Heliyon 7 (2021) e08198

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

A growth and biochemistry of ten high yielding genotypes of Pakistani rice (Oryza sativa L.) at maturity under elevated tropospheric ozone

Adnan Arshad [*]

Department of Botany, Government College University, Faisalabad, 3800, Pakistan

HIGHLIGHTS

- Air pollution in Pakistan could be serious threat to vegetation in near future.
- Ten Rice (Oryza sativa L.) genotypes were testified against ozone concentration of 120 pbb.
- Ozone exposure cause serious damages to morphological and chemical components of plants.
- Leaf injury proved a key factor for the determination of Plant health.
- Research findings could prove fruitful in developing ozone tolerant varieties.

ARTICLE INFO

Keywords:
Ozone
Rice genotype
Foliar injury
Biochemical and morphological attributes

ABSTRACT

Experimental studies were conducted to estimate the possible damage caused to ten rice (Oryza sativa L.) genotypes of Pakistan by tropospheric ozone. The experimental site is located at 31.4504° N and 73.1350° E, at an altitude of 184 m.a.s level with an average annual rainfall of 784 mm. A suitable and agile method was adopted to assess tolerance and susceptibility in rice genotypes at an early growth stage. Genotype Injury response, growth and biochemical parameters were measured to estimate possible effects of ozone, which was subsequently proclaimed as a criterion for ozone tolerance. Rice genotypes were subjected to ozone concentrations of 70 pbb (Current ambient) and 120 pbb (expected in near future) under a polytunnel. The findings indicated that ozone, an atmospheric pollutant, substantially harmed crop growth and metabolism, as well as inflicted a specific type of foliar injury that caused early leaf senescence. Rice genotype IR-9 followed by Punjab-Basmati and Ksk-434 appeared to be the most susceptible, whereas Basmati-515 followed by Basmati 2000 and super-Basmati were found to be Ozone-tolerant. Plant genotypes grown under elevated ozone showed 13.45% and 11.35% reduction in total root and shoot dry weight, and 25.54% and 6.6% decrease in plant leaf area and plant total length respectively compared to the control group. A significant interaction between treatment × chemical components and growth parameters was also found. The Present study confirms a direct relationship between visual response and growth as well as biochemical parameters. Declared results were statistically analyzed by using analysis of variance at confidence level of $p < 0.05$.

1. Introduction

The escalation of industrial work, uncontrolled city growth, and unchecked anthropogenic activities has changed the chemical composition of air. The effects of air pollutants such as ozone, heavy metals, oxides of carbon, nitrogen and sulfur, on growth, biomass, grain size and grain quality of various grain cultures have been widely reported in Asia and other regions of the world (Sitch et al., 2007; Akhtar et al., 2015; Ishii et al., 2004; Agarwal et al., 2002; Ainsworth 2008). It has been reported that out of total of 9% of the agricultural area, 91% is exposed to various abiotic stresses under both natural and agricultural conditions (Wahid et al., 1995; Kajla et al., 2015). The negative response of different cereal crops to air pollutants (Pleijel et al., 2006; Wahid, 2006; Rai et al., 2007); forecasts a need to take action in order to deal with future food production problems. According to the International Fund for Agriculture Development (IFAD), 70 percent of poor people from rural areas mainly depend on agriculture for their livelihoods (McGuire, 2015). Food scarcity is a global issue. It has been widely reported that around 800 million

---

[*] Corresponding author.
E-mail address: adnanarshhad413@gmail.com.

https://doi.org/10.1016/j.heliyon.2021.e08198
Received 28 May 2021; Received in revised form 3 August 2021; Accepted 14 October 2021

people suffer from malnutrition (Bertram, 1967). Rice is an important cereal crop that provides more energy than any other cereal crop. It is grown as a food source an economic tool in more than 95 countries in the world (Dawe et al., 2002). It holds a noteworthy position in Asia as it is adopted as the main calorie intake by more than 2 billion people (Usman et al., 2013). Rice belongs to the grass family Gramineae (poacea) and the genus Oryza (Gnanamanickam, 2009). Tropospheric ozone (O3), a highly reactive oxidizing gas, is one of the most harmful air pollutants (Leisner and Ainsworth, 2012). This allotropic form of oxygen formed in the presence of sunlight via complex chemical reactions between nitrogen oxides (NOx) and volatile organic compounds (VOX) (Cho et al., 2011). Contrary to worldwide efforts, the concentration of ozone in relation to the climate emergency has been rising from 0.5 to 2% annually, which may prove a persistent threat to various types of vegetation (Sitch et al., 2007; Cho et al., 2011; Vingarzan, 2004). Ozone poses a serious threat to different types of vegetation in the Indo-Gangetic plain in view to plant growth and food production (Wahid, 2006a, b). Experiment based studies such as Peng et al. (2018) from China, Bhatia et al. (2012) from India, Manigbas et al. (2010) from Korea, and Wahid (2006) & Ahmad et al. (2013) from Pakistan have documented the growth, biochemical, and injury responses of ozone in different rice cultivars at different concentration levels. It was reported that long-term, low-level ozone stress can affect both physiological and biochemical attributes of plants prior to any visible damage (Heath and Taylor, 1997; Sarkar and Agarwal 2010). In support of the present study, earlier research results have directly linked the ozone induced injury response with low root and shoot weight, reduction in leaf area, low photosynthetic activity and total biomass (Shi et al., 2009; Bhatia et al., 2013; Burkey et al., 2007; Frei et al., 2010). Ozone affects the photosynthetic apparatus and renders its physiological functions via oxidative damage to chloroplast and cell membrane, consequently weakens the plant and reduced dry matter production (Fuhrer, 2009; Tomer et al., 2015; Karberg et al., 2005). Peng et al. (2018) observed a gradual biomass loss of 17.6 % and 25.4% in Yangdao 6 and You 084 (rice cultivars in China) at mid and late stage. Ozone in its interaction with biochemical processes impaired by various chemical components (Krupa et al., 2001). A comparative analysis of ozone-damaged leaf extracts indicated a substantial decrease in photosynthetic and energy metabolism proteins in two Indian rice cultivars (Sarkar and Agrawal, 2012). An early analysis of the antioxidants response to ozone in soybeans reported a negative linear response (Oa et al., 2012). According to Phothi et al. (2016), an ozone concentration of 70 pbb has a deleterious effect on the total soluble sugar contents extracted from rice seedlings. During the development stage, excessive ozone exposure increased the concentrations of lignin and phenol in rice straw (Frei et al., 2011). In Pakistan, various studies have reported certain biotic (pests, microbes) and abiotic (drought, salinity) factors that are detrimental to rice productivity (Wahid et al., 1995; Akhtar et al., 2015; Bashir et al., 2007). A study reported a 42% and 37% yield loss in two rice cultivars due to bad air composition (Wahid and Campus, 1995). Since the rice plant is an important cereal crop, all problems related to its productivity must be properly addressed. In the course of the present work, we discovered a significant varietal difference between all genotypes with regard to their ozone response.

2. Experimental

2.1. Materials and method

2.1.1. Experimental site and design

A field experiment was conducted from April to July (kharif season) by installing a polythene polytunnel (7 ft. h, 35 ft. l, 16 ft. w) in the available research area of Government College University, Faisalabad. The subsurface soil at 1-meter depth comprises cohesive clay silt, non-cohesive silty sand, and an underlying dense poorly graded fine sand material (Kamal et al., 2015). Experimental structure was randomized a complete block design (RCBD) under split plot arrangements with three replications. Two main field blocks, Experimental & Controlled with Genotypes (variety 10 × replication 3) as the subplots were designed to overcome the experimental error. Soil in both dry direct-seeded rice main fields was dry-ploughed and harrowed, without puddling to meet agricultural conditions. Weeding process was done twice with hands as well as by weedicides.

2.1.2. Seed raising and sowing

During the seed raising process, 200 g seeds of each cultivar (Table 1) were dipped in zip lock bags containing sterilized distilled water for 48 h. Pre-soaked seeds were latterly sown (hand sowing) at the depth of 2–3 cm (20 plants/row) by following the direct seeding method (DSR) at a rate of 15–18 kg/hector. The average site temperature was recoded between the range of 40.5 °C and 26.9 °C.

2.1.3. Ozone fumigation process

Ozone treatments of 120 pbb were applied between 6:45 pm and 7:15 pm (half hour/day) for 10 days by deploying polyethylene tunnels. Following the protocol, polythene tunnels were only used at the time of the fumigation process. The first ozone treatment was applied by a high voltage discharge generator and monitored by an ozone meter (M400E with a range of 0-500pbb) on the 3rd of July 2019. All Ozone treatments were randomly distributed by introducing many pipe outlets in to the field at different positions. The outer end of each pipe was timely replaced with an ozone meter to ensure equal distribution of applied ozone concentration (Figure 1). The control group plants were managed in a free Ozone environment (ventilated with ambient air) under an open ended polythene polytunnel.

2.2. Evaluation

2.2.1. Ozone symptoms and morphological attributes

Leung et al. (2020) prescribed visible injury scoring system was adopted while recording leaf injury score. Following the health key, a final report of ozone damage was prepared by classifying the affected plants into five groups based on type & intensity of ozone symptoms. Injured leaves by percentile were assessed by Kanhar et al. (2017) prescribed method (Eq-1).

$$ozone\frac{damage}{genotype} = \frac{no.of\ affected\ plants}{Total\ no.of\ plants} \times 100 \tag{1}$$

Morphological attributes (leaf area, root and shoot length), as well as biomass production (Shoot and root fresh and dry weight) were calculated by collecting plant samples at the end of an 8 day treatment. The collected plants were fragmented into shoots and roots and fresh and dry weights were determined by using an electric balance. The dry weight calculations were done following the 48-hr oven-dried process at 70 °C. Characters recorded during visual analysis include, Plant genotype, Total no. of plants/genotype, Type and nature of Ozone induced injury

Table 1. The experiment was carried out by growing following local varieties of Pakistan.

