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EXPLORING THE HIDDEN THREAT OF GASEOUS POLLUTANTS USING RICE (*ORYZA SATIVA* L.) PLANTS IN PAKISTAN

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Abstract

Three rice (*Oryza sativa* L.) varieties (Basmati-Pak, Basmati-370 and IRRI-9) of Pakistan were grown for their entire life cycle in open top field chambers to investigate the impacts of ambient ozone (O_3), nitrogen dioxide (NO_2), and sulphur dioxide (SO_2) on the biomass, growth physiology, and yield. The treatments were charcoal filtered air (FA), unfiltered air (UFA) and ambient air (AA). O_3, NO_2, and SO_2 concentrations recorded at the experimental site were 75, 29 and 18 ppb as 8 h daily mean, respectively. Microclimate conditions inside and outside the chamber environment remained virtually similar. All the rice cultivars showed severe depression in biomass (20-30%) due to reductions in number of tillers (20-27%) and leaves per plant (21-29%) during vegetative growth phase with maximum reductions seen in both Basmati-370 and IRRI-9 in UFA treatments compared with FA. These reductions were primarily due to decreased transpiration rate (10-20%), stomatal conductance (12-23%), net photosynthetic rate (9-22%) and photosynthetic efficiency (6-12%) of plants in UFA. Yield losses in total seed weight per plant in UFA were more or less similar in IRRI-9 (37%) and Basmati-370 (36%), followed by lower reduction in cv. Basmati-Pak (29%). Reductions in number of panicles per plant (18-25%), filled seeds per panicles (8-11%), seeds weight per panicle (13-17%) and 1000-seeds weight (5-8%) in UFA contributed significantly towards greater yield depression. Percent seed sterility was also higher in UFA and AA treatment plants. Starch contents of seeds in UFA were reduced by 14%, 19% and 21% in Basmati-Pak, Basmati-370 and IRRI-9, respectively when compared to FA control.

Introduction

Pakistan is endowed with varying climatic regions and land resources, very conducive for growing different varieties of rice (*Oryza sativa* L.). Rice is grown as an irrigated crop under special ecological and diverse soil conditions on two million hectares (about 10%) of the total cultivated area in the country. Punjab is the dominant rice-growing province with more than 55% share in total cultivated area, and is famous for production of fine aromatic rice all over the world. Rice is a versatile cash crop and Pakistan exports more than one million tonnes of rice annually, which is 10% of the total world rice trade. Rice is one of the most important cereal crops after wheat, because more than half of the world's population depends on rice for its basic diet (Wahid, 2003, Emberson *et al*., 2009).

It is now well documented that ambient ozone (O_3) is an all pervasive phytotoxic secondary air pollutant (Krupa *et al*., 1994) and its production in the troposphere is greatly favoured by many primary pollutants like oxides of nitrogen (NOx) and volatile organic hydrocarbons (HCs) in bright sunlight with high temperature and relatively still air (Krupa and Manning, 1988; Bell, 1986). Unlike other air pollutants, its elevated concentrations are often reported in rural backgrounds than urban centres (Bell and Cox, 1975; Ball and Bernard, 1978; Ashmore *et al*., 1980) and under appropriate conditions, chronic exposure of O_3 can result in rice growth and yield reductions (Kobayashi *et al*., 1995; Wahid *et al*., 1995a; Maggs and Ashmore, 1998). O_3 is known to impair plant metabolism leading to yield reduction in agricultural crops (Pleijel *et al*., 1991; Grandjean-Grimm and Fuhrer, 1992). The influence of O_3 on vegetation is dependent on doses, genetic background and developmental phase of plants (Calatayud *et al*., 2004), and frequently has harmful effects on crops that reduces the net photosynthetic rate (Soja *et al*., 1998; Koch *et al*., 1998) by destroying the structure and function of biological membranes leading to electrolyte leakage (Soldatini *et al*., 1998; Guidi *et al*., 1999), which may alter the properties of thylakoids, thereby changing the yield of chlorophyll fluorescence (Reichenauer *et al*., 1998) and a decrease in fluorescence ratio (Fv/Fm) in ozone-treated plants.

Open top chambers (OTCs) have been widely used in Europe under European Open-top Chamber Programme (EOTC) and under National Crop Loss Assessment Network protocol (NCLAN) in the United States (Jager *et al*., 1993) and represent one of the best techniques for the study of the impact of ambient air pollutants on plant performance (Unsworth and Geissler, 1993) because they have the advantages of widespread use, portability, moderately cost and ease of maintenance (Bell and Ashmore, 1986). Most certainly many important advances in our understanding of the impact of air pollution on plants have arisen through the use of air-filtration and pollutants dose-response experiments involving OTCs (Ashmore and Bell, 1994; Wahid *et al*., 2001)

Air pollution has been a problem for centuries (Evelyn, 1661; Oliver, 1894; Bell, 1984), although it has recently been felt in many developing countries including Pakistan (Emberson *et al*., 2003; 2009; Wahid, 2006a & b). Atmospheric quality in major cities of Pakistan is rapidly deteriorating, because the infra structure development in big cities has not kept pace with rising population flux; thereby giving rise to poor planning, faulty traffic system and blatant disregard for motor vehicle maintenance (Faiz and Sturm, 2000). Thus, emission levels are rising rapidly in the region with significant yield reductions reported in economically important crops in Pakistan (Emberson et *al*., 2001).

The present investigation was undertaken after more than 15 years in order to clarify some uncertainties in a preliminary study on the yield of two rice varieties using OTCs in Lahore region during 1992 growth season by Wahid *et al*., (1995a). In this study, three newly released rice varieties were grown a the same site in Lahore using OTCs and a comprehensive vegetative growth analysis (biomass, transpiration rate, stomatal conductance, net photosynthetic rate and photosynthetic efficiency), yield and grain composition with respect to starch was carefully done. The main aim behind was to monitor the current levels of ambient air pollution in the region and to check the response of new rice varieties to ambient gaseous pollutants, and also to verify whether the elevated level of air pollution (especially ambient O_3) was primarily responsible for the greater yield losses or not in rice crop.

