Observations on the structural changes of embryos of *Paeonia rockii* L. by low-energy ion irradiation

D.M.Zhang, F.Z.Cui, and Y.B.Lin

Department of Materials Science and Engineering, Tsinghua University,
Beijing 100084, China.

Abstract

The mechanism of interaction between low energy ions and biological organisms has been paid much attention recently. In order to clarify the microstructural response to low energy ion irradiation, embryonic cells of *Paeonia rockii* L. implanted by Fe^{1+} ions with the energy of 80KeV were investigated by Optical Microscopy (OM), Scanning Electron Microscopy(SEM) and Transmission Electron Microscopy (TEM). At the dose of 1×10^{15} ions /cm^2, apparent cellular damage was observed in the outer several layers of the radicle. The shape of the cells was obviously deformed from regular polygon to irregular. The cell walls became obscure. SEM micrographs showed that the surface of the radicle was etched severely. It was observed by TEM that nucleus of the implanted cell was elongated and tended to fracture. Nuclear envelope lost its integrity. The implanted Fe^{1+} ions were detected by Energy Dispersive Spectroscopy (EDS). These observations showed that low energy ions could damage to the plant organisms with the thickness of about 30 ~ 50μm. The possible reasons for radiation damage in the biological organisms were discussed.

1. Introduction

In the middle of 1980's, the biological effects induced by ion implantation into the seeds have been investigated [1]. This stimulated the efforts to develop the low energy ion beam as a new mutagenic source. Since then, several new species of rice, wheat and microbes have been produced using low energy ion implantation. The biological effects induced by X-rays, γ-rays and UV have been widely investigated. However, ion implantation effects on the biological organisms are found to be different from the radiation effects of X-rays and γ-rays. Some new mechanisms are considered to be involved in the interaction between low energy ion beam and the organisms, such as charge exchanging and slowed-down ions depositing [2]. However, exact nature of the interaction is still unclear up to date.

The radiation effects on biological samples, such as crop seeds, cells and microbes, induced by ion implantation were rarely reported at cellular level [3]. In the present work, the morphology of the embryonic root (radicle) of *Paeonia rockii* L. before and after Fe^{1+} ions implantation was investigated by optical microscopy, scanning electron microscopy and transmission electron microscopy. The sample was chosen for the convenience to excise embryos from seeds.

2.Materials and Methods.

Materials

The seeds of *Paeonia rockii* L. were gratefully gifted by Forest research institute, Chinese Academy of Forestry. Embryos of the seeds were carefully excised using a

scalpel the day before the ion implantation. The embryos were held on moist filter papers in culture dishes and stored in refrigerator at 4 ℃.

Ion implantation

Ion implantation was performed on 400KeV heavy ion implanter in Beijing Normal University. The embryos were placed in a target plate to ensure the embryonic roots facing the ion beam. The bombardment energy of Fe^{1+} beam was 80 keV . The dose was 1×10^{15}ions/cm^2. Ion current was controlled below 10μA to minimize the increase in the substrate temperature. The working pressure was about 10^{-5} Torr. The controls were also placed in the chamber during the ion implantation.

Serial paraffin sections

Some implanted embryos and the controls were fixed in 70% ethanol solution. The sections were stained with 1% safranine O and observed under Olympus light microscope.

Transmission electron microscope(TEM)

For TEM, the samples were fixed with 2% glutaraldehyde in 0.1M phosphate buffered solution (pH 7.2) immediately after ion implantation, and postfixed by 1% osmium tetroxide in the same buffer. They were dehydrated in a graded acetone series, embeded with Epon 812. All blocks were carefully trimmed under a dissecting microscope. Ultrathin sections were cut on an ultrotome LKB-III, picked up on formvar-coated grids, stained with uranylacetate and lead citrate, and examined under a JEM-100CX electron microscope.

Scanning electron microscopy (SEM)

The samples were fixed with 2% glutaradehyde in 0.1M phosphate buffer (pH 7.2) after implantation, followed by 1% osmium tetroxide in the same buffer, and dehydrated in a graded acetone series. They were critical point dried with CO_2 in a HCP-2 Hitachi Critical Point Dryer, and gold-coated for examination under a CSM 950 scanning electron microscope.

