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Effects of $LaCl_3$ on the growth and photosynthetic characteristics of Fny-infected tobacco seedlings

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Abstract: Plant growth, gas exchange characteristics, chlorophyll fluorescence, disease index, and disease prevention efficiency of $LaCl_3$ in tobacco (*Nicotiana glutinosa*) plants infected by Fny-CMV (fny stain of cucumber mosaic virus) strain were determined. Leaf area, chlorophyll and carotenoid contents, maximum photosynthesis rate, apparent quantum yield, and carboxylation efficiency dramatically decreased after 5 weeks post inoculation. The plants infected by Fny-CMV only presented much severer symptom than those infected in the presence of appropriate concentration of $LaCl_3$. ETR (apparent rate of photosynthetic electron transport), NPQ (nonphotochemical quenching), qP (coefficient of photochemical quenching), and yield (II) in infected tobacco plants obviously reduced in higher light intensity after 5 weeks post inoculation. And these fluorescence parameters in Fny-infected plants obviously reduced compared with those in the plants infected by Fny in the presence of $LaCl_3$. Together with the growth status and disease index, it revealed that exogenously appropriate concentration of $LaCl_3$ could significantly alleviate the damage of tobacco seedlings caused by Fny-CMV.

Keywords: cucumber mosaic virus; *Nicotiana glutinosa*; chlorophyll fluorescence; photosynthesis; $LaCl_3$; rare earths

Lanthanum (La) is one of the lanthanides or rare earth elements (REEs), which had been used as fertilizer or growth stimulator in agriculture for quite a long time[1]. With the theoretical and empirical knowledge developing and safety assessed, the products and complexes of REEs would be applied to wider and wider range in bio-industry, which exhibits enormous economic and social benefits. REEs and their complexes were reported to exert antifungal, bactericidal, and anti-virus activity[2–5]. CMV (cucumber mosaic virus) is one of the most important plant viruses in the world, and has a very wide host range of infecting plants in 1241 species, 498 genera, 101 families[6]. Different host plants presented various symptoms after being infected by CMV[7]. However, as one of the most serious virus in agriculture, there were few effective, universal, and operational prophylactic measures against the harm of CMV to the crops.

So far, many studies have been conducted on the physiological effects related to the application of REEs in plants[8–10]. And the treatment of exogenous REEs could increase the resistance of plants under abiotic and biotic stresses to some extent[11,12]. But there are few reports about the research of anti-virus of REEs in infected plants as yet. To better understand the effect and physiological mechanisms of REEs on the physiological responses in virus-infected plants, we determined and analyzed the leaf gas exchange, chlorophyll fluorescence parameters, the disease indexes, and disease prevention efficiency of $LaCl_3$ in tobacco plants infected by Fny (a strain of CMV). The results of the experiment could provide some experimental and theoretical evidences for the application of REEs against the viral disease in crops.

1 Materials and methods

1.1 Tobacco seedlings culture and treatments

The experiment was conduced with tobacco (*Nicotiana glutinosa*) seedlings, which grew for 7 weeks from seeds on pots in a greenhouse at temperatures of 28/15 ºC (day/night) under natural light. At six leaf stages, the tobacco seedlings were first sprayed with 0.08 mmol/L $LaCl_3$ solution for 3 d, and then infected by Fny-CMV. The control and only Fny-infected plants were respectively sprayed by distilled water at the same time. The $LaCl_3$ content was 99.99%, and provided by the REACTON company. The application concentration of $LaCl_3$ (0.08 mmol/L) was from the pre-experiments.

Artificial inoculation of Fny-CMV was performed by placing 10 μl (contained 200 μg/ml Fny) on one newly expanded leaf, which was previously dusted with carborundum (600 mesh). Ten plants per treatment (control, Fny-infected, Fny plus $LaCl_3$ treatment) were used in the following experiments. The infective clone of Fny strain was presented by Peter Palukaitis from Scottish crop research institute.

