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Combining effects of ozone and carbon dioxide application on photosynthesis of Thai jasmine rice (*Oryza sativa* L.) cultivar Khao Dawk Mali 105

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Abstract

This research investigated the effects of elevated ozone and carbon dioxide on photosynthesis of rice (*Oryza sativa* L.) cultivar Khao Dawk Mali 105. Seedlings were kept in indoor climate control chambers which were set to typically background level of ozone (<10 ppb) by passing inlet air from outside through charcoal filter prior to enter to the chambers. Plant samples were fumigated by ozone concentration level at 40 ppb, 70 ppb and carbon dioxide concentration level at 700 ppm. For combined effects, elevated carbon dioxide concentration 700 ppm was given into two combination treatments of ozone concentration level at 40 and 70 ppb. Control groups were grown in charcoal-filter chambers with no additional ozone. Plant samples then were fumigated with ozone and carbon dioxide for 28 days at tillering stage (at the rice age of 42 to 70 days), and analyzed weekly for photosynthesis rate, leaf chlorophyll, total soluble sugar and biomass. The results showed that ozone significantly caused reduction in photosynthesis, leaf chlorophyll, total soluble sugar and total biomass of rice. The ozone concentration level of 70 ppb significantly ($p \leq 0.05$) affected rice more than 40 ppb treatment. Nevertheless, elevated carbon dioxide reduced the negative effects of ozone from both ozone concentration levels. Moreover, higher photosynthesis was observed in combined treatment, when compared with the control group. Finally, increasing of ozone caused reduction in rice photosynthesis; however, elevated carbon dioxide could significantly adverse the effects of ozone.

Keywords: ozone, carbon dioxide, rice, photosynthesis, chlorophyll, biomass, total soluble sugar.
Abbreviations: CF_charcoal-filter, $EO_3$40_elevated ozone concentration 40 ppb, $EO_3$70_elevated ozone concentration 70 ppb, ECO_2_elevated carbon dioxide concentration 700 ppm, $EO_3$40+ECO_2 _elevated ozone concentration 40 ppb combine with carbon dioxide concentration 700 ppm, $EO_3$70+ECO_2_elevated ozone concentration 70 ppb combine with carbon dioxide concentration at 700 ppm.

Introduction

Climate change was currently an important global problem which supposed to be more severe and affect people all around the world. Carbon dioxide is one of the main greenhouse gases which had been significantly increasing since the industrial revolution. Its concentration in the atmosphere was dramatically rising from 280 ppm in the last century to the present at 385 ppm. There was an attempt to hinder this situation by imposing an agreement of the world congress or The Kyoto Protocol (Vasser, 2009). However, the situation has not been improved enough in term of emission reduction; particularly form the industrial base countries and new developing countries. In contrast, there was a report from IPCC (Meehl et al., 2007) that predicted the carbon dioxide emission in 2050 would change to be 470 - 570 and climbs up to 730 - 1,020 ppm by year 2100. Not only carbon dioxide but also the other greenhouse gases, such as ozone, are anthropogenic pollutant, which are increasing significantly around the globe. This was due to high emission of precursor gases such as nitrogen dioxide and volatile organic compounds. Generally, ozone affects plants due to reactive oxygen species, when gas is uptaken through stomata and decrease plant growth and yield. Moreover, as a high free oxidative radical, the ozone gas is taken into plants and becomes more reactive with oxygen species (ROS) i.e. O_2^-, HO^- and H_2O_2 (Umponstira et al., 2006). Cell membrane damage will cause leaf visible injury and reduced chlorophyll content and photosynthesis. Ozone also inhibit carbon dioxide fixation in carboxylation process due to the decrease of stomata (Kumari et al., 2015) and also reduce the quantities and efficiency of enzymes in Calvin cycle (Vainonen and Kangasjärvi, 2015). Normally, the decreasing in photosynthesis would affect sugar collection and biomass of plants and eventually the productivity (Mafakheri et al., 2010). Several crops have been well studied for the effects of extra ozone such as wheat (Saitanis et al., 2014) potato (Lawson et al., 2001) soybean (Betzelberger et al., 2012) tomato (Calatayud and Barreno, 2001) and rice (Akhtar et al., 2010). Indeed, both carbon dioxide and ozone could affect plant metabolisms, growth and physiology in different ways (Al-Rawahy et al., 2013). Carbon dioxide affect many plant processes, primarily through direct effects on photosynthesis and stomatal function (Drake et al. 1997). Mostly, plants would gain benefit from elevated carbon dioxide by promoting growth and productivity because it was the basic source of carbon for plants and mainly utilized in synthesizing primary metabolites via photosynthesis (Mishra et al., 2013a). Long and Drake (1992) reported that across 32 studies involving plant growth under CO_2 enrichment, soluble carbohydrates increased by 52%, and across 62 studies starch content increased 160%. Feedback inhibition of photosynthesis to elevated carbon dioxide might result from carbohydrate source-sink imbalances that develop when photosynthetic rate exceeds the export capacity or the capacity of sinks (e.g. growing tissues and storage organs) to utilize the photosynthesis for growth (Gesch, 2001). Plant exposure to elevated carbon dioxide not only caused a significant increase in the soluble sugar and starch contents, but also an increase in the cellulose content (Nianjun et al., 2006). This could result in the accumulation of soluble carbohydrates in photosynthetic active source tissues (Farrar and Williams, 1991). The two most direct alterations of plants physiology at elevated carbon dioxide were the increase in photosynthesis and the decrease of stomatal conductance followed by reduced transpiration rate (Ainsworth and Mcgrath, 2010).