Materials and Methods

Site description: Impact of ambient air pollution on the growth and yield of rice (*Oryza sativa* L.) was studied in OTCs during 2007 rice growth season. Three locally grown, currently recommended cultivars of rice viz., Basmati-Pak, Basmati-370 and IRRI-9 was studied in this experiment using seed obtained from Ayub Agricultural Research Institute (AARI), Faisalabad. Equal sized healthier seeds of rice cultivars were carefully sorted from a fresh lot while discarding the infected ones.

Soil and pot preparation: Normal field soil collected from a cultivated field of Botanic Garden was sieved to remove roots and stones etc. Farm-yard-manure (FYM) and humus were air-dried and also sieved to remove stones or humps, or any decaying twigs etc. Sieved soil, FYM and humus were mixed in 8:1:1 ratio, respectively, and stored in a shed before use in the experiment. Selected plastic pots (30 cm diameter) were thoroughly cleaned with tap water before use in the experiment. Pots were also labelled with permanent-ink marker for the respective rice cultivar, air treatment, replicate number, and filled with equal amounts (approximately 10 kg) of specially prepared garden compost.

Seedling transplantation: Rice seedlings of all the cultivars were raised separately in plastic trays in the third week of May 2007, and healthier seedlings of equal length and vigour were then transplanted in the center of each pot during 1^{st} week of June 2007. Transplantation was carried out in the evening to avoid harsh daytime environmental conditions and pots were transferred to relevant OTCs and unchambered ambient air field plots on 10^{th} June 2007.

Experimental design and crop management: Twelve plots (1.5m diameter) were assigned to four replicates of three treatments; charcoal-filtered air (FA) unfiltered air (UFA) and unchambered ambient air field plots (AA). OTCs of the design by Bell and Ashmore (1986) were used in this study. The details of OTCs are given in Wahid *et al.*, (1995 a, b). Six replicate pots of each cultivar containing one plant per pot were placed in each plot on 10^{th} June 2007; giving 24 plants of each cultivar per treatment. Pots of each rice variety were rotated inside the chambers on weekly basis and between the replicate treatment chambers on monthly basis in order to minimize any effect of environmental variables especially of light intensity on plants. Three pots of each cultivar per treatment i.e., 12 plants of each cultivar per treatment were harvested after 10 weeks of treatment for assessment of biomass, while three pots of each cultivar (12 plants of each cultivar/treatment) were harvested after complete maturity. Plants were irrigated with equal amount of tap water on alternate days and any weeds or insects/pest appearing in the pots was removed manually. The use of chemical fertilizers, pesticides or herbicides was avoided through out the course of the experiment.

Measurements of growth physiology: Two days prior to mid-season harvest (10-weeks-old plants), growth physiology of 12 plants/cultivar/treatment were recorded for transpiration rate (E), stomatal conductance (g_s), and net photosynthetic rate (P_N), of youngest unfolded healthy leaf of main stem using an Infrared Gas Analyser (IRGA) LCA-2 attached to a Parkinson Broad Leaf Chamber (Analytical Development Company, Herts, U.K.). Measurements were carried out between 0900-1600 hours in the natural light conditions on a bright sunny day using 12 flag leaves per cultivar per treatment. Photosynthetic efficiency (Fv/Fm) of already selected leaves was also noted by using a portable Plant Efficiency Analyser (PEA, Hansatech Ltd., Kings Lynn, England). Leaves were dark adapted for 30 minutes using a light-excluding plastic clip with a shutter. The clip was then covered by a sensor head containing a fibre optic system. When the shutter was opened, the enclosed leaf area (circle of 5 mm diameter) was irradiated for 5 seconds with a flash of saturating light, strong enough to detect chlorophyll fluorescence data through an attached microprocessor.

Harvesting protocol: First destructive harvest (mid-season harvest) of 10-weeks-old plants was taken in order to assess the effects of air pollutants on vegetative growth parameters and biomass. Plants were carefully removed from the pots by giving them several washings under tap water, and any loss of roots was avoided by placing 2 mm sieve under the plants. Parameters such as tillers per plant, leaves per plant, shoot and root length, fresh weight of shoot and root was measured on an electric balance. Plants were then placed in labelled paper bags in an oven for drying at 80°C for 48 hours and then their dry weights were taken. At the approach of crop maturity (21-weeks-old plants), a detailed destructive harvest was made by cutting the plants at soil level and placed in pre-labelled paper bags and brought to the laboratory for measurements of various reproductive growth parameters viz., panicles per plant, filled and unfilled seeds per panicle & per plant, seeds weight per panicle & per plant (g), and 1000-seeds weight (g). Straw dry weights (g) were determined to find Harvest Index (ratio of total seed weight to total straw weight) according to Wahid *et al.*, (1995 a, b).

Starch analysis: The seeds obtained from various air-treatments were then checked for nutritional quality. Starch content was analysed enzymatically after alkaline hydrolysis following Boehringer (1986), which was based on the method described by Beutler (1978).

Microclimate and pollution climate monitoring: The OTCs were operated as closely as possible following Jager *et al*., (1993). This necessitated microclimatic measurements within and outside the chambers to assist in interpreting the results. Air temperature and relative humidity were measured by using a temperature humidity probe (Thermo-hygrometer, HI8564, Hanna Instruments, USA), while light intensity was measured with the help of a portable light meter (Horticultural Lux Meter, OSK2711, Ogawa Seiki Co, Tokyo, Japan). All these environmental variables were measured three times daily (0800, 1200 and 1600 hrs) at crop canopy height in the center of chambered and unchambered plots. In order to minimize the difference in light levels between inside and outside the OTCs, dust on the polythene walls of OTCs was washed on regular basis every day. O_3 concentrations were continuously recorded during 0900 to 1700 hrs daily using UV absorption O_3 analyser (Model 1100, Dylec Inc., Japan), while SO_2 and NO_2 concentrations were monitored using Enerac-2000 (Energy Efficiency System, Inc., Westbury, New York). O_3, SO_2 and NO_2 were determined in all treatments in the center of each plot at crop canopy height and as the crop grew up, monitoring heights were also adjusted accordingly.

