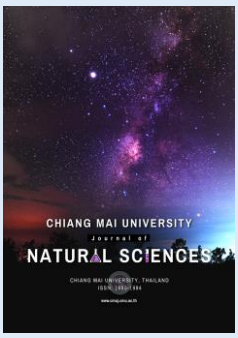

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Corresponding author:
Vichai Puripunyavanich,
E-mail: vichaipuri@gmail.com

Research article

Effects of Benzyladenine on *in vitro* 'Hom Rangsi' Rice and Induction of Aluminum Acid Tolerance Lines by Gamma Irradiation

Vichai Puripunyavanich*, Vararas Khamvarn, and Somjai Ngamjob

Thailand Institute of Nuclear Technology, Nakhon Nayok 26120, Thailand

Abstract 'Hom Rangsi' was the non-photoperiod aromatic mutant rice which derived from fast neutron radiation KDML 105. 'Hom Rangsi' seeds were cultured on MS solid medium without any supplemented for a week. And then, all explants were placed on MS (Murashige and Skoog, 1962) medium supplemented with 0, 5, 10, 15, 20, 25 mg/L BA (benzyladenine) for multiple shoot induction. The optimal concentration of BA for induced multiple shoot induction of 'Hom Rangsi' line was MS + BA 25 mg/L, the highest number of shoots were 5.38 shoot/seed. The following experiment was done, irradiated 'Hom Rangsi' seeds with 0, 100, 200, 300, 400 Gy gamma ray which cultured on MS solid medium supplemented with 400 mg/L Al^{3+} pH 2.9 were selected for acid tolerance lines. After six weeks cultured, the survivals of irradiated plantlets were 86.32, 77.78, 58.95, 58.95, 21.87% and the height of irradiated plantlets were 8.4, 8.3, 6.7, 6.6, 6.1 cm respectively without any shoot budding. All survival plantlets were transferred to suitable MS + BA 25 mg/L medium which discovered from the first experiment for multiple shoot budding. After six weeks cultured, the maximum of 5.24 shoots/plantlet were found from 300 Gy irradiation significantly and followed by 400, 200, 0 and 100 Gy irradiation treatments which gave 4.55 and 4.41, 4.37 and 4.31 shoots/planlet respectively..

Keywords: Acidic tolerance, Breeding, *In vitro*, Irradiation, Rice

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INTRODUCTION

'Hom Rangsi' rice was a mutant line derived from irradiated fast neutron on KDML105 (Puripunyavanich et al., 2018). 'Hom Rangsi' was similar to KDML105 but there were two major characteristics different from KDML105. The first one, 'Hom Rangsi' was photoinsensivity variety which can be planted all year round while KDML105 was strongly long day photosensitivity rice (Wangcharoen et al., 2015, Yano et al, 2000). The second one, 'Hom Rangsi' had short and rather wide plant compared with taller and narrower plant shape of KDML105. For aroma quality, various aromatic or fragrant rice including KDML105 and 'Hom Rangsi' contain a volatile substance called 2-Acetyl-1-pyrroline (2AP). In KDML105, one gram of aromatic white rice contains 2AP 0.40-0.90 ppm and 1.00-2.00 ppm in brown rice while one gram of white rice 'Hom Rangsi' contains 2AP 0.98-1.06 ppm and 0.99-1.07 ppm in brown rice. The milled rice is transparent and hard texture with good milling quality. The cooked rice is soft and contains a natural fragrant aroma. Anyway, Hom 'Rangsi' is susceptible to almost major diseases and insect pests as KDML105 (Rice Department, 2010; Chongkid et al, 2014). Also, drought, salty or acidic soil are devastating abiotic stress, since its effect on the crop may range from a minor reduction of 15% in yield to a loss of more than 50% as as KDML105 too. (Siangliw et al, 2007). Aroma level of 'Hom Rangsi' is similar to KDML 105 rice which both were highly influenced by environmental factors in the production area such as soil quality, soil salinity, mineral water, weather (Kong-ngern et al., 2011). While annual production of KDML105 is only 3–4 million tons of paddy rice, which is not enough for local market and world market demand (Pitiphunpong et al., 2011), therefore, any varieties which derived from KDML105 are need to support local and world market demand although the market price may cheaper than KDML105. In the present time 'Hom Rangsi' was on registration process in Plant Varieties Protection Office, Department of Agriculture.

