

Influences of calcium deficiency and cerium on the conversion efficiency of light energy of spinach

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Abstract Chloroplast absorbs light energy and transforms it into electron energy, and then converts it into active chemical energy and stable chemical energy. In the present paper, we investigated the effects of Ce^{3+} , which has the most significant catalytic effects and similar characteristics with Ca^{2+} , on light energy conversion of spinach chloroplasts under Ca^{2+} -deficient stress. The results illuminated that the Hill reaction activity, electron flow both photosystems and photophosphorylation rate of spinach chloroplasts reduced significantly under Ca^{2+} -deficient condition, and activities of Mg^{2+} -ATPase and Ca^{2+} -ATPase on the thylakoid membrane were severely inhibited. Meanwhile, the activity of Rubisco, which is the key enzyme of photosynthetic carbon assimilation, was also prohibited. However, Ce^{3+} decreased the inhibition of calcium deprivation the electron transport rate, the oxygen evolution rate, the cyclic and noncyclic photophosphorylation, the activities of Mg^{2+} -ATPase, Ca^{2+} -ATPase and Rubisco of spinach chloroplasts. All above implied that Ca^{2+} -depletion could disturb light energy conversion of chloroplasts strongly, which could be reversed by Ce^{3+} .

Keywords Ce^{3+} · Ca^{2+} -deficiency · Spinach · Chloroplast · Light energy conversion

Introduction

As an essential nutriment of plants, calcium plays an indispensable role during oxygen evolution, photophosphorylation and carbon assimilation (Buchanan et al. 2002). Rare earth elements (REEs) such as Ce^{3+} , is similar to Ca^{2+} with a radius of 100–118 ppm (coordination number = 6–9). So, there are possibilities for Ce^{3+} to replace Ca^{2+} and affect a series of physiological functions after being absorbed by plants (Ni 2002).

Dong et al. had proved that the addition of $LaCl_3$ to calcium-deficient media of cucumber could relieve its calcium-deficient symptoms and improve plant growth (Dong et al. 1993). It had been found that the absorption of Ca^{2+} and many other kinds of microelements in plants could be prompted since the addition of Eu^{3+} in a certain amount (Shtangeeva and Ayrault 2007). The researches of Zhang et al. showed that La^{3+} , Ce^{3+} and Nd^{3+} could accelerate oxygen-evolving and hydrogen-evolving of photosynthesis of *Spirulina platensis*, speculating that REEs treatment might affect the PS II activity of *Spirulina platensis* (Zhang et al. 1988). Chu et al. demonstrated that $CeCl_3$ could promote the formation and development of photosystem I (PS I) of yellow seedling of cucumber (Chu et al.

