RECONSTRUCTION OF 3D DIGITAL IMAGE OF WEEPING FORSYTHIA POLLEN

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Abstract: Confocal microscopy, which is a major advance upon normal light microscopy, has been used in a number of scientific fields. By confocal microscopy techniques, cells and tissues can be visualized deeply, and three-dimensional images created. Compared with conventional microscopes, confocal microscope improves the resolution of images by eliminating out-of-focus light. Moreover, confocal microscope has a higher level of sensitivity due to highly sensitive light detectors and the ability to accumulate images captured over time. In present studies, a series of Weeping Forsythia pollen digital images (35 images in total) were acquired with confocal microscope, and the three-dimensional digital image of the pollen reconstructed with confocal microscope. Our results indicate that it's a very easy job to analysis three-dimensional digital image of the pollen with confocal microscope and the probe Acridine orange (AO).

Keywords: confocal microscopy, pollen, 3D digital image, reconstruction

1. INTRODUCTION

Confocal microscope, which is one of the most exciting advances in optical microscope of the last century, has become a routine technique and indispensable tool for cell biological studies and molecular investigations

(Lichtman, 1994). Confocal microscope works by exciting fluorescence with a highly focused beam of laser light. The light emitted from the point out-of-focus is blocked by the pinhole and can not reach the detector, which is one of the critical features of the confocal microscope. Thus only an image of the fluorescence from the focal plane is observed. In addition, the laser can scan over the sample from point to point and a single two-dimensional image of the optical section is obtained (Lichtman, 1994). In order to collect a series of images, the focus is shifted by a fixed amount. Then the object is scanned at the different Z position and the next image produced. The series of images are stored and the 3D data set is built. Thus a full three-dimensional image of the sample can be reconstructed via collecting a series of optical sections at different focal planes (Webb, 1999).

Using confocal microscopes, cells can be seen in three dimensions with much more clarity than previously possible. There have been numerous scientific papers employing confocal microscope in plant biology since it was first introduced, and this technology is very important for plant science researchers (Hepler and Gunning, 1998; Taira et al., 2004; Meckel et al., 2007; Cañamero et al., 2006). Since the advantage of confocal microscope is the ability to produce 3-dimensional reconstructions of specimens as mentioned above, the aims of the present studies is to reconstruct 3D image of Weeping Forsythia pollen with confocal microscope.

2. MATERIALS AND METHODS

2.1 Plant material and reagents

Pollen was collected from the Weeping Forsythia plants grown in Shandong University of Technology. Acridine orange (AO) was purchased from Molecular Probes (Eugene, OR, USA). Barley pollen was placed in a 200ml micro-centrifuge tube and 20ml of 20μM AO was added. The micro-centrifuge tube was incubated in dark at 4℃ for 1h followed by incubation at 20℃ for 1h in dye free solution.

2.2 Laser scanning confocal microscopy

The pollen was observed with a 40×water immersion lens (NA = 1.2, Leica) and the images was captured by a confocal microscope, the microscope equipped with a 10×cular lens. A laser-scanning confocal microscope (Leica TCS SP2, Germany) with an air-cooled, argon-ion laser as the excitation source at 488nm was used to view the pollen. The images of

pollen were detected in the yellow channel. Moreover, the channel settings of pinhole, detector gain, amplification offset and gain, and laser transmission 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 figures.

2.3 Three-dimensional pollen images reconstruction

A series of pollen images (35 images in total) were acquired with the laser-scanning confocal microscope, and the software of Leica TCS SP2 is used to reconstruct three-dimensional images of the sample.

3. RESULTS AND DISCUSSION

Confocal microscope, which is a major advance upon conventional microscope, has been used in a number of scientific fields (Michalet et al., 2003). Using confocal microscope, one can visualize deep into cells and create images in three dimensions (Hibbs, 2000). Compared with conventional microscopes, confocal microscopes have some distinct advantages. Confocal microscope has a higher level of sensitivity, because it has highly sensitive light detectors and the ability to accumulate images (Rawlings and Byatt, 2002). In addition, the resolution of images can be improved by eliminating out-of-focus light (Rawlings and Byatt, 2002).

