PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES OF LOCALLY GROWN MALAYSIAN RICE CULTIVARS (*ORYZA SATIVA* L.) TO DIFFERENT OZONE CONCENTRATIONS

S. ISHII, F. M. MARSHALL* and J. N. B. BELL
Department of Environmental Science and Technology, Imperial College London, Silwood Park campus, Ascot, Berkshire, SL5 7PY, UK
(author for correspondence, e-mail: f.marshall@imperial.ac.uk; fax: +44 207 594 2339)*

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Abstract. Malaysian rice (*Oryza sativa* L.) cultivars MR84 and MR185 were grown in greenhouse chambers and exposed to four different levels of ozone from 28th August, 2001 to 22nd January, 2002. Four ozone levels were selected in close relation to the Malaysian peri-urban ambient level (approximately 30 ppb, 8 hr mean), the Malaysian guideline level (approximately 60 ppb) and possible future higher ozone levels (approximately 90 ppb). Both morphological and physiological parameters showed distinctive impacts of ozone treatments. The plants treated with the highest ozone concentration showed different morphological development, probably induced by severe foliar injury and physiological adaptation of the plants to the ozone stress. The physiological measurements revealed a high sensitivity at the early and late vegetative stages. It was concluded that MR84, which was found to be physiologically sensitive, responded to ozone relatively quickly and altered its morphology to compensate for effects on growth and yield, while MR185, found to be physiologically insensitive, responded to ozone stress slowly which resulted in more severe impacts on growth and yield parameters. Slight growth stimulation was observed at the lowest (30 ppb) ozone level for MR185, whilst negative impacts on growth occurred at both of the higher ozone levels. The study provided useful insights into the earlier findings of an open-top chamber filtration study on the same cultivars in the field at a peri-urban site in Malaysia. The present study proved that yield could be reduced substantially when other parameters associated with grain yield were affected, which was accompanied by increasing yellowing of leaves and premature senescence.

Keywords: chlorophyll fluorescence, closed fumigation chambers, Malaysia, rice, *Oryza sativa* L., ozone, photosynthetic rate, stomatal conductance

1. Introduction

Ozone (O_3) at ambient levels is widely known to damage the morphology and physiology of forest species, agricultural crops and natural vegetation (Heck, 1989; UNECE ICP Vegetation, 2001). Much of the knowledge concerning agricultural crop sensitivities has been determined as a result of coordinated research efforts in the United States (Adams *et al.*, 1988; Heck, 1989) and Europe (Mathy, 1988; UNECE ICP Vegetation, 2001). In Asia, a number of recent findings on increasing O_3 concentrations in several urban centres in Seoul (Jo *et al.*, 2000), Hong Kong (Lee *et al.*, 2002) and Tokyo (Wakamatsu *et al.*, 1999), raise the possibility of

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substantial impacts on crop yields in peri-urban areas, but of even more concern are the cities in developing counties where major deterioration of air quality, as well as increasing food demands, are taking place simultaneously (Ashmore, 1991; Bell *et al.*, 1993; Marshall *et al.*, 1997). Rice is undoubtedly the most important agricultural product in Asia. Several studies have been conducted in northeastern and southern parts of the continent such as in Japan (Asakawa *et al.*, 1981; Nishi *et al.*, 1985), China (Jin *et al.*, 2001; Feng *et al.*, 2003), India (Anbazhagan *et al.*, 1989) and Pakistan (Maggs and Ashmore, 1988; Wahid *et al.*, 1995). However research on the sensitivity of local cultivars under Southeast Asian climatic conditions is even of a greater importance, since a large number of people are heavily dependent upon rice farming in the region and any threat to rice production may have serious social, economic and environmental implications.

The present study follows on from an open-top chamber filtration study on local Malaysian rice cultivars which indicated that yield and growth parameters of Malaysian rice cultivars were adversely affected by the ambient O_3 levels in the southern part of the Klang Valley, in the rapidly developing area embracing Kuala Lumpur and other satellite cities (Ishii *et al.*, in press). Further investigations are described in this paper on the same Malaysian rice cultivars in the form of a greenhouse fumigation experiment. The morphological and physiological responses of these cultivars to different levels of O_3 were analysed in order to confirm the earlier findings and elucidate further the causal mechanisms.

2. Materials and Methods

2.1. CONTROLLED CHAMBER SYSTEM

The experimental chambers were made of Perspex® and wood, with dimensions of 0.7 × 0.7 × 1.3 m high and were installed in a greenhouse. Ambient air drawn from the air inlet was passed through charcoal filters (Emcel Filters Ltd., UK) and distributed equally to each chamber by a blower, giving an air exchange rate of more than once min^{-1}. O_3 was generated by an ozone generator (Wallace and Tierman, Tonbridge, England) using pure oxygen (BOC Gases, UK). Needle valves were used to distribute sufficient amounts of O_3 to be mixed to the supplied air for each chamber. The inlet of the air sample line inside the chamber was adjusted to plant height. Sampled air was handled by an air handling unit which consisted of solenoid valves and cam timers, allowing all chambers to be monitored continuously over equal intervals by a UV-photometric ozone analyser (Dasibi Environmental Corp., U.S.A.). Non-reactive PTFE tube was used for all tubing systems.

