotenoid (Car) content also increased while relative conductivity yield parameters significantly decreased. But under Elevated O_3 concentration, the SOD activity, Carotenoids decreased. The final result was decreased grain yield and plant biomass. Interactive effects of doubled CO_2 and O_3 concentrations on soybean were mostly counteractive. However, the beneficial effects of concentration-doubled CO_2 is more than compensate the negative effects imposed by doubled O_3 and the latter in its turn partly counteracted the positive effects of the former.

Key words: O_3, CO_2, Drought stress, Photosynthesis, Lipid peroxidation, Antioxidative ability, Growth and Yield.

INTRODUCTION

Two aspects of global climate change that directly influence plant physiology, growth and productivity; increasing in concentrations of ambient ozone (O_3) and carbon dioxide (CO_2)[1,2]. Atmospheric CO_2 is projected to continue rising to at least 550 ppb by 2050[3]. The current annual average (O_3) is predicted to continue increasing by 0.5-2% per year over the next century, mainly due to increases in precursor emissions from anthropogenic sources[4,5].

Greenhouse effect is one of the important concern in present global change and the increase of concentrations of greenhouse gases is the main reason which resulted in the enhancement in the greenhouse effect. CO_2 is the most important greenhouse and carbon source for plant photosynthesis. O_3 in troposphere is essentially a pollutant6 and it restricts the growth of plant severely. The concentrations of CO_2 and O_3 have been increased continually and the responses of plant to them are regarded increasingly.

Ozone diffuses into the leaf apoplast via the stomata where it is rapidly converted into other reactive oxygen species (ROS) that signal a diverse metabolic response (Long and Naidu, 2002; Kangasjarvi *et al*., 2005; Hassan, 2006). Stress may promote the formation of harmful reactive oxygen species (ROS) which have the capacity to initiate chlorophyll bleaching, lipid peroxidation, protein oxidation, and injury to nucleic acids (Kangasjarvi

et al., 2005). The effect of CO_2 and O_3 on plant growth and productivity has been determined separately for a large number of plant species, but very little work has focused on their interactive effect[2,6,7,8].

The studies on combination effects of CO_2 with temperature, moisture[6,7] and effects of O_3 with SO_2 or NO_2[8,9] on plant have been reported. Interactive effects of CO_2 and O_3 on winter wheat[10], potato[11, 12], aspen and birch[13]. However, the research on interactive effects of CO_2, O_3 and drought, are seldom[15]. Although many studies addressed the effects of CO_2, O_3 and /or drought stress on plants in the developed world, no such study, to the best of knowledge, was conducted in developing world.

In this paper, respective and interactive effects of doubled CO_2 and O_3 concentration on wheat yield and biomass production, Photosynthesis, stomatal conductance, anti-oxidative ability and cell membrane lipid peroxidation of leaves in the context of free radical biology were studied in open-top chambers (OTC).

Though several previous studies report evocation of oxidative stress by water deficit stress in case of wheat[4,9,26] at individual level, information on their comparative response to same degree of stress in terms of their stress sensitivity and functional variation is lacking. In order to fill this gap of knowledge, this study was undertaken to evaluate oxidative response to O_3, CO_2 and/or drought singly and in combination of wheat plants. The hypothesis behind the work was the sensitivity of plants to water stress might be associated with different abilities for their carbon fixation, and also these stresses may affect to the net assimilation through their impact on stomatal conductance as well as antioxidant enzymes.

Therefore the aims of the present study were to describe interaction of enhanced O_3, elevated CO_2 and water stress on growth, seed yield, photosynthetic activities, photosynthetic pigments, antioxidant enzymes such as glutathione (GR), Peroxidase (POD), superoxide dismutase (SOD) and ascorbate Peroxidase (APX) as well as molecular biomarkers in durum wheat (*Triticum aestivum L.*) plants. Moreover, to study the importance of the accumulation of photosynthetic pigments which could change during exposure to multiple stresses, and this is the novelty of this experiment was a study of triple interaction of the mentioned factors.

MATERIALS AND METHODS

Plant materials, growth conditions and Experimental design

Grains of wheat (*Triticum aestivum L.*) plants were sown in pots with 20x20cm^2 filled with soil collected from top soil in the field. There were five plants/pot. They were transferred to four Open-Top Chambers (OTCS)[19] when second foliage leaf appeared. The treatments were: (a) control (FA), (b) O_3 without CO_2 (O_3), (c) FA with CO_2 (CO_2), (d) O_3 and CO_2 (O_3 +CO_2).