1.2 Measurement of photosynthetic gas exchange

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Net photosynthetic rate (Pn) are determined according to the method described by Guo et al.[13] with an LCPro+ photosynthetic system (ABD, England) under artificial light source. Pn of the third completely expanded leaf from the top of each plants was measured at a temperature of 25 ºC under the given light intensity, relative humidity 45%, and given CO_2 concentration. Leaf temperature was controlled by the leaf cuvette with a temperature control system (ABD, England). Light response curves were obtained with the LCPro+ photosynthetic system under 400 μl/L CO_2 concentration and different light intensities (0–1500 μmol/(m^2·s). The initial slope of the line represented apparent quantum yield of photosynthesis (AQY) of determined leaf.

CO_2 response curves were obtained with the LCPro+ photosynthetic system under 1,300 μmol/(m^2·s) light intensity and different CO_2 concentrations. The initial slope of the line represents carboxylation efficiency (CE) of determined leaf.

1.3 Measurements of chlorophyll fluorescence parameter

Chlorophyll fluorescence parameters of tobacco leaves were determined at room temperature (25 ºC) and ambient CO_2 concentration with an Imaging-PAM chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) after the leaves were dark-adapted for 30 min. The experiment was accomplished according to the method described by the Genty et al[14] and Guo et al.[13]. The following chlorophyll parameters were calculated with an PAMWIN Data Acquisition System (Heinz Walz GmbH, Effeltrich, Germany): Yield (effective PSII quantum yield)=(Fm′–F)/Fm′; ETR (apparent rate of photosynthetic electron transport)=0.5×Yield×PAR×0.84, which is assumed that 50% of the absorbed PAR is distributed to PSII, and 84% of the incident photons of photosynthetically active radiation is absorbed by the leaf; NPQ (nonphotochemical quenching)=(Fm-Fm′)/Fm′; qP (coefficient of photochemical quenching)=(Fm–F)/(Fm′–Fo′). The measurements were made between 8:00 and 11:00 a.m. And 5 AOIs (area of interest) were selected in each plant leaf.

1.4 Measurements of chlorophyll and careteniod contents

0.5 g of the third leaf from different plants was extracted with 80% cold acetone. Then the contents of carotenoid and chlorophyll a, b, a+b, a/b were calculated by the method described by Lichtenthaler et al.[15].

1.5 Disease index（DI）statistics and the calculating of disease prevention efficiency (DPE)

The incidences of the diseases in infected tobacco seedlings were observed and counted after inoculation. The symptoms of infected tobacco plants were divided into four grades according to the description in Table 1. The disease index was calculated as described by Fu et al.[7]

2 Results and analysis

2.1 Effects of $LaCl_3$ on the growth of tobacco seedlings infected by Fny-CMV

The change of plant growth is the distinct performance of the energy conversion efficiency in photosystem II under biological and abiotic stress. The size of leaves in the two group of virus-infected tobacco plants obviously dwindled compared with those in control plants after 5 weeks post inoculation (wpi) (see Fig. 1). The symptom of virus-infected plants became more and more serious with the lengthening of time of culture. The results in Fig. 1 display that $LaCl_3$ could partly alleviate the symptom of Fny infection. The growth differences of infected tobacco plants are shown in Table 2. The tobacco seedlings inoculated with Fny-CMV obviously dwindled. The presence of $LaCl_3$ made plants

Table 1 Grades of symptoms on tobacco plants infected by Fny-CMV

Grades	Symptoms on tomato seedlings
0	No symptoms
1	Systemic mottle or mild mosaic on unfolded leaves
2	Obvious mosaic, mild shrinking or slight malformation on systemic leaves
3	Severe mosaic, malformation on systemic leaves, or plants stunt slightly

DI=[Σ(the number of diseased plants×grades of symptoms)/ (total number of plants × the highest grades of symptoms)] ×100%

DPE=(DI of Fny-infected plants–DI of Fny-infected plants in the presence of $LaCl_3$)/DI of Fny-infected plants×100%

Fig. 1 Symptom of Fny-infected tobacco seedlings in the presence and absence of $LaCl_3$ (The left is the control plants. The middle is Fny-infected plants in the presence of $LaCl_3$. And the right is Fny-infected plants. All these plants were grown for 5 weeks after virus infection. And the growing period of control plants and infected plants were identical)

Table 2 Comparison of growth in tobacco seedlings infected by Fny-CMV in the presence and absence of $LaCl_3$ after 5 wpi (Each value is mean±S.E. of randomly selected seven plants. Values followed by asterisks indicate the significant difference between Fny-treated plants and Fny plus $LaCl_3$-treated plants)

