Research Paper

# Effect of CO, NO$_x$ and SO$_2$ on ROS production, photosynthesis and ascorbate–glutathione pathway to induce *Fragaria × annasa* as a hyperaccumulator[☆]

Sowbiya Muneer [a], Tae Hwan Kim [b], Byung Chul Choi [c], Beom Seon Lee [d], Jeong Hyun Lee [a,*]

[a] *Department of Horticulture, College of Agricultural Life Sciences, Chonnam National University, 300 Young Bong-Dong Buk-Gu, Gwangju 500-757, Republic of Korea*
[b] *Department of Animal Science, Institute of Agricultural Science and Technology, College of Agriculture & Life Science, Chonnam National University, 300 YoungBong-Dong Buk-Gu, Gwangju 500-757, Republic of Korea*
[c] *School of Mechanical Systems Engineering, College of Engineering, Chonnam National, University, 300 Young Bong-Dong Buk-Gu, Gwangju 500-757, Republic of Korea*
[d] *Dayung GS Co., Ltd., Damyang, Jeonnam 517-922, Republic of Korea*

## ARTICLE INFO

## ABSTRACT

A study was conducted to determine the effect of carbon monoxide (CO), nitroxide (NO$_x$) and sulfur dioxide (SO$_2$) on ROS production, photosynthesis and ascorbate–glutathione pathway in strawberry plants. The results showed that both singlet oxygen (O$_2^{-1}$) and hydrogen peroxide (H$_2$O$_2$) content increased in CO, NO$_x$ and SO$_2$ treated strawberry leaves. A drastic reduction of primary metabolism of plants (photosynthesis), with the closure of stomata, resulted in a reduction of protein, carbohydrate and sucrose content due to production of reactive oxygen species (ROS) under prolonged exposure of gas stress. The resulting antioxidant enzymes were increased under a low dose of gas stress, whereas they were decreased due to a high dose of gas stress. Our results indicate that increased ROS may act as a signal to induce defense responses to CO, NO$_x$ and SO$_2$ gas stress. The increased level of antioxidant enzymes plays a significant role in plant protection due to which strawberry plants can be used as a hyperaccumulator to maintain environmental pollution, however, the defense capacity cannot sufficiently alleviate oxidative damage under prolonged exposure of CO, NO$_x$ and SO$_2$ stress.

## Introduction

Environmental pollution from industrial effluents and other organic pollutants has become a major environmental and human concern worldwide [1,2]. The major hazardous gases released from industries are abundant in number, among which carbon monoxide (CO) is a piousness gas to mammals, and has many similar qualities and effects to those of carbon dioxide (CO$_2$), except for its flammability at high concentration [3]. As an important signaling molecule in physiological activity, CO has a similar toxic effect to that of cyanide, the inhibitory effect of a higher ratio of CO to O$_2$ (above 4:1) on cytochrome *c* oxidase in the mitochondrial electron transport chain at the cellular level or the effect on the detoxification enzyme P-450 in the monoxygenase system and multiprotein complex proteins in chloroplasts. CO can have important signaling roles as well as toxic effects, but this depends upon the amount produced and context, as plants also contain a variety of heme moiety-containing proteins (catalase, peroxidase, cytochromes, etc.), and produce a number of important, biliverdin-related tetrapyrrole pigments, such as phycocyanobilin [4] and phytochromes [5].

On the other hand nitrogen oxides (NO$_x$) are produced by combustion processes and NO$_x$ is usually > 90% nitric oxide (NO), with the balance being composed of nitrogen dioxide (NO$_2$). In the atmosphere, chemical reactions convert NO to NO$_2$, which can further react to produce O$_3$. While NO$_x$ emission rates vary from plant to plant, largely according to the design of the plant and the characteristics of the fuel, an uncontrolled emission rate of 1.0 b mmBtu$^{-1}$ is typical for coal-fired generators. NO$_x$ is a free radical gas which transfers electrons across biological membranes [6].

In general the electron acceptor is oxygen and the product of the electron transfer reaction is superoxide. The biological function of $NO_x$ is therefore the generation of reactive oxygen species (ROS) [6]. The physiological generation of ROS can occur as a byproduct of other biological reactions. ROS generated as a byproduct occurs within mitochondria, peroxisomes and other cellular elements [7].

Sulfur dioxide ($SO_2$) is one of the most common harmful air pollutants. The phytotoxicity of $SO_2$ depends on its concentration and on the duration of exposure [1] and is influenced by sulfur status in plants [8]. The low dose of sulfur even can be useful to plants since sulfur is important for plants, and can help to alleviate other nutrient deficiencies in plants, such as that of Fe [9]. Sulfur is also a structural component of amino acids, proteins, vitamins and chlorophyll [8,10,11]. Sulfur enhances the development of nodules and nitrogen fixation, and also affects carbohydrate metabolism. However, exposure to high doses can lead to leaf chlorosis and necrosis, growth inhibition and plant death [12]. The concentration of $SO_2$ in the atmosphere has increased in many countries during the past few decades. It is estimated that parts of global plants exposed to $SO_2$ have prompted widespread attempts to evaluate defense responses.