The experiment was split plot Latin square, one chamber was equipped with charcoal filter (FA) and the other was ventilated with FA + Target O_3 (78 ppb/h) and the third was ventilated with FA +Target CO_2 (450 ppm), while the fourth OTC was supplied with O_3 (78 ppb) and CO_2 (450 ppm).

There were 12 pots/chamber. Pots were distributed in a completely randomized block design (CRBD). Half of pots were irrigated to the field capacity while the other half was water-stressed to 0.5 MP[10, 12]. They were rotated within each chamber every week.

Biomass and grain yield

5 plants per pot were harvested for determination of grain and seed yield and yield attributes. The grains harvested were air-dried and the shoots, leaves and roots were dried at 80Cfor 72h. Number of grains per ear was counted. Yield per ear, yield per plant, and 1000-grain weight were determined.

Stomatal conductance and Net photosynthetic measurements

Net CO_2 assimilation rate (A) and stomatal conductance (gs) were measured using Infrared Gas Analyzer (IRGA,ciras-1PP System, Hitchin UK). Measurements were carried out on ten attached leaves per treatment on weekly basis.

Measurement of Photosynthetic Pigments

Photosynthetic pigments, chlorophyll a & b and carotenoids were extracted and from flag leaves and were determined by UV-spectrophotometer (LKB, UK.)[35, 36].

Antioxidant enzymes assays

Extractions of antioxidant enzymes from the leaves of the four treatments were performed[21]. Leaves were cut from each treatment and immersed in liquid nitrogen and kept in a deep freezer at 80°C until the analyses were performed at University of Newcastle, UK.

Samples were weighed and ground at about °C in 25 m Tris-HCl buffer containing 3 mM $MgCl_2$, then the homogenates were centrifuged at 20 000 for 15 min (Centrifuge17 S/RS, Heraeus Sepatech). The supernatants were used for the enzyme assays and the results were expressed on protein basis[35].

All assays were performed using a final volume of 1 mL, with at least duplicate assays undertaken on each sample. Moreover, the assays were end-point determinations.

SOD (EC 1.15.1.1) activity was monitored[21]. The extraction mixture contained 50 mM phosphate buffer solution (pH 7.8), 13 mM L-methionine, 63 lM nitro blue tetrazolium and 2 lM riboflavin. The ability of the extract to inhibit the photochemical reduction of nitro blue tetrazolium was determined at 560 nm (Schimadzu UV-1201 spectrophotometer). The amount of the extract resulting in 50% inhibition of nitro blue tetrazolium reaction is defined as one unit of SOD activity.

Catalase (EC, 1.11.1.6) activity was assayed in enzyme extract reaction mixture containing 50 mM phosphate buffer (pH 7.4). The reaction was started by adding 10 mM H_2O_2, and the reduction in absorbance was determined at 240 nm[14, 36].

GPX (EC, 1.11.1.7) activity was determined by adding 50 mM phosphate buffer (pH 6.1), 1% H_2O_2 and 1% guaiacol to the extract, and the absorbance was determined at 470 nm.

APX (EC, 1.11.1.11) activity was determined according to Maehly & Chance (1954). The reaction mixture contained 50 mM potassium phosphate, 0.5 mM ascorbate, 0.1 mM ethylenedimethyl tartaric acid (EDTA) and 0.1 mM H_2O_2, and the absorbance was determined at 290 nm.Protein concentrations of leaf extracts were determined as described earlier[26,37].

Data analysis

Data were subjected to three-way analysis of variance (ANOVA), using O_3, CO_2 and drought treatments as factors, followed by a least significant difference test, and P values ≤ 0.05 were considered significant (using the STATGRAPHICS statistical package, Package 3, UK) based on plot means.

RESULTS

Effects on visible injury symptoms

Visible injury symptoms appeared on the upper surface of flag leaves as point brown spots and by the end of experiment.

