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EFFECT OF NEODYMIUM ION ON MITOCHONDRIAL METABOLISM OF RICE (*ORYZA SATIVA*)

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Abstract

Mitochondria were isolated from the F_1 Hybrid Rice-Shanyou 63 and the metabolic and thermogenic effect of Nd(III) (0, 8, 15, 20, 40, 60ug·L^{-1}) was determined by the use of ampoule method at 30°C. The thermogenic curves were used to calculate the rate constant (K_1), the maximum heat production (P_m) and the total heat production (Q) of mitochondria during activity recovery phase after treatment with Nd(III). The result shows that low concentration Nd(III) stimulated metabolism in the mitochondria which was suppressed by high Nd (III) concentration treatments. The observed concentration-dependent effect of Nd (III) on mitochondrial metabolism is similar to plant growth response to rare earth elements. Based on these results, Nd (III) could have primarily interfered in mitochondrial metabolism which would subsequently affect plant growth.

Introduction

Since the 70's in the 20th century, soluble rare earth elements are widely used as fertilizer supplements (Yang *et al*., 2000; Liu *et al*., 2009). These elements are distributed in whole plant and more than 80% is mainly present in plant roots (Hu *et al*., 2000; Wei *et al*., 2001). Although the ion radius is similar to Ca^{2+}, rare earth elements have stronger coordinating capability. Therefore, these elements have widespread physiological and biological effect on various organisms (Yang *et al*., 2000; Qiu *et al*., 2008; Liu *et al*., 2001). On the other hand, recent studies have demonstrated that rare earth elements can bind to large biological molecules to form a complex which will enter intracellular spaces and organelles (Zheng *et al*., 2000; Chen *et al*., 2001). As the mitochondria are the center of energy metabolism and serve essential functions in living cells, it is very important to understand the impact of rare earth elements on the metabolism in the plant mitochondria.

Neodymium (Nd) is a rare earth element that benefits plant growth. In this research, mitochondria were isolated from hybrid rice variety Xianyou 63. The *In vitro* metabolism of the mitochondria under treatments of different Nd^{3+} concentrations was monitored by a LKB-2277 bioactivity monitor to produce a metabolic thermogenic fingerprint. This study shows that low concentration of Nd^{3+} enhanced mitochondrial metabolism, whereas high concentration treatments became inhibitory.

Materials and Methods

Materials and instruments: The hybrid rice variety Xianyou 63 seeds were provided by Hubei Seed Company. The reagents and $NdCl_3$ were all analytical grades and manufactured by Shanghai Reagent Company. The TAM air Microcalorimeter (manufactured in Sweden) was placed in 30°C incubator and the thermogenic signal was recorded by a computer.

Cultivation of experimental materials: Experiments were prepared with rare earth elements into $NdCl_3$ concentrations (0, 8, 15, 20, 40, 60ug·L^{-1}) of the stock precursor solution, culture medium of rice formula in Table 1.

Table 1. The concentrations of various nutritive salts and trace elements in liquid culture medium.

Nutritive salt	**KNO_3**	**$Ca(NO_3)_2$**	**$MgSO_4·7H_2O$**	**KH_2PO_4**	**Fe EDTA**
Concentration /mg·L^{-1}	51	82	49	13.6	5.57
Trace elements	**H_3BO_3**	**$CuSO_4·5H_2O$**	**$ZnSO_4·7H_2O$**	**$MnCl_2·4H_2O$**	**$H_2MoO_3·4H_2O$**
Concentration /μg·L^{-1}	2.86	0.08	0.02	1.81	0.09

Cultivate seedlings of the Hybrid rice Shanyou 63 (F_1): Rice seeds that have been soaked in 2% NaClO solution for 30 minutes were cultivated under 30°C. Three days later seeds with large germinations were chosen and transferred to the nylon mesh which were placed in plastic containers containing 1L medium. Medium was replaced after every 3 days; the pH of medium was adjusted by 0.1 M HCl and 0.1M NaOH solutions and all the process was carried out at 25°C culture.

Isolation of the mitochondria and microcalorimeter measurement: Roots of the seedlings were excised and isolation of the root mitochondria was done under sterile condition following the method of Mei (Mei *et al*., 1990). Protein content of the mitochondrial samples was determined by *Biuret method*. The protein concentration was 40ug·L^{-1}.

The microcalorimetry measurement was done using the ampoule method to record mitochondrial metabolic thermogenic curve, using mitochondrial samples without $NdCl_3$ treatment as the control. $NdCl_3$ solution was diluted into final concentrations of 0, 8, 15, 20, 40, 60ug·L^{-1}. The treated and untreated mitochondrial samples were incubated at 30°C in an incubator in which a TAM air Microcalorimeter was installed and the thermogenic process was measured and recorded by a computer.