The overall toxicity of these hazardous gases CO, $NO_x$ and $SO_2$ is derived from toxic molecular species such as those from the combustion of $CO_2$ to CO, $NO_x$ to $NO_2$, as well as $O_3$, sulphite ($SO_3^{2-}$) and bisulphite ($HSO_3^-$). The detoxification reaction of these compounds further leads to the production of reactive oxygen species, such as hydroxyl radical (OH•) and hydrogen peroxide. Increased production of ROS can attack bio macromolecules and results in oxidative damage to nucleic acids, proteins and lipids [13,14]. However, plants can scavenge excess ROS by invoking the antioxidant defense system to avoid oxidative damage. Many studies have shown that some antioxidant enzymes are involved in plant response under various abiotic stresses. The induction of antioxidant enzymes is thought to be a protective reaction of plants against abiotic stress, but the exact defense mechanism is not clear.

Strawberry plants are the richest grown plants in greenhouses which are often fertilized by direct $CO_2$ enrichments fertilizers for heating. The $CO_2$ boilers/direct heating systems used in greenhouse however, often lead to incomplete combustion which results in formation of hazardous gases such as carbon monoxide (CO), nitroxide ($NO_x$) and sulfur dioxide ($SO_2$) and other hydrocarbons. In the present study, we selected hazardous gases to conduct an investigation of alterations of ROS production, photosynthetic measurements and the ascorbate–glutathione pathway in strawberry plants exposed to different concentrations of CO, $NO_x$ and $SO_2$, assessing strawberry plants as a hyper-accumulator of these gasses.

## Methods

### Plant material and treatments

Young strawberry (*Fragaria × anansa*) plants were propagated on rock–wool cubes ($10 \times 10 \times 6.5$ cm) after germination at 25 °C under fluorescent light (300 µmol m$^{-2}$ s$^{-1}$ of photosynthetic photon flux density) for three weeks. The rooted strawberry plants were grown on the cubes with a hydroponic nutrient solution containing (m mole per litter for the macro elements): N 9.5, P 1.5, K 5.5, Ca 5.0, S 1.5, P 1.5 Mg 1.5 and (µmole per litter for the micro elements): Fe 33, Mn 22, B 10, Zn 5, Cu 1.0, Mo 0.5. The nutrient solution supplied was regularly determined for its electrical conductivity (EC) (1.0 dS m$^{-1}$) and pH was maintained at 6.5–7.0. Fluorescent light was supplied at 150 µmol m$^{-2}$ s$^{-1}$ at the canopy height for 16 h day$^{-1}$. Six-week-old plants were transferred to air

tight glass bottles (3 l) and were supplied with 133 (low dose), 267 (medium dose) and 533 (high dose) ppm of carbon monoxide (CO), 25 (low dose), 50 (medium dose) and 199 (high dose) ppm of nitroxide ($NO_x$), and sulfur dioxide ($SO_2$). Upon 24 h of treatment, the leaves were separated from the order of antigenic appearance and young leaves were used for the study. Plant samples were immediately frozen in liquid $N_2$ and were stored in a deep−freezer ($-80$ °C) prior to further analysis.

### Determination of $H_2O_2$ and $O_2^{-1}$

$H_2O_2$ concentration was measured calorimetrically as described by Lin and Kao [15] using titanium sulfate. $H_2O_2$ concentration was calculated using the extinction coefficient 0.28 mM$^{-1}$ cm$^{-1}$ and was expressed as nmol g$^{-1}$ tissue fresh weight. For $O_2^{-1}$ determination fresh tissue was extracted in phosphate buffer (pH 7.5) and centrifuged at 10,000g for 5 min. The resultant extract was added to 1 ml of 10 mM $NH_2OH$–HCl and phosphate buffer (pH 7.8) and incubated at 25° for 1 h. After incubation the mixture was added with 0.5 ml of 17 mM alpha aminobenzene sulphonic acid and 0.5 ml of 7 mM alpha naphthylamine solution and was incubated again for 20 min at 25° and absorbance was recorded at 530 nm.

### Measurement of net photosynthetic activity and $F_V/F_m$ ratio

Photosynthetic rate was measured using a portable photosynthesis measurement system (LI-6400XT. LI-COR, Inc., Lincoln, NE). Chlorophyll fluorescence ($F_V/F_m$) was measured by using a chlorophyll fluorescence meter (PAM 2000; Heinz Walz GmbH, Effeltrich, Germany). The leaves were adapted to dark conditions for 30 min before the measurement. The maximum fluorescence ($F_m$) and minimum fluorescence ($F_0$) were determined by applying a saturating light pulse (20 kHz) of 1100 µmol m$^{-2}$ s$^{-1}$ PPF for 3 µs. The maximum PS II quantum yield ($F_V/F_m$) was calculated as $F_V/F_m = (F_m - F_0)/F_m$ for 24 h at different concentrations of CO, $NO_x$ and $SO_2$.