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1991; Chu and Mou 1994). Their studies on *Spirulina platensis* also indicated that Ce^{3+} was useful to the formation of PS I and light energy transfer from PS II to PS I, and La^{3+} , Ce^{3+} and Nd^{3+} could accelerate oxygen-evolving of net photosynthesis in *Spirulina platensis* (Chu et al. 1994). Zeng et al. (2000) proved that La^{3+} inhibited electron transport, and photoreduction activity of DCIP (2, 6-dichlorophenol indophenol) of PS I and PS II and oxygen-evolving activity of cucumber, and increased contents of protein–pigment complex of PS II (Zeng et al. 2000). Chen et al. illuminated that REEs in low concentration could improve the carboxylase activity of tobacco Rubisco (Chen et al. 2000, 2001). It had been found that La^{3+} could increase the activities of Hill reaction, Mg^{2+} -ATPase and the rate of photophosphorylation in chloroplast of tobacco seedlings (Chen et al. 2001). Wang et al. confirmed that La^{3+} could entered the chloroplasts of spinach and replaced Mg^{2+} , forming a more stable bilayer sandwich structure by coordinating with atom N and exocyclic β -diketone (Wang et al. 2001). Hong's researches proved that La^{3+} or Ce^{3+} could obviously promote growth, increase chlorophyll contents and photosynthetic rate in spinach. La^{3+} or Ce^{3+} could substitute Mg^{2+} in chlorophyll formation of spinach grown in Mg^{2+} -deficient Hoagland's solution, and promote PS II formation significantly more than PS I formation. Further study indicated that the entering of La^{3+} or Ce^{3+} to chloroplasts not only made the Mg^{2+} -chlorophyll contents increased, but also formed REE-chlorophyll. By the spectroscopy method, it was found that La^{3+} or Ce^{3+} bound to porphyrin rings of chlorophyll, and formed bilayer sandwich structure (Hong et al. 2002a, b). Pan et al. indicated that La^{3+} and Pr^{3+} could obviously enhance the activity of Hill reaction, photophosphorylation rate and activity of Mg^{2+} -ATPase of spinach chloroplasts (Pan et al. 2003). REEs could not only promote the formation of chlorophyll and light energy absorbance, but also intensify the function of P680⁺ of D1/D2/Cytb559 as primary electron donor (Liu et al. 2004; Hong et al. 2003, 2005). Researches showed that La^{3+} , Ce^{3+} and Nd^{3+} could induce the formation of Rubisco–Rubisco activase super-complex and increase the value of Ω and activity of Rubisco activase significantly, in which Ce^{3+} treatment had a most effective influence (Hong et al. 2005; Liu et al. 2006).

Recently, we studied comparatively about the effects of La^{3+} , Ce^{3+} and Nd^{3+} on the absorption,

distribution and transformation of light energy and the activity of PS II in chloroplasts of spinach. The results showed that the most significant one among the three REEs was Ce^{3+} on light absorption enhancement, promotion of energy transfer from LHC II to PS II and excitation energy distribution from PS I to PS II, electron transport rate and photochemical activities of spinach chloroplasts (Liu et al. 2007a). Ce^{3+} treatment could also accelerate the energy transport among amino acids and even from tyrosine residue to chlorophyll *a* in protein–pigment complex of PS II (Liu et al. 2007b).

In conclusion, REEs can promote the absorbance and transfer of light energy obviously, but does the fact that REEs substitute calcium or make up the effects of its deficiency then promote the Hill reaction, photophosphorylation and carbon assimilation of chloroplasts can attribute to the similar characteristics of REEs and calcium? So far, there is no systematical research.

In this research, Ce^{3+} , which has the most significant catalytic effect and similar characteristics with Ca^{2+} , was used to study its effects on the light energy conversion of Ca^{2+} -deficient spinach and the relative mechanisms to account for the promotion of REEs on photosynthesis.

Materials and methods

Material culture

Experimental material was *Spinacia oleracea*. The seeds were purchased from a local seed company. Spinach seeds were soaked with 15 mM CeCl_3 solution for 48 h at 10°C, and with deionized water for control. And the seeds were whole surrounded with the solution. 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) calcium-deficient Hoagland's nutrient solution. Hoagland's nutrient solution and calcium-deficient Hoagland's nutrient solution were prepared as described in Shanghai Plant Physiology Society (1999). These were placed in a glasshouse under sunlight ($1,200 \mu\text{M m}^{-2} \text{s}^{-1}$ of light intensity) for 5 weeks. Spinach seedlings at the age of two leaves and four

leaves were sprayed with 15 μM CeCl_3 solution, and deionized water for control.

Preparation of chloroplasts

The spinach leaves grown various medium 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 3,000g 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).

Assay of Hill reaction activity

The rate of oxygen evolution of chloroplasts was measured with an Oxygraph oxygen electrode (Hansatech instruments, UK) according to the manufacturer's instructions. Red actinic light, up to an intensity of 700 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, was provided by a Hansatech LS2H light box and was passed through neutral density filters and a Melles Griot OG 590 sharp cut-off glass filter. A 1 mol l^{-1} solution of sodium carbonate was present in the gas-phase chamber to ensure that the CO_2 concentration did not limit the observed rates of oxygen evolution. The reduction rate of $\text{K}_3\text{Fe}(\text{CN})_6$ (FeCy) of the chloroplasts was measured photometrically by following the absorbance change at 420 nm, using a dual-beam spectrophotometer (UV-3010, Hitachi Co., Japan). The above measurement methods were described in Allen and Holmes (1986).

