# Effect of $^{211}$At treating pollen and stigma on generative cells and seed setting of rice

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**Abstract** Low specific radioactivity (7.4 kBq/ml) $^{211}$At treating pollen and stigma can obviously affect morphological structures and physiological functions of pollen, stigma and ovule or embryo sac cells, and cause injury. Results showed that because of the radiation effects the seed setting rate of rice was decreased, and the development of some embryos were affected and others became abnormal.

**Keywords** $^{211}$At, Rice, Generative cells, Seed setting rate, Induced variation

## 1 Introduction

Traditional $\gamma$-ray is still used as a main mutagenic source for the radiation induced mutation research in plants. In the meantime, there are new developments in utilizing neutron, laser, $\beta$-ray, X-ray and electron beam as mutagenic sources. Recent years, the low energy ion implantation, as a new mutagenic factor, has made great advances in improving crops in China.[1] However, the application of short-lived $\alpha$-emitter in the research of induced biological variation in plants is a new attempt. The cyclotron-produced $\alpha$-emitter $^{211}$At has a half-life of 7.2 h and decays by the emission of 6.8MeV average energy $\alpha$-particles which have tissue ranges of 55~ 80$\mu$m.[2] It is very meaningful to do exploratory research of induced variation by using $^{211}$At in crop plants. Here we reported the radiobiological actions of $^{211}$At on pollen, stigma and ovule cells of rice, and the preliminary results on the effect of $^{211}$At treating pollen and stigma on rice production.

## 2 Materials and method

### 2.1 Crop plant materials

Materials used in experiments were obtained from cultivated hybrid rice varieties, of which some materials were male sterile line. The florets were emasculated and bagged before flowering. Fresh pollen was collected during florescence from rice plants, and immediately used, or stored at low temperature.

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### 2.2 $^{211}$At test solutions

$^{211}$At was produced by irradiating a Bi-target with 27 MeV $\alpha$-particles using the $^{209}$Bi ($\alpha$, 2n) $^{211}$At reaction at the 1.2 m cyclotron of Sichuan University. $^{211}$At was isolated from the target by thermochromatography as described previously.[2] The produced free $^{211}$At solution were used for labeling pollen protein or directly in experiments. The $^{211}$At labeling pollen proteins of rice and sorghum were performed by conjugation with diazo salt of *p*-phenylenediamine intermediate.[3]

### 2.3 Tests for radiation effects on cells

The pollen was treated by soak with $^{211}$At test solution (7.4~74 kBq/ml). The stigma was treated with 3$\mu$l $^{211}$At test solution by means of microinjection. The radiation effects on cells were tested with different methods, such as fluorescein diacetate (FDA) for checking cell viability, including the permeability of cell membranes and the activity of esterase[4], aminonaphthylene sulfonic acid (ANS) for surface proteins of pollen and stigma[5], chlorotetracycline fluorescence (CTC) for $Ca^{2+}$ reaction on pollen surface,[6] ethidium bromide (EB) for DNA of pollen sperm nuclei[7], and Herr's clearing-squash technique[8] and the paraffin section for embryological observation.

### 2.4 Effect of $^{211}$At on seed setting of rice

Following tests were performed and compared.

2.4.1 Pre-pollination irradiation test. During flowering, pollination was first carried out, and

1~2 h later the pollinated stigma was treated with 3$\mu$l $^{211}$At test solution by means of microinjection.

2.4.2 Delayed pollination irradiation test. Stigmas of emasculated or male sterile florets were treated with 3$\mu$l $^{211}$At solution as the above, and 2~18 h later pollinated with fresh pollen of rice or sorghum.

2.4.3 Non-pollination irradiation test. Stigmas of emasculated or male sterile florets were treated with 3$\mu$l $^{211}$At solution as above, and bagged individually without pollination.

2.4.4 Using irradiated pollen pollination test. During flowering, fresh pollen were soaked with $^{211}$At solution, and pollination was carried out immediately.

## 3 Results

### 3.1 Radiation effects of $^{211}$At on pollen and stigma cells

The observations presented in Table 1 reveals that at a lower radioactivity (7.4 kBq/ml), $^{211}$At has obvious radiation effects on pollen and stigmatic cells of rice. This can be found from that the changes have taken place in the pollen viability (including structure and permeability of cell membrane, esterase activity), the surface protein properties of pollen and stigma, $Ca^{2+}$ distribution and reaction on pollen surface, and the conformation and property of DNA in the pollen sperm cells.

