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Influences of magnesium deficiency and cerium on antioxidant system of spinach chloroplasts

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Abstract Magnesium-deficiency conditions applied to spinach cultures caused an oxidative stress status in spinach chloroplast monitored by an increase in reactive oxygen species (ROS) accumulation. The enhancement of lipids peroxide of spinach chloroplast grown in magnesium-deficiency media suggested an oxidative attack that was activated by a reduction of antioxidative defense mechanism measured by analysing the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, and glutathione reductase, as well as antioxidants such as carotenoids and glutathione content. As the antioxidative response of chloroplast was reduced in spinach grown in magnesium-deficiency media, it caused a significant reduction of spinach plant weight, old leaves turning chlorosis. However, cerium treatment grown in magnesium-deficiency conditions decreased the malondialdehyde and ROS, and increased activities of the antioxidative defense system, and improved spinach growth. Together, the experimental study implied that cerium could partly substitute for magnesium and increase the oxidative stress-resistance of spinach chloroplast grown in magnesium-deficiency conditions, but the mechanisms need further study.

Keywords Cerium · Magnesium-deficiency · Spinach · Chloroplast · Antioxidant system

Introduction

It is well known that magnesium (Mg) is one of the essential elements for plant growth which is the central atom of the chlorophyll (chl) molecule, and fluctuations in its levels in the chloroplast regulate the activity of key photosynthetic enzymes such as ribulose carboxylase (Rubisco), fructose-1, 6-bisphosphatase, and phosphoribulokinase. Mg is the most abundant free divalent cation in the plant cytosol. The functions of Mg in plants (as well as in other organisms) are mainly related to its capacity to interact with nucleophilic ligands (Orit 2002). Moreover, Mg can promote the transport of phosphate in vivo, and is involved in the lipid metabolism and the synthesis of Vitamin A and Vitamin B. The researches indicated that reactive oxygen species (ROS) injury under the stress of Mg deficiency could cause the decrease of chl content and leaves chlorosis (Li et al. 1998, 2000), the reduction of protein and nucleic acid in plants. And Mg deficiency accelerated ageing of plants (Li et al. 1998; Candan and Tarhan 2003). Marschner et al. found that highlight significantly

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reduced the chl content and caused chlorosis of bean subjected to Mg deficiency, but it did not obviously influence Mg content in bean (Marschner and Cakmak 1989). By Mg deficiency, the activities of superoxide dismutase (SOD), ASA-POD, GR and the contents of antioxidants such as ASA, SH compounds were increased and ROS such as $O_2^{\cdot-}$ and OH were accumulated in bean leaves (Cakma 1994; Cakma et al. 1994; Poller et al. 1994; Wang and Chu 1998).

Chloroplast is a site of photosynthesis. The damage of Mg deficiency on the photosynthesis may be closely related to oxidative stress in chloroplasts. But it needs to be confirmed by experimental study.

Hong et al. proved that La^{3+}, Ce^{3+}, Nd^{3+} could obviously promote growth, increase chl contents and photosynthetic rate of spinach. La^{3+}, Ce^{3+}, and Nd^{3+} might substitute Mg^{2+} for chl formation of spinach without Mg^{2+}, bind to chl and thus form Ln-chl, and improved significantly photosystem II (PS II) formation and enhanced electron transport rate of PS II of spinach (Hong et al. 2002a, b, 2003). The effects of Ce^{3+} on the chloroplast senescence of spinach under light were studied, showing that Ce^{3+} significantly decreased ROS accumulation and malonyldialdehyde (MDA), and maintain stability of membrane structure of chloroplasts under light, which protected chloroplasts from aging for long time illumination (Yang et al. 2005). Huang et al. proved that Ce^{3+} could relieve the inhibition of calcium deprivation the electron transport, the oxygen evolution, the photophosphorylation, the activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase and Rubisco of spinach chloroplasts (Huang et al. 2008). However, whether Ce^{3+} could improve antioxidant defense of chloroplasts, or decrease oxidative damage of chloroplasts subjected to Mg deficiency? It also needs to be studied.