Electron transfer rate of two photosystems

PS II electron transport

The photoreduction activities of PS II DCPIP (H_2O to DCPIP, DPC to DCPIP) were assayed by the method

of Tang and Satoh (1985). The rate of PS II photoreduction of DCPIP, either with or without the added artificial electron donor, DPC, was measured photometrical ($\Delta\text{A}580$, 12.9 $\text{mM}^{-1} \text{cm}^{-1}$) at 25°C in a medium containing 130 mM NaCl, 30 μM DCPIP, 50 mM CaCl_2 and 50 mM MES–NaOH (pH 6.0). The concentration of DPC was 150 μM and that of chloroplast isolated from spinach was equivalent to 10 $\mu\text{g Chl ml}^{-1}$. Actinic light was provided by an incandescent lamp combined with heat-absorbing filter and a red cut-off filter.

PS I electron transport

The photoreduction activities of PS I of chloroplast isolated from spinach (DCPIP to MV) were determined according to the method described by Tang and Satoh (1985), Neelima et al. (1991). The assay mixture contained 20 mmol/l HEPES (pH 7.5), 100 mM sucrose, 10 mM NaCl, 2 mM MgCl_2 , 0.6 mM ascorbate, 1 mM sodium azide, 0.5 mM MV and 5 μM DCMU and 100 μM DCPIP as the electron donor.

Assay of photophosphorylation rate

Photophosphorylation (PSP) activity of chloroplasts was assayed by using the luciferin-luciferase method to measure the amount of ATP synthesized within 2 min at saturating irradiance of about 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 25°C according to Allnutt et al. with some modification (Allnutt et al. 1991). Cyclic photophosphorylation (c-PSP) activity was determined in 1 cm^3 of reaction mixture containing 50 mM Tricine–KOH (pH 8.0), 2 mM MgCl_2 , 1 mM ADP, 5 mM phosphate (Pi), 0.05 mM phenazine methosulfate (PMS), and the chloroplasts containing about 10 μg chlorophyll. Non-cyclic photophosphorylation (nc-PSP) activity was assayed similarly to c-PSP except that PMS was replaced by 1 mM FeCy. By putting the test tubes for 3 min into boiling water the reactions were stopped.

Assay of the activity of Mg^{2+} -ATPase and Ca^{2+} -ATPase

Mg^{2+} -ATPase on the thylakoid membranes was extracted and assayed according to McCarty's method (McCarty and Racker 1968). 0.6 ml activated

solution was added into 0.4 ml suspension of chloroplasts (containing 50 μM Tris–HCl pH 8.0; 50 μM NaCl; 5 μM MgCl_2 ; 5 μM dithiothreitol (DTT)), then activated it with light of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 6 min in room temperature. About 0.5 ml reaction solution (containing 50 μM Tris–HCl pH 8.0; 5 μM MgCl_2 ; 20 μM ATP) was added into 0.5 ml activated suspension of the chloroplasts above, and incubated at 37°C for 10 min, then 0.1 ml 20% trichloroacetic acid (TCA) was added to stop the reaction. The inorganic phosphorus content was determined by Ames's method (Ames 1966).

Ca^{2+} -ATPase was extracted from spinach chloroplasts according to Li's method (Li et al. 1978) and assayed with Shi et al. method (Shi et al. 2001). About 100 μl of Ca^{2+} -ATPase samples were mixed with 1 ml of reaction buffer (50 mM Tris–HCl, 5 mM ATP, of 5 mM CaCl_2 , 25% CH_3OH , 20 mM NaCl, pH 8.8) and incubated at 37°C for 2 min. The reaction was stopped by adding 0.2 ml 20% TCA and the inorganic phosphorus content was determined by Ames's method (Ames 1966).

