Effects of nitrogen ion implantation on lily pollen germination and the distribution of the actin cytoskeleton during pollen germination

REN Haiyun[1], HUANG Zhonglian[1], CHEN Zhiling[2], YUAN Ming[2] & LU Ting[3]

1. Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education, Beijing Normal University, Beijing 100875, China (e-mail: hren@bnu.edu.cn);
2. Key Laboratory of Plant Physiology and Biochemistry of Ministry of Agriculture, China Agricultural University, Beijing 100094, China;
3. Institution of Low Energy Nuclear Physics, Beijing Normal University, Beijing 100875, China

Abstract The effects of low energy nitrogen ion implantation on lily (*Lilium davidii* Duch.) pollen germination and the distribution of the actin cytoskeleton during pollen germination have been studied. Preliminary results showed that the ratio of pollen germination increased from (16.0± 1.6)% to (27.0±2.1)% when implanted with nitrogen ions by 100 keV and a dose of 10^{13} ions/cm^2. Further experiments were performed by staining the actin filaments in pollen with rhodamine-phalloidin and detected by using laser confocol microscopy. After hydration for 10 h, the actin filaments in ion implanted pollen grains tended to form thick bundles oriented in parallel or ring shape at the germinal furrow, indicating that the effect of nitrogen ion implantation on the germination of pollen might be mediated by reorganization of the actin cytoskeleton.

Keywords: **nitrogen ion implantation, lily pollen, microfilament.**

Physiological and genetic variations can be caused by implanting ions with low energy (30 — 200 keV) into organisms due to the electronic, energetic and qualitative effects of ions[1]. Significant success has been achieved in rice breeding by applying the method of low energy ion implantation[2]. But how ions penetrate into organisms, what depth they can reach, what the distribution of penetrated ions is in the organisms and the mechanism for the genetic variation induced by ion implantation are still far from clear. By now, most of the ion implantation work has been focused on their effects at physiological and chromosome level in crop breeding. Little is known about their effects at subcellular level[3—5]. A pollen grain is composed of one vegetative cell and two or three generative cells. Under proper conditions, pollen germinates, which results in the growth of a pollen tube known as a type of tip growth, in order to transport the male gametes in function to complete fertilization. The pollen is commonly used as a modal plant material to study cell structure and function because of its relatively simple structure and tip growth pattern[6]. There is also plenty of work concerning the roles of the actin cytoskeleton in the processes of pollen germination and pollen tube growth[7, 8]. To study the mechanism of biological effects of low energy ion implantation, their effects on the ratio of pollen germination and the

distribution of the actin cytoskeleton during lily pollen germination have been studied in the present note.

1 Materials and methods

(i) Plant material. Mature lily (*Lilium davidii* Duch.) pollen grains were collected from Lanzhou, Gansu Province. After air dried, the pollen was stored at –20℃ for use.

(ii) Ion implantation. Ion implantation was performed on a 400-keV implanter located at the Institute of Low Energy Nuclear Physics at Beijing Normal University. Pollen grains were attached with glue onto a 70 mm-in-diameter round aluminum foil and located perpendicularly to the ion beams in a sample chamber vacuumized to 1 mPa. The energy of the ion used for the experiments was 75 and 100 keV, with implantation dose of 10^{11}, 10^{13} and 10^{15} ions/cm^2 respectively.

(iii) Pollen hydration and germination. Pollen grains were hydrated in a dish covered with a wet gauze at 4℃ for 10 h, and recovered at room temperature for 2 h, then germinated in germination buffer (15% sucrose, 0.01% KNO_3, 0.02% $MgSO_4$, 0.01% H_3BO_3, 0.03% $Ca(NO_3)_2$, pH 5.6), shaking at 110 r/min at room temperature. The ratio of germination was calculated in 10 h. At least 100 samples were taken randomly in each experiment and repeated 3 times.

(iv) Fluorescent staining of the microfilaments of pollen grains and pollen tubes. Pollen grains and pollen tubes were fixed in 4% polymethanal, 50 mmol/L pipes buffer for 1 h and washed 3 times with 50 mmol/L pipes buffer. The fixed samples were then stained with 1 μmol/L rhodamine-phalloidin (Molecular Probes) in PBS buffer (0.137 mol/L NaCl, 2.68 mmol/L KCl, 8 mmol/L $NaHPO_4 \cdot 7H_2O$, 1.47 mmol/L KH_2PO_4, pH 7.4) at room temperature for 2 h.

