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Impact of Radiation on Rice Calli and Subsequent *In vitro* Regeneration

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ABSTRACT

Aromatic rice has high commercial importance with greater economic return. Among the aromatic rice varieties, Kataribhog is a famous local variety that is mostly cultivated in the Dinajpur district northern part of Bangladesh. The yield performance of Kataribhog is lower (<2.50 t/ha) and has shown lodging tendency. Under these circumstances, a research program was taken to improve the existing problem through in vitro culture including nuclear irradiation techniques. Gamma irradiation (Co60) is a powerful tool in crop improvement, and its effects on callus development from kataribhog rice were explored in this research. Three types of media, PT-100G, PT-011G, and PT-100, were utilized to induce callus, which were then subjected to three different doses of radiation (10Gy, 12Gy, and 15Gy). Among the tested media, PT-100G was the most effective, boasting an impressive average of Callus initiation of 79.27% and embryogenic callus induction percentage of 32.93%. In contrast, PT-011G and PT-100 lagged with 52.46% and 44.44% callus initiation; 27.87% and 11.11% embryogenic callus induction, respectively. The radiation dose of 10 Gy was optimal for calli survival, showing an average percentage of 80.00%. 10 Gy is the most effective dose for promoting plant regeneration.

Keywords: Kataribhog rice; In vitro regeneration; gamma irradiation; Embryogenic callus; agricultural biotechnology.

INTRODUCTION

“Rice is a member of the Gramineae, or grass family. The genus *Oryza*, which includes cultivated rice, most likely evolved 130 million years ago. The genus *Oryza* contains twenty-one wild species and *Oryza* genus has two cultivated species. The Asian cultivated rice, *Oryza sativa*, is grown all over the world. In West Africa, the African-cultivated rice, *O. glaberrima*, is grown on a modest scale” (Dai et al., 2012).

Rice cultivation and production have significant economic implications. It is a major agricultural commodity, contributing substantially to the global economy and providing livelihoods for millions of people (FAO, 2021). Rice holds cultural and social significance in many countries, particularly in Asia. It is often intertwined with religious ceremonies, cultural traditions, and social customs (Kiminami et al., 2021).

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Rice is a crucial source of nutrition, providing essential nutrients like carbohydrates and micronutrients to a significant portion of the world's population. It provides a significant portion of daily calorie intake (Hussain, 2020). Efforts in biofortification aim to enhance its nutritional value further (Bouis & Welch, 2010). Rice cultivation systems are highly vulnerable to climate change. Research explores ways to make rice production more resilient and sustainable, addressing environmental concerns and ensuring future food security (Wassmann et al., 2009). Rice cultivation is a crucial source of livelihood for millions of farmers in Bangladesh. The rice sector contributes significantly to the country's agricultural GDP (Sarker et al., 2017). Rice is essential for ensuring food security in Bangladesh, where a large portion of the population depends on rice as their primary source of nutrition (Dawe et al., 2002). "Worldwide 503.17 MT rice is produced where China produces 29.5% of the total, followed by India (23.8%), Bangladesh (7.0%), Indonesia (6.9%), Vietnam (5.4%), and Thailand (3.7%)" (USDA, 2020).

"Rice is also the staple food in Bangladesh and accounts for approximately 78 per cent of the country's total net cropped areas cultivation. The country achieves an autarky to meet the rice demand for its 169.04 million people from 11.55 million hectares of cultivated gross area" (Nasim et al., 2021 & Kabir et al., 2020). "In Bangladesh, food security is equivalent to rice security" (Kabir et al., 2020). "Rice is cultivated in three seasons namely Aus, Aman and Boro throughout the year. Since independence, rice production has increased threefold from approximately 11 MT in 1971–72 to about 36.6 MT in 2019–20" (BBS, 2020). This revolution has transformed the country from a so-called "Bottomless Basket" to a "Full Food Basket". "After a long period, rice production in Bangladesh rose significantly after 1990–1991, especially during two periods: 1996–1997 and 2000–01, as well as from 2009–10 to 2013–14. Improved loan distribution policies (credit deposits directly to farmers' 10 Taka bank accounts), well-organized fertilizer supplies, availability of high-quality seeds by the public and commercial sectors, and technical interventions (e.g. genetic improvements of varieties for favourable and unfavourable ecosystems) make it possible to make Bangladesh as one of the largest contributors of rice in the world" (Kabir et al., 2020 & Rabbi et al., 2020). "Bangladesh recently placed the third position worldwide in rice production, behind China and India, with a production volume of 3.6 core tons" (Rahman et al., 2021).

"Comparing the three seasons, Aman rice is more significant commercially due to its high value and volume of trade. In general, this time of year is very beneficial for growing aromatic rice. High commercial importance and greater economic return are associated with fine and aromatic rice. It is noteworthy that Bangladesh has approximately 54 aromatic and fine rice varieties that are grown throughout the nation. 12.5% of the area is covered by aromatic rice during the Aman season and their yield based on clean rice was nearly 2.0 per hectare" (Islam et al., 2018 Islam et al., 2017). Among the varieties of aromatic rice, Kataribhog is a well-known regional variety that is primarily grown in the Dinajpur district. Delicious, flattened rice is made famous by Kataribhog. The flattened rice from Kataribhog has a sweet flavour and is a bright white colour. In the entire nation, boiled rice and Polau rice from Kataribhog are very well-liked. The performance of Kataribhog's yield is a little higher than Bangladesh's typical yield of aromatic rice. The yield of Kataribhog was calculated by Islam et al., (2017) to be 2.54 t/ha. "The production of aromatic rice is heavily concentrated in the Dinajpur district due to its geographic and environmental advantages. In Dinajpur, several rice processing businesses, including ACI, Pran Group, and Square Company Limited, have built aromatic rice processing facilities. About 15,540 hectares of land, or 5.6% of the total area of Aman, were planted to Kataribhog rice in 2017–18. From this land, 36,460 tons of clean rice were produced with an average yield of 2.37 t/ha in this district" (DAE 2017). Due to its pleasant aroma and fine grain quality, Bangladeshi aromatic rice is anticipated to enjoy a healthy market demand on a global scale. Due to increased demand on the global market and low production costs, Bangladesh may have a good opportunity to export aromatic and fine-grain rice.

