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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet

Asian Network for Scientific Information

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (2): 332-335, 1999 Research Article

Effect of O_3 on Ethylene Biosynthesis and Yield of Egyptian Cultivar of Wheat (*Triticum aestivum* L.)

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Abstract
An Egyptian cultivar of wheat (*Triticum aestivum* L.) was exposed to 60 nl l^{-1} O_3 (8 h d^{-1}) in open-top chambers for 61 d. Ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC), N-malonyl 1-aminocyclopropane-1-carboxylic acid (MACC) chlorophyll contents, visible injury symptoms and yield were measured in this study. O_3 was found to increase ethylene, ACC and MACC contents by 22, 29 and 38 percent, respectively, while chlorophyll content and yield were decreased by 29 and 61 per cent, respectively. The potential relationships between these parameters are discussed in the context of O_3 induction of accelerated senescence. These results have significance in terms of the maintenance of agricultural yields as O_3 rises in Egypt.

Introduction
Ozone (O_3) plays an important role in the earth's atmosphere and changes in its concentrations are of concern for several reasons: increased penetration of UV radiation, a contribution to global warming and direct toxic effects on terrestrial vegetation (Ashmore and Bell, 1991). Ambient concentrations of O_3 in Egypt are high enough to cause negative effects on sensitive crops (Hassan *et al*., 1995). O_3 is a strong oxidant, injuring plant tissues and disturbing the anatomical, physiological and biochemical functions of plants (Heath *et al*., 1984; Nast *et al*., 1993; Reddy *et al*., 1993; Mortensen *et al*., 1995). Although phytotoxic effects of O_3 are well known, very little is yet known regarding its possible effects on crop species in Egypt (Elkiey and Ormrod, 1987; Hassan *et al*., 1994; 1995; Hassan, 1998).
When plants are exposed to O_3, ethylene (C_2H_4) is produced (Taylor *et al*., 1988; Langebartels *et al*., 1991; Mehlhorn *et al*., 1991). It is known that biosynthesis of phytohorrnone ethene (ethylene) can reduce gene expression during fruit ripening, plant senescence and plant stress (Mehlhorn *et al*., 1991; Reddy *et al*., 1993; Hippeli and Elstner, 1996). Whether the emission of C_2H_4 regulates senescence-related responses to O_3 awaits further investigations.
Fuhrer and Grandjean (1988) observed that chlorophyll loss (yellowing) of wheat leaves was associated with increases in the synthesis of ethylene and its precursors 1-aminocyclopropan-1-carboxylic acid (ACC) and N-malonyl 1-aminocyclopropane-1-carboxylic acid (MACC) when plants were stressed by O_3.
The aim of the present study was to quantify the impact of O_3 on Egyptian cultivar of wheat (*Triticum aestivum* L.) in terms of growth, yield, ethylene and ethylene precursors.

Materials and Methods
Plant material and experimental design: Grains of an Egyptian cultivar of wheat (*Triticum aestivum* L.) were purchased from a commercial source in Alexandria. They were washed with tap water to remove excess fungicides and then imbibed with water over night. Wheat was sown in pots filled with loamy clay soil with pH 7.1 , at about 26/15°C day/night temperature in a glasshouse in Alexandria in 8/5/96. After emergence, seedlings were transferred in plastic bags to Germany on 29/6/1996 where they were thinned to one plant/pot. Pots were transferred to four open-top chambers (OTCs) of the design of Weight and Jager (1988) on 1/7/1996. There were 15 pots/chamber. Plants were irrigated twice a week with tap water.
Four OTCs were used in this experiment in a split plot design (for more details of 0_3 fumigation and experimental design (Hassan, 1998).

Visible injury assessment: Leaf injury symptoms we assessed on day 115 of fumigation by counting the number of injured leaves and estimating the percentage of each leaf's area showing chlorotic or necrotic spots (0 ' injury' to 5' 100%' injury).

Destructive harvest and chlorophyll determination: A single destructive harvest was performed at maturity on day 110 after sowing, with measurements of number of ears/plant number of grains/plant, grain weight and 1000-grain weight. Chlorophyll was extracted in 80 percent ethanol and determined on double beam spectrophotometer according to Knudson *et al*. (1977).