There have been numerous scientific studies employing confocal microscope in pollen research. Confocal microscopy was used for morphological analysis and imaging purposes to organic-walled microfossils from the Middle Jurassic (Feist-Burkhardt and Pross, 1998). Yamaoka and Leaver (2008) reveal that mitochondrial morphology is influenced by MIRO1 and plays a vital role during embryogenesis and pollen tube growth with laser confocal microscopy. McInnis et al (2006) investigate reactive oxygen species and NO accumulation with confocal microscopy using ROS probes DCFH2-DA and DAF-2DA, respectively.

It has been shown that confocal microscope is a very important tool in the morphological analysis of fossil palynomorphs such as pollen, spores and dinoflagellate cysts without special sample preparation (Feist-Burkhardt and Pross, 1998; Feist-Burkhardt and Monteil, 2001; Hochuli and Feist-Burkhardt, 2004; Thouand et al., 2005). In present studies, a series of Weeping Forsythia pollen digital images (35 images in tolal) were acquired with confocal microscope (Fig.1). Furthermore, the three-dimensional digital

image of the pollen was reconstructed with confocal microscope software, and the clear 3D image of the pollen was acquired (Fig.2). It has been found that AO is the most popular fluorochrome for studies on whole blood, reticulocyte counting, and identification of nucleic acids (Anderson, 1957; Armstrong, 1956, 1957). Here we applied AO to dye the pollen of Weeping Forsythia, and the clear 3D image of the pollen was acquired. Our results indicate that it's a very easy job to analysis pollen with confocal microscope and probe AO.

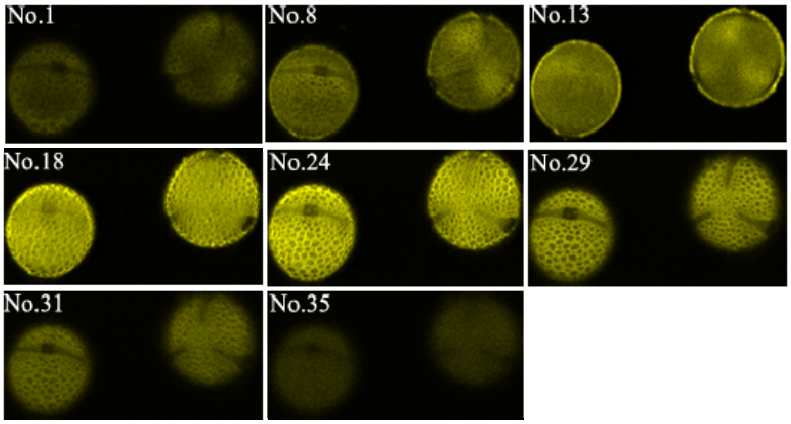

Fig.1. A series of confocal microscope digital images of Weeping Forsythia pollen. Pollen was labeled with AO. Thirty-five images were acquired with the laser-scanning confocal microscope, and eight images (from No.1 image to No.35 image) were chosen in the figures. (×400).

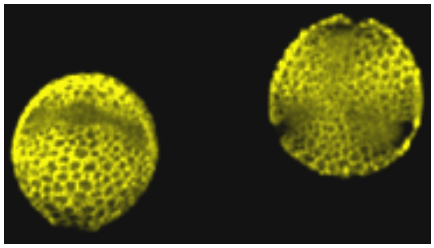

Fig.2. A full three-dimensional digital image of the pollen was reconstructed with confocal microscope software. (×400)

4. CONCLUSION

Confocal microscope, which is a major advance upon conventional microscope, has been used in a number of scientific fields. Using confocal

microscope, one can visualize deep into cells and create images in three dimensions. Our results indicate that it's a very easy job to analysis three-dimensional digital image of the pollen with confocal microscope and probe Acridine orange (AO).

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