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Response of Malaysian Local Rice Cultivars Induced by Elevated Ozone Stress

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Abstract

Ground-level ozone affects agricultural crops and trees, including rice plants through reduction in antioxidant activities, physiological performance and yield production. For that reason, it is essential to identify the most tolerance rice cultivar as the ozone concentration in Malaysia is on the rise. In this study, four Malaysian local rice cultivars representing old cultivars, current dominant planted cultivar and newly introduced cultivar were exposed to two regimes of elevated ozone i.e. 60 and 120 ppb to determine their biochemical and yield response. For the biochemical aspect, superoxide dismutase (SOD) and glutathione reductase (GR) expression were examined because of their role as the first line of defence system and tolerance indicator towards elevated ozone exposure while yield performance was determined by total grain weight. Each of the cultivars exhibits statistically significant ($P<0.05$) susceptibility towards elevated ozone exposure in the range of 5-15% yield reduction with the old cultivar (*Mahsuri*) as the most tolerant towards ozone stress.

Keywords: biochemical expression; yield response; ground level ozone; local rice plants; Malaysia

1. Introduction

Ground level ozone or surface ozone (O_3) is one of the major air pollutants that negatively affecting human health and crops. O_3 is a secondary air pollutant produced by the reaction between two primary pollutants i.e. nitrogen oxides (NO_x) and volatile organic compounds (VOCs) under the influence of sunlight. Increasing emissions of NO_x and VOCs in Malaysia from activities such as motor vehicles, chemical and petroleum industries, power plants and industrial processes have caused formation of ozone to be amplified; becoming a major threat on crops. Moreover, Malaysia is located in the equatorial region and is at greater risk because its climate is characterised by high levels of solar radiation which can promote the formation of photochemical pollutants such as ozone (Ashmore and Bell, 1991). Premature leaf senescence, decrease in chlorophyll content and reductions of yield are among the negative effects of ozone stress (Biswas *et al*., 2008; Feng *et al*., 2008,). Studies conducted also found that crops i.e. maize, soybean, wheat, potatoes, and rice had experienced metabolism injury when exposed to elevated ozone stress. Ozone attacked antioxidants activities i.e. superoxide dismutase (SOD) and glutathione reductase (GR) which lead to physiological damage and reduction of yield in the range of 5% to 20% (Ishii *et al*., 2004; Pleijal *et al*., 2006; Agrawal *et al*., 2008). SOD and GR are two important antioxidants which play a role as the first line of defence system within cells and as tolerance indicator for ozone exposure.

In this study, rice was selected because 97% of Malaysian populations take rice as their major source of carbohydrate (Norimah *et al*., 2008). Furthermore the demand for rice is increasing parallel with the increasing population. The increase in production is targeted to come from higher productivity in the existing granary areas since there is no plan by the government to increase the paddy cultivation area. In fact, the total granary area in Peninsular Malaysia has experienced a loss of about 5% of its irrigated area as a result of conversion to urban land use during the past 20 years and is projected to lose another 3% by the year 2015 (MANCID, 2003). Hence, there is a significant need for this study to be conducted considering the ongoing increase in the Malaysian population which requires high production of rice to meet the projected domestic demand.

2. Materials and Methods

2.1. Plant material and experimental design

The experiment was conducted at the Universiti Malaysia Terengganu, Kuala Terengganu, Malaysia (5.94^{o}N; 103.42^{o}E) with four rice (*Oryzae sativa* L.) cultivars (cv. MR263, cv. MR219, cv. MR84 and cv. Mahsuri) obtained from the Malaysia Agricultural Research and Development Institute (MARDI) from