Tobacco seedlings	Plants height average/cm	Plants mass average/g	Leaves area/ cm^2	Roots mass/ g	Stem diameter/ cm
CK	20.3±0.97	79.5±0.77	22.3±0.88	3.9±0.39	1.3±0.08
Fny-infected	8.7±0.46**	31.6±0.38**	7.6±0.39**	1.3±0.17**	0.46±0.04**
Fny+$LaCl_3$	13.6±1.08	49.5±0.38	12.1±0.98	2.1±0.23	0.78±0.08

* means $P<0.05$, and ** means $P<0.01$

height, plants mass, leaves area, roots mass, and stem diameter in Fny-CMV infected tobacco seedlings increase by 56.3%, 56.6%, 59.2%, 61.5%, and 69.6%, respectively. It further demonstrated that $LaCl_3$ could lighten the physiological damage caused by Fny-CMV in tobacco seedlings to some extent.

2.2 Disease prevention efficiency (DPE) of $LaCl_3$ in Fny-treated tobacco seedlings at different weeks post inoculation

The disease indexes (DI) of Fny-infected tobacco seedlings were observed and measured in the presence and absence of $LaCl_3$. And then the DPE of $LaCl_3$ in infected tobacco was calculated (Table 3). The results showed that the DI in infected tobacco seedlings notable increased with lengthening of culture. However, the presence of $LaCl_3$ remarkably alleviated the symptoms of infected tobacco plants caused by Fny-CMV. DPEs of $LaCl_3$ were 32.4%, 35.6%, 36.1%, 38.7%, and 41.9% at 1–5 wpi, respectively. It implied that the treatment of $LaCl_3$ could mitigate the harm of Fny-CMV to the tobacco seedlings.

2.3 Effects of $LaCl_3$ on the contents of chlorophyll and carotenoid in the leaves of Fny-infected tobacco seedlings

As showed in Table 4, the total chlorophyll content, the ratio of chlorophyll a/b, and the content of carotenoid continuously decreased with the development of symptom in Fny-infected tobacco seedlings. The content of chlorophyll and carotenoid, and the chlorophyll ratio in the two kinds of infected plants significantly reduced compared with those in control plants after 3 wpi. However, the presence of $LaCl_3$ obviously prevented the pigments from being degraded in leaves of tobacco seedlings caused by Fny-CMV.

2.4 Light and CO_2 response curves of photosynthesis (Pn) in the leaves of Fny-infected tobacco plants in the presence and absence of $LaCl_3$

Figs. 2 and 3 show that the light and CO_2 response curves of Pn in tobacco leaves were much affected by the virus infection. Compared with the healthy tobacco plants, the net photosynthetic rate (Pn) in Fny-CMV-infected plants dramatically declined in different light and CO_2 supply at 5

Table 3 Disease indexes of tobacco seedlings inoculated with Fny-CMV and disease prevention efficiency of $LaCl_3$ (Each value is mean±S.E. of randomly selected seven plants. Values followed by asterisks indicate the significant difference)

Index	Tobacco seedlings	Weeks post inoculation 1	2	3	4	5
DI	Fny	3.1±0.06	10.4±0.78	29.6±1.92	43.9±1.68	85.3±1.94
	Fny+$LaCl_3$	2.1±0.07*	6.7±0.08*	18.9±0.65*	26.9±0.96*	49.6±1.83*
DPE of $LaCl_3$/%		32.4	35.6	36.1	38.7	41.9

* means $P<0.01$

Table 4 Contents of chlorophyll and carotenoid in leaves of Fny-infected tobacco seedlings at different stages (unit: mg/g FW) (Each value is mean±S.E. of seven plants. Values followed by asterisks indicate the significant difference between different treated plants and the control using the Tukey test)