Assay of Rubisco carboxylase activity

Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylase activity assay procedure was performed by following Lan's method (Lan and Mott 1991).

Statistical analysis

The presented data are the average of the five independent experiments.

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 analysis

Hill reaction activity of chloroplasts

The electron transport rate and oxygen-evolving rate are two important indexes of chloroplast Hill reaction activity. As shown in Figs. 1, 2, the electron transport rate (Fecy reduction rate) and oxygen evolution rate

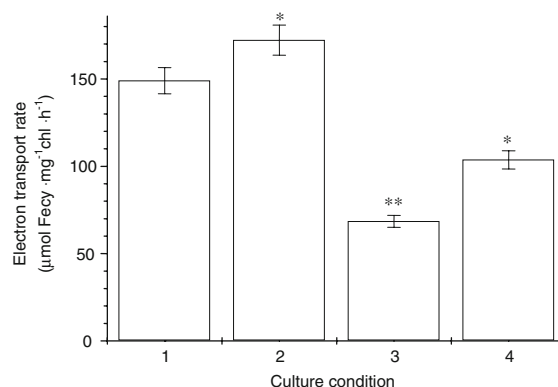


Fig. 1 Effect of Ce^{3+} on electron transport rate of spinach chloroplasts under Ca^{2+} -deficient conditions. *Note:* 1. Hoagland's solution; 2. Hoagland's solution with Ce^{3+} ; 3. Ca^{2+} -deficient Hoagland's solution; 4. Ca^{2+} -deficient Hoagland's solution with Ce^{3+} . Bars marked with a star or double stars means it is significantly different from Hoagland's solution at the 5 or 1% confidence level. Bars indicate mean and error bars are SE data

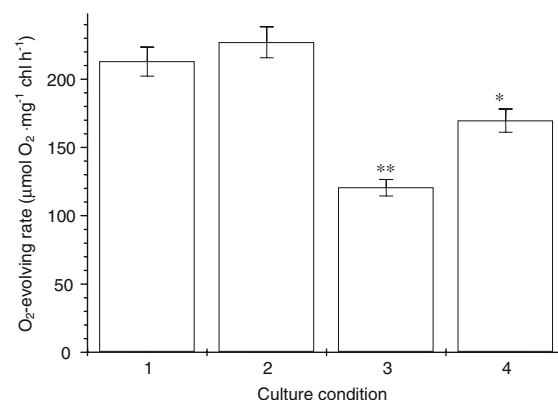


Fig. 2 Effect of Ce^{3+} on the oxygen-evolving rate of spinach chloroplasts under Ca^{2+} -deficient conditions. *Note:* 1. Hoagland's solution; 2. Hoagland's solution with Ce^{3+} ; 3. Ca^{2+} -deficient Hoagland's solution; 4. Ca^{2+} -deficient Hoagland's solution with Ce^{3+} . Bars marked with a star or double stars means it is significantly different from Hoagland's solution at the 5 or 1% confidence level. Bars indicate mean and error bars are SE

from Ce^{3+} -treated spinach under Hoagland's media increased by 15.56 and 6.62%, compared with those of the control (grown in Hoagland's media); Ca^{2+} -deficient groups decreased by 54.09 and 43.40% compared with those of the control groups, which were significantly different at the 1% confidence level. But Ce^{3+} alleviated the impairment of Ca^{2+} deficiency, making the upper indices decreased by 30.4 and 20.35%, compared with those of the control.

Electron flow of two photosystems

Chloroplast is capable of light-induced charge separation, electron transfer between PS II to PS I, water photolysis and oxygen evolution, which is a site of producing photoreduction reaction. Therefore, the experiment studied the effects of Ca^{2+} deprivation and Ce^{3+} treatment on photoreduction reaction activities of two photosystems of spinach chloroplast.