March 2011 until July 2012. MR84 and Mahsuri are old cultivars, MR219 is the current cultivar. MR263 is the just introduced future cultivar; and each has a life cycle from germination to maturity of 137 days, 138 days, 111 days and 95 days, respectively. Two close-top chambers (CTCs) of cylindrical type were constructed and used in this study. Each CTC is 1 meter in height, 1.5 meter in diameter, $0.77m^2$ in base area and $1.77m^3$ in capacity, fabricated from stainless steel covered with transparent Plexiglas sheet. The air-conditioner was applied as a mechanical ventilation system inside each chamber in order to ensure air exchange, temperature and relative humidity could be controlled to mimic the ambient condition. One of the CTCs was supplied with elevated ozone, i.e. 60 parts per billion and 120 parts per billion (ppb) for exposure process and the other one as a control. Microclimatic parameters, i.e. temperature and relative humidity were measured both inside and outside the CTCs. The mean temperature range was 26.8 - 39.4°C while relative humidity range 56.5 - 81.8%. During the whole rice plant growth period, weeds, diseases, and insect pests were absent, water and fertilizers were in abundance, there were no adverse soil conditions, and no extreme weather event, such as typhoons occurred.

All seeds were manually sown based on agronomic practice suggested by MARDI. Seeds were soaked with water and germinated within seven days, then being planted in 30 pots per cultivar (3 plants per pot), located in a greenhouse. Fourteen days before panicle initiation (P.I.) for each cultivar, 10 pots were randomly selected and placed in the OTC; of which 5 pots were exposed to elevated ozone concentrations, i.e. 60 and 120 parts per billion (ppb), for 8 hours per day for 3 consecutive days; while the remaining pots were used as a control.

2.2. Biochemical compound determinations

2.2.1. Superoxide dismutase (SOD)

Activities of superoxide dismutase were determined as described by Fridovich (1974). A 0.1 gram fresh sample from each selected rice plant was ground using mortar and pestle with 2 ml of phosphate buffer, pH 7.0. The samples were then centrifuged at 10,000 rpm at 4°C for 10 minutes. Then, 100 µl of crude extract was added to 1.0 ml of the reaction mixture. The reaction mixture was prepared using the method of Beauchamp and Fridovich (1971) which consists of 50 mM phosphate buffer with 0.1 mM EDTA (pH 7.8), 0.1 mM nitroblue tetrazolium (NBT), 0.048 mM xanthine oxidase and 0.05 mM xanthine. SOD activities were measured spectrophotometrically at wavelength 560 nm. One unit of SOD is defined as the amount of enzyme that slows down the rate of NBT reduction by 50%. Absorbance of the wavelength and unit of SOD activity were then calculated using equation developed by Giannopolitis and Ries in 1977:

$$\textit{Percentage of absorbance} = \frac{\Delta \text{ Absorbance}}{\textit{Initial}}\ x\ 100\%$$
$$= a\ \% \qquad (1)$$

Where; Δ Absorbance: Initial absorbance - final absorbance; a %: Percentage of absorbance Thus,

$$\textit{SOD activity} = \frac{\text{a }\%}{50\%} = U \qquad (2)$$

2.2.2. Glutathione reductase (GR)

Glutathione reductase (GR) activities were measured according to Calberg and Mannervick, 1985. A 0.15 gram fresh sample from each selected rice plant was ground up with 1.0 ml of 50 mM phosphate buffer (pH 7.0) in pre-chilled mortar and pestle at 0-4°C. The ground samples were then centrifuged at 10,000 rpm at 4°C for 10 minutes. Then, 200 µl of enzyme extract was added in 1.0 ml of reaction mixture. The reaction mixture consists of 100 mM phosphate buffer (pH 7.0), 1.0 mM oxidized glutathione (GSSG), 1.0 mM EDTA and 0.1 mM NADPH. Measurement of GR activities was performed by changes in absorbance at wavelength 340 nm. Glutathione reductase was calculated using the formula developed by Bergmeyer (1965):

$$\textit{GR activity} = \frac{\left(\frac{\Delta \text{ Absorbance}}{\textit{minute}}\right)(\textit{vol. of assay mixture})\ (60\ \textit{minutes})}{(\varepsilon)\ (\textit{vol. of assay mixture})}$$
$$= \frac{\textit{units}}{\textit{ml}}\ \textit{of sample} \qquad (3)$$

Where; ε: molar absorption coefficient of 6. 22 $mM^{-1}\ cm^{-1}$

2.3. Yield measurement

Grains of each cultivar were collected at ripening stage. Panicles were harvested using hand threshing and submerged in tap water in order to identify filled, half and empty spikelets. Then filled spikelets were dried in the oven at 70°C to constant weight and calculated according to Peng *et al*. (2004).