Statistical analysis: Duncan's multiple range test (at 5% significance level) was carried out using statistical software Costat version 3.02 between treatment means based on chamber/plot means, while % reductions, standard errors and graphics were made in Symphony & Microsoft Excel, respectively.

Results

Ambient and microclimate: Daily means of ambient air temperature, light intensity and relative humidity were collected from the data recorded three times a day (i.e. 0800, 1200 and 1600 hrs) at the experimental site and further processed to calculate monthly and seasonal means. Light intensity at the start of the experiment in June showed steady decline with the passage of time till the termination of the experiment in November. However relative humidity showed an opposite trend of lowest values at the beginning of experiment and maximum at the end (Fig. 1) Seasonal mean ambient climatic conditions (outside chamber environment) for air temperature and light intensity were 34°C and 49 Klux, respectively, while relative humidity was 44% during rice season (Table 1). Looking at the microclimatic conditions (inside chambers) from Table 1, the results showed little impact of chambers on the growing environment of crop and the differences between inside and outside the OTCs remained more or less similar from month to month with seasonal mean air temperature increased by only 1°C inside, light intensity reduced by 3% and relative humidity was slightly higher (1%). This slight increase in temperature inside OTCs was primarily due to heating effects of motor and fan system.

Air pollution climate: Air pollutants concentrations remained virtually similar in both UFA and AA treatments and were appreciably much higher than lower concentrations recorded through out experimentations in FA. The 8 h seasonal mean concentrations for O_3, NO_2 and SO_2 were 75, 29 and 18 ppb, respectively in both UFA and AA treatments, while in FA, the concentrations of O_3, NO_2 and SO_2 were 8, 9 and 3 ppb, respectively. Overall pattern of air pollution climate in UFA during the experimental season is shown in Fig. 2. It is interesting to know that O_3 concentrations decreased from month to months as light and temperature decreased, but NO_2 concentrations showed an opposite trend. However, SO_2 concentrations remained more or less similar with minor fluctuations in different months. Filtration efficiencies were 89% for O_3, 83% for SO_2 and 69% for NO_2 during the course of experiment.

Biomass assessment: First detailed mid-season harvest of plants was taken in order to investigate the impact of ambient air pollution on plant biomass after 10-weeks of treatment when the pants had completed their vegetative growth phase (Table 2). Plants from both UFA and AA treatments were almost similar with stunted growth and higher rate of their leaf senescence as compared to comparatively healthier and better developed with lush green, relatively longer and more expanded leaves of plants from FA treatment in all the cultivars. It is evident from the Table 2 that the values of all the parameters of vegetative growth in plants from FA were higher and statistically significant than that of their counterparts in both UFA and AA treatments. Increased tillering in all the rice cultivars in FA resulted in more leaves per plant while reduced tillering in UFA and AA led to less number of leaves per plant. In UFA, reduction in total number of tillers per plant was highly pronounced in IRRI-9 (27%) than that of Basmati-370 (24%) and Basmati-Pak (20%) as compared to FA. Total number of leaves per plant was reduced by 29%, 24% and 21%, respectively for IRRI-9, Basmati-370 and Basmati-Pak in UFA. Shoot and root lengths were reduced significantly in all the cultivars, with higher reductions found in IRRI-9. Total plant fresh weight was comparatively lower in IRRI-9 (29%) than both Basmati-Pak and Basmati-370 (25% in each), while magnitude of reduction in total plant dry weight was 31%, 28% and 26%, respectively for IRRI-9, Basmati-370 and Basmati-Pak in UFA than that of FA. The reductions in various parameters of vegetative growth in AA compared to UFA were much less and remained non-significant in most cases. In general, the magnitude of reduction in biomass was highly pronounced in UFA treatment than FA control plants (Fig. 3).

Table 1. Ambient climate; mean monthly and seasonal summaries during rice growth season.

Months	Days	Temperature (°C)			Light intensity (Klux)			Relative humidity (%)		
		Inside	Outside	Diff.	Inside	Outside	Diff.	Inside	Outside	Diff.
Jun.	10	42.41	41.23	+1.09	59.39	60.92	-1.53	32.14	30.86	+1.28
Jul.	31	39.43	38.32	+1.11	54.20	55.76	-1.56	38.57	37.31	+1.26
Aug.	31	36.21	35.09	+1.12	52.83	54.38	-1.55	42.51	41.27	+1.24
Sep.	30	34.89	33.76	+1.13	46.85	48.42	-1.57	49.37	48.13	+1.24
Oct.	31	31.07	29.94	+1.13	40.94	42.52	-1.58	53.24	52.02	+1.22
Nov.	12	28.09	26.95	+1.14	31.40	32.99	-1.59	57.51	56.32	+1.19
Seasonal:	**145**	**35.35**	**34.22**	**+1.12**	**47.60**	**49.16**	**-1.56**	**45.56**	**44.32**	**+1.24**

Table 2. The performance (average per plant per pot) of 3 varieties of rice grown in open-top chambers with or without filtered air at mid season harvest (10-week-old plants).

