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# Effect of gamma irradiation pretreatment on embryo structure and long-term germinating characteristics of rice seed**

*J. Wang* and Y. Yu*

Department of Biosysterms Engineering, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, PR China

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A b s t r a c t. After rice seed was irradiated, the embryonal structure (structure of embryo bud and radicle) was affected by $\gamma$-ray. The result revealed that the outline and venations of embryo and radicle were unclear with the increasing dose. These changes of embryonal structure caused by increasing irradiation dose were due to the increasing breakage degree of embryo (including embryo bud and radicle) cells. The influence of γ-ray irradiation on the structure of radicle was more significant than that of embryo. The results were confirmed by a germination experiment, which showed that the lengths of buds of irradiated rice were diminished, the roots of irradiated rice were found to disappeare, and the germination of irradiated rice was 0%. After long-term storage, the germination rate of irradiated rice seed increased and that of some irradiated samples were found to reach to comparatively high values after different storage time.

K e y w o r d s: gamma irradiation, rice, embryo, germination, micrology observation

## INTRODUCTION

Irradiation is a suitable method for crop preservation because it can effectively destroy moulds and insects without any pollution. The crop seeds are easily infected with insect and pests during storage. To combat these problem cereals, pulses and other stored products were preserved with chemical fumigation. Their use has created problems relating to health and environment. The use of irradiation has been proven to be very useful for the effective control of pests and reduce mass loss during post-harvest storage (Pietruszewski and Kania, 2010; Zepeda-Bautista, *et al*., 2010).

However, it was reported that the irradiation of wheat seeds reduces shoot and root lengths upon germination. Selvan and Thomas (1999) have evaluated the rooting characteristics and rate of root elongation in irradiated onions and shallots. It was testified by Yu and Wang (2005, 2006) that cells of rice and wheat could be destroyed by gamma irradiation, and it may affect the germination of rice and wheat seed. The effect of gamma irradiation on wheat is to inhibit the germination (Barros *et al*., 2002; Wang and You, 2000) and especially inhibits the development of root. It is clear that gamma irradiation is a kind of processing methods which affect the germination of grain.

In the former reports on the effects of gamma irradiation on germination of grain mentioned above, the germination experiments were concluded in three months after irradiation at farthest, and their research were mainly focus on low irradiation dose (0-1.0 kGy). Many researches in these reports demonstrated that the irradiated organism could self-repair in long term ( Boubriak *et al*., 1997; Martini and Almouzni, 1997; Mustonen *et al*., 1999). Little research has been reported on the long-term germination characteristics of irradiated rice (*Oryza sativa* L.) seed and the effect of gamma irradiation on embryo structure of rice seed.

The long-term germination characteristics of irradiated rice seed were investigated and microstructure observation was performed to explain the effect of gamma irradiation on the germination of rice seed.

*Corresponding author's e-mail: jwang@zju.edu.cn

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MATERIALS AND METHODS

Rice seed (Zhenong 1, late-cropping season japonica rice) harvested in October, 2003, from the Experimental Farm of Agronomy, Zhejiang University, was used. The moisture content of rice seed was 30% d.b., which was determined by drying five samples at 105°C till the mass of samples became constant (GB/5497-85, National Standard of China).

Five samples (500 g each sample) were irradiated before dried. After irradiation, experiment of micrology observation was carried out. Then the samples were dried and packed in polyethylene bags and stored in refrigerator at 5°C.

Rice seed samples were exposed to $^{60}Co$ source at an ambient condition at the Institute of Nuclear-Agriculture Sciences, Zhejiang University. The samples were exposed to controlled doses of 0, 2, 5, 8, and 10 kGy, respectively, with a dose rate of 1 kGy $h^{-1}$.