Nowadays, callus initiation and the use of mutagen for induction of mutation in the callus is one of the proven tools employed by plant breeders for creating variability in crop plants. This technique known as in vitro mutagenesis is a sudden change in the heritable characters of an organism which serves as a source of

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creating variability for better selection in a short time. Tissue culture as well as callus production followed by irradiation is an important tool for creating variants and could play an important role in crop improvement (Bansal et al., 1990). Somaclonal variation within the group of non-irradiated controlled plants was less than that of irradiated plants. Many research workers have attempted to exploit somaclonal variation for crop improvement particularly treated by gamma radiation. Considerable works have been done on induced mutation in rice callus (Zhenyu and Danian, 1999; Mullin et al., 2021). However, significant research work in this field is still limited in Bangladesh.

LITERATURE REVIEW

This study focused on the initiation of callus from kataribhog rice embryos and the effect of gamma radiation on callus growth and its subsequent impact on plant regeneration in rice. In the world, rice is one of the most important cereal crops in many countries, due to the growing population, there is an increasing demand for rice production. The rice productivity is affected by various environmental stresses. Further, the nutritional improvement of rice can help in decreasing the evil of malnutrition. The use of biotechnological tools is the most workable option to develop such rice varieties. However, the lack of a simple and efficient protocol for embryogenic callus induction and quick plant regeneration in this cereal crop is a major constraint. Most of their interesting findings have been published in scientific journals, bulletins, the internet etc. Although some similar research has been conducted in various nations throughout the world, research linked to the current topic is scarce in Bangladesh. Thus, the relevant literature has been examined and presented in this chapter under the following headings and subheadings:

2.1 Concept of Rice Tissue Culture

Stroud et al. (2013) described that most transgenic crops are produced through tissue culture.

2.2 Initiation of Callus

Woo (1989) reported that “callus induction occurred from the somatic cells of the ovary wall, immature and mature embryos, immature endosperm, stem nodes, and seedlings. Many researchers were successful in callus initiation from different explants of various genotypes in different combinations of growth regulators. The literature which is most relevant to callus initiation is reviewed here”.

2.2.1 Influence of explants

Ferdous et al. (2013) reported that “different concentrations of 2, 4-D (0.0, 1.5, 2.5, 3.5, 4.5 mgL^{-1}) in MS medium were used for callus induction. Among the concentrations of 2, 4-D, 2.5 mgL^{-1} was the best concentration for callus induction (95.83%). Different concentrations of Kinetin (0.0, 8.0, 10.0, 12.0, 14.0 mgL^{-1}) with a constant concentration of NAA (0.5 mgL^{-1}) were used for plant regeneration from callus. The combination of 10.0 mgL-1 Kinetin and 0.5 mgL^{-1} NAA was found to be the best for shoot regeneration (77.50%)”.

Hoque & Mansfield (2004) reported that “the immature explants are not available for a short period of the growth cycle of rice plants. So, other explants such as mature embryos, leaves and roots which are available year-round, are more suitable for rice tissue culture and provide that a high frequency of plant regeneration can be achieved from them”.

2.2.2 Influence of culture media on callus induction and regeneration

Khatun et al. (2003) reported that “the media composition, explant source and age, and culture environment all affected callus induction and regeneration efficiency in rice plants”.

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Aditya (2004) reported that "the genotype and nutrient composition of the medium were the most important factors for efficient rice plant regeneration and appropriate media composition can increase regeneration efficiency".

Lee et al. (2002) used "three based media LS, MS and N6 to induce calli of japonica rice cultivars and reported that number, colour, size, shape, and appearance time of the induced embryogenic calli varied among the rice cultivars depending on the type of basal medium".

Visarada (2002) studied "the effect of four culture media on callus induction, regeneration and number of plants per unit culture of five indica rice genotypes from mature seeds as explants. They found that NBKNB medium is suitable for in vitro culture of all indica genotypes under test".

Nouri-Delawar (2001) cultured "the immature embryos of 18 rice genotypes on 3 different culture media (MS, LS and N6) and recorded callus diameters callus water content, and callus fresh and dry weight. They found that MS and N6 media were suitable for in vitro culture of immature embryos".

Rashid et al. (1996) reported that "the callus induction frequency of Super Basmati on MS media was 23.9% and on N6 47.7%. Comparing 6 callus induction and N6 media proved to be better".

2.2.3 Influence of growth regulators

Various combinations and concentrations of auxins and cytokinins in the culture medium have been tried by several scientists to follow their effects on both callus initiation and plant regeneration in rice. The most relevant literature related to callus induction has been reviewed here.

Chauhan & Kothari (2004) obtained "a high frequency of callus initiation achieved on MS medium supplemented with 11.31 mM 2, 4-D, 0.5 mM $Fe_2(SO4)_3$ and 0.1 mM Na_2EDTA".

Krishnan et al. (2018) noted "the highest callus induction on MS medium supplemented 2.0 mgL-1 2, 4-D and 0.5 mgL^{-1} Kn from coleoptiles of ARC 15759 and ARC18214 lines".

Gomez & Kalamani (2001) studied "callus growth and plant regeneration of landraces rice (Oryza sativa L.) on MS medium supplemented with 2 mgL^{-1} 2,4-D + 0.5 mgL^{-1} Kn and observed this combination good for callus induction and observed this combination good for callus induction and callus growth".

Gul et al. (2000) studied "four rice cultivars including Basmati-385, Jp-5, Pakhal and Swatt-II cultured in MS medium and different growth regulators were used and reported that 2.0 mgL^{-1} 2,4-D with 0.2 mgL^{-1} Kinetin was best for callus induction".

Mosavi et al. (2001) studied "callus induction from mature embryos of six indica rice cultivars and reported that maximum callus initiation was observed on MS medium supplemented with 2.0 mgL^{-1} 2, 4-D in all cultivars".

Azria & Bhalla (2000) cultured matured embryos of four Australian varieties (Amaroo, Millin, Pelde and Longi) of rice on MS and N6 media and indicated that MS medium supplemented with 0.5-2.0 mgL^{-1} 2, 4-D was suitable for callus formation for those varieties.