Parameters	Basmati-Pak			Basmati-370			IRRI-9		
	FA	UFA	AA	FA	UFA	AA	FA	UFA	AA
Tillers per plant	21.8a	17.5b	16.8b	19.4a	14.7b	13.9b	29.9a	21.7b	20.5b
Leaves per plant	89.9a	71.4b	67.3c	77.3a	58.6b	53.8c	113.6a	81.2b	75.5c
Shoot length (cm)	68.7a	63.4b	62.6b	66.5a	60.3b	59.3b	43.5a	37.7b	36.8b
Root length (cm)	63.6a	58.0b	57.5b	61.2a	55.2b	54.7b	39.4a	33.4b	33.0b
Shoot fresh wt. (g)	196.4a	148.5b	147.1b	164.5a	123.5b	122.3b	194.5a	138.6b	137.2b
Root fresh wt. (g)	152.7a	114.0b	113.6b	129.6a	96.1b	95.1b	161.6a	114.2b	113.4b
Total plant fresh wt. (g)	349.1	262.5	260.7	294.1	219.6	217.4	356.1	253.1	250.6
Shoot dry wt. (g)	58.9a	43.5b	43.0b	53.2a	39.2b	38.7b	61.7a	43.1b	42.2b
Root dry wt. (g)	40.6a	29.7b	29.3b	35.7a	24.7b	24.3b	45.6a	31.2b	30.2b
Total plant dry wt. (g)	99.5	73.2	72.3	88.9	63.9	63.0	107.3	74.3	72.4
Root shoot ratio	0.69a	0.68b	0.68b	0.67a	0.63b	0.63b	0.74a	0.72b	0.71b

Treatment means followed by different letters in the same row are significantly different from one another according to Duncan's multiple range test at $p=0.05$. FA: Filtered Air, UFA: Unfiltered Air, AA: Ambient Air.

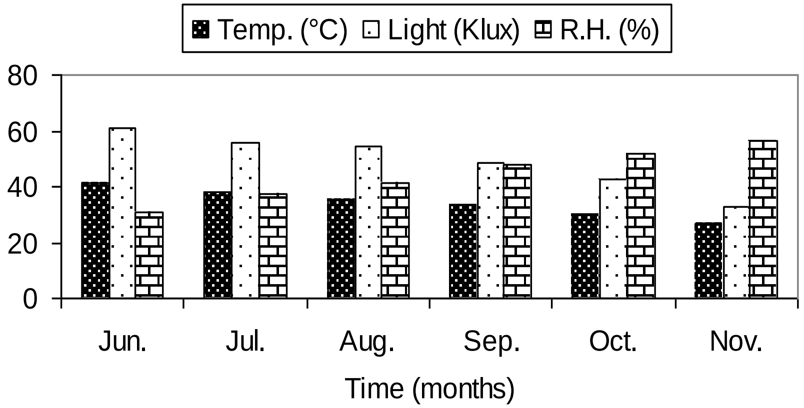

Fig. 1. Ambient climatic conditions at the experimental site at Lahore during 2007 rice season.

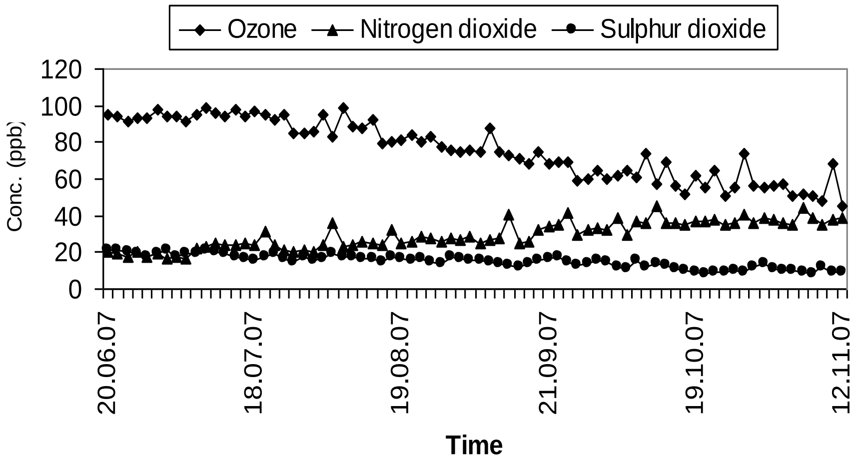

Fig. 2. Ambient levels of O_3, NO_2 and SO_2 in UFA during rice season 2007. [Concentrations represents 8-h daily mean].

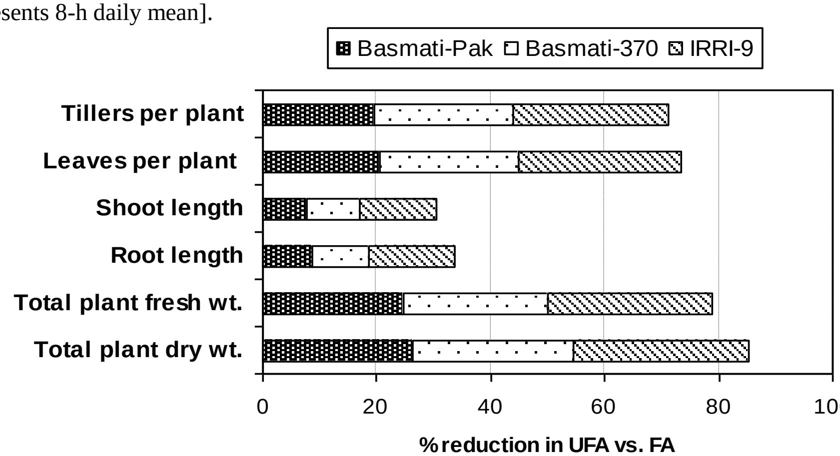

Fig. 3. Biomass reduction (%) in rice varieties in UFA as compared to FA controls at the prime of vegetative growth (10-weeks-old plants).

Growth physiology: Plants grown in FA treatment showed higher values for various parameters of growth physiology than that of both UFA and AA treatments (Fig. 4). The magnitude of reduction in UFA compared with FA for transpiration rate (*E*) was highest in IRRI-9 (20%), followed by Basmati-370 (19%) and comparatively less in Basmati-Pak (10%). Stomatal conductance (g_s) was reduced by 12%, 19% and 23% respectively for Basmati-Pak, Basmati-370 and IRRI-9. Similar pattern of reduced net photosynthetic rate (P_N) and photosynthetic efficiency (Fv/Fm) was found in plants grown in UFA. Overall, P_N were reduced by 9-22% and Fv/Fm by 6-12% in different cultivars following the same trend of highest reductions in IRRI-9 and lowest in Basmati-Pak. However, plants grown in AA and UFA treatments were almost alike but far different from FA control (Fig. 4).