Energy dispersive spectrum (EDS)

EDS analysis was performed to detect the presentation of the implanted element of Fe. The acceleration voltage was 20KV. The elemental information was obtained within several micrometers on the surface of the materials.

3. Results

3.1 Optical microscopy

Figure 1 shows the cellular morphology of the embryonic root in light microscope. cellular damage on the surface of the implanted radicle was evident (Fig. 1b) compared with the control (Fig. 1a). The cellular morphology of the control was regularly polygonal, and the cell walls were clear. In contrast, in the implanted radicle, the cell deformed, and the cell walls became obscure. The damaged thickness was estimated of about 30-50μm in the outer 3-5 cellular layers of the embryonic root.

3.2 SEM morphology and EDS analysis

Figure 2 is the surface morphology of the radicle under SEM. The shape of the cells on the surface of the control (Fig. 2a) was regular polygonal. After Fe^{1+} implantation at the dose of 1×10^{15} ions/cm^2, the surface was etched severely (Fig. 2b). The cell walls were destroyed completely and the cytoplasm exuded as shown in Figure 2c.

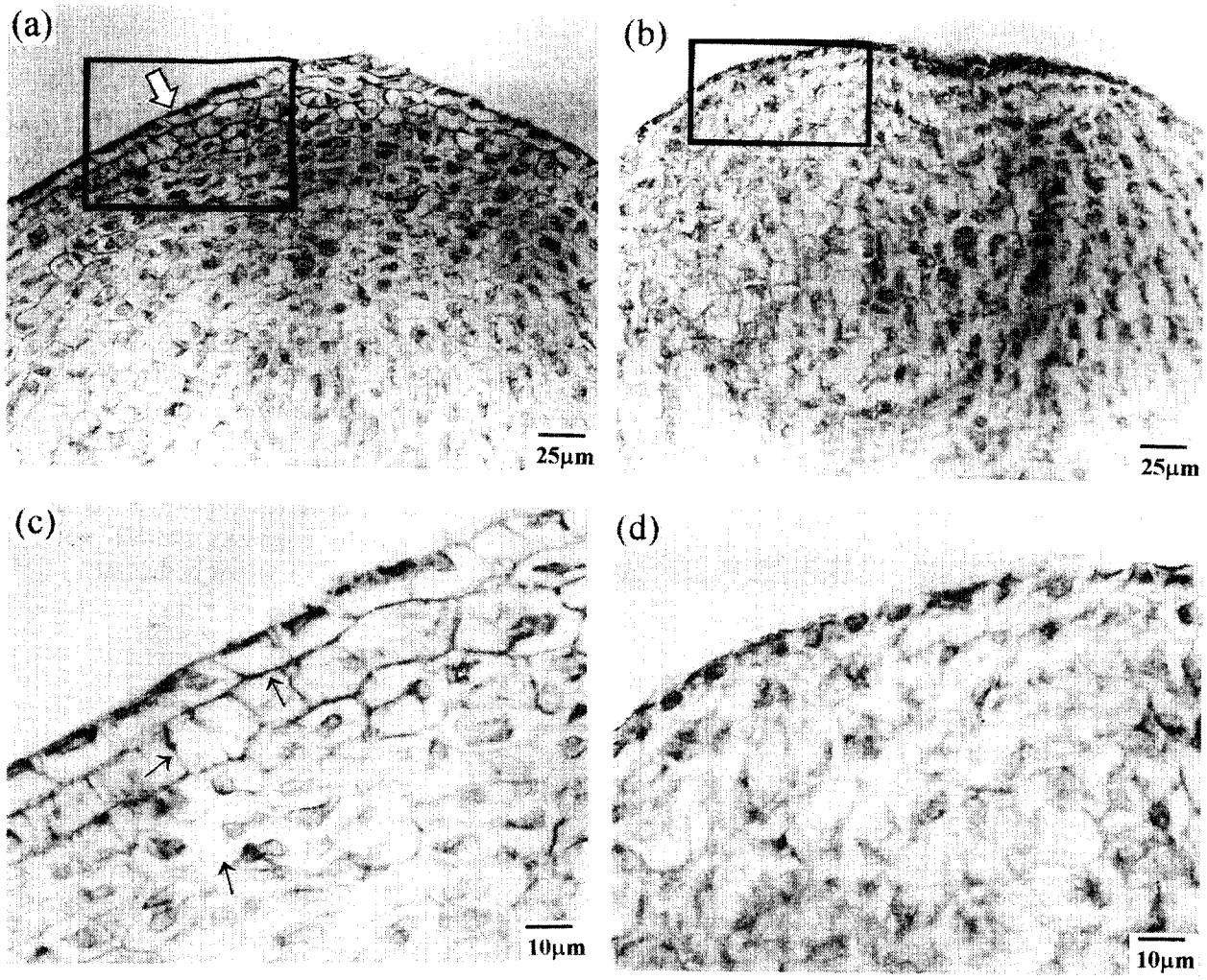
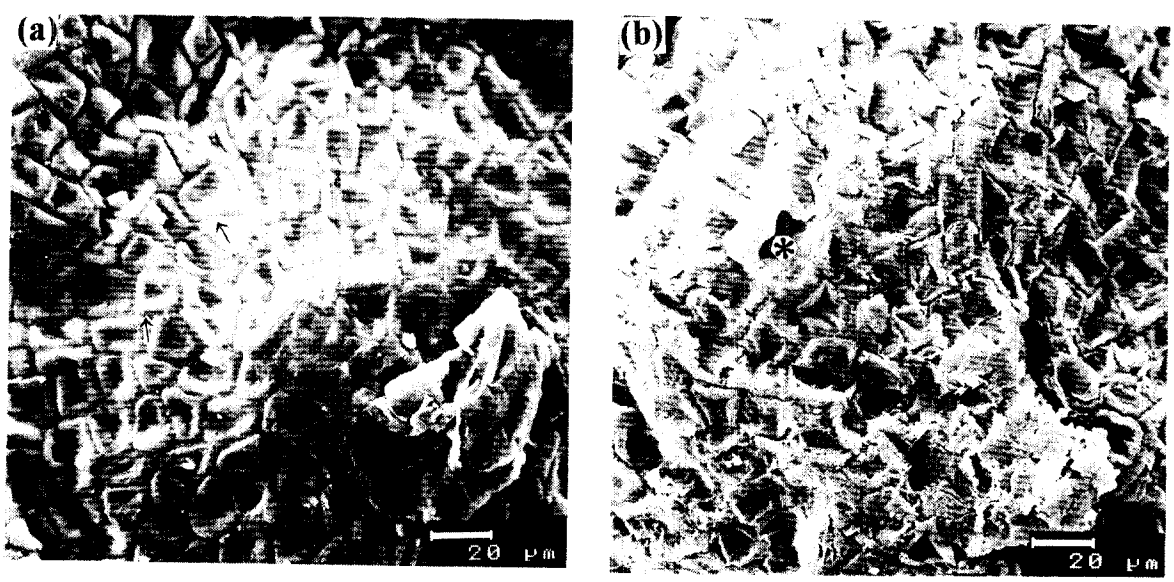


Fig. 1 cellular morphology of the embryonic root of *Paeonia rochii* L.. (a) is the control without ion implantation. The open arrow indicated the normal cells on the surface. (b) is embryo root implanted by Fe^{1+} ions with the energy of 80keV . The dose was 1×10^{15} ions/cm^2. (c) is the enlargement of (a) showing clear cell walls (solid arrows). (d) is the enlargement of (b). The cells on the surface deformed and the cell wall became obscure.