### Photosynthetic pigments and stomatal observation

The content of chlorophyll and carotenoid was estimated by the method of Hiscox and Israclstam [16]. The fresh leaves were collected in glass vials, to which 10 ml dimethyl sulfoxide (DMSO) was added, after which samples were kept in an oven at 65 °C for complete leeching of pigments for 1 h. Optical density was recorded at 480, 645, 520 and 663 nm. The content of total chlorophyll and carotenoid was calculated using the formulae given by Arnon [17].

For stomatal observation, thin layers of leaf tissues were carefully cut and were laid on glass slides, covered with cover slips by adding few drops of water, and were observed under a light microscope (Leica DM4000 M) at $40 \times$ magnification.

### RuBisCO determination by SDS-PAGE

Leaf tissues were homogenized at 4°C in 100 mM Tris buffer (pH 7.5) containing 5 mM of DTT, 2 mM iodoacetate and 5% (v/v) glycerol at a leaf; buffer ratio of 1:5–10 (g:ml). For this extraction, a buffer without sodium or potassium ion was recommended for SDS-PAGE analysis because those cations reduce the solubility of DS (dodecyl sulfate). Before centrifugation, a TritonX100 (25%, v/v) was added to a portion of leaf homogenate to make a final concentration of 0.1% (v/v). An addition of TritonX100 was effective for the extraction of RuBisCO bound to the membrane fraction [18]. The homogenates were centrifuged at 5000g for 3 min at 4 °C. A lithium DS solution (25% w/v) and 2-mercaptoethanol were added to the supernatant fluid to a final concentration of 1.0%

(w/v) and 1% (v/v), respectively. This preparation was immediately treated at 100 °C for 1 min, and was then stored at −30 °C until the analysis of SDS-PAGE. The samples containing 2–10 μg RuBisCO were loaded onto 12% polyacrylamide gel. After electrophoresis, the gels were stained in 0.25% (w/v) CBB-R. The stained bands corresponding to larger and smaller subunits of RuBisCO were cut out of the gels with a razor blade and were eluted in 1–2.5 ml of formamide in a stoppered amber test tube at 50 °C for 5 h with shaking. The absorbance of the resultant solution was read at 595 nm with a spectrophotometer. RuBisCO content was determined by using the standard curve calculated from the absorbance of a known amount of purified RuBisCO.

*Total carbohydrate and sucrose content*

The carbohydrate content was determined by anthrone method [19]. One hundred milligrams of dried leaf tissue were added to a boiling tube. The tissues were hydrolyzed in a boiling tube by keeping them in a water bath for 3 h with 5 ml of 2.5 N-HCl, and were then cooled at room temperature. The solution was then neutralized with solid sodium carbonate until effervescence ceased, and samples were then centrifuged. After centrifugation 1 ml of supernatant had added to it 4 ml of anthrone reagent. The solution was then heated for 8 min in a boiling water bath and absorbance at 630 nm were recorded after cooling down at room temperature. The corresponding concentration of carbohydrate was determined against a standard curve prepared by using glucose solution.

For sucrose content 0.1 g of dried leaf was taken in 10 ml of ethanol (90%). The vials were incubated at 60 °C for 1 h and volume was made up to 25 ml by adding 15 ml of 95% ethanol. A 1 ml aliquot had added to it 1 ml of 5% phenol, and the solution was thoroughly mixed. Five ml of concentrated $H_2SO_4$ was added and the solution was allowed to cool at room temperature, after which the absorbance was recorded at 485 nm using a Beckman DU 640 spectrophotometer. The corresponding concentration of sucrose was determined against a standard curve which was prepared using glucose solution.

*Antioxidant enzyme assays*

Superoxide dismutase (SOD) activity was determined by the method of Dhindsa et al. [20] with minor modifications. Fresh tissues (200 mg) were extracted in phosphate buffer (pH 7.3) and were then centrifuged at 15,000g. SOD activity in the supernatant was assayed for its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) by reading the absorbance at 560 nm. One unit of enzyme activity was defined as the amount of enzyme required to inhibit 50% of the NBT photo reduction in comparison with tubes lacking the plant extract.

Catalase (CAT) activity was determined by the method of Aebi [21]. About 500 mg of grounded tissues was extracted in phosphate buffer (pH 7.3), and were then centrifuged at 15,000g for 20 min. The decrease in absorbance at 240 nm was recorded as a result of $H_2O_2$ degradation (extinction coefficient of $36 \, mM^{-1} \, cm^{-1}$). One unit of enzyme is defined as the amount necessary to decompose 1 μm of $H_2O_2$ per min.

Glutathione reductase (GR) activity was determined by the method of Rao [22]. About 500 mg of fresh tissues was extracted in phosphate buffer (pH 7.0), and were centrifuged at 15,000g for 10 min. The supernatant was immediately assayed for GR activity through the glutathione-dependent oxidation of NADPH at 340 nm. One unit of enzyme is defined as the amount necessary to decompose 1 μmol of NADPH per min.

Ascorbate peroxidise (APX) activity was estimated by measuring the decrease in absorbance at 290 nm (extinction coefficient of absorbance $2.8 \, mM^{-1} \, cm^{-1}$ for ascorbate) according to Nokano et al. [23]. One hundred milligrams of fresh tissue were extracted in 100 mM K-phosphate (pH 7.0), and were then centrifuged at 15,000g for 10 min. One unit of enzyme was expressed as the amount necessary to decompose 1 μmol of ascorbate in 1 min.

