Eco. Env. & Cons. 30 (August Suppl. Issue) : 2024; pp. (S487-S491)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i05s.074

Effect of Gamma Irradiation on Anther Derived Calli of Indica Rice Varieties

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(Received 9 March, 2024; Accepted 12 May, 2024)

ABSTRACT

The anther culture derived calli of rice varieties IBD-1 and MTU1010 were exposed to doses of 15 Gy and 25 Gy gamma irradiations along with control (without irradiations) to study effect of gamma irradiation on survivability of calli. The calli from the both varieties that had been exposed to gamma radiation were placed on MS media supplemented with BAP, Kin NAA and 3% sucrose. In the control of (without irradiations) IBD-1 and MTU1010 exhibited the high calli survival rate *i.e.* 70% and 56% respectively. But in case of IBD-1 calli, gamma ray dose of 15 Gy was causing 46% reductions in survival rate and in case of the MTU1010 calli the survival rate was reduced to 38% for 15 Gy dose. At higher dose of 25 Gy gamma ray, the survival rate of calli is very much affected in the variety IBD-1 with 22% reduction and 16% reduction in MTU1010 variety. The callus from IBD-1 and MTU1010 showed the decreased survival rate after increased gamma irradiation. In the comparison of both varieties the clear pattern of proportional decrease of survival rate of calli to increased dosage of gamma irradiation was observed. The doses of 15 Gy of gamma radiation were found to be the near about 50% inhibition dose for callus survival in IBD-1 and MTU1010. The irradiated calli from both the varieties showed the survival rate near to 50% upon the 15 Gy treatment of gamma radiation indicating that the treatment of 15 Gy was better for mutagenic studies in rice. The results also exhibiting that the genus of the genotype is responsible for the callus sensitivity to gamma irradiation. The present study successfully demonstrates the use of *in vitro* mutagenesis and tissue culture to create genetic variations is the first step in any rice improvement programme.

Key words: *Rice, Anther culture, Callus and Gamma ray*

Introduction

Rice (*Oryza sativa* L.) is a traditional food crop that has gained global importance due the presence of nutritional content and frequently used in most of the countries for satisfying human diet. In last few decades the consumption of rice has been increasing drastically due to the increase in human population (Kumari *et al.,* 2023). On the other hand the production of rice is going down year by year due to various biotic and abiotic stresses. Therefore, to make the rice crop tolerant against various stresses the researchers are interested in development of new cultivars. But with the help of conventional breeding methods it is not possible to develop new cultivars within a short period of time (Islam *et al.,* 2020). To develop biotic and abiotic stress tolerant cultivars researchers are adopting modern technique such as plant transformation and gene editing (Panzade *et al.,* 2020; Panzade *et al.,* 2024). Recently, to develop

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biotic and abiotic stress resistant cultivars researchers adopting modern technique such as induced mutagenesis on calli by using gamma radiation (Hosain and Alam, 2001 and Hasbullah *et al.,* 2012). It was found that the researchers get a better source of variability upon the application of low gamma radiation and (Islam *et al.,* 2020). The high scope of improvement mostly depends upon the variability of a population that can lead to the effective selection. In recent study the induced mutation with callus has been performed by researchers and it was found that the survival rate of callus exhibit significant effect upon the various doses of gamma irradiation (Islam *et al.,* 2020).

Double haploid technique is an advanced tissue culture approach that provides excellent selection opportunities for mutants using novel and extended genetic diversity. The effective selection and the scope for improvement can be achieved through the variability present in the population (Hosain and Alam, 2001). The cause of genetic variation and its recognition can be facilitate through haploid tissue of the individuals. In the plant breeding for enhancement of germplasm can be possible through the haploid systems that provide several advantages for crop improvement. After mutagenic treatment the selection of both dominant and recessive progeny in first generation is easily possible via anther culture derived callus mutagenesis. Within a short period of time this technique also enables the fixation of mutated genotypes that leads to the pure mutant lines production. Further, the lack of chimerism and zygotic versus gametic segregation ratios can cause the enhancement of the selection capability of interested mutants (Szarejko, 2003). Considering the background information, in present investigation anther culture derived callus mutagenesis was employed. The generated calli were mutated using gamma (γ) irradiation. After irradiation the anther culture derived calli were subjected to regeneration for production of doubled haploids and investigate the response of anther culture derived calli for its survival upon various treatments of gamma radiation.

