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# Effects of $GeO_2$ on chlorophyll fluorescence and antioxidant enzymes in apple leaves under strong light

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## Abstract

In this study, we chose apple leaf as plant material and studied effects of $GeO_2$ on operation of photosynthetic apparatus and antioxidant enzyme activities under strong light. When exogenous $GeO_2$ concentration was below 5.0 mg $L^{-1}$, maximum photochemical quantum yield of PSII and actual quantum yield of PSII photochemistry increased significantly compared with the control under irradiances of 800 and 1,600 μmol(photon) $m^{-2}$ $s^{-1}$. Photosynthetic electron transport chain capacity between $Q_A$–$Q_B$, $Q_A$–PSI acceptor, and $Q_B$–PSI acceptor showed a trend of rising up with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ and declining with 10.0 mg($GeO_2$) $L^{-1}$. On the other hand, dissipated energy *via* both ΔpH and xanthophyll cycle decreased remarkably compared with the control when $GeO_2$ concentration was below 5.0 mg $L^{-1}$. Our results suggested that low concentrations of $GeO_2$ could alleviate photoinhibition and 5.0 mg($GeO_2$) $L^{-1}$ was the most effective. In addition, we found, owing to exogenous $GeO_2$ treatment, that the main form of this element in apple leaves was organic germanium, which means chemical conversion of germanium happened. The organic germanium might be helpful to allay photoinhibition due to its function of scavenging free radicals and lowering accumulation of reactive oxygen species, which was proven by higher antioxidant enzyme activities.

*Additional key words*: chlorophyll fluorescence; irradiance; photodamage; photosynthetic electron transport chain.

## Introduction

Germanium (Ge), a rare element, belongs to the fourth group of the periodic table along with carbon (C), silicon (Si), tin (Sn), and lead (Pb). It exists in the Earth's crust (approximately 1.6 mg $kg^{-1}$) and has dual nature of metal and nonmetal. Ge can be divided into general inorganic compounds including elemental germanium, $GeO_2$, and organic compounds including Ge-132, spiro-germanium and proxi-germanium (Adams and Thomas 1994, Kaplan *et al*. 2004). Among various kinds of Ge, organic Ge has been confirmed to possess therapeutic attributes including fighting cancer and improving the immune system in human and animals and it was further concluded that the change was attributed to raising radical scavenging activity (Goodman 1988, Tang *et al*. 1997, Yang and Kim 1999).

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*Abbreviations*: ABS – absorption flux; ABS/RC – absorption flux per reaction center of PSII; $ABS/CS_0$ – absorption flux per sample cross section; APX – ascorbate peroxidase; CAT – catalase; CS – phenomenological energy fluxes per excited cross section; DHAR – dehydroascorbate reductase; $DI_0/CS_0$ – dissipated energy flux per sample cross section; $DI_0/RC$ – dissipated energy flux per reaction center of PSII; DM – dry mass; FM – fresh mass; $F_M$ – maximal fluorescence; $F_0$ – initial fluorescence; $F_v/F_M$ – maximum photochemical quantum yield of PSII; $F_V'/F_M'$ – actual photochemical efficiency; $F_S$ – steady-state fluorescence; GPX – guaiacol peroxidase; GR – glutathione reductase; MDHAR – monodehydroascorbate reductase; NPQ – nonphotochemical quenching coefficient; OJIP – fast chlorophyll fluorescence transients; PETC – photosynthetic electron transport chain; POD – peroxidase; $q_P$ – photochemical quenching coefficient; RC – specific energy fluxes per active PSII reaction center; ROS – reactive oxygen species; SOD – superoxide dismutase; $TR_0/CS_0$ – flux of energy trapping per sample cross-section; $TR_0/RC$ – flux of energy trapping per reaction center of PSII; $V_I$ – relative variable fluorescence at the I-step; $V_J$ – relative variable fluorescence at the J-step; $\Phi_{NO}$ – quantum yield of non-light-induced nonphotochemical fluorescence quenching; $\Phi_{NPQ}$ – quantum yield of light-induced ΔpH and zeaxanthin-dependent; $\Phi_{PSII}$ – actual quantum yield of PSII photochemistry.
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Ge exists in all biomaterials including plants and animals (Rosenberg 2009, Sparks *et al*. 2011). A few studies have reported the effects of Ge on plant growth and mineral nutrition uptake in barley (Halperin *et al*. 1995), rice (Seo *et al.* 1995), lettuce (Cheong *et al*. 2009), Chinese cabbage (Han *et al*. 2007), and ginseng seedlings (Yu *et al*. 2005). Barley seedlings accumulated Ge in roots and shoots, and the shoots accumulated Ge linearly as an exogenous Ge concentration increased (Halperin *et al*. 1995). In hydroponically-grown lettuce treated with a low concentration of $GeO_2$, Ge was taken up by roots and barely moved to shoots. Furthermore, with all types of Ge treatment, plants showed no changes in their phenotype of leaf shape and color, including necrosis or chlorosis (Han *et al.* 2007).

Although several previous studies have reported the toxicity and uptake of Ge in plants and explored the effects of Ge on photosynthesis and antioxidant enzyme systems in leaves (Tarakhovskaya *et al*. 2012, Liu *et al*. 2016), there is still no research on the operation of photosynthetic apparatus. Various studies have demonstrated ROS accumulating more under high light intensity and excess ROS causing photoinhibition (Takahashi and Murata 2008). In biological systems, the antioxidant enzyme system plays an important role in resistance to oxidative stress and is helpful to maintain the normal biological structure and physiological activities (Apel and Hirt 2004).

Apple, one of the most important fruits in the world, has been confirmed containing Ge in our preliminary study. In consideration of therapeutic attributes of Ge, apple may have the effect of improving antioxidant ability for humans; it indicates the urgency of the study on influences of exogenous Ge on apple trees. In this study, we chose apple leaf as the test material and tried to reveal the effects of $GeO_2$ on operation of photosynthetic apparatus and antioxidant enzyme system under two different light intensities. Graphite furnace atomic absorption spectrometry was used to investigate the uptake, accumulation, chemical conversion of Ge in apple leaves with exogenous $GeO_2$. Furthermore, through modulated chlorophyll (Chl) fluorescence and Chl fluorescence transients (OJIP), more information about the $P_{680}$, electron transport between $P_{680}$ and $P_{700}$, and $P_{700}$ (PETC) and partitioning of absorbed light energy *via* photochemistry and thermal dissipation processes can be obtained. Moreover, in order to explain the influence of exogenous $GeO_2$ on apple photosynthetic apparatus, we determined activities of seven antioxidant enzymes and contents of hydrogen peroxide ($H_2O_2$) and superoxide anion ($O_2^{\bullet-}$) in order to explain how exogenous $GeO_2$ influences apple photosynthetic apparatus.

## Materials and methods

**Plant material and growth conditions**: Potted three-year-old dwarf apple (*Malus domestica* Borkh. cv. Red Fuji)/M26 (*Malus pumila* Mill.) trees were selected as test material. The experiments were conducted at Northwest A&F University, Yangling City (34°20'N, 108°24'E), Shaanxi Province, China. All plants were grown in plastic pots (height of 38 cm, diameter of 23 cm) filled with a mixed soil (volume ratio of field topsoil to organic matter was 2:1, pH 7.5, relative soil water content of 75%). Prior to the start of our trials, all trees were well watered and regulated daily to avoid water deficiency, diseases, and insect pests.