2.4. Statistical analysis

All the data were entered into a spreadsheet i.e. MS-Excel® for analysis. Fitness to normal distribution and homogeneity of variance of the data were examined by employing the Kolmogorov-Smirnov test for goodness of fit and Cochran's test for homogeneity of variances (Day and Quinn, 1989). Where normality and homogeneity of variance of the data were confirmed, single classification analysis of variance (ANOVA) *F test* was performed to compare biochemical compounds and yields at different ozone level exposure among the cultivars using SPSS-20 software.

3. Result and Discussion

3.1. Superoxide dismutase (SOD)

SOD activity clearly showed significant increments in expression response when ozone concentrations increased as shown in Fig. 1. Meanwhile, the average SOD expression for each cultivar is depicted in Fig. 2. From the four selected Malaysian local rice cultivars, MR219 has the highest SOD expressions when exposed to 60 ppb and 120 ppb, i.e. from 1.971 to 1.976 U/g (60 ppb) and from 1.976 to 1.980 U/g (120 ppb) while average SOD expressions at 60 ppb and 120 ppb exposure are 1.957±0.0115 U/g and 2.005±0.0083 U/g, respectively. MR263 rice cultivar has the second highest expressions, ranging from 1.944 - 1.974 U/g for 60 ppb with an average of 1.974±0.0018 U/g; and 1.996 - 2.017 U/g with an average of 1.978±0.0015 U/g when exposed to elevated ozone regime of 120 ppb. This is followed by Mahsuri and MR84, the two older cultivars; of which MR84 has the lowest expressions. The range of SOD expressions for Mahsuri is from 1.794 to 1.798 U/g with an average of 1.794 ±0.0014 U/g when exposed to 60 ppb and from 1.801 to 1.818 U/g with an average of 1.807±0.0054 when exposed to 120 ppb, while for MR84 rice cultivar, the increment of SOD expressions is from 1.320 to 1.429 U/g for 60 ppb exposure with an average of 1.380±0.0429 U/g and when exposed to 120 ppb, the expressions are from 1.600 to 1.780 U/g with an average 1.668±0.0785 U/g. Overall, SOD expressions are significantly different when exposed at different regimes of ozone concentration and among exposed and non-exposed rice cultivars. In addition, SOD expressions are also significantly different (*P<0.05*) between each rice cultivar.

3.2. Glutathione reductase

The expression of GR also increased significantly parallel to ozone concentration increment as shown in Fig. 3, while Fig. 4 illustrates the average GR activities for each cultivar. Mahsuri has the highest expression which ranges from 10.85 to 11.41 U/ml with an average of 11.180±0.2764 U/ml for 60 ppb exposure; and from 12.71 to 13.53 U/ml with average expression of 12.922±0.3419 U/ml for 120 ppb exposure. MR84 rice cultivar has the second highest expression, ranging from 10.06- 10.44 U/ml with an average of 10.230±0.1670 U/m g for 60 ppb and 10.18-11.38 U/ml with an average of 11.036±0.3121 U/ml for 120 ppb exposure. This is followed by MR263 and MR219; of which MR219 is the current dominant cultivar having the lowest