Dode et al. (2000) studied the effects of growth regulators (2,4-D, Kn, BAP and NAA) on callus induction from mature seeds of 10 rice cultivars and reported that seeds incubated on MS medium containing 2,4-D showed the highest callus induction frequency. (Gonzalez, 2000) cultured the seeds of rice cv. Amisted 82 and reported that there was no callus formation in the absence of 2, 4-D. Gandhi & Khurana (1999) studied mature seed embryos of 5 genotypes of indica rice (IR-72, IR-36, Rasi, Mashuri and Jaya) on MS medium

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supplemented with 2,4-D and observed a high frequency of callus induction (100%). Mostly 2, 4-D has been used as the only growth regulator in callus induction media.

Katiyar et al. (1999); Zhenyu et al. (1999); and Al-Khayri et al. (1996) showed that genotype, sugar type and the concentrations of kinetin, 2, 4-D and NAA influenced callus induction and plant regeneration. They observed that plant regeneration was optimal on media containing 0.5 mgL-1 2, 4-D. They also found that higher concentrations of 2, 4-D inhibited plant regeneration. High-frequency callus production was achieved when mature seeds of indica rice were cultured on MS-based media supplemented with strong auxins, such as 2, 4-D.

2.2.4 Influence of pH

Murashige (1973) observed that the pH of plant tissue culture media is generally adjusted to pH 5.5 to 6.0. Below 5.5, the agar will not have gel properties and above 6.0, the gel may be too film.

Lee et al. (2003) experimented and reported that pH 5.8 was suitable for both callus initiation and plant regeneration culture media.

2.3 Organogenesis

Lai & Liu (1982) studied plant regeneration is of crucial importance in the realization of the potential of cell and tissue culture techniques for plant improvement. In previous studies, we discovered that shoot regeneration frequency was magnanimously different among rice callus induced from different varieties. Different researchers have reported the differentiation of plants from callus culture. Regeneration of plants has drawn less attention than callus induction. However, literature closely related to the *in vitro* regeneration of rice is enumerated here.

Khalequzzaman et al. (2005) studied on genotypic effect observed for embryogenic callus induction and subsequent plant regeneration in 15 indica-type Bangladeshi rice (*Oryza satiava* L.) landraces. The highest frequency (55.6%) of shoot regeneration from embryogenic callus was observed in Hashikalmi with the supplementary hormone concentration at 2.0 mgL^{-1} BAP, 1.0 mgL^{-1} NAA and 1.5 mgL^{-1} Kn in MS medium

Saharan et al. (2004) studied on high frequency of plant regeneration from mature seed-derived embryogenic calli of two recalcitrant indica cv. HKR-46 and HKR-126 and maximum shoot regeneration frequency (63%) were observed in the MS medium supplemented with 2 mgL^{-1} Kn + 0.5 mgL^{-1} NAA + 30 gL^{-1} gelrite.

Asaduzzaman et al. (2003) studied five rice verities (viz. BR-5, BR-31, BR-34, BR-37 and BR-38) were culture for callus induction, plant regeneration and observed in MS medium supplemented with 2.0 mgL^{-1} BAP+ 0.5 mgL^{-1} + 1.0 mgL^{-1} NAA was the highest percentage (33.32%) of green plantlet regeneration from calli.

Fatima et al., (2002) found that plant regeneration (*Oryza satiava* L. cv. Basmati) showed in MS medium supplemented with 2 mgL-1 2, 4-D and 0.5 mgL^{-1} Kn.

Deepti et al. (2001) observed plant regeneration from matured embryos of six indica rice cultivars and observed that MS medium supplemented with 0.5 mgL^{-1} BAP was optimum for the regeneration of plantlets from all test cultivars Rashid et al. (2001) found that MS medium with 2 mgL^{-1} 2, 4-D is best for callus induction and combination with 1 mgL^{-1} NAA + 5 mgL^{-1} BAP showed the best plant regeneration frequency.

Shankhdhar et al. (2001) examined plant regeneration from mature seed-derived callus in six indica rice and

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reported that 0.5 mgL^{-1} BAP in combination with IAA was found to be optimum for the regeneration of all cultivars Vijayalaxmi & Reddy (1997) showed that plantlet regeneration was related to endogenous plant growth regulators of embryo-derived callus. They reported that the balance of endogenous growth regulators through adjusting exogenous growth regulators of culture media was an effective means of increasing culture efficiency. Plant regeneration 25% was obtained on MS medium supplemented with 1 mgL^{-1} NAA, 2 mgL-1 BAP, 0.5 mgL-1 Kn and 3% sucrose.

Usha Rani and Reddy (1996) reported that plant regeneration was maximum (92%) when media were supplemented with 2, 3 and 1 mgL^{-1} of IAA, BAP and Kn, respectively.

Marassi et al. (1996) observed shoot regeneration in the medium supplemented with 0.5 or 1 mgL^{-1} 2, 4-D with cytokinins. They reported that the best medium for plant regeneration was MS with 1.5 mgL^{-1} BAP.

Mandal & Gupta (1995) found that maximum green plant regeneration was obtained in calli induced in the presence of BAP and later transferred to a regeneration medium supplemented with 0.5 mgL^{-1} NAA and 2.0 mgL^{-1} Kn.

Peyachoknagul et al. (1994) cultured embryo-derived calluses and found that about 30% of these calluses were regenerated to plantlets on a medium containing 2.0 mgL^{-1} Kn. They also observed that the best shoot formation was given by the media containing 10 mgL^{-1} BAP.

Seraj et al. (1991) reported regeneration of rice plantlets on media using 0.37 mgL^{-1} NAA and 10.7 mgL^{-1} Kn in combination with YE and CH.

2.4 Effect of Irradiation on Callus and Plant Regeneration

Different workers observed the effect of gamma irradiation on the callus of rice at different doses. Some of the important information closely related to the present study is reviewed below:

Jianjun et al. (1994) examined that induced mutagenesis serves as a source of variability for better selection. Many research works have attempted to exploit somaclonal variation for crop improvement particularly treated with gamma irradiation. Considerable work has been done on induced mutation in rice callus.