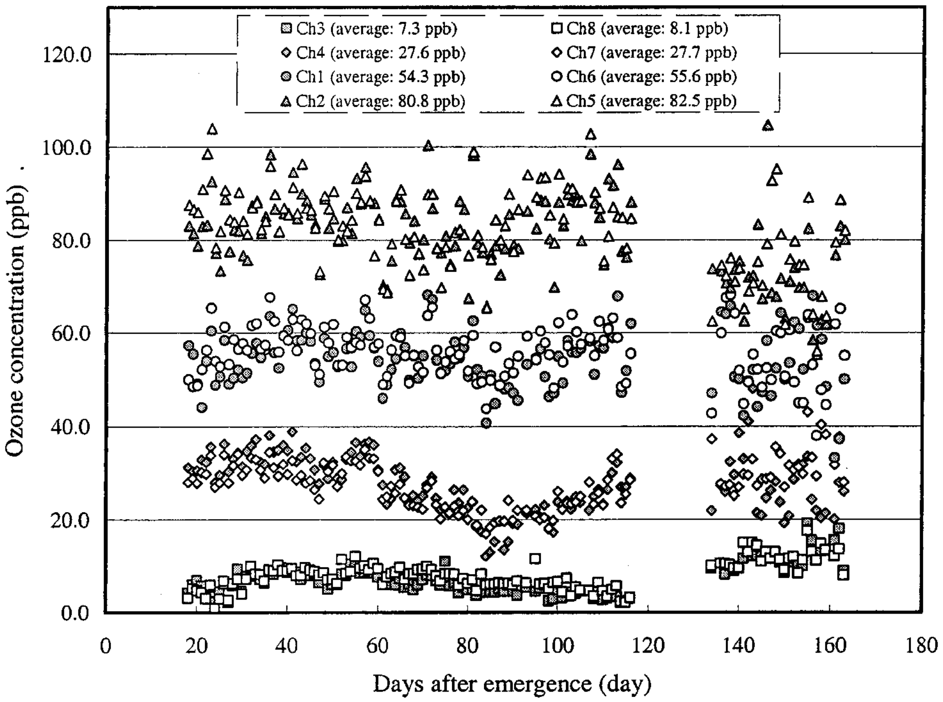

Figure 1. 8 hr mean O_3 concentrations for each chamber during the experimental period *(Note: recording was absent for 22 days in total including 117-133DAE).*

2.2. MICROCLIMATE MEASUREMENT AND OZONE FUMIGATION

Air temperature was recorded in all chambers by copper-constantan thermocouples (RS Components Ltd., UK) and relative humidity was monitored by a humidity probe (Vaisala HMP-111) but only in one of the chambers due to limited availability of the instrument. After installing heating equipment during later autumn and winter period, the day (7:00–19:00 hr) and night (19:00–7:00 hr) hourly temperature differences across the chambers were within a standard deviation of ± 0.71 °C and ± 0.47 °C, respectively. Average hourly day and night temperature and humidity throughout the experimental period were 24.1 ± 2.21 °C and 20.8 ± 3.01 °C (± standard deviation) for temperature and 63.4 ± 10.9% and 47.1 ± 7.2% (± standard deviation) for relative humidity, respectively. The photoperiod was 12 hr (7:00–19:00 hr) (Son-T agro lamps, Phillips, U.S.A.).

The four levels of O_3 were selected as appropriate for a study relevant to Malaysian ambient conditions. At the end of the experiment, the actual levels recorded were the clean air control of 7.7 ppb (8 hr mean), a low O_3 treatment of 27.7 ppb close to the field level found in the previous open-top chamber experiment, a medium O_3 treatment of 55.0 ppb close to the Malaysian air pollution guideline for O_3 (60 ppb, 8 hr mean), and a high O_3 treatment of 81.7 ppb, as 1.5 times higher than the medium O_3 treatment level (Figure 1). Differences between replicate chambers were kept within a maximum difference of 1.7 ppb (8 hr mean). The AOT40 value (accumulated O_3 exposure of hourly concentration over a threshold of 40 ppb during daylight hours) (Grünhage *et al.*, 1999) for the medium and for high ozone treatments were 17778.8 and 47689.5 ppb·h, respectively after replacing 22 days of missing data with the average hourly concentration. O_3 concentration in the low O_3 treatment exceeded 40 ppb occasionally, resulting in the AOT40 value of 194.7 ppb·h.

2.3. PLANT CULTURE AND EXPERIMENTAL DESIGN

Seeds of rice cultivars, MR84 and MR185 were obtained from the Department of Agriculture Malaysia and the Malaysian Agricultural Research and Development Institute. On 12th August 2001, pre-soaked seeds were germinated. At 14 days after emergence (14 DAE) when the seedlings were at the third leaf growth stage, uniform sized healthy seedlings of each cultivar were selected and transplanted individually to 4L plastic pots (17.5 cm diameter) filled with John Innes compost No.2. The plants were acclimatised to the chamber for two days and fumigation commenced on 28th August 2001 (18DAE).

Eight chambers were used for four O_3 treatments. 16 pots (8 plants per cultivar × 2 cultivars) were placed in each chamber and treated as a block. A total of 128 plants were used for the experiment (8 plants × 2 cultivars × 4 treatments × 2 replicate chambers). The plants were watered daily with tap water and standard local Malaysian agricultural practice was used. The plants were shuffled daily within the block in addition to moving the whole block to other replicate chambers every 8 days.

The heading and flowering of the experimental plants was delayed and a final harvest was extended by approximately 40 days, as compared to an average growth period of 125 days (cultivar MR185) and 138 days (cultivar MR84) for field plants (Department of Agriculture Malaysia, 1999). This delay was thought to be due to a low average nighttime temperature of below 20 °C, recorded from the heading and panicle development stage (80DAE) onwards (Collinson *et al.*, 1995). It is known that the flower development and panicle initiation stages of rice plants are the most sensitive phases with respect to low air temperature (Nishiyama, 1983) and as little a difference as 0.8 °C can delay heading approximately 3 to 10 days (Collinson *et al.*, 1995). Due to the problems associated with this low temperature fumigation ceased on 22nd January 2002 (165DAE) at grain filling stage and the plants were transferred to another greenhouse in order to complete their full maturity and final harvest (180DAE).