Sr. No.	Variety	Released by	Year
1	KSK-282	Rice Research Institute, KSK, Lahore, Pakistan	1983
2	BS-2000	Rice Research Institute, KSK Lahore, Pakistan	2001
3	BSM-515	Rice Research Institute, KSK, Lahore, Pakistan	1996
4	Chenab-BS	Rice Research Institute, KSK, Lahore, Pakistan	2016
5	KSK-434	Rice Research Institute, KSK, Lahore, Pakistan	2014
6	SUPER-BS	Rice Research Institute, KSK, Lahore, Pakistan	1996
7	KSK-133	Rice Research Institute KSK, Lahore, Pakistan	2006
8	PK-386	Rice Research Institute, KSK, Lahore, Pakistan	2013
9	PUNJAB-BS	Rice Research Institute, KSK, Lahore, Pakistan	2016
10	IR-9	NIAB, Faisalabad, Pakistan	2001

Figure 1. Test plants grown under a closed polytunnel with applied ozone treatment (left) and compared with a control group grown under an open-ended polytunnel with ambient air (right).

symptoms appearance sites, Plant health (No. Of affected plants/replica & no. of affected plants/genotype).

2.2.2. Key for the health of plant

Ozone damage with the cause and extent of injury (percentage) to each genotype was categorized into single score adopting the following scheme.

Injury = A + B + C + D + E

A: % leaves with no ozone injury (Tolerant)
B: % leaves with injury of 1–5 percent (mild)
C: % leaves with injury of 5–25 percent (moderately affected)
D: %leaves with injury >25 percent (Severely affected)
E: % Number of dead/senesced leaves which remain on the plant. (Dead)

2.3. Determination of chemical assays

To analyze chemical components, representative plants of each genotype were carefully removed from the field. It was impossible to start the chemical analysis immediately after the collection, so for analysis ease, purposed leaves of each genotype were freeze at -80 °C during lab work.

2.3.1. Photosynthetic pigments

Arnon (1949) described methodology was followed to find out the contents of chlorophyll ‘a’, ‘b’, carotenoids and total chlorophyll (Eq-2, 3, 4). The change in absorbance was recorded at 645, 663 and 480 nm using a spectrophotometer (Hitachi-U2001). The contents of Chlorophylls' a ’and' b’ were determined following formula was considered.

$$contents\ of\ chlorophyll = [12.7(\mathrm{O.D663nm}) - 2.69(\mathrm{O.D645n})]\frac{volume}{1000} \times weight \tag{2}$$

$$contents\ of\ chlorophyll\ b = [22.9(\mathrm{O.D663nm}) - 4.69(\mathrm{O.D663n})] \times \frac{volume}{1000} \times weight \tag{3}$$

$$carotenoies'\left(mg\ ml^{-1}\right) = \frac{A.carotenoides}{EM\ 100\%} \times 100 \tag{4}$$

$$A\ Car(carotenoid) = (OD480) + 0.114(OD663) - 0.638(OD645)$$

Extracted material volume (milliliter) = V Leaf tissues fresh weight (gram) = W Total

Chlorophyll content means total contents of chlorophyll ‘a’ and chlorophyll ‘b’.

2.3.2. Total free amino acid & protein (Bradford, 1976)

Amino acid contents were quantified by adopting the Hamilton and Hamilton and Van Slyke (1943) protocol at 570 nm absorbance change (Eq-5).

$$Total\ free\ amino\ acid = \frac{Absorbance \times V \times DF}{Weight\ of\ fresh\ sample} \times 1000 \tag{5}$$

Activity for the quantification of protein contents was performed by the following approach (Emami Bistgani et al., 2017). Series of protein reference (200–1900 mg/g) were followed to measure total soluble protein spectrophotometrically at the wavelength of 595 nm.

2.3.3. Phenolic (Julkunen-Titto, 1985) & soluble sugar

As per Julkunen-Tiitto (1985) prescribed protocol, refrigerated leaves Samples (0.05 g) of each genotype were grounded with 80% acetone solution. To obtain precipitate, acetone extract was latterly centrifuged at 10,000 g, for 10 min. 100 μl supernatant, 1 μl Folin– Ciocalteau's phenol reagent and 2.0 mL of distilled water mixed in a test tube. Obtained solution was mixed with 5.0 ml of 20 % $Na2CO_3$ and total volume increased by adding 10 ml of distilled water. At the end, with fine shaking the spectrophotometry was done at 750 nm. Following the method prescribed by Blenkinsopp (1999) soluble sugar content was calculated and absorbance was recorded at 625 nm using spectrophotometer ((Hitachi-U2001) and the concentration was calculate by stander method.

2.4. Statistical analysis

Collected data was statistically analyzed by following the two-way ANOVA (analysis of variance) technique with the use of CoStat (Version 6.303, PMB 320, Monterey, CA, 93940 USA) software. Difference between cultivars of main plots (Experimental & control) was declared statistically significant at the confidence level of $p \leq 0.05$ for all parameters.

3. Results and discussion

3.1. Ozone induced injury

The potential hazards of ozone were carefully evaluated by observing the nature of symptoms of ozone induced foliar injury (Table 2). Early

Table 2. Genotype injury score and nature of symptoms of O3-induced foliar injury.

Sr. No.	Genotype	Score	Nature of symptoms
1	KSK-282	B	Pale yellow interveinal chlorotic mottles which laterally turn into chlorotic streaks, small size orange red to gray brown bronzing stipples associated with white flecking, vein turn white, injury Progress downward from tip to base hen bronze spots fuse into each other.
2	BSM-2000	C	Very fine light yellow chlorotic streaks, Nib sized to large, Orange brown to dark brown stipples, large size blotches of Bronzing followed by dark purple bifacial necrotic lesions, Injury mostly appear near edges of leaves. Mostly leaves remained fresh and green.
3	BSM-515	B	Low intensity yellow chlorotic blotches firstly appear close to tip of leaves laterally converted into orange brown to dark gray irregular stipples on the entire surface of leaf which cause Death of small veins, injury mostly appear near edges. Necrosis with mild lesion, white flecking mottles.
4	Chenab-BSM	C	Initial doze of ozone resulted in interveinal yellow chlorotic streaks and white gray appearance of veins (flecking), orange brown to dark gray uniform stipples streaks from base to tips. Bronzing, necrotic spots Injury mostly appear near the edges of leaf.
5	KSK-434	D	Chronicle response characterize by orange red to dark brown bifacial necrotic areas in the middle of leaf causing death of interveinal tissues. Initially dark yellow chlorotic streaks spread from tip to base followed by orange brown to dark brown irregular blotchy spots. With the increase in ozone concentration blotchy spots grow in size ultimately spread throughout the leaf surface and resulted in bow appearance of.
6	Super-BSM	D	Tipburn, led by red orang to gray dark stippling later on turning into red brown to brown dark necrotic blotches, necrotic blotches encompass the entire surface of leaf by diffusing into each other following the prolong ozone exposure. Injury first appear close to tip of leaf; leaf bronzing characterizes by unevenly distributed dark brown blotches which eventually led to wilting of leaves.
7	KSK-133	D	Unevenly distributed Orange brown to gray dark interveinal Mottles, with associated fine light yellow chlorotic streaks which increase in density as the concentration of ozone increased, finally tuning into bronzing streaks, injury spread upward from base to tip. Venial white Flecking. Mostly older and small size leaves show their susceptibility to ozone.
8	PK-386	D	Very fine light yellow chlorotic streaks, venial white flecking, Bronzing characterize by red orange to brown black blotches followed by interveinal severe necrosis. Necrotic areas diffuse into each other and spread more vigorously which accelerated leaf senescence.
9	Punjab-BSM	E	chronicle response characterizes by wilting of leaves. Chlorotic streaks followed by orange red to brown purple interveinal stippling and bronzing which turn into severe blotchy gray brown gray brown necrotic spots. Necrotic spots cracks as the concentration of ozone increased which laterally cause premature leaf senescence.
10	IR-9	E	Stippling evolve in the form of red brown to dark brown color followed by bronzing and chronic necrotic areas. Necrotic spots with diffusing borders severely damage the interveinal tissues, Severe ozone induced injury resulted in dead white brown areas. Injury spread from tip to downward causing burning of tips, ultimately death of entire leaf.