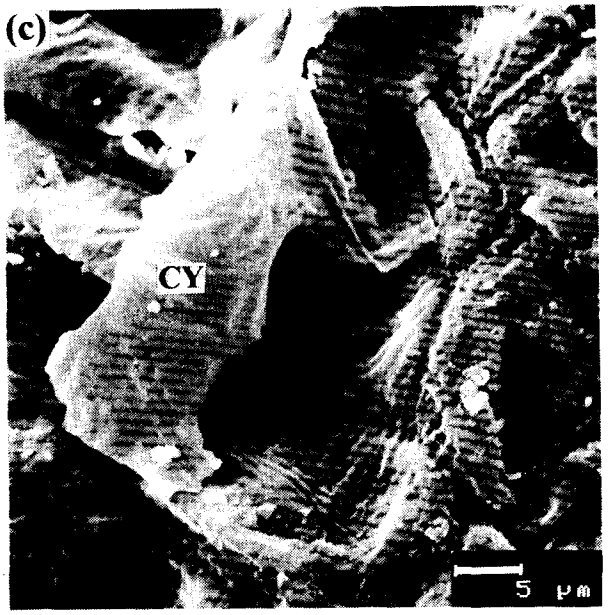
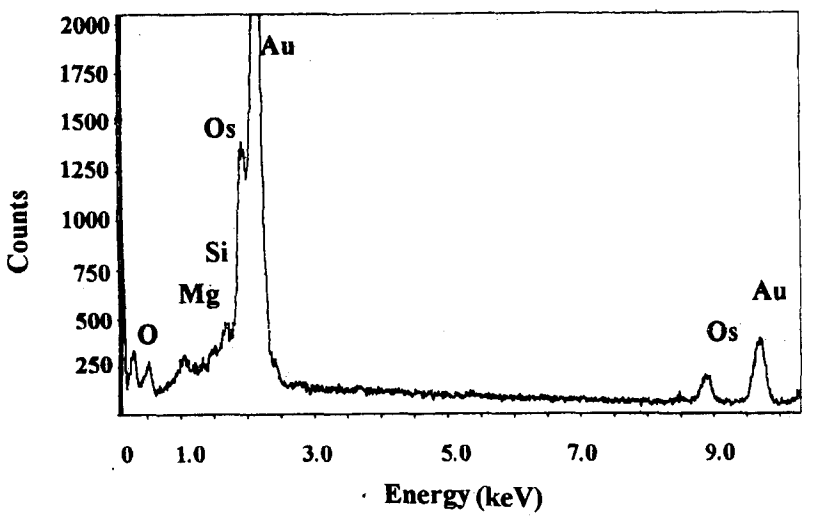
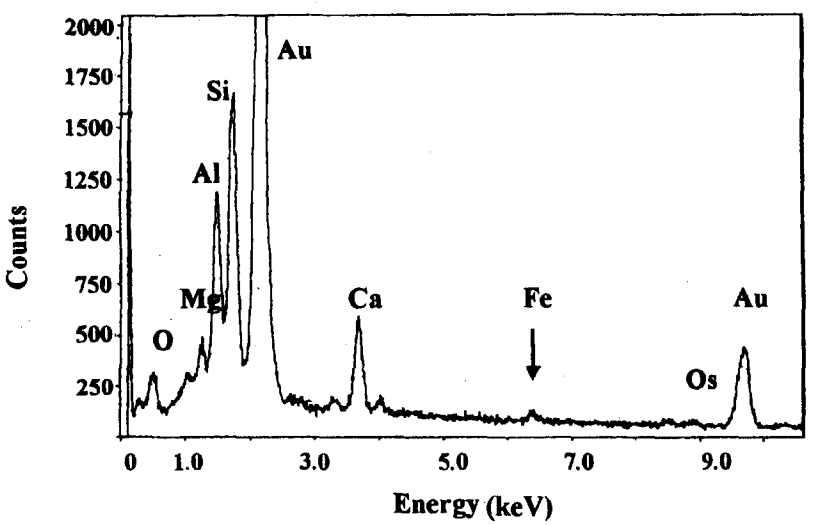


Fig.2 surface observation of the radicles under SEM. (a) normal polygonal-shape cells (arrows) on the surface of the control. (b) destroyed surface by ion implantation with deformed cells. (c) enlargement of a cell in (b) (asterisk) showed that cytoplasm (CY) outside of the cell.