Materials and Methods

Two mega rice varieties namely IBD-1 and MTU1010 which are suitable to different rice ecologies of Chhattisgarh were taken for the study. The varieties IBD-1 and MTU1010 were released by State Variety Release Committee of Chhattisgarh and Andhra Pradesh Rice Research Institute respectively. In the year 2020 the study was carried out at the Department of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwavidyala, Raipur, Chhattisgarh. The anther culture derived calli were developed from panicles of primary and secondary tillers of booting stage. For callus induction the panicles were treated with cold treatment at 10 °C for 10 days in a refrigerator (Naik *et al.,* 2017; Kharate *et al.,* 2021). Callus initiation by using anthers as explants was performed in N6 medium (Chu *et al.,* 1975) added with BAP (0.5 mg/L), 2,4-D (2 mg/L) and maltose (3%) (Naik *et al.,* 2017).

The calli at the stage of 21 days were used for two doses (15 Gy and 25 Gy) of gamma radiation along with control (Calli without gamma radiation). At the each level of 15 Gy and 25 Gy radiations, a set of 50 calli (5 calli per plate) were irradiated at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. After gamma radiation treatment the calli were transfer into the MS medium fortified with BAP (1.5 mg/l), NAA (0.5 mg/l), kinetin (0.5 mg/l) and sucrose (3%) (Naik *et al.,* 2017). The sub-culturing was performed after 15 days of interval to develop green spot in irradiated white calli, where continuous proliferation and differentiation into shoot will taken place. The completely randomized design (CRD) was used to analyse the data collected for survival rate of calli. The analysis of variance (ANOVA) and duncan's multiple range test (DMRT) were performed with OPSTAT software programme, where data was subjected to statistical analysis at 1% and 5% level of significance (Sheoran *et al.,* 1998; Panzade *et al.,* 2021).

Results and Discussion

Initiation of callus from the anthers of rice plant is a most crucial step in this experiment. The callus initiation from anthers was started after 5 days of inoculation. It also appeared that the callus initiation efficiency from the anthers of IBD-1 and MTU1010 was better in the N6 medium with BAP (0.5 mg/l), 2,4-D (2 mg/l) and maltose (3%) (Naik *et al.,* 2017). After 21 days of anther inoculation calli were well developed and used for gamma irradiation treatments. Simultaneously the treated calli were transferred into MS medium contained BAP (1.5 mg/l), NAA (0.5 mg/l), kinetin (0.5 mg/l) and sucrose (3%) (Naik *et al.,* 2017) to observe the effect of gamma ir-

radiation on survival percentage. After the 20 days of gamma irradiation treatment the irradiated calli of IBD-1 and MTU1010 were clearly depicted the variation in survival percentage on different treatments of gamma radiation along with control.

Survival rate of IBD-1 calli was 70%, 46% and 22% in control, 15 Gy and 25 Gy respectively (Fig. 1). The analysis of variance data suggests that the survival rate of IBD-1 calli upon various treatments of gamma irradiation showed significant differences at 1 % level of significance (Table 1). It was observed that the calli of IBD-1 with control (calli without gamma irradiation) showed highest survival rate of 70% with an average of 3.50 ± 1.95 survived calli followed by 15 Gy treatments which showed 46% survival rate with 2.30 ± 1.16 average survived calli. The lowest 22% survival rate was observed for 25 Gy treatments with 1.10 ± 1.10 average survived calli (Table 2). The IBD-1 calli survival percentage was 46% with 15 Gy treatments and it reduced to 22% with increased 25 Gy treatments.

