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 ≤ 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, EO₄₀ elevated ozone concentration 40 ppb, EO₇₀ elevated ozone concentration 70 ppb, ECO₂ elevated carbon dioxide concentration 700 ppm, EO₄₀+ECO₂ elevated ozone concentration 40 ppb combine with carbon dioxide concentration 700 ppm, EO₇₀+ECO₂ 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. O₂-, HO· and H₂O₂ (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 Barreño, 2001) and rice (Akhtar et al., 2010). Indeed, both carbon dioxide and ozone could affect plant metabolisms, growth and physiology in different ways (Al-Rawahi 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 CO₂ 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⁻²s⁻¹ (Table 1) compared to control group (17.56±0.61 µmol m⁻²s⁻¹). Indeed, less effect was observed in elevated ozone fumigated plants at 40 ppb as value as 13.01±0.99 µmol m⁻²s⁻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⁻²s⁻¹, respectively, which were still higher 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⁻²s⁻¹, when elevated ozone concentrations were 40 and 70 ppb, respectively. Similarly, the study of Kumar 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 (Paolletti and Gruulke, 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 (EO₃40) and 70 ppb (EO₃70) carbon dioxide 700 ppm (ECO₂) 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 ≤ 0.05.

<table>
<thead>
<tr>
<th>Days of exposure O₃</th>
<th>CF</th>
<th>EO₃40</th>
<th>EO₃70</th>
<th>ECO₂</th>
<th>ECO₂ + EO₃40</th>
<th>ECO₂ + EO₃70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis</td>
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<td>(µmol m⁻² s⁻¹)</td>
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</tr>
<tr>
<td>7</td>
<td>21.36±0.83cd</td>
<td>19.17±1.43d</td>
<td>11.26±1.18e</td>
<td>28.64±0.98a</td>
<td>25.87±0.72ab</td>
<td>23.17±0.72bc</td>
</tr>
<tr>
<td>14</td>
<td>17.56±0.51c</td>
<td>14.24±1.43d</td>
<td>9.28±0.88e</td>
<td>27.78±0.65a</td>
<td>25.84±0.66ab</td>
<td>23.35±1.02b</td>
</tr>
<tr>
<td>21</td>
<td>17.17±0.73c</td>
<td>9.15±0.44d</td>
<td>7.67±0.55d</td>
<td>31.44±1.22a</td>
<td>30.70±1.55a</td>
<td>27.01±1.54b</td>
</tr>
<tr>
<td>28</td>
<td>14.17±0.48d</td>
<td>9.46±0.50c</td>
<td>7.27±0.85e</td>
<td>25.51±0.89a</td>
<td>22.33±1.33b</td>
<td>17.79±0.73c</td>
</tr>
<tr>
<td>Average</td>
<td>17.56±0.61d</td>
<td>13.01±0.99e</td>
<td>8.87±0.53f</td>
<td>28.34±0.63a</td>
<td>26.18±1.82b</td>
<td>22.83±0.84c</td>
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<td>Leaf chlorophyll</td>
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<tr>
<td>7</td>
<td>43.97±0.29a</td>
<td>41.58±0.60b</td>
<td>39.63±0.56c</td>
<td>45.00±0.35a</td>
<td>39.52±0.54c</td>
<td>40.98±0.52bc</td>
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<tr>
<td>14</td>
<td>42.52±0.69ab</td>
<td>39.23±0.88cd</td>
<td>37.67±0.86ed</td>
<td>44.53±1.38a</td>
<td>41.13±0.63bc</td>
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<tr>
<td>21</td>
<td>41.42±0.38a</td>
<td>38.63±0.48bc</td>
<td>34.72±0.42d</td>
<td>41.67±1.17a</td>
<td>39.40±0.39b</td>
<td>37.17±0.60c</td>
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<td>28</td>
<td>38.47±1.04a</td>
<td>35.98±0.41b</td>
<td>32.10±1.22c</td>
<td>40.58±1.18a</td>
<td>37.33±0.53b</td>
<td>36.15±0.62d</td>
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<tr>
<td>Average</td>
<td>41.59±0.52a</td>
<td>38.86±0.51b</td>
<td>36.03±0.71c</td>
<td>42.95±0.64a</td>
<td>39.35±0.37b</td>
<td>38.43±0.48b</td>
</tr>
<tr>
<td>Total soluble Sugar (mg g⁻¹ FW)</td>
<td>70.37±1.30ab</td>
<td>62.19±0.67ab</td>
<td>59.02±0.98b</td>
<td>70.44±5.30ab</td>
<td>71.47±5.58a</td>
<td>65.37±3.96ab</td>
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<td>14</td>
<td>70.35±4.72a</td>
<td>59.62±4.05a</td>
<td>59.99±12.30a</td>
<td>75.47±1.43a</td>
<td>69.60±4.02a</td>
<td>60.89±3.61a</td>
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<tr>
<td>21</td>
<td>82.04±2.92ab</td>
<td>65.62±3.20c</td>
<td>59.57±4.79c</td>
<td>88.89±5.03a</td>
<td>69.94±1.82bc</td>
<td>66.97±5.87c</td>
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<tr>
<td>28</td>
<td>69.07±3.48ab</td>
<td>61.79±2.64bc</td>
<td>55.49±0.98c</td>
<td>75.74±2.15a</td>
<td>66.95±1.31ab</td>
<td>61.17±5.52bc</td>
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<tr>
<td>Average</td>
<td>72.96±2.07ab</td>
<td>62.30±1.40dl</td>
<td>58.52±2.88d</td>
<td>77.63±2.64a</td>
<td>69.49±1.62bc</td>
<td>63.60±2.21cd</td>
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</tbody>
</table>