two-day fumigation led in very minor foliar damage, but in response to the third ozone treatment, several genotypes expediently showed their vulnerability to high ozone (120 pbb) with light yellow to brown red spots (Figure 1). These findings are consistent with earlier research results that plant cultivars are more susceptible to high ozone concentrations (Ahmad et al., 2013; Hur 2014). The presence of distinct and severe ozone damage, i.e. slightly yellow, tiny and thin brown patches on lower and older leaves, was also previously reported by Manigbas et al. (2010). A remarkable varietal difference among rice genotypes in response to ozone treatment could be seen in present study. Rice genotype KSK-282 shows its resistance to elevated ozone with 1–5% leaf injury, while genotypes Basmati-2000, KSK-133, BSM-515, and Chenab-basmati show average response as they appear with late and less developed ozone symptoms with 5–25% leaf injury. A significant interaction between ozone treatment and rice genotype PK-386, Chenab-basmati, Super-basmati, and Punjab basmati was observed as they show their susceptibility (>25 % leaf injury) by eliciting early ozone induced symptoms which latterly become severe and cause early leaf senescence. The index of ozone induced foliar damage was much higher in the case of rice genotype IR-9 as it appears with few of it leaves dead. Rice genotypes KSK-282, Basmati-2000, KSK-133 and BSM-515 show their resistance to elevated ozone levels as they appear with late and less developed ozone symptoms. Investigating the ozone stress response of two Indian rice cultivars, Sarkar and Agrawal (2010) reported interveinal yellowing and chlorotic stipples on the leaf surface. The study also declared rice plant as an inexpensive biomarker to detect the high concentration of ozone. Ishii et al. (2004) reported a specific type of foliar injury in rice plants, grown under different ozone concentrations. A very few studies have been conducted yet to evaluate the response of rice cultivars to their genotypic differences to ozone (Shi et al., 2009). Other plant species have also been investigated for ozone resistance (Krupa et al., 2001). Dumont et al. (2014) discovered that ozone treatment of different concentrations caused chlorosis, stippling, and necrosis in three genotypes of Euramerican poplar, Carpaccio, Cima, and Robusta, respectively. Leung et al. (2020) reported the appearance of a specific type of bronze red spots on different locations of examined bean plant leaves (Phaseolus vulgaris L.). It is a noteworthy fact that ozone exposure time and concentration greatly influenced the nature of symptoms, as in the present study, early days ozone exposure resulted in light yellow to brown red spots (bronzing) which stayed tuned with ozone concentration and appeared to be indicative of chlorotic streaks and necrosis. Foliar injury in rice is a cumulative effect of ozone concentration and exposure time (Cho et al., 2011; Ishii et al., 2004). In some understudy rice genotypes, leaf injury in its most forms appeared on both the adaxial and abaxial surfaces of leaves (mostly in older & middle-aged leaves). Manigbas et al. (2013) identified leaf browning as one of the most prevalent signs that may be utilized to assess ozone tolerance in different rice genotypes. Ozone induced visual symptoms have been reported as a suitable tool for the classification of different rice genotypes into a certain categorical groups (Dumont et al., 2014). On a results basis, all genotypes were classified into the following categories, Tolerant, Mild, moderately affected, severely affected and Senesced or Dead. Table 2 & Figure 2 shows complete description of different types of foliar injury and secured injury score by understudy rice genotypes in response to ozone treatment (120 pbb).

3.2. Plants damage percent and leaves damage percent

Under study, all genotypes of rice showed a statistically significant difference in their plant damage percent and leaf damage percent (Figures 3 and 4). Average plant damage percentage was observed to be 41 % higher for plants grown under elevated ozone (120pbb) than those grown under ambient ozone level. The leaf damage percent value for the experimental group (120 pbb) was observed to be 29 % higher than control group (ambient ozone). The data presented shows that the rice genotype *IR-9* followed by *ksk-434* and *Punjab-Basmati* showed maximum value for plant damage percent and the rice genotypes *IR-9* followed by *Punjab-Basmati* and *ksk-434* showed maximum leaf damage percent. The rice genotypes *KSK-282 and Basmati-515* hold the lowest value for plant damage percent and leaf damage percent respectively, which show their resistance to elevated ozone. Rice genotypes × ozone treatments interaction was also found statistically significant.

3.3. Plant growth attributes

Data collected for plant total length, total no. of leaves/plant, leaf area shoot and root dry weight (Figures 5, 6, 7, and 8) showed a statistically

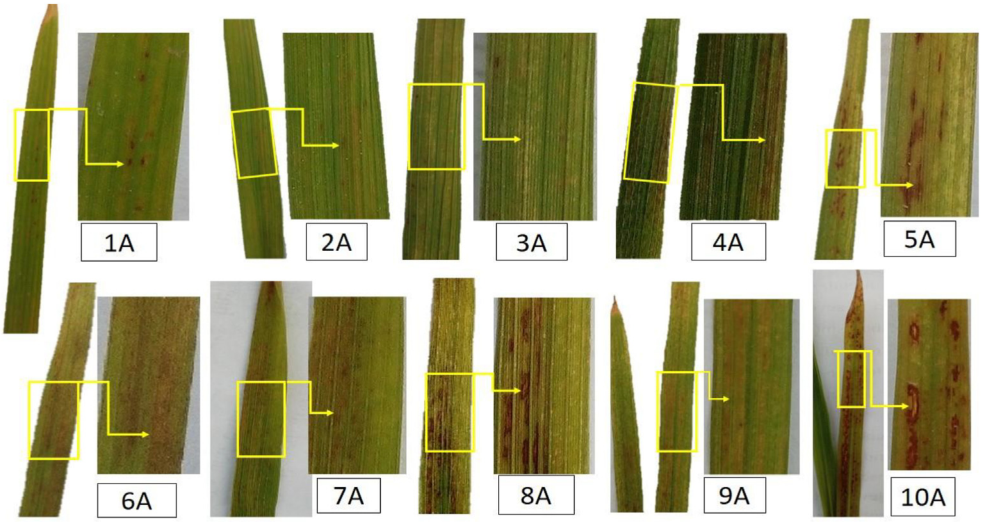

Figure 2. Foliar injury response to ozone in ten genotypes of Pakistani rice (Oryza Sativa): A1) KSK-282, A2) Basmati-200, A3) Basmati-515, A4) Chenab-Basmati, A5) KSK-434, A6) Super- Basmati, A7) KSK-133, A8) PK-386. A9) Punjab-Basmati, A10) IR-9.

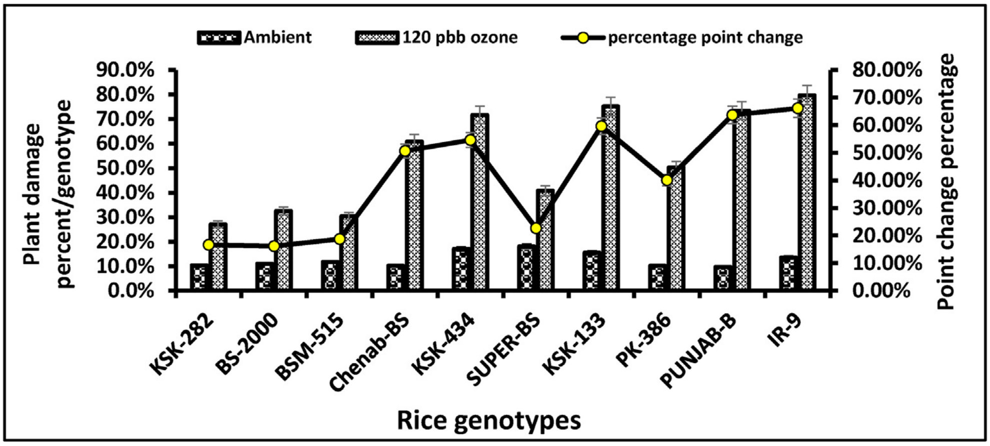

Figure 3. Ozone induced plant damage (percentage)/genotype.

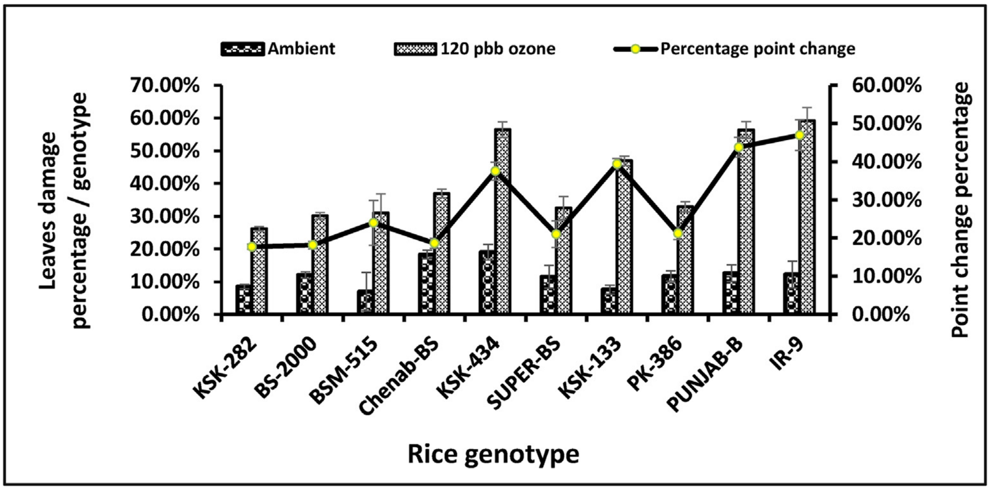

Figure 4. Ozone induced Leaves damage (percentage)/genotype.

