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Effect of simulated microgravity on aged pea seed vigour and related physiological properties

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Abstract

We used simulated microgravity to treat aged pea seeds, in order to observe its effect on seed vigour and related physiological properties. The result shows that microgravity obviously promotes the germination rate, vitality index, germination index, and keeps the activity of protective enzyme, with conductivity and the content of MDA decreased. These effects gradually disappeared for a certain period after the microgravity administration.

Keywords: Microgravity; Vitality index; Germination index

1. Introduction

Ageing exists widely in the storage of seeds. Some of them even start to age after maturity. Indeed, aged seeds are lower in activity and their seedlings grow slower too. In order to reduce the disbenefit to agriculture, people started studying the method to promote the activity of aged seeds. According to Tao's introduction [1], a theory that aged seeds can rejuvenate, has become widely accepted recently. However, research on the methodology are quite limited. On the other hand, since 1987, China has sent hundred kinds of seeds to space via satellite. It is found that activity of some of them was promoted [2,3], whereas the environment of space is complex, with other factors, i.e. radiation effects. To further understand the effect of microgravity on the activity and related physiological properties of aged pea seeds, we used simulated microgravity in the laboratory, providing more evidence of the promotion.

2. Materials and method

2.1. Materials

Seeds of *Vigna acoitifolia*, stored for 4 years, were selected, marinated for 12 h, and then treated with simulated microgravity. A circumgyration device was used to produce simulated microgravity, with seeds fixed on the gyroscope wheel, at a speed of 2 r/min, for 36 h. The control was placed in the same environment for 36 h.

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Table 1
Effect of microgravity on seed vigor and seeding growth

	Germination rate (%)	Vigor index	Germination index	Plant height (cm/stem)
Control	41	24.12	245.18	4.1
Microgravity	59*	33.22*	231.06**	5.8*

Germination index = $\Sigma\ Gt/Dt$. Gt, number germination in t days; Dt, number of days. Vigour index = germination index × height of seedlings; $^{*}P < 0.05$; $^{**}P < 0.01$.

2.2. Germinative test measurement of seed vigour

A germinative test was performed according to Gu's methods [4], repeated four times. Each time we placed 50 seeds in humid culture dish (10 cm in diameter), in light culture at 25 °C.

2.3. Measurement of conductivity

Fifty seeds prepared for the measurement were washed three times with de-ionized water, surface dried, and put into 50 ml de-ionized water for 5 h at 25 °C. DOS-11 conductometer was used to measure the conductivity.

2.4. Measurement of POD (peroxidase) and SOD (superoxide dismutase)

According to the photoreduction of nitroblue tetrazolium (NBT), an inhibitor of SOD, the unit of SOD activity was represented as 50% of the inhibition of NBT photoreduction. We used 3 ml reaction solution, containing 13 mmol/l methionine, 75 umol/l NBT, 16.7 mmol/l lactoflavin, 0.1 mmol/l EDTA and 50 mmol/l sulfuric acid buffer solution (pH 7.8), reacting in 4000 × light intensity for 15 min.

Activity of POD was measured according to Kochba's method [5]. The unit of activity was represented as 0.1 of the increase of light intensity (470 nm) per mg protein per min. We set the reacting time for 2 min.

2.5. Measurement of MDA (malondialdehyda)

Protein extracting solution (0.5 ml) and 0.5 ml distilled water were added into 3 ml 0.5% thiosulbarbital sodium, mixed and boiled for 15 min. The solution was then cooled quickly, centrifuged at 3000 r/min for the 10 min. The supernatant layer was selected and the OD value at 600 nm was measured using standard MDA solution as operation curve to calculate the content of MDA.

3. Results

3.1. Effect of microgravity on germination rate

As shown in Table 1, microgravity obviously promotes the germination rate, vitality index, germination index, and seedling height, indicating that microgravity has an obvious promotion effect on the activity and growth of aged seeds. Microgravity promotes the activation of the seed's cells, wakes up dormant cells, and increases the seed's absorption of nutritive material in growth.