Reproductive growth: At the culmination of reproductive growth, a final destructive harvest was taken after 21 weeks of treatment and the data on various parameters of yield are summarized in Table 3. Yield parameters were higher and statistically significant in all the cultivars in plants from FA than that of both UFA and AA treatments. In UFA, yield losses were highly pronounced showing reductions in total seed weight per plant by 37% for IRRI-9, 36% for Basmati-370, and 29% for Basmati-Pak. This yield reduction in UFA compared to FA was basically due to combined reductions in number of panicles per plant (18-25%), number of filled seed per panicle (8-11%), seed weight per panicle (13-17 %), and 1000-seed weight (5-8%) in different cultivars with highest reduction seen in cultivar IRRI-9 (Fig. 5). Increased % seed sterility in UFA was also recorded in all the cultivars. The magnitude of reduction for straw weight and harvest index was significantly higher in all the cultivars grown in UFA compared with counterparts grown in FA. The results of plants in AA when compared with UFA treatments showed more or less similar and negligible effects on yield and yield components but sometimes differences between them were statistically significant in case of certain parameters (Table 3). Seed obtained from different cultivars showed higher starch contents in FA than both UFA and AA (Fig. 6). Starch content was reduced by 14% in Basmati-Pak, followed by 19% and 21% reductions observed in Basmati-370 and IRRI-9 respectively.

Discussion

Open-top chamber system proved to be robust and relatively low cost technique to ascertain the effects of ambient air pollution of crops in the developing countries like Pakistan. Ambient climate showed a progressive decline in light levels and temperature from June to November during this study while relative humidity showed an opposite trend as shown in Fig.1. The temperature inside the OTCs was increased only by 1.1°C which is consistent with the earlier studies (Colls *et al*., 1993; Wahid *et al*., 1995a, b), light levels were only slightly reduced (3%) inside the OTCs which is also supported by many workers (DeTemmermann and Traenniers, 1993; Wahid *et al*., 1995a, b), while relative humidity was 1.2% higher inside the OTCs in present study - a reflection of higher evapotranspiration rate under hot and dry climate at the site, and is consistent with Wahid *et al*., (1995a, b). An inevitable consequence of the OTCs design which allows incursion of ambient air through the open top is that the filtration efficiency of the chambers operating with charcoal is not 100%, and the charcoal filters are most efficient in absorbing O_3 and SO_2 than NO_2 (Unsworth & Giessler, 1993). Filtration efficiencies for O_3 and SO_2 were 89% and 83% respectively, and remained much higher than NO_2 (54%) in this study, and are well consistent with Fangmeier *et al*., (1994), Wahid *et al*., (1995a, b) and Gruters *et al*., (1995). Thus OTCs design proved extremely effective under climatic conditions of Pakistan.

Table 3. Yield components (average per plant per pot) of 3 cultivars of rice grown in open-top chambers, with or without filtered air after final harvest (21-weeks-old plant).

Parameters	Basmati-Pak			Basmati-370			IRRI-9		
	FA	UFA	AA	FA	UFA	AA	FA	UFA	AA
Panicles per plant	22.5a	18.5b	17.7b	20.8a	15.8b	14.5b	31.4a	23.5b	22.7b
Filled seeds per panicle	163.6a	148.9b	145.4b	154.1a	136.7b	135.1b	160.5a	147.9b	145.9b
Unfilled seeds per panicle (% sterility)	11.2b (6.8)	22.6a (15.2)	24.1a (16.6)	12.6b (8.2)	26.8a (19.6)	26.7a (19.8)	9.9b (6.2)	21.2a (14.3)	22.2a (15.2)
Filled seeds per plant	3685a	2749b	2566c	3198a	2161b	1961c	5046a	3440b	3315c
Seed weight per panicle (g)	4.7a	4.1b	4.0b	4.5a	3.8b	3.7b	5.4a	4.5b	4.5b
Seed weight per plant (g)	105.7a	75.3b	70.1c	92.3a	59.5b	53.9c	169.2a	106.1b	101.1c
1000-seed weight (g)	28.7a	27.4b	27.3b	28.9a	27.5b	27.4b	33.5a	30.8b	30.5b
Straw dry weight (g)	85.3a	61.9b	57.4c	78.2a	56.9b	51.9c	76.5a	52.5b	50.2b
Harvest index	1.24a	1.22b	1.22b	1.18a	1.04b	1.04b	2.21a	2.02b	2.01b

Treatment means followed by different letters in the same row are significantly different from one another according to Duncan's multiple range test. Legend as in Table 2.