*Statistical analysis*

A completely randomized design was used with three biological replicates for five treatments. An individual Student's *t* test was employed to compare the means of separate replicates by using software SAS (version 9.1, USA).

## Results and discussion

*CO, $NO_x$ and $SO_2$ induced ROS generation*

Reactive oxygen species generation was observed by determination of hydrogen peroxide ($H_2O_2$) and singlet oxygen ($O_2^{-1}$). Strawberry leaves which were exposed to CO, $NO_x$, and $SO_2$ gases exhibited high concentration of $H_2O_2$ and $O_2^{-1}$ (Fig. 1A and B). The concentration of $H_2O_2$ and $O_2^{-1}$ was observed highest by more than 90 and 80% respectively under high dose of CO, $NO_x$ and $SO_2$ compared to control.

In plant cells, ROS are unavoidable by-products of aerobic metabolism. Under normal growth conditions, the amount of ROS is modest, and cells experience only mild oxidative stress, whereas, many stresses enhance ROS production [13,24,25]. The results of our study clearly show that CO, $NO_x$ and $SO_2$ triggered the rapid induction of $O_2^{-1}$ and $H_2O_2$ generation in strawberry leaves. This enhanced production of ROS under stress can pose a threat to cells and can also act as a signal to activate stress response pathways such as the ascorbate–glutathione cycle [13,26].

*Photosynthetic parameters*

The net photosynthetic rate in plants exposed under low doses of hazardous gases (CO, $NO_x$ and $SO_2$) showed a slight reduction of net photosynthesis, however, at the medium dose of gas stress the net photosynthetic rate decreased (Fig. 2A) and the reduction of photosynthesis was observed to be highest in plants under high doses of CO, $NO_x$ and $SO_2$.

Similarly, the ratio between $F_V/F_m$ under CO, $NO_x$ and $SO_2$ treatment was not different between control plants, and those exposed to a low dose of gas stress (Fig. 2B). After a medium dose of gas stress, the ratio between $F_V/F_m$ decreased, and the lowest ratio was observed under a high dose of gas stress by 13%, 24% and 11%, respectively, when compared to the control.

We also observed that photosynthetic pigments (total chlorophyll and carotenoid) under CO, $NO_x$ and $SO_2$ were reduced slightly under a low dose of gas stress, whereas under a medium dose of hazardous gas stress the photosynthetic pigments reduced significantly and the highest reduction was observed in plants under a high dose of gas stress, reducing total chlorophyll by 54%, 60% and 59% (Fig. 3A) and carotenoid content by 66%, 80% and 70% (Fig. 3B) compared to control.

The present results show that photosynthetic parameters such as net-photosynthesis, $F_V/F_m$ ratio and photosynthetic pigments (chlorophyll and carotenoid) were reduced under CO, $NO_x$ and $SO_2$, which represents that there might be a transfer of lesser electrons and lesser energy investment in the biochemical machinery of strawberry. The reduction of net photosynthesis and ratio between $F_V/F_m$ has been also observed under salt stress in green algae [27] and in *Vigna radiata* under mineral deficiency [24,25]. The decline in net photosynthesis and $F_V/F_m$ under hazardous gas stress might be due to the inactivation of reaction centers of
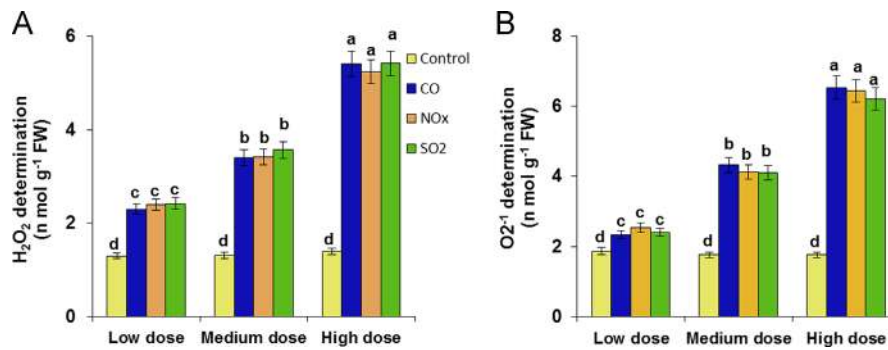
**Fig. 1.** Production of reactive oxygen species (ROS) (A) $H_2O_2$ and (B) $O2^{-1}$ determination, as affected by low, medium and high dose of CO, $NO_x$, and $SO_2$. Vertical bars indicate $\pm$ SE by means with $n=3$. Means denoted by different letters are significantly different at $p < 0.05$ according to the Tukey's studentized range test.
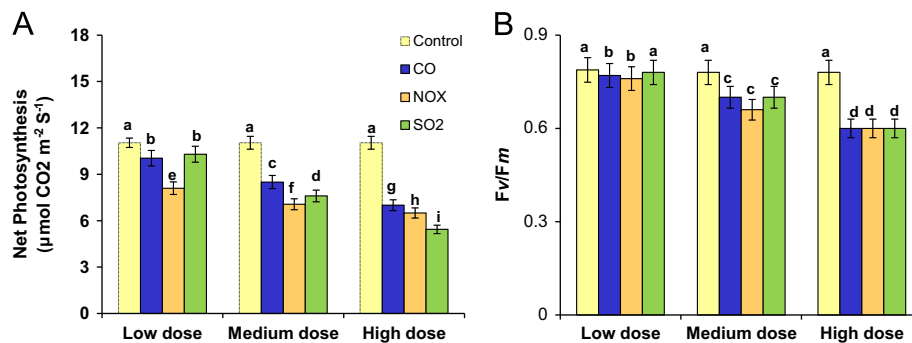