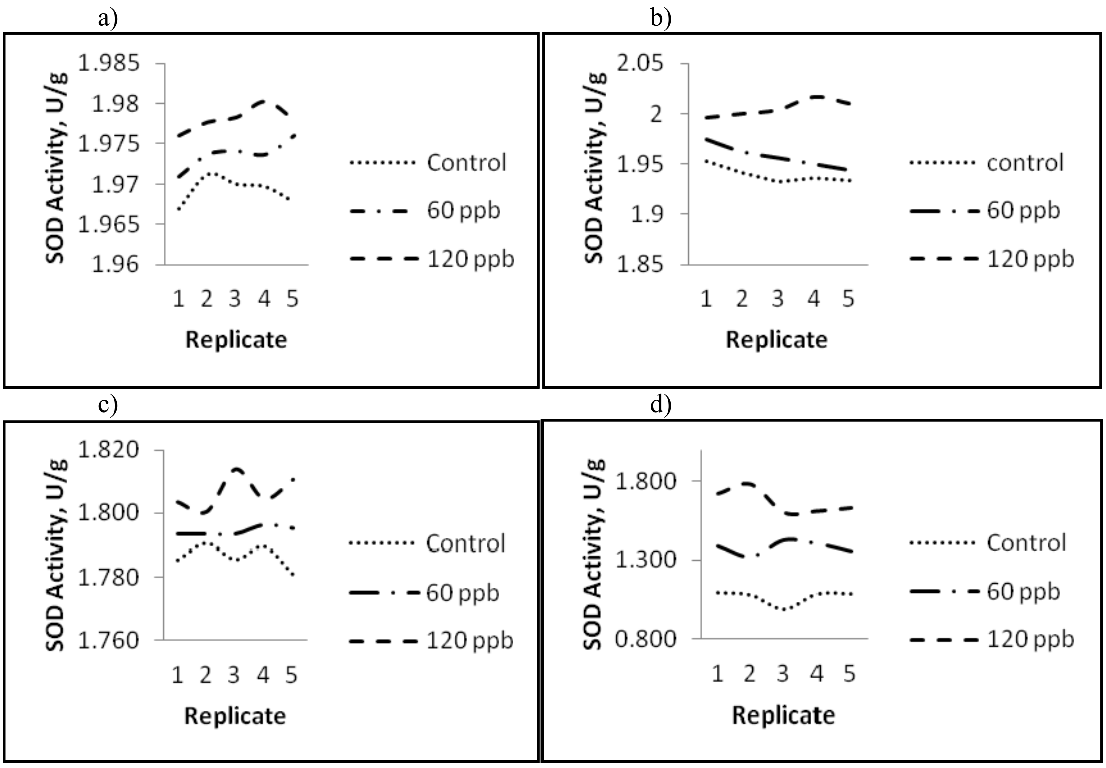

Figure 1. SOD activities of four selected rice cultivars at different ozone concentrations a) MR219 b) MR263 c) Mahsuri and d) MR84