Table 1 showed that Ca^{2+} deprivation significantly inhibited photoreduction activity of two photosystems. For example, when spinach is grown in Ca^{2+} -deficient media, the DCPIP photoreduction of PS II reducing side (H_2O to DCPIP) and oxidative side (DPC to DCPIP) was decreased by 36.45 and 58.88%, and the DCPIP photoreduction of PS I (DCPIP to MV) was lowered by 25.08% compared to those grown in Hoagland's media, respectively. The results suggested that the main inhibited site of Ca^{2+} deficiency on DCPIP photoreduction occurred in PS II of chloroplast, and the reduction of photoreduction activity of PS II reducing side was lower than that of PS II oxidative side. However, the photoreduction activities of Ce^{3+} -treated spinach grown in Ca^{2+} -deficient media were 86.09, 89.42 and 85.19% of that of the control grown in Hoagland's media, respectively. Ce^{3+} -treated spinach grown in Hoagland's media were increased by 65.87, 40.49 and 5.45%, respectively, suggesting that Ce^{3+} could repair the damage of photosystems caused by Ca^{2+} deprivation or improve the bound state of various compositions of photosystems, which therefore made much more energy distributed to PS II, accelerated charge separation of PS II reaction center, and greatly

promoted the photochemical reaction activity of PS II.

Photophosphorylation rate of chloroplasts

Non-cyclic photophosphorylation requires that both of two photosystems are excited. The electron transport pathway of noncyclic photophosphorylation is that electron coming from water photolysis transfers through PS I, PS II and a succession of electron carriers, then transfers to NADP^+ and NADP^+ is reduced to NADPH eventually. Since adding NADP^+ and Hill oxidizing agent Fecy to the reaction media, the electron transport follows the non-cyclic pathway, thus the photophosphorylation assayed is called to the non-cyclic photophosphorylation.

Cyclic photophosphorylation occurs only when PS I is excited whereas PS II is not. By adding PMS, a cyclic electron carrier, to the reaction media, the electron transport then follows the cyclic pathway, without oxygen evolving and NADP^+ reduction. The photophosphorylation assayed is called to the cyclic photophosphorylation.

The non-cyclic and cyclic photophosphorylation rate of spinach chloroplasts are shown in Fig. 3. The non-cyclic and cyclic photophosphorylation rate of Ce^{3+} -treated chloroplasts grown in Hoagland's media were elevated by 12.41 and 18.0%, respectively, compared with those of the control; Ca^{2+} -deficient chloroplasts were significantly inhibited, which decreased by 30.18 and 24.52%, compared with those of the control; however, Ce^{3+} -treated groups grown in Ca^{2+} -deficient media showed 11.02 and 9.56% reduction, respectively. The results are consistent with the electron transfer rate of two photosystems.

Table 1 Effects of Ce^{3+} on electron transport rate of spinach chloroplast under Ca^{2+} -deficient conditions

Culture condition	PS II reducing side activity ($\mu\text{mol DCIP mg}^{-1} \text{ Chl h}^{-1}$)	PS II oxidized side activity ($\mu\text{mol DCIP mg}^{-1} \text{ Chl h}^{-1}$)	PS I activity ($\mu\text{mol DCIP mg}^{-1} \text{ Chl h}^{-1}$)
1	150.21 \pm 7.85	127.09 \pm 6.76	133.67 \pm 6.71
2	249.15 \pm 11.16**	178.55 \pm 8.61*	140.96 \pm 6.96
3	95.46 \pm 4.35**	56.26 \pm 2.65**	100.15 \pm 5.20*
4	129.32 \pm 6.37	113.65 \pm 5.48	113.88 \pm 5.54