The oxidative damage caused by Mg deprivation in spinach chloroplasts and antioxidative stress of chloroplasts from $CeCl_3$-treated spinach grown in Mg-deficiency conditions were studied in the paper. The results showed that Mg deprivation in spinach chloroplasts increased ROS and the level of lipid peroxidation, and decreased activities of the antioxidant enzymes and non-enzymatic system (antioxidants). However, cerium treatment cultivated in Mg-deficiency media decreased ROS and MDA, and increased activities of the antioxidative defense systems, suggesting that on cerium added to Mg-deficiency media in the spinach plants could substitute for Mg and increase oxidative stress-resistance.

Materials and methods

Material treatment and culture

Experimental material was *Spinacia oleracea*. The seeds were purchased from a local seed company. Spinach seeds were soaked with 15 μM $CeCl_3$ solution for 48 h at 10°C, and with deionized water for control. And the seeds were whole surrounded with the suspension. Then, the seeds were carefully selected and planted in a perlite-containing pot and placed in porcelain dishes, which were respectively, added with 500 ml of the following culture solutions: (1) Hoagland's nutrient solution; (2) Magnesium-deficiency Hoagland's nutrient solution. Hoagland's nutrient solution and Magnesium-deficiency Hoagland's nutrient solution were prepared as described in ref (Heath and Packer 1968). These were placed in a glasshouse under sunlight (1,200 μmol m^{-2} s^{-1} of light intensity) for 4 weeks. Spinach seedlings at the age of two leaves and three leaves were sprayed with 15 μM $CeCl_3$ solution, and deionized water for control.

Assay of physiological and biochemical indexes

Plant weight

The fresh weight and dry weight of spinach were weighted at 28th day.

Chloroplast preparation

The leaves of spinach were homogenized in a prechilled mortar and pestle in ice-cold isolation buffer, which contained 400 mM sucrose, 10 mM NaCl, and 20 mM Tricine (pH 7.8). The slurry was filtered through five layers of cheesecloth, and the chloroplasts were sedimented at 3,000×*g* for 5 min at 4°C. The supernatant was carefully discarded and the pellet retained. The pellet was washed and resuspended in a small volume of chilled suspension buffer that contained 100 mM sucrose, 10 mM NaCl, 2 mM

$MgCl_2$, and 20 mM HEPES pH 7.5. Care was taken that the whole procedure was completely done in ice-cold conditions as quickly as possible to inactivate and prevent the degradation of chloroplast by proteolytic enzymes. Chlorophyll was extracted in chilled 80% acetone and estimated spectrophotometrically (Arnon 1949; Shanghai Plant Physiology Society 1999).

ROS assay of chloroplast

Superoxide ion ($O_2^{\cdot-}$) was measured as described by Able et al. (1998), by monitoring the reduction of XTT in the presence of $O_2^{\cdot-}$, with some modifications. The chloroplasts were homogenized with 2 ml of 50 mM Tris–HCl buffer (pH 7.5) and centrifuged at 5,000×*g* for 10 min. The reaction mixture (1 ml) contained 50 mM Tris–HCl buffer (pH 7.5), 20 mg chloroplast supernatant proteins, and 0.5 mM sodium, 3′-[1-[phenylamino-carbonyl]-3,4-tetrzolium]-bis (4-methoxy-6-nitro) benzenessulfonic acid hydrate (XTT). The reaction of XTT was determined at 470 nm for 5 min. Correction were made for the background absorbance in the presence of 50 units of SOD. The production rate of $O_2^{\cdot-}$ was calculated using an extinction coefficient of 2.16×10^4 M^{-1} cm^{-1}.

H_2O_2 were extracted according to Wang and Luo (1990). Chloroplasts were homogenized in 3 ml of ice-cold acetone. The homogenate was centrifuged at 30,000*g* for 10 min and the supernatant was used for assays of the contents of H_2O_2. The reaction mixture contained 0.1 ml of 5% $Ti(SO_4)_2$, 0.2 ml of ammonia solution, and 1 ml of the H_2O_2 extract, which was centrifuged at 30,000×*g* for 10 min. The precipitate was repeatedly washed with acetone until pigments were completely removed; it was then dissolved with 5 ml of 2 M H_2SO_4, and the contents of H_2O_2 was measured at 415 nm(Wang and Luo 1990).