Samples	wpi	1	2	3	4	5
CK	Chl.a+b	1.99±0.018	1.98±0.031	2.26±0.036	2.45±0.009	2.43±0.034
	Chl.a/b	2.71±0.015	2.55±0.026	2.46±0.028	2.43±0.021	2.33±0.029
	Car.	0.416±0.009	0.392±0.004	0.378±0.006	0.373±0.006	0.358±0.007
Fny+La^{3+}	Chl.a+b	1.97±0.015	1.98±0.017	1.89±0.013*	1.96±0.027*	2.01±0.046*
	Chl.a/b	2.68±0.023	2.49±0.021	2.41±0.021	2.37±0.014	2.27±0.013
	Car.	0.411±0.007	0.387±0.005	0.331±0.008*	0.313±0.009*	0.302±0.006*
Fny	Chl.a+b	1.87±0.029	1.56±0.019*	1.34±0.011**	1.26±0.008**	1.17±0.012**
	Chl.a/b	2.65±0.016	2.31±0.023	2.13±0.021*	2.11±0.014*	1.99±0.011*
	Car.	0.403±.011	0.346±0.008*	0.269±.007**	0.152±.010**	0.141±.006**

* means $P<0.05$; ** means $P<0.01$

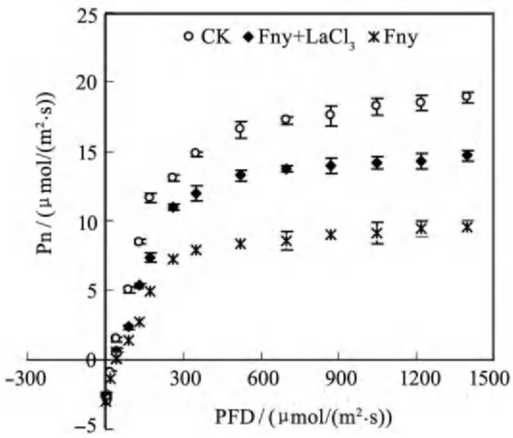

Fig. 2 Light response curves of photosynthesis in infected and control tobacco seedlings at 5 wpi (Measurements were made by the LCPro+ photosynthetic system at the condition of CO_2=400 μl/L (ppm), t_a=28 ºC. Each value represents the mean ±S.E. of five repetitions. PFD (photo flux density))

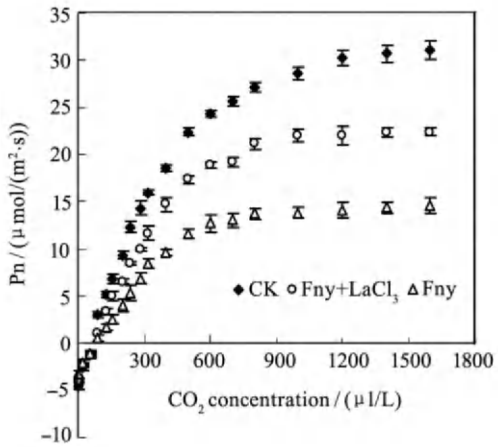

Fig. 3 CO_2 response curve of photosynthesis in infected and control tobacco seedling at 5 wpi (Measurements were made by the LCPro+ photosynthetic system at the condition of PFD=400 μmol/(m^2·s), t_a=28 ºC. Each value represents the mean ±S.E. of five repetitions)

wpi. Furthermore, the data in Table 5 show that the maximum photosynthetic rate (A_{max}), carboxylation efficiency (CE) and apparent quantum yield of photosynthesis (AQY) also obviously descended at 5 wpi. However, the photosynthetic parameters in Fny-infected plants changed more remarkably than those in the Fny-infected plants in the presence of $LaCl_3$. The above results have revealed exogenous

Table 5 Photosynthetic characteristics in infected and control tobacco seedling at 5 wpi. Values followed by asterisks indicate the significant difference between different treated plants and the control using the Tukey test

	CK	Fny+$LaCl_3$	Fny
A_{max}/(μmol/(m^2·s))	30.99	22.38 *	14.56**
AQY/(CO_2·photon^{-1})	0.059	0.052	0.032**
CE/(μmol/(m^2·s))	0.067	0.053 *	0.035**

Note: A_{max}–Maximum photosynthetic rate with saturated light and CO_2 concentration; AQY–Apparent quantum yield of photosynthesis; CE: Carboxylation efficiency; * means P<0.05, ** means P<0.01

$LaCl_3$ might have the mitigative effect on the photosynthetic damage in Fny-infected tobacco plants.