Lee et al. (2003) studied rice in vitro mutagenesis and various (30, 50, 70 and 90 Gy) doses of gamma-ray were applied to investigate the effect of irradiation on callus formation and green plant regeneration, and reported that 30 and 50 gamma rays had significant effects on callus formation and plant regeneration.

Sarowar (2003) studied the effect of gamma irradiation (0, 1, 2, 3, 4, 5, 6 and 7) on callus and subsequent regeneration of three aromatic rice verities (Kalizira, BRRI dhan34 BRRI dhan38) and found that shoot regeneration and root induction was significantly affected by gamma-ray and decreased gradually with the increase in irradiation dose.

Min et al. (1991) studied that the callus induction percentage decreased with the increase of the radiation dosage given to germinating embryos. The callus induction frequency was 53.1%, 40.7%, and 37.5% for 2.5, 5.0 and 10.0 KR dosage treatment respectively, while that of the control was 57.4%. It is suggested that the low dosage irradiation treatment (2.5-5.0 KR) to callus with green spots is worthy of adoption in rice improvement.

Chen et al. (2001) applied various doses (10.50 Gy) of gamma rays to investigate the effect of radiation on callus formation and green plant regeneration and reported that 20 Gy gamma rays had a significant stimulation effect on the regeneration of green plants from rice anther culture.

Hossain & Alam (2001) studied regenerable embryo-derived calli of four rice varieties exposed to 0 to 6 Gy

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of gamma rays to determine their effect on growth and plant regeneration capacity. Both growth and regeneration capacity decreased with increasing levels of gamma rays, however, plant regeneration capacity was more sensitive to gamma rays than growth. The 50% inhibition dose for callus growth and plant regeneration was approximately 5.0 Gy of gamma irradiation in the Binnatoa variety. But in other varieties, it was 4.0 Gy for callus growth and 2.0 Gy for regeneration.

Mullin et al. (2021) cultured seeds of 4 Basmati rice cultivars on MS medium. Calli were sub-cultured and irradiation with 0, 1, 2, 3, 4 and 5 KR gamma radiation subsequent plants were regenerated on MS medium containing 1 and 2 mg BAP, 0.5 mgL^{-1} Kn and 1.0 mgL^{-1} NAA. They reported that Basmati 370 had the highest regeneration frequency and 3 KR was determined as the best irradiation dose.

Gao et al. (1992) experimented to determine the effect of gamma irradiation and reported that 2.5-5.0 KR was the optimal dose range for mature embryos of rice for their regeneration.

Maddumage et al. (1990) observed the callus derived by culturing mature grains of rice 3 varieties on modified MS medium were treated with gamma ray. They reported that regenerative ability was appreciably reduced by gamma irradiation.

2.5 Induction of Root

In vitro root induction of rice was reported by several research workers. The information which is closely related to root induction is reviewed here:

Ahmed (2004) studied three aromatic rice cultivars and the rooting medium used (1.0, 2.0, 2.5 and 3.0 mgL^{-1}) MET and reported that 2.5 mgL^{-1} MET was suitable for root induction with an increased number of roots per plant.

Islam et al. (2004) & Hossain (2000) studied indica rice (*Oryza sativa* L. cv. Kataribhog) used 2.5 mgL^{-1} MET + 0.5 mgL^{-1} NAA for root induction in MS medium and reported that maximum root induction and root length occurred with those hormonal combinations. Studied on four rice cultivars and used MET (1.0, 1.5, 2, 2.5 and 3.0 mgL^{-1}) for root inducing in MS medium and reported that 2.5 mgL^{-1} MET was best for producing root induction, number of roots per plant and length of roots.

Min et al. (1991) reported that application of MET with NAA, IAA and Ethrel enhanced root formation as well as strengthening the roots from regenerated plantlets.

Sahrawat & Chand (2001) reported that regeneration shoots of indica rice (*Oryza sativa* cv. Kasturi) were rooted on an MS basal medium containing 4.9 μM IBA and plants were successfully transferred to soil and grown to maturity.

METHODOLOGY

This chapter discusses the different materials used and the methodology used during the research period.

3.1 Experimental Design

CRD (Completely Randomized Design) was followed in this experiment. Samples were collected randomly from Dinajpur district rural area. The experiment was carried out from June 2022 to February 2023 at the Tissue Culture Laboratory of the Plant Breeding Division, BINA, Mymensingh, Bangladesh. The experiment used mature rice grain seeds as material. Kataribhog rice was used as the grain in this experiment.

