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The Three-dimensional Images and Intracellular Calcium Analysis of Weigela Floridacv and Lonicera Japonica Thunb Pollen

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Abstract

Confocal microscope, which is a major advance upon normal light microscope, has been used in a number of scientific fields. Moreover, the three-dimensional images of specimens can be reconstructed with confocal microscope. It is ideal to analyze the three dimensional specimens for the non-destructive, non-invasive nature of the confocal microscope. In the present studies, a series of Weigela floridacv and Lonicera japonica thunb pollen optical sections were acquired with confocal microscope. Then the three-dimensional images of the pollen were reconstructed with the software of confocal microscope. In addition, intracellular calcium in the pollens was detected with the probe Fluo-3 AM, and the distribution of calcium in the pollens was analyzed with confocal microscope. Our results indicate that it is a very easy job to analyze the three-dimensional digital images of the pollen and intracellular calcium in the pollens with confocal microscope and the probes Acridine orange (AO) and Fluo-3 AM.

Keywords: Confocal microscope; Pollen; Three-dimensional image; Reconstruction; Calcium

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1. Introduction

Confocal microscope, which is one of the most exciting advances in optical microscope, has become a routine technique and indispensable tool for cell biological studies and molecular investigations [1]. Compared with conventional microscope, the specimens can be analyzed in three dimensions with much more clarity with confocal microscope. In addition, thick and opaque specimens that can not be observed in a conventional light microscope are excellent specimens for a confocal microscope. Confocal microscope is ideal to analyze the three dimensional specimens because its non-destructive, non-invasive nature allows imaging of a sample at a sequence of depths without physical sectioning.

The potential roles of Ca^{2+} in pollen have received considerable attention in the recent years. The signals of Ca^{2+} are thought to play an important role in plant growth and development, including key aspects of pollen tube growth and fertilization [2]. It has been reported that pollen germination and pollen tube growth has significantly regulated by the transport of Ca^{2+} [3-4]. Confocal laser scanning microscope has been widely used to study calcium in the pollen tubes [5]. However, there are fewer reports about the distribution of Ca^{2+} in pollens.

There have been numerous scientific researches employing confocal microscope in the pollen studies, such as magnitude and direction of vesicle dynamics in the growing pollen tubes, mitochondrial morphology in pollen, and the actin cytoskeleton of the pollen [6-8]. Since the primary value of the confocal microscope is its ability to produce optical sections through a 3-dimensional specimen, the aims of the present studies

were to reconstruct the 3-dimensional images of Weigela floridacv and Lonicera japonica thunb pollens. Furthermore, the distribution of Ca^{2+} in the pollens was investigated in the present studies.

2. Materials and methods

2.1 Plant material and reagents

Pollen was collected from the Weigela floridacv and Lonicera japonica thunb plants grown in Shandong University of Technology. The pollens were placed in a 5.0 mL micro-centrifuge tube and 4.0 mL of 20 μM Acridine orange (AO) (Molecular Probes, Inc) was added. The micro-centrifuge tube was incubated in dark at 4°C for 1 h followed by incubation at 20°C for 1 h in dye free solution.

To analyze Ca^{2+} level, pollens were loaded with 5 μM Fluo-3 AM (Molecular Probes, Inc) at 37°C for 30 min. Excess dye was eliminated by washing the disks several times in phosphate buffer.

2.2 Laser scanning confocal microscopy

To reconstruct the three-dimensional images, the pollen was observed with a 40× lens and the images were captured with a confocal microscope. A laser-scanning confocal microscope (Leica TCS SP2, Germany) with an air-cooled, argon-ion laser as the excitation source at 488 nm was used to view the pollen. The series images of pollen were detected in the yellow channel. In addition, the excitation source at 488 nm was used to view the sites of Ca^{2+}, and the distribution of Ca^{2+} were detected in the green channel. The channel settings of pinhole, detector gain, amplification offset and gain were adjusted to provide an optimal balance of fluorescence intensity of the targeted pollen and background. Data were collected by a computer attached to the instrument, stored on the hard drive, processed with a Leica TCS Image Browser, and transferred to Adobe Photoshop 6.0 for preparation of Figure.