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Results

Thermogenic curve: Fig. 1 is the metabolic thermogenic curve of the isolated mitochondria from the hybrid rice variety XIanyou 63. The thermogenic curves were generated under treatments of $NdCl_3$ at 0, 8, 15, 20, 40, 60ug·L^{-1} (Fig. 2).

Thermodynamics: According to the thermogenic curves (Figs. 1 and Fig. 2) the mitochondrial metabolism was divided into four stages: the stagnant stage, the activity recovery stage, the stably increasing stage and the decaying stage. Then the mitochondrial samples treated with different Nd^{3+} concentrations were compared to the control, the thermogenic ratio was designated as P_0 at time 0 and Pt at *t/ min*. During the recovery and decaying stages, energy-time curve fits into the exponential formula as:

$$P_t = P_0 \exp(k\,t)$$
$$\text{Or}$$
$$\ln P_t = \ln P_0 + k\,t \qquad (1)$$

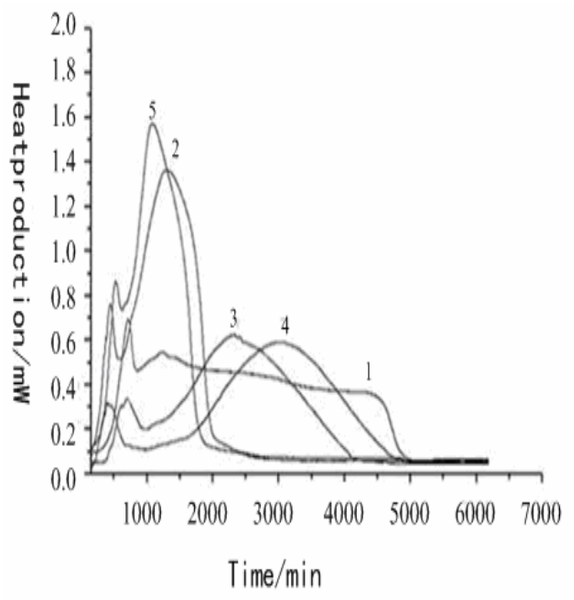

Fig. 1. Metabolic thermogenic curve of the mitochondria under different concentration $NdCl_3$ treatments.The curve number show respective $NdCl_3$ concentration of 8, 15, 20, 40 and 60 μg·L^{-1}.

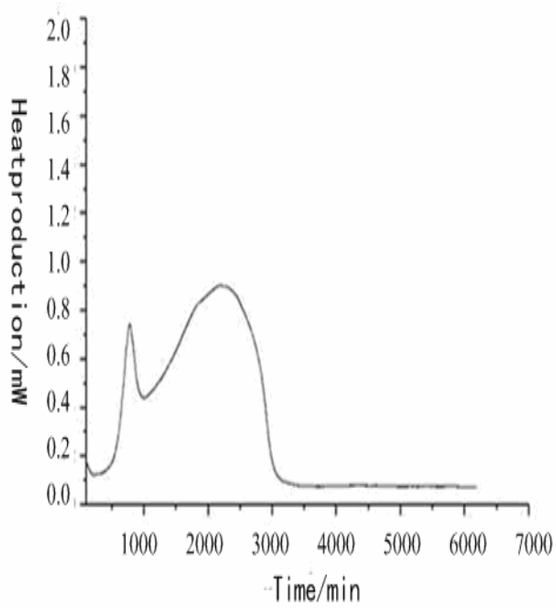

Fig. 2. Metabolic thermogenesis curve of mitochondria without $NdCl_3$ treatment.

The formulas were used to calculate the ratio constant *K* at the activity recovery stage, they also gave the values for two very valuable parameters i.e., the *Pm* (maximum thermogenic value) and the *Q* (total thermogenic value). All the corresponding values are presented in Table 2

Table 2. Metabolic parameters of hybrid rice 63 (F1).

$C_{Nd\,(III)}$ /μg/L^{-1}	*k* /$10^{-3}$$min^{-1}$	R	*Pm*/mW	*Q* /J
0	3.59	0.999 8	0.508 0	79.68
8.00	3.99	0.999 7	0.584 4	79.26
15.00	5.83	0.999 7	0.777 0	83.88
20.00	6.02	0.999 4	0.828 9	83.94
40.00	5.81	0.999 5	0.525 2	89.46
60.00	1.61	0.999 0	0.216 4	32.16

Note: $C_{Nd\,(III)}$ /μg/L^{-1}: experiments were prepared with rare earth elements into $NdCl_3$ concentrations (0, 8, 15, 20, 40, 60ug·L^{-1})
k: the rate constant in the activity recovery stage; R: the relative coefficient of *k*
P_m: the maximum amount of heat produced; *Q*- the total heat produced

The relationship between the thermogenic rate constant and concentrations of Nd^{3+} during the activity recovery stage: Addition of $NdCl_3$ at low concentrations resulted in higher *K* value, which was reduced significantly at high $NdCl_3$ concentrations (Fig. 3).