After irradiation, microstructures of samples were observed for determining the effect of irradiation on the embryonal structure. Micrology observation was done in the Institute of Plant-Protection Sciences, Zhejiang University, with the electronic microscope (XL30-ESEM, Philip, Holand) and the ion coater (IB-5 ION COATER, Philip, Holand). The rice embryo (including embryo bud and radicle) samples used in this experiment were separated from rice grain with knife (Fig. 1a). Then, under optical microscope, the embryos were cut into two parts with thin knife along the middle of embryo (Fig. 1b), that parts with whole shape of embryo bud and radicle were used in micrology observation (Fig. 1c).

Irradiated samples were symmetrically placed on a sifter drying with an air velocity of 0.5±0.1 m $s^{-1}$ and a low drying temperature of 40°C (Inprasit and Noomhorm, 2001). The samples were dried until it reached a final moisture content of 14.5±0.1% d.b., which represented the safe moisture value for rice storage.

For each sample, which had been dipped in water for one hour, in a 15 cm diameter Petri dish, one hundred seeds were symmetrically placed on two filter papers. Seeds were incubated under 12 h light and 12 h darkness for 14 days in growth chambers at 30°C (GB/T 3543.4-1995, National Standard of China). Seed germination was checked daily and the length of root and sprout were measured at the 14th day. Seeds were considered germinated when the root was 5 mm long (Bai *et al*., 2003). All germination experiment had there replications. The data in the figures are average values, and the standard errors are less than 12.46% of average values.

After irradiated at 0, 2, 5, 8, and 10 kGy, the first set of germination tests were commenced immediately, and the other sets of tests were done at intervals of two months. The experiments were conducted within two years.

RESULTS AND DISCUSSION

The effect of irradiation dose on embryo structure was shown in Fig. 2. The embryo structure of non-irradiated rice grain was intact. The outline and venations of embryo bud and radicle were clear and easy to distinguish (Fig. 2a). With the increasing irradiation dose, the venations of embryo bud were decreased and blurry gradually (Fig. 2b-e). Similar changes were more evidently represented in radicle. After irradiation with 2 kGy dose, the outline and venations of radicle were unclear, and those were hard to distinguish when the dose was higher than 5 kGy (Fig. 2b-e). It could also be seen from Fig. 2 that the influence of $\gamma$-ray irradiation on the structure of radicle was more significant than that of embryo bud.

The changes of venations of embryo bud and radicle caused by different irradiation dose were more distinctly in Figs 3 and 4. The venations of embryo bud and radicle were actually composed of the gaps of adjacent two layers of cells. The cells of embryo bud and radicle were destroyed by $\gamma$-ray irradiation, and the cytoplasm was found to flow out of cell and filled in those gaps. With the increasing irradiation dose, the breakage degree of cells was increased and more cytoplasm flowed out of cells and filled in those gaps. It was likely to be the reason for the changes of embryo structure caused by increasing $\gamma$-ray irradiation.

