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# *In vitro* response and effect of gamma irradiation on four local *indica* rice varieties

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## ABSTRACT

*In vitro* response of four local *Indica* rice cultivars viz. Sadamota, Kachamota, Moulata and Dudhkalam was evaluated. The aim of this study is to develop an efficient protocol for callus induction, plant regeneration and to observe the effect of gamma radiation on plant regeneration for creating possible genetic variability. In Different concentration of 2,4-D and growth regulators were supplemented with MS medium (Murashige and Skoog's) to observe their callus induction frequency using mature embryo as explant. Among the cultivars, the highest primary callus (92.55%) as well as embryogenic callus induction (56.26%) was showed in sadamota at 3.0 mg<sup>-1</sup> 2,4-D and 10 mg<sup>-1</sup> kinetin under dark condition. Twenty one days old embryogenic calli were exposed to 0, 2, 4 and 6 Gy of gamma rays and transferred to regeneration medium. Both callus growth and regeneration capacity were found to be decreased with increasing level of exposure to gamma rays. The doses of 4 Gy of gamma radiation were found to be the 50% inhibition dose for callus growth and plant regeneration in sadamota and kachamota, respectively whereas the 50% inhibition dose for moulata and dudhkalam at 2 Gy. This results indicate that sensitivity of gamma radiation on rice callus depends on genotype of a genus.

**KEYWORDS:** *Indica* rice, gamma radiation, callus growth, plant regeneration

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## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops, which supplies food for more than half of the world population [1]. In the coming 30 years, the world will require 70% more rice than that it requires today. According to conservative estimates, 800 million tons of rice will have to be grown with considerable reduction in the input of agrochemical under sustainable conditions [2]. In Bangladesh, rice is the most dominant and main food crop, which grows in all the three crop growing seasons of the year [3].

The southern coastal belt of Bangladesh (about 2.0 m.ha) i. e. the greater Barisal region is crisscrossed with innumerable rivers, rivulets, creeks and canals. Lands along these rivers are subject to inundation due to cyclic tidal flooding. Monsoonal tidal flooding and consequential inundation of land and often submergence of crop is the major problem of growing rice. Soil submergence and frequent crop inundation due to high tides twice daily over a period of 4-8 months (April-November) is the characteristic feature of tidal floodplain ecosystem. About 80% of the cultivable land of greater Barisal and Patuakhali districts is inundated up

to a range of 6-90 cm during monsoon presenting difficulty in growing modern varieties of transplanted T. aman rice. Farmers use tall statured, photosensitive, long duration, traditional cultivars tolerant to tidal flooding. A few cultivars namely Sadamota, Moulata, Lalmeta and Dudkalam dominate for cultivation in T. aman cropping season in these areas [4]. Having endured the climatic variability and environmental hazards, these cultivars are being grown in the region over ages. However, the yield of the traditional varieties is low (<2.0 t/ha) and also long duration (>170 days). Therefore, agricultural scientists to develop varieties which are capable to produce more yield both in submerged and flood tide conditions. But the traditional methods of rice breeding are not sufficient enough to meet the demand of developing rice varieties in the shortest possible time in twenty first century. Many researchers have attempted to exploit many options for rice varieties development out of this induced mutagenesis on embryogenic calli by treatment with low gamma radiation serves as a better source of variability [5,6,7]. The variability in the population leads the chance of effective selection and increases the scope of improvement [8]. Considerable works have been done on induced mutation in rice callus by many workers [8,9,10] applied low doses of gamma ray treatments to rice callus and found

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significant effect on callus induction. Considering the background information, the present study was undertaken to investigate the extent of variability on callus production and plant regeneration at different doses of gamma radiation of four local rice cultivars.

## MATERIALS AND METHODS

The experiment was conducted during the period of January 2018 to November 2018 at the Tissue Culture Laboratory of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. Four local *Indica* rice cultivars viz., Sadamota, Moulata, Dudhkalam and Kachamota were used in this study. The materials used in the experiment were obtained from larger Barisal region by the courtesy of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA). Mature rice embryos were used as explants for callus initiation and subsequent plant regeneration.

The dehusked seeds were then washed five times with autoclaved distilled water. Then one minute washed with 70% ethanol. After then it washed three times with autoclaved distilled water. The floating dehusked seeds were discarded. The surface sterilization of these dehusked seeds was carried out in the aseptic conditions of a laminar air flow cabinet. The seeds were taken into a sterile tube and immersed into 3.5 % NaOCl with 2 drops of Tween twenty solution for 15 minutes with shaking followed by 10 times rinsed in autoclaved distilled water to remove traces of NaOCl and Tween twenty. After that seeds were dried about 5 minutes and sterilized seeds were cultured in MS medium with 3 mg/l 2,4-D and 10 mg/l Kn growth regulator combination, 30 g/l sucrose and 5.6% gelrite to initiate callus and incubated under dark condition.

