Photosynthetic characteristics of diploid honeysuckle (*Lonicera japonica* Thunb.) and its autotetraploid cultivar subjected to elevated ozone exposure

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Abstract

In order to investigate the effect of chromosome doubling on ozone tolerance, we compared the physiological responses of a diploid honeysuckle (*Lonicera japonica* Thunb.) and its autotetraploid cultivar to elevated ozone (O_3) exposure (70 ng g^{-1}, 7 h d^{-1} for 31 d). Net photosynthetic rate (P_N) of both cultivars were drastically (P<0.01) impaired by O_3. Although there were significantly positive correlation between P_N and stomatal conductance (g_s) in both cultivars under each treatment, the decreased g_s in O_3 might be the result rather than the cause of decreased P_N as indicated by stable or increasing the ratio of intercellular to ambient CO_2 concentration(C_i/C_a). P_N under saturating CO_2 concentration (P_{Nsat}) and carboxylation efficiency (CE**)** significantly decreased under O_3 fumigation, which indicated the Calvin cycle was impaired. O_3 also inhibited the maximum efficiency of photosystem II (PSII) photochemistry in the dark-adapted state (F_v/F_m), actual quantum yield of PSII photochemistry (Φ_{PSII}), electron transport rate (ETR), photochemical quenching coefficient (q_P), non-photochemical quenching (NPQ), the maximum *in vivo* rate of Rubisco carboxylation (V_{cmax}) and the maximal photosynthetic electron transport rate (J_{max}) which demonstrated that the decrease in P_N of the honeysuckle exposed to elevated O_3 was probably not only due to impairment of Calvin cycle but also with respect to the light-harvesting and electron transport processes. Compared to the diploid, the tetraploid had higher relative loss in transpiration rate (E), (g_s), (P_{Nsat}), V_{cmax} and J_{max}. This result indicated that the Calvin cycle and electron transport in tetraploid was damaged more seriously than in diploid. A barely nonsignificant (P=0.086) interaction between O_3 and cultivar on P_N suggested a higher photosynthetic sensitivity of the tetraploid cultivar.

Additional key words: air pollution; chlorophyll *a* fluorescence; chromosome doubling; gas exchange; *Lonicera japonica*; ozone sensitivity.

Introduction

Tropospheric ozone (O_3) is a key air phytotoxic pollutant, formed by the reaction between reactive oxidized nitrogen (NO_x) and volatile organic compounds (VOCs) which are emitted from the industrialization and other human activities (Fowler *et al.* 1998, Placet *et al.* 2000). The background concentrations of O_3 are continuously rising year by year (IPCC 2001). Ozone not only harms the health of human being, but also seriously damages the

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Abbreviations: CE – carboxylation efficiency; Chl – chlorophyll; C_i – intercellular CO_2 concentration; C_i/C_a – the ratio of intercellular to ambient CO_2 concentration; E – transpiration rate; ETR – electron transport rate; F_v/F_m – maximum efficiency of photosystem II photochemistry in the dark-adapted state; g_s – stomatal conductance; J_{max} – the maximal photosynthetic electron transport rate; NPQ – non-photochemical quenching; PAR – photosynthetically active radiation; P_N – net photosynthetic rate; P_{Nsat} – P_N under saturating CO_2 concentration; PSII – photosystem II; Φ_{PSII} – actual quantum yield of PSII photochemistry; q_P – photochemical quenching coefficient; V_{cmax} – the maximum in vivo rate of Rubisco carboxylation.
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growth and development of plants (Lippmann 1989, Fagnano *et al*. 2009). It has been documented that elevated O_3 caused serious damage to agricultural productivity, forest health and seminatural ecosystems (Adams *et al.* 1989, Fuhrer *et al.* 1997, Ashmore 2005).