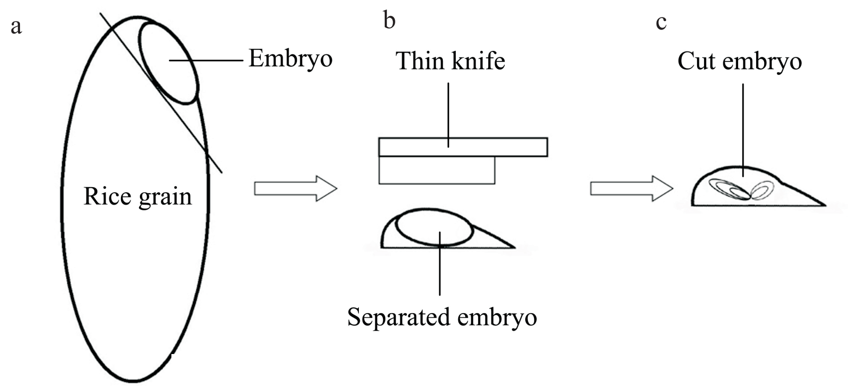


**Fig. 1.** Sketch of process of making sample for micrology observation.

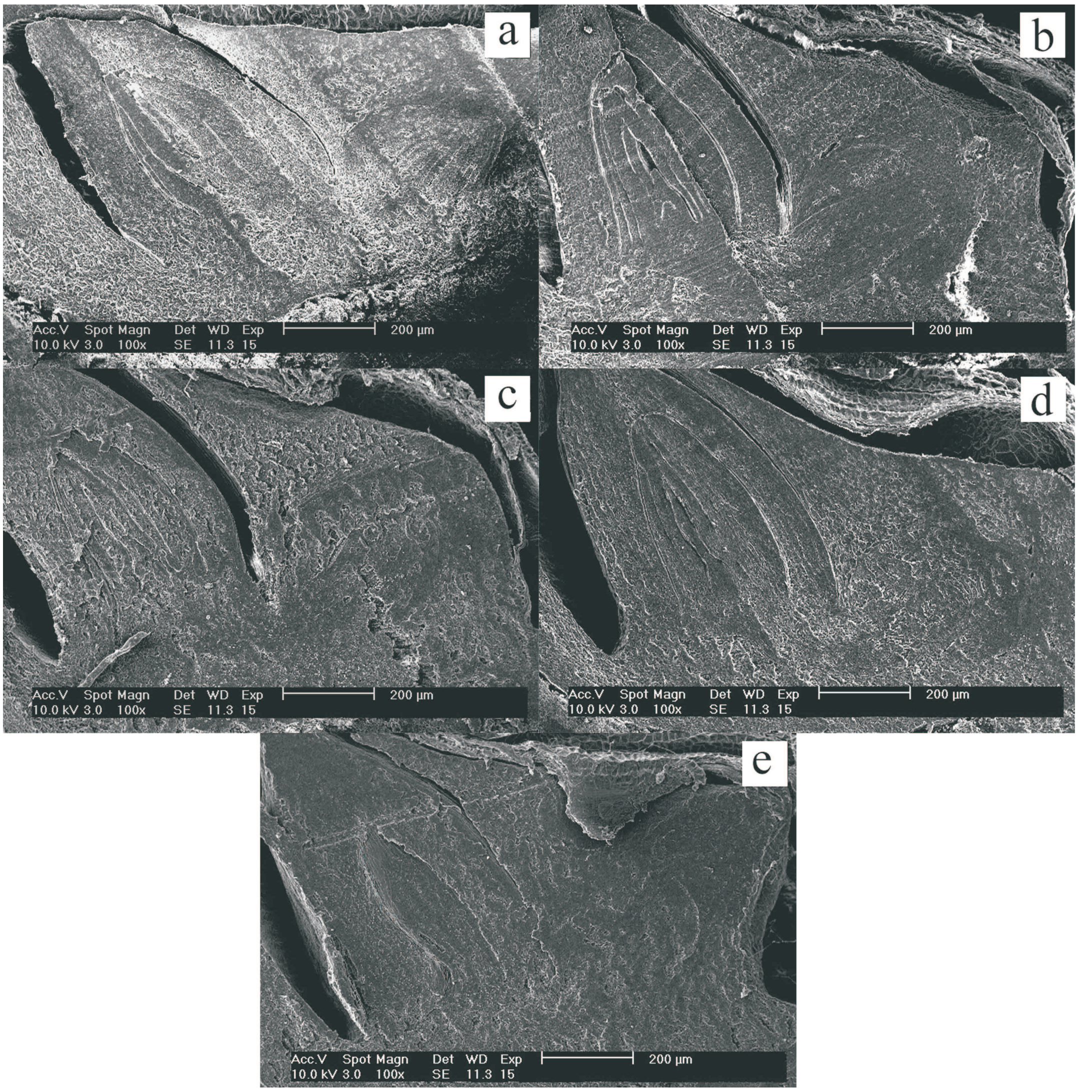


**Fig. 2.** Effect of irradiation dose on the structure of whole rice embryo: a – 0, b – 2, c – 5, d – 8, e – 10 kGy.

The results of germination experiment which commenced immediately after irradiation are shown in Table 1 (data measured at 14th day). It was evident that the germination rate of rice seed was significantly affected by gamma irradiation, and the germination rates were decreased to 0 % when dose was higher than 2 kGy. The effects of different dose on germination characteristics were represented in bud occur rate (the percentage of rice grain owning bud). With the increasing dose, the bud rates decreased from 87 to 52%, and the mean length of bud decreased from 0.6 to 0.4 cm. The bud rate and mean length of bud of irradiated samples evidently decreased than that of non-irradiated rice sample (98%, 6.9 cm). The root occur rate and mean length of root, however, decreased to 0% and 0 cm, respectively, when dose was higher than 2 kGy. The conclusion draw from data of Table 1 was that the influence of $\gamma$-ray irradiation on root was more significant than that on bud, and this conclusion is collaborated by the micrology observation mentioned above. Because the seed were considered germinated when the root was 5 mm long, the effects of $\gamma$-ray irradiation on germination rate were mainly due to the effects on root.

The results of long-term germination experiment are shown in Table 2 (data measured at 14th day). Firstly, the germination rates of irradiated samples could increase from 0% after different terms storage. Secondly, the terms needed for the irradiated samples to increase from 0% were prolonged with the increasing dose. The terms needed for the samples irradiated at 2, 5, 8, and 10 kGy were about 12, 16, 20 and 22 months, respectively. Thirdly, after the germination rate reaching a certain value (95, 90, 85% for 0, 2, 5 kGy, respectively), the germination rates of irradiated samples decreased like non-irradiated rice sample, and this certain germination rate was comparatively high. For example, the germination rate of samples irradiated at 2 and 5 kGy could increase to about 90 and 85% in two years, and higher

