Effect of Proton Beam Irradiation on M₁ Seeds and Seedling Growth in Rice

Joohyun Lee¹, A-Rim Lee², Soon-Wook Kwon^{2*}

¹Department of Applied Bioscience, Konkuk University, Seoul 05029, Korea

²Department of Plant Bioscience, Pusan National University, Miryang 50463, Korea

ABSTRACT This study was carried out to evaluate effect of proton beam irradiation on M₁ seed germination and seedling growth. For dosage effect, mature and healthy Supersami2 seeds were irradiated with 0, 204, 395, 502, and 700Gy. The traits for germination were not affected by dosage effect of proton beam irradiation. Germination rate evaluated at 7 days after imbibition ranged from 93.3% to 98.7%; germination vigor ranged from 59.3% to 68.7% where in the dose of 700Gy showed the lowest value of 59.3%. The average days of germination ranged from 1.36 to 1.48. The seedling growth was affected by the dosage. Withered rate (withered plants after germination) was increased as the dose increased. The withered rate of 53.9% was detected in 395Gy and no plant survived in 700Gy. In the ~400Gy treatment, the sensitivity of the traits of germination among Dianxi4, Jeogjinju, MS11(Maligaya Special 11), and Superjami2 was not different while the withered rate was different: 9.7% in MS11, 32.1% in Dianxi4, 53.9% in Superjami2, and 59.7% in Jeogjinju. Based on the germination rate and withered rate, it can be suggested that 350Gy to 450Gy is a starting point for applying proton beam irradiation to rice seed for mutation breeding.

Keywords Rice, Mutant, Proton beam Irradiation

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major food crops and food for more than half of the world's population (Singh *et al.* 2005). There is large variation in rice, especially in the two subspecies, *japonica* and *indica*, showing differences in plant architecture, agronomic and physiological features (e.g., stress resistance, cold tolerance, and seed quality) (Hu *et al.* 2014). Recently rice breeders have faced various challenges. Customers demand diverse functional and healthful rice (Ham *et al.* 2015). Climate change also threatens stable rice production. For rice breeders, it is a continuous task to find the new genomic variation that can be directly used in rice breeding programs.

The general method of creating new strains and developing new rice varieties is the cross-breeding method. However, cross-breeding is limited in developing new and diverse traits of crop cultivars for the following reason. It is

limited to exchanges between the same or very closely related species because of crossing barriers. In some cases, the desired characteristics of interest frequently do not exist in any related species. Mutation-breeding is one of the complementary methods to generate a new variation then develop new varieties. Generally, mutation is induced by radiation such as gamma-rays, X-rays, ion irradiation and chemicals such as ethyl methane sulphonate (EMS), n-methyl nitrosourea (MNU), and sodium azide (SA). These mutagens have been widely used to induce a great number of functional variations in rice and others crop (Talebi *et al.* 2012; Bhat *et al.* 2007).

According to FAO/IAEA Mutant Variety Database, 815 varieties are officially registered as mutants of rice (*Oryza sativa* L.) in 2015. The effect and efficiency of the mutagens are various and, in rice the generally used mutagens are gamma ray irradiation (Kawai and Inoshita 1965), EMS (Rajarajan *et al.* 2014), SA (Awan *et al.* 1980), and ion

Received October 21, 2015; Revised November 10, 2015; Accepted November 11, 2015; Published December 31, 2015

*Corresponding author Soon-Wook Kwon, swkwon@pusan.ac.kr, Tel: +82-55-350-5506, Fax: +82-55-350-5509

beam irradiation (Hayashi *et al.* 2007).

Recently, proton beams have attracted attention in the fields of plant breeding (Shikazono *et al.* 2003). Proton beams had higher energy than gamma rays and worked with localized strength. A characteristic feature of ion beams is their ability to deposit high energy on a target, densely and locally, as opposed to low linear energy transfer (LET) radiation such as gamma rays and X-rays (Yang and Tobias 1979; Tanaka 1999). Mutation induction with ion beams, using various plants, has been attempted since the 2000s in Korea (Tanaka *et al.* 1997).

In this study, by treating proton beam irradiation to several rice cultivars, we evaluated the effects of proton beam irradiation on M₁ plant for growth characteristics.