2.5 Effects of $LaCl_3$ on the chlorophyll fluorescence parameters in Fny-infected tobacco seedlings

Fig. 4 display the typical light curve of ETR, qP, NPQ, and yield(II) in leaves of Fny-infected tobacco plants after 5 wpi. When the PAR was more than 400 μmol/(m^2·s), ETR and qP in Fny-infected tobacco seedlings remarkably reduced compared with those in the control. Similarly, NPQ and yield(II) in Fny-infected seedlings obviously decreased compared with those in the control when the PAR was more than 100 μmol/(m^2·s). It was implied that virus could restrain the quantum efficiency of PSII and depress the ability of heat dissipation in tobacco seedlings under high light intensity, whereas $LaCl_3$ could alleviate the damage of photosystem caused by Fny-CMV.

3 Disscussion

Plant growth inhibition is the visible characteristics that distinguish the healthy plants from the injured ones under biotic and abiotic stresses. So the reduction of leaf area in virus-infected tobacco seedlings could attribute to the virus inoculation. The infection of Fny resulted in a mild mosaic on systemic leaves at the early stage. Furthermore, shrinking and malformation emerged in systemic leaves of Fny-infected tobacco plants after 4 wpi. However, the systemic leaves of tobacco infected by Fny-CMV did not represent any symptom of shrinking and malformation after 5 wpi in the presence of $LaCl_3$ (see Fig. 1). The results suggested that exogenously appropriate concentration of $LaCl_3$ could alleviate the physiological damage caused by Fny-CMV to a certain extent. A very few publications were available on anti-virus properties of rare earth. It was reported that rare earth complexes markedly displayed inhibitory effect on hepatitis B virus replication *in vitro*[16], and some compounds containing Ln (La, Ce, Pr, Nd, Sm and Gd) had high disease resistance ability from tomato mosaic virus with cure rate 70%–95%[17]. In order to understand the anti-virus role of rare earth to plants disease, a systematic study would be undertaken in our coming work.

The improvement of growth indexes was the physiological phenomenon, but not the physiological reason of anti-virus in Fny-infected tobacco seedlings in the presence of $LaCl_3$. The treatment of $LaCl_3$ dramatically made the maximum photosynthetic rate (A_{max}), carboxylation efficiency (CE) and apparent quantum yield of photosynthesis (AQY) elevate in infected plants at 5 wpi (see Table 5). The virus which spread out after inoculation, could disrupt the chloroplast structure, restrain the lamellar development or membrane vesiculation[18], and make the enzymes in Calvin cycle tend to be inactive[19]. The tissues and photosynthetic system were seriously damaged by the accumulation of excessive active oxygen in the sugarcane plants[20]. Our previous work showed that feasible concentration of La^{3+} could enhance the