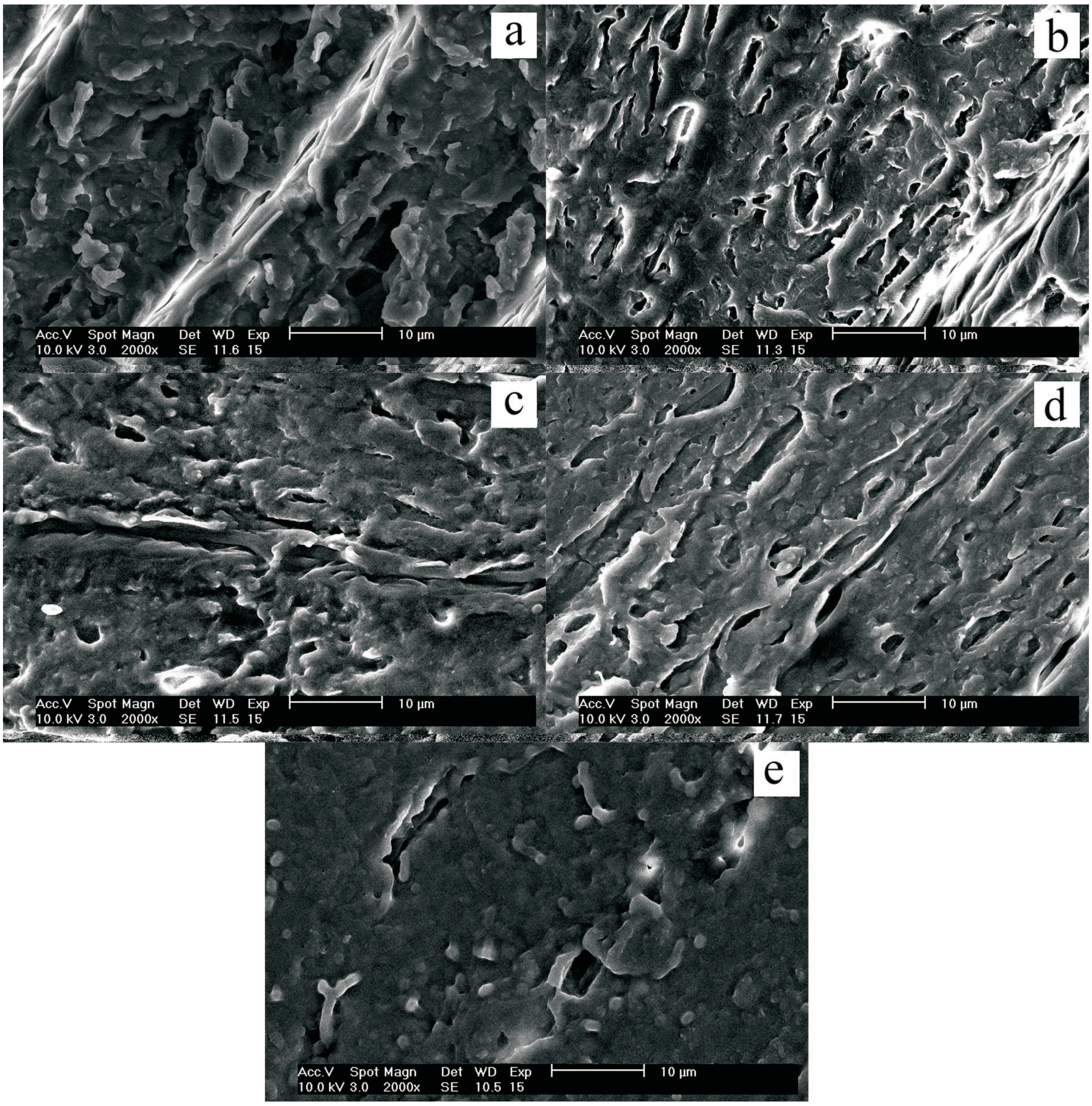


**Fig. 3.** Effect of irradiation dose on the structure of embryo bud: a – 0, b – 2, c – 5, d – 8, e – 10 kGy. The greyish strias in (a) is the venations of embryo bud, and from (b) to (e), the venations is unclear with the increasing dose.

**T a b l e 1.** Results of germination experiment commenced immediately after irradiation (measured at 14th day)

| Parameter | Irradiation dose (kGy) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 5 | 8 | 10 |
| Germination rate (%) | 97 | 0 | 0 | 0 | 0 |
| Bud occur rate 1 (%) | 98 | 87 | 77 | 65 | 52 |
| Mean length of bud (cm) | 6.9 | 0.6 | 0.5 | 0.5 | 0.4 |
| Root occur rate 2 (%) | 97 | 0 | 0 | 0 | 0 |
| Mean length of root (cm) | 5.6 | 0 | 0 | 0 | 0 |

1 – bud occur rate: the percentage of rice grain owning bud, 2 – root occur rate: the percentage of rice grain owning root.