Note: 1. Hoagland's solution; 2. Hoagland's solution with Ce^{3+} ; 3. Ca^{2+} -deficient Hoagland's solution; 4. Ca^{2+} -deficient Hoagland's solution with Ce^{3+} . Ranks marked with a star or double stars means it is significantly different from Hoagland's solution at the 5 or 1% confidence level, respectively. Ranks indicate mean and error \pm are SE

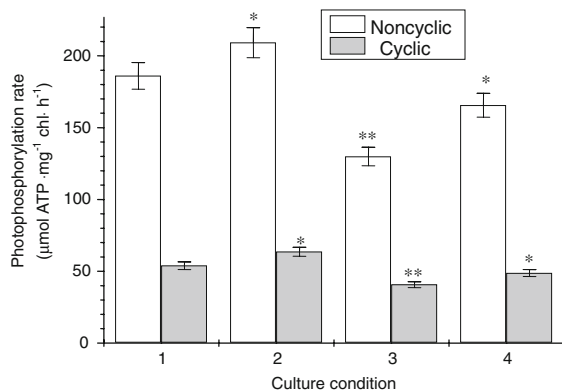


Fig. 3 Effect of Ce^{3+} under Ca^{2+} -deficient conditions on cyclic and noncyclic photophosphorylation of chloroplasts of spinach. *Note:* 1. Hoagland's solution; 2. Hoagland's solution with Ce^{3+} ; 3. Ca^{2+} -deficient Hoagland's solution; 4. Ca^{2+} -deficient Hoagland's solution with Ce^{3+} . Bars marked with a star or double stars means it is significantly different from Hoagland's solution at the 5 or 1% confidence level. Bars indicate mean and error bars are SE

Mg^{2+} -ATPase and Ca^{2+} -ATPase activities of chloroplasts

It is observed in Fig. 4 that the activities of Mg^{2+} -ATPase and Ca^{2+} -ATPase of Ce^{3+} -treated groups grown in Hoagland's media increased only by 1.61 and 2.66% contrasted to those of the control. But under Ca^{2+} -deficient condition, the activities of

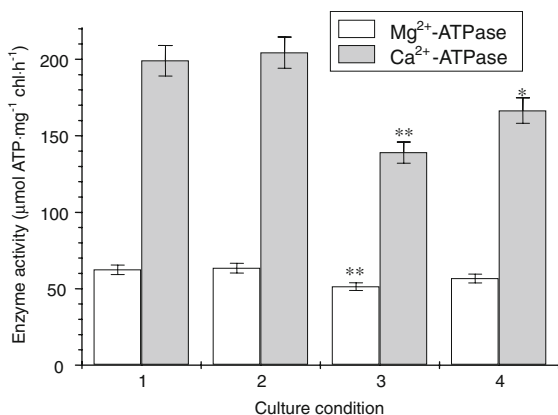


Fig. 4 Effect of Ce^{3+} under Ca^{2+} -deficient conditions on the Mg^{2+} -ATPase activity and Ca^{2+} -ATPase activity of spinach chloroplasts. *Note:* 1. Hoagland's solution; 2. Hoagland's solution with Ce^{3+} ; 3. Ca^{2+} -deficient Hoagland's solution; 4. Ca^{2+} -deficient Hoagland's solution with Ce^{3+} . Bars marked with a star or double stars means it is significantly different from Hoagland's solution at the 5 or 1% confidence level. Bars indicate mean and error bars are SE

Mg^{2+} -ATPase and Ca^{2+} -ATPase were declined by 17.64 and 30.15%. Furthermore, the two enzymes activity of Ce^{3+} -treated groups grown in Ca^{2+} -deficient media were 10.26 and 19.73% higher than those of Ca^{2+} -deficient condition. It was apparently that Ca^{2+} -deficiency exerted more inhibition on Ca^{2+} -ATPase activity than that of Mg^{2+} -ATPase, and Ca^{2+} -ATPase displayed more activity recovery since Ce^{3+} was added.