The relationship between the maximum thermogenic ratio Pm and Nd^{3+} concentration: Addition of $NdCl_3$ caused changes in the maximum thermogenic ratio *Pm* (Fig. 4). Low concentration $NdCl_3$ treatment actually resulted in higher *Pm* in the mitochondria. The high $NdCl_3$ concentration treatments caused significant reduction in *Pm* value.

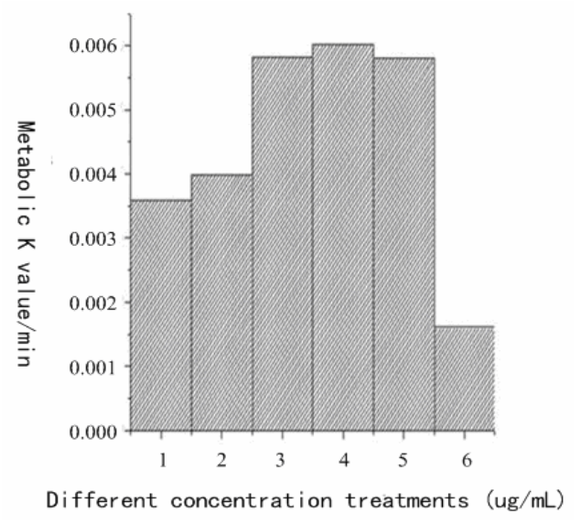

Fig. 3. Metabolic *K* value of mitochondria and its relationship with applied $NdCl_3$ concentrations. (1-0μg·L^{-1}, 2-8μg·L^{-1}, 3-15μg·L^{-1}, 4-20μg·L^{-1}, 5-40μg·L^{-1}, 6-60μg·L^{-1}).

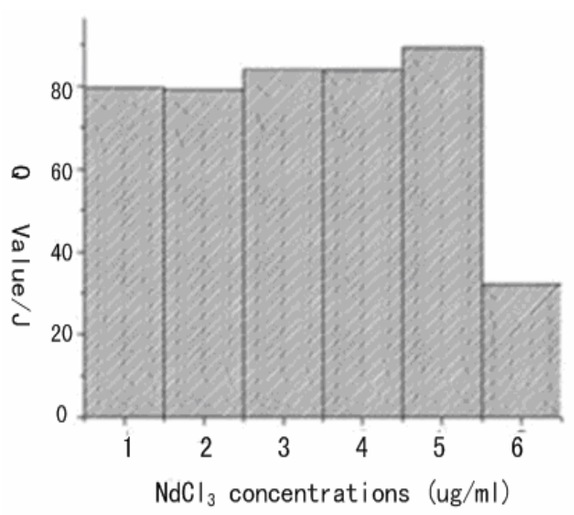

Fig. 5. Histogram of the relationship between total mitochondria metabolic thermogenic value *Q* and $NdCl_3$ concentrations. (1-0μg·L^{-1}, 2-8μg·L^{-1}, 3-15μg·L^{-1}, 4-20μg·L^{-1}, 5-40μg·L^{-1}, 6-60μg·L^{-1}).

Discussion

In this study we have generated a mitochondrial metabolic thermogenic fingerprint in response to $NdCl_3$ treatment. We have found that $NdCl_3$ treatments caused alteration in mitochondrial metabolic thermogenic curves. Next the thermodynamics method was used to analyze several important thermodynamics parameters (*K_1*, *Pm*, *Q*). It was observed that low concentration of Nd^{3+} enhanced mitochondrial metabolism whereas high concentration treatments became inhibitory. For instance *K1* and *Pm*, the two mitochondrial metabolism parameters, were induced by 0-20μg·L^{-1} Nd^{3+} treatments, they reached the highest level at 20μg·L^{-1} (Table 2). Continuously raising Nd^{3+} concentration led to reduction of *K_1* and *Pm*. In the 60μg·L^{-1} Nd^{3+} treatment, *K1* was reduced to 44.8%, and *Pm* was reduced to 42.5%, of the control level.

It has long been known that rare earth elements at low concentration promote plant growth whereas they will damage plant metabolism when the concentration exceeds the threshold level. But it is not clear how this happens at cellular organelle level. This study observed similar effect of Nd^{3+} on the mitochondria which is the centre of cellular metabolism. The results supported the hypothesis that rare earth elements have some function in promoting plant growth, by affecting metabolic processes in the mitochondria.

