Biological effects of implantation and penetration of nitrogen ion beams on wheat seeds*

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Abstract The biological effects of wheat seeds irradiated by 48 MeV/u and 20 MeV/u $^{14}N^{7+}$ beams are studied, respectively. The results show: both penetration and implantation of heavy ions can significantly inhibit growing power (height of plant stem) of wheat seeds. The greater dose is, the weaker growing power is, and the implantation influence on growing power is stronger than the penetration; a great variety of chromosomal aberrations were observed in root tip cells of irradiated seeds and the aberration frequencies were several hundred times higher than that of the corresponding control; it has been proved by ^{3}H-TdR incorporation test that unscheduled DNA synthesis exists in irradiated wheat seed embryos. It is found by ESR spectroscopy that the variation of free radical concentration with total dose has different features for the penetrated and implanted seeds.

Keywords Radiation biological effect, Wheat seeds, Nitrogen ion beam, Penetration, Implantation, Chromosomal aberration, Free radicals

1 Introduction

Studies on biological effects in plants and crop breeding by ultralow energy (<200keV) ion implantation were started in China in the 1980's and many excellent results have been obtained.[1] However, studies on biological effects of plants irradiated by medium energy (10~100MeV/u) ions are still rare. Especially with respect to experimental comparisons between implantation (20 MeV/u) and penetration (48 MeV/u) there is no report until now. In this experiment, we studied the biological effects of wheat seeds treated by the penetration and implantation of $^{14}N^{7+}$ beams.

2 Materials and methods

2.1 Plant materials

Wheat seeds, Dingxi-24 and 82-579, were provided by Institute of Grain Crops, Gansu Provincial Academy of Agricultural Science and their moisture mass fraction was 0.03 as compared with 40 h-dried seeds.

2.2 Irradiations

The wheat seeds were irradiated by 48 and 20 MeV/u $^{14}N^{7+}$ beams facing their embryos, respectively. For LET(linear energy transfer), the former is 65 keV/μm, the latter 120 keV/μm. Fluences of the beams were determined with air ionization chambers. The fluences were 5×10^{6}, 1×10^{7}, 1×10^{8}, and 1×10^{9} cm^{-2}, respectively.

2.3 Treatments of postirradiation

In a Petridish 50 grains of irradiated seeds are evenly arranged, adding just the right amount of water, germinating at 25°C, changing water once a day, observing and registering germination states. Until wheat seeds grow the seventh day, height of plant stem is measured and the average value is defined as their growing power (cm). In chromosomal aberration ex-

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periment, 2~3 strips of root tips are taken out in every group, and hydrolyzed for 15 min in 1 mol/L HCl at 60°C, then dyed with Schiff agent, finally sealed with coverslips. At last chromosomal aberration types and frequencies in root tip cells are counted. The DNA synthesis was traced by ^{3} H-TdR labeling method. For ESR spectral determination of wheat seeds irradiated by $^{14}N^{7+}$ ion Varian E-115 EPR spectrometer is applied in ~9.5GHz wave band and 100 kHz field tuning at room temperature. The distance (6.81 mT) from the third EPR spectral line to the fourth in $ZnS{\cdot}Mn^{2+}$ and DPPH(g =2.0036) were employed as standard of determining sample EPR parameters.

3 Results and discussion

3.1 Influence of $^{14}N^{7+}$ penetration or implantation on growing power

It can be seen from Table 1 that growing powers of both penetration and implantation decrease with increasing radiation dose; influence of implantation on growing power is stronger than that of penetration.

Table 1 Dependence of growing power for wheat seeds on $^{14}N^{7+}$ penetration and implantation

Irradiated method	Fluence/cm^{-2}	Testable seeds	Total height of plant stem/cm	Growing power/cm	Relative growing power
Control	0	50	424.0	8.48	1
Implantation	5×10^{6}	50	116.8	3.34	0.394
Implantation	1×10^{8}	50	158.3	3.17	0.374
Implantation	1×10^{9}	50	92.4	1.85	0.218
Penetration	5×10^{6}	50	276.7	5.53	0.652
Penetration	1×10^{8}	50	200.0	4.00	0.472
Penetration	1×10^{9}	50	101.3	2.03	0.239

3.2Chromosomal aberrations in root tip cells

In root tip cells of irradiated seeds, a great varicty of chromosomal aberrations were observed such as micronucleus (c), chromosome bridge (d), free chromosome (b) and backword chromosome (a) (see Fig.1). It can be seen from Table 2 that the aberration frequencies are several hundred times higher than those of the corresponding control.