As a gaseous pollutant, O_3 initiates toxicity in plants mainly through absorption by the foliage (Runeckles 1992). CO_2 assimilation can be suppressed under O_3 fumigation by the reduction of stomatal conductance (Reich and Lassoie 1984) and decrease of contents and/or activity of Rubisco (Pell *et al.* 1997, Reichenauer and Goodman 2001). Meanwhile, O_3 enhances respiration rate with respect to detoxification and repair processes (Amthor 1988, Biswas *et al.* 2008b). In the long term, therefore, O_3 inhibits growth and decreases productivity (Grimm and Fuhrer 1989). Although there are a lot of reports about the responses of different plant species to elevated O_3 (e.g. Burkey *et al*. 2000, Calvo *et al.* 2007, He *et al.* 2007, Feng *et al.* 2007, Feng *et al.* 2008, Imai *et al.* 2008), information about the responses of polyploidy to O_3 is limited (Biswas *et al.* 2008a).

Specific sensitivity to O_3 can be found among different plant species or cultivars (Paoletti *et al.* 2009), and most importantly, such O_3 sensitivity is heritable. We found in the previous study that higher sensitivity to O_3 could be induced by higher stomatal conductance, lower rate of respiratory process and larger decrease of antioxidative capacity (Biswas *et al.* 2008b). Chromosome number genetically influences morphological traits such as stomata size and number and physiological processes such as antioxidative ability which may together determine sensitivity of plants to O_3. Compared with diploids, chromosome doubling (polyploidy) is often associated with increases in mesophyll cell volume, leaf thickness and area, the amounts of photosynthetic enzymes, net assimilation and numbers of shoots and flowers (Warner *et al.* 1987, Aranda *et al.* 1997, Xiong *et al.* 2006). These morphological and physiological variations generally lead to different ecological adaptations, *e.g.*, drought tolerance (Pustovoitova *et al.* 1996, Al Hakimi *et al.* 1998, Xiong *et al.* 2006) or heat tolerance (Chinnusamy and Khanna-Chopra 2003). To our knowledge, however, much less is known about how chromosome doubling affects plant sensitivity to O_3. We hypothesized that chromosome doubling would enhance plant tolerance to O_3 since the increased photosynthetic enzymes and net CO_2 assimilation may supply higher reducing power and more carbon skeletons for detoxification and repairing (Dizengremel *et al.* 2008).

Honeysuckle (*Lonicera japonica* Thunb.), whose dried flower buds (known as *Flos Lonicerae*) have been utilized as traditional Chinese medicine for over 1,000 years (Li *et al.* 2003, Chen *et al.* 2005), is commonly cultivated as a highly valued medicinal and garden plant in East Asia, particularly in China (Leatherman 1955, Chai *et al.* 2005). To meet the great demand for medical uses and urban greening, scientists in China had induced an autotetraploid cultivar (Jiufengyihao) from the diploid honeysuckle (Damaohua) by colchicines (Tan *et al.* 2005). Besides increasing flower yield, the new tetraploid cultivar also has stronger resistance to environmental stresses such as heat and drought (Li 2007, Li *et al.* 2009). We wondered whether morphological and physiological modification *via* chromosome doubling would increase the resistance of honeysuckle against O_3 pollution since such species might be widely used in greening cities where O_3 is quite a common pollutant.

The objectives of this research therefore were (*1*) to determine how chromosome doubling would affect physiological responses in terms of gas exchange and chlorophyll fluorescence in honeysuckle to O_3, and (*2*) to reveal any potential mechanisms that induce their different sensitivities to O_3.

Materials and methods

Plant materials: Two honeysuckle cultivars, 'Damaohua' (diploid) and 'Jiufengyihao' (tetraploid), were obtained from Jiujianpeng Agricultural Technology Limited Company (Pingyi, Shangdong, China). Both honeysuckle cultivars were propagated using stem cuttings. After sprouting and growing for over one year, 40 healthy and uniform seedlings per cultivar were potted in 35 cm diameter crockery pots filled with a mixture of vermiculite, peat, and field soil (1:3:6, v/v/v). During the experiment, water and nutrients were supplied sufficiently to avoid potential nutrient deficiency and drought stress.