The agricultural area of Nakhon Nayok province is mainly rice fields which covers an area of approximately 75,920 rai but the soil type in this area is the Ongkharak soil set. The pH range is 4.0 or lower, which is a barrier to the absorption of phosphorus and nitrogen of plants. In addition, the Ongkharak soil also contains high amounts of iron and aluminum which will be toxic to plants growth. The aluminum caused the plants to produce less roots and the roots become unusually swollen and not able to absorb enough nutrients resulting produce low yield crops. In another aspect, the selection of aroma rice varieties test planting in experimental plots requires a long time. Therefore, tissue culture techniques will be used paralleled with mutation breeding (Hutchinson et al, 1985). To screen in laboratory along with the test in the greenhouse and finally, convert the acid soil tolerance lines to the target field area. The selection of acid soil tolerance rice varieties in the tissue culture laboratory could be done using acid addition and aluminum compounds into the rice tissue culture media. Then selected acid soil tolerance rice that can grow in acidic conditions in the tissue culture laboratory. After laboratory scale, it will be tested at the greenhouse level and on a larger experimental field plot.

One of the benefits of gamma radiation is that it can be used for plant mutation breeding, which leads to create new characters such as disease resistance, blast resistance, flood tolerance, drought tolerance, salt tolerance and acid tolerance. The objective of this research project was to solve the problem of aroma rice production in the acid soil area in Nakhon Nayok and Chachoengsao provinces by using gamma irradiation and tissue culture techniques. At the first experiment, study on appropriate BA (benzyladenine) concentration on the growth of 'Hom Rangsi' rice *in vitro*. After that, study the appropriate dose of gamma ray on 'Hom Rangsi' rice *in vitro* and selected the survival acid tolerance plantlets after treated by liquid $AlCl_3$ at 400 mg/L concentration. Due to the location of Thailand Institute of Nuclear Technology is in Nakhon Nayok, any research project which is targeted directly to farmers in the area considered to be the greatest benefit to the public relationship.

MATERIALS AND METHODS

Effects of various concentrations of BA on the growth of 'Hom Rangsi' rice *in vitro*

Seeds of 'Hom Rangsi' rice were surface-sterilized and cultured on MS solid medium without hormone supplements for a week. Then, the explants were transplanted on to MS medium supplemented with BA at 0, 5, 10, 15, 20, and 25 mg/L for multiple shoot induction. This medium was used throughout the study with BA and without any growth regulators incorporated. The pH was adjusted to 5.8. All cultures were incubated in a culture room maintained at 25°C ± 2°C under 16 h/8 h light/dark cycle.

Effects of various doses of gamma ray on 'Hom Rangsi' rice *in vitro*

Hom-Rangsi seeds were irradiated by gamma radiation (GIC Multipurpose Irradiator, Paul Stephens Consultancy Ltd, England) at Irradiation Center, Thailand Institute of Nuclear Technology. One hundred seeds of 0, 100, 200, 300, 400 Gy irradiated 'Hom Rangsi' rice were surface-sterilized and cultured on MS solid medium without any hormone for a week. Then, the explants were transplanted on to MS solid medium supplemented with 400 mg/L liquid AlCl3 without any plant growth regulators. The pH was adjusted to 2.9 for screening of acidic tolerance among various doses of gamma irradiation treatments. All cultures also were incubated in a culture room maintained at 25°C ± 2°C under 16 h/8 h light/dark cycle. The experiment was performed with a completely randomized design of 5 treatments and 3 replications.

Shoot induction of the surviving acidic-tolerant plantlets

All surviving acidic-tolerant plantlets from the second experiment were transplants onto the suitable medium MS supplemented with 25 mg/L BA from experiment 1 for multiple shoot induction. All cultures also were incubated in a culture room maintained at 25°C ± 2°C under 16 h/8 h light/dark cycle. Numbers of shoot budding, growth rate and plantlet color were recorded.

RESULTS

Effects of various concentrations of BA on the growth of 'Hom Rangsi' rice *in vitro*

Seeds of rice were surface-sterilized and cultured on MS solid medium without any hormone supplements for a week. Then, the explants were transplanted on to MS medium supplemented with BA at 0, 5, 10, 15, 20, and 25 mg/L for multiple shoot induction. After 8 weeks, shoots were successfully regenerated with the average yields of 2.00, 3.55, 3.55, 4.34, 4.45 and 5.38 shoots/seed, respectively. The optimal concentration of BA in MS medium for multiple shoot induction of 'Hom Rangsi' rice was MS + BA 25 mg/L; the highest average regeneration rate was 5.38 shoots/seed (Table 1).