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 (ECO₂ ; ■), ozone 40 ppb (EO₃40 ; □), ozone 40 ppb + carbon dioxide 700 ppm (EO₃40+ECO₂ ; □), ozone 70 ppb (EO₃70 ; □) ozone 70 ppb + carbon dioxide 700 ppm (EO₃70+ECO₂ ; □). The data represent the mean ± SE. Different letters indicate significant differences among treatments at p ≤ 0.05.

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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$_3^{40}$) and 70 ppb (EO$_3^{70}$) 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 ≤ 0.05.

<table>
<thead>
<tr>
<th>Biomass (g)</th>
<th>Shoot</th>
<th>Root</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>23.91±0.62c</td>
<td>17.75±0.38b</td>
<td>41.66±0.83b</td>
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<tr>
<td>EO$_3^{40}$</td>
<td>18.17±0.53d</td>
<td>13.17±0.37c</td>
<td>31.34±0.61d</td>
</tr>
<tr>
<td>EO$_3^{70}$</td>
<td>14.83±0.58c</td>
<td>9.30±0.32d</td>
<td>24.13±0.64c</td>
</tr>
<tr>
<td>ECO$_2$</td>
<td>30.26±1.02a</td>
<td>20.64±1.08a</td>
<td>50.90±1.30a</td>
</tr>
<tr>
<td>ECO$_2$+EO$_3^{40}$</td>
<td>26.47±0.87b</td>
<td>16.66±0.52b</td>
<td>43.13±0.90b</td>
</tr>
<tr>
<td>ECO$_2$+EO$_3^{70}$</td>
<td>23.89±1.01c</td>
<td>14.69±0.51c</td>
<td>38.58±1.47c</td>
</tr>
</tbody>
</table>

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$_3^{40}$; □), ozone 40ppb + carbon dioxide 700 ppm (EO$_3^{40}$+ECO$_2$; □), ozone 70 ppb (EO$_3^{70}$; □), ozone 70 ppb + carbon dioxide 700 ppm (EO$_3^{70}$+ECO$_2$; □). The data represent the means±SE. Different letters indicate significant differences among treatments at p ≤ 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 (O) 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 77.6±2.64 mg g⁻¹ FW, when rice was exposed 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 ppm 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 other plants such as barley and tomato (Jukyns 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 factorial, 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) (Elblait 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 (EO40), elevated ozone concentration at 70 ppb (EO70), elevated carbon dioxide concentration at 700 ppm (ECO2), elevated ozone concentration at 40 ppb combine with carbon dioxide concentration at 700 ppm (EO40+ECO2) and elevated ozone concentration at 70 ppb combine with carbon dioxide concentration at 700 ppm (EO70+ECO2). 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 CO₂ 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 °C and 28 °C 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 leaf 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 °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 °C 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<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