The oxidative phosphorylation in mitochondria occurs in the catalytic complex consisting of five functional enzymes. These enzymes have one or more metal ions in the reaction centers (Saraste 1999). According to the reported studies, rare earth elements can function synergistically with Ca^{2+} and other metal ions in these reaction centers to affect metabolism of enzyme proteins and nucleic acids in biological systems (Yang *et al*., 2000; Qiu *et al*., 2008). This might be because mitochondrial metabolism was enhanced by low Nd^{3+} concentration. By enhancing enzyme activity rare earth elements will absolutely activate metabolism in the mitochondria.

Relationship between Nd^{3+} concentrations and the total thermogenic value Q: Fig. 5 indicates that addition of 0-40μg·L^{-1} of Nd^{3+} did not have any obvious effect on total thermogenic *Q*, but when Nd^{3+} concentration was increased to 60μg·L^{-1}, mitochondrial *Q* value dropped.

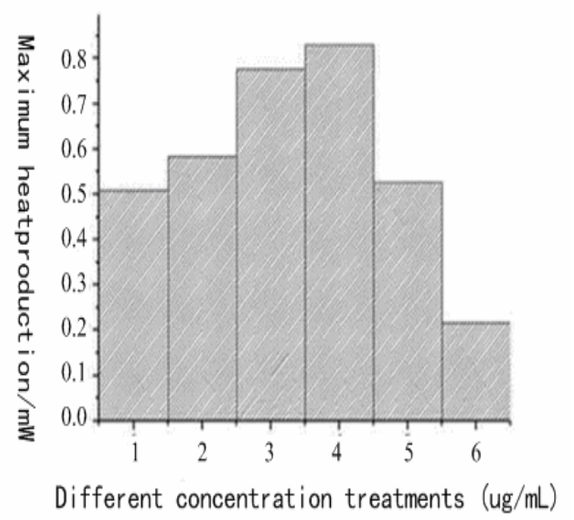

Fig. 4. Histogram of the relationship between mitochondrial metabolic value *Pm* and $NdCl_3$ concentrations.(1-0μg·L^{-1}, 2-8μg·L^{-1}, 3-15μg·L^{-1}, 4-20μg·L^{-1}, 5-40μg·L^{-1}, 6-60μg·L^{-1}).

Previously researches have studied the interaction between the rare earth elements and large biological molecules such as proteins (Draper, 1985; Liu *et al*., 2006). The rare earth elements have higher electric charge and binding affinity and capacity to oxygen than Ca^{2+}. At high concentration treatments, the rare earth element can form complex with enzyme proteins. The binding could disrupt the balance in the surface charge of enzyme proteins and ligand distribution, the resultant damage in the configuration of protein complex will disrupt normal cellular activity and functions. Because of this effect, high Nd^{3+} concentrations treatments suppressed metabolic activity in the mitochondria.

Total metabolic thermogenic value *Q* was also affected by Nd^{3+} concentration, but differently from K_1, and *Pm*. As shown in Fig. 5, no significant change in *Q* was found at $NdCl_3$ 0-40μg·L^{-1} concentration range. In the real situation, the metabolic heat generated in the isolated mitochondria came from two sources. One is the heat released during the oxidative phosphorylation reaction; the second is during the mtDNA replication process. The mtDNA replication complex is comprised of 13 peptides which occasionally generate some heat (Liu *et al*., 2001). Under normal condition, the well coordinated function ensures that most of the nutrients are consumed (or converted into other molecules) in the mitochondria, which could correspond to the situation of 0-40μg·L^{-1} Nd^{3+} treatments, therefore *Q* remained relatively constant. When the Nd^{3+} concentration was raised to 60μg·L^{-1}, the mitochondrial DNA system was badly damaged. Due to disruption in the thermogenic process, the *Q* value dropped sharply.

Conclusions

The result of this research shows that the low concentration of Nd^{3+} promote the metabolic activity of mitochondria *In vitro* and the high concentration Nd^{3+} depress it. As shown in Table 2, Fig. 3 and Fig. 4, the rate constants of activity recovery phase (k) and the maximum heat production (*Pm*) increased sharply while the concentration of $NdCl_3$ increased over the range of 0-20ug·L^{-1}.

As the concentration of $NdCl_3$ continued to increase over the range of 20-60ug·L^{-1}, the obvious decrease were observed especially in 60ug·L^{-1}, in which the value *k* of *dropped* to 44.8% of the control and the value of *Pm* dropped to 42.6% of the control.

Since *k* and the *Pm* both are basic parameters of metabolic activity of mitochondria, the results demonstrate that Nd^{3+} can change the metabolic mechanism of mitochondria as well as the normal function of mitochondria. Nd(III) could have primarily interfered in mitochondrial metabolism which would subsequently affect plant growth, Nd(III) application in rice should be controlled within the beneficial range of concentrations.

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