Thailand, the largest rice producer and exporter for many decades in the Southeast Asia, would be under threat of climate change, particularly increasing of ozone at ground level. In 2013, 95% of 55 monitoring stations in Thailand detected ozone level were higher than the standard limit. The maximum of an hour average was found to be 73 - 190 ppb and the maximum of average 8 hour to be 60 - 142 ppb (Pollution Control Department, 2013). This had become serious air pollution problem in Thailand due to the increase of volatile organic compounds (VOCs) and nitrogen oxides (NOx) generate from traffic, industries and open field burning. The large areas of rice production in the central of the country such as Ayutthaya, Pathum Thani and Nakhon Sawan provinces had been also reported with ozone levels frequently higher than the standard limit, which certainly increase the risk of getting affected from ozone.

This research will investigate the effects of elevated ozone and carbon dioxide and combining effects of both gases on Thai jasmine rice cultivar Khao Dawk Mali 105. Rice was subjected to elevated ozone and carbon dioxide fumigation for 28 days. Photosynthesis, leaf chlorophyll, total soluble sugar and biomass were continuously analyzed during the experiment on a weekly basis.

Results and Discussion

Effects of ozone and carbon dioxide on photosynthesis

Both elevated ozone concentrations at 40 and 70 ppb caused significant reduction of photosynthesis after 28 days of the exposure, when compared with the control group (Table 1). Comparison between ozone fumigation and the control group, the average highest reduction value of photosynthesis was found under elevated ozone concentration at 70 ppb as 8.87±0.53 μmol $m^{-2}s^{-1}$ (Table 1) compared to control group (17.56±0.61 μmol $m^{-2}s^{-1}$). Indeed, less effect was observed in elevated ozone fumigated plants at 40 ppb as value as 13.01±0.99 μmol m^{-2} s^{-1}. Elevated carbon dioxide fumigated plants shown beneficiary on promoting photosynthesis since beginning of day 7, 14, 21 until last at day 28 of fumigation period with values of 28.64±0.98, 27.78±0.65, 31.44±1.22 and 25.51±0.89 μmol $m^{-2}s^{-1}$, respectively, which were still greater than the control group. There were no negative effects from ozone when combining ozone fumigation with carbon dioxide even though the severe effect commonly found under single exposure of high ozone concentration at 70 ppb. The results showed that average photosynthesis from combination of two gases is still as high as 26.18±0.82 and 22.83±0.84 μmol $m^{-2}s^{-1}$, when elevated ozone concentrations were 40 and 70 ppb, respectively. Similarly, the study of Kumari et al. (2015) in potato found that photosynthesis increased by 57.93% under elevated carbon dioxide and by 47.17% under elevated carbon dioxide and ozone, when compared with ambient carbon dioxide and ozone. There have been total significant differences of photosynthesis among groups of all treatments (Fig. 1a). Our results found different dose of ozone dramatically caused photosynthesis reduction of 27.28% and 49.63% by elevated ozone concentration at 40 and 70 ppb, respectively. Plants defense mechanisms naturally react to ozone by generate more antioxidant substances and reduced stomatal conductance. It consequently leads to carboxylation reaction which directly causes less carbon fixation in the main part of Calvin cycle (Ainsworth et al., 2012). Therefore, the reduction of photosynthesis was observed under extra ozone levels which has been well studied in several cereal crops such as Bangladesh rice (Akhtar et al., 2010), Indian rice (Sarkar and Agrawal, 2012) and wheat (Sarkar et al., 2010). Notably, the results of combining carbon dioxide with the ozone could alter the effect of ozone. Furthermore, increasing of high concentration carbon dioxide could directly supply enough carbon dioxide to carboxylation reaction of plant photosynthesis process during photochemical reaction when light energy was converted into ATP and NADPH (Ashraf and Harris, 2013). Also, increasing of intercellular carbon dioxide (Ci) under elevated carbon dioxide may reduce rubisco oxygenation as same as enhance rubisco carboxylation, resulting in an increase of photosynthesis (Kumari et al., 2015). The effect of ozone on photosynthesis of plant could be explained by reduction of stomatal conductance because of elasticity of guard cells damaged (Paoletti and Grulke, 2005), consequently decreasing carbon dioxide uptake capability. Nevertheless, plants spend shorter time to uptake carbon dioxide under high concentration of carbon dioxide, which allows less amount of ozone absorbing through stomata. Another study, combining carbon dioxide with the other factors such as soil and water temperature found that elevated carbon dioxide in each treatment could increase photosynthesis during the growing stage (Adachi et al., 2014). Similarly, the study of Vu et al. (1997) found increasing of net photosynthesis of rice when grew under elevated carbon dioxide at 660 ppm rather than elevated temperature.