Analysis of ethylene and its biosynthesis: Ethylene and its precursors were determined after 20 minutes of incubation of small pieces of flag leaves (from the final harvest) in closed vials in the dark at room temperature. Use of this period removes any problem caused by the production of wound ethylene which starts to be produced about 25 minutes after excision. Ethylene emission was determined by gas chromatography according to Reddy *et al*. (1993). For 1-aminocyclopropane-1-1 carboxylic acid (ACC) and N-malonyl ACC (MACC) determinations, wheat leaves were ground to a fine powder in liquid nitrogen and homogenized in 10 cm^3 g 80 percent ethanol. The extract was centrifuged at 2800 rpm for 40 min at room temperature and the supernatant dried. The residue was dissolved in 1 cm^3 of water, partitioned against 1 cm^3 of chloroform and the aqueous phase assayed for ACC according to Langebartels *et al*. (1991). MACC was determined after neutralization of the extract with NaOH according to Reddy *et al*. (1993).

Statistical analysis: Data were subjected to one-way analysis of variance based on pot means using STATGIRAPHICS statistical package. There were no covariates used in ANOVA.

Results

O_3 was found to increase the number of injured leaves and degree of injury by 3-fold each (Table 1).

Table 1: Effect of O_3 on visible injury symptoms

Parameter	Treatment	
	FA	O_3
No. of injured leaves	0.10±0.02	0.40±0.08**
Degree of injury	0.12± 0.3	0.53±0.07***

FA = Characoal-filtered air

Table 2: Ethylene, ACC, MACC and chlorophyll content of foliage of wheat plants treated with either 60 nl l^{-1} O_3 or FA

Parameter	Treatment	
	FA	O_3
Ethylene (nmol/g fw)	0.09 ± 0.06	0.11 ±0.08*
ACC (nmol/g fw)	0.71 ±0.11	1.02 ± 0.17**
MACC (nmol/g fw)	0.94 ± 0.00	1.51 ±0.19"
Chlorophyll (mg/g fw)	5.83 ± 0.79	4.12 ± 0.58**

fw = fresh weight, FA = Characoal-filtered air

Ethylene emission increased by 22 percent in O_3 in the flag leaves (Table 2). Similar results were observed for ACC and MACC concentrations; their concentrations rose in response to fumigation with 60 nl l^{-1} O_3 by 29 and 38 percent, respectively (Table 2). Table 2 also showed that chlorophyll content decreased by 29 percent after exposure to O_3.

Table 3 summarises the effects of O_3 on yield and the major yield components. A close examination of this table indicates that the greatest overall contribution to the yield reduction was the reduction in the number of ears/plant (24%). There were larger effects on number of grains/ear (48%) and 1000-grain weight (20%) and this accounted for the large effect on yield (61% reduction) (Table 3).

Table 3: Effect of 0$_3$ on final yield of wheat

Yield parameter	Treatment		
	FA	O_3	% Reduction
No. of ears/plant	4.01	3.15	24
	(1.03)	(0.97)**	
No. of grains/ear	79.7	41.32	48
	(6.45)	(4.311***	
Grain dry wt./plant (g)	30.14	10.75	61
	(4.01)	(2.10)***	
1000-grain weight (g)	88.95	71.19	20
	(9.78)	16.541**	

FA = Characoal-filtered air, Values in parenthesis are±SE

Discussion

The present study showed the susceptibility of the cultivar chosen for visible injury symptoms from O_3 (both foliar injury parameters were increased by 3-fold each).

The chlorotic and necrotic spots reflect chlorophyll bleaching as a result of reactions involving O_2 activation, and this could be a response of physiological and biochemical impairments due to exposure to O_3. The percentage reduction in chlorophyll content was highly correlated to the degree of injury (R^2 = 0.89) and this is in agreement with the results of Knudson *et al*. (1977) and Fuhrer and Grandjean (1988).