**Treatments**: In order to ensure $GeO_2$ entry into apple leaves, the marked mature leaves were cut at the petiole and immediately dipped into water solution containing 0, 1.0, 2.0, 5.0, and 10.0 mg($GeO_2$) $L^{-1}$, respectively, and maintained at 25°C under irradiance of 20 μmol(photon) $m^{-2}$ $s^{-1}$ for 24 h. Subsequently, the leaves in each treatment were divided into two parts and exposed under two light intensities of 800 and 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ for 4 h before the determination of the parameters.

**Chl content in apple leaves**: Fresh leaves (0.1 g) were ground in 80% acetone to extract both Chl *a* and Chl *b*. The samples were kept in the dark for 24 h at 4°C, and the extracts were centrifuged for 2 min at 3000 rpm and then analyzed by a spectrophotometer (*UV–2800*, *Unic*, Shanghai, China). The absorbance readings were performed at 665 nm for Chl *a*, at 649 nm for Chl *b*. Pigment quantities were calculated according to Lichtenthaler *et al*. (1987).

**Ge content**: The content of organic and inorganic Ge in apple leaves was determined using graphite furnace atomic absorption spectrometry, as described by McMahon *et al*. (2004). Plant samples were cleaned with distilled water, then oven-dried at 70°C for 72 h and ground to a fine powder with a mortar. The powder with particle size below 100 mm were collected for analysis. Powder (0.1 g) from each blade was fully carbonated and then was transformed into muffle furnace to be ashed at 600°C for 4 h. Through this process, all forms of Ge in apple leaf converted into elemental Ge and its content can be determined as the total Ge content using graphite furnace atomic absorption spectrometry. Then free inorganic Ge in apple leaf could be obtained from another 0.1 g of leaf powder through water bath (100°C) and its content can be determined using graphite furnace atomic absorption spectrometry. The content of organic Ge in each blade can be calculated by the difference between the content of total Ge and the content of inorganic Ge.

**Modulated Chl fluorescence parameters** were measured at room temperature of 25°C with a pulse amplitude modulation fluorometer (*PAM-2500*, *Walz*, Germany) after leaves were dark-adapted for 20 min. The fluorometer was connected to a trifurcated fiber-optic (*2010-F*) and to

a computer with data acquisition software *(PAM Win 3.02*). The experimental protocol was basically followed according to Genty *et al*. (1989).

The minimum fluorescence ($F_0$) and maximum fluorescence ($F_M$) were determined, respectively, using measuring light [<1 μmol(photon) $m^{-2}$ $s^{-1}$] and a 0.8-s saturating pulse at 6,000 μmol(photon) $m^{-2}$ $s^{-1}$. Actinic light of 619 μmol(photon) $m^{-2}$ $s^{-1}$ was used. After about 5 min, the steady-state value of fluorescence ($F_S$) was thereafter recorded and a second saturating pulse at 6,000 μmol(photon) $m^{-2}$ $s^{-1}$ was imposed to determine $F_M$ in the light-adapted state ($F_M$'). $F_0$' was basal fluorescence after 5 μmol(photon) $m^{-2}$ $s^{-1}$ of far-red irradiation at 720–730 nm for 4 s, which excites PSI and oxidizes the plastoquinone and $Q_A$ pools associated with PSII. With these original parameters above, the maximum photochemical quantum yield of PSII ($F_v/F_M$), efficiency of excitation capture by PSII ($F_v'/F_M'$), actual quantum yield of PSII photochemistry ($\Phi_{PSII}$), coefficient of photochemical fluorescence quenching ($q_P$), coefficient of nonphotochemical fluorescence quenching *via* ΔpH ($q_N$), coefficient of nonphotochemical fluorescence quenching *via* xanthophyll cycle (NPQ), quantum yield of non-light-induced nonphotochemical fluorescence quenching ($\Phi_{NO}$), quantum yield of light-induced ΔpH and zeaxanthin-dependent nonphotochemical fluorescence quenching ($\Phi_{NPQ}$) were calculated as defined by previous studies (Kitajima and Butler 1975, Schreiber *et al*. 1986, Genty *et al*. 1989, Bilger and Björkman 1990, Kramer *et al*. 2004).

**OJIP transients and analysis of relative parameters**: The Chl *a* fluorescence transients (OJIP-test) were also measured at room temperature (25°C) with a pulse amplitude modulation fluorometer (*PAM-2500*, *Walz*, Germany) after the leaves were dark-adapted for 20 min. Each Chl *a* fluorescence transient was analyzed according to the JIP-test utilizing original data: (*1*) the minimum fluorescence intensity [the fluorescence intensity at 20 μs, $F_0$]; (*2*) the maximum fluorescence intensity ($F_M$); (*3*) the fluorescence intensity at 2 ms (J-step, $F_J$) and 30 ms (I-step, $F_I$) (Stirbet and Govindjee 2011).

The relative variable fluorescence intensity at J-step ($V_J$) and I-step ($V_I$) were calculated as: $V_t = (F_t - F_0)/(F_M - F_0)$. In JIP-test, $ET_0/TR_0 = 1 - V_J$, reflecting the probability that a PSII trapped electron is transferred from $Q_A$ to $Q_B$; $RE_0/TR_0 = 1 - V_I$, reflecting the probability a PSII trapped electron is transferred until PSI acceptors; and $RE_0/ET_0 = (1 - V_I)/(1 - V_J)$, reflecting the probability an electron from $Q_B$ is transferred until PSI acceptors. In addition, in the JIP-test, $F_v/F_M \times ET_0/TR_0$, $F_v/F_M \times RE_0/TR_0$, $F_v/F_M \times RE_0/ET_0$ reflect the quantum yield of the electron transport flux from $Q_A$ to $Q_B$, quantum yield of the electron transport flux until the PSI electron acceptors, quantum yield of the electron transport flux from $Q_B$ until PSI acceptors, respectively.

The parameters reflecting absorbed photon flux were calculated as follows: $ABS/RC = (M_0/V_J) \times [1/(F_v/F_M)]$, quantifying average absorbed photon flux per PSII reaction center (RC); $ABS/CS_0 = F_0$, quantifying absorbed photon flux per cross section (CS). The parameters that reflect trapped exciton flux were calculated as follows: $TR_0/RC = M_0/V_J$, quantifying maximum trapped exciton flux per PSII RC; $TR_0/CS_0 = F_v/F_M \times F_0$, quantifying maximum trapped exciton flux per CS. The parameters that reflect energy dissipation were calculated as follows: $DI_0/RC = ABS/RC - TR_0/RC$, quantifying dissipated energy flux per active PSII RC; $DI_0/CS_0$ (t = 20 μs) = $ABS/CS_0 - TR_0/CS_0$, quantifying dissipated energy flux per CS.

**$H_2O_2$ and $O_2^{\bullet-}$ contents** were determined according to Chen (2013). Fresh leaf powder of 0.1 g was homogenized with 1.8 ml of 5% (w/v) trichloroacetic acid and then centrifuged at 16,000 × *g* for 10 min. The supernatant was neutralized to pH 7.5 with 17 M $NH_4OH$, and used for assay immediately.

For $O_2^{\bullet-}$ assay, the extract was divided into two aliquots of 100 μl. Superoxide dismutase [SOD, 50 U, 10,000 U $mg^{-1}$(protein), *Sigma*, USA] was added to one aliquot (blank). Both the blank and the other aliquot without SOD were added to 0.9 ml with 50 mM Tris-HCl (pH 7.5), and then kept at room temperature for 10 min, before adding 100 μl of 5 mM 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt. The reaction mixture was incubated at room temperature for 10 min, and then the absorbance at 470 nm was monitored. The difference on absorbance between the blank and the other aliquot was used to calculate the concentration of $O_2^{\bullet-}$ using an extinction coefficient of 21.6 $mM^{-1}$ $cm^{-1}$.