Number of injured leaves were increased by 2-fold due to exposure to O_3, while exposure to both CO_2 and O_3 increased it by 31% (Table 1). There was no significant effect ($p > 0.05$) of either CO_2 or drought stress and their interaction on number of injured leaves and on degree of injury. Degree of injury increased by 6-fold when exposed to O_3 and 4-fold due to exposure to both O_3 and CO_2. Drought stress and CO_2 protected plants against toxic effects of O_3. Plants exposed to D had 39% less leaf injury while exposure to both CO_2 and D had 50% lower leaf injury than plants exposed to O_3 alone (Table 1).

Effect on growth and yield

O_3 had greater effect on the numbers of ears per plant and the number of grains per ear than drought as these parameters was reduced by 20 and 26% respectively (Table 2). However, CO_2 caused increased by 26% and 18% in these parameters respectively. Moreover, the percentage reduction in 1000 grain weight due to O_3 (40%) was greater than that due to drought(29%),CO_2 alone increased it by 23%,interaction between stresses was less than additive. O_3 and Drought caused

significant reductions in all yield parameters measured in this experiment (Reductions reached maximum of 63% in dry mass and 54% in 1000 grains weight).

Effect on stomatal conductance and photosynthetic rates

It was clear that both O_3 and drought had the greatest negative effects on stomatal conductance (g_s) caused reduction by 41 and 50% respectively (over the entire period the experiment) (Fig.1). Moreover, interaction between O_3 and drought was great reduction more than additive (58% reduction). On the other hand CO_2 had a synergistic effect it caused increases to 12%, while it ameliorates toxic effects of O_3 and drought when applied with other stresses. The interactive effects of CO_2 and O_3 are contradictory caused greater reduction in g_s than CO_2 and drought by 17 and 11%, respectively. Interactions between different treatments were less than additive (21% reduction). After 10 weeks O_3 and drought exposure negative impacts were evident for Wheat plants revealed a 58% decline in stomatal conductance (g_s).

It was clear that O_3 and drought had the greatest effect on net photosynthetic rates (A) (Fig.2). O_3 and drought caused reduction in net photosynthetic rates (A) by 70% and 80%, respectively. On the other hand, CO_2 increased net photosynthetic rates A by 6%. Interaction between O_3 and CO_2 decreased it by 8%, while O_3 and drought decreased it to 12%. Exposure of Wheat plants to O_3 and drought simultaneously had the greatest decline effect on the net photosynthetic rates A by 88%. Drought had more pronounced negative effects than those other parameters. CO_2 mitigates toxic effects of drought and O_3, it mitigated O_3 and reduced its toxicity by to 23 % and drought to 19 %. Interactions between different treatments were less than additive.

Effects on antioxidant enzymes

A significant increase was observed in POD by 1.5-fold in Wheat plant treated with elevated CO_2; in contrast, elevated CO_2 had a reducing effect on GR and SOD by 11and 9 %, respectively. There was no significant (Pd≤0.05) effect of all treatments on APX.

O_3 caused increases in activities of GR, POD by 18 and 36 % respectively, while SOD was decreased by 11%. Drought had more pronounced effect on these enzymes as it caused increases by

Table 1: Effect of CO_2 and O_3, drought, singly and in combination on foliar injury symptoms of Wheat leaves in year 2005. (Means not followed by the same letter in each row are significantly different from each other at P≤0.05) (n=40).

Parameter	FA	O_3	CO_2	O_3+ CO_2	D	O_3+D	CO_2+D	O_3+CO_2+D
Number of injured leaves	3^a	18^c	4^a	14^{bc}	5^a	10^c	6^a	15^b
Degree of injury	0.14^a	0.82^d	0.12^a	0.52^c	0.15^a	0.61^c	0.56^c	0.43^b

Table 2: Effects of different treatments on yield parameters of Wheat (Triticum aestivium L.) plants grown under field conditions in open top chambers(OTCS).FA (346 ppm CO_2 + CFA); O_3(ambient CO_2+75 ppb O_3 7hd^{-1}(10.00-17.00 h); elevated CO_2 (702 ppm^1CO_2 +CFA) elevated CO_2 +O_3 (elevated CO_2+78 ppb O_3 7hd^{-1}). Plants were harvested 70 d after transfer to open top chamber (OTCs). (*mean P≤ 0.05; P < 0.01;*** P < 0.001).**