**Fig. 2.** Changes in photosynthetic parameters (A) photosynthesis rate, (B) $F_v/F_m$ ratio, as affected by low, medium and high dose of CO, $NO_x$, and $SO_2$. Vertical bars indicate $\pm$ SE by means with $n=3$. Means denoted by different letters are significantly different at $p < 0.05$ according to the Tukey's studentized range test.
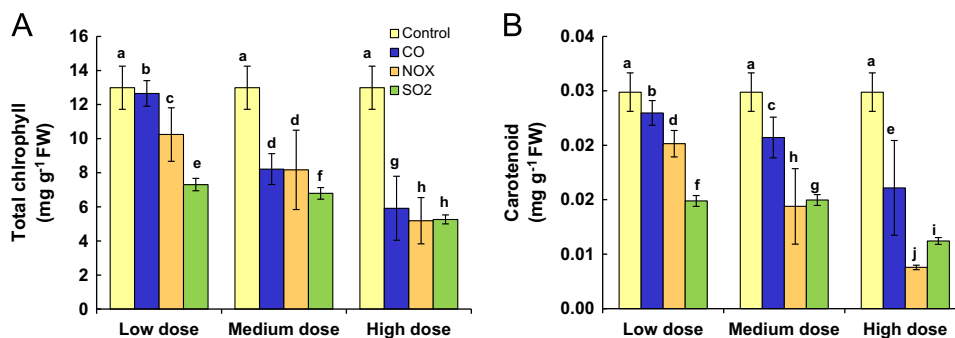


**Fig. 3.** Changes in the content of photosynthetic pigments (A) total chlorophyll (B) carotenoid, as affected by low, medium and high dose of CO, $NO_x$, and $SO_2$. Vertical bars indicate $\pm$ SE by means with $n=3$. Means denoted by different letters are significantly different at $p < 0.05$, according to Tukey's studentized range test.

photosystems which receive an initial amount of light energy and cannot be exploited efficiently due to the presence of oxidative stress. The ineffective energy exploitation [28,29] leads to an extreme increase of dissipated energy, and therefore, decreases photosynthesis. In addition to this the loss of carotenoids might be due to the loss of chlorophyll, because carotenoids absorb light energy for use in photosynthesis, and they protect chlorophyll from photo-damage. Such a decrease in chlorophyll content may also be attributed to a reduction of hazardous gases for the formation of precursor molecules, δ-aminolevulinic acid and protochlorophyllide [30].

### Observation of stomata under microscope

Microscopic observations ($40\times$) have shown that stomata did not show any change in behavior in plants under low dose of CO, $NO_x$, or $SO_2$ treatment (Figs. 4 and 5). With increased doses of gas exposure the stomata gradually closed and guard/subsidiary cells

were damaged compared to those of the control, which was found to be exaggerated at high dose of gas exposed after 24 h.

As we know, stomata play an important regulatory role in leaf physiological processes, as the primary pathway for the exchange of gases between internal leaf surfaces and the atmosphere [31]. The closing of stomata as a result of exposure under hazardous gas has been reported in several species such as *Vicia faba* [32] and *Pisum sativum* [31] under $SO_2$ stress. The closure of stomata under high concentrations of CO, $NO_x$ and $SO_2$ demonstrated that certain key enzymes involved in stomatal metabolism might have been affected, which are also involved in several other metabolic activities in plants such as malate formation and metabolism in epidermis, and turgor pressure of guard cells. The destructive damage of guard cells and density might also occur due to defoliation of leaves during growth conditions of strawberry, and indeed, due to high temperatures under hazardous gas conditions [33], which might have resulted in the induction of palisade and sponge parenchyma cell length and thickness.