2.4. PARAMETERS MEASUREMENT

2.4.1. *Growth and Yield Parameters*

Plant height, leaf and tiller number, and visible injury were recorded on a weekly basis. Type and degree of injury were examined visually and scored from 1 to 5. At final harvest, the plants were separated into leaf blade, leaf sheath, stem, panicle and root and dry weights were determined after drying in an oven for 5 to 7 days until the weights became constant. Due to the delayed flowering and the potential influence of low temperature in the final stage of experiment, the yield parameter measurement was restricted to the total grain number per plant, which is known to be determined during the vegetative growth stage at 20 to 24 days before heading (Takeoka *et al.*, 1993).

2.4.2. *Gas Exchange of the Plants (Photosynthetic Rate and Stomatal Conductance)*

Gas exchange parameters, namely photosynthetic rate and stomatal conductance of the most recently expanded young leaf of the mother tiller were measured on 30, 40, 50, 75, 91, 105 and 125 DAE. Readings were taken from randomly selected plants and measurements were made strictly between 10:00 and 14:00 hrs to avoid unnecessary experimental error. After the first three measurements, the instrument had to be changed from LCA-1 portable infrared gas analyser (ADC, Hoddesdon, UK) to CIRAS (PP systems, Hearts, UK) due to mechanical failure. Accordingly, the number of samples had to be reduced from four samples$\cdot$cultivar$^{-1}\cdot$chamber^{-1} (64 samples in total) to two samples$\cdot$cultivar$^{-1}\cdot$chamber^{-1} (32 samples in total).

2.4.3. *Light Harvesting and Utilisation Efficiency by the Plants (Chlorophyll Fluorescence)*

Chlorophyll fluorescence parameters, namely minimal initial fluorescence (F_o), maximal fluorescence (F_m) and reaction time to maximal fluorescence (T_m), were measured *in situ* on 32, 42, 54, 64, 72, 83, 103, and 127 DAE in order to investigate the Kautsky effect by means of a plant efficiency analyser (Hanstech Instruments Ltd. Norfolk, UK) (Bolhar-Nordenkampf and Oquist, 1993). A variable fluorescence (F_v) was calculated by subtracting F_o from F_m, and an induced chlorophyll fluorescence rate (F_r) was calculated from F_v divided by T_m (Barnes *et al.*, 1988; Reiling and Davison, 1992). Approximately 30 mm from the apex of the most recently expanded young leaf of the mother tiller were firstly dark adapted for 30 min and then illuminated by red light with a peak wavelength of 650 nm. Four samples cultivar$^{-1}\cdot$chamber^{-1} were randomly selected, giving a total sample number of 64 in all measurements. The measurement was also carried out strictly between 10:00 and 14:00 hr.

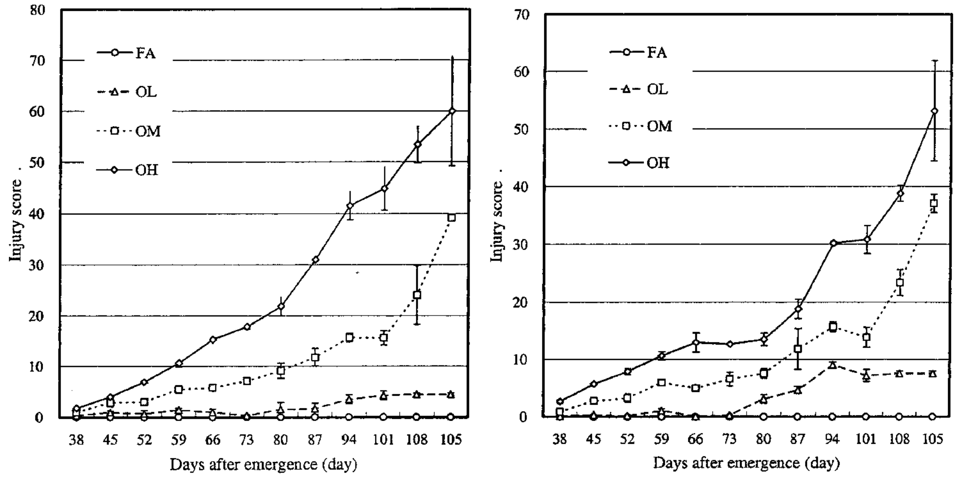

Figure 2. Cumulative injury score of the rice plants, MR84 (left) and MR185 (right) *(error bars show standard error).*

2.5. STATISTICAL ANALYSIS

The datasets obtained were based on the block mean values and were subjected to statistical analysis. The ANOVA was carried out for detecting any O_3 impact on the parameters. If necessary, significant differences between the means of each O_3 treatment were tested by Tukey's honest significant difference test. All analyses were run by using software, STATISTICA® (version 5.5a StatSoft Inc., U.S.A.) and Microsoft Excel® (2000 Microsoft, U.S.A.).

3. Results and Discussion

3.1. VISIBLE INJURY

Both cultivars showed visible injury in all fumigation treatments and the degree of injury increased with duration of exposure and increase in concentrations of O_3 (Figure 2). Despite there being a wide range and types of sensitivities amongst rice cultivars to air pollution (Shiau *et al.*, 1993), the two cultivars showed a uniform injury type in the form of a reddish stipple, silvering and yellowing (chlorosis) of the leaves. In the present study, silvering and reddish stipple was associated with relatively little O_3 impact in terms of morphological and physiological responses. This could be an indication of isolated cellular level damage because these injuries were found mainly in the young plants of the low O_3 treatment. In contrast, yellowing was an indication of impacts on the basis of plant organ and whole plant physiology, because the injury was usually followed by impairment of leaf functions and early senescence as found in the other studies (Nouchi *et al.*, 1991 and

1995; Agrawal, 1982). The yellowing in the present experiment was mainly found on the plants treated with medium and high O_3 levels and large morphological and physiological damage. In addition, the yellowing also became increasingly dominant over the other two types of injury in the low O_3 treatment at the end of vegetative growth stage (about 80DAE). This might suggest that yellowing can be triggered and accelerated even by low levels of O_3 when plants are in their maturation stage. In the medium and high O_3 treated plants, the injury increased sharply after the end of vegetative growth and heading stage (Figure 2). The severity of injury at the higher two O_3 concentrations as compared with the low O_3 treatment at 108DAE was 5.9 and 4.2 times higher in the medium O_3 treated plants, and 11.9 times and 6.3 higher in the high O_3 treated plants for MR84 and MR185, respectively.