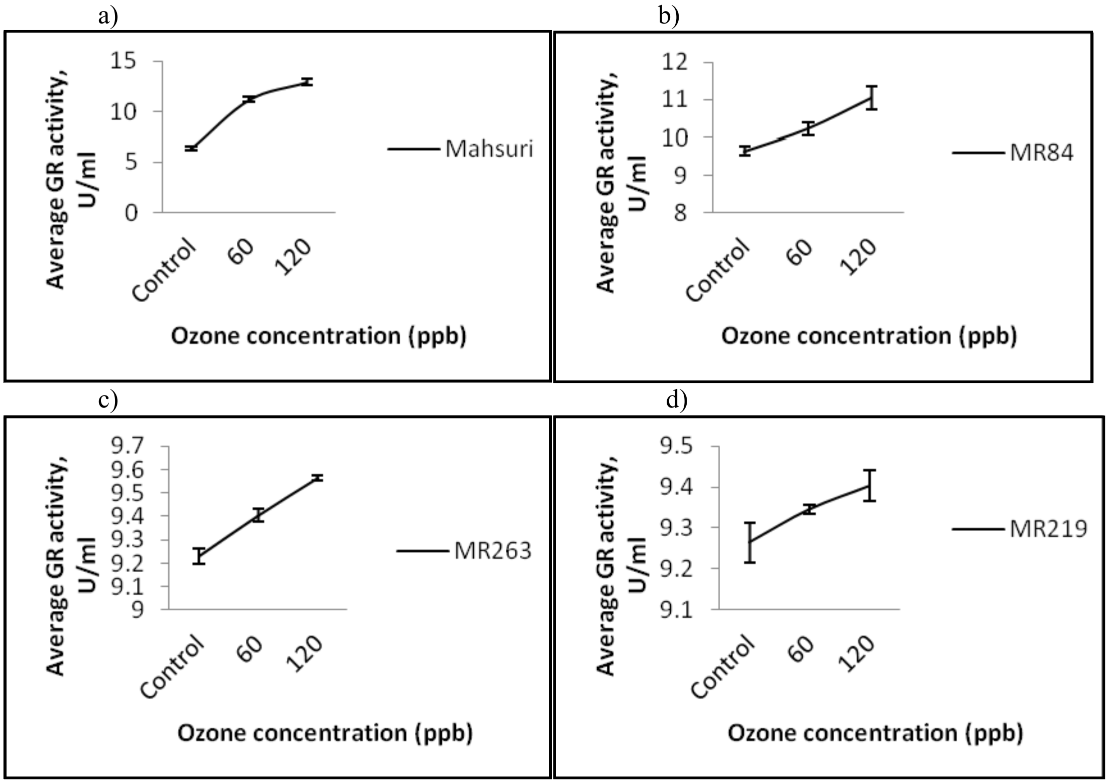

Figure 2. Average SOD activities of four selected rice cultivars at different ozone concentrations a) MR219 b) MR263 c) Mahsuri and d) MR84

expressions. The range of GR expressions for MR263 is from 9.36 to 9.43 U/ml with an average of 9.404±0.0270 U/ml when exposed to 60 ppb and from 9.55 to 9.58 U/ml with an average of 9.564±0.0114 U/ml when exposed to 120 ppb. Meanwhile, for MR219 rice cultivar, the increment of GR expressions is from 9.33 to 9.35 U/ml with an average of 9.346±0.0114 U/ml; and from 9.38 to 9.47 U/ml with an average of 9.404±0.0378 U/ml for