Lipid peroxide content (MDA) assay of chloroplast

The level of lipid peroxidation was measured as 2-thiobarbituric acid-reactive metabolites (TBA), mainly malonyldialdehyde (MDA), following the modified method of Heath and Packer (1968). Frozen chloroplasts were homogenized in a pre-chilled mortar and pestle with two volumes of ice-cold 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged for 15 min at 15,000×*g*. Assay mixture containing 1 ml aliquot of supernatant and 2 ml of 0.5% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid (TCA) was heated to 95°C for 30 min and then rapidly cooled in an ice-bath. After centrifugation (10,000×*g* for 10 min at 4°C), the supernatant absorbance (532 nm) was read and values corresponding to non-specific absorption (600 nm) were subtracted. MDA concentration was calculated using its extinction coefficient (155 mM^{-1} cm^{-1}).

Antioxidant enzyme activity assays of chloroplast

The chloroplasts were homogenized in 1 ml of ice-cold 50 mM sodium phosphate (pH 7.0) that contained 1% polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 30,000*g* for 30 min and the supernatant was used for assays of the actives of superoxide dismutase (SOD), catalase (CAT), ascobate peroxidase (APX) and guaiacol peroxidase (GPX).

The activity of SOD was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Each 3-ml reaction mixture contained 50 mM sodium phosphate (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 100 μM EDTA, and 200 μl of the enzyme extract. Monitoring the increase in absorbance at 560 nm followed the production of blue formazan (Ginnopolitis and Rice 1977). One unit of SOD was defined as the quantity of enzyme required to inhibit the reduction of NBT by 50% in a 1-ml reaction volume.

The activity of CAT was determined by measuring the rate of disappearance of H_2O_2 at 240 nm. Each 3-ml reaction mixture contained 50 mM sodium phosphate (pH 7.8), 12.5 mM H_2O_2, and 200 μl of enzyme extract (Prasad 1997). One unit of enzyme activity was defined as a decrease in absorbance of 0.001 min^{-1} at 240 nm.

Ascobate peroxidase activity was assayed using the method described by Reuveni et al. (1992). A reaction mixture consisting of 100 μl supernatant, 17 mM H_2O_2 (450 μl), and 25 mM ascorbate (450 μl) was then assayed for 3 min at 290 nm. Activity was measured as disappearance of ascorbate. One unit of enzyme activity was defined as a decrease in absorbance of 0.001 min^{-1} at 290 nm.

Guaiacol peroxidase activity was measured using the method described by Reuveni et al. (1992). A

reaction mixture consisting of supernatant (100 µl), 17 mM H_2O_2 (450 µl), and 2% guaiacol (450 µl) was then assayed for 3 min at 510 nm. Activity was measured as appearance of tetra-guaiacol. One unit of enzyme activity was defined as an increase in absorbance of 0.001 min^{-1} at 510 nm.

Reduced glutathione (GSH) assay

In order to perform the GSH assay, chloroplast homogenized as described above. However, supernatants were not diluted fivefold as described in the case of the antioxidant enzyme assays. Reduced glutathione (GSH) content was estimated using the method of Hissin and Hilf (1976). The reaction mixture contained 100 µl of supernatant, 100 µl *o*-phthaldehyde (1 mg ml^{-1}), and 1.8 ml phosphate buffer (0.1 M sodium phosphate, 0.005 M EDTA, pH 8.0). Fluorometry was performed using a F-4500 fluorometer (F-4500, Hitachi Co., Japan) with excitation at 350 nm and emission at 420 nm. The activity of glutathione reductase (GR) was determined by measuring the rate of disappearance of NADPH at 340 nm. Each 3-ml reaction mixture contained 50 mM sodium phosphate (pH 7.8), 12.5 µM NADPH, and 200 µl of enzyme extract (Hissin and Hilf 1976). One unit of enzyme activity was defined as a decrease in absorbance of 0.01 min^{-1} at 340 nm.

Activities of the oxygen evolution of chloroplast

The oxygen evolution of chloroplasts isolated from spinach was measured with an Oxygraph oxygen electrode (Hansatech instruments, UK). The assay medium contained 0.5 M sorbitol, 10 mM KCl, 0.5 mM $MgCl_2$, 0.05% (w/v) BSA, 10 mM $NaHCO_3$, and HEPES-KOH (pH 7.6). The concentration of the artificial electron acceptor was 2 mM for $K_3Fe(CN)_6$ (FeCy). The concentration of the chloroplast was equivalent to about 20 µg of chlorophyll every ml.

