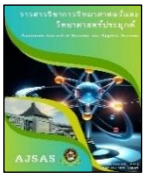
Faculty of Science and Technology, Uttaradit Rajabhat University

Academic Journal of Science and Applied Science

Journal homepage: https://ajsas.uru.ac.th/



Effects of elevated ozone levels on photosynthesis and physiological response in rice (*Oryza sativa* L.)

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ARTICLE INFO

Article History

Received: 8 May 2024

Revised: 26 June 2024

Accepted: 27 June 2024

Available online:

28 June 2024

Keywords:

ozone

photosynthesis

chlorophyll

dry weight

rice

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ABSTRACT

Elevated tropospheric ozone (O_3) has become a major threat to agriculture and crop production in several countries. This research aimed to investigate the effects of elevated ozone on photosynthesis, stomatal conductance, chlorophyll, and the dry weight of Thai rice cultivar RD47. Rice samples were fumigated with ozone concentrations of 40 ($EO_3$40) and 70 nmol/mol ($EO_3$70). Plants were kept in climate-controlled chambers for 28 days. Elevated ozone was applied according to the different treatments as per the experimental design. According to the results, elevated ozone at 70 nmol/mol significantly caused severe damage to rice RD47 as photosynthesis, stomatal conductance, chlorophyll, shoot, root, and total dry weight decreased by 44.42%, 28.47%, 16.17%, 19.41%, 49.53%, and 37.05%, respectively, compared to the control group ($p < 0.05$). The higher ozone concentration of 70 nmol/mol obviously caused more effects than the ozone concentration of 40 nmol/mol. Finally, the intrinsic physiological response involving the stomata aperture was determined to be one of the key effects of ozone. Photosynthesis mechanisms were severely disrupted, resulting in a reduction in carbon assimilation through dry weight loss.

1. Introduction

Tropospheric ozone has been reported to be increasing around the world because of the high emissions of precursor gases such as nitrogen dioxide and volatile organic compounds from industries and traffic [1]. It has been predicted that the concentration could rise on average from 60 to 100 nmol/mol in 2050 [2]. In Thailand, ozone concentration at ground level has been gradually increasing; for instance, in 2013, the ozone concentration was 45 nmol/mol, which was recorded in Nakhon Sawan province, i.e. the central ricegrowing region of Thailand. Also, several areas in the central and northern parts of Thailand found ozone concentrations exceeding above 40 nmol/mol during 8 hours from the annual report and the highest 8 hours mean value was about 60-142 nmol/mol observed from 55 air monitoring stations, which only 2 stations found the highest concentrations less than 70 nmol/mol. Thailand was considered at risk from ozone effects [3]. The effects of ozone have been well studied in several plants, including cell metabolism and physiology responses, which affected the growth and yield of agricultural plants [4]. Normally, ozone reduced plant height, leaf area [5], causing leaf injury [6], leaf senescence and premature defoliation [7]. Moreover, decreasing of chlorophyll and photosynthetic parameters were commonly found [8]. There were large areas around the world experience the effect of elevated ozone on growth and yield of crops [9]. A decline in the photosynthesis from ozone could relate to several mechanisms, for instance, reduction of stomatal conductance, chlorophyll and Rubisco activity. When ozone entered to the intercellular space of the mesophyll [10] and become reactive with oxygen species [6] cell membrane was perhaps injured [11]. Also ozone induced inhibition carbon dioxide fixation in

carboxylation process due to stomata closure [12], cell death, changes in gene expression, enzyme activities and metabolic profiles also destroy Rubisco enzyme in Calvin cycle [1]. This research was to investigate the effects of elevated ozone on photosynthesis, stomatal conductance, chlorophyll and dry weight of rice. Thai rice cultivar RD47 was selected due to non-photo sensitivity and being introduced to the farmer recently. The effects of elevated ozone on rice photosynthesis and physiology have been investigated. The knowledge obtained from this study could be applied as a practical strategy for suitable rice cultivar selection in climate change and rice plantation area selection.

2. Research Methodology

2.1 Experiment facility

The experiment was conducted in the climate control chambers. The facility was located at Air Pollution Laboratory, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Thailand.

2.2 Plant materials

Rice seeds were germinated in 21 x 26 cm in plastic tray. Seedlings two weeks old were transferred to 12 inch plastic pot fill with clay soil preparing from the paddy field and proper fertilizer was applied during the experimental period. Eight pots of each different treatment were required which number of total plant sample was 24 pots.