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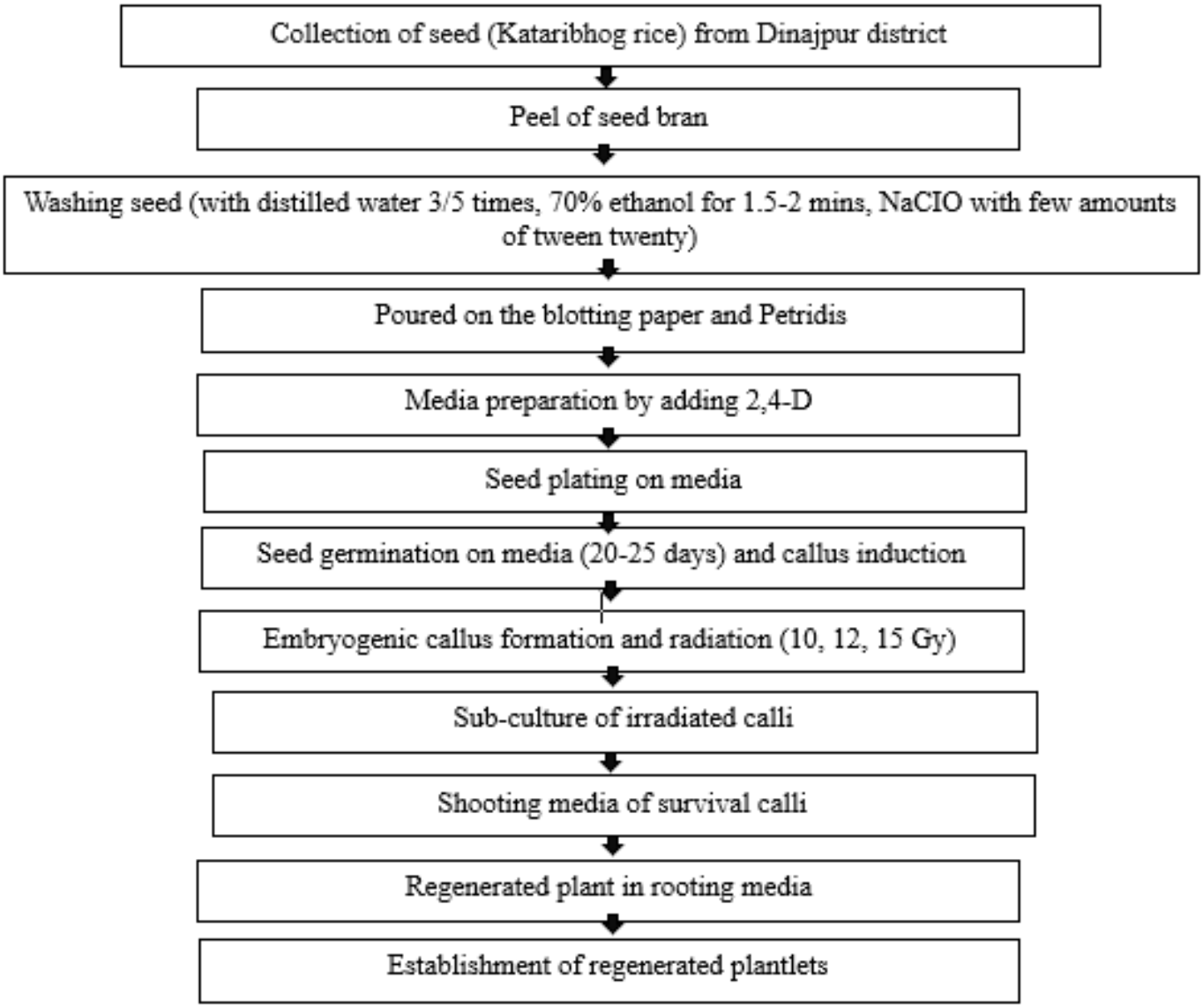

Fig. 1. Layout of the experimental design

RESULTS AND DISCUSSION

The present study was conducted with 3 types of media namely PT-100 G, PT-011G and PT-100. These media were used for embryogenic callus formation from callus. The results of different steps of the experiment are described under the following heads.

4.1 Callus Initiation from Mature Embryos

A callus is largely unorganized, with the continued proliferation of undifferentiated parenchyma cells from parent tissue on clearly defined semi-solid media. One rice cultivar (Kataribhog) in MS medium supplemented with different concentrations and combinations of hormones was used to achieve the ultimate goal of embryogenic callus formation via calli. Mature embryos of this cultivar were used as explants. The results of different steps of the experiment for callus initiation from seeds are described below:

4.1.1 Callus initiation response of seeds on MS Medium Supplemented with 2,4-D

The seeds of one rice cultivar (Kataribhog) were inoculated on MS medium supplemented with 2,4-D (2.0

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mgL^{-1}) to observe the callus formation response. Callus formation was invariably developed from the scutellum region of the seeds and was visible within 14 days. Results on the callus initiation performance of the rice cultivar (Kataribhog) are presented in Table 1. Among the treatments, high callus initiation (%) was observed in T_1 (PT-100G) followed by T_2 (PT-011G) and T_3 (PT-100) in all concentrations. The average of callus initiation of cultivar (%) was 58.72%. Initiation of embryogenic calli is considered the most critical step. Several different media including MS medium have been used for rice tissue culture and mostly 2,4-D has been used as the only growth regulator in callus initiation media (Katiyar et al., 1999; Zhenyu et al., 1999). In most of the early reports, it was observed that different concentrations of 2, 4-D were widely used in MS medium for sufficient callus in rice (Briside et al., 1990; Azira and Bhalla, 2000).

The present findings also showed concurrency with the findings of Gao and Huang (1999) and Aditya et al. (2004). They also reported 2.0 mgL^{-1} 2,4-D as the suitable concentration for callus initiation. Higher levels of 2,4-D showed inhibitory effects on callus induction and growth probably by damaging the cells. High-frequency callus production was achieved when mature seeds of Kataribhog rice were cultured on MS based medium supplemented with strong auxins, such as 2,4-D (Seraj et al., 1997) (Fig. 5).