All the experiments were independently performed at 25°C and the presented data are the average of the recordings from five independent experiments.

Statistical analysis

The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences ($P < 0.05$) among the treatment means and the Scheffe test was performed to compare among experimental groups for significant differences.

Results and discussion

Growth of spinach

As shown in Fig. 1, cerium could improve spinach growth. The single fresh weight of the cerium-treated groups was enhanced by 16.17% (column 2) compared to the control (column 1). However, the fresh weight of single plant cultivated in Mg^{2+}-deficiency conditions were much lower than that grown in Hoagland's solution, suggesting 32.34% reduction (column 3) and Ce^{3+} treatment grown in Mg^{2+}-deficiency media suggested 16.59% reduction (column 4), respectively. In the experimental study, we also observed that old leaves of spinach plants developed Mg^{2+}-deficiency symptoms such as chlorosis, plants were short under culture with Mg^{2+}-deficiency conditions. While all leaves treated by cerium grown in Mg^{2+}-deficiency media kept green. From Table 1, Ce^{3+} could significantly promote the formation of chl and Mg^{2+}-deficiency greatly inhibited the formation of chl of spinach, particularly chl-*a*,

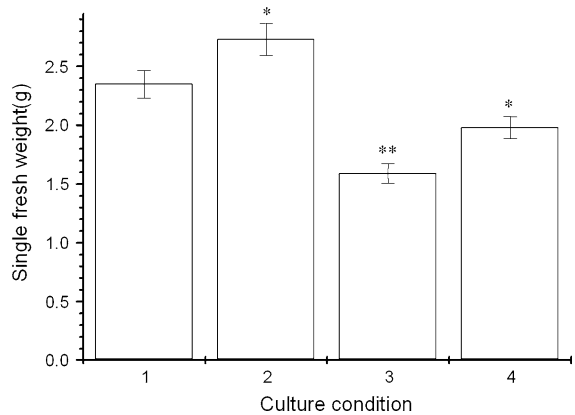

Fig. 1 Effect of Ce^{3+} on spinach plant weights cultivated in Mg^{2+}-deficiency media. *1* Hoagland's solution; *2* Hoagland's solution + Ce^{3+}; *3* Mg^{2+}-deficiency Hoagland's solution; *4* Mg^{2+}-deficiency Hoagland's solution + Ce^{3+}. *Bars* marked with an *star* and *double stars* were different from the others in that *panel* at the 5% confidence level and at the 1% confidence level, respectively. *Bars* indicate mean and *error bars* are SE

Table 1 Effects of Ce^{3+} on chlorophyll synthesis, lipid peroxide level, ROS accumulation and oxygen evolution of spinach chloroplast in Mg^{2+}-deficient media

Index	1	2	3	4
Chl *a* (mg/g FW)	0.62 ± 0.03	0.71 ± 0.04*	0.34 ± 0.02**	0.60 ± 0.03
Chl *b* (mg/g FW)	0.16 ± 0.01	0.21 ± 0.01*	0.12 ± 0.01*	0.16 ± 0.01
O_2-evolving rate (μmol/mg chl h)	275.00 ± 13.75	309.12 ± 15.45*	189.23 ± 9.46**	255.08 ± 12.75
MDA (nmol/mg chl)	415.70 ± 20.80	327.42 ± 16.37*	645.47 ± 32.28**	563.78 ± 28.19*
$O_2^{\cdot-}$-producing rate (μmol/mg chl min)	80.14 ± 4.01	64.30 ± 3.22*	133.01 ± 6.65**	98.04 ± 4.90**
H_2O_2 producing rate (μmol/mg chl min)	50.58 ± 2.53	18.53 ± 0.93*	87.79 ± 4.39**	52.75 ± 2.64

Lines marked with a star and double stars were different from Hoagland's solution in that panel at the 5% confidence level and at the 1% confidence level, respectively. Lines indicate mean and standard errors ± are SE

1 Hoagland's solution; *2* Hoagland's solution + Ce; *3* Mg^{2+}-deficient Hoagland's solution; *4* Mg^{2+}-deficient Hoagland's solution + Ce^{3+}

that is the chl-*a*, *b*, and the ratio of chl-*a*/chl-*b* cultivated in Mg^{2+}-deficiency conditions suggesting 44.93, 23.71%, and 1.07 reduction as compared with those in the control, respectively, but chl-*a*, and *b* contents from Hoagland's solution + Ce^{3+} groups increasing by 14.46%, and 27.32% as compared with those in the control and in Mg^{2+}-deficiency Hoagland's solution + Ce^{3+} groups having 76.54 and 71.04% enhancement, and the ratio of chl-*a*/chl-*b* increasing by 0.92 as compared with those in Mg^{2+}-deficiency Hoagland's solution groups, respectively.