MATERIALS AND METHODS

Plant material

Mature and healthy rice seeds harvested in 2014 were used. The varieties used in this study are Superjami2 (Ham *et al.* 2015); a japonica type pigmented rice variety, MS11 (Maligaya Special 11); a japonica rice, Jeogjinju (Lee *et al.* 2001); a japonica type pigmented rice variety, and Dianxi4; a japonica type Chinese variety.

Proton beam irradiation

Rice seed samples were irradiated by using 57-MeV protons the 100 MeV proton linear accelerator in the Korea Multi-purpose Accelerator Complex (KOMAC) in Gyeongju city. The Bragg peak of proton beam was spread out by the ridge filter-type modulator designed to obtain uniform depth-dose distributions (Jung *et al.* 2013). The peak beam current was 1 mA, repetition rate was 1 Hz, and the beam pulse width was 100 µsec. The irradiation dose rate was 0.6 Gy/pulse. The radio-chromic film (Gafchromic, HD-V3) was used for the absorbed dose measurement of each sample. The absorbed doses ranged from 100 to 700Gy. The dose uniformity was under ±10% in a 60-mm-diameter culture plate.

Germination test

After seed disinfection, M₁ rice seeds were placed on a

sheet of tissue paper in 90 × 15 mm petri dishes (SPL life sciences, 10090). Fifty seeds were placed in one petri dish. Then, 20 mL of distilled water was poured into each petri dish. Seeds were germinated at 25°C in the dark in an incubator for seven days. Experiments were replicated three times. A seed was counted as germinated daily if it had a radical >1 mm long (Lee *et al.* 1998). Germination rate, germination vigor and average days of germination were measured as described below.

Germination rate = (total number of seeds germinated by 7days/number of total seeds placed) × 100

Germination vigor = (total number of seed germinated by the day when the most seeds germinated/number of total seeds) × 100

Average days of germination =

Σ (number of the germinated seeds × the accumulated days for seed germination) / the number total of germinated seeds

Three replications were conducted and SAS software was used for ANOVA.

Investigations on growth characteristics

All of the germinated M₁ seeds were carefully placed on a 40-cell plug pot (50 × 50 × 140 mm cell). A seed was placed in each single plug. The commercial soil specially designated for rice seedlings was used. The general cultivating method for M₁ rice seedlings followed the conventional method by Pusan National University obtained. After sowing, withered plants were counted every day until 10 days after sowing. The leaf age which is the number of leaf except coleoptile and plant height were measured at 20 days after sowing.

RESULTS

Effect of proton ion beam radiation on rice seed germination

For the evaluation of dosage effect, four different doses of ion beam irradiated to the superjami2 (Table 1). For the germination rate, the germination rate for all doses including control, ranged from 96% to 99%. At the 502Gy treatment, the germination rate showed the lowest value of

Table 1. Dosage effect of proton beam irradiation on the germination traits of superjami2.

Cultivars	Dose (Gy)	Germination rate (%)	Germinating vigor (%)	Average days of germination
Superjami2	0	98.7±2.3	68.7±11.4	1.37±0.14
	204	97.3±1.2	66.7±3.1	1.36±0.09
	395	98.7±1.2	66.0±3.5	1.38±0.02
	502	93.3±6.4	60.0±14.4	1.41±0.18
	700	96.0±2.0	59.3±7.0	1.49±0.07

Table 2. Effect of proton beam irradiation on the germination traits.

Cultivars	Dose (Gy)	Germination rate (%)	Germinating vigor (%)	Average days of germination
Superjami2	395	98.7±1.2	66.0±3.5 ^{c*}	1.38±0.02 ^a
MS11	440	100	100 ^a	1 ^c
Jeogjinju	390	100	95.3±1.2 ^b	1.05±0.01 ^b
Dianxi 4	420	98.7±2.3	98.7±2.3 ^{a,b}	1 ^c

*Mean with same letters are no significantly different in DMRT ($p < 0.05$).

Table 3. Dosage effect of proton beam irradiation on the seedling growth of superjami2.

Cultivars	Dose (Gy)	Withering rate (%)	Average Leaf age
Superjami2	0	10.0	3.9
	204	12.6	3.9
	395	53.9	2.3
	502	83.1	0.8

93%. However this result was not associated with the dosage because the germination rate in the higher dose of 700Gy showed 96% and the difference of the germination rate among the treated dose and control was not statistically significant. For germination vigor, they ranged from 59% to 68% wherein the dose of 700Gy showed the lowest value of 59%. The average days of germination ranged from 1.36 to 1.48. As the dose was increased, the average days of germination were more required.