Table 1. Effect of various concentrations of BA on the growth of 'Hom Rangsi' in MS medium supplemented with 0, 5, 10, 15, 20 and 25 mg/L after 8 weeks cultured.

	MS medium					
BA (mg/L)	**0**	**5**	**10**	**15**	**20**	**25**
Total seeds	40	40	40	40	40	40
Number of shoots/ seeds	42/40	137/40	137/40	167/40	171/40	215/40
Number of shoots/ seed	1.05	3.43	3.43	4.18	4.28	5.38

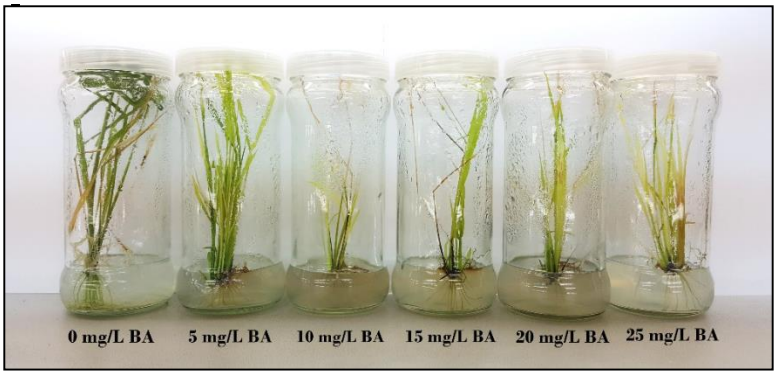

Figure 1. Number of multiple shoots on MS medium supplemented with 0, 5, 10, 15, 20, 25 mg/L after 8 weeks cultured.

Effects of various doses of gamma ray on 'Hom Rangsi' rice *in vitro*

After 6 weeks of being cultured and studied on the effects of Al^{3+} on the growth of irradiated Hom Rangsi planlets, it was found that the plantlets which were treated by gamma irradiation at 0, 100, 200, 300 and 400 Gy showed the survival percentages of 86.32%, 77.78%, 58.95%, 58.95% and 21.87%. The plant heights were 8.40, 8.30, 6.70, 6.60 and 6.10 cm, respectively. Plantlets which were treated by 0 and 100 Gy were green while plantlets which were treated by 200, 300 and 400 Gy became pale green (Table 2). All surviving plantlets were transferred to MS + 25 mg/L BA + 0.25% phytagel medium to induce new multiple shoots.

Table 2. Effect of various doses of gamma ray on 'Hom Rangsi' rice after 6 weeks cultured in MS + 400 ppm Al3+ medium.

Gamma dose on 'Hom Rangsi' plantlets	0 Gy	100 Gy	200 Gy	300 Gy	400 Gy
Total plantlets	100	100	100	100	100
Number of survivals (%)	86.32^{a}	77.78^{b}	58.95^{c}	58.95^{c}	21.87^{d}
Plant height (cm)	8.40^{a}	8.30^{a}	6.70^{b}	6.60^{b}	6.10^{c}
Plant color	green	green	pale green	pale green	pale green

Note: F-test ($P > 0.05$)

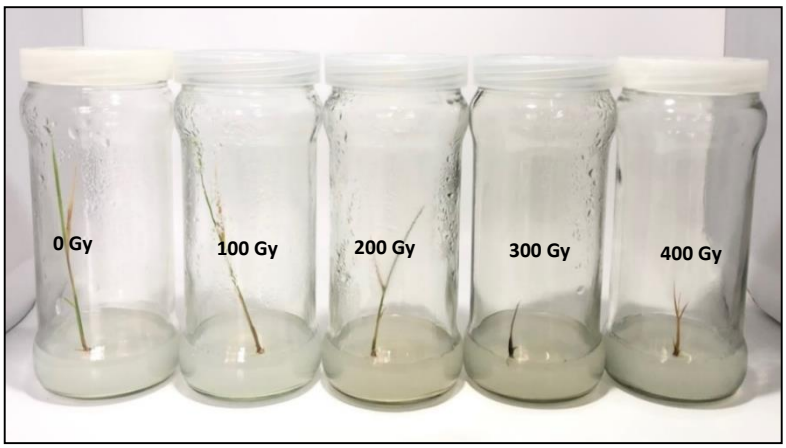

Figure 2. Effect of gamma ray on 'Hom Rangsi' rice plantlet after 6 weeks cultured in MS + Al^{3+} medium.

Shoot induction of the survival acid tolerance plantlets.