For $H_2O_2$ assay, the extract was divided into two aliquots of 100 μl. Catalase [CAT, 20 U, 20,000 U $mg^{-1}$(protein), *Sigma*, USA] was added to one aliquot (blank). Both the blank and the other aliquot without CAT were added to 0.5 ml with 50 mM Tris-HCl (pH 7.5) buffer, and then kept at room temperature for 10 min, following the addition of 0.5 ml of colorimetric reagent. The colorimetric reagent was made daily by mixing 1:1 (v/v) 0.3 mM potassium titanium oxalate and 0.3 mM 4-(2-pyridylazo)resorcinol monosodium salt. The assay mixture was incubated at room temperature for 15 min and then the absorbance at 508 nm was monitored (*UV–2800*, *Unic*, Shanghai, China). The difference on absorbance between the blank and the other aliquot was used to calculate the concentration of $H_2O_2$ using an extinction coefficient of 39.4 $mM^{-1}$ $cm^{-1}$.

**Antioxidant enzymes activities**: Fresh tissue samples (0.1 g each) were homogenized with 1.8 ml of 100 mM phosphate buffer saline PBS (pH 7.0) containing 5 % (w/v) polyvinylpyrrolidone, 1.0 mM EDTA and 1% *Triton X-100*. The homogenates were centrifuged at 13, 000 × *g* for 20 min at 4°C and the supernatants were used for enzyme assays. All enzyme activities were calculated on fresh mass basis.

CAT (EC 1.11.1.6) activity was determined by monitoring the decrease in absorbance at 240 nm due to decomposition of $H_2O_2$ (Díaz-Vivancos *et al*. 2008). The 1.0 ml of reaction mixture contained 50 mM PBS (pH 7.0), 10 mM $H_2O_2$, and 20 µl of enzyme extract. This reaction was initiated by adding $H_2O_2$. One CAT unit was defined as the amount of enzyme necessary to decompose 1 mM($H_2O_2$) $min^{-1}$ under the above-mentioned assay conditions. The specific CAT activity was expressed as U $g^{-1}$(FM) $min^{-1}$.

Peroxidase (POD, EC 1.11.1.7) activity was measured by the increase in absorbance at 470 nm due to guaiacol oxidation (Nickel and Cunningham 1969). The reaction mixture of 1.0 ml contained 10 mM guaiacol, 10 mM $H_2O_2$, and 20 µl of enzyme extract. The reaction was started by adding $H_2O_2$. One unit of POD activity was defined as the amount of the enzyme causing a change in absorbance at 470 nm of 0.01 per min. The specific POD activity [U $g^{-1}$(FM) $min^{-1}$] = ($\triangle A_{470} \times V$)/($M \times V_s \times 0.01 \times t$), in expressions, $\triangle A_{470}$ was the change of $A_{470}$ during every minute; V was total volume of crude enzyme solution; $V_s$ was volume of crude enzyme used in the determination; M was mass of fresh materials; t was reaction time in min.

SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), according to the methods of Rao and Sresty (2000). The 1.0 ml of reaction mixture contained 50 mM PBS (pH 7.8), 6.5 mM methionine, 50 µM NBT, 10 µM EDTA, 20 µM riboflavin, and 20 µl of enzyme extract. A reaction mixture lacking enzyme served as the control. All mixtures were stirred under darkness in small glass test tubes, and then irradiated for 5 min by fluorescent lamps [160 µmol(photon) $m^{-2}$ $s^{-1}$] before measured at 560 nm. The mixture that lacked enzyme was used to zero the absorbance at 560 nm (*UV–2800*, *Unic*, Shanghai, China). One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions. The specific SOD activity was expressed as U $g^{-1}$(FM).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was evaluated by monitoring the decrease in absorbance at 290 nm as ascorbate was oxidized (Nakano and Asada 1981). The 1.0 ml of reaction mixture contained 50 mM PBS (pH 7.6), 0.1 mM EDTA, 0.5 mM reduced ascorbate (AsA), 1 mM $H_2O_2$, and 20 µl of enzyme extract. The reaction was initiated by adding $H_2O_2$. One unit of APX activity was defined as the amount of the enzyme causing a change in 1.0 µmol AsA was oxidized per min. The specific APX activity [U $g^{-1}$(FM) $min^{-1}$] = ($\triangle A_{290} \times V$)/($2.8 \times M \times V \times t$), in expressions, $\triangle A_{290}$ was the change of $A_{290}$ during 30 s; V was total volume of crude enzyme solution; 2.8 $mM^{-1}$ $cm^{-1}$ was an extinction coefficient; M was mass of fresh materials; t was reaction time, 0.5 min.

Dehydroascorbate reductase (DHAR, EC 1.8.5.1) activity was determined by monitoring the increase in absorbance at 265 nm due to AsA formation (Nakano and Asada 1981). The 1.0 ml of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 2.5 mM reduced glutathione (GSH), 0.1 mM EDTA, 0.2 mM dehydroascorbate (DHA), and 20 µl of enzyme extract. The reaction was initiated by adding DHA. One unit of DHAR activity was defined as the amount of the enzyme causing a change in 1.0 µmol AsA formation per min at absorbance at 265 nm. The specific DHAR activity [U $g^{-1}$(FM) $min^{-1}$] = ($\triangle A_{265} \times V$)/($M \times V_s \times 5.42 \times t$), in expressions, $\triangle A_{265}$ was the change of $A_{265}$ during every minute; V was total volume of crude enzyme solution; $V_s$ was volume of crude enzyme used in the determination; M was mass of fresh materials; 5.42 $mM^{-1}$ $cm^{-1}$ was an extinction coefficient; t was reaction time, 3.0 min.

Monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) activity was assayed by monitoring the decrease in absorbance at 340 nm due to NADH oxidation (Nakano and Asada 1981). The 1.0 ml of reaction mixture contained 50 mM PBS (pH 7.6), 2.5 mM AsA, 0.1 mM EDTA, 0.1 mM NADH, 0.5 units of AsA oxidase (EC 1.10.3.3), and 20 µl of enzyme extract. The reaction was initiated by adding AsA oxidase. One unit of MDHAR activity was defined as the amount of the enzyme causing a change in 1.0 µmol NADH oxidation per min. The specific MDHAR activity was expressed as U $g^{-1}$(FM) $min^{-1}$.

Glutathione reductase (GR, EC 1.6.4.2) activity was measured by monitoring the decrease in absorbance at 340 nm due to NADPH oxidation (Zhang and Kirkham 1996). The 1.0 ml of reaction mixture contained 50 mM PBS (pH 7.0), 0.2 mM EDTA, 0.1 mM NADPH, 0.25 mM oxidized glutathione (GSSG), and 100 µl of enzyme extract. The reaction was initiated by adding NADPH. The GR activity was calculated by measuring the change in absorbance at 340 nm for GSSG-dependent oxidation of NAPDH. One unit of GR activity was defined as the amount of the enzyme causing a change in 1.0 µmol NADPH oxidation per min. The specific GR activity [U $g^{-1}$(FM) $min^{-1}$] = ($\triangle A_{340} \times V$)/($M \times V_s \times 6.22 \times t$), in expressions, $\triangle A_{340}$ was the change of $A_{340}$ during every minute; V was total volume of crude enzyme solution; $V_s$ was volume of crude enzyme used in the determination; M was mass of fresh materials; 6.22 $mM^{-1}$ $cm^{-1}$ was an extinction coefficient; t was reaction time, 3.5 min.