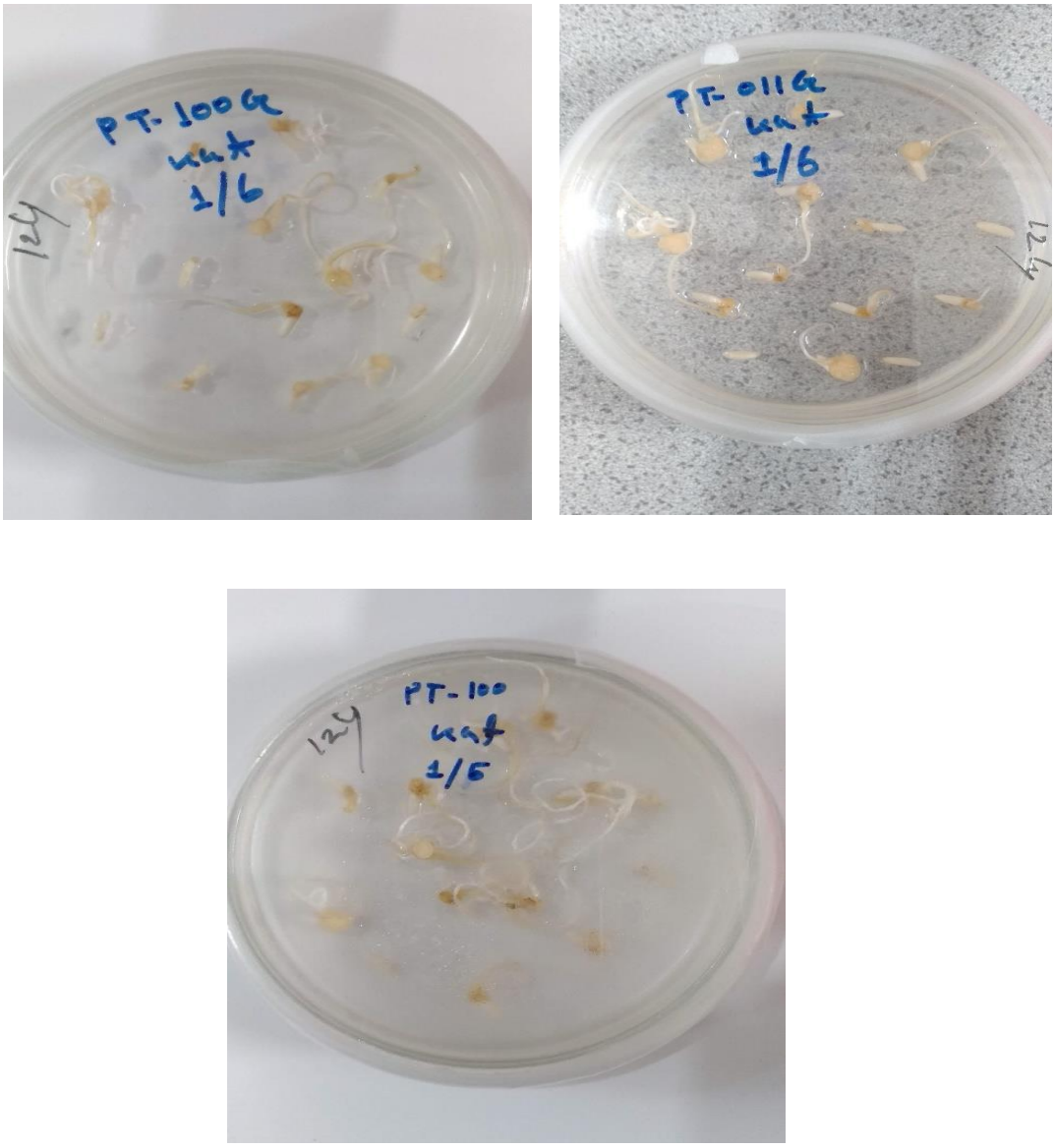

Fig. 2. Callus initiation efficiency from mature embryos of rice (kataribhog) on MS medium supplemented with 2,4-D on three different types of media (PT-100 G, PT-011G and PT-100)

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Table 1. Callus initiation efficiency from mature embryos of Kataribhog rice cultivar on MS medium supplemented with 2,4-D

Cultivar	Growth regulators (mg L^{-1}) 2,4-D	Treatment	No. of Explants Inoculated	No. of Explants Showing Callus Initiation	Callus Initiation (%)	Average of Callus Initiation of Cultivar (%)
Kataribhog	2.0	T_1(PT-100G)	82	65	79.27	58.72
	2.0	T_2(PT-011G)	61	32	52.46	
	2.0	T_3(PT-100)	63	28	44.44	

3.1.2 Embryogenic callus initiation response of mature embryos of kataribhog rice cultivar on various treatment

The mature embryos of the rice cultivar (Kataribhog) were inoculated on various treatments to observe the embryogenic callus formation response. Embryogenic callus formation invariably develops within 21 days. Results on embryogenic callus initiation performance of local rice cultivar (Kataribhog) are presented in Table 2.

Among the treatments, high embryogenic callus initiation (%) was observed in T_1 (PT-100G) (32.93%) followed by T_2(PT-011G) (27.87%) and T_3(PT-100) (11.11%). Average of embryogenic callus initiation of the cultivar (%) was 23.97%. Initiation of embryogenic calli in rice is considered the most critical step. (Fig 6)

Table 2. Embryogenic callus initiation response of mature embryos of Kataribhog rice cultivar on various treatments

Cultivar	Treatment	No. of Explants Inoculated	No. of Explants Showing Embryogenic Callus Initiation	Embryogenic Callus Initiation (%)	Average of Embryogenic Callus Initiation of Cultivar (%)
Kataribhog	T_1(PT-100G)	82	27	32.93	23.97
	T_2(PT-011G)	61	17	27.87	
	T_3(PT-100)	63	7	11.11	

ANOVA and CRD data analysis for NSP, NCI and NEC

For data analysis here single factor CRD has been followed.

Here,

>Trt=as. factor (r$ Treatment)

Data sheets for NSP, NCI and NEC are given in Appendix 3 4 and 5.

3.1.3 The results of the group table for NSP, NCI and NEC

From the NSP groups (Table 3) it was clear that treatments had no significant differences. The treatment

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mean was equal for all of the treatments.

From the NCI groups (Table 4) it was clear that treatments had no significant differences. The highest treatment mean was observed in T_1(PT-100G) (50) and the lowest was observed in T_3(PT-100) (31).

From the NEC groups (Table 5) it was clear that all treatments had significant differences. The highest treatment mean was observed in T_1(PT-100G) (21) and the lowest was observed in T_3(PT-100) (14).

Note: NSP (Number of Seed Plating), NCI (Number of Callus Induction), NEC (Number of Embryogenic Callus)