Effects of ozone and carbon dioxide on leaf chlorophyll

The elevated ozone concentration at 40 and 70 ppb significantly decreased SPAD value. The highest average value was 42.95±0.64 (Fig. 1b) when rice was subjected to elevated carbon dioxide without ozone. This value was slightly higher than normal ambient carbon dioxide or the control group with average SPAD value of 41.59±0.52. After fumigation by elevated ozone concentration at 40 and 70 ppb for 28 days, the SPAD values were significantly dropped to 38.86±0.51 and 36.03±0.71, respectively. However, combining ozone with carbon dioxide fumigation, the average SPAD values were slightly improved to 39.35±0.37 and 38.43±0.48, where ozone concentrations were 40 and 70 ppb, respectively. Indeed, comparison between single ozone fumigation and carbon dioxide combination the average SPAD value was still greater than both single ozone concentration at 40 and 70 ppb. A correlation of photosynthesis and chlorophyll content was observed, when ozone treatments with and without carbon dioxide were compared (Fig. 3a). In presence of extra ozone the chlorophyll content was significantly damaged and caused reduction of photosynthesis, which affected photochemical

Table 1. Photosynthesis, leaf chlorophyll and total soluble sugar of rice. Plant samples were fumigated by ozone and carbon dioxide for 28 days; ozone 40 ppb (EO_340) and 70 ppb (EO_370) carbon dioxide 700 ppm (ECO_2) and the control group (CF). The data represent the mean± SE (n = 6; photosynthesis and leaf chlorophyll n=3 total soluble). Different letters indicate significant differences among treatments at $p \leq 0.05$.

	Days of exposure O_3	CF	EO_340	EO_370	ECO_2	ECO_2 + EO_340	ECO_2 + EO_370
Photosynthesis	7	21.36±0.83cd	19.17±1.43d	11.26±1.18e	28.64±0.98a	25.87±0.72ab	23.17±0.72bc
(μmol $m^{-2}s^{-1}$)	14	17.56±0.51c	14.24±1.43d	9.28±0.88e	27.78±0.65a	25.84±0.66ab	23.35±1.02b
	21	17.17±0.73c	9.15±0.44d	7.67±0.55d	31.44±1.22a	30.70±1.55a	27.01±1.54b
	28	14.17±0.48d	9.46±0.50e	7.27±0.85e	25.51±0.89a	22.33±1.33b	17.79±0.73c
	Average	17.56±0.61d	13.01±0.99e	8.87±0.53f	28.34±0.63a	26.18±0.82b	22.83±0.84c
Leaf chlorophyll	7	43.97±0.29a	41.58±0.60b	39.63±0.56c	45.00±0.35a	39.52±0.54c	40.98±0.52bc
(SPAD value)	14	42.52±0.69ab	39.23±0.88cd	37.67±0.86d	44.53±1.38a	41.13±0.63bc	39.43±0.54cd
	21	41.42±0.38a	38.63±0.48bc	34.72±0.42d	41.67±1.17a	39.40±0.39b	37.17±0.60c
	28	38.47±1.04ab	35.98±0.41b	32.10±1.22c	40.58±1.18a	37.33±0.53b	36.15±0.62d
	Average	41.59±0.52a	38.86±0.51b	36.03±0.71c	42.95±0.64a	39.35±0.37b	38.43±0.48b
Total soluble	7	70.37±1.30ab	62.19±0.67ab	59.02±0.98b	70.44±5.30ab	71.47±5.58a	65.37±3.96ab
Sugar (mg g^{-1} FW)	14	70.35±4.72a	59.62±4.05a	59.99±12.30a	75.47±1.43a	69.60±4.02a	60.89±3.61a
	21	82.04±2.92ab	65.62±3.20c	59.57±4.79c	88.89±5.03a	69.94±1.82bc	66.97±5.87c
	28	69.07±3.48ab	61.79±2.64bc	55.49±0.98c	75.74±2.15a	66.95±1.31ab	61.17±5.52bc
	Average	72.96±2.07ab	62.30±1.40d	58.52±2.88d	77.63±2.64a	69.49±1.62bc	63.60±2.21cd