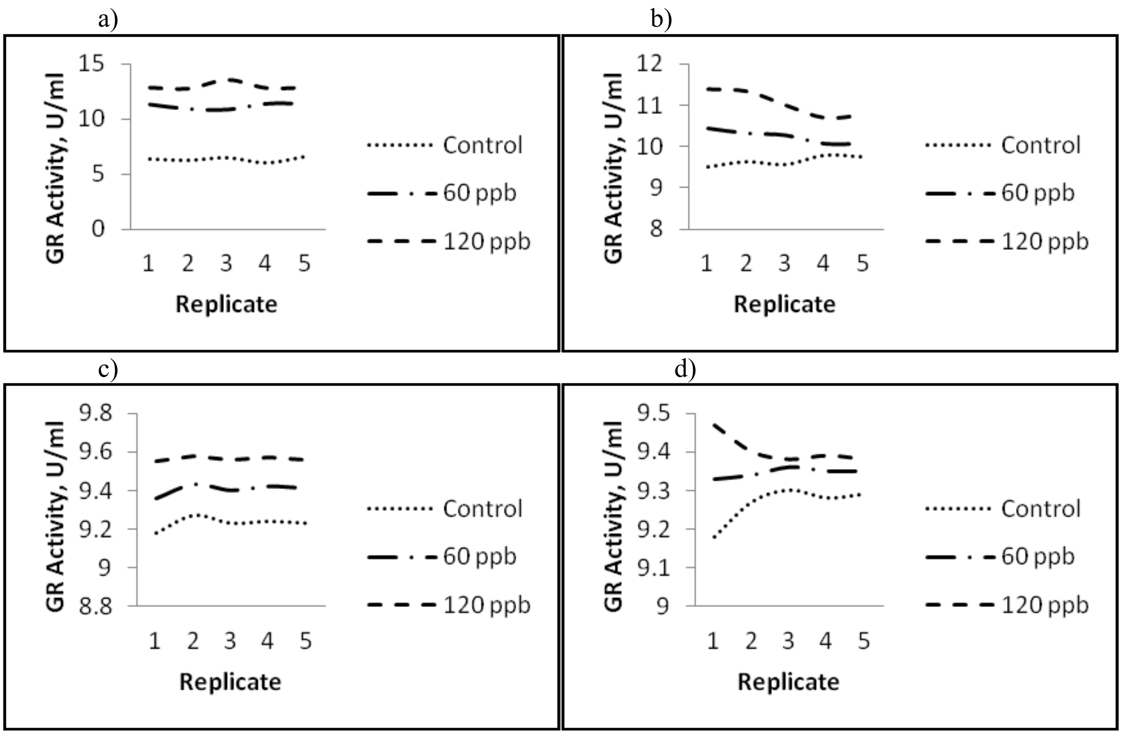

Figure 3. GR activities of four selected rice cultivars at different ozone concentrations a) Mahsuri b) MR84 c) MR263 and d) MR219.

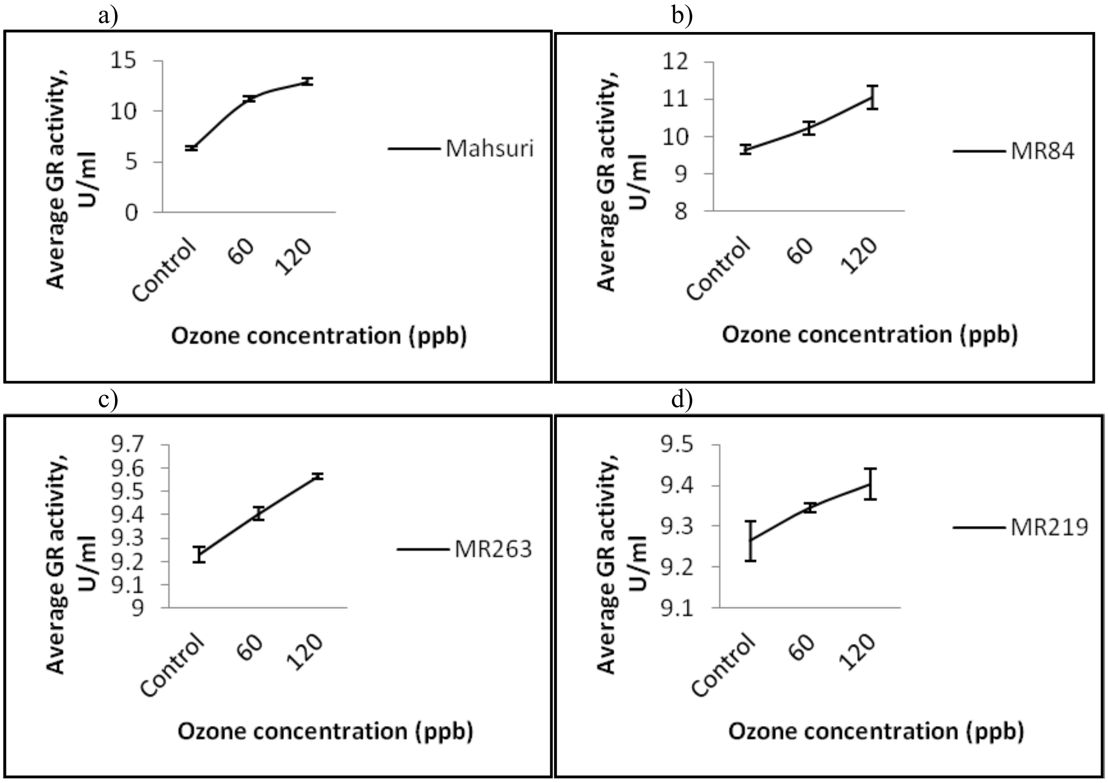

Figure 4. Average GR activities of four selected rice cultivars at different ozone concentrations a) Mahsuri b) MR84 c) MR263 and d) MR219

60 ppb and 120 ppb, respectively. On the whole, GR expressions are significantly different (*P<0.05*) when exposed to different regimes of ozone concentration among the rice cultivars and also among exposed and non-exposed of rice plants of each cultivar.