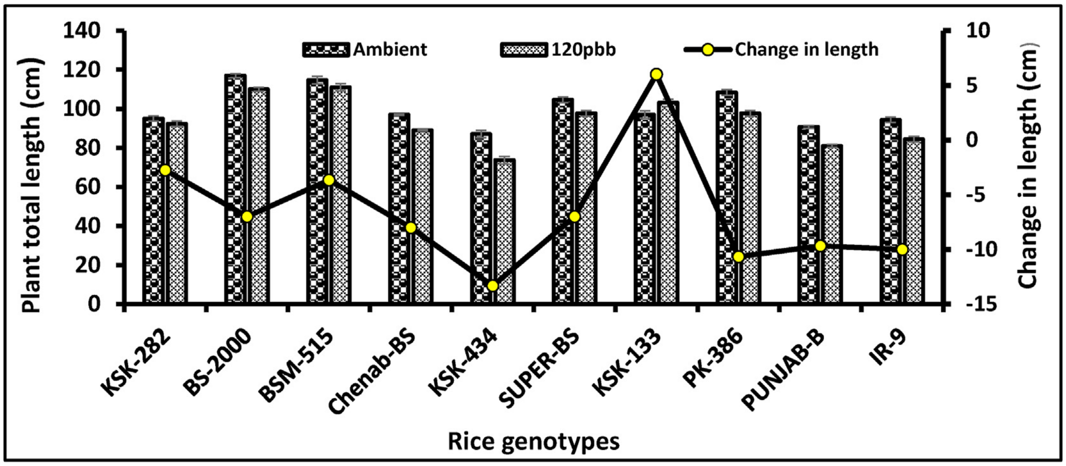

Figure 5. Plant total length.

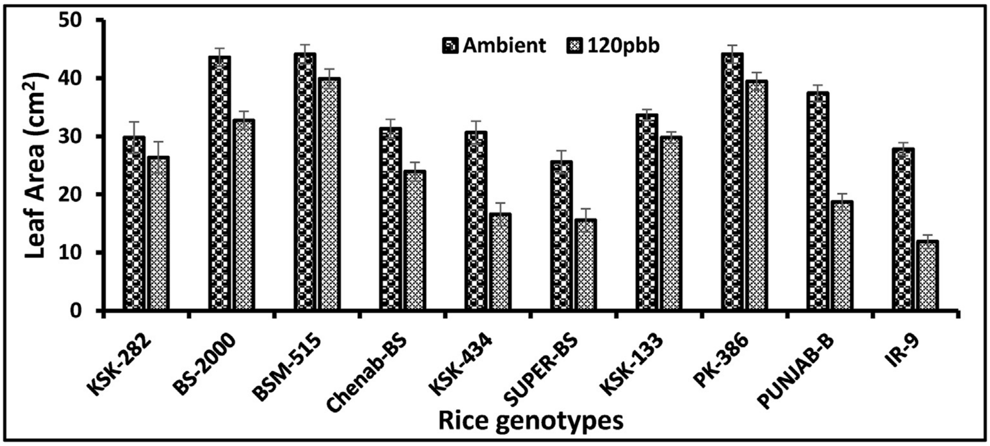

Figure 6. Leaf area.

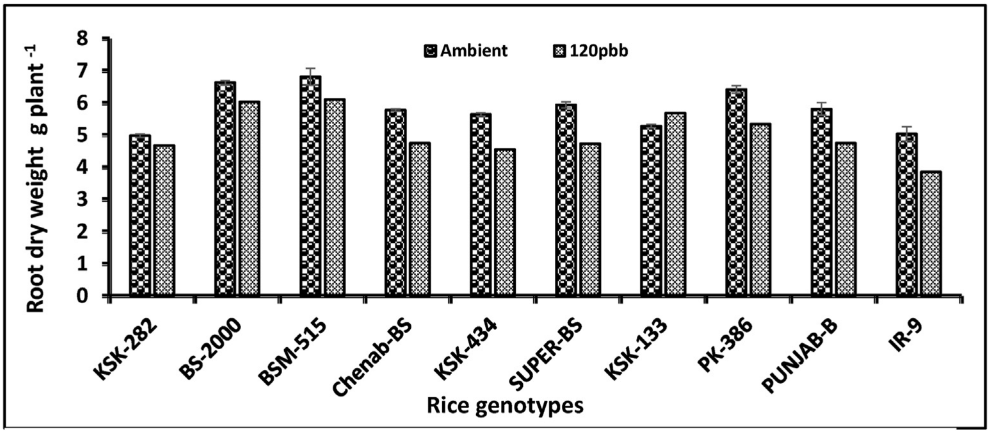

Figure 7. Plant Root dry weight.

significant difference for two levels of ozone (Table 3). The total length of each genotype was measured as the length of ozone treated plants. The interaction between applied Treatment × Replicate appeared non-significant. Ozone treated genotypes showed a 6.5 % reduction in total plant length compared with the control group. Rice genotype Basmati-515 followed by Basmati-2000 and ksk-133 appeared with maximum while Ksk-434 followed by Punjab-Basmati and IR-9 Showed lowest value for plant total length. plant total length may have decreased due to low stomatal conductance to water vapours and photosynthetic rate (Frei et al., 2008). In a study on ozone response in wheat cultivars, Tomer et al. (2015) discovered that decreased stomatal conductance led to low photosynthetic activity, resulting in lower biomass production. This has also been explored in prior studies that found ozone stress and rice cultivar interaction greatly influenced plant height and plant total biomass. Peng et al. (2018) used two rice varieties, Yangdao 6 and indica II you 084 in their experiment and observed a significant effect on

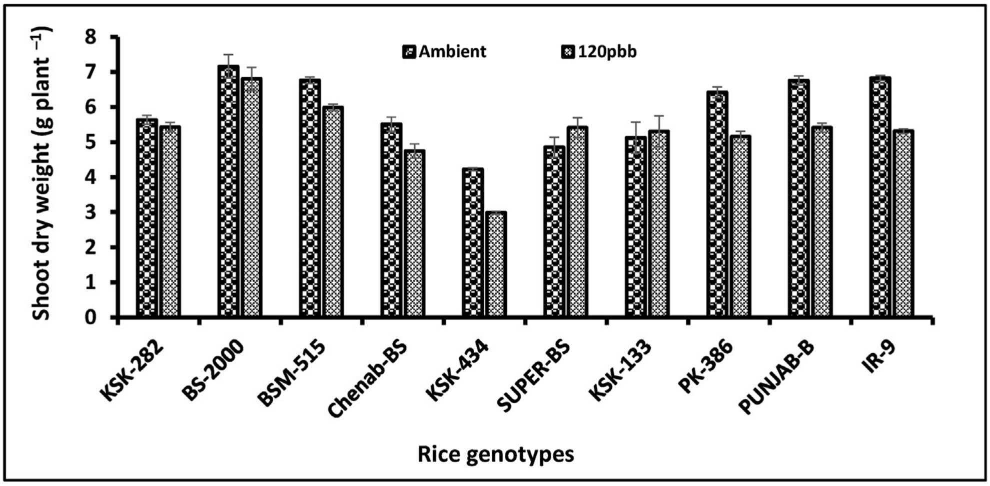

Figure 8. Plant Shoot dry weight.

Table 3. Growth parameters (mean values ±SD) in response to ozone (120 pbb) compared with control, in ten Pakistani genotypes of rice (Oryza sativa).

Genotype		Root dry weight (g/plant)	shoot dry weight (g/plant)	Leaf Area (cm^2)	Plant total length (cm)	Plant damage (%)	Leaf damage (%)
KSK-282	Control	4.98 ± 0.09	5.64 ± 0.23	29.76 ± 2.70	95 ± 2.44	10.37 ± 0.52	8.49 ± 0.01
	120 Ozone	4.68 ± 0.11	5.43 ± 0.58	26.36 ± 1.53	92 ± 1.79	27.05 ± 0.56	26.19 ± 0.01
BS-2000	Control	6.63 ± 0.47	7.16 ± 0.16	43.60 ± 1.66.	117 ± 3.26	11.00 ± 0.96	12.04 ± 0.10
	120 Ozone	6.03 ± 0.07	6.80 ± 0.35	32.73 ± 1.06	110 ± 0.81	32.50 ± 2.04	30.16 ± 0.02
BSM-515	Control	6.81 ± 0.09	7.76 ± 0.07	44.10 ± 1.95	114 ± 3.29	11.70 ± 1.36	6.94 ± 0.05
	120 Ozone	6.11 ± 0.16	5.99 ± 0.49	39.9 ± 1.98	111 ± 2.44	30.42 ± 4.12	30.95 ± 0.14
Chenab-BSM	Control	5.78 ± 0.09	5.51 ± 0.77	31.29 ± 0.97	97 ± 3.26	10.13 ± 3.85	18.33 ± 0.02
	120 Ozone	4.75 ± 0.21	4.74 ± 0.28	23.93 ± 1.54	89 ± 2.44	60.75 ± 1.06	36.94 ± 0.02
KSK-434	Control	5.64 ± 0.35	4.22 ± 0.23	30.63 ± 1.40	87 ± 1.41	17.05 ± 2.09	18.98 ± 0.04
	120 Ozone	4.56 ± 0.39	2.99 ± 0.13	16.55 ± 1.14	73.66 ± 2.49	71.67 ± 2.36	56.51 ± 0.10
SUPER-BSM	Control	5.94 ± 0.21	4.86 ± 0.65	26.69 ± 2.41	104.66 ± 3.39	18.11 ± 1.14	11.48 ± 0.04
	120 Ozone	4.73 ± 0.26	5.42 ± 0.60	15.35 ± 0.79	97.66 ± 1.24	40.82 ± 1.65	32.50 ± 0.06
KSK-133	Control	5.28 ± 0.24	5.13 ± 0.46	33.63 ± 1.01	97.00 ± 3.74	15.51 ± 4.55	7.57 ± 0.01
	120 Ozone	5.69 ± 0.12	5.30 ± 0.41	29.77 ± 1.73	103.45 ± 2.44	75.13 ± 1.47	46.95 ± 0.14
PK-386	Control	6.42 ± 0.38	6.42 ± 0.26	44.11 ± 1.48	108.33 ± 3.39	10.17 ± 0.24	11.71 ± 0.02
	120 Ozone	5.35 ± 0.26	5.15 ± 0.27	39.45 ± 0.49	97.66 ± 2.86	50.24 ± 2.66	32.92 ± 0.10
PUNJAB-BSM	Control	5.81 ± 0.08	6.75 ± 0.10	37.42 ± 1.33	90.66 ± 1.88	9.75 ± 6.37	12.62 ± 0.03
	120 Ozone	4.72 ± 0.04	5.42 ± 0.50	18.69 ± 0.88	81 ± 1.63	73.40 ± 5.07	56.39 ± 0.11
IR-9	Control	5.04 ± 0.07	6.83 ± 0.39	27.77 ± 2.89	94.33 ± 2.05	13.59 ± 8.35	12.21 ± 0.03
	120 Ozone	3.86 ± 0.06	5.50 ±	11.87 ± 0.54	84.33 ± 3.39	79.69 ± 2.68	59.20 ± 0.12
Blocks		.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0164 *
Replicate		.9571 ns	.6354 ns	.6858 ns	.2515 ns	.6988 ns	.8528 ns
Treatment		.0009 ***	.0136 *	.0000 ***	.0040 **	.0001 ***	.0000 ***
Treatment × Replicate		.6249 ns	.2328 ns	.8802 ns	.5267 ns	.6527 ns	.1927 ns