Experimental design: The experiment was conducted in four open top chambers (OTCs, 1.8 m in diameter and 2.4 m in height) at the Botanical Garden, Institute of Botany, the Chinese Academy of Sciences, Beijing, China. On 1 April 2007, 4 pots per cultivar were randomly placed in each OTC for adaptation for one week. During this period, all plants received charcoal-filtered air [CF, < 5 ng $g^{-1}(O_3)$]. After adaptation, two chambers were injected with O_3 and maintained concentration at 70 ± 5 ng g^{-1} (09:00 – 16:00) for 31 d, while the other two were still injected with CF air. Ozone was generated by electrical discharge using charcoal-filtered ambient oxygen with an O_3 generator (*CF-KG1, Beijing Sumsun EP Hi-Tech., Co. Ltd.*, Beijing, China) and bubbled through distilled water before entering the elevated O_3 chambers. Manual mass flow controllers were applied to regulate the flow of O_3-enriched air to the OTCs. Ozone concentrations in the OTCs were continuously monitored at approximately 10 cm above the plant canopy using an O_3 analyzer (*Model 205, 2B Technologies Inc.*, Boulder, Colorado, USA). In order to diminish chamber

effects, pots were rotated between the chambers and randomized within the chambers every day.

Gas exchange: Instant gas exchange was measured twice on the latest fully expanded leaves using gas exchange and fluorescence systems (*GFS3000, Heinz Walz,* Effeltrich, Germany) on the 15th and 24th d after the treatment, respectively. Three plants of each cultivar in O_3 or in CF were randomly selected for the measurement. The *GFS3000* system was connected with a PC with data acquisition software (*GFS-Win*) and calibrated to zero point prior to measurements. The cuvette condition was set with a light intensity of 1,500 μmol m^{-2} s^{-1} provided by the special artificial light (*LED-Array/PAM-Fluorometer 3055-Fl, Heinz Walz*, Effeltrich, Germany), with relative humidity and air temperature being set at 60±5 % and 25±0.5 °C, respectively. Gas exchange was measured using ambient CO_2 concentration (380±10 μmol mol^{-1}). Net CO_2 and H_2O exchange rates were stored after 3~5-min adaptation. P_N, g_s, and intercellular CO_2 concentration (C_i) were automatically calculated following the program based on the theory of von Caemmerer and Farquhar (1981). On the 20th d after O_3 exposure, the diurnal gas exchange was studied. The light intensity, air temperature, relative humidity and CO_2 concentration were set as the actual ambient values. Measurements were taken for 12 times from 06:00–18:00, each time lasting for 1 h. P_N and g_s were automatically calculated.

Three plants of each cultivar in O_3 or in CF were randomly selected for P_N-C_i curve measurement. On the 27th d after O_3 exposure, P_N-C_i curve was measured with the leave cuvette condition being set with relative humidity of 60±5 %, air temperature at 25±0.5 °C, and PFD at 1,500 μmol m^{-2} s^{-1}. The steady-state rate of net photosynthetic rate (P_N) was determined at external CO_2 concentrations of 400, 300, 200, 100, 50, 400, 400, 600, 800 μmol mol^{-1}, respectively. Every CO_2 step lasted for 4 min and data were recorded for 6 times. The data obtained for each leaf were analyzed using a curve-fitting program (*Photosynthesis Assistant*, *Dundee Scientific*, Dundee, UK) to obtain the parameters of the curve.

Chlorophyll (Chl) *a* fluorescence was measured twice on the latest fully expanded leaves randomly using gas exchange and fluorescence systems (*GFS3000, Heinz Walz*, Effeltrich, Germany) on the 15th and 24th d after the treatment, respectively. After 40 min dark adaptation, the minimum (F_o) and maximum fluorescence (F_m) were determined with a modulated irradiation (<0.1 μmol m^{-2} s^{-1}) and a 0.8-s saturating pulse (>8000 μmol m^{-2} s^{-1}), respectively. The yield of variable fluorescence (F_v) was calculated as $F_v = F_m - F_o$. After 2 min of dark re-adaptation, actinic white light (400 μmol m^{-2} s^{-1}) was switched on. The steady state fluorescence (F_s) was reached within 5 min and a second saturating pulse was imposed to determine maximum fluorescence in the irradiation-adapted state (F_m'). Minimum fluorescence in the irradiation-adapted state (F_o') was determined during a brief interruption of actinic illumination in the presence of far-red light. The maximum efficiency of photosystem II (PSII) photochemistry in the dark-adapted state (F_v/F_m) and the non-photochemical quenching (NPQ) were calculated by $F_v/F_m = (F_m - F_o)/F_m$ and $NPQ = F_m/F_m' - 1$, respectively (Bilger and Björkman 1990, Schreiber 2004). The actual quantum yield of PSII photochemistry (Φ_{PSII}), photochemical quenching coefficient (q_P), and electron transport rate (ETR), were calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$, $q_P = (F_m' - F_s)/(F_m' - F_o')$, and $ETR = \Phi_{PSII} \times PAR \times 0.5 \times 0.84$, respectively (Genty *et al.* 1989).