3.2. PHYSIOLOGICAL PARAMETERS

In the present study, initial fluorescence (F_o) remained unaffected for all measured plants (data not shown), suggesting that the O_3 levels used had no impacts on the initial stage of the light harvesting system of the plants, and that the antenna pigments or the efficiency of excitation trapping at the light harvesting complexes (LHC) were not affected (Krause and Weis, 1984; Calatayud and Barreno, 2001). This also meant that variability found in the variable fluorescence (F_v) was attributed mainly to the maximal fluorescence (F_m).

Physiological sensitivity of both cultivars to O_3 was observed, especially during the young vegetative stage (30- 32DAE) and the late vegetative to heading stage (72-91DAE) (Figures 3, 4 and 5), but different physiological mechanisms were involved in each of these growth stages. In the young vegetative growth period, the photosynthetic rate at 30DAE was significantly suppressed by 62.1 and 59.7% (high O_3 treatment) for MR84 and MR185, respectively. In this stage, neither impacts nor any detectable trends were found for maximal chlorophyll fluorescence (F_m) and thus the variable fluorescence (F_v) (Figures 4 and 5). The only parameter affected in this stage was T_m, which showed a decreasing trend on 32 and 42DAE for MR84 and on 32DAE for MR185 (Figures 4 and 5). The impact in T_m in the present study was still unclear since a lower T_m value is known to be accompanied with decreasing F_v (Barnes *et al.*, 1990). Therefore, the cause of photosynthetic inhibition in the present study might be due to stomatal closure but less related to the light harvesting system of the plant. Although it was not statistically significant, a trend of reducing stomatal conductance was observed in this stage (Figure 3). A similar sensitivity of gas exchange parameters of the young plants has been found in another O_3 fumigation study on the Japanese cultivar Nihonbare (50 and 90ppb, 8 hr mean) (Nakamura and Saka, 1978). The inhibition of photosynthetic rate in the young plant development stage was not observed in the subsequent active vegetative stage in the present study.

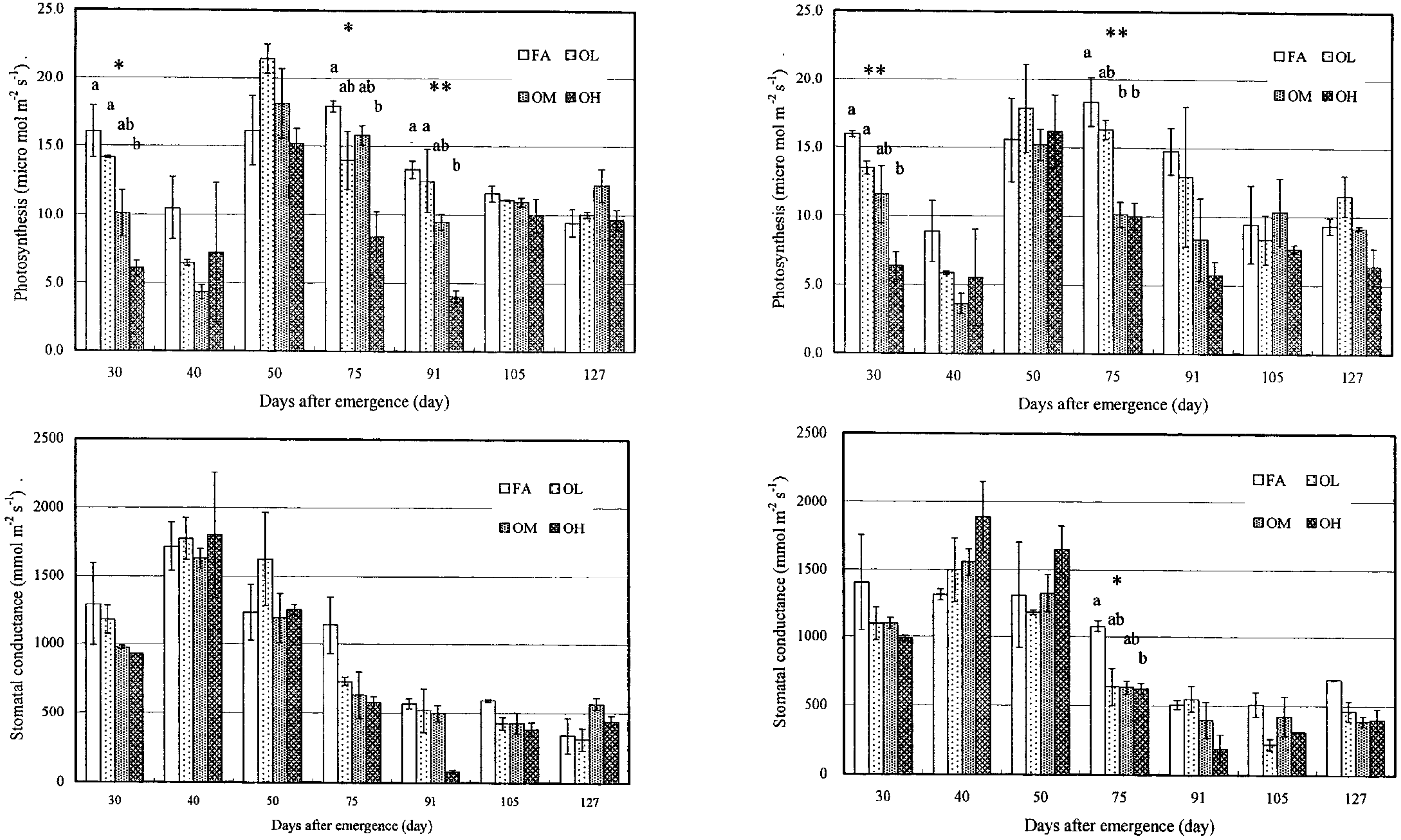

Figure 3. Photosynthetic rate (top) and stomatal conductance (bottom) of cultivar MR84 (left) and MR185 (right) during experimental period *(graph shows mean and error bars indicate standard error; different letters indicate statistically significant difference between treatment means by Tukey's HSD test ($p < 0.05$)) *, ** shows statistical significance of of O_3 treatment in overall treatment $p < 0.05$, $p < 0.01$ level)).*