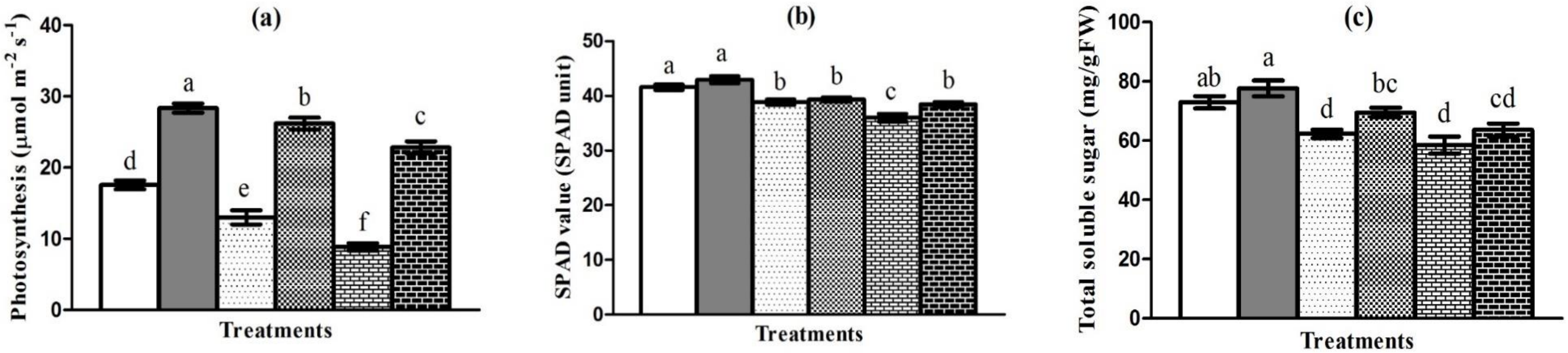

Fig 1. Photosynthesis (a), leaf chlorophyll (b), and total soluble sugar (c) of rice. Plant samples were allocated in six treatments; control (CF ;), elevated carbon dioxide (ECO_2 ;), ozone 40 ppb (EO_340 ;), ozone 40 ppb + carbon dioxide 700 ppm ($EO_340+ECO_2$;), ozone 70 ppb (EO_370 ;) ozone 70 ppb + carbon dioxide 700 ppm ($EO_370+ECO_2$;). The data represent the mean ± SE. Different letters indicate significant differences among treatments at $p \leq 0.05$.

Table 2: Shoot, root and total biomass of rice. Plant samples were fumigated by ozone and carbon dioxide for 28 days; ozone 40 ppb (EO_340) and 70 ppb (EO_370) carbon dioxide 700 ppm (ECO_2) and the control group (CF). The data represent the mean ±SE (n = 5). Different letters indicate significant differences among treatments at $p \leq 0.05$.

Biomass (g)	Shoot	Root	Total
CF	23.91±0.62c	17.75±0.38b	41.66±0.83b
EO_340	18.17±0.53d	13.17±0.37c	31.34±0.61d
EO_370	14.83±0.58e	9.30±0.32d	24.13±0.64e
ECO_2	30.26±1.02a	20.64±1.08a	50.90±1.30a
ECO_2+EO_340	26.47±0.87b	16.66±0.52b	43.13±0.90b
ECO_2+EO_370	23.89±1.01c	14.69±0.51c	38.58±1.47c

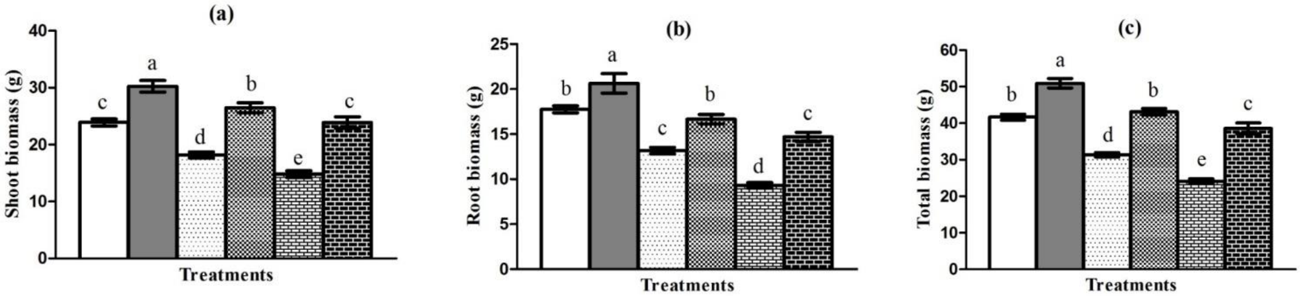

Fig 2. Shoot biomass (a), root biomass (b), and total biomass (c) of rice. Plant samples were allocated in six treatments; control (CF ;), elevated of carbon dioxide (ECO_2 ;), ozone 40 ppb (EO_340 ;), ozone 40ppb + carbon dioxide 700 ppm (EO_340+ECO_2 ;), ozone 70 ppb (EO_370 ;) ozone 70 ppb + carbon dioxide 700 ppm (EO_370+ECO_2 ;). The data represent the means±SE. Different letters indicate significant differences among treatments at $p \leq 0.05$.