To evaluate the different effect among the varieties, the germination traits for Superjami2, MS11, Jeogjinju, and Dianxi4, three varieties MS11 at the ~400Gy were evaluated (Table 2). No difference was detected in germination rate. Even though MS11 and Dianxi4 showed better germination vigor and the average days of germination compared to those of Superjami2, this result does not indicate that the Superjami2 is more sensitive to

proton beam irradiation because the performance of Superjami2 in the control showed similarities with those of Superjami2 in the 395Gy. Thus, the different germination performance resulted not from the sensitivity to proton beam irradiation, but from the genetic differences.

Effects of proton ion beam irradiation on rice seedling growth

Unlike germination traits, dosage effect of the irradiation was clearly detected for the traits of seedling growth (Table 3). As we described previously, only germinated M₁ seeds were sown. For the Superjami2, it showed 10% withering rate even in the control. The withering rate was severely increased. At the dose of 204Gy, the withering rate was 12% showing a similar rate to that of the control where the withering rates were 53.9% at 395Gy, 83% at 502Gy, and 100 at 700Gy. All the seedlings were measured to calculate 20 day old seedling leaf age. The control at the dose of 204Gy showed 3.9 and the leaf ages of all of the individual seedlings were similar, while the leaf age was 2.3 at 395Gy and 0.8 at 502Gy. At 395Gy and at 502Gy, many of the rice seedlings showed retarded growth and eventually withered. Some seedlings showed a leaf age of 3-5 days like the control, however, unlike the control seedlings and those dosed with 204Gy, in both at the dose of 395Gy and 502Gy.

Fig. 1. Effect of proton beam irradiation of ~ 400 Gy on rice seedling growth.

To compare sensitivity among the varieties, the withered rate was compared among Superjami2, Jeogjinju, MS11, and Dianxi4 at ~ 400 Gy (Fig. 1). Interestingly, the withered rate was only 9.7% for MS11 and that of Dianxi4 was 32.1% while Superjami2 and Jeogjinju showed 53.9% and 59.7%, respectively.

DISCUSSION

In the mutagen treatment and mutation breeding program, the survival rate is important. Here, we measure the survival rate in terms of the withered rate. According to the results of the germination rate in the ranges of the treated dose, proton beam irradiation did not affect the germination rate significantly. However, the withered rates indicate that even the germinated seeds were lethal in the developmental seedling stage. In this report, we used the term withered rate instead of survival rate or lethal rate because the survival rate and lethal rate include the rice seeds whose germination ability was affected by mutation. The germination rate was not affected in our experiment.

Interestingly, we did not detect sensitivity among the tested varieties in the germination rate. Moreover, the dosage effect was not significant even at 700Gy where all of the germinated seedlings were lethal during seedling stage. This result may suggest that there are relatively few genes involved in the germination process, thus the chance

of mutation on these genes is low. In mutation breeding with gamma ray irradiation, a survival rate of 40-60% in comparison to the control plants is taken as a criterion for a promising radiation treatment (Van Harten 1998). The withered rate of 53.9% was detected at 395Gy and increase of the withered rate was positively correlated to the increase in dose. Similar results have been reported in rice (Cheema and Atta 2003; Sareen and Koul 1999). The leaf age was used as an index in determining the biological effects of various physical irradiations in rice (Konzak *et al.* 1972). At above 200Gy, the average leaf age significantly decreased, but there was large variation, representing that the damage in the biological process varied among the individuals. Based on the criterion of the lethal rate, ~ 395 Gy, is the possible suggestion in rice, however we also this suggestion has limitation because of the fact that our results only include evaluation of M_1 seedling stage, the fertility of M_1 and the ratio of emerging mutant in the M_2 generation cannot be predicted. In addition, we detected that MS11 variety showed less than 10% withered rate at 440Gy, suggesting that the sensitivity among the variety is relatively large. In conclusion, for applying proton beam irradiation to rice seed for mutation breeding, 350Gy to 450Gy is suggested as a starting point then adjust the dose depending on the breeder's purpose and variety.

ACKNOWLEDGMENTS

This work was supported by National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP:Ministry of Science, ICT and Future Planning, No. NRF-2015M2B2A4033154) and Pusan National University Research Grant, 2013.