__Sign presented__; at bottom indicates how growth components of ozone treated plants are different from control group in view of statistical approach at a confidence level of $P < 0.05$…, 0.01…**, 0.001…*** ns = non-Significance level.

phenology and plant height of indica II you 084 compared to Yangdao 6. Ozone due to its oxidative nature, significantly influenced the height of different rice genotypes (Agrawal et al., 2002; Sarkar and Agrawal, 2010).

Shoot and root dry weight of each ozone treated genotype was measured and considered as the weight of plants in experimental group. Statistical analysis revealed that ozone concentrations of 120 pbb substantially reduced root and shoot dry mass in ozone-treated. In agreement with previous results (Frei et al., 2008; Inada et al., 2008) the present research work also observed 12 % and 14 % reduction in average total shoot and root dry weight respectively. Shoot dry weight significantly dropped in response to ozone stress in genotype IR-9, followed by Punjab-Basmati and Pk-386, indicating sensitivity to applied ozone concentrations. The plant genotype Super-Basmati, followed by IR-9 and Ksk-434, had the lowest value for average total root dry weight compared to the control group. Ksk-133 was the most tolerant genotype, with an increase in average root dry mass. Wahid et al. (1995) observed same trend in two Pakistani cultivars, IRRI-6 and Basmati-38, in terms of reduction in straw weight and root mass. Manigbas et al. (2010) observed the significant depression in shoot dry weight when they exposed 15–30 day old rice seedlings to 0.3 and 1 ppm ozone concentration for 7 h/day for ten days. It is also well acknowledged that shoot biomass is not directly associated with leaf bronzing. Previous studies have shown that rice plants subjected to 0.120 ppm (120ppb) ozone concentration for 18 days showed a reduction of 24 % in plant total biomass (Frei et al., 2010). Previous studies have also linked reduction in root and shoot dry weight with low photosynthesis rate, cell membranes damage, especially chloroplast damage (Karberg et al., 2005; CAO et al., 2009; Noormets et al., 2010; Rai et al., 2010). Plant height and stem dry weight are greatly influenced by the rice cultivar × ozone stress. Revealing the response of two Chinese rice variety Peng et al. (2018) reported that ozone stress reduced the stem dry weight by 11.11 %–12.7 % respectively.

Negative response found in total leaf area among different rice genotypes under applied ozone concentration. The decrease in average leaf area index was observed more in genotype Punjab-Basmati followed by IR-9 and Super-Basmati compared with control group plants. There was a 29% decrease in average leaf area in ozone exposed genotypes compared to unexposed. It has earlier been reported that the decrease in leaf area in soybean is correlated with ozone triggered earlier leaf senescence process (Dermody et al., 2006). Sarkar and Agrawal (2011) found that two Indian rice cultivars, Malviya dhan 36 and Shivani, reduced their total leaf area by 30% and 27%, respectively, in response to ambient and elevated ozone level. Agrawal et al. (2005) experiment on mung bean also reported significant reductions in total leaf area and biomass at Allahabad, India. Evaluating the response of two wheat cultivars grown under high ozone concentration Tomer et al. (2015) explained that the total no. of leaves and leaf area reduces following ozone-induced premature leaves senescence. Previous research reports characterized chronicle ozone damage by low photosynthetic activity and reduced leaf green area Ashmore (2005); Fuhrer (2009). Bhatia et al. (2013) in their two-year research project found that maize crops grown in charcoal filtered air chambers with elevated ozone levels (E0, E1) showed a 12–15% and 13–15.4% decrease in leaf area respectively. The increased ozone level of 120pbb decreased relative growth rates of plant by 68% in Alfalfa (M. sativa) (Al-Rawahy et al., 2013).

3.4. Chemical analysis

3.4.1. Chlorophyll contents

Rice being an ozone sensitive crop (Sarkar and Agrawal, 2010), appears to have a differential response in view of different chemical contents (Table 4). The chlorophyll content of each genotype grown under both ambient and ozone levels showed a statistically significant difference from each other. Plants grown under elevated ozone level showed low value for chlorophyll a & b, ratio of Chl a & b, and total chlorophyll contents compared to ambient. Rice genotype Pk-386 followed by IR-9 and Punjab-Basmati showed the highest difference for chlorophyll ‘a’ while rice genotype Basmati-2000 followed by Ksk-133 and Basmati-515 showed the lowest difference compared to genotypes grown under ambient ozone level. Ozone treated genotypes showed chlorophyll ‘a’ content ranged from 0.95 to 1.25 mg g^{-1} with an average of 1.09 mg g^{-1}. In Pakistan, deleterious effects of ambient ozone (>60 pbb) on onion, potato and cotton plant growth have been reported in the north-west parts (Ahmad et al., 2013). A significant damage to carotenoids contents was also observed. Average Carotenoids contents reduced by 8.4 % in the case of ozone treated plants as compared to plants grown under ambient conditions. Previous literature has also revealed the same trend, Phothi et al. (2016) when exposed rice plants to high ozone concentration for 28 days, found significant damage to chlorophyll contents which ultimately reduce the photosynthesis rate. Sawada et al. (2016) study reported that increased ozone levels decrease chlorophyll content in the leaves of rice plants. Experimental analysis of Japanese rice has revealed that an ozone concentration level of 100 pbb reduces the chlorophyll content of lower leaves (Inada et al., 2008). In the present study, total chlorophyll content and chl. a/b showed their range from 1.40 to 1.88 mg g^{-1} with an average of 1.65 mg $g^{-1}$and 0.66 to 1.5 mg g^{-1} with an average of 1.02 mg g^{-1} respectively. Decrease in average total chlorophyll contents compared to ambient found 8.2 %. Statistical approach reported a non-significant effect of Treatment × replicate on total chlorophyll and the ratio of Chl a to b. Tomer et al. (2015) observed a significant loss in total chlorophyll contents at the flowering stage in wheat cultivars grown under elevated ozone levels. It has also been reported that ozone exposure of 80 pbb can reduce 20.5–47.6% of the total chlorophyll content of rice (cv Ratna). The value of total chlorophyll contents the increased in case of rice genotype super basmati. Previously, Debski et al. (2017) reported the same trend that a high dose of ozone resulted in a non-significant effect on chlorophyll ‘a’ & ‘b’ content in buckwheat. The rice genotype Punjab-basmati followed by IR-9 and KSK-282 showed the lowest value for Chl a/b while Basmati-515 followed by Basmati-2000 and KSK-133 appeared with the maximum chlorophyll a/b value. Experimental plants showed an average of 5.2 % less chlorophyll a/b ratio. High ozone concentration significantly damages the chlorophyll content, which ultimately slows the photosynthesis rate and affects certain photochemical reactions (Sarkar and Agrawal, 2010). A study explained that loss of chlorophyll content and lipid peroxidation trigger the process of premature leaf senescence in plants (Li et al., 2008). Murchie et al. (2002) reported that ozone majorly affects the older leaves of plants, which may be a natural consequence of ageing.

3.4.2. Total free amino acids contents

The present study confirms a decrease in total free amino acid contents in response to elevated ozone. A significant difference was found between total free amino acids contents between the two levels of ozone. Loss of total free amino acid contents was observed more in the plant genotype Punjab-Basmati, followed by IR-9 and Ksk-434 compared with the control group. In response to ozone exposure, the content of total free amino acids increased in the case of plant genotypes Ksk-282 ($p = 0.54$) and Ksk-133 ($p = 0.24$). While examining ozone induced loss in two-week old rice seedlings (24 h ozone treated), CAO et al. (2009) revealed that production of antioxidants and ozone triggered repair process led to overproduction of amino acids. This has also been explored in a prior study by Dumont et al. (2014) that found ozone has a negative effect on amino acid content because the most abundant amino acid, aspartic acid, and total amino acid contents significantly decreased in three Euramerican poplar genotypes in response to ozone treatment. An experiment based on the open air ozone fumigation process reported that the concentration of total free amino acids was higher in ozone treated pine foliage compared to ambient (Holopainen et al. 1997).