2.3 Three-dimensional pollen images reconstruction

Sixty-three Weigela floridacv pollen optical sections and fifteen Lonicera japonica thunb pollen optical sections were acquired with the laser-scanning confocal microscope, respectively. Then the software of Leica TCS SP2 was used to reconstruct the three-dimensional images of the Weigela floridacv and Lonicera japonica thunb pollen.

3. Results and discussion

Confocal microscope is a relatively new light microscopical imaging technique, which has been widely used in the biological sciences. One of the important functions to the biologist is its ability to produce optical sections through a three-dimensional specimen. Furthermore, the three-dimensional images are accurate representations of the data and can be handled and manipulated to evaluate surface details and morphology. There is a pinhole aperture in the confocal microscope, which is confocal with the specimen point. The pinhole blocks light from other points in the specimen and permits the imaging of a well-defined spot deep inside the specimen. With moving mirrors and motion of the objective, a three-dimensional image can be constructed by scanning this spot [9]. Then a series of optical sections can be transformed into a three-dimensional volume. The volume permits visualization of the stack from viewpoints, which are different from the plane containing the individual optical sections [10]. In addition, the reconstruction of three-dimensional image by optical sectioning is faster and more precise than the mechanical sectioning. Another thing, the interior of relatively thick histological sections can be analyzed with confocal microscope [11-15]. Thus the morphology and differentiation of thicker sections can be provided [14, 15].

In the present studies, sixty-three Weigela floridacv pollen optical sections and fifteen Lonicera japonica thunb pollen optical sections were acquired with the laser-scanning confocal microscope, respectively (Figure 1 and 2). Then the three-dimensional images of the Weigela floridacv and Lonicera japonica thunb

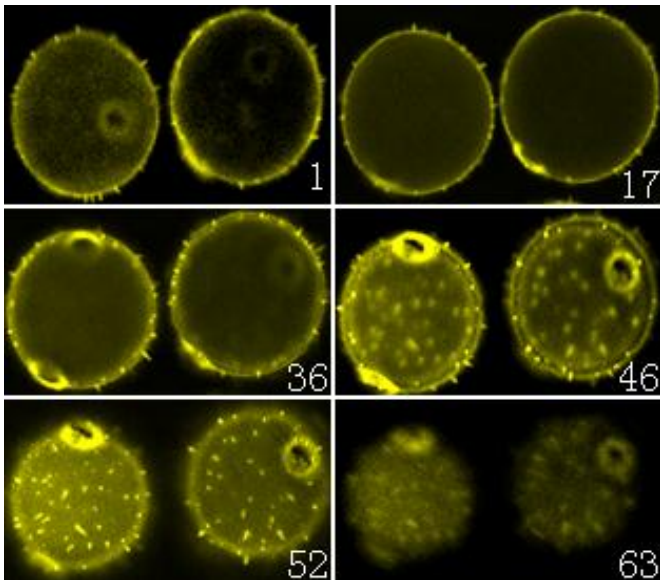

Figure 1. A series of confocal microscope optical sections of Weigela floridacv pollen. Pollen was labeled with AO. Sixty-three optical sections were acquired with the laser-scanning confocal microscope, and six images were chosen in the Figures (from No.1 to No.63 image). (×400).

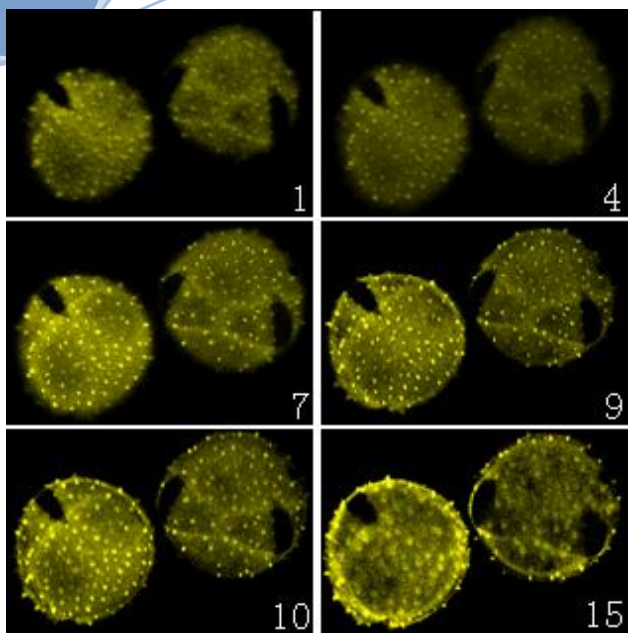

Figure 2. A series of confocal microscope optical sections of Lonicera japonica thunb pollen. Pollen was labeled with AO. Fifteen optical sections were acquired with the laser-scanning confocal microscope, and six images were chosen in the Figures (from No.1 to No.15 image). (×400).