**Table 1** Cytochemical test for radiation effects of $^{211}$At (7.4~74 kBq/ml)

| Test methods | Test content | Experimental groups | Control group |
|---|---|---|---|
| FDA | Pollen | Lower or no viability | High viability |
| | Stigmatic cells | Viability (low dose) or no viability (high dose) | High viability |
| ANS | Surface proteins of pollen and stigma | negative reaction | positive reaction |
| CTC | $Ca^{2+}$ on pollen surface | negative reaction | positive reaction |
| EB | DNA in sperm cells | negative reaction | positive reaction |

### 3.2 Effect of $^{211}$At treatment on seed setting rates of rice

Groups I and II were treated with $^{211}$At-labeled pollen proteins of sorghum and rice, respectively, radioactivity was 14.8 kBq/ml. Groups III and IV were used free $^{211}$At solution, radioactivity was 7.4 and 14.8 kBq/ml, respectively. In group V, rice pollen was treated with 7.4 kBq/ml free $^{211}$At solution. In control group normal pollination was used without any treatment. All experimental groups in four tests were pollinated with rice pollen except group I of delayed pollination irradiation which was pollinated with sorghum pollen. The obtained seed setting rates from different experimental groups were given in Table 2.

It can be seen from Table 2 that seed setting rates of various experimental groups by treating pollen or stigma with 7.4 or 14.8 kBq/ml of $^{211}$At were notably lower than that of control group. The seed setting was seriously inhibited in three tests, delayed pollination irradiation, non-pollination irradiation and using irradiated pollen pollination, so far no seeds were produced in some tests.

**Table 2** Effect of $^{211}$At treating pollen and stigma on seed setting rates (%) of rice

| Test methods | Experimental groups | | | | | Control group |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| Pre-pollination irradiation | – | 48.3 | 34.9 | 30.9 | – | |
| Delayed pollination irradiation | 9.1 | 8.3 | 14.0 | 0 | – | 57.0 |
| Non-pollination irradiation | 0 | 4.1 | 0 | 0 | – | |
| Using irradiated pollen pollination | – | – | – | – | 1.3 | |

It was noteworthy that the pollen used for pollination on the stigma of rice was from sorghum and seeds could be produced normally in group I of delayed pollination irradiation test. But in other tests, setting seeds were resulted from pollination with rice pollen. In non-

pollination irradiation test, a few seeds were produced in group II. These seeds might originate from parthenogenetic reproduction. But this guess has yet to be definitely proved by the further research.

### 3.3 Preliminary cyto-embryological observation

Preliminary cyto-embryological observation indicated that the morphological structures of pollen and stigmatic cells treated by $^{211}$At had changed in varying degrees, and there were some abnormally expanded cell masses to be similar to embryo form in the embryo sacs. Also there was an abnormal ovule structure or not a normally developed embryo sac structure in some ovaries. However, it was found that there were normal embryos in most seeds produced from the delayed pollination and non-pollination irradiation tests. Seed germination tests indicated that these seeds could germinate to form seedlings. But there were some seeds which did not germinate at all, or developed into buds without roots. An interesting phenomenon was that some seeds treated by $^{211}$At, especially those from delayed pollination and non-pollination tests, showed black powder (possibly infected by rice blast fungus), while seeds from the control group did not.

## 4 Discussion

Some researchers have succeeded in inducing parthenogenetic plants in nicotiana using $\gamma$-ray irradiating pollen[9], and introducing exogenous DNA and gene transfer can also be carried out in the same way.[10,11] Compared with $\gamma$-ray, $\alpha$-particles emitted by $^{211}$At possess the characteristics of short range and strong ionization effect in tissue. It is possible to use $^{211}$At to specifically or orientationally act on some tissues and cells in plants, thus to produce more biological inducing effects. For example, when $^{211}$At enters into rice ovule, it may specially damage or inhibit the egg cell and proembryo development, stimulate and promote the development and formation of the adventitious embryo alone. All these are because the irradiation range of $^{211}$At is close to the size of egg, zygote and proembryo, and its half-life is approximate to the time required for the fertilization process in rice, which is generally between 5~7 h after flowering. Additionally, it is possible that the $^{211}$At internal irradiation technique may be used to induce the offsprings which have characters of androgenesis in plants. This may be achieved by pollination with fresh pollen after eggs or zygotes in the embryo sacs are killed by $^{211}$At firstly. In this case, after the male gamete cells enter the embryo sac they can probably develop into androgenetic embryos. Of course, parthenogenesis may be induced.

To apply short-lived $\alpha$-emitter $^{211}$At to study induced variation in plants is a new attempt. This work is only the exploratory experimental research. From the tests of radiation effect on cells and the effects of different irradiation treatments with $^{211}$At on seed setting and embryo development of rice , it has been demonstrated that $^{211}$At has injury effects on morphological structures and physiological functions of pollen, stigmatic cells and ovule or embryo sac cells, and has inhibition effect on seed setting. The abnormally expanded cell masses are observed really in some embryo sacs, especially, some seeds are obtained in non-pollination test. These results indicated that $^{211}$At can produce an obvious biological inducing effect on rice, and it is possible to apply $\alpha$-emitter $^{211}$At on the research and application of induced variation in plants.

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