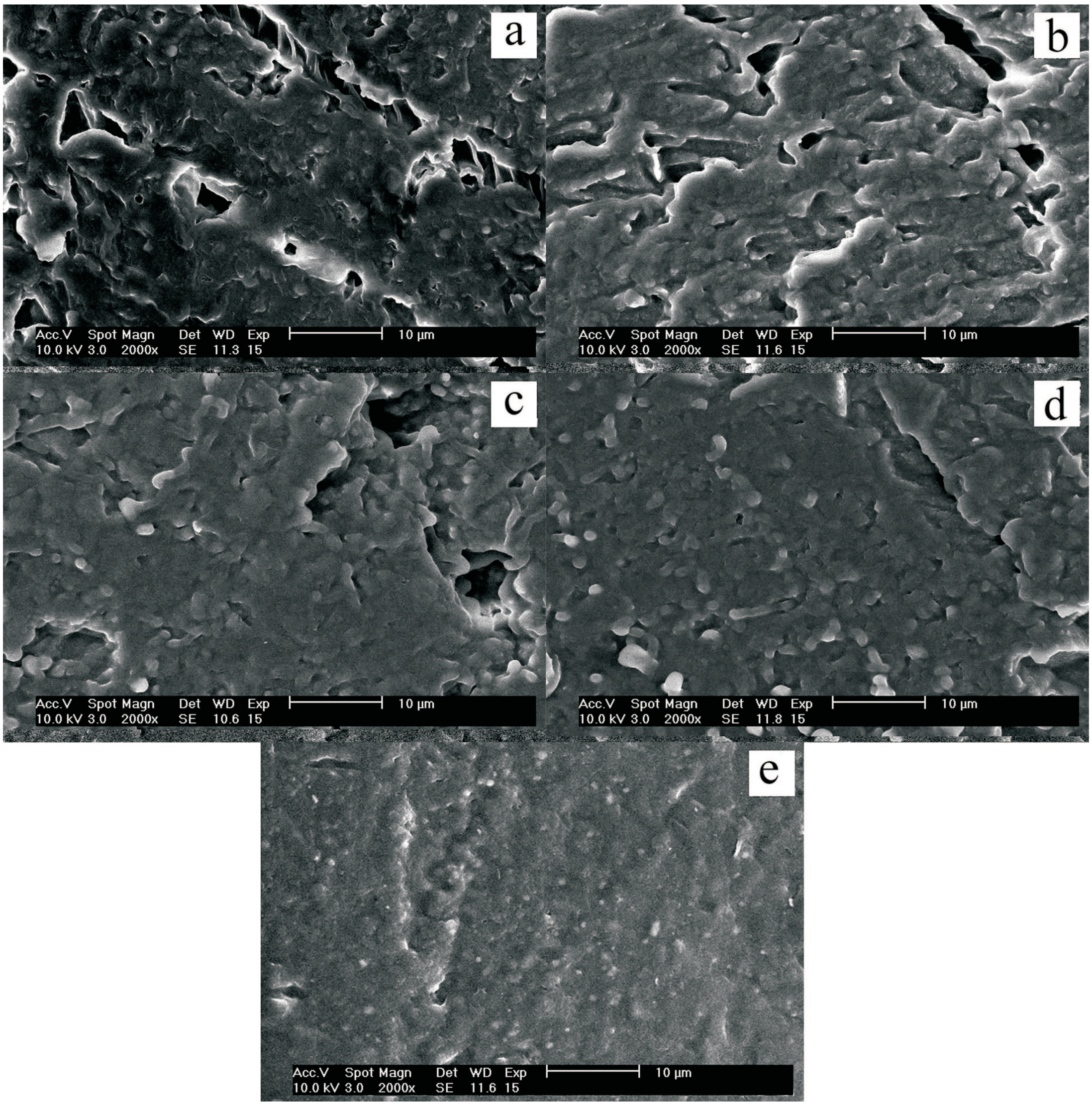


**Fig. 4.** Effect of irradiation dose on the structure of radicle: a – 0, b – 2, c – 5, d – 8, e – 10 kGy. The greyish strias in (a) is the venations of radicle, and from (b) to (e), the venations is unclear with the increasing dose.

germination rates may emerge from two adjacent experiments periods. Fourthly, after long-term storage, germination rates of some irradiated samples were higher than that of non-irradiated rice sample. For example, after 22 months storage, the germination rates of irradiated at 2 and 5 kGy (86 and 84%, respectively) were higher than that of non-irradiated sample (73%). Those changes of germination rates of irradiated samples might be due to the self-repair of irradiated organism (Boubriak *et al.*, 1997; Martini and Almouzni, 1997; Mustonen *et al.*, 1999).

The effect of storage time on germination characteristics of non-irradiated rice sample and 2 kGy irradiated rice sample were shown in Fig. 5. The points in these figures express the number of germinated seeds in each day during germination period. For the non-irradiated rice sample, it was evident that, with the increasing storage time from 0 to 24 months, the peak value of germinated seeds decreased and the time needed to reach the peak value increased (Fig. 5a). For the 2 kGy irradiated rice sample, and for an the increasing storage time from 14 to 18 months, the peak value of germinated seeds increased and the time needed to reach the peak value decreased (Fig. 5b). With an increased storage time from 18 to 22 months, the peak value of germinated seeds decreased. Figure 6 also shows that the germination characteristics may be similar to that of non-irradiated rice sample if storage time was prolonged.