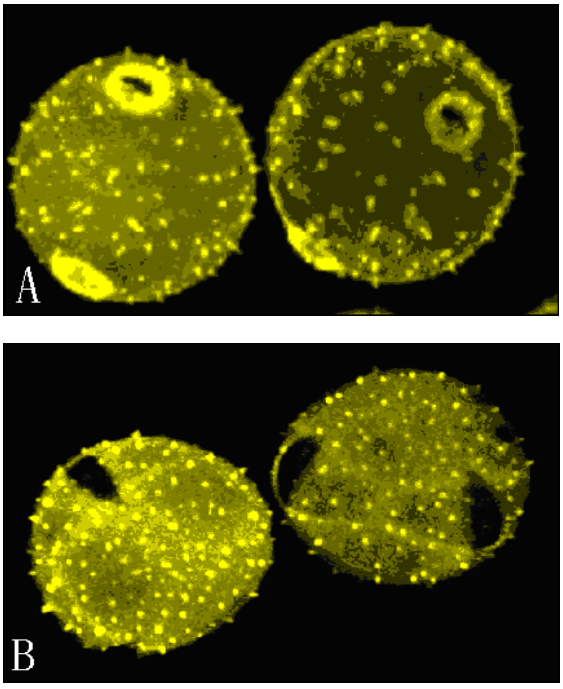

Figure 3. The three-dimensional images of the pollen were reconstructed with confocal microscope software. (A) The three-dimensional images of the Weigela floridacv pollen. (B) The three-dimensional images of the Lonicera japonica thunb pollen. (×400).

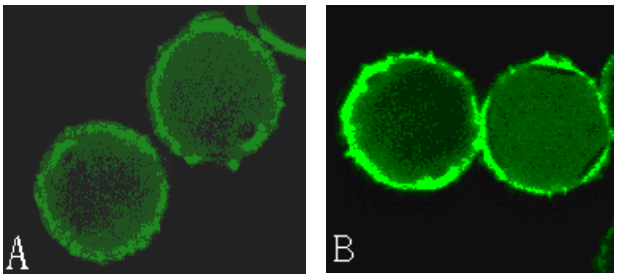

Figure 4. The distribution of calcium in the pollens. (A) The distribution of calcium in the Weigela floridacv pollens. (B) The distribution of calcium in the the Lonicera japonica thunb pollens. (×400)

pollen were reconstructed with confocal microscope software (Figure 3, A and B). Here we applied AO to dye the pollens of Weigela floridacv and Lonicera japonica thunb, and the clear 3-dimensional image of the pollen was acquired (Figure 3, A and B). Our results indicate that it's a very easy job to analysis pollen with confocal microscope and the probe AO.

Confocal microscopy provides the means to localize molecules in living cells with high spatial and temporal resolution. Since calcium is an intracellular messenger, the techniques for measuring cytosolic free Ca^{2+} concentrations have been essential. Fluo-3 fluorescence depends on concentration of free Ca^{2+} [16, 17]. Using Fluo-3 AM as probes, the distribution of Ca^{2+} can be clearly detected in the pollens of Weigela floridacv and Lonicera japonica thunb (Figure 4, A and B). Moreover, the results indicated that there is more Ca^{2+} distribution in the periphery of Weigela floridacv and Lonicera japonica thunb pollens (Figure 4, A and B).

Taken together, our results indicate that it is a good method to research the pollens with confocal microscope. With the fluorescence probes AO and Fluo-3 AM, not only the 3-dimensional image of the pollen can be reconstructed, but also the fluorescence of Ca^{2+} can be analyzed. It's a very easy job to analyze three-dimensional images of the pollen and intracellular calcium in the pollens with confocal microscope and the probes AO and Fluo-3 AM.

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