3.3. Yield of Selected Rice Cultivar

Effects of elevated ozone on yield of rice were determined by measuring dry weight of grains. There are significant differences (*P<0.05*) of yield among the rice cultivars and results showed that Mahsuri is the most resistant, i.e. having the least grains weight reduction, followed by MR84, MR263 and MR219 as in Fig. 5. There are also significant differences among non-exposed and exposed rice plants for each selected rice cultivar. Average yield for non-exposed rice plants of Mahsuri cultivar is 25.11±0.04 g which reduces to 23.85±0.03 g when exposed to 60 ppb and 23.10±0.04 g when exposed to 120 ppb. For MR84, the average yield for non-exposed rice plants is 25.21±0.04 g and being reduced to 23.69±0.04 g and 22.69±0.01 g when exposed to 60 ppb and 120 ppb of ozone concentration, respectively. For non exposed rice plants, the average yield of MR263 is 26.18±0.01 g, and starts to decline when exposed to elevated ozone, having average yield of 23.56±0.01 g and 22.77±0.01 g for exposure to 60 ppb and 120 ppb, correspondingly. For non-exposed rice plants, average yield of MR219 is the highest compared to other three selected rice cultivars which is 26.19±0.01 g. However, when exposed to the elevated ozone, yield of MR219 produced is less compared to the other three cultivars, with average yield of 22.78±0.01g and 22.40±0.01 g for 60 ppb and 120 ppb, respectively.

4. Discussion

Ozone enters the leaf through stomata which are located in the cell walls of plant cells. Then ozone, which is highly reactive, reacts within the apoplast (i.e. a space of diffusion) and produces free radicals and other reactive oxygen species (ROS) which lead to destruction of the structure and function of biological membranes (Rao, 2000; Heath, 2008). Hydrogen peroxide (H_2O_2) also has been reported as one ROS that is produced after the ozone exposure (Moeder *et al.*, 2002; Pellinen *et al.*, 2002; Oksanen *et al.*, 2003). Based on previous studies (van Hove *et al.*, 2001; Conklin and Barth 2004; Chen *et al.*, 2007), two initial factors which are reduction of antioxidants and rapid entry of ozone have been identified causing protection mechanism to decline and this leads to membrane injury. Superoxide dismutase is one of the essential antioxidants due to the facts that its role as a first line defence mechanism in living cell walls including rice plants. The role of SOD is detoxifying superoxide anion radical to