2.3 Ozone exposure and climate control

The experiment was conducted in 3 treatments: control group (CF; Charcoal Filter: O_3<10 nmol/mol), elevated ozone 40 nmol/mol ($EO_3$40) and elevated ozone 70 nmol/mol ($EO_3$70). Inlet air from outside the chamber was passed through charcoal filter to eliminate background ozone before enter to the chambers. The control group grew in the chambers which no elevated ozone given. Plant samples were subjected to elevated ozone at 40 and 70 nmol/mol for 8 hours/day (09.00 – 17.00 hours) for 28 days. Elevated ozone was generated by an ozone generator, model OZ 3020 (Belle, Ltd., Bangkok, CO, Thailand) and supply to the chamber through the stainless steel ball valve controller. Ozone concentration was daily monitored by an Ozone Analyzer model 49 C (Thermo Environmental Instruments, USA). Temperature was controlled at 28 °C and 35 °C at night and day. Relative humidity was recorded by Testo 608-H1 – Thermo-hygrometer (Testo Limited, UK) as 80-95 %. Light was supplied for 12 hours/day by two 400 watt bulbs of metal-halide.

2.4 Analysis of photosynthesis, stomatal conductance, chlorophyll and dry weight

Photosynthesis and stomatal conductance were analyzed by a portable photosynthesis system (LI- 6400, Li-Cor, Lincoln, NE, USA) with an open system and logged at carbon dioxide concentration at 400 μmol/mol in the leaf chamber where a constant air flow rate of 500 μmol/s was set. The photosynthetic photon flux density (PPFD) was maintained at 1500 μmol/m^2/s by an artificial light source [13].

Chlorophyll was analyzed by non-destructive method chlorophyll meter model SPAD-502 (Minolta Camera Co., Osaka, Japan). Second leaves were selected in each measurement and three SPAD replicate readings were taken around the middle of each leaf.

Plant samples were subjected to the final harvest at day 28 of ozone exposure. Shoots and root were collected separately and washed then subsequently dried in a hot air oven at temperature of 70 °C for 72 hours before weighing.

2.5 Statistical analysis

The data was analyzed by Analysis of variance: one way ANOVA with different between group was perform by Duncan's New Multiple Range Test (DMRT).

3. Results and Discussion

3.1 Photosynthesis

Ozone exposure showed significantly reduction of the photosynthesis of rice. The photosynthesis values of ozone fumigating plants at 70 nmol/mol were gradually declined as 12.73±1.04, 12.59±0.76, 10.12±0.77 and 9.60±0.99 μmol/m^2/s, respectively. Nevertheless, in the control group, the photosynthesis values were greater than ozone fumigating plants and slightly declined as 21.62±1.17, 21.30±0.29, 19.65±0.72 and 18.47±0.53 μmol/m^2/s from day 7, 14, 21 and 28, respectively. The results showed ozone 70 nmol/mol caused high reduction of photosynthesis as 41.13, 40.92, 48.48 and 48.01% (Table 1). Similarly, ozone fumigating plant at 40 nmol/mol, the photosynthesis values were less than the control group as 18.23±1.08, 16.45±0.50, 16.07±0.87 and 15.87±1.36 μmol/m^2/s from day 7 to day 28, respectively and photosynthesis values was significantly decrease 15.65, 22.77, 18.24 and 14.08% when compared with the control group. This result was similar to several studies in rice which found decreasing of photosynthesis by ozone, for instance, Indian rice photosynthesis and stomatal conductance were reduced by ozone [5] and several

Bangladesh rice cultivars fumigated by different dose of ozone from 60-100 nmol/mol [14]. In the different crop, wheat also showed the decreasing of photosynthesis, stomatal conductance and chlorophyll fluorescence kinetics. Mostly, major leaf photosynthetic protein e.g. Rubisco and important energy metabolism protein e.g. Adenosine triphosphate (ATP) synthase, aldolase and phosphoglycerate kinase were drastically reduced [15].