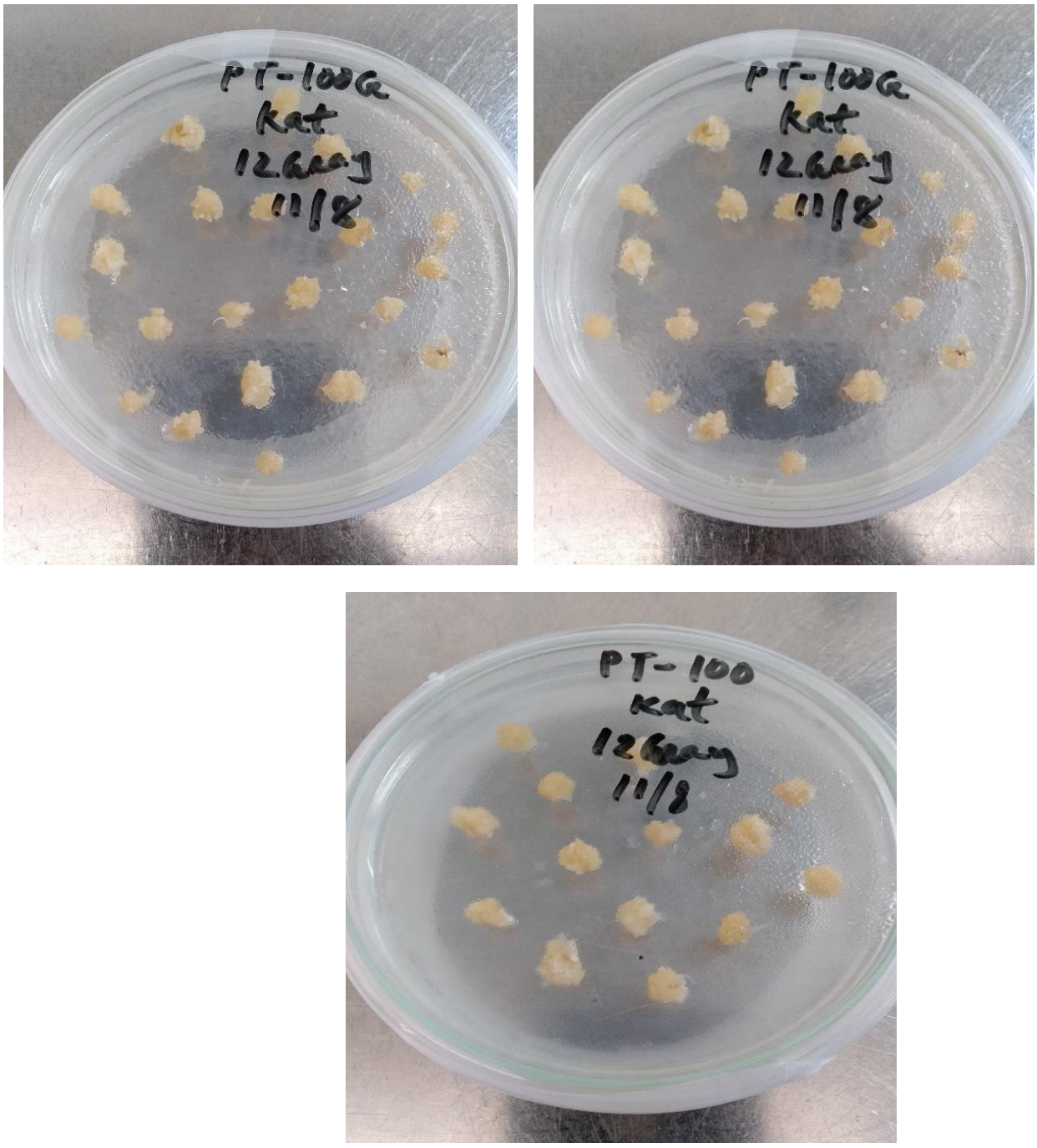

Fig. 3. Embryogenic callus initiation from mature embryos of kataribhog rice cultivar on three different

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types of culture media (PT-100 G, PT-011G and PT-100)

Table 3. Treatment-wise table for NSP groups of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Treatment	Mean	NSP groups
T1(PT-100G)	120	a
T2(PT-011G)	120	a
T3(PT-100)	120	a

Table 4. Treatment-wise table for NCI groups of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Treatment	Mean	NCI groups
T1(PT-100G)	49.6	a
T2(PT-011G)	35.6	ab
T3(PT-100)	31.0	b

Table 5. Treatment-wise table for NEC groups of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Treatment	Mean	NCI groups
T1(PT-100G)	21.4	a
T2(PT-011G)	13.6	b
T3(PT-100)	13.6	b

Note: NSP (Number of Seed Plating), NCI (Number of Callus Induction), NEC (Number of Embryogenic Callus)

3.1.4 The results of mean value, CV and LSD for treatment

For NSP (Table 9) the mean value (120), CV (3.552714e-14) and LSD was 5.874777e-14. For NCI (Table 10) the mean value (38.73), CV (31.54) and LSD was 16.83, For NEC (Table 11) the mean value (16.2), CV (29.26) and LSD was 6.53. That is presented in Appendix VI.

3.2 Effect of Irradiation of Callus on Shoot Regeneration

Different doses of gamma irradiation (10, 12, 15 Gy) were applied in the calli of the rice cultivar (Kataribhog) to see the effect of gamma rays on plant regeneration. The irradiated calli were then transferred to MS medium supplemented with 1.5 mgL^{-1} NAA + 2.5 mgL^{-1} BAP to initiate plantlets.

After 21 days of irradiation, it was observed (Table 12) that the extent of regeneration ability varied from different irradiation doses. In the genotype (Kataribhog), regeneration percentages were found to be higher in the 10 Gy (80%) followed by 15 Gy (71.43%) and 12 Gy (66.67%). (Table 6).

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3.3 Effect of Gamma Irradiation on Root Induction from Shoot

The regenerated shoots of the local rice cultivar (Kataribhog) required sufficient roots to establish them in the soil. MS media supplemented with 0.5 mgL^{-1} IBA was used to see the rooting response of the regenerated shoots. Root induction (%) and number of roots per plant were studied in this experiment.

Table 6. Effect of irradiation on callus for shoot regeneration

Cultivar	Dose (Gy)	No. of Irradiated Calli Sub-culture	No. of Calli Survive	No. of Survived Calli Showing Shoot Induction	Shoot Regeneration (%)
Kataribhog	10	18	15	12	80
	12	23	3	2	66.67
	15	41	7	5	71.43