3.4.3. Total soluble sugar contents

The statistical approach revealed two levels of ozone concentration as important influencing parameters on total soluble sugar content at 0.05 significance level. Experimental group plants showed a 17.69% average reduction in total soluble sugar content compared to the control group. The negative influence of ozone was high in the plant genotype Punjab-Basmati, followed by Ksk-434 and IR-9. Plant genotype Super-basmati followed by Ksk-282 and did not appear to have a significant difference compared to the control group. Similar results have also been mentioned in different research papers. An investigation has reported that ozone concentrations of 40 pbb and 70 pbb significantly decrease the total soluble sugar content extracted from the leaves of rice seedlings (Phothi et al., 2016). The total soluble sugar response of ozone in view of its negative nature was also reported in other crops; Meyer et al. (2000) wheat, Köllner and Krause (2000) potatoes, and Keutgen et al. (2005) strawberries.

3.4.4. Total proteins contents

A number of questions regarding the impact of high ozone concentration on the protein profile of rice plants remain to be addressed. Limited research studies have reported the loss of proteins involved in metabolism, stress related, and most commonly, RuBisCO (Sarkar and Agrawal, 2010; Feng et al., 2008; Agrawal et al., 2002). An investigation reported that rice seedlings exposed to an ozone concentration of 200 ppb showed clear damage to their protein profile with specific ozone induced foliar injury (Agrawal et al., 2002; Agrawal et al., 2005). In the present study, a 12.72 % decrease in average total protein content was found in ozone fumigated plants, compared to ambient. Ozone induced

Table 4. Biochemical components concentration (mean values ±SD) in response to ozone (120 pbb) compared with control, in ten Pakistani genotypes of rice (Oryza sativa).

Genotype		Chl a	Chl b	Carotenoids	Total Chl. (mg/g FW)	chl a/chl b	Amino acids (mg/g FW)	Sugar (mg/Kg FW)	Protein (mg/Kg FW)	Phenolic (mg/g)
Ksk-282	Control	1.24 ± 0.01	0.44 ± 0.01	0.03 ± 0.002	1.68 ± 0.00	2.84 ± 0.08	11.15 ± 0.33	1694.0 ± 28.4	702.0 ± 61.7	348.15 ± 15.66
	120 Ozone	1.13 ± 0.00	0.42 ± 0.01	0.035 ± 0.002	1.55 ± 0.01	2.67 ± 0.06	11.69 ± 0.59	1498.8 ± 6.9	569.7 ± 43.3	252.02 ± 6.46
Bas-2000	Control	1.39 ± 0.01	0.38 ± 0.00	0.049 ± 0.00	1.77 ± 0.00	3.71 ± 0.06	5.02 ± 1.60	1622.9 ± 30.2	756.2 ± 70.7	150.84 ± 37.31
	120 Ozone	1.26 ± 0.02	0.37 ± 0.01	0.045 ± 0.001	1.62 ± 0.00	3.44 ± 0.18	3.53 ± 2.57	1411.0 ± 107.2	588.2 ± 48.5	87.00 ± 6.70
Bas-515	Control	1.37 ± 0.01	0.49 ± 0.01	0.046 ± 0.002	1.86 ± 0.01	2.80 ± 0.07	12.67 ± 1.81	1726.0 ± 15.6	696.5 ± 54.7	350.08 ± 9.56
	120 Ozone	1.26 ± 0.04	0.48 ± 0.01	0.044 ± 0.001	1.74 ± 0.02	2.63 ± 0.15	10.80 ± 0.70	1461.1 ± 5.7	673.9 ± 87.0	293.22 ± 7.41
Chenab-BSM	Control	1.21 ± 0.01	0.41 ± 0.01	0.046 ± 0.001	1.62 ± 0.00	2.92 ± 0.08	12.48 ± 2.89	1790.1 ± 12.1	731.8 ± 32.1	±408.80 ± 14.65
	120 Ozone	1.05 ± 0.00	0.38 ± 0.00	0.043 ± 0.000	1.44 ± 0.00	2.74 ± 0.07	7.12 ± 1.30	1474.9 ± 32.6	664.3 ± 61.3	313.93 ± 13.03
Ksk-434	Control	1.31 ± 0.01	0.31 ± 0.01	0.041 ± 0.001	1.62 ± 0.01	4.26 ± 0.01	7.46 ± 1.11	1500.8 ± 9.1	764.4 ± 23.1	406.50 ± 12.73
	120 Ozone	1.10 ± 0.00	0.26 ± 0.01	0.035 ± 0.003	1.36 ± 0.01	4.20 ± 0.01	1.43 ± 0.47	1133.8 ± 21.9	756.5 ± 116.3	251.73 ± 6.23
Super-BSM	Control	1.26 ± 0.04	0.42 ± 0.01	0.049 ± 0.001	1.69 ± 0.02	2.98 ± 0.15	13.12 ± 2.79	1638.0 ± 17.0	723.6 ± 13.2	369.61 ± 18.93
	120 Ozone	1.15 ± 0.01	0.40 ± 0.00	±0.047 ± 0.000	1.54 ± 0.00	2.88 ± 0.08	10.49 ± 2.74	1471.9 ± 32.6	670.5 ± 23.6	265.46 ± 4.30
Ksk-133	Control	1.32 ± 0.01	0.35 ± 0.00	0.041 ± 0.001	1.66 ± 0.01	3.77 ± 0.08	12.91 ± 0.80	1798.2 ± 20.5	787.0 ± 24.2	391.06 ± 21.15
	120 Ozone	0.99 ± 0.17	0.27 ± 0.07	0.039 ± 0.003	1.26 ± 0.10	3.88 ± 1.43	13.15 ± 0.12	1458.6 ± 23.4	708.5 ± 43.7	256.62 ± 3.16
Pk-386	Control	1.26 ± 0.01	0.49 ± 0.00	0.033 ± 0.002	1.75 ± 0.00	2.58 ± 0.06	17.14 ± 5.97	1427.6 ± 15.5	789.4 ± 26.9	373.69 ± 29.32
	120 Ozone	1.08 ± 0.01	0.45 ± 0.02	0.030 ± 0.002	1.53 ± 0.02	2.39 ± 0.11	11.63 ± 0.69	1164.5 ± 27.8	654.0 ± 54.1	286.17 ± 7.70
Punjab-BSM	Control	1.30 ± 0.02	0.53 ± 0.01	0.037 ± 0.002	1.83 ± 0.03	2.47 ± 0.05	19.62 ± 2.91	1456.5 ± 21.3	779.2 ± 28.9	380.15 ± 17.79
	120 Ozone	1.02 ± 0.02	0.43 ± 0.01	0.034 ± 0.001	1.46 ± 0.00	2.36 ± 0.12	12.25 ± 0.40	1058.3 ± 8.7	662.6 ± 71.3	241.85 ± 33.86
IR-9	Control	1.30 ± 0.01	0.45 ± 0.02	0.034 ± 0.002	1.75 ± 0.02	2.90 ± 0.19	16.75 ± 13.66	1462.1 ± 10.6	757.2 ± 52.3	384.38 ± 11.81
	120 Ozone	0.96 ± 0.01	0.36 ± 0.01	0.027 ± 0.001	1.32 ± 0.00	2.67 ± 0.10	10.00 ± 1.47	1133.8 ± 20.3	586.5 ± 66.9	247.49 ± 5.38
Replicate		.5184 ns	.2803 ns	.1324 ns	.7226 ns	.3929 ns	.1889 ns	.5288 ns	.4643 ns	.5541 ns
Treatment		.0001 ***	.0021 **	.0003 ***	.0001 ***	.0030 **	.0000 ***	.0035 **	.0006 ***	.0000 ***
Treatment × Replicate		.6458 ns	.2540 ns	.7049 ns	.7061 ns	.3229 ns	.8021 ns	.4897 ns	.3542 ns	.6361 ns

*__Sign presented__; at the bottom indicates how chemical components of ozone treated plants are different from the control group in view of statistical approach at a confidence level of $P < 0.05$…. *,0.01…**, 0.001…*** ns = non-Significance level.

total protein content loss was observed high in plant genotypes Basmati-2000 followed by IR-9 and Punjab-Basmati. Plant genotypes ksk-133 followed by Basmati-515 and Chenab-basmati show their susceptibility to ozone treatment in view of ozone total protein content. It was reported in literature that rice seedlings exposed to 40, 80, and 120 pbb appeared to have clear damage to proteins (Feng et al., 2016). The literature pertaining to ozone's effect on protein content (Alyemeni, 2016) strongly co-relates the protein damage with the concentration level of ozone. Rice genotype ksk-434 showed its peculiar behavior by showing its resistance to damage in protein content despite high foliar injury. This contrary behavior was previously explained by (Plessl et al., 2007). They reported that tomatoes plants when exposed to an ozone concentration of 40–120 pbb appeared to have increased protein and fat content.

3.4.5. *Total phenolic contents*

The Anova results revealed a significant varietal difference in total phenolic and flavonoids content among all rice genotypes. A non-significant effect of treatment replicate was observed at $p = 0.636$. A reduction of 29% was recorded in average total phenolic content. Rice genotype Ksk-434 followed by ksk-133 and Punjab basmati highly reduced their total phenolic content in comparison to the control group. The average percentage value for phenolic content showed a low difference compared to the control group in the case of genotype Basmati-515, followed by Basmati 2000 and Pk-386. Reduction in phenolic content in response to ozone has some previous records, as Betzelberge et al. (2012) reported a significant loss in phenolic content in response to increased ozone concentration in soybean leaves. A study reported that wheat cultivars exposed to elevated ozone concentration showed a significant difference in phenolic content at different growing stages (Feng et al., 2016). In contrast, an increase in total phenolic content in response to ambient ozone level was also observed in two Indian wheat cultivars (Rai and Agrawal, 2014).