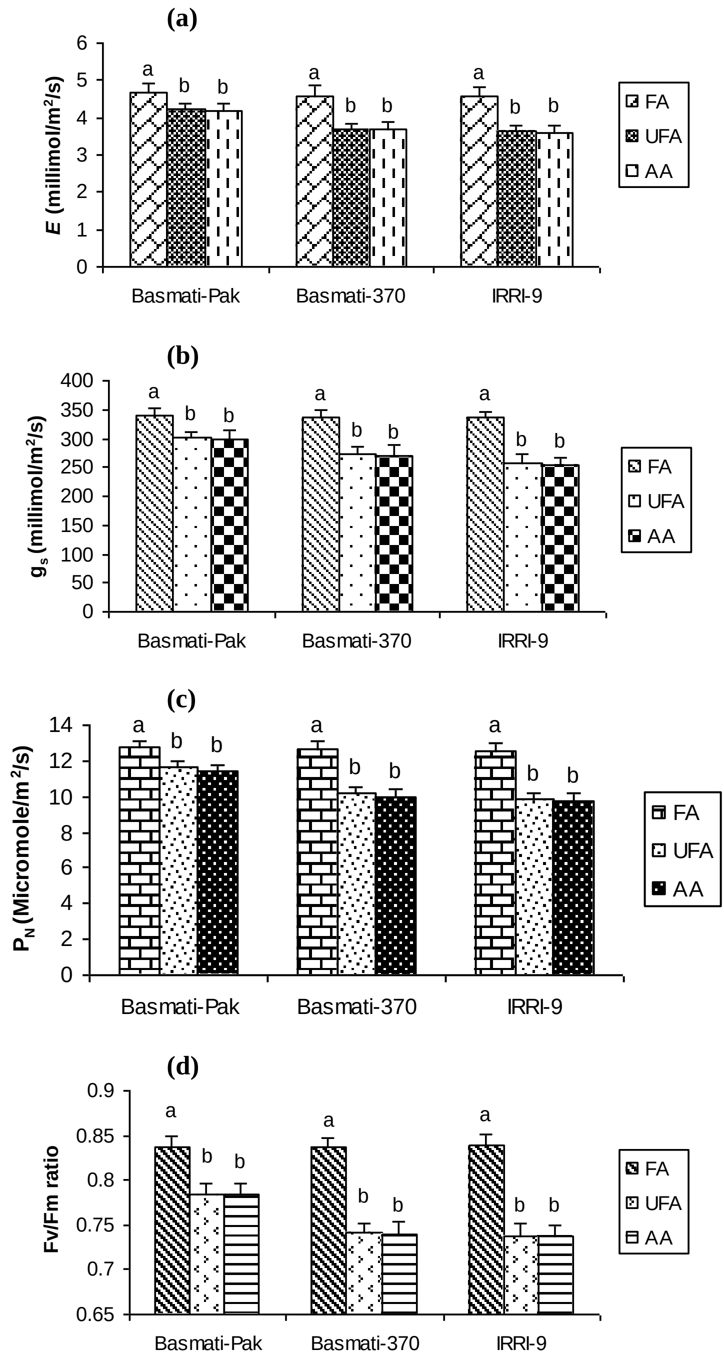

Fig. 4. Impact of air pollution on (a): transpiration rate (*E*), (b): stomatal conductance (g_s), (c): net photosynthetic rate (P_N) and (d) photosynthetic efficiency (Fv/Fm) of 10-weeks-old rice plants. Bars with different letters are significantly different according to Duncan's Multiple Range Test at P=0.05. ± represents standard error.

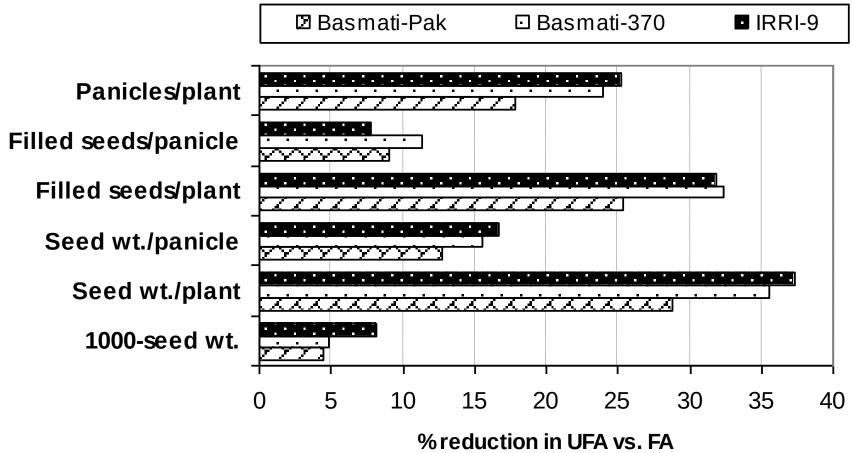

Fig. 5. Yield reduction (%) in rice varieties in UFA compared with FA counterparts after final harvest.

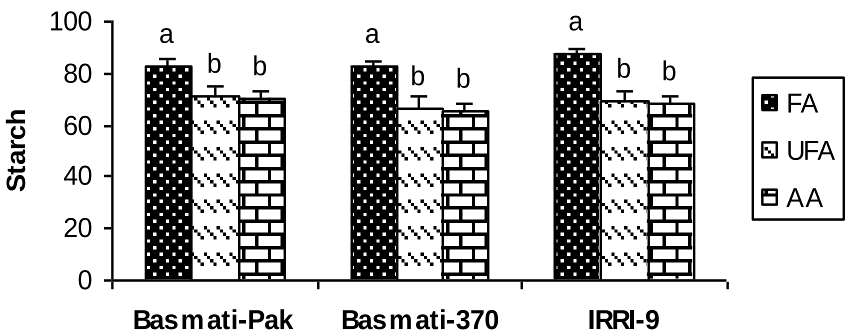

Fig. 6. Influence of air pollution on starch contents of rice seeds (on % dry weight basis) after final harvest. Bars with different letters are significantly different according to Duncan's Multiple Range Test at P=0.05. ± represents standard error.

The effects of ambient air pollution on the vegetative growth of rice cultivars after 10 weeks of exposure in OTCs were highly significant in UFA as compared to FA (Table 2). Parameters of biomass assessment showed lower values in UFA compared with FA treatment. The most obvious effects found were on the number of tillers per plant which were reduced by 20-27%, number of leaves per plant by 21-29%, total plant fresh weight by 25-29%, and total plant dry weight by 26-31%, for different rice cultivars (Fig. 3) and are constantly reported by many researchers (Wahid *et al.*, 1995a; Kobayashi *et al.*, 1995; Nouchi *et al.*, 1995). A more elaborated study by Maggs and Ashmore (1998) has reported results of two experiments on rice cultivars viz., IRRI-6 and Basmati-385. In their first experiment, IRRI-6 was exposed to a mean O_3 concentration of 43 ppb as 8 h/day for 133 days, while in another experiment; they fumigated rice cultivars with O_3 at 40-42 ppb (8 h/day) and NO_2 at 21-23 ppb (24 h/day), both singly and in combination. According to them, O_3 was more toxic than NO_2 and when used in combination their synergistic effects were additive in case of many parameters. They also reported that O_3 caused earlier leaf senescence in UFA and reductions in many parameters i.e.