REFERENCES

- Awan MA, Konzak CF, Rutger JN, Nilan RA. 1980. Mutagenic effects of sodium azide in rice, *Crop Science*. 20: 663-68.
- Bhat RS, Upadhyaya NM, Chaudhury A, Raghavan C, Qiu F, Wang H, Wu J, McNally K, Leung H, Till B, Henikoff S, Comai L. 2007. Chemical- and irradiation-induced mutants and tilling, p.148-180. In: *Rice Functional Genomics*. Springer, Newyork.
- Cheema AA, Atta BM. 2003. Radio-sensitivity studies in basmati rice. *Pak. J. Bot.* 35: 197-207.
- Ham TH, Kwon SW, Ryu SN. 2015. A rice variety ‘Superjami 2’, with large-grain and high contents of cyaniding 3-glucoside. *Korean J. Breed. Sci.* 47: 299-305.
- Hayashi Y, Takehisa H, Kazama Y, Ichida H, Ryuto H, Fukunishi N, Abe T. 2007. Effects of ion beam irradiation on mutation induction in rice. *Cyclotrons and their applications*. 18th International Conference. Giardini Naxos, Italy.
- Hu C, Shi J, Quan S, Cui B, Kleessen S, Nikoloski Z, Tohge T, Alexander D, Guo L, Lin H, Wang J, Cui X, Rao J, Luo Q, Zhao X, Fernie AR, Zhang D. 2014. Metabolic variation between japonica and indica rice cultivars as revealed by non-targeted metabolomics. *Sci. Rep.* 4: 5067.
- Jung MH, Ra SJ, Kim KR. 2013. Design of a ridge filter for spread-out Bragg-peak production in a pulsed 100-MeV proton beam. *Korean Phys. Soc. Journal.* 63: 1441-1445.
- Kawai T, Inoshita T. 1965. Effects of gamma ray irradiation on growing rice plants—I: Irradiations at four main developmental stages. *Radia. Bot.* 5: 233-IN11.
- Konzak CF, Wickham IM, De Kock MJ. 1972. Advances in methods of mutagen treatment. pp 95-119. In: *Induced mutations and plant improvement*. IAEA. Vienna.
- Lee JH, Jeong KH, Choi YK, Hwang HG, Moon HP, Choi HC, Yang SJ, Kim MK, Cho SY, Hwang KH, Yea JD, Baek MK, Hong HC, Jung OY, Shin YS, Ryu HY. 2001. A New Early-Maturing, Reddish-Brown Pigmented Rice ‘Jeogjinju-byeo’. *Treat. Crop Res.* Vol.2: 33-40
- Lee SS, Kim JH, Hong SB, Yun SH. 1998. Effect of humidification and hardening treatment on seed germination of rice. *Korean J. Crop Sci.* 43: 157-160.
- Rajaraman D, Saraswathi R, Sassikumar D, Ganesh SK. 2014. Effectiveness and efficiency of gamma ray and ems induced chlorophyll mutants in rice ADT(R) 47. *G. J. B. A. H. S.* 3: 211-218.
- Sareen S, Kaul V, Koul AK. 1999. Resource allocation in gamma ray treated plants of *Plantago ovata* Forsk. *Crop Improvement.* 25: 38- 45.
- Singh N, Kaur L, Sodhi NS, Sekhon KS. 2005. Physicochemical, cooking and textural properties of milled rice from different Indian rice cultivars. *Food Chemistry.* 89: 253-259.
- Talebi AB, Talebi AB, Shahrokhifar B. 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in malaysian rice (cv. MR219) for lethal dose determination. *Am. J. Plant Sci.* 3: 1661-1665.
- Tanaka A. 1999. Mutation induction by ion beams in *Arabidopsis*. *Gamma Field Symposia* 38: 19-28.
- Tanaka A, Tano S, Chantes T, Yokota Y, Shikazono N, Watanabe H. 1997. A new *Arabidopsis* mutant induced by ion beams affects flavonoid synthesis with spotted pigmentation in testa. *Genes Genet. Syst.* 72: 141-148.
- Van Harten AM. 1998. *Mutation breeding*. Cambridge University Press. Cambridge. p.207.
- Yang TC, Tobias CA. 1979. Potential use of heavy ion radiation in crop improvement. *Gamma Field Symposia.* 18: 141-151.