Exposure to O_3 led to enhanced production of ethylene (C_2H_4), with higher accumulation of ACC and MACC. Excess C_2H_4 production occurs in response to a number of environmental stresses, including air pollutants (Stan and Schicker, 1982; Fuhrer *et al*., 1988; Mehlhorn *et al*., 1991; Reddy *et al*., 1993; Yin *et al*., 1994; Schraudner *et al*., 1994; Hippeli and Elstner, 1996; Finlayson and Reid, 1996). Ethylene is a phytohormone associated with senescence. The increase in C_2H_4 observed in this study is consistent with many earlier reports in the literature (e.g. Taylor *et al*., 1988; Langebartels *et al*., 1991; Yin *et al*., 1994). O_3 could be inducing C_2H_4 formation through a regulated stimulation of a precursor (e.g. stimulation of ACC synthase leading to formation of ACC). The results of the present study supports this possibility as there was an increase in ACC in wheat plants stressed by O_3. Alternatively free radicals a needed to convert ACC to C_2H_4 and O_3 could b mediating this step (Yang and Hoffman, 1984). MACC is formed when there is excess ACC which is not converted to C_2H_4. In O_3-stressed leaves the increase in C_2H_4, ACC and MACC correlates significantly with the reduction in chlorophyll content (R^2 = 0.46, 0.72, 0.67, respectively),(n = 30) and this is consistent with the results of Fuhrer and Grandjean (1988) with wheat leaves and Reddy *et al*. (1993) with potato leaves.

All yield component parameters measured in this study showed significant reductions following to fumigation with 60 nl l^{-1} O_3 (20-38%) and the reduction in total grain weight were attributed to the combined effects of reductions in the number of ears/plant, number of grains/ear and the 1000-grain weight. These are of great interest in that the results showed the sensitivity to O_3 of Egyptian cultivar of wheat and this is consistent with the results of many other workers e.g. Wahid *et al*. (1995), who reported 20 percent reductions in number of ears/plant, 19 percent reduction in number of grains/ear and 16 percent reductions in 1000-grain weight. Moreover, the reduction in 1000-grain weight is consistent with observations of Heagle *et al*. (1979), Mulchi *et al*. (1986), Fuhrer *et al*. (1989, 1992) and De Temmerman *et al*. (1992) after exposure to O_3.

Reduction in grain dry weight by 38 percent in plants fumigated with 60 nl l^{-1} O_3 compared with plants grown in charcoal-filtered air is considerably greater than nearly all those reported from OTC studies with wheat at similar concentrations in Europe, North America and Pakistan (e.g. Kohut *et al*., 1987; Fuhrer *et al*., 1989; De Temmerman *et al*., 1992; Nussbaum *et al*., 1995; Wahid *et al*., 1995). This may be due to difference in cultivar sensitivity, climate or experimental conditions.

The results indicate the potential for substantial yield losses and ethylene emission caused by O_3, in a region where yields needed to be increased to feed the rapidly increasing population and where pollution emissions are also increasing rapidly and this must be a matter of great concern. If such impacts are widespread, the implications for agricultural production are considerable not only for Egypt but also for many other developing countries. Moreover, the concentration used in this experiment (60 nl l^{-1}) was slightly lower than that recently recorded in rural area in Egypt (99 nl l^{-1}, as annual mean) (Hassan, 1999). Since the O_3 level is likely to continue to rise over the coming decades in many developing countries, it is clear that its potential impacts on agriculture need urgent consideration. Moreover, wheat plant has been the major crop plant to establish critical levels for O_3 (level I) in recent years, and in the present study 20000 nl l^{-1} h 0_3 in total was applied which is far above AOT 40 level of 3000 nl l^{-1} h. It would have been interesting in future investigation to determine biochemical parameters, symptoms and growth at the time point when the dose of 3000 nl l^{-1} h was recorded.

Acknowledgements

This work was jointly supported with fellowship visit. The Royal Society of London and C1M0 of Finland I.A.Hassan. Part of this project was supported with D.A scholarship to IAH. We would like to express our thanks Professor S. Barakat (Faculty of Science, Alexandria University) for his critical suggestions and revising manuscript. Our special thanks to Prof. H. Helal at FA his help in biochemical analysis.

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