Rubisco carboxylase activity of chloroplasts

Rubisco, that is ribulose-1, 5-bisphosphate carboxylase/oxygenase (EC4.1.1.39), is the key enzyme of photosynthetic carbon assimilation. It regulates the photosynthesis and photorespiration and plays an important role in the conversion from active chemical energy such as ATP and NADPH, generated from electron transport and photophosphorylation, to stable chemical energy.

As represented in Fig. 5, Rubisco carboxylase activity of spinach grown in Hoagland's media with Ce^{3+} treatment was 10.07% higher than that of the control, the difference between them was significant ($P < 0.05$). The activity grown in Ca^{2+} -deficient condition was 18.2% lower than that of the control, which also got a level of significant difference ($P < 0.05$). Since adding Ce^{3+} to Ca^{2+} -deficient spinach, the carboxylase activity was as 93.35% as the control.

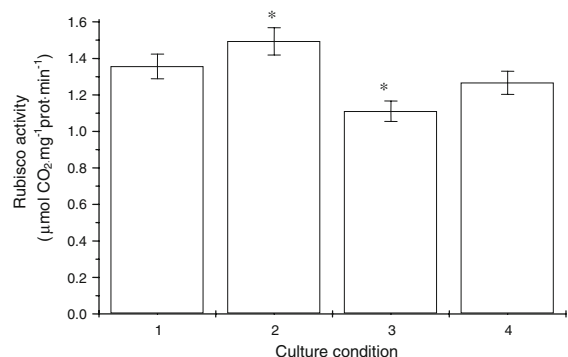


Fig. 5 Effect of Ce^{3+} on the Rubisco activity of spinach chloroplasts under Ca^{2+} -deficient conditions. *Note:* 1. Hoagland's solution; 2. Hoagland's solution with Ce^{3+} ; 3. Ca^{2+} -deficient Hoagland's solution; 4. Ca^{2+} -deficient Hoagland's solution with Ce^{3+} . Bars marked with a star means it is significantly different from Hoagland's solution at the 5% confidence level. Bars indicate mean and error bars are SE

Discussions

Green plants transform light energy into chemical energy and restore it in the organism by photosynthesis in chloroplasts. The whole Photosynthesis is three stages including electron transfer, photophosphorylation and carbon assimilation. During the primary reaction, plants absorb light energy and transport and transform it into electric energy, then convert electric energy to active chemical energy such as ATP and NADPH by electron transfer and photophosphorylation. Finally, the newly generated ATP and NADPH are used to assimilate CO_2 and reduce nitrate, transforming them into stable chemical energy.

There is a oxygen evolution central complex in photosystem II (PS II) where the primary electronic donor H_2O was oxidized and oxygen was released in the primary reaction. The central complex combined with a characteristic Ca^{2+} which served an essential function in the water oxidative process (Buchanan et al. 2002). Our work revealed that the Hill reaction activity from Ca^{2+} -deficient spinach decreased sharply by 40% and the electron flow both two photosystems was significantly inhibited, which had a serious impact on the electron-harvesting from the primary reaction in PS II or PS I, thus reduced the conversion efficiency from light energy to electric energy. The recovery of Hill reaction activity and the electron flow both two photosystems to some extent since the addition of Ce^{3+} suggested that Ce^{3+} had the similar function as Ca^{2+} in oxygen evolution center so that it could decrease the inhibition caused by Ca^{2+} deficiency. Our previous researches always implied that Ce^{3+} could accelerate the electronic transfer and oxygen evolution of chloroplasts or PS II of spinach grown in Hoagland's media or field (Hong et al. 2003, 2005; Liu et al. 2007a, b).