It had been reported that $CeCl_3$ could improve reaction of changing yellowing seedlings into green seedlings, which suggested that Ce^{3+} catalyzed the change protochlorophyll into chlorophyll, and chlorophyll contents enhanced by 24% over that of control (Liu et al. 2004a, b), indicating that the effect of REE in chlorophyll contents of spinach was that REEs increased absorption of nitrogen and phosphorus, and induced greatly synthesis of pre-compounds of chlorophyll and REEs are some catalysts and play the indirect role for chlorophyll formation (Liu et al. 2004a, b; Hong et al. 2002a, b). The results of experiments also showed that Ce^{3+} could accelerate synthesis of chlorophyll, meanwhile, Ce^{3+} could partly replace Mg^{2+} to form REE-chl, which was confirmed by our previous researches (Liu et al. 2004a, b; Hong et al. 2002a, b). Meanwhile, we consider that the reduction and/or the enhancement of spinach growth and chlorophyll caused by Mg^{2+}-deficiency and/or by cerium treatment maybe related to oxidative damage and/or antioxidative stress of chloroplast.

Oxygen evolution activity of chloroplast

It can be seen from Table 1 that the various culture media had a great effect on the oxygen evolution rate of spinach chloroplast. The oxygen evolution rate of those grown in Hoagland's media was elevated by 12.36% due to the Ce^{3+} treatment, and those grown in Mg^{2+}-deficient media was reduced by 31.27% and that of Ce^{3+}-treated spinach chloroplast grown in Mg^{2+}-deficient media decreased by 7.27%, respectively. The results imply that Mg^{2+} deprivation may impair oxygen-evolving center of PSII, while Ce^{3+} treatment may improve the oxygen-evolving center damaged by Mg^{2+} deficiency, which is consistent with the changes of oxidative stress of chloroplast. It had been conformed that Mg^{2+} could regulate light energy distribution between PS II and PSI, inhibit excitation energy transfer from chl *a* of PS II to chl *a* of PS I in chloroplast (Murata 1969), distributed much more energy to PS II, and activated PS II reaction center (Rurainski and Mader 1977), which led to the oxygen evolution of PS II. Therefore, the water photolysis in chloroplast was inhibited by Mg^{2+} deprivation, thus led to the reduction of oxygen evolution rate of spinach chloroplast. However, Ce^{3+} treatment could accelerate oxygen evolution rate of spinach grown in Mg^{2+}-deficient media, implying that Ce^{3+} might substitute for Mg^{2+} in chloroplast to some extent.

Lipid peroxide level of chloroplast

Lipid peroxide level of chloroplast is a very important index for damage of membrane structure. It can

be seen in Table 1 that MDA content of spinach chloroplast grown in Mg^{2+}-deficiency conditions was 55.27% higher than that grown in Hoagland's solution, Ce^{3+} treatment grown in Mg^{2+}-deficiency media was 35.62% higher than that grown in Hoagland's solution. However, MDA content of spinach treated by Ce^{3+} grown in Hoagland's solution was reduced by 21.24%. The changes of MDA content are consistent with plant growth above mentioned.

Due to magnesium deprivation, the obvious enhancements of MDA content of spinach chloroplast were observed in the experimental study, implying that plasma membrane structure of spinach chloroplast was damaged and its photosynthesis function was easily lost, which led to the reduction of spinach growth (Hong et al. 2002a, b, 2003, 2005; Liu et al. 2004a, b). In the results, the reduction of MDA content of spinach chloroplast was observed by cerium treatment grown in Mg^{2+}-deficiency media, suggesting that cerium could repaire membrane structure damaged in magnesium-deficiency conditions, thus improve spinach growth.