Calli of twenty-one-day old were subjected to 4 different doses (0, 2, 3, 5, and 6 Gy) of gamma rays from <sup>60</sup>Co source. About 50-65 calli were exposed to each radiation level. Irradiated calli were transferred to MS medium supplemented with 0.5 mg/l NAA and 10 mg/l Kn fo. Repeated sub-culturing was done at 15 days interval for callus proliferation and organogenesis in same regeneration medium. Those irradiated calli continued to be proliferated and differentiated into shoots. When these shoots grew to about 3-4 cm height, these were separated aseptically from each other and transferred to freshly prepared rooting medium (half strength MS + 0.5 mg/l IBA) and kept in growth room at 25±2°C. Data were recorded on survival rate, plant regeneration and number of regenerated shoots per callus. The collected data were analysed statistically as completely randomized design following the analysis of variance (ANOVA) technique and the mean differences were adjusted with Duncan's Multiple Range Test (DMRT) using the statistical computer package programme, MSTAT-C.

## RESULTS AND DISCUSSION

The mature embryos of four local rice cultivars were inoculated on various treatments to observe the callus formation response. Callus formation invariably developed within 21 days. Among the cultivars, high average callus initiation (%) was observed in Sadamota (92.55 %) followed by Kachamota (91.96%). Initiation

of embryogenic calli in rice is considered most critical step. It also appeared that Kachamota and Sadamota had better callus initiation efficiency in comparison to Moulata and Dudhkalam.

The result was indicated that gamma radiation had inverse effects on survival rate, plant regeneration and number of regenerated shoots per callus (Table 1).

It was clearly observed that survivability of twenty one days irradiated calli varied on different range of gamma radiation. Survival rate of callus varied from 81% to 21%. In all cases, The growth regeneration capability of calli were found to be higher in control than in the irradiated calli. Plant regeneration percentage was ranged from 0%- 33.53% in Moulata, 48.86% to 20.73% in Sadamota, in Dudhkalam 44.03% to 15.73% in Kachamota 0% to 30%. Increasing the doses of gamma radiation decreased the shoot regeneration capacity. The calli could be viable in Shadamota and Kachamota by 50% at 6 Gy of radiation, whereas, in Moulata and Dudhkalam, was reduced to 50% at 4 Gy of radiation.

The percentage of plant regeneration decreases from 48.86% to 0% while number of regenerated shoots callus ranged from 5.6% to 0%. A few numbers of shoots were produced from the Sadamota irradiated with 4 Gy as compared to control. Both growth and regeneration capacity decreased with increasing levels of gamma rays; however, plant regeneration capacity was more sensitive to gamma rays than growth [5,11]. observed that 2.5-5.0 Gy is the optimal dose range for mature embryos of rice.

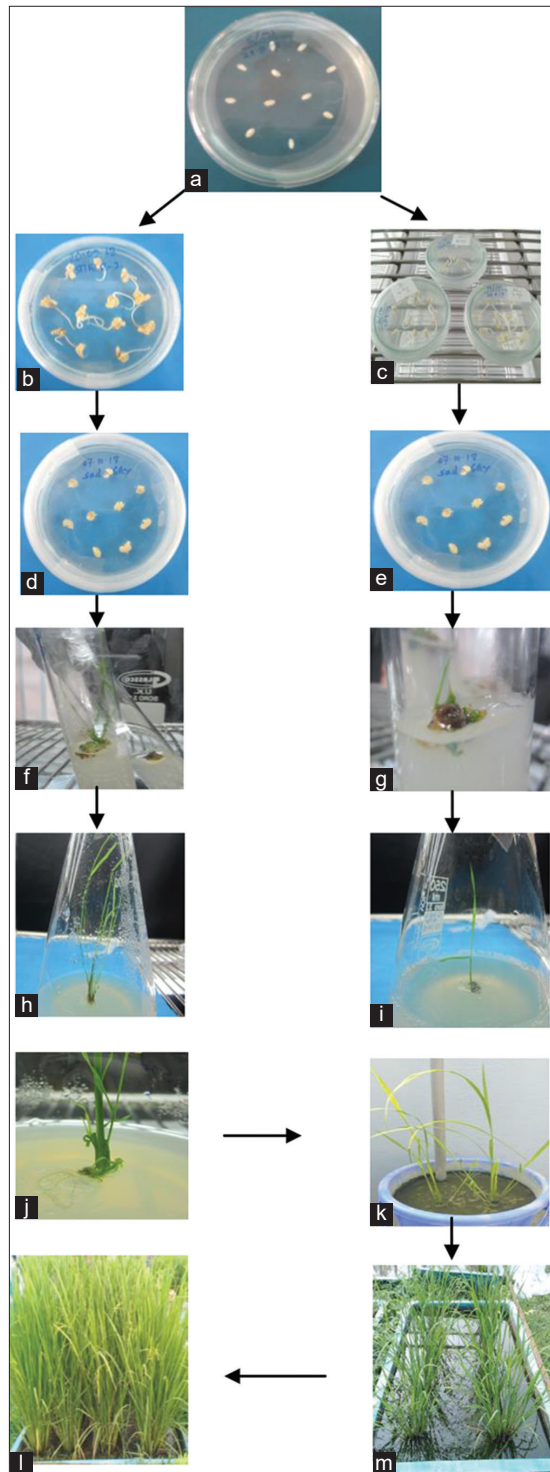
*In vitro* regeneration of both control and irradiated (4 Gy) calli from mature embryo of Sadamota are shown in Figure 1.