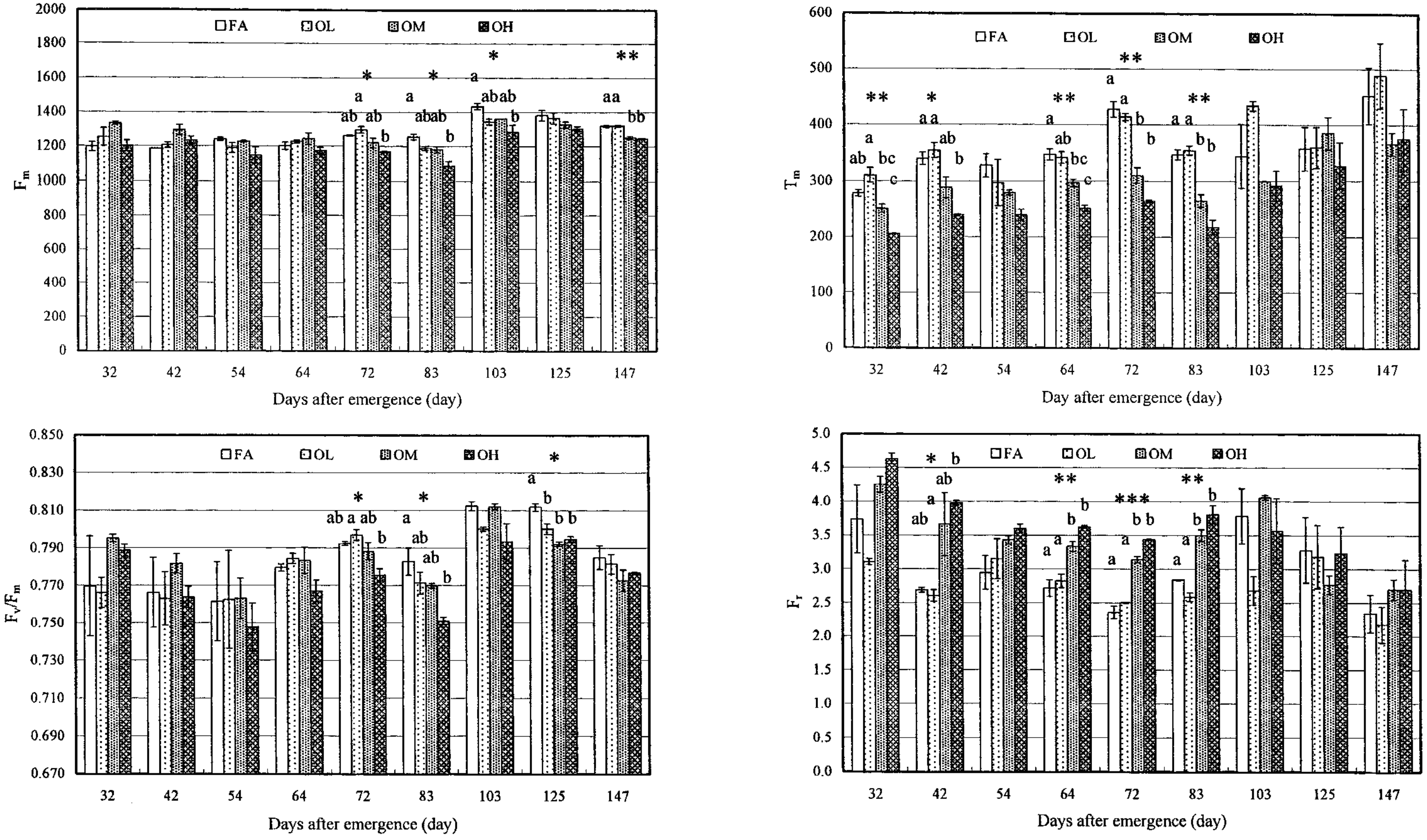

Figure 4. Chlorophyll fluorescence parameters of MR84 during the experimental period *(Note: same flag leaves might be measured in 103, 125 and 147DAE; Figure shows mean value and standard error; different letters indicate statistically significant difference between treatment means by Tukey's HSD test ($p <$ 0.05))*; *,**, *** *show statistical significance of O_3 treatment by $p < 0.05$, 0.01, 0.001 level).*