**Statistical analysis**: All statistical analysis were conducted with the *SPSS* software (*Version 19, SPSS Inc.*, USA). Analysis of variance (*ANOVA*) were used to evaluate the effects of treatment. Significant differences between plants with different treatment were calculated at $p<0.05$ level, using *Duncan*'s tests. Values were presented as the means of ten replicates ± standard error (SE). Figures were finished with the *Origin Pro* software (*Version 7.5, OriginLab Inc.*, USA).

## Results

**Ge accumulation**: Compared with the control, exogenous $GeO_2$ application significantly increased endogenous Ge contents of both inorganic and organic germanium form in leaves (Table 1). Specifically, inorganic germanium content in leaves treated with 1.0, 2.0, 5.0, and 10.0 mg($GeO_2$) $L^{-1}$ was 2.6, 11.2, 14.2, and 21.9 times higher than the control, respectively; organic Ge content in leaves was 30.7, 44.8, 61.8, and 102.8 times higher than the control, respectively. Although the exogenous Ge was applied in its inorganic form ($GeO_2$), in apple leaves, organic Ge content was significantly higher than that of inorganic form.

**Change of Chl content in apple leaves**: Chl *a* content increased with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ treatment compared with control, but there was no remarkable difference between 10.0 mg $L^{-1}$ and control (Table 2). The total Chl (*a*+*b*) content changed similarly as Chl *a*. Chl *b* content did not increase as much as Chl *a*, although its content with 5.0 mg $L^{-1}$ was significantly higher than that with 1.0 and 10.0 mg($GeO_2$) $L^{-1}$ treatments and control. For Chl *a*/*b*, only the value after 10.0 mg $L^{-1}$ treatment was significantly lower than others.

Table 1. Contents of total Ge, inorganic Ge, and organic Ge in apple leaves with treatments of different $GeO_2$ concentrations. Each value is the mean ± standard error of ten independent measurements. Means ± SE ($n$ = 6). Different *lowercase letters* indicate significant differences between treatments ($P$<0.05).

| $GeO_2$ [mg $L^{-1}$] | Total Ge [mg $kg^{-1}$(DM)] | Inorganic Ge [mg $kg^{-1}$(DM)] | Organic Ge [mg $kg^{-1}$(DM)] |
|---|---|---|---|
| 0 | 1.53 ± 0.22$^{e*}$ | 0.19 ± 0.06$^{d}$ | 1.34 ± 0.18$^{e}$ |
| 1.0 | 43.23 ± 4.14$^{d}$ | 0.70 ± 0.19$^{c}$ | 42.53 ± 3.95$^{d}$ |
| 2.0 | 63.70 ± 3.90$^{c}$ | 2.32 ± 0.22$^{b}$ | 61.39 ± 3.99$^{c}$ |
| 5.0 | 87.06 ± 16.28$^{b}$ | 2.88 ± 0.75$^{b}$ | 84.18 ± 17.03$^{b}$ |
| 10.0 | 143.41 ± 10.18$^{a}$ | 4.35 ± 0.35$^{a}$ | 139.06 ± 10.52$^{a}$ |

**Modulated Chl fluorescence parameters**: In apple leaves with 1.0, 2.0 and 5.0 mg($GeO_2$) $L^{-1}$, the Chl fluorescence parameters, such as $F_v/F_M$, $F_v'/F_M'$, $q_P$, $q_L$, and $\Phi_{PSII}$, increased significantly under both 800 and 1, 600 μmol(photon) $m^{-2}$ $s^{-1}$ (Table 3). These five parameters declined after 10.0 mg($GeO_2$) $L^{-1}$ treatment. On the other hand, the value of NPQ and $q_N$, both quantifying the energy dissipation, declined in apple leaves with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ and rose at the concentration of 10.0 mg $L^{-1}$. Meanwhile, the change of NPQ and $q_N$ affected the value of $\Phi_{NPQ}$, but not $\Phi_{NO}$. $\Phi_{NPQ}$ decreased with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ and increased in 10.0 mg($GeO_2$) $L^{-1}$, while $\Phi_{NO}$ was maintained stable compared with control.

**OJIP transients and relative parameters**: Clearly, in Chl fluorescence transient, exogenous $GeO_2$ changed the momentary maximum fluorescence intensity of J-step and I-step (Fig. 1). The parameters including $1 - V_J$, $1 - V_I$, $(1 - V_I)/(1 - V_J)$, $F_v/F_M \times (1 - V_J)$, $F_v/F_M \times (1 - V_I)$, and $F_v/F_M \times (1 - V_I)/(1 - V_J)$ all showed a rising trend along with the increase of $GeO_2$ concentration and reached a peak when it was 5.0 mg $L^{-1}$, then all declined at 10.0 mg $L^{-1}$ (Fig. 2). A significant difference in $1 - V_J$ between 800 and 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ was observed as well as in $F_v/F_M \times (1 - V_J)$, while other four parameters with same $GeO_2$ treatment were almost equal under the two light intensities.

We also determined partitioning of absorbed light energy *via* photochemical reaction and thermal dissipation. The change of parameters including ABS/RC, $ABS/CS_0$, $TR_0/RC$, $TR_0/CS_0$, $DI_0/RC$, $DI_0/CS_0$ were shown in Fig. 3. The light absorption per residue active RC (ABS/RC) and per CS ($ABS/CS_0$) in control and at 10.0 mg $L^{-1}$ were higher than that at 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ (Fig. 3*A*,*D*). Moreover, the values of ABS/RC and $ABS/CS_0$ under 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ were both lower than that under 800 μmol(photon) $m^{-2}$ $s^{-1}$. $TR_0/RC$ and $TR_0/CS_0$ did not change remarkably with different concentrations of exogenous $GeO_2$ solutions and different light intensities (Fig. 3*B*,*E*). $DI_0/RC$ and $DI_0/CS_0$, dissipated energy flux per PSII RC and CS, decreased at 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ (Fig. 3*C*,*F*), meaning lesser dissipation of excess excitation energy in these chloroplasts.

Table 2. Effect of different $GeO_2$ concentrations on chlorophyll (Chl) content in apple leaves. Means ± SE ($n$ = 6). Different *lowercase letters* indicate significant differences between treatments ($P$<0.05).

| $GeO_2$ [mg $L^{-1}$] | Chl (*a*+*b*) [mg $g^{-1}$] | Chl *a* [mg $g^{-1}$] | Chl *b* [mg $g^{-1}$] | Chl *a*/*b* |
|---|---|---|---|---|
| 0 | 2.21 ± 0.07$^{d}$ | 1.64 ± 0.05$^{d}$ | 0.57 ± 0.01$^{c}$ | 2.85 ± 0.05$^{ab}$ |
| 1.0 | 2.38 ± 0.03$^{c}$ | 1.76 ± 0.03$^{c}$ | 0.62 ± 0.01$^{b}$ | 2.86 ± 0.03$^{a}$ |
| 2.0 | 2.48 ± 0.05$^{b}$ | 1.84 ± 0.02$^{b}$ | 0.64 ± 0.02$^{ab}$ | 2.87 ± 0.04$^{a}$ |
| 5.0 | 2.62 ± 0.04$^{a}$ | 1.94 ± 0.03$^{a}$ | 0.68 ± 0.02$^{a}$ | 2.86 ± 0.02$^{a}$ |
| 10.0 | 2.27 ± 0.03$^{d}$ | 1.67 ± 0.04$^{d}$ | 0.60 ± 0.02$^{bc}$ | 2.76 ± 0.04$^{b}$ |

Table 3. The effect of different $GeO_2$ concentrations on modulated chlorophyll fluorescence in apple leaves. Each value is the mean ± standard error of ten independent measurements. Means ± SE ($n$=6). Different *lowercase letters* indicate significant differences between treatments ($P$<0.05).