(a)



(b)

Fig. 3 EDS analysis of the radicles. (a) control without the element of Fe. (b) implanted radicle with Fe on the surface.

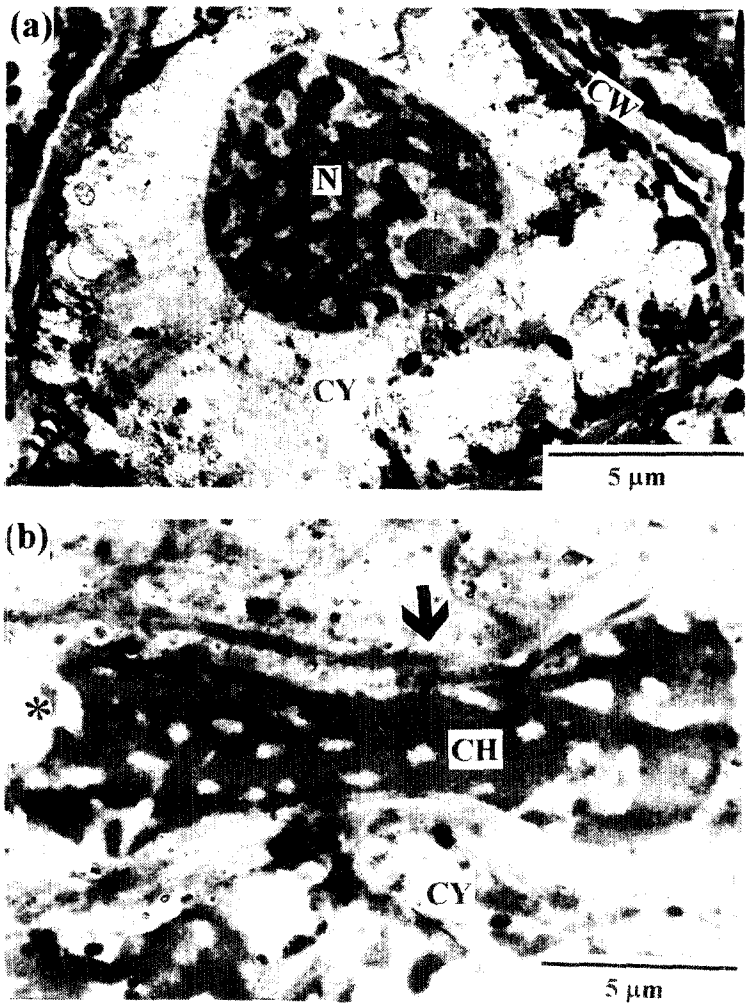


Fig. 4 TEM micrographs of the individual cell in embryo root. (a) is the control with normal morphology. (b) shows severely deformation of nucleus induced by Fe^{1+} implantation. The nuclear envelope was damaged and the chromatin became localized (asterisk). The nucleus was elongated and tended to fracture into two parts (black arrow). CH: chromatin, CY: cytoplasm, CW: cell wall, N: nucleus.

Figure 3 is the surface composition analysis of the radicle of *Paeonia rachii* L. by EDS. The element of Fe had not been detected in the control (Fig. 3a), but it was detected on the surface of the implanted radicle (Fig. 3b).

3.3 Ultrastructural observations under TEM

The transmission electron micrographs reveal some ultrastructural modifications in detail (Fig. 4). An individual cell of the control was shown in Figure 4a. Considerable changes were evident in the cell implanted by Fe^{1+} ions (Fig. 4b). Nuclear envelope was damaged to become obscure. The cytoplasm bacame loose and less uniform. The nucleus was elongated severely and had a tendency to fracture into two pieces. Chromatin became localized and seemed to lose its connection with the nuclear envelope at some sites . The chromatin became looser compared with the control.

4. Discussion

The embryo is the most important part of a seed and any biological damage will first be manifested at the time of germination. Therefore, the embryo was selected to be subjected to the ion implantation in vitro in this work.

Low energy ion implantation can lead to morphological changes of biological

organisms at cellular level. It was observed that the damaged depth is at least 30-50 μm. This fact suggested that the influence depth on the biological system of low energy ion beams was much farther than that on the solid materials. Meanwhile, the morphological observation at cellular level is suggested to be a method to investigate the effective range of the ion beams on the biological materials.