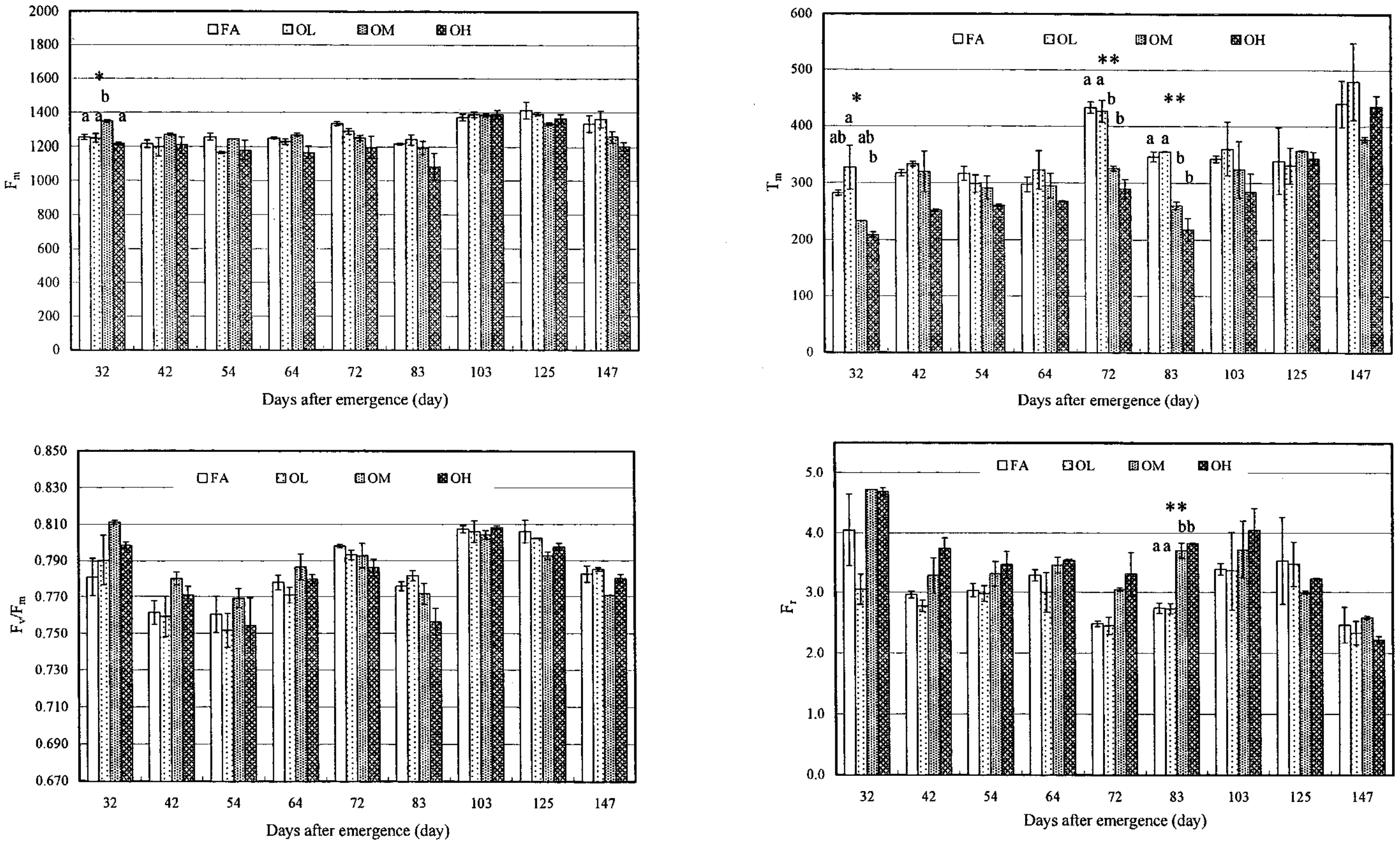

Figure 5. Chlorophyll fluorescence parameters of MR185 during the experimental period *(Note: same flag leaves might be measured in 103, 125 and 147DAE; Figure shows mean value and standard error; different letters indicate statistically significant difference between treatment means by Tukey's HSD test ($p < 0.05$)); *,**, show statistical significance of O_3 treatment by $p < 0.05$, 0.01 level).*

Comparatively more prominent impacts were evident in the late vegetative to heading stage of the plants. For MR84, which showed larger impacts of O_3 on the parameters, high O_3 treatment suppressed photosynthetic rate by 53.3 and 69.9% on 75DAE and 91DAE, respectively. Meanwhile the chlorophyll fluorescence parameter F_m also declined on 83DAE and 103DAE, including a negative impact on F_v/F_m ratio on 83DAE. This indicated increased non-photochemical and/or decreased photochemical quenching by O_3 (Reiling and Davison, 1994). The real reason for declining variable fluorescence emission is still unclear but O_3 could either impair an electron transport involving a recombination reaction between oxidized P680 and reduced phaeophytin (Phaeo^-) within photosystem II (PSII) (Krause and Weis, 1984; Calatayud and Barreno, 2001) or affect directly a PSII antenna system (Barber *et al.*, 1989). Barnes *et al.* (1988) also mentioned a possibility of inactivation of the water-splitting system and/or PSII reaction centre as a cause of declining fluorescence emission. Since impacts on stomatal conductance for MR84 were less indicative in comparison to the chlorophyll fluorescence parameters in the present study, it might be extrapolated that the light harvest system could react firstly to O_3 stress before stomatal conductance responds. If this is the case, impacts on photosynthesis of the Malaysian rice cultivars could be attributed largely to a reduced efficiency of light energy utilisation and this could cause subsequent retardation of the carbon assimilation cycle, leading to stomatal closure.

However, this hypothesis should be noted with caution because MR185 showed similar but not identical responses. The high O_3 treatment significantly suppressed photosynthetic rate and stomatal conductance by 45.5 and 42.5%, respectively, without any significant impacts on the chlorophyll fluorescence parameters, F_m and F_v/F_m. Although some decreasing trend was observed for these chlorophyll fluorescence parameters, it would suggest the possibility of the other physiological reaction to O_3 in this cultivar. It could be viewed that the response of stomata was dominant over the chlorophyll parameters for MR185. The increase in stomatal resistance restricts CO_2 uptake and/or affects enzyme activities adversely, leading to increase of leaf internal CO_2 concentration. This can result in retardation of the carbon assimilation cycle as well as an electron transport chain, which causes a reduction in the amount of open PS II as an electron acceptor (Barnes *et al.*, 1988). Indeed, the inhibition of photosynthetic activity of the plant was unlikely to be derived from a single path of physiological reaction such as limiting light harvesting activity or causing a decline in stomatal conductance. It is more plausible that there are simultaneous responses of various plants parts and physiological pathways as a result of a widespread effect of O_3. The present results suggested that O_3 could affect both the gas exchange apparatus and the light harvesting activities simultaneously and degree of impacts was dependant on the sensitivity of cultivars.