Statistical analysis: The experiment consisted of two randomized blocks of two treatments with four plants per replicate. Data were analyzed using the *General Linear Models* Procedure in the SAS package (*SAS Institute, Inc.*, Cary, NC, USA). One-way analysis of variance (*ANOVA*) was used to identify differences between treatments in each cultivar in gas exchange and Chl fluorescence parameters. Difference was considered significant when $P<0.05$. Interaction between treatment and cultivar were analyzed using two-way analysis of variance (*ANOVA*) in the *General Linear Model*. For P_N-C_i curve parameters, least significant differences between means of different treatments in each cultivar were estimated at 95% confidence level. Two-way analysis of variance (*ANOVA*) was also used to identify the interaction between treatment and cultivar. Linear regression of P_N on g_s was calculated and graphed using the *SigmaPlot 8.0* (*Aspire Software Intl.*, Ashburn, VA, USA).

Results

Gas exchange: P_N, E, and g_s significantly decreased after 14 d of O_3 fumigation (AOT_{40}, 2940 ng g^{-1} h) (Fig. 1, Table 1). However, there were no significant differences in the ratio of intercellular CO_2 to ambient CO_2 concentration (C_i/C_a) between O_3 and CF treatments (Table 1). No considerable differences between cultivars were noted in P_N and C_i/C_a in CF or O_3-treated plants (Table 1). However, E and g_s significantly differed between diploid and tetraploid honeysuckles. The tetraploid honeysuckle had higher g_s and E than the diploid cultivar (Table 1).

There were significant interactions between treatment and cultivar in E and g_s after 23 d of O_3 fumigation (AOT_{40}, 4830 ng g^{-1} h) (Fig. 1, Table 1). In tetraploid honeysuckle, E (P=0.002) and g_s (P=0.002) decreased significantly under O_3 exposure. However, the diploid cultivar was not significantly affected by the O_3 exposure. Ozone fumigation significantly decreased P_N but

Table 1. Effects of treatment and cultivar and their interactions on gas exchange parameters (P_N – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i/C_a – the ratio of intercellular CO_2 to ambient CO_2 concentration) and chlorophyll fluorescence parameters (F_o – the minimum fluorescence; F_m – the maximum fluorescence; F_v/F_m – the maximum efficiency of PSII photochemistry in the dark-adapted state; Φ_{PSII} – the actual quantum yield of PSII photochemistry; ETR – electron transport rate; q_P – photochemical quenching coefficient; NPQ – the nonphotochemical quenching) after 14-d (AOT_{40}, 2940 ng g^{-1} h) and 23-d treatment (AOT_{40}, 4830 ng g^{-1} h), respectively. The values in the table are the P values of the two-way *ANOVA* test.

		P_N	E	g_s	C_i/C_a	F_o	F_m	F_v/F_m	Φ_{PSII}	ETR	q_P	NPQ
AOT_{40} (2940)	Treatment	0.005	<0.0001	0.001	0.267	0.020	0.011	0.577	0.341	0.340	0.144	0.031
	Cultivar	0.240	0.005	0.005	0.381	0.494	0.472	0.830	0.894	0.893	0.874	0.521
	Treatment × cultivar	0.847	0.933	0.180	0.822	0.863	0.736	0.992	0.217	0.218	0.346	0.078
AOT_{40} (4830)	Treatment	<0.001	0.007	0.007	0.009	<0.001	0.069	<0.001	0.005	0.005	0.030	0.015
	Cultivar	0.978	0.640	0.534	0.805	0.304	0.281	0.023	0.814	0.809	0.599	0.538
	Treatment × cultivar	0.086	0.029	0.028	0.184	0.979	0.265	0.104	0.107	0.106	0.430	0.107