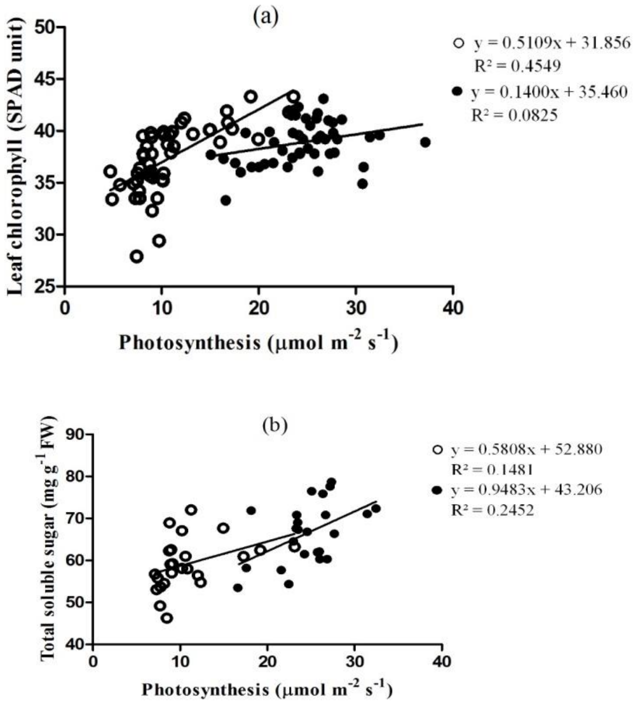

Fig 3. The relationship between leaf chlorophyll (a) and total soluble sugar (b) with photosynthesis of rice. Plant samples were fumigated by elevated ozone (○) and elevated ozone with carbon dioxide (●)

reactions (Sarkar and Agrawal, 2012). These results were similar to that, which evaluated effect of ozone exposure on chlorophyll content in rice leaves (Sawada et al., 2012), indicating SPAD values in leaves were significantly lower during ozone exposure. However, Mishra et al. (2013b) found chlorophyll content of tropical wheat was increased under elevated carbon dioxide with and without ozone. Under elevated carbon dioxide concentrations, the green leaf area index of the main shoot was increased, largely due to an increase in green leaf area duration (Mulholland et al., 1997). Moreover, elevated carbon dioxide increased chloroplast number, width and profile area, and starch grain size and number, but reduced the number of grana thylakoid membranes (Nianjun et al., 2006). The results showed elevated carbon dioxide would enhance the anti-oxidative system to prevent the action of reactive oxygen species produced by ambient and elevated ozone (Kumari et al., 2015).

Effects of ozone and carbon dioxide on total soluble sugar

The highest average value of total soluble sugar in the rice leaves was 77.63±2.64 mg g^{-1} FW, when rice was subjected to elevated carbon dioxide without ozone. This value was higher than the control group but not significantly different (Fig. 1c). Likewise photosynthesis and chlorophyll content, the total soluble sugar values were significant drop to 62.30±1.40 and 58.52±2.88 mg g^{-1} FW after expose to both ozone concentration levels at 40 and 70 ppb, respectively. Therefore, the effect of ozone could be described by decreasing of the green leaf area or in the rate of net carbon assimilation, which resulted in a lower production of carbohydrates (Gelang et al., 2001). Similarly, Sun et al. (2014) found that the total soluble sugar of soybean decrease under elevated ozone. Furthermore, the accumulation of carbohydrate and the decrease of photosynthesis were directly related to the opening and closing of stomata, which was affected by ozone exposure. The decreased volume of sugar was associated with glucose 6-phosphate, the source of NADPH, which was necessary to create the antioxidants. In addition, the activity and the amount of rubisco, enzyme involved in the photosynthesis were also reduced (Keutgen et al., 2005). The total soluble sugar was significantly improved when ozone combined with elevated carbon dioxide. The value improved to 69.49±1.62 and 63.60±2.21 mg g^{-1} FW when ozone treated at 40 ppb and 70 ppb combine with carbon dioxide, respectively. An increase in soluble carbohydrates often accompanies photosynthetic acclimation to long term growth under elevated carbon dioxide (Gesch, 2001). Similarly, this has been reported by Vu (2005) that found the total soluble sugar of peanut increased under elevated carbon dioxide. The correlation of photosynthesis and total soluble sugar production was clearly observed by two treatments of ozone combining with and without elevated carbon dioxide (Fig. 3b)

Effects of ozone and carbon dioxide on biomass

Like the other parameters, shoot, root and total biomass were decreased when rice was exposed to elevated ozone for 28 days. The highest reduction value of shoot, root and total biomass were found when fumigation was done by ozone concentration at 70 ppb, with the values of 14.83±0.58, 9.30±0.32 and 24.13±0.64 g, compared to the control group as 23.91±0.62, 17.75±0.38 and 41.66±0.83 g (shoot, root and total biomass), respectively (Fig. 2). As normal, less impact was observed under ozone concentration at 40 ppb with value of 18.17±0.53, 13.17±0.37 and 31.34±0.61 g, respectively. Therefore, ozone dose response to the effect was related to photosynthesis and the total soluble sugar. This was due to the suppression of photosynthesis by elevated ozone, reduced rice biomass and yield (Imai and Kobori, 2008). Similarly, in cowpea the biomass was significantly decreased under ozone concentration at 40 and 70 ppb (Umponstira et al., 2006). In wheat biomass was 16% decreased under ozone 43 ppb when compared to the control group (Feng et al., 2008). Interestingly, rice grew under the elevated carbon dioxide concentration at 700 ppm, gained shoot, root and total biomass with values of 30.26±1.02, 20.64±1.08 and 50.90±1.30 g, respectively, which was almost double of rice under elevated ozone concentration at 70 ppb. The results of combining effects from elevated carbon dioxide and ozone at 70 ppb, the shoot, root and biomass values were slightly less than sole elevated carbon dioxide treatment but still greater than the single ozone group (Table 2). Moreover, fumigation by combining elevated carbon dioxide and ozone 40 ppb had no effect on shoot, root and total biomass of rice. Under the condition of elevated carbon dioxide, photosynthesis would achieve its process, which significantly increases biomass accumulation in both above ground and below ground (root) biomass (Roy et al., 2012). This was similarly found in the other plants such as barley and tomato (Juknys et al., 2012).