The study confirmed that Malaysian rice cultivars also became physiologically most sensitive to O_3 at the end of the vegetative to heading stage (Nouchi *et al.*, 1991). In this particular growth stage, the plant might be very vulnerable to en-

TABLE I

Maximum plant height, leaf number and tiller number of MR84 and MR185

Parameters	Cultivar	Treatment				Overall
		FA	O_L	O_M	O_H	d.f. = (3,4)
Plant height	MR84	968.6 ± 5.88a	942.4 ± 10.69a	898.7 ± 4.94b	878.5 ± 5.63b	**
	MR185	931.1 ± 14.19a	933.9 ± 5.44a	861.8 ± 9.31b	826.8 ± 9.63b	**
Leaf number	MR84	82.4 ± 0.38a	72.5 ± 2.88ab	65.1 ± 2.00b	72.6 ± 1.19ab	*
	MR185	75.1 ± 0.63a	69.6 ± 0.19a	48.8 ± 0.00b	55.3 ± 3.75b	**
Tiller number	MR84	10.9 ± 0.31a	9.8 ± 0.06ab	8.6 ± 0.50b	9.5 ± 0.13ab	*
	MR185	10.7 ± 0.31a	9.9 ± 0.38a	6.8 ± 0.13b	7.4 ± 0.63b	**

*, **, *** shows statistical significance of overall treatment at $p < 0.05$, 0.01, 0.001 level; different letters indicate statistically significant difference between treatment means ($p < 0.05$) by Tukey HSD test; the values were expressed as arithmetic mean ± standard error.

vironmental stresses as the plants shift from a vegetative stage to a reproductive stage. The finding also supports the above discussion of why yellowing of the leaf in both cultivars was accelerated in this stage (Figure 2). In addition, an onset of chlorophyll pigment disruption is a main cause of the yellowing of leaves, which is always linked with reduction in photosynthetic activities on these leaves (Agrawal *et al.*, 1982; Welfare *et al.*, 1996).

It has been mentioned that F_r (= F_v/T_m) could be a good indicator of O_3 impacts (Barnes *et al.*, 1988, 1990; Reiling and Davidson, 1994). The present results suggested T_m (sometimes measured as a half value of reaction time as $t_{1/2}$) to be potentially a better indicator than F_r. Yet further clarification is necessary to investigate the implication of T_m, because the reliability of the parameter was questioned by Reiling and Davidson (1992). Moreover, T_m seemed to be less correlated with F_v (= F_m) in this study, although reduction in T_m was accompanied with lowered F_v (Barnes *et al.*, 1990).

3.3. MORPHOLOGICAL PARAMETERS

The significant adverse impacts of O_3 were especially evident in both medium and high O_3 treated plants in a number of morphological parameters (Tables I, II, and III). The present result confirmed the hypothesis from the previous open-top chamber field filtration study that sink organs were generally more sensitive (Welfare *et al.*, 1996) and sink strength in shoot apices was higher than the root. It was shown that the root dry weight of MR185 was suppressed by 69.2 and 65.4% in comparison with the shoot, which was affected by 50.2 and 54.2% in the medium and high O_3 treatments, respectively (Table II). Among the sink organs in the shoot, the stem had weaker sink strength than the leaf sheath so that the inhibition of leaf blade, leaf sheath and stem, respectively, was in the order of 37.7, 43.5 and

TABLE II

Dry weight of plant components of MR84 and MR185

Parameters	Treatment				Overall d.f.
	FA	O_L	O_M	O_H	= (3,4)
		< MR84 > (g)			
Leaf blade	12.2 ± 0.37a	12.0 ± 0.26a	7.6 ± 0.41b	9.6 ± 0.24b	**
Leaf sheath	10.8 ± 0.07a	10.2 ± 0.26a	6.1 ± 0.49b	6.7 ± 0.04b	***
Stem	6.0 ± 0.05a	6.2 ± 0.20a	2.9 ± 0.24b	3.1 ± 0.04b	***
Panicle	7.5 ± 0.03a	5.7 ± 0.14b	7.3 ± 0.17a	4.5 ± 0.03b	**
Root	3.1 ± 0.02a	3.4 ± 0.04a	1.6 ± 0.16b	2.1 ± 0.14b	***
Shoot	36.4 ± 0.01a	34.1 ± 0.03a	23.9 ± 0.11b	24.0 ± 0.10b	***
Total	39.5 ± 0.01a	37.5 ± 0.02a	25.5 ± 0.08b	26.1 ± 0.07b	***
		< MR185 > (g)			
Leaf blade	9.5 ± 0.03a	10.7 ± 0.15a	4.5 ± 0.20b	5.3 ± 0.52b	***
Leaf sheath	8.3 ± 0.10a	9.8 ± 0.34b	3.4 ± 0.08c	3.7 ± 0.35c	***
Stem	4.2 ± 0.08a	5.4 ± 0.17b	1.4 ± 0.07c	1.3 ± 0.20c	***
Panicle	8.1 ± 0.06a	6.6 ± 0.12a	5.7 ± 0.05b	3.5 ± 0.14c	**
Root	2.6 ± 0.02a	3.2 ± 0.10a	0.8 ± 0.02b	0.9 ± 0.20b	***
Shoot	30.1 ± 0.01a	32.6 ± 0.07a	15.0 ± 0.02b	13.8 ± 0.14b	***
Total	32.6 ± 0.01a	35.7 ± 0.05a	15.8 ± 0.01b	14.7 ± 0.10b	***

, * *shows statistical significance of overall treatment at $p < 0.01$, $p < 0.001$ level; different letters indicate statistically significant difference between treatment means ($p < 0.05$) by Tukey HSD test*; the values were expressed as arithmetic mean ± standard error.