3.2 Stomatal conductance

The stomatal conductance was significantly ecreased from 18.93 to 36.40% (Table 1) when compared with the control group during 28 days of exposure to ozone 70 mol/mol. Nevertheless, there had no significant difference of stomatal conductance when plants were fumigated by ozone 40 nmol/mol. The average of stomatal conductance value were 0.36±0.07 (CF), 0.34±0.01 ($EO_3$40) and .26±0.02 ($EO_3$70) mol $H_2O/m^2/s$ (Table1). Several researches have been investigating the effect of ozone on stomatal conductance, for instance, Kangasjärvi J. et al. [16] found the decreasing of stomatal conductance because of ozone enter to the leaves through the stomata and impaired of guard cells. Indeed, ozone destroyed plasma membrane and guard cells lost plasmodesmatal connections. Photosynthesis change in stomatal conductance after expose to elevated ozone had been imputed to a direct effect of ozone on photosynthesis due to stomata closure for plant protection mechanisms. At the same time it showed the negative result to photosynthesis process due to decline in carbon dioxide fixation [17]. However, some studies in wheat found low dose of ozone induced reduction in photosynthesis which had less effect on stomatal conductance called non-stomatal factors. This is similar to our results which low dose of ozone concentration at 40 nmol/mol did not alter the stomatal conductance but still causing effect on photosynthesis.

3.3 Chlorophyll

Chlorophyll damaged by the elevated ozone could be measured by decreasing of SPAD which affected efficiency of photochemical reaction [18].The results showed average SPAD values of the control group was 45.39±0.44 unit (Table 1). After exposure to ozone 40 and 70 ppb for 28 days average SPAD values were significantly decreased as 42.97±0.48 and 38.05±0.48 unit. The results showed average values of SPAD were declined 5.33 and 16.17% when compared with the Control group This results was similar to Sawada et al. [19] found that the SPAD values in rice leaves were significantly lower during ozone exposure. In the different

Table 1 Photosynthesis, stomatal conductance and chlorophyll of rice under control groups, elevated ozone concentration at 40 nmol/mol ($EO_3$40) and 70 nmol/mol ($EO_3$70).

	Days of exposure ozone	**CF**	**$EO_3$40**	**%change when compared with CF**	**$EO_3$70**	**%change when compared with CF**
Photosynthesis	7	21.62±1.17a	18.23±1.08b	-15.65	12.73±1.04c	-41.13
(μmol/m^2/s)	14	21.30±0.29a	16.45±0.50b	-22.77	12.59±0.76c	-40.92
	21	19.65±0.72a	16.07±0.87b	-18.24	10.12±0.77c	-48.48
	28	18.47±0.53a	15.87±1.36a	-14.08	9.60±0.99b	-48.01
	Average	**20.26±0.44a**	**16.65±0.51b**	**-17.79**	**11.26±0.51c**	**-44.42**
Stomatal	7	0.41±0.01a	0.37±0.02ab	-10.47	0.31±0.04b	-25.08
conductance	14	0.37±0.02a	0.35±0.01a	-4.83	0.30±0.02b	-18.93
(mol $H_2O/m^2/s$)	21	0.31±0.04a	0.32±0.02a	+5.35	0.20±0.03b	-36.40
	28	0.37±0.03a	0.31±0.02ab	-15.00	0.24±0.03b	-35.32
	Average	**0.36±0.07a**	**0.34±0.01a**	**-6.12**	**0.26±0.02b**	**-28.47**
Chlorophyll	7	47.38±0.47a	44.27±0.28b	-6.56	40.80±0.67c	-13.88
(SPAD value)	14	43.85±0.51a	40.10±0.58b	-8.55	36.28±0.43c	-17.26
	21	43.48±0.59a	43.03±0.46a	-1.04	37.92±0.80b	-12.79
	28	46.83±0.60a	44.48±1.12a	-5.02	37.18±0.86b	-20.61
	Average	**45.39±0.44a**	**42.97±0.48b**	**-5.33**	**38.05±0.48c**	**-16.17**

*The data represent the mean ± SE (n = 6). Different letters indicate significant differences among treatments at $p < 0.05$.

Table 2 Shoot, root and total dry weight of rice under control groups, elevated ozone concentration at 40 nmol/mol ($EO_3$40) and 70 nmol/mol ($EO_3$70).