After 6 weeks of being cultured on MS + 25 mg/L BA medium, most plantlets were healthy. The plantlets from 300 Gy treatment gave the highest multiple shoot budding of 5.24 shoots/plantlet, while the plantlets from 0, 100, 200 and 400 Gy gave 4.37, 4.31, 4.41 and 4.55 shoots/plantlet, plants height of the plantlets from 0, 100, 200, 300 and 400 Gy treatment were non significance at 8.30, 8.30, 8.10, 8.20, 8.20 and 8.20 cm respectively. All plantlets were green and became strong and were prepared for acclimatization in greenhouse experiment (Table 3).

Table 3. Shoot formation of the survival acid tolerance rice plantlets *in vitro* after 6 weeks cultured.

'Hom Rangsi' plantlets	Gamma-ray dose rate (Gy)				
	0	**100**	**200**	**300**	**400**
Number of survival plantlets	41	33	42	38	11
Number of shoots/plantlets	179/41	142/33	185/42	199/38	50/11
Ratio of shoot/plantlet	4.37^{b}	4.31^{b}	4.41^{b}	5.24^{a}	4.55^{b}
Plant height (cm.)	8.30^{ns}	8.30^{ns}	8.10^{ns}	8.20^{ns}	8.20^{ns}
Plant color	green	green	green	green	green

Note: F-test ($P > 0.05$)

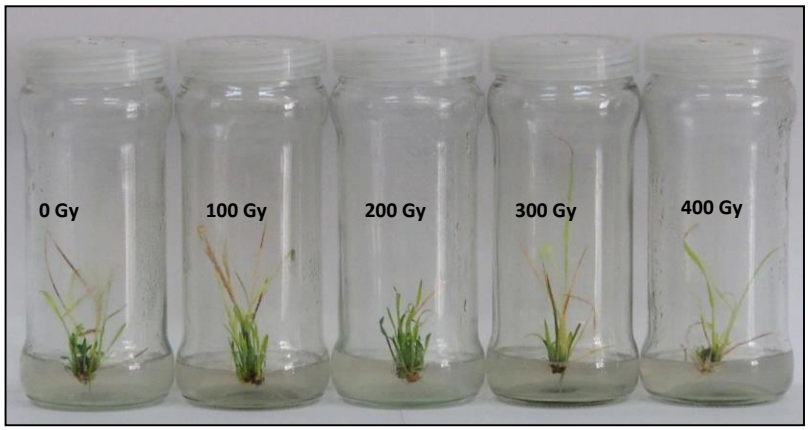

Figure 3. Shoot proliferation of the survival irradiated acid tolerance 'Hom Rangsi' rice plantlets *in vitro* after 6 weeks cultured.

DISCUSSION

Because 'Hom Rangsi' rice was derived from KDML105 rice, the cultured media should be similar. The first experiment indicated that the optimal concentration of BA for multiple shoot induction of Hom Rangsi was 25 mg/L in MS medium which was similar to the optimal media for multiple shoot induction of KDML105 rice (Sripichitt et al, 1992). After irradiation, 'Hom Rangsi' seeds were cultured in MS + 25 mg/L BA supplemented with 400 ppm Al^{3+} for acid-resistance screening. The result showed that the non-irradiated seeds showed the highest survival rate (86.32%), while the 400-Gy irradiated seeds showed the lowest survival rate (21.87%). This phenomenon followed the irradiation theory which stated that the survival rate was inversely correlated with the radiation dose received. However, the observed acid resistance might be caused by physiological adaptation in addition to mutation induction. After the surviving plantlets were transplanted onto the optimal MS + 25 mg/L BA, all of them became green and healthy. All plantlets will be transplanted and grow into mature plants and screened for acid soil tolerance in greenhouse and natural acidic field experiment in the near future.

CONCLUSION

'Hom Rangsi' aromatic rice seeds were cultured on MS solid medium supplemented with various concentrations of BA. The suitable media for multiple shoot induction was MS + 25 mg/L BA, which produced 5.38 shoots/seed. Later, irradiated 'Hom Rangsi' seeds cultured on MS solid medium supplemented with 400 mg/L Al3+, pH 2.9, were selected for acidic tolerance. The survivals of irradiated plantlets were studied. The non-irradiated seeds showed highest survivals (86.32%) while the 400 Gy irradiated seeds showed the lowest survivals (21.87%). All survival plantlets were transferred to MS + BA 25 mg/L medium. After six weeks of being cultured, the maximum of 5.24 shoots/plantlet were found from 300 Gy irradiation treatment.

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