Similar findings have been reported earlier by many workers [12,5,6,7]. However, the 50% inhibition dose (LD50)

**Table 1: Survival rate, plant regeneration and number of regenerated shoots per callus of irradiated calli developed from indica rice**

Survival rate %	0 Gy (Control)	2 Gy	4 Gy	6 Gy
Moulata	71.36 <sup>bc</sup>	67.00 <sup>c</sup>	53.00 <sup>c</sup>	23.46 <sup>c</sup>
Sadamota	81.00 <sup>a</sup>	76.93 <sup>a</sup>	72.43 <sup>a</sup>	48.00 <sup>a</sup>
Dudhkalam	69.00 <sup>c</sup>	66.50 <sup>c</sup>	49.93 <sup>c</sup>	21.00 <sup>c</sup>
Kachamota	77.50 <sup>ab</sup>	72.06 <sup>b</sup>	62.63 <sup>b</sup>	37.00 <sup>b</sup>
Level of sig.	**	**	**	**
<b>Regeneration%</b>				
Moulata	33.53 <sup>b</sup>	26.46 <sup>b</sup>	15.00 <sup>c</sup>	0.00 <sup>c</sup>
Sadamota	48.86 <sup>a</sup>	41.10 <sup>a</sup>	33.00 <sup>a</sup>	20.73 <sup>a</sup>
Dudhkalam	30.00 <sup>b</sup>	18.00 <sup>c</sup>	7.200 <sup>d</sup>	0.00 <sup>c</sup>
Kachamota	44.03 <sup>a</sup>	39.00 <sup>a</sup>	27.83 <sup>b</sup>	15.73 <sup>b</sup>
Level of sig.	**	**	**	**
<b>Shoot/callus</b>				
Moulata	5.00	5.33 <sup>a</sup>	2.26 <sup>bc</sup>	0.00 <sup>c</sup>
Sadamota	5.60	5.10 <sup>b</sup>	4.46 <sup>a</sup>	3.10 <sup>a</sup>
Dudhkalam	4.53	4.10 <sup>c</sup>	1.46 <sup>c</sup>	0.00 <sup>c</sup>
Kachamota	5.80	1.73 <sup>d</sup>	2.53 <sup>b</sup>	1.33 <sup>b</sup>
Level of sig.	NS	**	**	**

In a column, within treatment, figure(s) having the same letter(s) do not differ significantly as per DMRT at P≤0.05; \*\* Significant at 1% level of probability; NS- not significant



**Figure 1:** *In vitro* regeneration from mature embryo of irradiated embryogenic callus of rice cultivars Sadamota (a-m). Schematic representation of mature embryo culture on callusing medium (a), 21 days old Embryogenic Calli (b), 21 days old Irradiated Embryogenic Calli (c), 21 days Sub-cultured Embryogenic Calli on shooting Medium (d), 21 days Irradiated Calli Sub-culture on shooting Medium (e), established selected calli on shooting media (f), Irradiated established selected calli on shooting media (g), Shoot regenerated from non-irradiated embryo (h), Shoot regenerated from non-irradiated embryo (i), regenerated plant transferred on rooting media (j), Established Regenerated plants in pot (k), then the mature plants established on soil (l), finally regenerated plants were flowering (m)

for plant regeneration, 4 Gy of gamma radiation would be upper limit in the, sadamota and Kachamot whereas 2 Gy would be for remaining varieties.

DNA damages and mutation in Chromosomes caused by irradiation may be responsible for slow growth and reduction in regeneration rate. It was mentioned that the growth and regeneration ability of irradiated calli was negatively affected with an increasing the level of gamma doses. Moreover, these results indicate that sensitivity of gamma radiation depend on genotypes of a genus.

The results of this study revealed that 6 Gy of gamma ray might be considered as LD50 for sadamota and Kachamota. The LD50 for Moulata and Dudhkalam were found to be 4 Gy as these varieties showed 50% survivability after irradiation of calli.

## CONCLUSIONS

The results of this study revealed that 6 Gy of gamma ray might be considered as LD50 for sadamota and Kachamota. The LD50 for Moulata and Dudhkalam were found to be 4 Gy as these varieties showed 50% survivability after irradiation of calli.

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