ROS accumulation of chloroplast

The effects of various culture media on the production rate of $O_2^{\cdot-}$ and H_2O_2 of chloroplast in spinach are shown in Table 1. It can be seen that ROS grown in Mg^{2+}-deficiency conditions rose sharply, i.e., $O_2^{\cdot-}$ and H_2O_2 generating rates increased by 65.97 and 73.57% as compared to grown in Hoagland conditions, respectively, suggesting that exposure to Mg^{2+}-deficiency media caused a strong oxidative stress in spinach chloroplast. While Ce^{3+} treatments significantly decreased oxidative damage, i.e., $O_2^{\cdot-}$ and H_2O_2 generating rates grown in Mg^{2+}-deficiency conditions showed 22.34 and 4.29% enhancement, and grown in Hoagland's media suggested 19.76 and 63.36% reduction, respectively.

Oxidative stress, resulting from the generation of ROS, is a common phenomenon in many stress responses, such as drought, cold shock, photoinhibition and hypo-osmotic stress. ROS generation leads to cellular damage and ultimately to cell death, primarily through damage to the PS II reaction centre and to membrane lipids (Chris and Robert 2000). The results showed that the obvious accumulation of ROS such as $O_2^{\cdot-}$, H_2O_2 occurred in spinach chloroplast grown in Mg^{2+}-deficiency media (Table 1), it was consistent with the significant enhancement of lipid peroxide level, implying that magnesium deprivation caused the oxidative stress in spinach chloroplast, thus conduced to the damage of plasma membrane structure. However, cerium treatment could decrease ROS accumulation (Table 1), showing that cerium could remove ROS and increase the oxidative stress-resistance of spinach. Hong et al. proved that the increase of the aged seed (rice, spinach) vigor by La^{3+}, Ce^{3+}, Nd^{3+} pretreatment was closely related to ROS removal, and Ce^{3+} treatment was better (Hong et al. 2000, 2002a, b, 2003). We think that the enhancement and/or the reduction of ROS caused by Mg^{2+}-deficiency and/or by cerium treatment maybe related to the reduction and/or the increase of antioxidant defense of chloroplast.

Antioxidant defense of chloroplast

It can be seen in Table 2 that the activities of SOD, CAT, APX, GPX, and GR enzymes of chloroplast cultivated in Mg^{2+}-deficiency media were decreased by 39.68, 44.44, 66.58, 17.15, and 37.74% as compared to culture of Hoagland's solution, respectively; these enzymes activities from Ce^{3+} -treated spinach grown in Mg^{2+}-deficient media were 18.06, 16.65, 33.20, 8.55, and 17.27% lower than those grown in Hoagland's solution, respectively. But the activities of the antioxidant enzymes from Ce^{3+}-treated groups grown in Hoagland's media were 65.38, 15.21, 33.13, 11.42, and 18.12% higher than those of control.

The effects of various culture media on non-enzymatic antioxidants such as carotenoids and reduced glutathione (GSH) of chloroplast are presented in Table 2. The carotenoids, GSH content and the ration of GSH/GSSG of chloroplast grown in Mg^{2+}-deficient conditions were as 62.61, 40.42, and 70.59% as grown in Hoagland's solution, Ce^{3+} treatment grown in Mg^{2+}-deficient media were as 98.70, 85.42, and 88.24% as grown in Hoagland's solution. But carotenoids, GSH content and the ration of GSH/GSSG of Ce^{3+}-treated groups grown in Hoagland solution were 4.78, 6.25, and 5.88% higher than that of control.

The generation of oxidative stress in plant cell is because an imbalance between ROS and their removal makes macromolecules and membranes

Table 2 Effects of Ce^{3+} on antioxidant defense of spinach chloroplast in Mg^{2+}-deficient media

Index	1	2	3	4
SOD (unit/mg chl min)	20.54 ± 1.03	33.97 ± 1.70**	12.39 ± 0.62**	16.83 ± 0.84**
CAT (unit/mg chl min)	68.45 ± 3.42	78.86 ± 3.94**	38.03 ± 1.90**	57.05 ± 2.85**
APX (unit/mg chl min)	147.56 ± 7.38	196.45 ± 9.82**	49.32 ± 2.47**	98.57 ± 4.93**
GPX (unit/mg chl min)	133.15 ± 6.66	148.36 ± 7.42*	110.32 ± 5.52**	121.77 ± 6.09**
GR (nmol GSSG/mg chl min)	4.69 ± 0.23	5.54 ± 0.28*	2.92 ± 0.15**	3.88 ± 0.19*
Caroteroids (mg/g FW)	2.30 ± 0.11	2.41 ± 0.12*	1.44 ± 0.07**	2.27 ± 0.11
GSH (μg/mg chl)	0.48 ± 0.02	0.51 ± 0.03	0.29 ± 0.01**	0.41 ± 0.02*
GSSG (μg/mg chl)	2.82 ± 0.14	2.81 ± 0.14	2.42 ± 0.12*	2.79 ± 0.14
GSH/GSSG ratio	0.17 ± 0.03	0.18 ± 0.04	0.12 ± 0.01*	0.15 ± 0.02