Materials and Methods

Plant materials

Thai jasmine rice cultivar Khao Dawk Mali 105 provided by Surin Rice Research Center and was selected as the plant sample. This rice cultivar is an important breed in Thailand. It has been popular both domestically and internationally for its good quality. Rice seeds were germinated in a 21 × 26 cm plastic tray. The 14-day-old seedlings were then transferred into a 12 inch plastic pot filled with clay soil from the paddy field. Fertilizers were applied during the experiment. Forty eight pots were prepared and divided into 6 different treatments. To allow plants adapt themselves into the new environment, plant samples were kept in the fumigating chambers for 28 days before ozone and carbon dioxide was given. Plant samples then were fumigated with ozone and carbon dioxide for 28 days at tillering stages (at the rice age of 42 to 70 days) before analyzing the photosynthesis rate, leaf chlorophyll, total soluble sugar and biomass.

Experiment facility

The experiment was conducted in the indoor climate control chambers, which inlet air passed through a charcoal-filter to eliminate ambient ozone before entering the chambers. The facility was located at Air Pollution Laboratory, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand.

Ozone and carbon dioxide exposure and climatic control chambers

The experiment was arranged as 2×3 factorials, 2 carbon dioxide level (ambient and elevated) and 3 dose of ozone in Completely Randomized Design (CRD). Ozone in indoor climate control chambers were set to typically background level (<10 ppb) (Ehhalt et al., 2001) by passing inlet air from outside through charcoal filter prior to enter to the chambers. These chambers were allocated in to six different treatment as the control group (CF; Charcoal-Filter: no additional ozone), elevated ozone concentration at 40 ppb ($EO_3$40), elevated ozone concentration at 70 ppb ($EO_3$70), elevated carbon dioxide concentration at 700 ppm (ECO_2), elevated ozone concentration at 40 ppb combine with carbon dioxide concentration at 700 ppm ($EO_3$40+ECO_2) and elevated ozone concentration at 70 ppb combine with carbon dioxide concentration at 700 ppm ($EO_3$70+ECO_2). The control group grew in the chambers with no elevated ozone and carbon dioxide given. 42-day-old plant samples were subjected to elevated ozone concentration at 40 and 70 ppb for 8 hours day^{-1} (09.00 – 17.00 hours) and elevated carbon dioxide concentration at 700 ppm for 12 hours day^{-1} (06.00 – 18.00

hours) for 28 days. Notably, elevated carbon dioxide fumigation hour period was set following the light operation due to photosynthesis duration. The fumigating chambers were carefully calibrated to deliver elevated ozone and carbon dioxide concentration before plants were transferred inside. Ozone and carbon dioxide were generated by an ozone generator model OZ 3020 (Belle, Ltd., Thailand) and CO_2 cylinder. Ozone and carbon dioxide concentration levels were daily monitored by an ozone analyzer model 49C (Thermo Environmental Instruments, USA) and carbon dioxide analyzer model 41 C (Thermo Environmental Instruments, USA). Temperature was controlled at 35 ºC and 28 ºC during day and night in the air conditioning room. Relative humidity was maintained and recorded at 80-90% by Testo 608-H1-Thermohygrometer (Testo Limited, UK). Light was supplied for 12 hours day^{-1} by two 400 W metal-halide bulbs which provided 600 µmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD).

Measurements of photosynthesis, leaf chlorophyll, total soluble sugar and biomass

Photosynthesis, leaf chlorophyll and total soluble sugar were analyzed once a week at day 7, 14, 21 and 28 during ozone and carbon dioxide exposure. Photosynthesis analyzed by a portable photosynthesis system (LI- 6400, LiCor, Lincoln, NE, USA) with an open system and logged at carbon dioxide concentration of 400 µmol mol^{-1} in the leaf chamber with a constant air flow rate of 500 µmol s^{-1}. The photosynthetic photon flux density (PPFD) was maintained at 1500 µmol m^{-2} s^{-1} by an artificial light source (Shimono et al., 2004).

Leaf chlorophyll was measured by a non-destructive method chlorophyll meter (SPAD) (SPAD-502, Soil and Plant Analysis Development), Minolta Camera Co., Osaka, Japan). Six leave samples were selected for each measurement and three SPAD replicate readings were taken around the middle of each leaf. The mean of SPAD ± SE was calculated from total 18 readings of each measurement (Wang et al., 2012).