Parameter	FA	O_3	CO_2	D	O_3+CO_2	CO_2 +D	O_3+D	O_3+D+CO_2
no. of ears /plant	3.72*	3.02**	4.68**	3.00**	3.32*	3.52*	2.79*	2.92*
no. of grains /ear	41.5*	33.6**	49.86***	31.5**	39.4*	40.03*	30.21**	32.5**
1000 grain Wt (g)	56.7**	34.1**	61.3**	30.6**	46.10*	44.3*	28.2***	30.03**
Dry mass of grain/g	3.98**	1.92***	4.26**	1.80*	2.31**	4.01**	1.72*	1.79*

21% in POD while GR and SOD were decreased by 11 and 15%, respectively. The antioxidant enzymes; GR and SOD were significantly decreased in Wheat plants exposed to elevated CO_2 + elevated O_3 by 15 and 50%, respectively than in plants grown under normal conditions. CO_2 followed the same pattern of O_3. Interaction between O_3, CO_2 and drought and their multiple interactions were less than additive.

Table 3: Activities of glutathione reductase(GR).guaicol peroxidase (POD),superoxide dismutase (SOD) and Ascorbic peroxidase (APX) in extract Wheat leaves (70 DAP)

Treatment	GR activity (nmol $cm^{-2}S^{-1}$)	POD activity (nmol $cm^{-2}S^{-1}$)	SOD activity (μ cm^{-2})	APX (μ cm^{-2})
Control	0.28	5.62	4.61	0.32
O_3	0.33	7.63	4.12	0.29
D	0.25	6.80	3.92	0.30
CO_2	0.25	8.21	4.23	0.34
O_3+$C0_2$	0.24	5.82	2.35	0.31
O_3+D	0.25	4.39	2.02	0.29
CO_2+D	0.27	5.03	2.21	0.32
O_3+D+CO_2	0.30	6.12	2.98	0.30

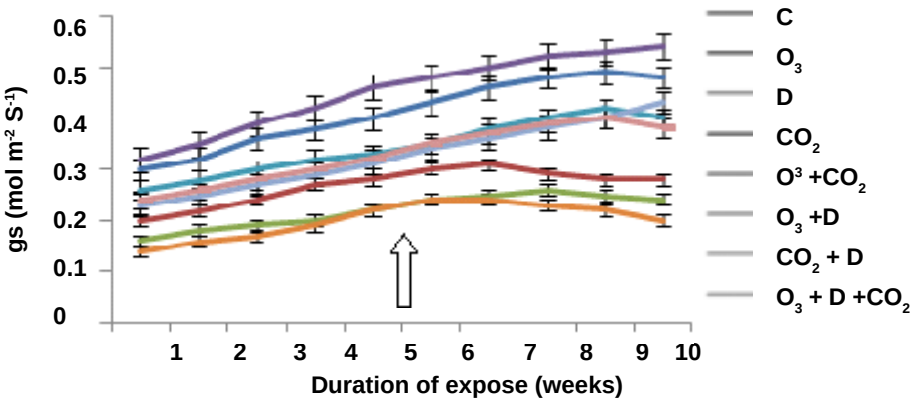

Fig. 1:Effects of different treatments on stomatal conductance (gs) mmol $m^{-2}s^{-1}$ of Wheat plants grown under field conditions in open top chambers(OTCS). Values are means of 10 replicates ± 1SE. An arrow indicates data of re irrigation of water -stressed plants.

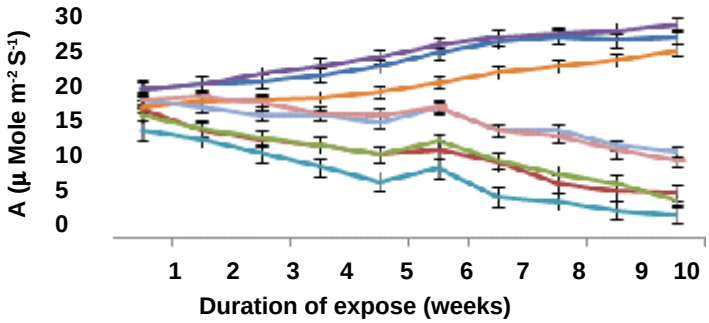

Fig. 2: Effects of different treatments on net photosynthetic rates (A) μmol m^{-2} s^{-1} of Wheat plants (*Triticum aestivum L.*) grown under field conditions in open top chambers(OTCS).