photosynthetic capacity, tiller numbers per plant, plant height, total plant dry weight. However, the reductions were of lower magnitude as reported in this study. Maggs and Ashmore (1998) emphasized that O_3 exposure caused reduction in photosynthetic rate for the youngest leaves by 8% compared to reductions of 25% and 35% for leaves of increasing age. They also reported reduction in various growth parameters due to O_3 + NO_2 interactions. For instance, plant height, number of tillers per plant and onset of earlier leaf senescence was found due to O_3 + NO_2. These O_3 + NO_2 behaviours showed similar interaction reported by Bender and Weigel (1993) confirming the synergistic action of these pollutants. In general, results of present study showed similar reductions in biomass of rice varieties of Pakistan due to 8-h mean O_3 concentration of 75 ppb along with NO_2 concentrations of 25 ppb as that reported in fumigation studies by Maggs and Ashmore (1998) using 8 h/day O_3 concentrations of 43 ppb and NO_2 around 21-23 ppb.

Kats *et al*., (1985), Nouchi *et al*., (1991), Kobayashi *et al*., (1995) and Welfare *el al*., (1996) have reported reduction in plant height, increased rate of leaf senescence and reduction in total number of leaves per plant along with reduced plant biomass in responses to O_3 and SO_2, although the responses in the present study are much greater than the work of the above reported studies. Kats *et al*., (1985) using OTCs reported reductions in total plant dry weight (0.5-7.0%) at 50 ppb O_3 of three California rice varieties after 68 days of exposure for 5 h/day for 5 days/week. Similarly, Nouchi *et al*., (1995) reported total plant biomass reduction of 17% in Japanese rice varieties grown in culture solution at 50 ppb O_3 after 68 days of exposure 8 h/day for 7 days/week in environmentally controlled glass chambers. Jin *et al*., (2001) working with OTCs experiments in China demonstrated that O_3 has significant effect on growth of rice crop with more reductions in root dry weight than shoot fresh weight due to elevated O_3 exposures (50-200 ppb). According to them O_3 caused 14%, 31% and 52% decrease in total plant biomass per plant due to O_3 concentrations of 50, 100 and 200 ppb. These comparisons showed that Pakistani rice cultivars might be slightly more sensitive to O_3 than USA and Japanese or Chinese rice cultivars.

Growth physiology of 10-weeks-old rice plants has also revealed a significant reduction in photosynthesis parameters such as *E*, g_s, P_N and Fv/Fm (Fig. 4). The reduction in growth physiology parameters in response to ambient gaseous pollutants (O_3, NO_2, SO_2) in the present study is in agreement with a number of studies on cereal crops (Farage *et al*., 1991; McKee *et al*., 1995; Plazek *et al*., 2000). According to Ojanpera *et al*., (1998), exposure of wheat cv. Satu in OTCs during the periods of anthesis (45 ppb O_3, 8-h/day for 4 weeks) reduced the rate of photosynthesis of flag leaves by 40%. A decrease in flag leaf and canopy photosynthetic rate of 44% and 23%, respectively, was reported (Grandjean-Grimm & Fuhrrer, 1992) in wheat cv. Albis after season-long exposure to ozone (35-50 ppb) in OTCs along with partial closure of stomata in response to high O_3 concentrations. Plazek *et al*., (2000) working with cereals in Poland also reported accelerated rate of senescence and yellowing of leaves in barley under ozone stress of 180 µg kg^{-1}. They reported decrease in net photosynthetic rate (P_N) of 15% in barley than control, along with reduced photosynthetic efficiency (F-v/F-m) due to O_3 treatments. Ishii *et al*., (2004a, b) found reduced morphological responses in two Malaysian rice cultivars (MR84 and MR185) due to O_3 (30-90 ppb) in OTCs. In a recent study, Ariyaphanphitak *et al*., (2005) reported reduced biomass in Thai Jasmine rice cultivars in OTC experiments in Japan. They reported most severe damage of high O_3 (100 ppb and 150 ppb) on photosynthetic components, namely chlorophyll, carotenoids, and net photosynthesis. O_3 actually causes closure of stomata and strongly affects photosynthesis and respiration resulting in poor growth of plants and any decline in photosynthetic efficiency (Fv/Fm ratio) indicates chronic photoinhibition (Maxwell & Johnson, 2000; Calatayud *et al.*, (2004).