4. Conclusion

For the first time in history, ten different Pakistani rice genotypes in view of their foliar damage, biochemical and morphological attributes were tested against ozone treatment. Study findings declare remarkable changes in understudy parameters which indicate a clear varietal difference among different rice cultivars. Studies do confirm a strong relationship between foliar damage and morphological & chemical changes. In view of future ozone concentration and its possible effects on regional vegetation, present findings provide enough evidence which could help the concerned authorities in deciding the future status of the genotype as a bio indicator of ozone pollution. Research findings could also prove fruitful in developing ozone tolerant rice varieties.

Declarations

Author contribution statement

Adnan Arshad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Mr., Adnan Adnan Arshad was supported by Higher Education Commission, Pakistan.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to thank Rice Research Institute, KSK, Lahore, Pakistan for experimental work and the Head of Botany department, Government College University Faisalabad, Dr. Naeem Iqbal, for technical support.

References

Agrawal, Kumar, Ganesh, Rakwal, Randeep, Yonekura, Masami, Kubo, Akihiro, Saji, Hikaru, 2002. Rapid induction of defense/stress-related proteins in leaves of rice (Oryza sativa) seedlings exposed to ozone is preceded by newly phosphorylated proteins and changes in a 66-KDa ERK-type MAPK. J. Plant Physiol. 159 (4), 361–369.

Agrawal, S.B., Singh, Anoop, Rathore, Dheeraj, 2005. "Role of Ethylene Diurea (EDU) in assessing impact of ozone on Vigna Radiata L. Plants in a Suburban area of Allahabad (India). Chemosphere 61 (2), 218–228.

Ahmad, M.N., Büker, P., Khalid, S., Van Den Berg, L., Shah, H.U., Wahid, A., Ashmore, M., 2013. Effects of ozone on crops in north-west Pakistan. Environ. Pollut. 174, 244–249.

Ainsworth, Elizabeth A., 2008. Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Global Change Biol. 14 (7), 1642–1650.

Akhtar, M., Akhtar, M.S., Haider, Z., 2015. PK 386: a new high yielding, early maturing, long grain rice (Oryza sativa L.) variety. J. Agric. Res. 53 (3), 321–330.

Al-Rawahy, Salim H., Sulaiman, Hameed, Farooq, Sardar A., Karam, M.F., Sherwani, Neelam, 2013. Effect of O3 and CO2 levels on growth, biochemical and nutrient parameters of Alfalfa (Medicago sativa). APCBEE Procedia 5, 288–295.

Alyemeni, Mohammed, 2016. Effect of ozone on the quality of two Legume crops (Vicia Faba, and Pisum Sativum) around Riyadh city. Life Sci. J. January 2013.

Arnon, Daniel I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. Plant Physiol. 24 (1), 1–15.

Ashmore, M.R., 2005. Assessing the future global impacts of ozone on vegetation. Plant Cell Environ. 949–964.

Betzelberger, A.M., Yendrek, C.R., Sun, J., Leisner, C.P., Nelson, R.L., Ort, D.R., Ainsworth, E.A., 2012. Ozone exposure response for US soybean cultivars: linear reductions in photosynthetic potential, biomass, and yield. Plant Physiol. 160 (4), 1827–1839.

Bashir, K., Khan, N.M., Rasheed, S., Salim, M., 2007. Indica rice varietal development in Pakistan: an overview. Paddy Water Environ. 5 (2), 73–81.

Bertram, G.C., 1967. The State of Food and Agriculture, Vol. 59, 1966.

Bhatia, A., Kumar, V., Kumar, A., Tomer, R., Singh, B., Singh, S., 2013. Effect of elevated ozone and carbon dioxide interaction on growth and yield of maize. Maydica 58 (3–4), 291–298.

Bhatia, A., Tomer, R., Kumar, V., Singh, S.D., Pathak, H., 2012. Impact of tropospheric ozone on crop growth and productivity–a review. J. Sci. Ind. Res. 71 (2).

Blenkinsopp, A., 1999. A good year. Int. J. Pharm. Pract. 7 (4), 197.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254.

Burkey, K.O., Booker, F.L., Pursley, W.A., Heagle, A.S., 2007. Elevated carbon dioxide and ozone effects on peanut. II. Seed yield and quality. Crop Sci. 47, 1488–1497.

Cao, Ling, Ji, Wang, Liang, Zeng, Qing, Liang, Jing, Tang, Hao Ye, Xie, Zu Bin, Liu, Gang, Zhu, Jian Guo, Kobayashi, Kazhuhiko, 2009. Characteristics of photosynthesis in wheat cultivars with different sensitivities to ozone under O3-free air concentration enrichment conditions. Acta Agron. Sin. 35 (8), 1500–1507.

Cho, Kyoungwon, Tiwari, Supriya, Agrawal, S.B., Torres, N.L., Agrawal, Madhoolika, Sarkar, Abhijit, Shibato, Junko, Agrawal, Ganesh K., Kubo, Akihiro, Rakwal, Randeep, 2011. Tropospheric ozone and plants: absorption, responses, and consequences. Rev. Environ. Contam. Toxicol. 212.

Dawe, D.C., Hardy, B., Hettel, G.P., 2002. Rice almanac: source book for the most important economic activity on earth. Rice Almanac: Source Book Most Import. Econ. Activ. Earth.

Dębski, Henryk, Wiczkowski, Wiesław, Szawara-Nowak, Dorota, Bączek, Natalia, Piechota, Małgorzata, Horbowicz, Marcin, 2017. The effect of tropospheric ozone on flavonoids and pigments content in common buckwheat cotyledons. Ecol. Chem. Eng. S 24 (3), 457–465.

Dermody, Orla, Long, Stephen P., DeLucia, Evan H., 2006. "How does elevated CO2 or ozone affect the leaf-area index of soybean when applied independently?". New Phytol. 169 (1), 145–155.

Dumont, Jennifer, Keski-Saari, Sarita, Keinänen, Markku, Cohen, David, Ningre, Nathalie, Kontunen-Soppela, Sari, Baldet, Pierre, Gibon, Yves, Dizengremel, Pierre, Noëlle Vaultier, Marie, Jolivet, Yves, Oksanen, Elina, Thiec, Didier Le, 2014. Ozone affects ascorbate and glutathione biosynthesis as well as amino acid contents in three Euramerican poplar genotypes. Tree Physiol. 34 (3), 253–266.

Emami Bistgani, Zohreh, Ataollah Siadat, Seyed, Bakhshandeh, Abdolmehdi, Ghasemi Pirbalouti, Abdollah, Hashemi, Masoud, 2017. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of thymus Daenensis Celak. Crop J. 5 (5), 407–415.

Feng, Yan Wen, Komatsu, Setsuko, Furukawa, Toshiko, Koshiba, Tomokazu, Kohno, Yoshihisa, 2008. Proteome analysis of proteins responsive to ambient and elevated ozone in rice seedlings. Agric. Ecosyst. Environ. 125 (1–4), 255–265.

Feng, Zhaozhong, Wang, Liang, Pleijel, Håkan, Zhu, Jianguo, Kobayashi, Kazuhiko, 2016. Differential effects of ozone on photosynthesis of winter wheat among cultivars depend on antioxidative enzymes rather than stomatal conductance. Sci. Total Environ. 572, 404–411.

Frei, M., Kohno, Y., Wissuwa, M., Makkar, H.P., Becker, K., 2011. Negative effects of tropospheric ozone on the feed value of rice straw are mitigated by an ozone tolerance QTL. Glob. Change Biol. 17 (7), 2319–2329.

Frei, Michael, Makkar, Harinder P.S., Becker, Klaus, Wissuwa, Matthias, 2010. Ozone exposure during growth affects the feeding value of rice shoots. Anim. Feed Sci. Technol. 155, 74–79.

Frei, Michael, Pariasca Tanaka, Juan, Wissuwa, Matthias, 2008. Genotypic variation in tolerance to elevated ozone in rice: dissection of distinct genetic factors linked to tolerance mechanisms. J. Exp. Bot. 59 (13), 3741–3752.

Fuhrer, Jürg, 2009. Ozone risk for crops and pastures in present and future climates. Naturwissenschaften 173–194.

Gnanamanickam, S.S., 2009. Rice and its Importance to Human Life.

Hamilton, Paul B., Van Slyke, Donald D., 1943. The gasometric determination of free amino acids in blood filtrates by the Ninhydrin-carbon Dioxide method. J. Biol. Chem. 150 (1), 231–250.

Heath, R.L., Taylor, G.E., 1997. Physiological processes and plant responses to ozone exposure. In: Forest decline and ozone. Springer, Berlin, Heidelberg, pp. 317–368.

Holopainen, Jarmo K., Kainulainen, Pirjo, Oksanen, Jari, 1997. Growth and reproduction of aphids and levels of free amino acids in Scots pine and Norway spruce in an open-air fumigation with ozone. Global Change Biol. 3 (2), 139–147.

Hur, Jae-seoun, 2014. Indicative responses of rice plant to atmospheric ozone. Plant Pathol. J. (October).

Inada, Hidetoshi, Yamaguchi, Masahiro, Satoh, Ryohei, Hoshino, Daiki, Nagasawa, Aki, Negishi, Yoh, Izuta, Takeshi, Nouchi, Isamu, Kobayashi, Kazuhiko, 2008. Effects of ozone on photosynthetic components and radical scavenging system in leaves of rice (Oryza sativa L.). J. Agric. Meteorol. 64 (4), 243–255.