| Treatments | | Modulated chlorophyll fluorescence parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Light intensity [μmol(photon) $m^{-2}$ $s^{-1}$] | $GeO_2$ [mg $L^{-1}$] | $F_v/F_M$ | $F_v'/F_M'$ | $q_p$ | $q_L$ | $q_N$ | NPQ | $\Phi_{PSII}$ | $\Phi_{NPQ}$ | $\Phi_{NO}$ |
| 800 | 0 | 0.708 ± 0.004$^{c*}$ | 0.538 ± 0.017$^{c}$ | 0.590 ± 0.011$^{c}$ | 0.427 ± 0.012$^{bc}$ | 0.676 ± 0.011$^{d}$ | 1.120 ± 0.056$^{d}$ | 0.318 ± 0.018$^{cd}$ | 0.361 ± 0.019$^{c}$ | 0.322 ± 0.012$^{a}$ |
| | 1.0 | 0.746 ± 0.008$^{b}$ | 0.582 ± 0.010$^{b}$ | 0.637 ± 0.018$^{ab}$ | 0.443 ± 0.012$^{b}$ | 0.625 ± 0.018$^{e}$ | 0.983 ± 0.043$^{e}$ | 0.371 ± 0.017$^{b}$ | 0.311 ± 0.018$^{d}$ | 0.319 ± 0.011$^{a}$ |
| | 2.0 | 0.769 ± 0.005$^{a}$ | 0.606 ± 0.012$^{a}$ | 0.654 ± 0.013$^{ab}$ | 0.446 ± 0.016$^{b}$ | 0.611 ± 0.011$^{e}$ | 0.968 ± 0.048$^{e}$ | 0.393 ± 0.021$^{ab}$ | 0.298 ± 0.012$^{d}$ | 0.309 ± 0.011$^{a}$ |
| | 5.0 | 0.780 ± 0.006$^{a}$ | 0.614 ± 0.007$^{a}$ | 0.665 ± 0.012$^{a}$ | 0.473 ± 0.010$^{a}$ | 0.603 ± 0.016$^{e}$ | 0.933 ± 0.057$^{e}$ | 0.408 ± 0.014$^{a}$ | 0.286 ± 0.014$^{d}$ | 0.306 ± 0.008$^{a}$ |
| | 10.0 | 0.710 ± 0.012$^{c}$ | 0.509 ± 0.015$^{c}$ | 0.587 ± 0.016$^{c}$ | 0.467 ± 0.018$^{ab}$ | 0.690 ± 0.011$^{d}$ | 1.131 ± 0.058$^{d}$ | 0.295 ± 0.007$^{d}$ | 0.374 ± 0.013$^{c}$ | 0.322 ±0.010$^{a}$ |
| 1,600 | 0 | 0.696 ± 0.008$^{c}$ | 0.449 ± 0.016$^{d}$ | 0.574 ± 0.009$^{c}$ | 0.400 ± 0.010$^{d}$ | 0.745 ± 0.013$^{b}$ | 1.423 ± 0.053$^{ab}$ | 0.256 ± 0.010$^{fg}$ | 0.427 ± 0.017$^{ab}$ | 0.317 ± 0.007$^{a}$ |
| | 1.0 | 0.699 ± 0.010$^{c}$ | 0.463 ± 0.017$^{d}$ | 0.590 ± 0.016$^{c}$ | 0.423 ± 0.008$^{bc}$ | 0.744 ± 0.015$^{b}$ | 1.366 ± 0.042$^{b}$ | 0.271 ± 0.010$^{f}$ | 0.419 ± 0.013$^{bc}$ | 0.310 ± 0.010$^{a}$ |
| | 2.0 | 0.695 ± 0.010$^{c}$ | 0.465 ± 0.014$^{d}$ | 0.598 ± 0.007$^{c}$ | 0.430 ± 0.016$^{bc}$ | 0.745 ± 0.011$^{b}$ | 1.365 ± 0.030$^{b}$ | 0.276 ± 0.011$^{ef}$ | 0.416 ± 0.011$^{bc}$ | 0.308 ± 0.009$^{a}$ |
| | 5.0 | 0.695 ± 0.010$^{c}$ | 0.479 ± 0.015$^{d}$ | 0.632 ± 0.010$^{b}$ | 0.434 ± 0.013$^{bc}$ | 0.731 ± 0.014$^{bc}$ | 1.281 ± 0.030$^{c}$ | 0.300 ± 0.016$^{e}$ | 0.393 ± 0.010$^{c}$ | 0.307 ± 0.010$^{a}$ |
| | 10.0 | 0.650 ± 0.015$^{d}$ | 0.402 ± 0.013$^{e}$ | 0.595 ± 0.014$^{c}$ | 0.413 ± 0.012$^{cd}$ | 0.771 ± 0.010$^{a}$ | 1.451 ± 0.041$^{a}$ | 0.240 ± 0.011$^{g}$ | 0.447 ± 0.011$^{a}$ | 0.313 ± 0.011$^{a}$ |

**$H_2O_2$ and $O_2^{\bullet-}$** contents were similarly affected by $GeO_2$ application in apple leaves (Fig. 4). Under two light conditions, $H_2O_2$ contents in the leaves significantly decreased with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$. For $O_2^{\bullet-}$, its contents descended with above three concentrated $GeO_2$ solutions under 1,600 μmol(photon) $m^{-2}$ $s^{-1}$, but under 800 μmol(photon) $m^{-2}$ $s^{-1}$, it only continually descended significantly with 1.0 and 2.0 mg($GeO_2$) $L^{-1}$ and did not show significant difference between 2.0 and 5.0 mg($GeO_2$) $L^{-1}$. However, the accumulation of $H_2O_2$ and $O_2^{\bullet-}$ significantly increased by application of 10.0 mg($GeO_2$) $L^{-1}$. And furthermore, apple leaves generated more $H_2O_2$ and $O_2^{\bullet-}$ under 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ than that under 800 μmol(photon) $m^{-2}$ $s^{-1}$. These results indicate Ge could decrease the accumulation of ROS in the apple leaves and alleviate ROS-induced membrane damage.

**Activities of CAT, POD and SOD** were enhanced in apple leaves along with the rise of the exogenous $GeO_2$ concentration and reached a peak at the concentration of 5.0 mg($GeO_2$) $L^{-1}$ and then the activities of these three enzymes declined after the treatment by 10.0 mg($GeO_2$) $L^{-1}$ (Fig. 5). For the two light intensities, 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ stimulated the activities of CAT and POD noticeably compared to those under 800 μmol(photon) $m^{-2}$ $s^{-1}$. Similar difference was not found for the activity of SOD with $GeO_2$ application.

**Activities of ascorbate–glutathione cycle enzymes**: Exogenous $GeO_2$ application significantly changed the activities of antioxidant enzymes including MDHAR, DHAR, APX, and GR under both light intensities (Fig. 6). Among them, MDHAR and DHAR were influenced by exogenous $GeO_2$ in a similar way. Activities of the two enzymes increased with the increasing concentration of $GeO_2$ solution compared with the control and the rising trend slowed down when the concentration was above 5.0 mg $L^{-1}$. The activities of APX and GR in apple leaves with $GeO_2$ treatment were enhanced along with the increase of exogenous $GeO_2$ concentration, but started to decline when the concentration was above 5.0 mg $L^{-1}$. In addition, activities of four enzymes of MDHAR, DHAR, APX, and GR under 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ were higher than that under 800 μmol(photon) $m^{-2}$ $s^{-1}$ after $GeO_2$ treatment.