51.7% in the medium O_3 treatment and by 21.3, 38.0 and 48.3% in the high O_3 treatment for MR84. A similar but even larger degree of suppression was observed for MR185 and the findings agreed with the other studies on rice plants (Kobayashi *et al.*, 1995; Nouchi *et al.*, 1991 and 1995; Maggs and Ashmore, 1998).

TABLE III

Total grain number of MR84 and MR185

Cultivar	Treatment				Overall d.f.
	FA	O_L	O_M	O_H	= (3,4)
MR84	659 ± 34.2*a*	517 ± 10.1*ab*	482 ± 6.4*b*	402 ± 44.1*b*	*
MR185	702 ± 37.1*a*	557 ± 43.8*ab*	445 ± 2.5*bc*	332 ± 19.3*c*	**

*, ** *shows statistical significance of overall treatment at $p < 0.05$, $p < 0.01$ level; different letters indicate statistically significant difference between treatment means ($p < 0.05$) by Tukey HSD test*; the values were expressed as arithmetic mean ± standard error.

The same stimulation in dry weights of MR185 was observed in the low O_3 treatment (Table II). This was only evident for the leaf sheath (by 17.6%) and the stem (by 28.6%) dry weights, probably suggesting that those plants somehow stored more photosynthate in these sink organs. The reason for the stimulation remained unclear since there was no indication of increasing photosynthetic activities on these plants (Figures 4 and 5). Growth stimulation has been found in the case of exposure to low O_3 level (45–50 ppb, 7 $\text{hr}\cdot\text{day}^{-1}$ seasonal mean) on the resistant common bean (*Phaseolus vulgaris*) cultivar Dwarf Horticultural but a large yield suppression was found at higher O_3 concentrations so that O_3 tolerance in beans occurs over a fairly narrow range (Heck *et al.*, 1988). Thus, the stimulation in low concentration might be caused by a mechanism inherent in tolerant crops since rice is known to be relatively insensitive to air pollution compared with many other agricultural crops (Katase *et al.*, 1983; Heagle, 1989). However, it was also noted that the stimulation found in the present study turned into a relatively large reduction in slightly higher O_3 levels, similarly to the case in common bean.

The plants treated in the high O_3 concentration tended to have more leaves and tillers than the ones treated in the medium O_3 concentration (Table I). This suggested that the heavy foliar damage could have triggered the plants to respond to the stress by replacing damaged leaves in order to compensate for reduced photosynthetic activity. Thus, the high O_3 treated plants produced more leaves (by 11.5 and 13.3% for MR84 and MR185, respectively) and tillers (by 10.5 and 8.8% for MR84 and MR185, respectively) than the medium O_3 treated plants (Table I) and therefore they have more leaf blade dry weight (by 20.8 and 15.1% for MR84 and MR185, respectively) and leaf sheath dry weight (by 9.0 and 8.1% for MR84 and MR185, respectively) (Table II).

Consequently, the present study extrapolated that MR84 was physiologically more sensitive and thus, reacted to O_3 stress quickly. The plants displayed severe foliar injury and premature senescence in leaves, and responded to excessive damage induced by the high level of O_3 by producing more photosynthetic organs to compensate the growth. As a result, the degree of adverse impacts on growth and yield parameters was less than MR185. MR185, in contrast, was physiologically insensitive, so the plants displayed less foliar injury in comparison to MR84. Therefore, MR185 showed the growth stimulation in the low O_3 treatment but because it was slow to respond to O_3 attack, the cultivar was highly vulnerable to the medium and high O_3 concentration and displayed a greater degree of negative impacts on the growth and yield parameters.

An indication of potential for large yield reductions was demonstrated in this study. Actual degree of yield reduction remained unclear because of a lack of data on the grain sterility, which was determined as the most sensitive parameter for the same rice cultivars in the previous field open top chamber study in Malaysia (Ishii *et al.*, under a second revision WATE3818). The total grain number showed a reduction of 26.9% (medium) and 39.1% (high) for MR84 and of 36.6% (medium) and 52.7% (high) for MR185 (Table III) and the higher sensitivity of MR185 agreed with the previous findings. The yield reduction of 6.3% induced by ambient air in the field of Malaysia (O_3 concentration 32.5 ppb, 8 hr mean) in the previous study was attributed predominantly to an increase in grain sterility but the present findings also showed that other yield related parameters could be affected negatively by the higher levels of O_3. Nevertheless, the limitation of the greenhouse chamber study potentially overestimates the impacts for two reasons. Firstly the exposure protocol of O_3 in the present study was the 'square type', which begins with an abrupt change of O_3 concentration from the baseline level to the target level in the morning and maintains throughout the day until the end of the exposure. In contrast, in the field, the plants experience the 'mountain type' exposure protocol, where the O_3 concentration increases gradually from the morning, reaches its peak in the middle of the afternoon and declines gradually towards evening. Musselman *et al.* (1994) found that impacts were larger in the former exposure protocol. Secondly, the plants grown in the chambers are known to be more sensitive to O_3 due to a constant disturbance of the leaf boundary layer, resulting in increased pollution uptake by the plants (Fuhrer, 1994). Despite these constraints, what was clear from the present findings was that there is a threat of adverse impacts on a number of parameters under the Malaysian air quality guideline level of O_3 (medium O_3 level treatment) and this could lead to substantial yield reduction, especially on MR185.

4. Conclusion

The present study presented a detailed investigation of the morphological and physiological responses of Malaysian cultivars to four different levels of O_3. The present study, confirmed results from open top chamber experiments with these two Malaysian rice cultivars in the field, that suggested the most sensitive stages of growth and development were at the end of vegetative growth and during the heading stage. Impacts on the total grain number from the present study clearly showed that the Malaysian guideline level of O_3 has the potential to induce substantial impacts on these cultivars. The threshold to trigger these impacts lies between the low and medium O_3 treatments in the study and the significant yield losses observed have important implications for air pollution management. Detailed air pollution data analysis in local agricultural field could be useful to ameliorate impacts on local agricultural productivities.

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