| Low Dose | Medium Dose | High Dose |
|---|---|---|
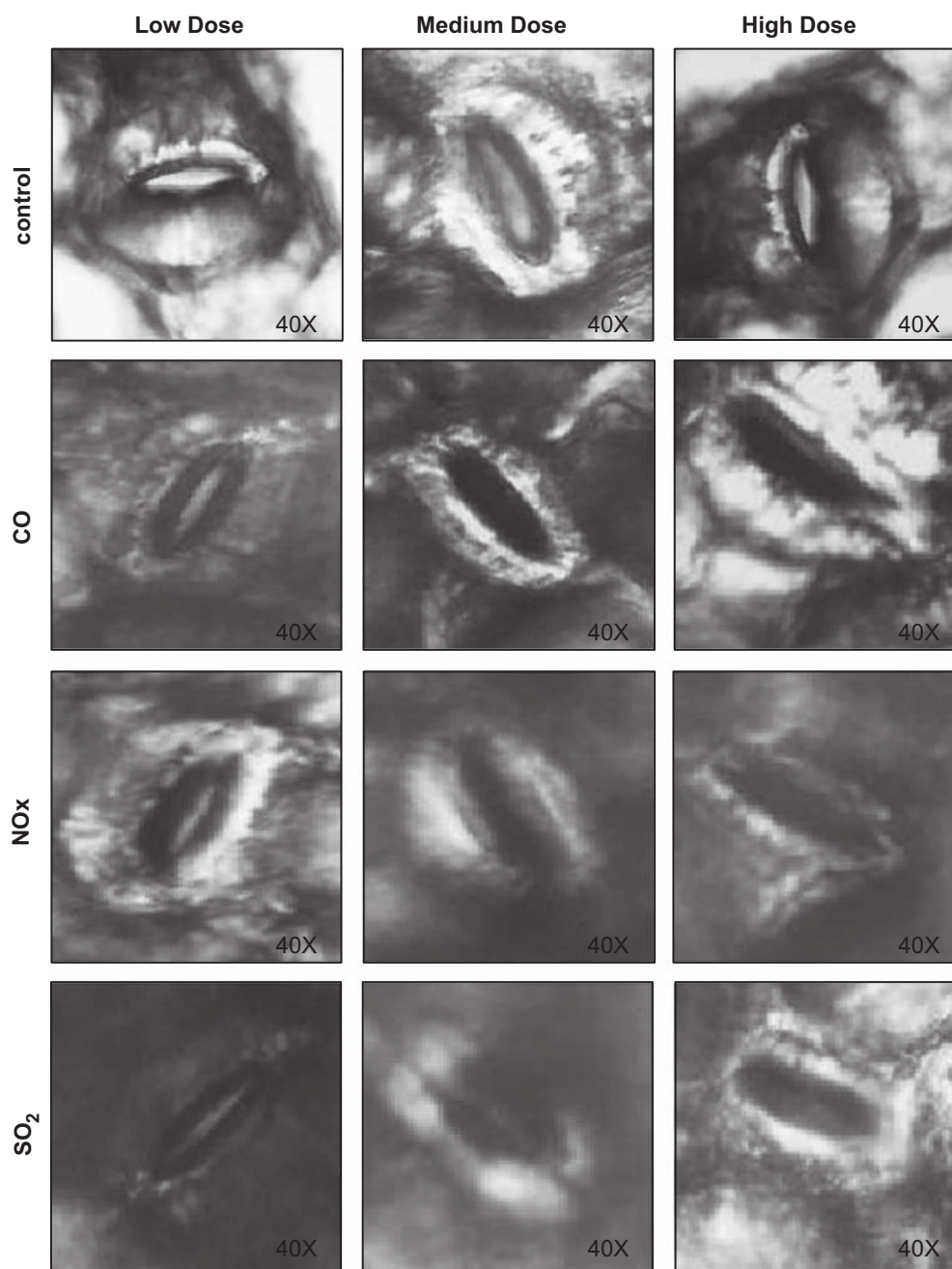


**Fig. 4.** Representative images of stomata, as affected by low, medium and high doses of CO, $NO_x$, and $SO_2$. Thin layer of leaf outer covering were peeled off carefully and laid on a glass slide, covered with a cover slip, and were observed under a light microscope (Leica CME) at $40 \times$ magnification.

*RuBisCO contents*

Fig. 4 shows that the RuBisCO content determined by SDS-PAGE, the RuBisCO content in plants under low dose CO, $NO_x$ or $SO_2$ showed great change in expression, and statistically significant results, as generated by a spectrophotometer, have shown a huge change even early stages of hazardous gas treatment. With increasing hazardous gas doses the reduction of RuBisCO content was observed to be highest in plants exposed to low doses of gas stress when compared to that of the control.

The changes in RuBisCO content in response to various abiotic stresses has been reported in many species, such as in *Trifolium alexandrinum* [34] under salt stress conditions. The maintenance of a higher RuBisCO content in plants has been correlated with a higher rate of photosynthesis in certain genotypes, and a lower content of RuBisCO is correlated with lower rates of photosynthesis under abiotic stresses [35]. In the present study, the reduction of RuBisCO content under hazardous gas stress (CO, $NO_x$ and $SO_2$) coincides with a reduction of photosynthesis (Fig. 2). In recent studies we observed the proteomic data of *Brassica napus* under Fe-deficiency to reduce most proteins classified as photosynthetic precursors, including RuBisCO content [10]. The loss of RuBisCO protein might be due to the progressive depletion of biochemical pathways associated with signal transduction and gene regulation, and in particular, might be involved in protein synthesis [36,37], and also might be associated with an excessive production of ROS which leads to incorrect folding or assembly of proteins, and consequent protein degradation [38].