**T a b l e 2.** Germination rate of long-term germination experiment (%)

| Months after irradiation | Irradiation dose (kGy) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 5 | 8 | 10 |
| 2 | 95 | 0 | 0 | 0 | 0 |
| 4 | 94 | 0 | 0 | 0 | 0 |
| 6 | 94 | 0 | 0 | 0 | 0 |
| 8 | 91 | 0 | 0 | 0 | 0 |
| 10 | 89 | 0 | 0 | 0 | 0 |
| 12 | 85 | 2 | 0 | 0 | 0 |
| 14 | 83 | 27 | 0 | 0 | 0 |
| 16 | 82 | 65 | 6 | 0 | 0 |
| 18 | 79 | 90 | 31 | 0 | 0 |
| 20 | 74 | 87 | 68 | 9 | 0 |
| 22 | 73 | 86 | 84 | 27 | 3 |
| 24 | 70 | 82 | 85 | 59 | 22 |

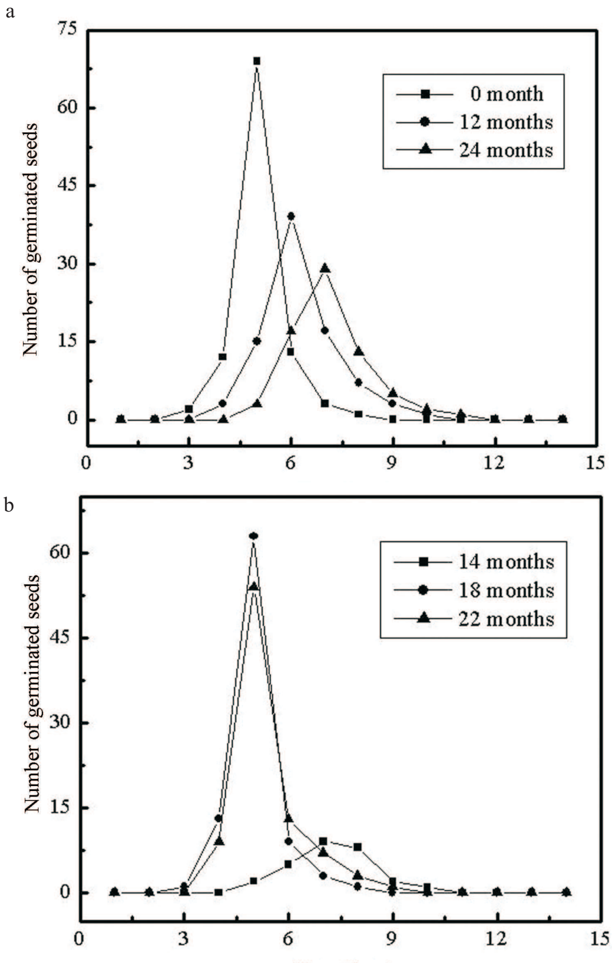


**Fig. 5.** Effect of storage time on germination characteristics of: a – non-irradiated rice, b – 2kGy irradiated rice.

## CONCLUSIONS

1. The embryo structure of rice was affected by $\gamma$-ray irradiation. The result shows that the outline and venations of embryo bud and radicle were unclear. These changes of embryo structure caused by increasing irradiation dose were due to the increasing breakage degree of embryo cells.

2. The germination experiment commenced immediately after irradiation, the lengths of buds of all irradiated rice minished consumingly, the roots of all irradiated rice were disappeared, and the germination rate of irradiated rice was all considered 0%.

3. After long-term storage, the germination rates of irradiated samples increased, and that of some irradiated samples could reach to the comparatively high values in two years after different store periods.

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