Plants convert electric energy to active chemical energy in the form of ATP and NADPH by non-cyclic and cyclic photophosphorylation, which couple electronic transfer and photophosphorylation on thylakoid membrane. It was revealed that the efficiency in the two photophosphorylation pathways was inhibited in spinach chloroplasts grown in Ca^{2+} -deficient media, as 70 and 76% as the control. Such state was improved since the addition of Ce^{3+} . ATP synthase which was the terminal point of the whole photophosphorylation, including Mg^{2+} -ATPase and

Ca^{2+} -ATPase, was inhibited in the groups grown in Ca^{2+} -deficient media. The reduction of ATP synthase activities might attribute to the Ca^{2+} deprivation, particularly the reduction of Ca^{2+} -ATPase content on the thylakoid membrane. It was obvious that the inhibition of the two kinds of photophosphorylation caused by Ca^{2+} deficiency had a close relationship with the couple level of chloroplasts. And Ca^{2+} deprivation also led to the changes of the conformation and function of PS II reaction central complex, which exerted an effect on the electron transport among PS II, PS I and Cytb_6f compound and lowered the efficiency of the whole photophosphorylation. In conclusion, Ce^{3+} decreased the descend of photophosphorylation efficiency caused by Ca^{2+} deprivation to some extent for the reason of having similar structure as Ca^{2+} , especially to the activity of Ca^{2+} -ATPase which recovered to 83.62% of the control. Chen and Pan et al. added LaCl_3 and PrCl_3 to pot cultured tobacco and spinach, demonstrating that REEs could promote the cyclic and noncyclic photophosphorylation of chloroplasts, and improve the couple level of chloroplasts and activate Mg^{2+} -ATPase and Ca^{2+} -ATPase which acts as a coupling factor of chloroplasts (Chen et al. 2001; Pan et al. 2003). Their results were in accordance with ours.

The assimilatory power generated by photophosphorylation will be used to assimilate CO_2 . Rubisco is the only enzyme to transform inorganic substance CO_2 to organic carbohydrates directly in the C_3 pathway of carbon assimilation, and it is also the key enzyme of plants to change the light energy absorbed to the stable chemical energy stored in organic carbohydrates. Our previous work proved that REEs could significantly enhance the activity of Rubisco activase, induce the formation of Rubisco–Rubisco activase complex, and increase the value of Ω , thus promote the carboxylation of Rubisco (Hong et al. 2005; Liu et al. 2006). The experimental research showed that the activity of Rubisco carboxylation was descend greatly in spinach grown in Ca^{2+} -deficient media, and it would be recovered almost to the level of the control since the addition of Ce^{3+} . It is known that only the activated Rubisco had the ability to catalyze the carboxylation of RuBP, and Rubisco is activated by Rubisco activase which utilizes ATP produced by photophosphorylation in chloroplasts. We believe the influence of Ca on Rubisco activity is indirectly. Because of the inhibition of Ca^{2+}

deficiency on Ca^{2+} -ATPase and photophosphorylation and the reduction of ATP synthesis, it was bound to cut down the activation of Rubisco activase to Rubisco, reduced the carboxylation of Rubisco and slowed the conversion from light energy to stable chemical energy at last. The enhancement of the photophosphorylation rate since the addition of Ce^{3+} to Ca^{2+} deficient cultured spinach was in accordance with the recovery trend of carboxylation activity of Rubisco, suggesting that both Ce^{3+} and Ca^{2+} could promote the transformation from active chemical energy to the stable one.

Conclusions

As an important ingredient of constitutional units in chloroplasts, calcium functioned as a key role during photosynthesis. It could regulate physiological functions by calmodulin (CaM), or as a signal molecule (second messenger). If calcium was deficient to plants, the whole light transformation would be inhibited strongly. Experiments showed that Ce^{3+} could partially recover some physiological index of the Ca^{2+} -deficiency plants, thus we infer Ce^{3+} might replace Ca^{2+} in certain position or substitute the physiological function of Ca^{2+} in some degree. Since Ce^{3+} was similar to Ca^{2+} in radius and coordination number, it served as Ca^{2+} in chloroplasts to a certain extent, thereby relieve the damage of calcium deficiency on light energy absorption and transformation. However, Ce^{3+} could not substitute for Ca^{2+} absolutely, and its mechanism still needs further research.

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