DISCUSSION

The results support the previous reports[22,23,24,25] that biomass and yield of crop plant were significantly increased under high CO_2 level but decreased under high O_3 level. In our case, the increased yield and biomass under doubled CO_2 were more than sufficient to eliminate the doubled O_3-induced yield decrease as shown in the treatment of the combination of doubled CO_2 and O_3 concentration.

It is known that enriched CO_2 increases photosynthesis[1,12] by providing more carbon source. This is the basis for the increase in yield and biomass. The increased photosynthesis also provides more reducing power and more biosynthesis of chlorophyll and carotenoids as well as enhancement of antioxidants concentrations. This would enhance the resistance of the plant to environment stresses, such as exposure to high O_3. The enhancement of the antioxidative ability is especially important since O_3 itself is a strong oxidant. Enriched CO_2 also decrease stomata conductance of the leaves. This would reduce the flux of O_3 into leaves though stomata. Take these two factors together, the effect of enriched CO_2 in amelioration of the harmful effect of O_3 is reasonable.

O_3 as a strong oxidant, is highly injurious to the plant tissues. It inhibits photosynthesis[1,2,12], decrease yield and biomass production[35,36,38]. It directly attacks the cell membranes inducing increase in lipid peroxidation and ion leakage. By inhibiting photosynthesis, the biosynthesis of the antioxidants or active oxygen scavengers may also be affected. The imbalance between the generation and scavenge of active oxygen would decrease the stress resistance of the plant.

It is interesting to note that when plants exposed to O_3 was less than 20 days, they responded in a way that it induced higher SOD activity, higher chlorophyll and carotenoids content. This indicates that small dosage O_3 or a short duration of exposure might not result in damage but induce acclimation response of the plant. But soon the accumulated dosage had increased to the level that intolerable to the plant and became injurious as indicated by the fast decrease in SOD activity, pigments content and increase in MDA accumulation and ion leakage[3,5,31]. The decrease in the anti-oxidative ability and increased production induced by high O_3 exposure is an indication of senescence. This is consistent to the fact that the plant exposed to doubled O_3 shed their leaves 7-8 days earlier than others.

It was found in the present study that elevated CO_2 confer some protection against O_3, and it was clear that stress caused by CO_2 predisposed leaves to injury caused by O_3 and not vice versa and this explain how they interact to alleviate foliar injury[39].

In the present study, increased GR concentration and POD activity in ambient air can be interpreted as response to oxidative stress imposed by O_3 (Chernkova *et al.* 2000). However, the lack of significant changes in activities of SOD and AA in ambient air, which also observed in other O_3 studies[40], differed from studies where activities of these enzymes increased in response to O_3[41]. The variability in the response of antioxidants to elevated O_3 and CO_2 among studies reflects differences in the magnitude of the perceived oxidative stress, the species-specific mechanisms involved in responses to changes in redox status, the plant capacity to cope with additional stress(s), experimental protocols and environmental conditions. Therefore, further studies are needed to further the understanding of the response of antioxidant metabolism to elevated O_3, CO_2 and drought singly and in combination[17,18,24,25,27,30].

CONCLUSIONS

Ozone exposure reduced corn grain yield in response to O_3 damage during the flowering process. Both O_3 and CO_2 had a major impact on physiological processes that are independent on PAR absorption. Therefore, radiation use efficiency (RUE) in response to gases treatments, in wheat, was significantly increased in response to CO_2 enrichment and significantly reduced in response to O_3-induced stress. While water use efficiency (WUE) was reduced in O_3- stressed plants grown under water stress and elevated CO_2. Similar results were observed for the control treatment and the

high-O_3/enriched-CO_2 treatment, indicating that the damaging effect of 0_3 air pollution was counteracted by the beneficial effect of CO_2 enrichment without any interactive effects between the two gases for the measured variables except for grain yield, during the first wheat experiment, where the CO_2-enriched environment more than overcame the O_3-induced stress.

Stomata closed under water stress treatment and decreased influx of O_3 and CO_3, which would affect growth, yield and physiology of both crops. Closure of stomata is beneficial as less O_3 influx but reduction in CO_2 influx would reduce photosynthetic rates of both crops and hence lowering yield

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