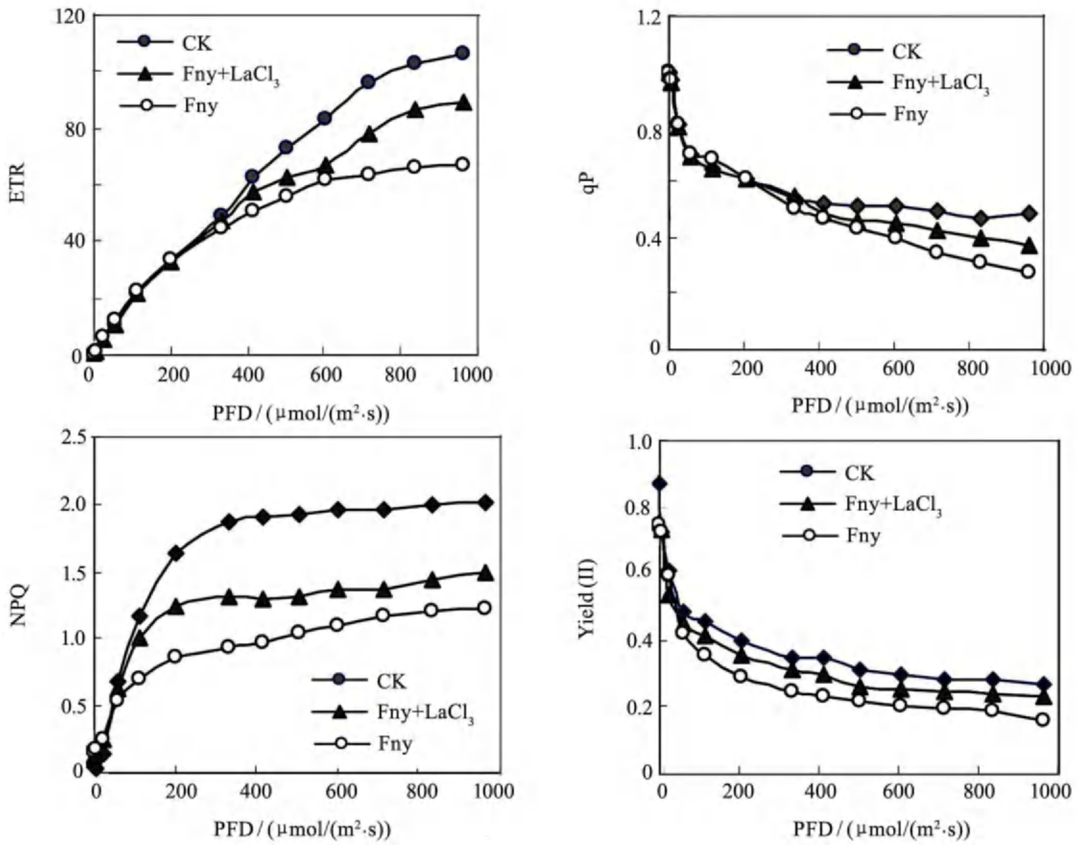

Fig. 4 Apparent electron rate (ETR), coefficient of photochemical quenching (qP), nonphotochemical quenching (NPQ), and yield(II) in tobacco plants after 5 wpi (Measurements were made by the Imaging-PAM fluorometer. Each value represents the mean±S.E. of five replicates)

ability of scavenging reactive oxygen species (ROS) in plants under abiotic stress[21]. It was be speculated that $LaCl_3$ could prevent the chloroplast structure, membrane vesiculation, and the key enzymes in Calvin cycle from the oxidative damage of overproduction of ROS in photosynthetic chain after treated by CMV.

Photochemical reactions, fluorescence quenching, and heat dissipation were the three kinds of whither of the light energy absorbed by chlorophyll molecules in leaves. The proportion and allotted amounts of the three energy consumption were commonly used to evaluate the running status of PSII and the resistance to environmental stress in plants. $LaCl_3$ treatment made apparent electron rate (ETR), coefficient of photochemical quenching (qP), nonphotochemical quenching (NPQ), and yield(II) obviously increase in infected tobacco plants at 5 wpi (see Fig. 4). A relatively high level of qP, ETR, and yield(II) signified that more excitation energy captured by antenna pigment could be used to propel the photosynthetic electron transfer. $LaCl_3$ could affect the electron transfer in biochemical pathway in plants leaves under environmental stress[22]. However, the improvement of non-photochemical quenching (NPQ) indicated the increased thermal dissipation, which could avoid photo damage at the pigment level. Corresponding to the change of NPQ, the content of carotenoid significantly increased in Fny-infected tobacco plants in the presence of $LaCl_3$ (see Table 4). Carotenoid plays an important role in antioxidant and photoprotection in PSII[23, 24]. So the induction of carotenoid synthesis by $LaCl_3$ in infected plants would strengthen the ability of eliminating free radicals, and protect the PSII against over-oxidation in high light intensity. And the treatment of $LaCl_3$ could increase net photosynthesis via enhancing the efficiency of harvesting light energy in photosynthetic system[9]. The precise physiological and molecular mechanism of anti-virus of rare earth elements (La) would be further studied in our coming work.

4 Conclusions

In this study, the photosynthetic indexes, together with the condition of growth and disease indexes of Fny-infected tobacco seedlings, indicated that the treatment of exogenously appropriate concentration of $LaCl_3$ could promote plants growth, and alleviate the photosynthetic damage caused by Fny-CMV. The possible mechanism was that the presence of La^{3+} might restrain the CMV from replication, prevent photosynthetic system from the oxidative damage of overproduction of reactive oxygen species via enhancing the ability of scavenging system, and affect electron transfer to enhance the photosynthesis efficiency in Fny-infected tobacco plants. This work would provide a technological guide for the application of REEs in the crop protection against virus. Further studies on molecular basis of La-induced disease resistance might help to elucidate the precise mechanisms of La^{3+}

on the anti-virus regulating.

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