Total soluble sugar was determined by the phenol sulfuric acid method. 80% ethanol was added on fresh leaf and heated at 80^{o} C for one hour in water bath, crude extract was filtered and treated with 5% phenol and 98% sulfuric acid. The mixture was kept for one hour and then absorbance at 485 nm was determined by spectrophotometer (Dubois et al., 1956). Shoot and root of plant samples were finally harvested, washed and then subsequently dried in a hot air oven at 70 ºC for 72 hours before weighing.

Statistical analysis

The data was analyzed by analysis of variance (ANOVA) and difference between groups was performed by Duncan's New Multiple Range Test (DMRT) at $p \leq 0.05$. Six replications for photosynthesis and leaf chlorophyll, five replications for biomass and three replications for total soluble sugar analysis were applied. The data represents the mean value with standard error.

Conclusion

The study effects of elevated ozone and carbon dioxide in Thai jasmine rice cultivar Khao Dawk Mali 105 was indicated that the photosynthesis, chlorophyll, total soluble sugar and biomass of rice was decreased by ozone at both concentrations of 40 ppb and 70 ppb. The results from the combining gases fumigation showed that rice gain benefited from elevated carbon dioxide. There was compensation of elevated carbon dioxide to the effects of elevated ozone by enhancing photosynthesis, reducing chlorophyll damage, and promoting total soluble sugar and biomass production. These finding could be useful to improve and develop the new rice variety to cope with the atmospheric change in the future.

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References

Adachi M, Hasegawa T, Fukayama H, Tokida T, Sakai H, Matsunami T, Nakamura H, Sameshima R, Okada M (2014) Soil and Water Warming Accelerates Phenology and Down-Regulation of Leaf Photosynthesis of Rice Plants Grown Under Free-Air CO_2 Enrichment (FACE). Plant Cell Physiol. 55(2): 370-380.

Ainsworth EA, McGrath J (2010) Direct Effects of Rising Atmospheric Carbon Dioxide and Ozone on Crop Yields. In: Lobell D, Burke M (eds.) Climate Change and Food Security. Springer, Netherlands.

Ainsworth EA, Yendrek CR, Sitch S, Collins WJ, Emberson LD (2012) The Effects of Tropospheric Ozone on Net Primary Productivity and Implications for Climate Change. Annu Rev Plant Biol. 63(1): 637-661.

Akhtar N, Yamaguchi M, Inada H, Hoshino D, Kondo T, Fukami M, Funada R, Izuta T (2010) Effects of ozone on growth, yield and leaf gas exchange rates of four Bangladeshi cultivars of rice (*Oryza sativa* L.). Environ Pollut. 158(9): 2970-2976.

Al-Rawahy SH, Sulaiman H, Farooq SA, Karam MF, Sherwani N (2013) Effect of $O_3$and CO_2 Levels on Growth, Biochemical and Nutrient Parameters of Alfalfa (*Medicago Sativa*). APCBEE Procedia. 5: 288-295.

Ashraf M, Harris PJC (2013) Photosynthesis under stressful environments: An overview. Photosynthetica. 51(2): 163-190.

Betzelberger AM, Yendrek CR, Sun J, Leisner CP, Nelson RL, Ort DR, Ainsworth EA (2012) Ozone exposure response for U.S. soybean cultivars: Linear reductions in photosynthetic potential, biomass, and yield. Plant Physiol. 160(4): 1827-1839.

Calatayud A, Barreno E (2001) Chlorophyll a fluorescence, antioxidant enzymes and lipid peroxidation in tomato in response to ozone and benomyl. Environ Pollut. 115(2): 283-289.

Drake BG, Gonzalez-Meier MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2. Annu Rev Plant Phys. 48: 609-639.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem. 28: 350–356.

Ehhalt D, Prather M, Dentener F, Derwent R, Dlugokencky E, Holland E, Isaksen I, Katima J, Kirchhoff V, Matson P, Midgley P, Wang M (2001) Atmospheric Chemistry and Greenhouse Gases. In Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA (eds) Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, USA.

Farrar JF, Williams ML (1991) The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning. Plant Cell Environ. 14(8): 819–830.

Feng Z, Kobayashi K, Ainsworth EA (2008) Impact of elevated ozone concentration on growth, physiology, and yield of wheat (*Triticum aestivum* L.): A meta-analysis. Global Change Biol. 14(11): 2696-2708.

Gelang J, Selldén G, Younis S, Pleijel H (2001) Effects of ozone on biomass, non – structural carbohydrates and nitrogen in spring wheat with artificially manipulated source/sink ratio. Environ Exp Bot. 46(2): 155-169.

Gesch RW, Vu JCV, Allen JLH, Boote KJ (2001) Photosynthetic responses of rice and soybean to elevated CO_2 and temperature. Plant Physiol. 2: 125-137.

Imai K, Kobori K (2008) Effects of the interaction between ozone and carbon dioxide on gas exchange, ascorbic acid content, and visible leaf symptoms in rice leaves. Photosynthetica. 46(3): 387-394.