It has been reported that the free radicals induced by ionizing radiation in biological system play an important role in reactions between radical generation and the measurable biological damage[4-6]. One of the possible explanation for the collapse of the nuclear envelope, the damage of the cell walls was the effect of the free radicals. Free radicals were produced in the process of the ion implantation. The change of the activity of some membrane -bound enzymes and the content of the plasma membrane may also contribute to these damage. Till now, little information on the effects of low energy ion beam on the implanted biological materials, in particular on the plant materials is available. Further experiments are necessary to study the free radicals generated in the implanted materials and the activity of some enzymes.

It is thought that the nuclear lamina plays a crucial part in organizing both the nuclear envelope and the underlying chromatin [7]. The nuclear shape has close relationship with the nuclear lamina protein. The change of the nuclear shape is attributed to the conformational modification of the nuclear lamina protein. Some components associated with the lamina are believed to bind a specific sites on chromatin and thereby guide the interaction of chromatin with the nuclear envelope. The radiation lesion of nuclear lamina may explain the change of nuclear shape and the localization of the chromatin.

The chromatin in the nucleus is comprised of deoxyribonucleic acid (DNA), histones as well as nonhistones. The direct interaction of ions and indirect actions such as ionizing and charge exchange induced by ion implantation can destroy the interaction between the DNA and histone molecules, thus lead to the looseness of the chromatin in the electron micrograph. The looseness of the cytoplasm may also be correlated with the charge exchanging during the implantation. The various damage concerning with the nucleus indicated that the nucleus was very susceptive to the ion implantation. Any damage to the hereditary substance — DNA — within the nucleus will result in the damage, mutation, and even death of the organisms.

The morphological changes induced by low energy ion implantation is evident, but the mechanisms responsible for the phenomenon is far from understood. Further experiments should be made to find out the exact sites where the cells are damaged and to determine how far the low energy ion beam can destroy the cells.

Acknowledgment:

This study was supported in part by the National Natural Science Foundation of China. The authors gratefully thank Dr. W. Q. Qi for providing the seeds of *Paeonia rockii* L..

References:

[1] Chen Yuanru, Mo Shangwu and Qiu Shuhua. Effects of N^+ ion implantation on wheat growth. *Nucl. Intru. Meth. in Phys. Res.* B23(1987), 344-346.

[2] Huang Weidong, Zengliang Yu, Xiangyu Jiang and Xiaohua Yang. Study of mass and energy

deposit effects of ion implantation of NAA. *Radiat. Phys. Chem.* 48(1996), 319-323.

[3] Yu Zengliang, Yang Jianbo, Wu Yuejin, Chen Beijiu, He Jianjun and Huo Yuping. Transferring Gus gene into intact rice cells by low energy ion beam. *Nucl. Intru. Meth. in Phys. Res.* B80/81 (1993), 1328-1331.

[4] M. Koter, W. K. Surewicz. Radiation induced structural changes in model lipid membranes, an ESR and fluorescence study. *Studia. Biophysica.* 106(1985), 79-86.

[5] I. P. Edimecheva, I. P. Edimecheva, M. A. Kisel, O.I. Shadyro, A. P. Vlasov and I. L. Yurkova. The damage to phospholipids caused by free radical attack on glycerol and sphingosine backbone. *Inter. J. Radiat. Biol.* 71(1997), 555-560.

[6] Li wenjian, Wei Zengquan, Xie Hongmei, Han Guangwu, Li Qiang, Dang Bingrong, Yang Hanmin and Gao Qingxiang. The effects of free radicals induced with heavy ion irradiation on chromosomal aberration of wheat seeds. *Acta Agriculture Nucleatae Sinica.* 10(1996), 189-192. (in Chinese)

[7] Bruce Alberts, Dennis Bray, Julian Lewis, Martin Raff, Keith Roberts and James D. Watson. *Molecular biology of the cell.* pp.386-406. New York and London, Garland Publishing, Inc. 1982.