Similarly, in MTU1010 decreasing calli survival percentage of 56%, 38% and 16% with different treatments of control, 15 Gy and 25 Gy respectively was observed (Fig. 2). The statistical analysis exhibit that the significant differences at 5 % level of significance present in data derived from MTU1010 calli with various gamma irradiation treatments (Table 3). Highest survival rate, i.e. 56 % was reported in control treatment of MTU1010 with 2.80 ± 1.68 average survived calli which was followed by 15 Gy and 25 Gy treatments with survival rate 38% and 16% respectively. The average survived calli of 15 Gy treatment were 1.90 ± 1.72 whereas, 25 Gy treatment showed 0.80 ± 0.78 (Table 4). Here, also the calli exhibiting the high survival rate in control as compare

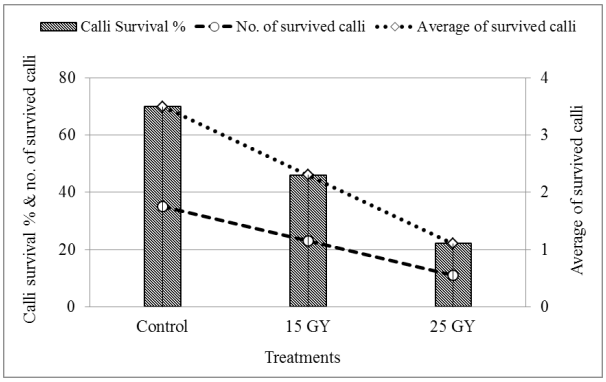


Fig. 1. Survival rate of IBD-1 calli

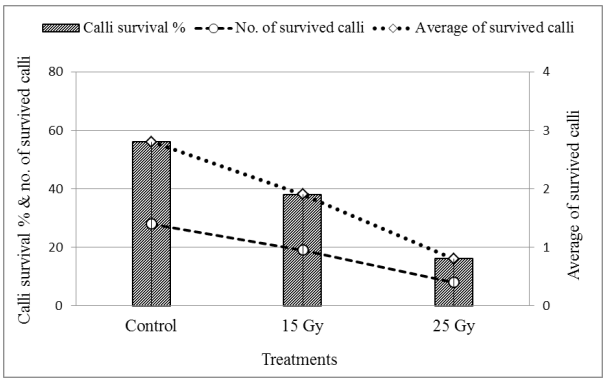


Fig. 2. Survival rate of MTU1010 calli

Table 1. CRD analysis of variance on survival rate of IBD-1 calli

Source of Variation	D.F.	Sum of Square	Mean Squares	F-value	P-value	CD (1%)	CD (5%)
Treatment	2	28.800	14.400	6.762	0.004	1.808	1.339
Error	27	57.500	2.130				
Total	29	86.300					

Note: The data was analysed by one way ANOVA, values are significant at $p \leq 0.01$.

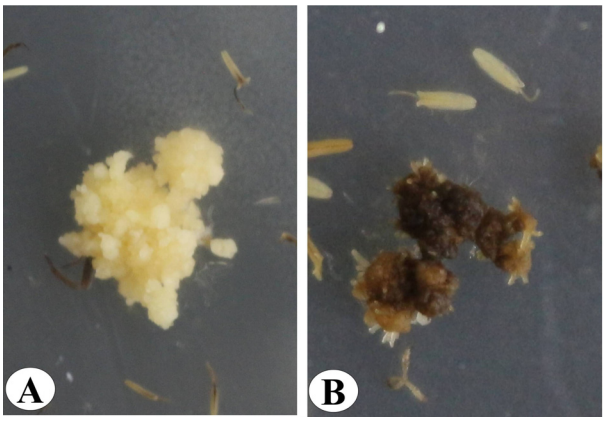
Table 2. Response of IBD-1 derived calli to gamma irradiation