3.3 DNA synthesis in wheat seed embryos

Unscheduled DNA synthesis is one of the most important items of measuring biological effects induced by mutagenic agents. Fig.2 is the change procedure of unscheduled DNA synthesis during the early period of germination in wheat seed embryos implanted and penetrated by $^{14}N^{7+}$ beam. This shows that $^{14}N^{7+}$ ion radiation can induce an unscheduled DNA synthesis (UDS), i.e., DNA damage repair synthesis in wheat seed embryos. The peaks of the UDS occurred at the 10 th h (penetration) and 8 th h (implantation) after seeds soaking, both being earlier than the peak (14 th h) of the normal DNA synthesis of seed embryos. The UDS of the implantation appears earlier and repairs more efficiently than that of penetration because the effect of implantation on DNA is greater than that of penetration. Compared with control, the delay of the normal DNA synthesis for the penetration group, which may result from the unscheduled DNA synthesis, affects next normal DNA synthesis because of a small interval between the UDS and the normal DNA synthesis. In order to test and verify the UDS further, the peak of the UDS in wheat seed embryos is eliminated after adding coffeine treatment (Fig.3).

3.4 Free radicals in wheat seeds

Many researchers found that medium energy heavy ion irradiation can induce chromosomal aberration in root tip cells of plant dry seeds, such as Wei Zeng-Quan *et al* [2]. For studies on physical mechanism of inducing the biological effects direct action is more remarkable

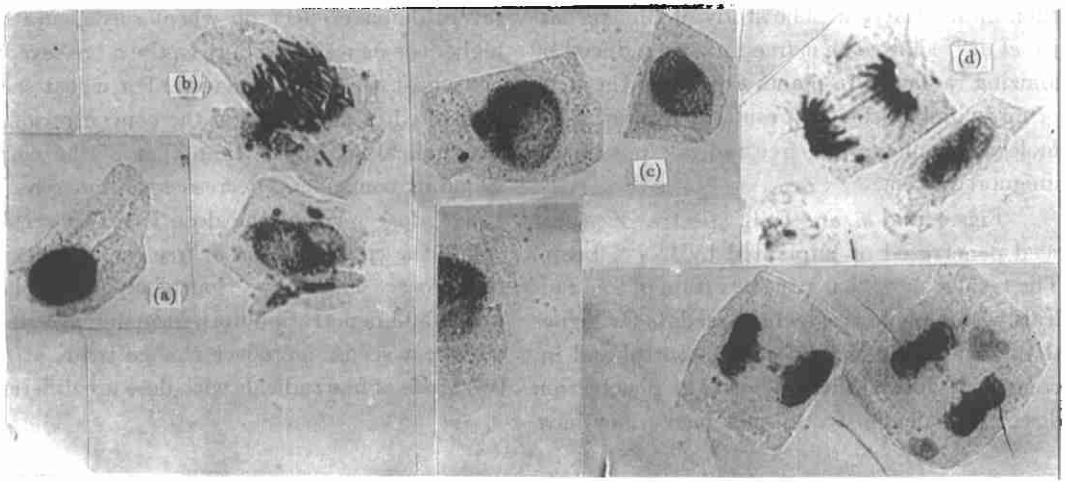

Fig.1 Chromosomal aberrations in root tip cells of irradiated seeds

Table 2 Dependence of micronucleus rates and aberration frequencies of wheat seeds on $^{14}N^{7+}$ dose for penetration and implantation

Irradiated method	Fluence/cm^{-2}	Observable cell No.	Micronucleus cell No.	Aberration cell No.	Micronucleus rate	Aberration frequency
Control	0	20000	9	12	4.5×10^{-4}	6×10^{-4}
Implantation	5×10^{6}	20000	3342	3516	0.1671	0.1758
Implantation	1×10^{7}	20000	5406	5624	0.2703	0.2812
Implantation	1×10^{8}	20000	6843	7023	0.3422	0.3512
Implantation	1×10^{9}	20000	5873	6117	0.2987	0.3058
Penetration	5×10^{6}	20000	2258	2375	0.1129	0.1188
Penetration	1×10^{7}	20000	3891	4106	0.1946	0.2053
Penetration	1×10^{8}	20000	6517	6837	0.3258	0.3419
Penetration	1×10^{9}	20000	4844	5003	0.2422	0.2502