*Carbohydrate and sugar content*

The total carbohydrate in plants under low dose of CO, $NO_x$ or $SO_2$ was reduced compared to control (Fig. 6A) and more reduction was observed in plants under medium dose of hazardous gas stress by 54%, 81% and 72% respectively. The reduction was observed to be highest under high dose of CO, $NO_x$ and $SO_2$, by 65%, 87% and 70% respectively.
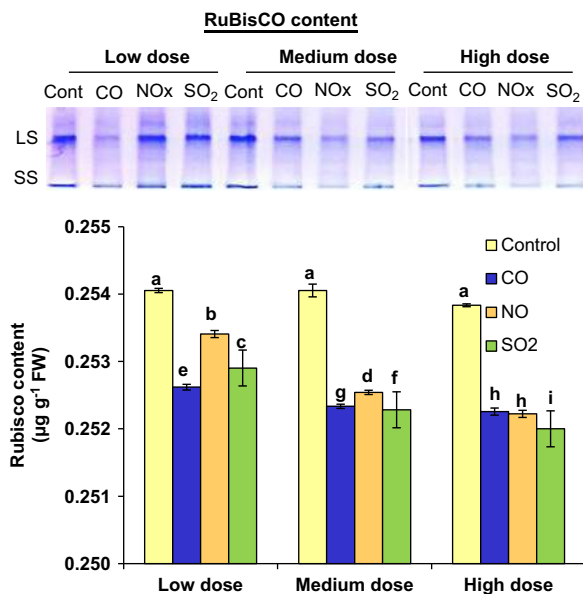
Sucrose content in plants under low dose of CO, $NO_x$ and $SO_2$ showed a slight reduction, which was greater (and significant) in medium dose plants (Fig. 6B), however, a high dose of CO, $NO_x$ and $SO_2$ reduced the sucrose content by 23%, 48% and 65%, respectively, when compared to control.

The decrease in total carbohydrates and sucrose content of damaged leaves probably corresponded with photosynthetic inhibition, or with the stimulation of a lower respiratory rate. Higher starch accumulation in damaged leaves may have resulted both in the higher resistance of their photosynthetic apparatus [39] and low starch export from the mesophyll. The negative effect of hazardous gases on carbon metabolism is a result of their possible interaction with the reactive center of ribulose bisphosphate carboxylase [40].
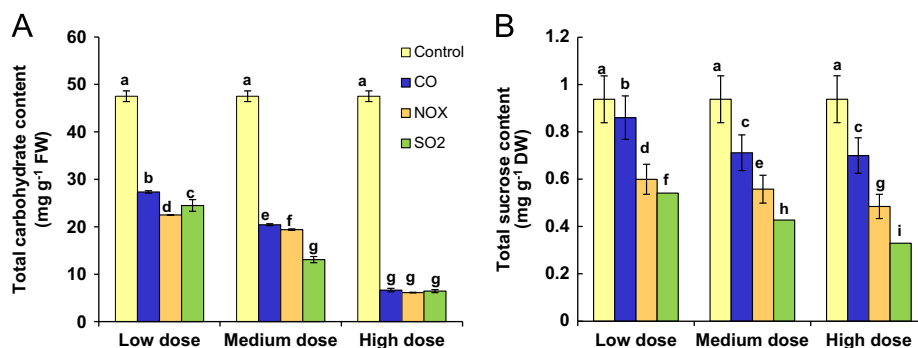


**Fig. 5.** Regulation of RuBisCO larger and smaller subunits to low, medium and high doses of CO, $NO_x$ and $SO_2$ (A) SDS-PAGE of leaf proteins from control and gas stressed plants, (B) quantification of the large subunit protein band. Vertical bars indicate $\pm$ SE by means with $n=3$. Means denoted by different letters are significantly different at $p < 0.05$ according to Tukey's studentized range test.

*CO, $NO_x$ and $SO_2$-induced antioxidative defense response*

We analyze the evolution of stress responses in strawberry plants exposed to low, medium and high doses of CO, $NO_x$ or $SO_2$ for 24 h. The treatment evokes physiological changes in the form of ascorbate glutathione cycle. There was a stark contrast between the responses of all antioxidative enzymes examined in this study. CO, $NO_x$ and $SO_2$ increased SOD, CAT, GR and APX, activity at low and medium doses of gas stress conditions (Fig. 7A–D), while at higher doses, SOD activity was decreased by 55%, 55.1% and 78%, catalase activity was decreased by 81%, 89% and 86%, GR activity was decreased by 69%, 30% and 47% and APX activity was decreased by 32%, 62% and 25% compared with control.