Lines marked with a star and double stars were different from Hoagland's solution in that panel at the 5% confidence level and at the 1% confidence level, respectively. Lines indicate mean and standard errors ± are SE

1 Hoagland's solution; *2* Hoagland's solution + Ce^{3+}; *3* Mg^{2+}-deficient Hoagland's solution; *4* Mg^{2+}-deficient Hoagland's solution + Ce^{3+}

damaged, thus leads to the reduction of plant growth. The control plant possesses its own active antioxidant defense systems (antioxidative enzymes such as SOD, CAT, APX, GPX, and GR, as well as non-enzymatic antioxidants such as carotenoids, glutathione, ascorbate and guaiacol et al.) through which production and removal of ROS is in balance. SOD can convert $O_2^{\cdot-}$ into H_2O_2 and O_2; moreover, CAT, APX, and GPX can reduce H_2O_2 into H_2O and O_2 (Lin et al. 1988). GR catalyze GSSG to reduce GSH (Hissin and Hilf 1976). Therefore, SOD, CAT, APX, and GPX can keep a low level of ROS and prevent ROS toxicity and protect cells (John and Scandalios 1993). In the experiments, we observed that the activities of SOD, CAT, APX, and GPX were significantly inhibited and the content of non-enzymatic antioxidants was decreased (Table 2), ROS was greatly accumulated in spinach chloroplast grown in Mg^{2+}-deficiency media, suggesting that exposure to Mg^{2+}-deficiency media caused an imbalance between ROS and their removal in spinach chloroplast. Cakma et al. (1994) reported that magnesium deficiency increased the activities of SOD, CAT, APX, GPX, and GR in bean plant. However, our study proved that cerium treatment could significantly increase the activities of SOD, CAT, APX, GPX and the contents of non-enzymatic antioxidants (Table 2), and led to the reduction of ROS accumulation of spinach chloroplast. Our previous researches demonstrated that La^{3+}, Ce^{3+} and Nd^{3+} treatments increased the activities of antioxidative enzymes, which led to ROS removal in aged seeds of rice and spinach (Hong et al. 2000, 2002a, b; Liu et al. 2004a, b). On the other hand, cerium treatment caused the reduction of ROS, which related to direct removal of $O_2^{\cdot-}$, it had been proved by Wang et al. (Wang et al. 1997). The mechanism was alleged to be: Ce^{3+} can reduce $O_2^{\cdot-}$ to H_2O_2 and oxidize itself to Ce^{4+} and Ce^{4+} can oxidize $O_2^{\cdot-}$ to O_2 while it reduces itself to Ce^{3+}.

Conclusion

Mg^{2+}-deficiency conditions applied to spinach cultures caused an oxidative stress status in spinach chloroplast monitored by an increase in ROS accumulation. The enhancement of lipids peroxide of spinach chloroplast grown in Mg^{2+}-deficiency media suggested an oxidative attack that was activated by a reduction of antioxidative defense mechanism measured by analysing the activity of SOD, CAT, APX, GPX, and GR enzymes, as well as antioxidants such as carotenoids and glutathione content. As the antioxidative response of chloroplast was reduced in spinach grown in a Mg^{2+}-deficiency media, it caused a significant reduction of spinach plant weight, old leaves turning chlorosis. However, Ce^{3+} treatment grown in Mg^{2+}-deficiency conditions decreased the MDA and ROS, and increased activities of the antioxidative defense system, and improved spinach growth. In the research, we speculate that Ce^{3+} could

substitute for magnesium and increase the oxidative stress-resistance of spinach chloroplast grown in Mg^{2+}-deficiency conditions, but the mechanisms need further study in future.

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