Treatments	Survival %	Survived Calli (Mean ± SD)	Std. Error
Control	70	3.500_{a} ± 1.95	0.619
15 Gy	46	2.300_{ab} ± 1.16	0.367
25 Gy	22	1.100_{b} ± 1.101	0.348
	CD at 0.01%	1.808	
	CD at 0.05%	1.339	

Note: Values are the means of ten replicates ± SD**.** The means with different Letters as superscripts are significant (P < 0.01). The means with same letters or having common letter(s) are not significantly different in Duncan's test.

to mutagenic treatment of gamma irradiation. Increasing the doses of gamma radiation from 15 Gy to 25 Gy also decreased the survival rate from 38% to 16% in MTU1010 calli.

The decreasing survival percentage of seed derived embryonic calli rice with increasing dose of gamma irradiation was also reported by researchers in last few decades (Hosain and Alam, 2001; Islam *et al.,* 2020; Kumari *et al.,* 2023). Increased gamma radiation on anther derived calli of IBD-1 and MTU1010 exhibit the inverse effects on survival rate. This inverse effect is due to the chromosomal damage caused by gamma irradiation where the high mutagenic dose leads to the high frequency of damage (Hasbullah *et al.,* 2012; Kumari *et al.,* 2023). The callus tissues were also affected due to cell wall and cell membrane damage caused by gamma irradiation (Photoplate 1). There are two main reasons behind the inhibition of callus growth upon higher gamma irradiation doses i.e. damage of the entire genome and during cell division the cell cycle get arrested at the phase of G2/M. (Preuss and Britt, 2003). Further, increased dose of gamma irradiation with decreasing callus survival percentage was also reported in the sugarcane, gerbera, stevia and citrus (Hasbullah *et al.,* 2012; Khalil *et al.,* 2015; Agisimanto *et al.,* 2016).



Photoplate 1. Effect of gamma irradiation on anther culture derived calli. A-Survived calli (without the treatment of gamma irradiation) and B- Dead calli (with the treatment of gamma irradiation)

Conclusion

The slow growth and reduction in survival rate of callus is the result of the DNA damage and mutation in chromosomes upon the treatment of gamma radiation. Increasing gamma radiation doses can cause the negative effect on the callus for its growth and survivability. Further, the results also ensure that the genus of a genotype that can lead the variation in sensitivity of callus upon the various treatments of gamma radiation. In the both varieties the survivability of calli close to the 50% was found with 15 Gy treatments of gamma rays. Hence, we can say that the 15 Gy treatment of gamma rays is optimal dose for IBD-1 and MTU1010 for mutagenesis.

Table 3. CRD analysis of variance on survival rate of MTU1010 calli

Source of Variation	D.F.	Sum of Square	Mean Squares	F-value	P-value	CD (1%)	CD (5%)
Treatment	2	20.067	10.033	4.663	0.018	N/A	1.346
Error	27	58.100	2.152				
Total	29	78.167					

Note: The data was analysed by one way ANOVA, values are significant at $p \leq 0.05$.

Table 4. Response of MTU1010 derived calli to gamma irradiation

Treatments	Survival %	Survived Calli (Mean ± SD)	Std. Error
Control	56	2.800_{a} ± 1.687	0.533
15 Gy	38	1.900_{ab} ± 1.729	0.547
25 Gy	16	0.800_{b} ± 0.789	0.249
	CD at 0.01%	N/A	
	CD at 0.05%	1.346	

Note: Values are the means of ten replicates ± SD. The means with different Letters as superscripts are significant ($P < 0.05$). The means with same letters or having common letter(s) are not significantly different in Duncan's test.

Acknowledgements

The authors gratefully acknowledge the gratitude for the support provided by Indira Gandhi Agriculture University, Raipur, Chhattisgarh (CG), India. We also thankful towards Bhabha Atomic Research Centre, Mumbai for flow cytometric analysis of callus samples, whenever required.

Conflict of interest: The authors declare no conflicts of interest associated with this publication. Additionally, there are no ethical issues infringed upon in this study.

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