Table 2. Effects of O_3 on P_N-C_i curve parameters (P_{Nsat} – net photosynthetic rate under saturating CO_2 concentration; V_{cmax} – the maximum *in vivo* rate of Rubisco carboxylation; J_{max} – the maximal photosynthetic electron transport rate; CE – carboxylation efficiency) of diploid (Daomaohua) and tetraploid (Jiufengyihao) honeysuckle after 26-d (AOT_{40}, 5460 ng g^{-1} h) treatment. % (+/–) indicated percent changes in O_3-exposed (O_3) relative to control (CF) plants, (O_3-CF)/CF. Data presented are means of 4 plants. $LSD_{0.05}$ – least significant difference at α=0.05. Asterisks denote significant difference between O_3-treated and control plant * <0.05, ** <0.01, *** <0.001.

Cultivar	Treatment	P_{Nsat} [μmol m^{-2} s^{-1}]	V_{cmax} [μmol m^{-2} s^{-1}]	J_{max} [μmol m^{-2} s^{-1}]	CE [mol m^{-2} s^{-1}]
Diploid	CF	21.17	13.35	56.65	0.029
	O_3	17.95	10.60	41.05	0.020
	%(+/–)	–15.21	–20.60	–27.54	–30.81
	$LSD_{0.05}$	3.58	4.44	18.84	0.015
Tetraploid	CF	34.30	17.50	68.95	0.035
	O_3	5.76	3.96	15.40	0.009
	%(+/-)	–83.22***	–77.37*	–77.67*	–73.10
	$LSD_{0.05}$	2.17	13.42	48.41	0.048
Treatment		0.031	0.008	0.005	0.041
Cultivar		0.655	0.491	0.331	0.717
Treatment × cultivar		0.017	0.030	0.035	0.222

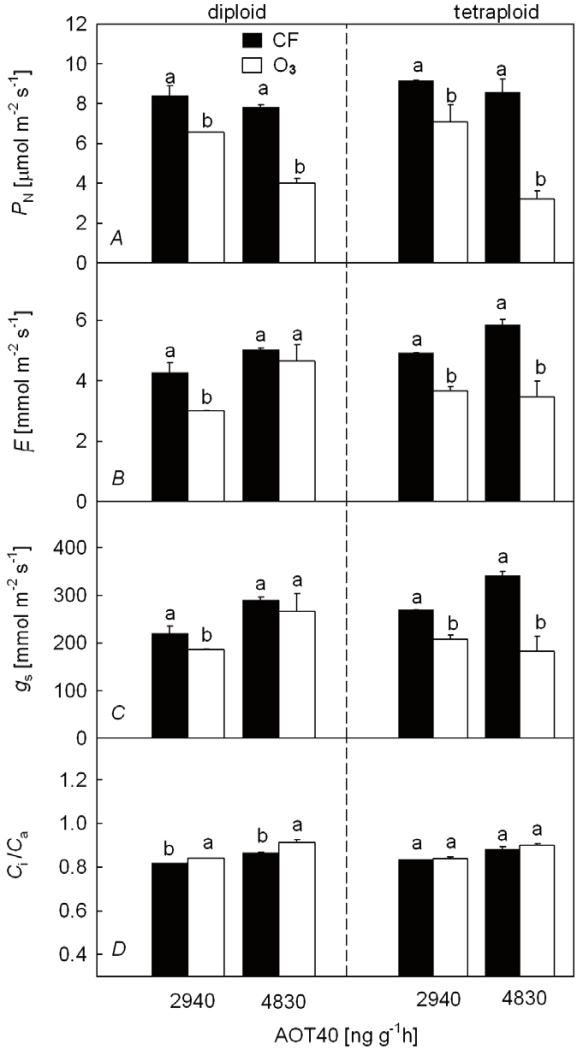

Fig. 1. Effects of O_3 on *A*: instantaneous net photosynthetic rate (P_N), *B*: transpiration rate (*E*), *C*: stomatal conductance (g_s), and *D*: ratio of intercellular CO_2 to ambient CO_2 concentration (C_i/C_a), of diploid (Daomaohua) and tetraploid (Jiufengyihao) honeysuckle after 14-d (AOT_{40}, 2940 ng g^{-1} h) and 23-d treatment (AOT_{40}, 4830 ng g^{-1} h), respectively. Error bars show SE, n = 3. One-way analysis of variance (*ANOVA*) was used to identify differences between treatments in each cultivar. Letters are comparable within treatments in each cultivar. Values with different letters are significantly different (P<0.05).