3.2. Effect of microgravity on electrolyte seepage velocity

As shown in Fig. 1, large quantities of electrolyte seepages demonstrated terrible permeability of the membrane and its faultiness. However, the conductivity of seeds was reduced significantly after microgravity administration, indicating the electrolyte seepages velocity was reduced. It can be concluded that the damaged membrane with its

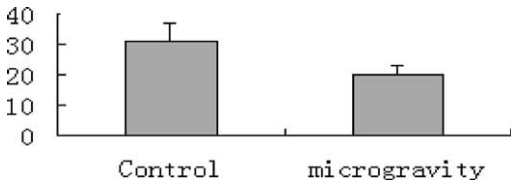

Fig. 1. Effect of microgravity on the rate of electrolyte leakage of seeds.

Table 2
Effect of microgravity on activity of SOD and POD and content MDA

	SOD (μm/g protein)	POD (U/min/mg protein)	MDA (nmol/mg protein)
Control	7.03±0.12	174.21±12.0	5.54±0.46
Microgravity	11.34±1.58*	321.32±16.0*	3.28±0.24*

* $P < 0.05$.

Table 3
The exchange of activity of SOD and POD and content of MDA after microgravity

Days		SOD (μm/g protein)	POD (U/min/mg protein)	MDA (nmol/mg protein)
3	Control	7.49±0.11	182.46±12.83	5.56±0.46
	Microgravity	11.92±0.58	234.56±16.09	3.87±0.24
6	Control	10.84±1.12	192.46±12.17	4.09±0.32
	Microgravity	13.26±2.31	240.53±18.14	3.12±0.84
9	Control	13.44±3.24	239.12±19.07	3.31±0.47
	Microgravity	14.10±2.63	242.83±21.74	3.42±0.33

selective permeability loss has been repaired after microgravity administration.

3.3. Effect of microgravity on SOD, POD activity and MDA contents

Former investigations indicate damage to membranes is a significant sign of the decrease of seed vigour. When seed ages, the increase of membrane permeability spoiled the cell regionalization leading to the terrible seepage of solute in cell during the imbibition. On the other hand, in the aging of seed, strength of active oxygen offence increases the oxidization of membrane lipid and results in the increase of toxic substances, and finally leads to the decrease of seed vigour.

Our experiment shows (Table 2) that the activity of SOD and POD increased significantly after microgravity. They both are part of seed's enzyme oxidization resistance system whose activities are responsible for the seed vigour. High activity of the protective enzyme system can clear the toxic substance produced by lipid peroxidation. MDA is this kind of lipid peroxidation whose content is a sign of the peroxidation of membrane lipid. The content of MDA of the microgravity administration group is much lower than the control, matching the conclusion that microgravity produced high activity POD and SOD effectively clearing the toxic substance.

We also measured the activity of protective enzyme and content of MDA after microgravity administration. The result shows (Table 3) that the difference between the administrated group and control on protective enzyme and MDA disappeared gradually after microgravity administration. It can be concluded that the physiological properties became normal, a certain period after the microgravity administration.

4. Discussion

Clearly, microgravity profoundly promotes the germination rate, vitality index, germination index, and seedling height, according to our experiment, showing an obvious promotion of aged seed vigour. Fu and coworker [6] indicated the activity

of SOD reduced significantly after the seed became aged. On the other hand, seed vigour is clearly positive-correlated to the activity of protective enzyme [7]. The deterioration of seed is related to the accumulation of free radicals in seed, which is the leading substance of the membrane lipid peroxidation and responsible for the degradation of membrane system, while protective enzymes can clear free radical and protect the membrane [8]. After microgravity administration, conductivity and content of MDA was reduced, indicating that the damaged membrane has been partially repaired. In addition, increase in the activity of SOD and POD shows their ability to clear free radical increase, which may be responsible for the decrease of membrane lipid peroxidation and electrolyte seepage. The repair of membrane keeps activities of the enzyme combined to the membrane, i.e. respiratory enzyme and synzyme, and therefore, benefits the growth of cell, which matches the result that microgravity administration profoundly, promotes the germination rate, vitality index and germination index.

Little research has been done to investigate the persistence of the effect of microgravity after administration. In our experiment, it is found that, 9 days after administration, activity of related enzyme and content of MDA approximately disappeared. It may be explained that microgravity does not change the DNA of cell, while only physiologically affected, which gradually disappeared after administration.

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