Juknys R, Januškaitienė I, Dikšaitytė A, Šliumpaitė I (2012) Impact of warming climate on barley and tomato growth and photosynthetic pigments. Biologija. 58(2): 87-97.

Keutgen AJ, Noga G, Pawelzik E (2005) Cultivar-specific impairment of strawberry growth, photosynthesis, carbohydrate and nitrogen accumulation by ozone. Environ Exp Bot. 53(3): 271-280.

Kumari S, Agrawal M, Singh A (2015) Effects of ambient and elevated CO_2 and ozone on physiological characteristics, antioxidative defense system and metabolites of potato in relation to ozone flux. Environ Exp Bot. 109: 276-287.

Lawson T, Craigon JIM, Tulloch AM, Black CR, Colls JJ, Landon G (2001) Photosynthetic responses to elevated CO_2 and O_3 in field grown potato (*Solanum tuberosum*). J Plant Physiol. 158(3): 309-323.

Long SP and Drake BG (1992) Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. In: Baker NR, Thomas H (eds) Crop Photosynthesis: Spatial and Temporal Determinants, Elsevier Science Publishers, Amsterdam.

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y (2010) Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust J Crop Sci. 4(8):580-585.

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao Z-C (2007) Global Climate Projections. In Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, USA.

Mishra AK, Rai R, Agrawal SB (2013a) Differential response of dwarf and tall tropical wheat cultivars to elevated ozone with and without carbon dioxide enrichment: Growth, yield and grain quality. Field Crop Res. 145: 21-32.

Mishra AK, Rai R, Agrawal SB (2013b) Individual and interactive effects of elevated carbon dioxide and ozone on tropical wheat (*Triticum aestivum* L.) cultivars with special emphasis on ROS generation and activation of antioxidant defence system. Indian J Biochem Bio. 50 (2):139-149.

Mulholland BJ, Craigon J, Black CR, Colls JJ, Atherton J, Landon G (1997) Effects of elevated carbon dioxide and ozone on the growth and yield of spring wheat (*Triticum aestivum* L.). J Exp Bot. 306 (48): 113-122.

Nianjun Teng, Jian Wang, Tong Chen, Wu Xiaoqin, Wang Yuhua, and Jinxing Lin. (2006) Elevated CO_2 Induces Physiological, Biochemical and Structural Changes in Leaves of Arabidopsis thaliana. New Phytol. 172 (1): 92-103.

Paoletti E, Grulke NE (2005) Does living in elevated CO_2 ameliorate tree response to ozone? A review on stomatal responses. Environ Pollut. 137(3): 483-493.

Pollution Control Department (2013) Thailand State of Pollution Report 2013. Ministry of Natural Resources and Environment. Bangkok, Thailand.

Roy KS, Bhattacharyya P, Neogi S, Rao KS, Adhya TK (2012) Combined effect of elevated CO_2 and temperature on dry matter production, net assimilation rate, C and N allocations in tropical rice (*Oryza sativa* L.). Field Crop Res. 139: 71-79.

Saitanis CJ, Bari SM, Burkey KO, Stamatelopoulos D, Agathokleous E (2014) Screening of Bangladeshi winter wheat (*Triticum aestivum* L.) cultivars for sensitivity to ozone. Environ Sci Pollut R. 21(23): 13560-13571.

Sarkar A, Agrawal SB (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 SB (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 Manage. 95: S19-S24.

Sawada H, Komatsu S, Nanjo Y, Khan NA, Kohno Y (2012) Proteomic analysis of rice response involved in reduction of grain yield under elevated ozone stress. Environ Exp Bot. 77: 108-116.

Shimono H, Hasegawa T, Fujimura S, Iwama K (2004) Responses of leaf photosynthesis and plant water status in rice to low water temperature at different growth stages. Field Crop Res. 89(1): 71-83.

Sun J, Feng Z, Ort DR (2014) Impacts of rising tropospheric ozone on photosynthesis and metabolite levels on field grown soybean. Plant Sci. 226: 147-161.

Umponstira C, Pimpa W, Nanegrungsun S (2006) Physiological and biochemical responses of cowpea (*Vigna unguiculata* (L.) Walp) to ozone. Songklanakarin J Sci Technol. 28(4): 861-869.

Vainonen JP, Kangasjärvi J (2015) Plant signalling in acute ozone exposure. Plant Cell Environ. 38(2): 240-252.

Vasser CP (2009) The Kyoto Protocol: Economic Assessments, Implementation Mechanisms and Policy Implications. Nova Science Publishers, New York.

Vu JCV (2005) Acclimation of peanut (*Arachis hypogaea* L.) leaf photosynthesis to elevated growth CO_2 and temperature. Environ Exp Bot. 53(1): 85-95.

Vu JCV, Aleen JLH, Boote KJ, Bowes G (1997) Effect of elevated CO_2 and temperature on photosynthesis and rubisco in rice and soybean. Plant Cell Environ. 20: 68-76.

Wang D, Xu C, Chen S, Tiao L, Zhang X (2012) Photosynthesis and Dry Matter Accumulation in Different Chlorophyll-Deficient Rice Lines. J Integr Agr. 11(3): 397-404.