(v) Confocal laser scanning microscopy. Confocal laser microscopy (MRC 1024, Bio-Rad) was employed to obtain the images of the actin cytoskeleton in pollen grains and pollen tubes using illumination from the 488 line of the Kr/Ar laser. 20—30 sections were acquired by collecting Kalman-filtered scans at individual steps of 1 μm. Images were reconstructed as projections by using the Confocal Assistant software.

2 Results

(i) Effect of nitrogen ion implantation on pollen germination. Pollen germination and pollen tube growth were observed after the pollen grains were implanted with different ion energies coupled with different doses. It was shown that ion implantation increased the ratio of pollen germination treated with proper dose. The ratios of pollen germination in samples treated with a dose of 10^{13} ions/cm^2 at 75 and 100 keV energy level implantation were increased to (20.0±3.6)% and (27.0±2.1)% respectively compared with (16.0±1.6)% of the control (table 1). But they did not exhibit much difference between different energy levels as the data shown in different dose manner. By either 75 or 100 keV ion implantation, a dose of 10^{11} ions/cm^2 had no effect on pollen germination. But when the dose was raised to 10^{13} ions/cm^2 (100 keV), the ratio of pollen germination increased obviously, from (16.0±1.6)% to (27.0±2.1)% (P<0.05). However, when the ion dose increased to 10^{15} ions/cm^2, ion implantation inhibits the germination ratio of pollen. In these experimental conditions, 100 keV with a dose of 10^{13} ions/cm^2 had most positive effect on pollen germination.

Table 1 The effect of nitrogen ion implantation on pollen germination

	75 keV			100 keV			Control
	10^{11} ions/cm^2	10^{13} ions/cm^2	10^{15} ions/cm^2	10^{11} ions/cm^2	10^{13} ions/cm^2	10^{15} ions/cm^2	
Average (%) ±SD (n=3)	17.0±2.4	20.0±3.6	6.3±1.8	15.0±2.3	27.0±2.1	7.5±1.2	16.0±1.6

(ii) Effect of nitrogen ion implantation on the organization of actin cytoskeleton.

Since the actin cytoskeleton plays an important role in pollen germination and pollen tube growth, we examined the distribution of the actin cytoskeleton in pollen grains and pollen tubes during pollen

germination and pollen tube growth after nitrogen ion implantation. The most effective conditions with energy of 100 keV and a dose of 10^{13} ions/cm^2 were employed in all experiments. The actin cytoskeleton was stained with rhodamine-phalloidin and the images were obtained with confocal laser microscopy. After hydration of pollen for 10 h, it was observed that the actin filaments in 70% of the treated pollen had formed long and thick bundles or rings in germinal furrow (fig. 1(a)). However, actin filaments located at germinal furrow in 67% of the control samples (germinating in the same conditions with no ion implantation treatment) were still in the organization of a thin network (fig. 1(b), table 2). The distribution of actin filaments in pollen tubes was observed with germinated pollen in 3 h immersing in germination buffer. The actin cytoskeleton in both treated and the control pollen tubes formed thick bundles parallel to pollen tubes (fig. 2). The treated and the control pollen tubes did not show much difference in their lengths.

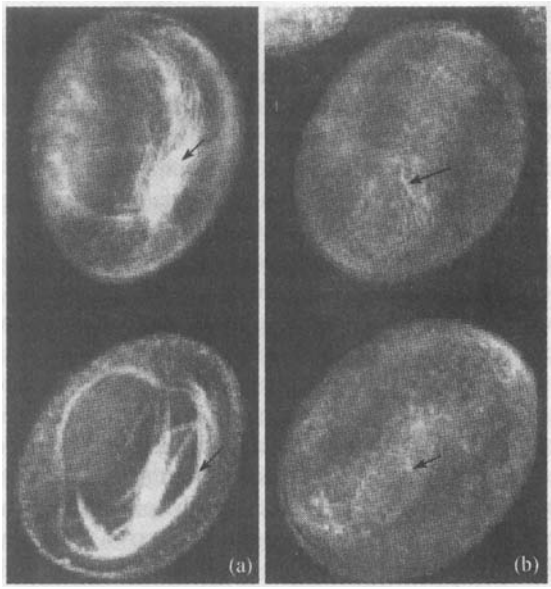

Fig. 1. Actin filaments organization in hydrated pollen grains. (a) Treated pollen. Arrows show the parallel bundles or ring structure of actin filaments; (b) control. Arrows show the network of actin filaments.