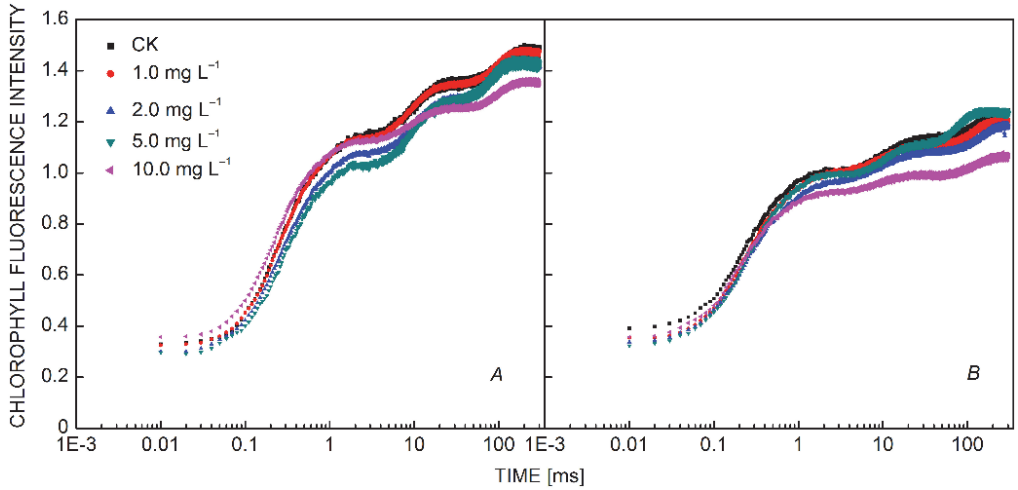


Fig. 1. Effect of different $GeO_2$ concentrations on chlorophyll fluorescence intensity in JIP-test under 800 µmol(photon) $m^{-2}$ $s^{-1}$ (*A*) and 1,600 µmol(photon) $m^{-2}$ $s^{-1}$ (*B*) in apple leaves.

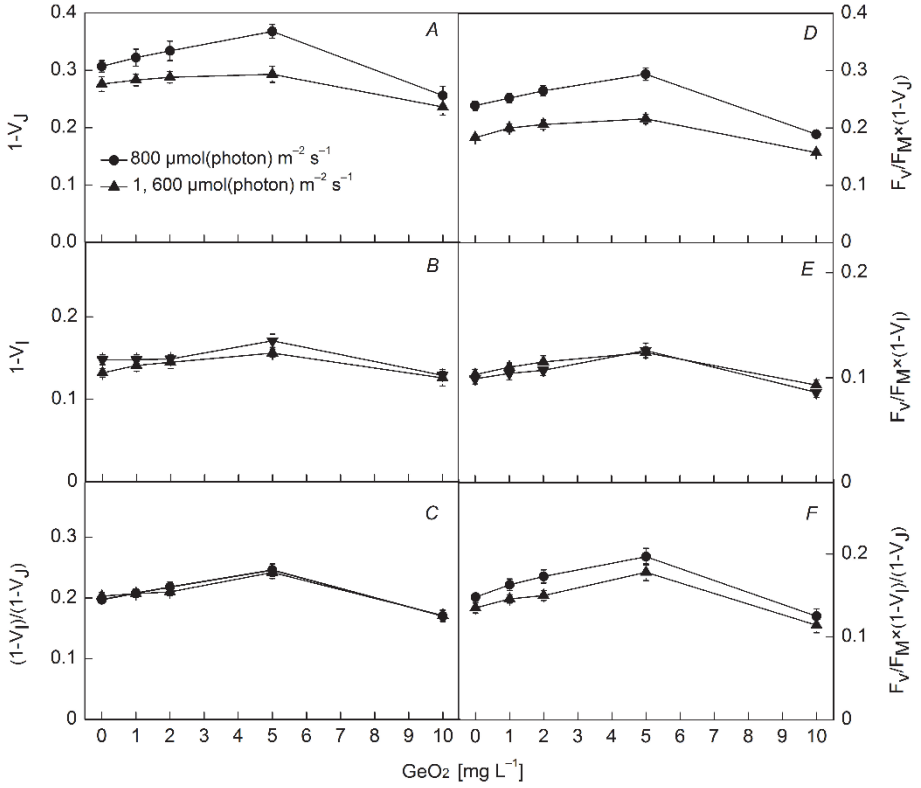


Fig. 2 Change in values of (*A*) 1 – $V_J$, (*B*) 1 – $V_I$, (*C*) (1 – $V_I$)/(1 – $V_J$), (*D*) $F_v/F_M$ × (1 – $V_J$), (*E*) $F_v/F_M$ × (1 – $V_I$), and (*F*) $F_v/F_M$ × (1 – $V_I$)/(1 – $V_J$) in apple leaves under 800 and 1,600 µmol(photon) $m^{-2}$ $s^{-1}$ for 4 h with different $GeO_2$ concentrations.

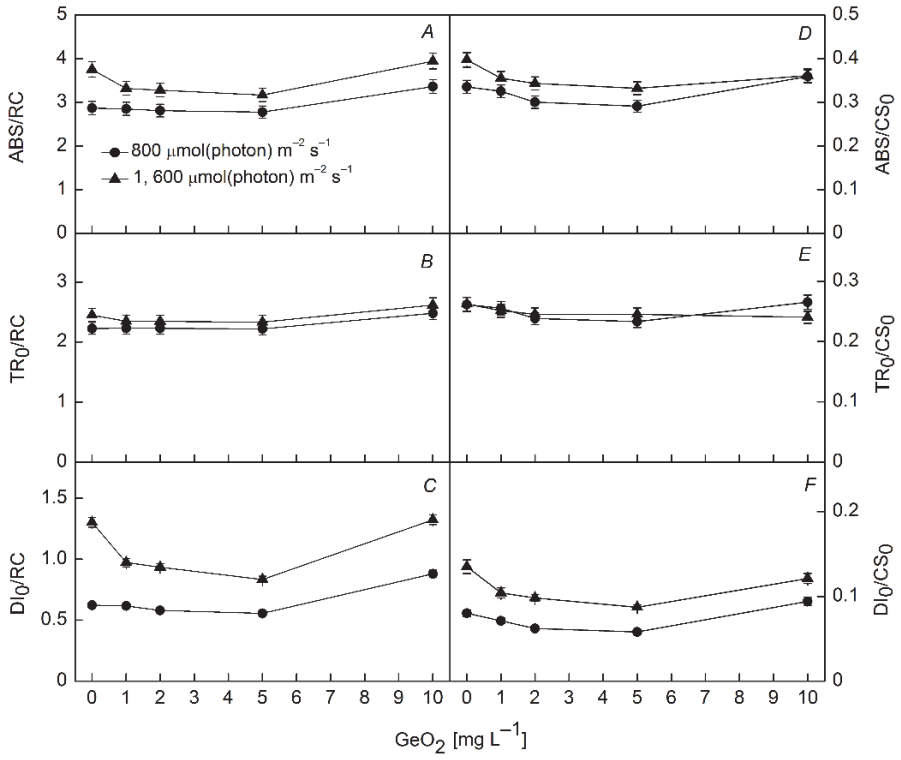


Fig. 3. Change in values of (*A*) ABS/RC, (*B*) $TR_0$/RC, (*C*) $DI_0$/RC, (*D*) ABS/$CS_0$, (*E*) $TR_0$/$CS_0$, and (*F*) $DI_0$/$CS_0$ in apple leaves under 800 and 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ for 4 h with different $GeO_2$ concentrations.