increased C_i/C_a ratio (Table 1). There were no differences in gas exchange between cultivars but there were differences in the responses to ozone exposure between cultivars. There was a barely non-significant (P=0.086) interaction between treatment and cultivar on P_N (Table 1). Higher relative loss in P_N (–62 %) was noted in tetraploid cultivar, together with a higher reduction in *E* (–41 %) and in g_s (–46%). The diploid cultivar had lower relative loss in P_N (–49 %) (Fig. 1).

There were significant interactions between ozone treatments and cultivars on many P_N-C_i curve parameters (Table 2). In tetraploid honeysuckle, P_{Nsat} (–83 %), V_{cmax} (–77 %), and J_{max} (–78 %) were significantly reduced under ozone exposure. However, those variables were not affected significantly by the ozone exposure in the diploid cultivar. Moreover, ozone significantly decreased carboxylation efficiency (CE) (P=0.041). There were no major differences between cultivars on CE in CF or in O_3 (Table 2).

Chl *a* fluorescence: Ozone exposure significantly increased F_o and F_m, while statistically significantly decreased NPQ after 14-d treatment (AOT_{40}, 2940 ng g^{-1} h) (Fig. 2,

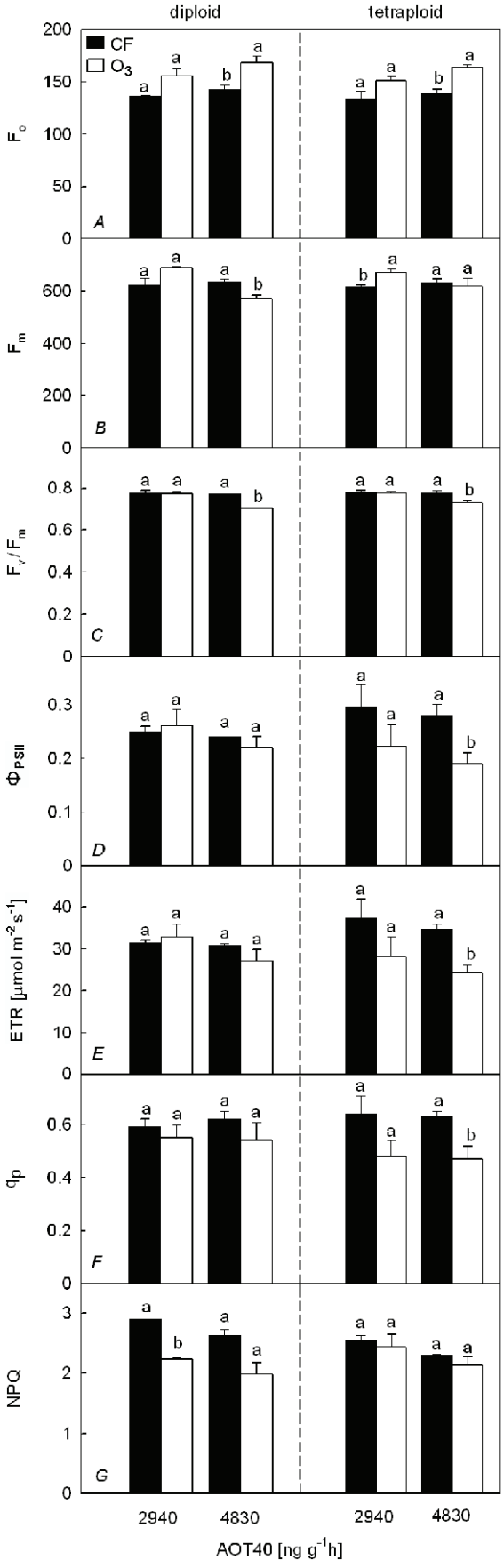

Fig. 2. Effects of O_3 on *A*: minimum fluorescence (F_o), *B*: maximum fluorescence (F_m), *C*: maximum photochemical efficiency of PSII (F_v/F_m), *D*: actual quantum yield of PSII (Φ_{PSII}), *E*: electron transport rate (ETR), *F*: photochemical quenching coefficient (q_P), *G*: and non-photochemical quenching (NPQ), of diploid (Daomaohua) and tetraploid (Jiufengyihao) honeysuckle after 14-d (AOT_{40}, 2940 ng g^{-1} h) and 23-d treatment (AOT_{40}, 4830 ng g^{-1} h), respectively. Error bars show SE, $n = 3$. One-way analysis of variance (*ANOVA*) was used to identify differences between treatments in each cultivar. Letters are comparable within treatments in each cultivar. Values with different letters are significantly different ($P<0.05$)

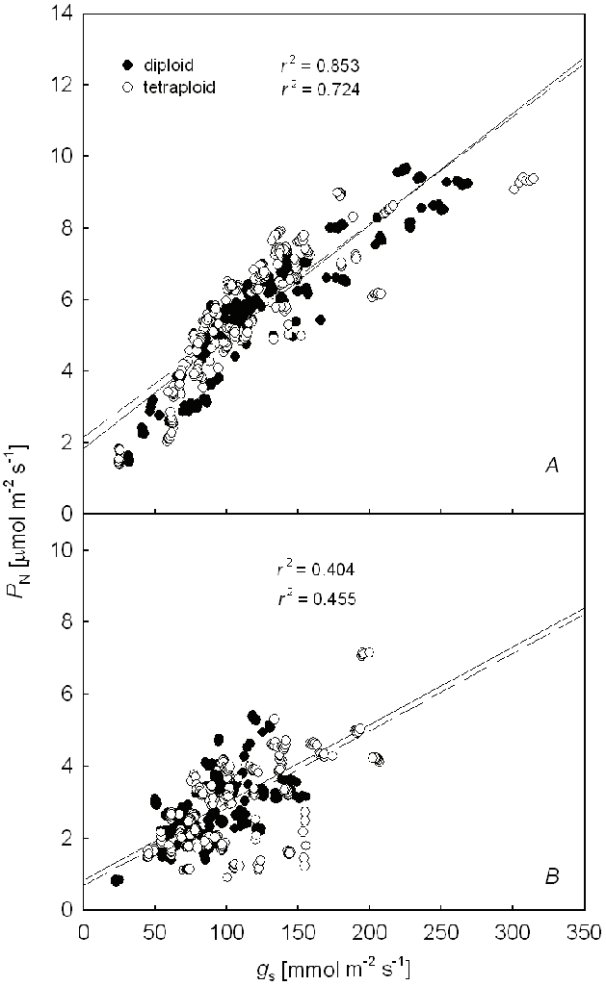

Fig. 3. The linear regression between net photosynthetic rate (P_N) and stomatal conductance (g_s) of diploid and tetraploid honeysuckle in CF (*A*) and in O_3 (*B*) after 20-d (AOT_{40}, 4200 ng g^{-1} h) treatment. The *solid line* means the linear regression of diploid and the long *dash line* means the linear regression of tetraploid honeysuckle. The regression equations of diploid in CF and in O_3 were $y = 1.831 + 0.0313\ x$ and $y = 0.854 + 0.0216\ x$, respectively, while the regression equations of tetraploid in CF and in O_3 were $y = 2.149 + 0.0298\ x$ and $y = 0.685 + 0.0215\ x$, respectively.

Table 1). Meanwhile, the F_v/F_m ratio, Φ_{PSII}, ETR, and q_P of two cultivars were not significantly affected by O_3 (Table 1).

F_o, F_v/F_m, Φ_{PSII}, ETR, q_P, and NPQ were all significantly affected by ozone after 23-d treatment (AOT_{40}, 4830 ng g^{-1} h) (Table 1). Ozone significantly increased F_o, but decreased F_v/F_m, Φ_{PSII}, ETR, q_P, and NPQ (Table 1, Fig. 2). Compared to diploid (–12 %), higher relative loss in Φ_{PSII} (–30 %) was noted in tetraploid cultivar, which exhibited a decrease in ETR (–30 %) and in q_P (–25 %). Compared to tetraploid (–4%), diploid cultivar had a relative higher loss in NPQ (–25 %), with F_v/F_m and F_m being decreased by 9 % and 10 %, respectively (Fig. 2).