Plant portion	Dry weight (g)		
	CF	**$EO_3$40**	**$EO_3$70**
Shoot	21.52±2.89a	20.11±1.03ab	14.83±0.87b
Root	10.23±1.45a	7.29±0.64ab	5.16±0.57b
Total	31.75±4.10a	27.40±1.52ab	19.99±1.25b

*The data represent the mean ± SE (n = 5). Different letters indicate significant differences among treatments at $p < 0.05$.

crop, wheat [20], winter wheat and flixweed [21] also showed the decreasing of chlorophyll. Another effect of ozone was observed by visible leaf injury which caused leaf senescence and result to reduce leaf area for photochemical reaction. Total chlorophyll was an indicator of the degradation of the photosynthetic apparatus [19] and related with the reduction of photosynthesis [5].

3.4 Shoot, root and total dry weight

After 28 days of ozone exposure the average of shoot dry weight value was significantly decreased (Fig.1) as 21.52±2.89 (CF), 20.11±1.03 ($EO_3$40) and 14.83±0.87 ($EO_3$70) g. Furthermore, root dry weight was decreased as 10.23±1.45 (CF), 7.29±0.64 ($EO_3$40) and 5.16±0.57 ($EO_3$70) g. Like shoot and root the total dry weight was significantly decreased as 31.75±4.10 (CF), 27.40±1.52 ($EO_3$40) and 19.99±1.25 ($EO_3$70) g (Table 2).

The results obviously showed rice dry weight was decreased by ozone 70 nmol/mol as 19.41, 49.53 and 37.05%; shoot, root and total dry weight respectively when compared with the control group (Fig. 2). Like the other measurement of this study found less impact of ozone concentration at 40 nmol/mol which reduced plant dry weight as 4.88, 28.74 and 13.72% (Fig. 2); shoot, root and total dry weight respectively when compared with the control group. Reduction of photosynthesis in previous measurement was certainly related to dry weight loss. Perhaps soluble sugar first product from photosynthesis process was also decrease due to ozone [10]. Similarly, Imai and Kobori [22] found that shoot, root and total dry weight of rice and yield were decreased by elevated ozone due to the suppression of photosynthesis. Significant decreasing of root dry weight was larger than shoot dry weight by ozone due to photosynthetic carbon assimilation was inhibited and limitation of carbohydrate translocate to roots. Considering root function and phloem transport was interrupted by ozone [23] or alternations in phloem loading and assimilate partitioning [14]. Rice was considered as another sensitive crop similar to wheat which showed reduction of dry weight even exposed to mild ozone 40 nmol/mol [20].

Figure 1 RD47 rice cultivar after exposure.

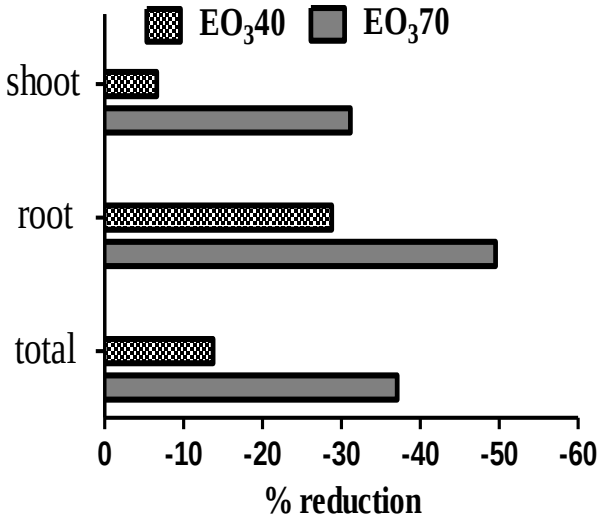

Figure 2 Percentage of the reduction of rice shoot, root and total dry weight under elevated ozone concentration at 40 nmol/mol ($EO_3$40) and 70 nmol/mol ($EO_3$70) compared with control groups.

4. Conclusion

This study showed the negative effects of elevated ozone on Thai rice cultivar RD47. Photosynthesis and total dry weight were those affected from reduction of Vcmax, Jmax, stomatal conductance and chlorophyll even though exposure to a low dose of ozone 40 nmol/mol. Notably higher dose of ozone has been considered retarding rice growth and providing severe damage to leaf stomata. Consequently, leaf gas exchange was reduced as shown by stomatal conductance reduction which significantly affected to the other physiological mechanisms. Finally, the effects of ozone could be described the link between photosynthesis and its mechanisms. This could be a risk and damage rice production of the country. The further research of rice physiological response and experiment in open top chambers with ozone was required to find out the other response mechanisms.

Acknowledgement

The authors would like to thank Faculty of Agricultural, Natural Resources and Environment, Naresuan University for facilities and equipments.

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