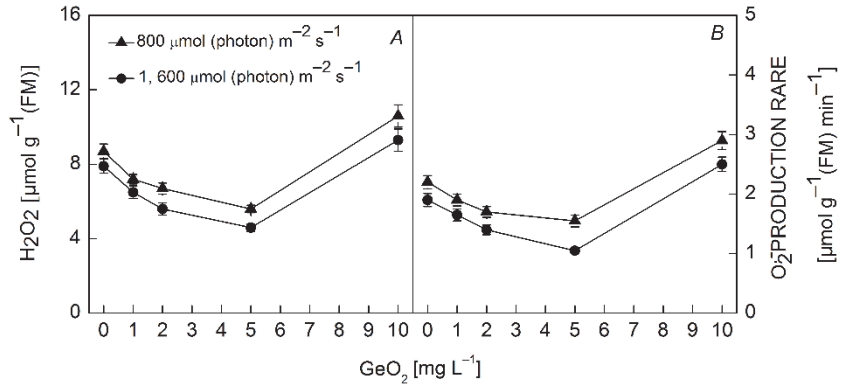


Fig. 4. Effect of different $GeO_2$ concentrations on ROS content in apple leaves including (*A*) $H_2O_2$ content and (*B*) $O_2^{\bullet-}$ content in apple leaves under 800 and 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ for 4 h.

## Discussion

Regarding to the effects of Ge on plants, previous studies focused on the accumulation of Ge in medicinal herb or herbaceous plants and tried to find a critical toxicity value of this element (Halperin *et al*. 1995, Yu *et al*. 2005, Han *et al*. 2007, Lim *et al*. 2008, Cheong *et al*. 2009). Previous studies also found Ge could be helpful to scavenge ROS (Munakata *et al*. 1987), but it remains unclear on how Ge influences photosynthetic apparatus, in which ROS generates under high light and environmental stress. In this study, in order to reveal effects of $GeO_2$ on photosynthesis and its mechanism, we monitored the response of apple leaves with different exogenous $GeO_2$ treatment using Chl fluorescence, concentration or formation rate of ROS and activities of antioxidant enzymes.

Compared with the control, contents of inorganic and organic Ge in leaves treated with exogenous $GeO_2$ increased significantly. After further analysis, we found that organic Ge content was the main form of Ge in apple leaves, although the exogenously applied Ge was in the inorganic form ($GeO_2$) (Table 1). It suggests that

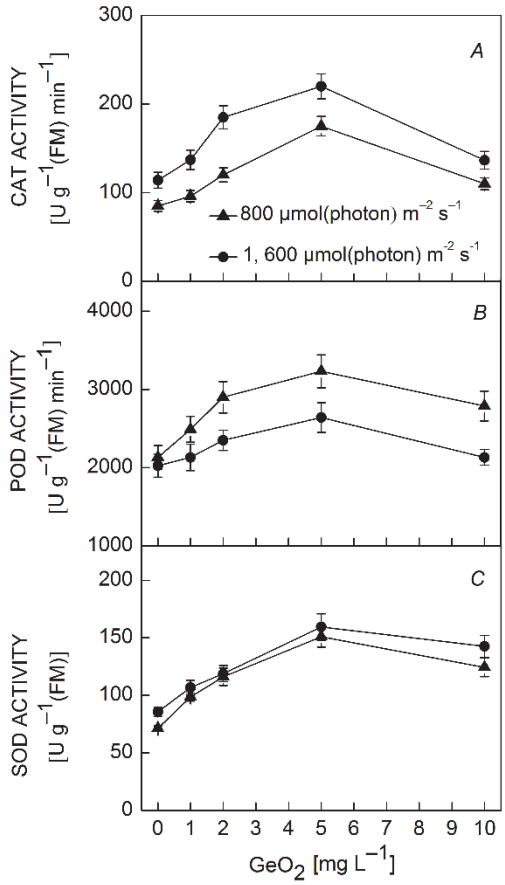


Fig. 5. Effect of different $GeO_2$ concentrations on (*A*) CAT activity, (*B*) POD activity, (*C*) SOD activity in apple leaves under 800 and 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ for 4 h.

exogenous $GeO_2$ converted into the organic form after entering into the apple leaves. The results are in accordance with studies in rice (Matsumoto *et al*. 1975). Therefore, we speculate that the *Malus* has the capacity to change free inorganic form into a bound organic form.

The change of Chl contents implies exogenous $GeO_2$ had a significant impact on Chl *a* content but not on Chl *b* content (Table 2). As Chl *a* has the most important role among photosynthetic pigments, exogenous $GeO_2$ may subsequently affect the function of photosynthetic apparatus which can be proved by modulated fluorescence parameters and fast fluorescence transient analysis.

As reported by previous researches, modulated fluorescence parameters could reflect photosynthetic performance including changes in PSII photochemistry efficiency, linear electron flux, and capacity of the Calvin cycle (Meyer *et al*. 2001). $F_v/F_M$, the maximum photochemical quantum yield of PSII, could provide a simple and rapid way to evaluate the photosynthetic performance (Henriques 2009, Zai *et al*. 2012, Zhang *et al*. 2014). In our study, $F_v/F_M$ of apple leaves with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ increased significantly and declined at 10.0 mg($GeO_2$) $L^{-1}$ under both 800 and 1,600 μmol (photon) $m^{-2}$ $s^{-1}$ (Table 3). Based on the changes of $\Phi_{PSII}$, $q_p$, $q_L$ and $F_v'/F_M'$, we suggest $GeO_2$ in appropriate concentration may facilitate the performance of photosynthetic apparatus. On the other hand, NPQ and $q_N$, quantifying energy dissipation through xanthophyll cycle and ΔpH (Jahns and Holzwarth 2012), both declined in apple leaves with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ and rose with 10.0 mg($GeO_2$) $L^{-1}$. Along with the rise of NPQ and $q_N$, $\Phi_{NPQ}$ decreased at 1.0, 2.0 and 5.0 mg($GeO_2$) $L^{-1}$ and increased at 10.0 mg($GeO_2$) $L^{-1}$, while $\Phi_{NO}$ remained at a steady level compared with control (Tab 1). If we consider $\Phi_{NPQ} + \Phi_{NO} + \Phi_{PSII} = 1$, the drop of $\Phi_{NPQ}$ with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ resulted in the rise of $\Phi_{PSII}$. Opposite response of photochemical capacity and energy dissipation indicates effects of $GeO_2$ on photosynthetic apparatus.

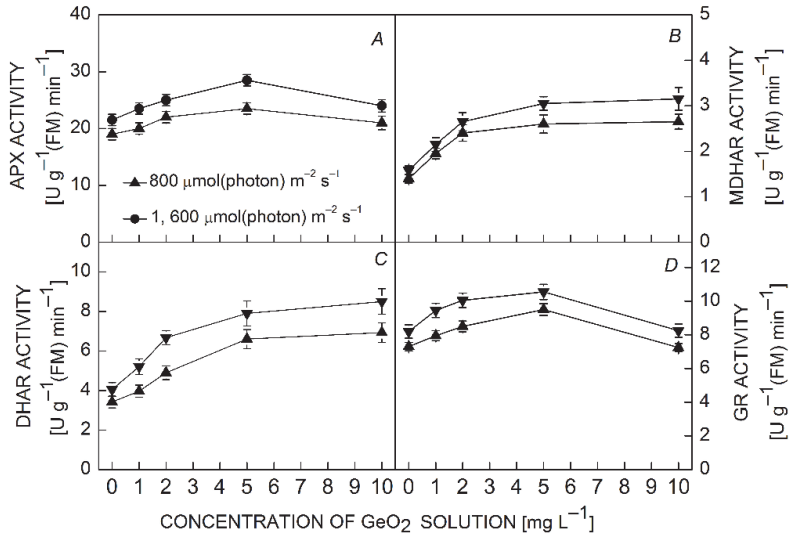


Fig. 6. Effects of different $GeO_2$ concentrations on ascorbate-glutathione cycle including (*A*) APX activity, (*B*) MDHAR activity, (*C*) DHAR activity, (*D*) GR activity in apple leaves under 800 and 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ for 4 h.