Discussion

Ozone exposure (23 d) in open top chambers significantly affected photosynthetic performance of honeysuckle cultivars. Ozone considerably depressed P_N, E, and, g_s, but increased C_i/C_a (Fig. 1, Table 1). Responses of the P_N-C_i curve parameters (Table 2) confirmed that the reduction in carboxylation efficiency (CE) played a major role in impairing photosynthesis. The decreases of V_{cmax} and CE for plants growing under O_3 were probably due to the reductions in activity and/or quantity of Rubisco. These results suggest that the reduction of P_N caused by elevated O_3 was primarily triggered by impairing mesophyll processes rather than stomatal limitation. Although g_s showed significant positive correlations with P_N (Fig. 3), the decreased g_s might be a response to an increased C_i/C_a which is a result of inhibition of P_N (Fiscus *et al.* 1997, Farage and Long 1999).

A barely nonsignificant (P=0.086) interaction between treatment and cultivar on P_N (Table 1) showed the responses to ozone exposure were different between cultivars. Ozone caused higher relative reduction in P_N and g_s in tetraploid cultivar than the diploid (Fig. 1), which probably indicated that the former was more sensitive to O_3 (Crous *et al.* 2006, Guidi *et al.* 2000). Therefore, our results do not support our hypothesis that chromosome doubling might increase plant O_3 tolerance. The relative higher loss of V_{cmax} was found in the tetraploid than the diploid under O_3 exposure (Table 2) suggested that the capabilities of Calvin cycle were impaired more seriously in the tetraploid honeysuckle.

After 23-d fumigation, O_3 significantly increased F_o while reducing the F_v/F_m ratio (Fig. 2, Table 1). The increasing F_o meant that photodamage or reversible inactivation of PSII centers happened. The decline of F_v/F_m ratio was probably due to an increase in protective non-radiative energy dissipation, photodamage of PSII centers or both (Osmond 1994). The depression of NPQ as the index of nonradiative energy dissipation indicated that the decline in F_v/F_m ratio in O_3-treated plants was mainly due to photodamage of PSII. This finding was similar with other results such as responses of plants to SO_2 (Deltoro *et al.* 1999). Ozone inhibited the Φ_{PSII}, q_P, and ETR of both cultivars (Fig. 2, Table 1). This result indicated that the photochemical efficiency of open PSII reaction centers during irradiation was depressed by O_3. Ozone also suppressed the ability to reduce the primary acceptor Q_A indicated by the depression of q_P (Calatayud *et al.* 1999, Calatayud and Barreno 2001). Our result demonstrated that decrease in P_N of the honeysuckle exposed to elevated O_3 was probably not only due to impairment of Calvin cycle but also with respect to the light-harvesting and electron-transport processes (Nie *et al.* 1993, Farage and Long 1999). Higher relative loss in Φ_{PSII}, ETR, and q_P were noted in tetraploid cultivar than in diploid cultivar (Fig. 2) which demonstrated that effects of O_3 on light-harvesting processes and electron transport were different in two chromosome cultivars. These results also proved that the light reaction of tetraploid was damaged more seriously than that of the diploid.

In conclusion, the autotetraploid honeysuckle was more sensitive to O_3 than the diploid cultivar, which might be caused by higher impairments of Calvin cycle and light reaction. Although O_3 has now been recognized as a major air pollutant, O_3 tolerance which is an inheritable character has not been considered as one of target traits in plant breeding/selection (Barnes *et al.* 1999). Plant breeding that integrates O_3 tolerance into selection strategies will be more and more necessary to obtain sustainable production (Booker *et al.* 2009). Although chromosome doubling increased the tolerance of the honeysuckle to many stresses such as drought, heat, and cold (Li 2007), O_3 tolerance was depressed. Therefore, the chromosome-doubling breeding of the ornamental plant might not be the ideal approach for preventing the ozone pollution or absorbing such kind of pollutant because of high photosynthetic sensitivity of polyploidy.

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