In this study, the activities of antioxidative enzymes (APX, SOD, CAT and GR) were found to be increased at low and medium doses of CO, $NO_x$ and $SO_2$ stress, whereas they were significantly decreased at high doses of gas stress. Several reviews have been published on different aspects of ascorbate and glutathione, ranging from their biosynthesis [41,42], to their roles in the transport system [43] and stress defense [44] in plants. It has been generally established that glutathione and ascorbate play a prominent role in non-enzymatic mechanisms to prevent the oxidation of cellular compounds [44], and are involved in the induction of enzymes and gene expression. SOD catalyzes the dismutation reaction of $O_2^{-1}$ and $H_2O_2$ for the initial period of stress conditions, as induction of these enzymes was observed in our study, whereas, after prolonged exposure the SOD could not dismutase these ROS into $H_2O_2$ and $H_2O$, which resulted in the reduction of enzyme activities of ascorbate glutathione cycle. Glutathione is involved as a substrate for glutathione peroxidase and is therefore necessary for the removal of lipid peroxidases, because GR reduces the oxidized glutathione, which is generated by glutathione peroxidase [42,45]. The observed increase in GR at initial period of stress resulted in the detoxification of oxidative damage, while a high dose of CO, $NO_x$ and $SO_2$ resulted in a disturbance in ascorbate glutathione pathways. CAT and APX are the primary $H_2O_2$ scavenging enzymes in plants. In this study, CO, $NO_x$ and $SO_2$ pronouncedly an increased APX activity and CAT activity in the initial period of stress, which indicates that APX and CAT are efficient enzymes for detoxifying ROS arising from hazardous gas treatments. Moreover the increased activity of these enzymes can contribute to resistance and enhancement since they participate in many other physiological processes in plant defense reactions.

## Conclusions

In conclusion, this is a systematic study which provided a specific insight into the changes in ROS production, photosynthesis



**Fig. 6.** Changes in content of (A) total carbohydrate and (B) sucrose content, as affected by low, medium and high dose of CO, $NO_x$, and $SO_2$. Vertical bars indicate $\pm$ SE by means with $n=3$. Means denoted by a different letter are significantly different at $p < 0.05$ according to Tukey's studentized range test.
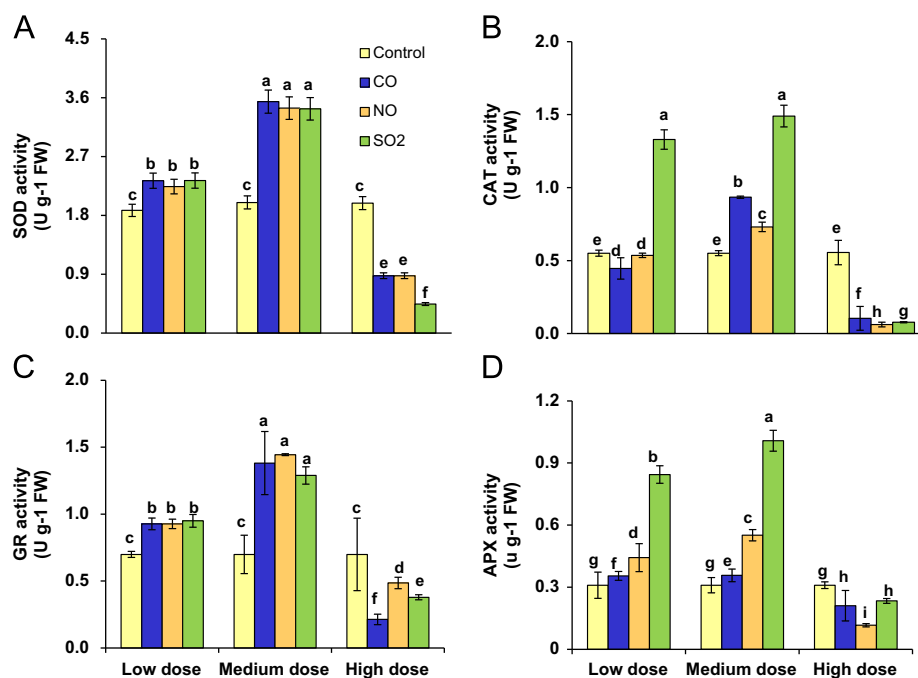
**Fig. 7.** Changes in antioxidant enzyme activities (A) SOD (B) CAT (C) GR and (D) APX as affected by low, medium and high dose of CO, $NO_x$, and $SO_2$. Vertical bars indicate $\pm$ SE by means with $n=3$. Means are denoted by different letters are significantly different at $p < 0.05$ according to Tukey's studentized range test.

physiology and antioxidant status in strawberry plants under CO, $NO_x$ and $SO_2$ stresses, which contribute the mechanism of plant adaptation and promote that strawberry plant, can act as hyperaccumulator to mitigate oxidative damage. Our results suggest that CO, $NO_x$ and $SO_2$ caused a high level of ROS production in the form of $O_2^{-1}$ and $H_2O_2$, as well as a reduction of photosynthetic physiology. Conversely, antioxidant enzymes of ascorbate glutathione pathways were induced to scavenge the negative effects of gas stresses. However, increased levels of lipid peroxidation products in strawberry plants exposed to CO, $NO_x$ and $SO_2$ for high dose demonstrated that the antioxidant defense system was no longer used to mitigate free radicals. Ascorbate glutathione cycle status may play a critical role in defense against CO, $NO_x$, and $SO_2$, as well as the maintenance of photosynthesis metabolism in plants, and will help to mitigate oxidative damage under abiotic stress. These results suggest that strawberry plants can act as a hyperaccumulator for detoxifying these gases.

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