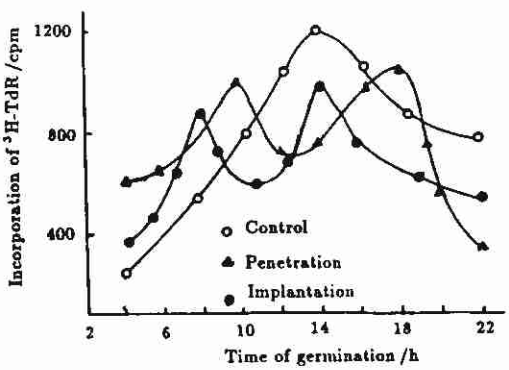

Fig.2 The incorporation of ^{3} H-TdR during the early period of germination in wheat seed embryos implanted and penetrated by $^{14}N^{7+}$ beam under the fluence 1×10^{7}cm^{-2}

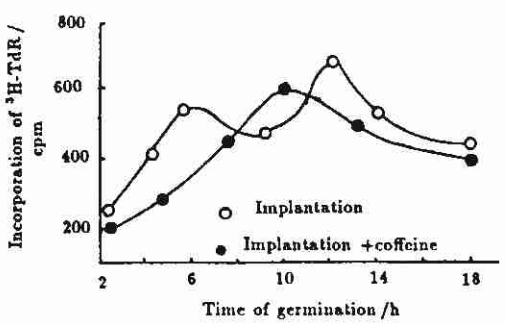

Fig.3 The change of DNA synthesis in wheat seed embryos implanted by $^{14}N^{7+}$ beam under fluence 1×10^{7}cm^{-2} following adding coffeine treatment

than indirect action. The study of Bhattacharjee *et al* [3] showed that free radicals induced by ionizing radiation in plants are one of the most primary indirect factors resulting in plant DNA molecule damage. So, free radicals possess an unignored effect.

Figs.4 and 5 are ESR spectra of wheat seed penetrated or implanted by $^{14}N^{7+}$ beam. The results show that concentration of free radicals within penetrated wheat seeds is far higher than that of the corresponding control and increases obviously with increase in penetration dose. This demonstrates that heavy ions have severe damage effect on wheat seeds, and the higher the penetration dose is, the more severely the wheat seeds are damaged. For wheat seeds implanted by nitrogen ion the concentration of free radicals is higher than that of the corresponding control and decreases with increase in $^{14}N^{7+}$ dose. When the dose is up to certain value, the concentration of free radicals begins to increase. This shows that heavy ion implantation and penetration both have damage effect on wheat seeds, moreover change tends of the two kinds of free radicals with dose are different.

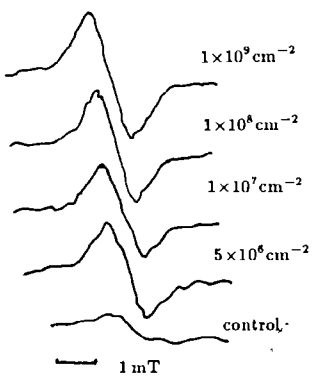

Fig.4 ESR spectra of wheat seeds penetrated by different fluences of $^{14}N^{7+}$

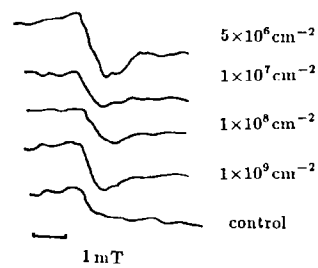

Fig.5 ESR spectra of wheat seeds implanted by different fluences of $^{14}N^{7+}$

4 Summary

a. Heavy ion irradiation has clear inhibitory action on growth of wheat seeds, and the inhibition effects of high LET ions surpass low LET ions.

b. Both ion implantation and penetration can induce chromosomal aberration in wheat seeds, and the aberration frequencies of implantation surpass those of the penetration.

c. It can be found that there is an UDS in wheat seed embryos implanted or penetrated by heavy ions. The UDS of the implantation occurs earlier and repairs more efficiently than that of the penetration.

d. Heavy ion irradiation can induce a lot of free radicals in wheat seeds. Moreover, the variation of free radical concentration with total dose has different features for the penetrated and implanted seeds.

Acknowledgement

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