Table 2 Actin filaments organization in pollen germinal furrow

Pattern of actin filament organization	Bundles or rings	Network	No obvious pattern
Ion implantation (n=30)	21	4	5
Control (n=30)	6	20	4

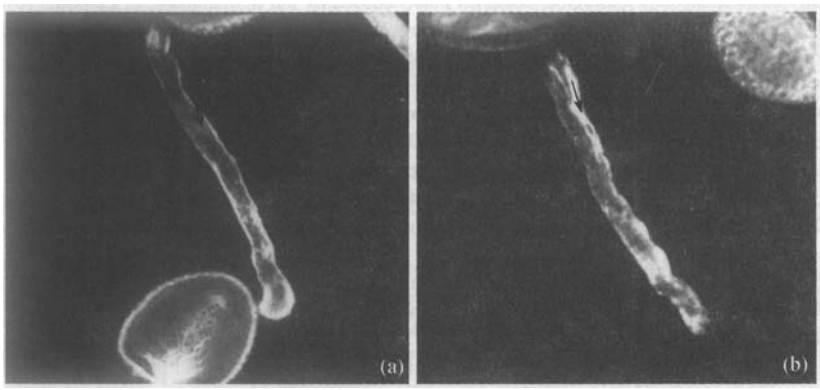

Fig. 2. Distribution of the actin cytoskeleton in pollen tubes. (a) Treated sample; (b) control sample. Arrow shows the actin filaments.

3 Discussion

In the present study, the ratio of pollen germination increased by nitrogenion implantation with proper energy and dose. Within a certain range, the effect became stronger with the energy increasing. But, the dose of ions seemed to be a more important factor to the effect: ions with low energy did not have distinct effect, while they had negative effect on germination with too high energy. Previous work showed that nitrogen ion implantation into seeds could also stimulate the growth of plants of the

contemporary generation[9, 10]. It seemed that they might have similar mechanism to accelerate cell growth. From hydration to germination, the physiological activities in pollen grain change greatly, especially with the striking, regular changes of the actin cytoskeleton organization which plays a key role in pollen germination[7, 8]. In the process of hydration, the organization of actin filaments changes significantly from permeating in the whole vegetative cell as a storage source to amassing together at the position of germinal furrow to form a thin, cross-linked network. During the time, the filaments located at the germinal furrow gradually form bundles parallel to the furrow to prepare for germination. After pollen germination, actin filaments were arranged to extend along the growing pollen tube as thick bundles. Nitrogen ion implantation experiment results showed that nitrogen ion implantation could cause the reorganization of actin filaments in vegetative cells from a cross-linked thin filament network to parallel thick bundles at the germinal furrow. It seemed that the effect of ion implantation on the actin cytoskeleton did not cause the variation of the regular pattern of actin filaments because such reorganization of the actin cytoskeleton occurs in normal pollen cells. But the actin cytoskeleton in ion implanted pollen reorganized much earlier than that in control cells. The actin filaments formed parallel thick bundles at the germinal furrow in ion implanted pollen while the actin cytoskeleton remained at its cross-linked thin filament network, and such rearrangement of actin cytoskeleton is essential for pollen germination, suggesting that the effect of nitrogen ion implantation on pollen germination might be due to its stimulating or accelerating the process of the activation of the actin cytoskeleton for pollen germination. Gu[11] also observed the effect of nitrogen ion implantation on the cytoskeleton in mung bean radicle cells with scanning electron microscopy, but did not show any physiological effect. Although the low energy ion implantation shows various physiological effects, its mechanism at the cell biology level remains unknown. The results obtained in the present study give the first indication that the actin cytoskeleton may mediate ion implantation and its effect on pollen germination. Since the actin cytoskeleton plays important roles in various cell biological processes, it could be a key factor for the cell biological effects of low energy ion implantation. The present study also provides a clue for further research of the physiological mechanism of low energy ion implantation.

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