The fast fluorescence transient analysis is a fast and nondestructive method to measure the response of PSII activity to environmental changes and has been used widely for characterizing the photochemical quantum yield of PSII photochemistry and electron transport activity (Stirbet and Govindjee 2011, Schansker *et al*. 2005). In the present study, the JIP-test was also used to assess the photosynthetic performance of apple leaves with different $GeO_2$ solutions under strong light (Fig. 1). The OJIP curves showed high light-induced decrease in apple leaves (Fig. 1*B*). This phenomenon was similar to Antarctic lichens under high light stress (Balarinová *et al*. 2014). The momentary maximum fluorescence intensity (J-step and I-step) represents the subsequent kinetic bottlenecks of the electron transport chain (Strasser *et al*. 2000, Lazár 2006). These limitations come from the exchange of a reduced plastoquinone molecule with an oxidized one at $Q_B$ site (J-step), and the reoxidation of plastoquinol ($PQH_2$, I-step) (Schansker *et al*. 2005). Such change of Chl fluorescence intensity in O–J, O–I, J–I phase can represent PETC activity between $Q_A$–$Q_B$, $Q_A$–PSI acceptor, $Q_B$–PSI acceptor, respectively (Wang *et al*. 2012). We found clearly the electron transport from $Q_A$ to $Q_B$, reflected by $1 - V_J$ and $F_v/F_M \times (1 - V_J)$, was affected more than the electron transport from $Q_B$ to PSI by exogenous $GeO_2$ and two light intensities (Fig. 2).

In order to elucidate the performance of photosynthetic apparatus in more details, we applied the parameters derived from the Chl *a* fluorescence transient including ABS/RC, $ABS/CS_0$, $TR_0/RC$, $TR_0/CS_0$, $DI_0/RC$, $DI_0/CS_0$, to give a further insight into partitioning of absorbed light energy in apple leaves treated with $GeO_2$. ABS/RC and $ABS/CS_0$, average absorbed photon flux per PSII RC and per CS, both presented a downward trend with 1.0, 2.0, 5.0 mg($GeO_2$) $L^{-1}$, and then rose with 10.0 mg($GeO_2$) $L^{-1}$ (Fig. 3*A*,*D*). ABS/RC and $ABS/CS_0$ can be regarded as a probe to understand the average amount of absorbing antenna chlorophylls per fully active ($Q_A$-reducing) reaction center, which offers a way to detect eventual inactivation of RCs. In the present study, those two parameters could tell that the number of $Q_A$-reducing RCs and the density of RCs per CS in control and 10.0 mg $L^{-1}$ group were lower than those at 1.0, 2.0 and 5.0 mg($GeO_2$) $L^{-1}$. Irradiance of 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ can more easily inactivate PSII RC than that of 800 μmol(photon) $m^{-2}$ $s^{-1}$ therefore under high irradiation, the number of $Q_A$-reducing RCs were lower in apple leaves. It can explain ABS/RC and $ABS/CS_0$ under 1,600 μmol(photon) $m^{-2}$ $s^{-1}$ were higher than those under 800 μmol(photon) $m^{-2}$ $s^{-1}$. $TR_0/RC$ and $TR_0/CS_0$ (Fig. 3*B*,*E*) in our study did not change remarkably with different exogenous $GeO_2$ solutions and different light intensities. Data of $DI_0/RC$ and $DI_0/CS_0$ (Fig. 3*C*,*F*) showed that dissipated energy per PSII RC and CS dropped significantly, This also suggests that high irradiation declines photoprotection capacity. Apparently, $GeO_2$ application in range of values of 1.0–5.0 mg $L^{-1}$ resulting in apple leaves absorbing less light energy and dissipating more energy per PSII RC and CS, which reduced the damage of strong light to photosynthetic apparatus. However, it also did not exclude the possibility for downregulation of photosynthetic excitation pressure (Li *et al*. 2015).

As the site of photosynthesis, chloroplasts are also the major source of ROS such as $O_2^{\bullet-}$ and $H_2O_2$. ROS can be generated by the direct transfer of the excitation energy from Chl to produce singlet oxygen or by oxygen reduction in the Mehler reaction in the chloroplasts, leading to membrane lipid peroxidation (Stepien and Klobus 2005, Gill and Tuteja 2010). In our study, we observed apple leaves treated with 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ accumulated less $O_2^{\bullet-}$ and $H_2O_2$ (Fig. 4), which could be responsible for the increase of photosynthetic performance.

During evolution, a serious of antioxidant enzymes are developed to scavenge ROS in plants. Concisely, SOD plays a central role in the enzymatic defense system in removing $O_2^{\bullet-}$ (Diao *et al*. 2014) and CAT has a potential to directly dismutate $H_2O_2$ into $H_2O$ and $O_2$, which is indispensable for ROS-detoxification (Garg and Manchanda 2009, Hasanuzzaman and Fujita 2011). So to figure out the reason for the decreased contents of ROS, we determined the activities of antioxidant enzymes. In our study, with low concentration of $GeO_2$ treatment, CAT, SOD and POD activities increased, especially the activities of CAT and POD were noticeably higher under high light intensity (Fig. 5). Apart from CAT, POD and SOD, it is well known that the ascorbate-glutathione cycle is the key mechanism to scavenge ROS in plant chloroplasts and its higher efficiency under abiotic and biotic stress is responsible for the alleviation of oxidant damage to chloroplasts (Nakano and Asada 1981). In this cycle, APX plays the most important role in removing $H_2O_2$. MDHAR, DHAR, and GR are mainly responsible for providing substrates for APX through the formation of reduced AsA and GSH. Our results showed that exogenous $GeO_2$ below 5.0 mg $L^{-1}$ promoted the activities of APX, MDHAR, DHAR and GR (Fig. 6). Such response of antioxidant defense system may be a protecting mechanism for apple leaves through improving the activity to clear excess ROS under strong light.

In this study, we investigated effects of $GeO_2$ on photosynthetic performance of apple leaves under strong light. With the application of $GeO_2$, the photosynthetic performance of apple leaves including maximum photochemical quantum yield of PSII, the actual photochemical quantum yield of PSII and photochemical quenching efficiency at 1.0, 2.0, and 5.0 mg($GeO_2$) $L^{-1}$ remarkably increased under both light intensities. In the meantime, dissipated energy through xanthophyll cycle and ΔpH decreased noticeably compared with the control when the $GeO_2$ concentration was below 5.0 mg $L^{-1}$. Also, PETC capacity was also found to be impacted by exogenous $GeO_2$ among $Q_A$–$Q_B$, $Q_A$–PSI acceptor, and $Q_B$–PSI acceptor, PETC happened in $Q_A$ to $Q_B$ was affected the most showing a rising trend with low concentration of $GeO_2$ teatment. The decrease in ROS accumulation and

increase of antioxidant enzymatic defense system might be the reason for the drop of dissipated energy and the promotion of photosynthetic performance in apple leaves treated with $GeO_2$ solution below 5.0 mg $L^{-1}$. As organic Ge has been reported to have a function scavenging free radicals, such response of antioxidant ability under strong light could be related to the transformation of Ge chemical form, from exogenous inorganic $GeO_2$ to organic Ge.

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