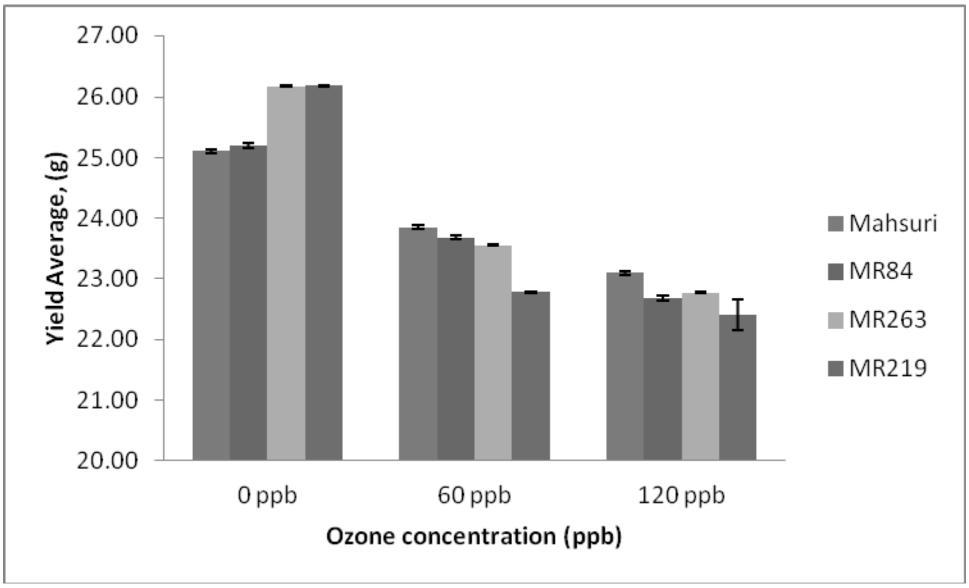

Figure 5. Average yield of each selected rice cultivar at different ozone concentrations

molecular oxygen and hydrogen peroxide (H_2O_2) which is then scavenged by ascorbate peroxidase (APX) and catalase (CAT). SOD expressions in all exposed rice plants increase when exposed to elevated ozone and this is similar to studies (Ariyaphanphitak *et al*., 2005; Agrawal *et al*., 2005; Mills *et al*., 2007; Feng *et al*., 2008) which found that SOD activity keeps increasing when ozone concentration increases and it shows that activity for detoxification of superoxide ions increases too. Meanwhile, response of GR activity towards elevated ozone is very important because of GR role as a tolerance indicator (Yousuf *et al*., 2012). The results of this study showed an incremental trend of GR expression when rice plants were exposed to elevated ozone concentrations. The trend is similar to studies conducted earlier (Nouchi, 1993; Calatayud and Barreno, 2004; Ariyaphanphitak *et al*., 2005; Biswas *et al*., 2008; Rai *et al*., 2010) which found that GR activity increased parallel to ozone concentrations in order to scavenge the oxidants and prevent injury which subsequently leads to yield losses. Mahsuri has been identified as the most resistant rice cultiva followed by MR84, MR263 and MR219. Eventhough SOD expressions of Mahsuri rice cultivar are low but due to high GR expressions, Mahsuri is determined as the most resistant because its detoxification of hydrogen peroxide and other ROS are high and efficient of ROS-scavenging capacity (Yousuf *et al*., 2012). Overall, reduction of yield that occurred to these four selected rice cultivars is in the range of 5% to 15% which is within the range of percentage from preceding studies (Ishii *et al*., 2004; Agrawal *et al*., 2005; Pleijal *et al*., 2006; Rai *et al*., 2010) where they found that reduction of yield of rice occurs in the range of 5% to 20% when exposed to elevated ozone concentration. The results clearly showed that there exists significant response of biochemical expression (i.e. SOD and GR) on selected Malaysian local rice cultivars when exposed to elevated ozone concentrations.

5. Conclusion

Interestingly, although the current dominant cultivar i.e. MR219 produced the highest yield in condition with no elevated ozone, it is by far the most susceptible to ozone attack. Moreover, this study has found that Mahsuri, which is an old variety, is the most resistant rice cultivar compared to the other Malaysian local rice cultivars. Therefore, it is suggested that in breeding new cultivars, plant breeders must design new hybrid varieties that are resistant to air pollution of which ozone is the overwhelming dominant pollutant.

The effects of ground level ozone on the susceptibility of the old and new varieties of Malaysian local rice cultivars have been identified and the results of this study show that at 60 ppb and 120 ppb of ozone concentrations, biochemical activities and yield of rice plants were affected whereby there exists reduction of the yield on exposed rice plants in the range of 5% to 15%. Thus from this study, it could be suggested that limits of 8- hour and 1- hour ozone exposure i.e. 60 ppb and 100 ppb should be reviewed by the government. Assessment of ozone impacts on Malaysian local rice plants and producing new cultivars that have resistance towards ozone stress are urgently needed.

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