Present study reports significant yield losses at 8 h mean concentrations of 75, 29, 28 ppb respectively for three pollutants like O_3, NO_2 and SO_2 during the rice season 2007. Total seed weight per plant was reduced by 37% in IRRI-9, 36% for Basmati-370 and 29% for Basmati-Pak in UFA compared with FA control plants. This yield reduction in UFA was primarily due to combined reductions in panicles per plant, filled seeds per panicle, seed weight per panicle and 1000-seed weight (Fig. 5). Increased percent seed sterility in UFA was also responsible for higher yield losses. Wahid *et al.*, (1995a) reported around 40% yield reduction in two rice cultivars at 6 h O_3 concentrations of 50 ppb in OTCs. A number of other workers have reported reduction in yield at higher O_3 levels. Nakamura *et al.*, (1976) reported reduced panicle number in rice cultivar grown in UFA as compared to FA. Asakawa *et al.*, (1981a, b) and Mayumi and Yamazoe (1983) found reduction in mature seeds and 1000-seed weight by fumigation in field chambers at O_3 concentrations of 75 ppb as seasonal mean. Nishi *et al.*, (1985) reported 60-80% reductions in yield, number of seeds per panicle and 1000-seed weight by growing plants in fumigation chambers at O_3 concentrations of 50-100 ppb as seasonal mean. Kats *et al.*, (1985) working in USA exposed rice cultivars M7, M9 and S201 in fumigation open-top filed chambers to O_3 concentrations of 50 ppb and 100 ppb (5 h/day and 5 days/week) reported reductions in seed weight of 3-7% at 50 ppb and 12-29% at 200 ppb while reduction in spikelets per panicle was 3-5% at 50 ppb and 13-20 at 200 ppb. They also reported increased seed sterility due to O_3 and reduction in 1000 seed weight and plant biomass. In another study, Nouchi *et al.*, (1991) reported 50% reduction in total plant dry weight and reduction in root shoot ratio by exposing rice cultivar Koshi-hikari at O_3 concentrations of 100 ppb for 8 weeks in controlled glass chambers. Similarly, Nouchi *et al.*, (1995) exposed the same variety in glass chambers to increasing O_3 concentrations of 50-100 ppb, 8 h/day for 7 weeks and found reduction in plant dry weight of 17% at 50 ppb and 47% at 100 ppb along with reduction in panicle dry weight. Kobayashi *et al.*, (1995) working in Japan exposed two rice cultivars Koshi-hikari and Nippon-bare to O_3 concentrations of 72-96 ppb as 7 h seasonal mean in field exposure chambers and reported 8-14% reduction in seeds per panicle and 1000 seed weight. They also reported significant reduced total dry matter production. Other workers have reported that O_3 effects are greater during the reproductive stage when panicles are initiated. Similar observation was recorded by Asakawa *et al.*, (1981a) that effects of ambient air pollution were higher during panicle development rather than seed filling stage. Kobayashi and Okada (1995) found that effects of O_3 on the light-use efficiency were greatest at the reproductive stage than in the vegetative stage by exposing two rice cultivars Koshi-hikari and Nippon-bare to O_3 (10-100 ppb seasonal mean) in field exposure chambers. In this study, at the onset of the reproductive stage, the O_3 concentration was well above 60 ppb and increased up to 90 ppb at the end of reproductive phase. Jin *et al.*, (2001) described O_3 induced decrease in the number of grains per plant, resulting from fewer ears per plant, fewer grains per ear and more unfilled grains per ear in rice varieties. Feng *et al.*, (2003) recorded grain yield reduction of 49%, 26% and 8% in rice varieties due to O_3 concentrations of 50-200 ppb in OTC experiments in China. Ishii *et al.*, (2004a, b) found reduced yield in two Malaysian rice cultivars of about 6.3% due to increased grain sterility in response to high O_3 (60-90ppb). Ariyaphanphitak *et al.*, (2005) reported strong effects of high O_3 (100-150ppb) on yield parameters of Thai Jasmine rice with reductions of 78% filled seeds per ear along with 12.3% reductions in 100-grain weight.

It is now well documented that increasing O_3 levels decreases cereals yield drastically (Ashmore, 1991; Skarby *et al*., 1993). According to Anon., (2000), at 40 ppb the more sensitive crops such as potatoes, pulses and wheat are affected, but above 45 ppb all the major crops (including rice) show significant yield reductions. In the present investigation, yield losses were primarily due to ambient O_3 and or NO_2/SO_2, but the involvement of other aerial pollutants including peroxyactylnitrate (PAN) or hydrogen peroxide (H_2O_2) and their synergistic actions cannot be ignored. In most of the previous studies dealing with mixtures of O_3 and NO_2 or SO_2 on cereal crops, a synergistic action of pollutants can cause severe reductions in plant growth and productivity (Reinert, 1984; Anon., 1984; Adaros *et al*., (1991; Barnes & Wellburn, 1998).

Comparison of responses of rice varieties grown in environments with either filtered or unfiltered air at a site in Lahore, Pakistan provide useful information on the growth physiology and tremendous losses in yield (29-37%) in three recently grown rice varieties and very high pollutant levels (75 ppb O_3, 29 ppb NO_2 and 18 ppb SO_2 as 8 h seasonal mean) in the region. It also demonstrated that rice varieties are very sensitive to noxious air pollutants with O_3 as a potential threat to crop production. It was interesting that beside the yield reductions, nutritional quality (starch) was also altered due to ambient air pollution. When compared to the earlier investigation on the impact of air pollution on only yield of two rice varieties (Wahid *et al*., 1995a), it showed more or less similar range of yield reductions (about 45%) but very high pollutants level were found, almost double (75 ppb O_3) than the in last decade (35-40 ppb O_3 as 6 h seasonal mean) during 1992 season. SO_2 was absent during 1992 growth season but the presence of significant concentrations of SO_2 (18 ppb) in the region is a matter of serious concern as well. This study also depicts the effectiveness of open-top chambers in assessing the impacts of ambient levels of atmospheric pollutants on agricultural crops in developing countries without any noticeable chamber effects on growth performance of crop as earlier supported by many European researchers (Miller, 1993; Ashmore & Bell, 1994). This study has confirmed that ambient levels of atmospheric pollutants (especially high O_3) are reducing the quantum yield of rice crop to an alarming extent in a country where there is already severe problems of food and shelter, and has also shown greater losses in biomass as well due to reduced physiological processes occurring in plants growing in stressful atmospheric conditions. In summary, this investigation highlights the importance of clean non-toxic atmospheric environment, necessary for the growth of plants, apart from soil, water, light, temperature and relative humidity. The yield losses attributable to the mix of pollutants, and experienced in the urban fringe of Lahore, are appreciably larger than expected. Their significance more widely in Pakistan needs to be assessed as a matter of priority, as population growth rates and emission levels are both rapidly increasing in the country (Wahid, 2003).

The evidences so far indicated need for monitoring of atmospheric pollutant levels in agricultural areas, particularly around major cities and in the vicinity of industries in the developing world. To avoid worsening human health, efforts should be directed to minimize the emission levels, and to feed an increasing human population in developing countries, air pollution resistant crops should be grown similarly as for insect/pest resistant crop varieties; this can be achieved by screening the agricultural crops for their sensitivities to major atmospheric pollutants in fumigation chambers followed by OTCs or antioxidant chemical protectant (EDU or benomyl) studies in the field conditions.

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