Ishii, S., Marshall, F.M., Bell, J.N.B., Abdullah, A.M., 2004. Impact of ambient air pollution on locally grown rice cultivars (Oryza sativa L.) in Malaysia. Water Air Soil Pollut. 154 (1–4), 187–201.

Julkunen-Tiitto, Riitta, 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. J. Agric. Food Chem. 33 (2), 213–217.

Kajla, M., Yadav, V.K., Khokhar, J., Singh, S., Chhokar, R.S., Meena, R.P., Sharma, R.K., 2015. Increase in wheat production through management of abiotic stresses: a review. J. Appl. Nat. Sci. 7 (2), 1070–1080.

Kamal, M.A., Arshad, M.U., Zaidi, B.A., 2015. Appraisal of geotechnical characteristics of soil for different zones of Faisalabad (Pakistan). J. Eng. Appl. Sci. 17 (August), 1–10.

Kanhar, K.A., Kanher, F.M., Panhwar, R., Tunio, S.A., 2017. Parasitoids associated with mango leaf miner, acrocercops syngramma (Meyrick) Lepidoptera: gracillariidae in Mango Orchard. J. Entomol. Zool. 5 (4), 1582–1588.

Karberg, N.J., Pregitzer, K.S., King, J.S., Friend, A.L., Wood, J.R., 2005. Soil carbon dioxide partial pressure and dissolved inorganic carbonate chemistry under elevated carbon dioxide and ozone. Oecologia 142 (2), 296–306.

Keutgen, Anna J., Noga, Georg, Pawelzik, Elke, 2005. Cultivar-specific impairment of strawberry growth, photosynthesis, carbohydrate and nitrogen accumulation by ozone. Environ. Exp. Bot. 53 (3), 271–280.

Köllner, B., Krause, G.H.M., 2000. Changes in carbohydrates, leaf pigments and yield in potatoes induced by different ozone exposure regimes. Agric. Ecosyst. Environ. 78 (2), 149–158.

Krupa, S., McGrath, M.T., Andersen, C.P., Booker, F.L., Burkey, K.O., Chappelka, A.H., et al., 2001. Ambient ozone and plant health. Plant Dis. 85 (1), 4–12.

Leisner, C.P., Ainsworth, E.A., 2012. Quantifying the effects of ozone on plant reproductive growth and development. Glob. Change Biol. 18 (2), 606–616.

Leung, Felix, Pang, Jacky Y.S., Tai, Amos P.K., Lam, Timothy, Tao, Donald K.C., Sharps, Katrina, 2020. Evidence of ozone-induced visible foliar injury in Hong Kong using phaseolus vulgaris as a bioindicator. Atmosphere 11 (3).

Li, Mei, Guo, Shi, Yi, Chen, Xin, 2008. Effects of elevated carbon dioxide and ozone on the growth and secondary metabolism of spring wheat. Chin. J. Appl. Ecol. 19 (6), 1283–1288.

Manigbas, Norvie L., Dong-soo, Park, Soo-kwon, Park, Sang-min, Kim, Woon-ha, Hwang, Hang-won, Kang, Yi, Gihwan, 2010. Development of a fast and reliable ozone screening method in rice (Oryza sativa L.). Crop Sci. 2 (August), 251–258.

Manigbas, N.L., Park, D.S., Park, S.K., Kim, S.M., Hwang, W.H., Kang, H.W., et al., 2010. Development of a fast and reliable ozone screening method in rice (Oryza sativa L.). J. Plant Breed. Crop Sci. 2 (8), 251–258.

McGuire, Shelley, 2015. FAO, IFAD, and WFP. The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress. Rome: FAO. Adv. Nutr. 6 (5), 623–624.

Meyer, U., Köllner, B., Willenbrink, J., Krause, G.H.M., 2000. "Effects of different ozone exposure regimes on photosynthesis, assimilates and thousand grain weight in spring wheat. Agric. Ecosyst. Environ. 78 (1), 49–55.

Murchie, Erik H., Hubbart, Stella, Chen, Yizhu, Peng, Shaobing, Horton, Peter, 2002. Acclimation of rice photosynthesis to irradiance under field conditions. Plant Physiol. 130 (4), 1999–2010.

Noormets, Asko, Olevi, Kull, Anu, Sôber, Kubiske, Mark E., Karnosky, David F., 2010. Elevated CO2 response of photosynthesis depends on ozone concentration in aspen. Environ. Pollut. 158 (4), 992–999.

Oa, Yield W., Betzelberger, Amy M., Yendrek, Craig R., Sun, Jindong, Leisner, Courtney P., Nelson, Randall L., Ort, Donald R., Ainsworth, Elizabeth A., Plant Biology, and Genomic Biology, 2012. Ozone exposure response for U. S. Soybean cultivars : linear reductions in photosynthetic potential, biomass. Plant Physiol. 160 (December), 1827–1839.

Peng, B., Feng, G.N., Zhen, Q., Qiu, M., 2018. Effects of ozone and density interaction on the growth, development and yield formation of rice. Appl. Ecol. Environ. Res. 16 (4), 4199–4215.

Phothi, Rutairat, Umponstira, Chanin, Sarin, Charoon, Siriwong, Wapakorn, Nabheerong, Nivat, 2016. Combining effects of ozone and carbon dioxide application on photosynthesis of Thai Jasmine rice (Oryza sativa L.) cultivar Khao Dawk Mali 105. Aust. J. Crop. Sci. 10 (4), 591–597.

Pleijel, H., Eriksen, A.B., Danielsson, H., Bondesson, N., Selldén, G., 2006. Differential ozone sensitivity in an old and a modern Swedish wheat cultivar—grain yield and quality, leaf chlorophyll and stomatal conductance. Environ. Exp. Bot. 56 (1), 63–71.

Plessl, Markus, Elstner, Erich F., Rennenberg, Heinz, Habermeyer, Johann, Heiser, Ingrid, 2007. Influence of elevated CO2 and ozone concentrations on late blight resistance and growth of potato plants. Environ. Exp. Bot. 60 (3), 447–457.

Rai, R., Agrawal, M., Agrawal, S.B., 2007. Assessment of yield losses in tropical wheat using open top chambers. Atmos. Environ. 41 (40), 9543–9554.

Rai, Richa, Agrawal, Madhoolika, 2014. Assessment of competitive ability of two Indian wheat cultivars under ambient O3 at different developmental stages. Environ. Sci. Pollut. Control Ser. 21 (2), 1039–1053.

Rai, Richa, Agrawal, Madhoolika, Agrawal, S.B., 2010. Threat to food security under current levels of ground level ozone: a case study for Indian cultivars of rice. Atmos. Environ. 44 (34), 4272–4282.

Sarkar, Abhijit, Agrawal, S.B., 2010. Elevated ozone and two modern wheat cultivars: an assessment of dose dependent sensitivity with respect to growth, reproductive and yield parameters. Environ. Exp. Bot. 69 (3), 328–337.

Sarkar, A., Agrawal, S.B., 2011. Evaluating the response of two high yielding Indian rice cultivars against ambient and elevated levels of ozone by using open top chambers. J. Environ. Manage. 95, S19–S24.

Sarkar, Abhijit, Agrawal, S.B., 2012. Evaluating the response of two high yielding Indian rice cultivars against ambient and elevated levels of ozone by using open top chambers. J. Environ. Manag. 95 (SUPPL.), S19–24.

Sawada, Hiroko, Tsukahara, Keita, Kohno, Yoshihisa, Suzuki, Keitaro, Nagasawa, Nobuhiro, Tamaoki, Masanori, 2016. Elevated ozone deteriorates grain quality of japonica rice cv. Koshihikari, even if it does not cause yield reduction. Rice 9 (1), 1–10.

Shi, Guangyao, Yang, Lianxin, Wang, Yunxia, Kobayashi, Kazuhiko, Zhu, Jianguo, Tang, Haoye, Pan, Shiting, Chen, Tao, Liu, Gang, Wang, Yulong, 2009. Agriculture, ecosystems and environment impact of elevated ozone concentration on yield of four Chinese rice cultivars under fully open-air field conditions. Agric. Ecosyst. Environ. 131, 178–184.

Sitch, S., Cox, P.M., Collins, W.J., Huntingford, C., 2007. Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. Nature 448 (7155), 791–794.

Tomer, Ritu, Arti, Bhatia, Vinod, Kumar, Amit, Kumar, Singh, Renu, Singh, Bhupinder, Singh, S.D., 2015. Impact of elevated ozone on growth, yield and nutritional quality of two wheat species in northern India. Aerosol Air Qual. Res. 3, 329–340.

Usman, Muhammad, Raheem, Zainabfishan, Ahsan, Taswar, Iqbal, Adeela, Noreen Sarfaraz, Zul, Haq, Zabinfatul, 2013. "Morphological, physiological and biochemical attributes as indicators for drought tolerance in rice (Oryza sativa L.). Eur. J. Biol. Sci. 5 (1), 23–28.

Vingarzan, R., 2004. A review of surface ozone background levels and trends. Atmos. Environ. 38 (21), 3431–3442.

Wahid, A., Quaid-e-azam Campus, 1995. Effects of air pollution on rice yield in the Pakistan Punjab. Environ. Pollut. 90 (3), 323–329.

Wahid, A., 2006a. Influence of atmospheric pollutants on agriculture in developing countries: a case study with three new wheat varieties in Pakistan. Sci. Total Environ. 371 (1-3), 304–313.

Wahid, A., 2006b. Productivity losses in barley attributable to ambient atmospheric pollutants in Pakistan. Atmos. Environ. 40 (28), 5342–5354.