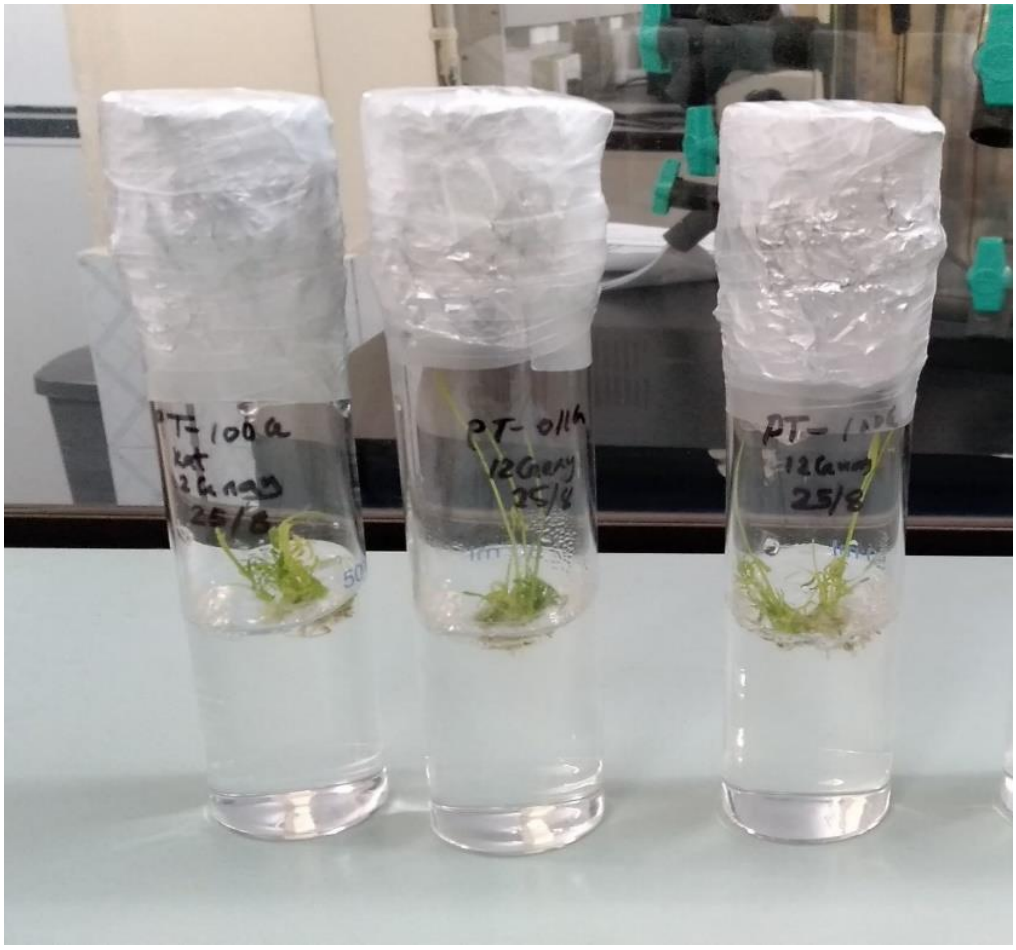

Fig. 4. Shoot regeneration from callus of local rice (Kataribhog) cultivars with gamma irradiation (12Gy) treatment 3 types of media (PT-100 G, PT-011G and PT-100)

After 15 days, it was observed (Table 13) that the extent of root induction ability varied from shoots that were differentiated due to different doses of irradiated calli. In the genotype (Kataribhog), root induction percentages were found to be higher in the 15 Gy (60%) followed by 10 Gy (58.33%) and 12 Gy (50%). (Table 7)

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Fig. 5. Root induced from shoot of local rice (kataribhog) cultivars on MS + 0.5 mgL^{-1} IBA 3 types of media (PT-100 G, PT-011G and PT-100)

Table 7. Effect of gamma irradiation on root induction

Cultivar	No. of Survived Calli Showing Shoot Induction	No. of Shoot Showing Root Induction	Root Induction (%)
Kataribhog	12	7	58.33
	2	1	50
	5	3	60

Fig. 6. Established plants of local rice cultivars in earthen plot on 3 types of media (PT-100 G, PT-011G and PT-100)

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3.4 Establishment of Regenerated Plantlets

After sufficient development of the root system, the small plantlets were taken from the culture vessels without damaging the roots. Excess agar around the roots was washed off by tap water to prevent microbial infection. Then the plantlets were transplanted in small plots. When the plantlets grew to a height of above 10 cm and sufficient roots were proliferated, those were transferred to earthen pots following the procedure described in materials and methods. The growth condition, the tillering capacity of plantlets and the survival rate of the plantlets in the plot were satisfactory.

CONCLUSION

To determine the ideal culture conditions for callus initiation from mature embryos of an aromatic rice cultivar, Kataribhog as explants and to examine the impact of gamma rays on callus and subsequent regeneration, a comparative study was conducted. From June 2022 to February 2023, the experiment was carried out in the Plant Breeding Division's Tissue Culture Laboratory at BINA, Mymensingh. The following list provides a summary of the study's findings.

A variety of hormone doses and combinations were added to the MS medium to cultivate mature embryos from test cultivars. The use of 2,4-D alone was made for callus initiation hormonal therapies. The largest callus initiation (41.67%) was seen among the hormonal therapies in T1(PT-100G) with 2.0 mgL^{-1} 2,4-D, while the lowest callus initiation (25.83%) was seen in T3(PT-100). We can conclude from this study that growth regulators had a varying impact on callus initiation efficiency.

To examine their ability to regenerate shoots, initiated calli test cultivars were grown on MS medium supplemented with 1.5 mgL^{-1} NAA + 2.5 mgL^{-1} of hormones. Regeneration percentages in the genotype (Kataribhog) were found to be greater in the 10 Gy (80%), followed by the 15 Gy (71.43%), and the 12 Gy (66.67%).

On the other hand, irradiated calli (by 10, 12 and 15 Gy) derived shoots were collected and cultured onto MS medium supplemented with 0.5 mgL^{-1} IBA to observe the effect of gamma rays on root induction (%). Among the different treatments of gamma rays, the 15 Gy (60%) showed better performance than the treated ones.

The following recommendation could be made based on the above experiments:

2. The local rice cultivar (Kataribhog) responded well to callus induction using 2.0 mgL^{-1} 2,4-D.
3. For shoot differentiation 5 mgL^{-1} NAA + 2.5 mgL^{-1} BAP was effective for all the cultivars.
4. For root induction 0.5 mgL^{-1} IBA was effective for all the cultivars.

Based on the discussion above, it may be inferred that a technique for in vitro regeneration using the callus-forming process and plantlet regeneration was created from mature embryos. The procedure created would be used in the future to improve local rice in other exotic and local cultivars.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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APPENDIX

Appendix I.

Function of MS medium (PT-100G, PT-011G, PT-100):

Macro-elements

1. Potassium nitrate (KNO_3); Potassium functions as osmoregulators, cation-anion balance, and pH stabilization.
2. Ammonium nitrate (NH_4NO_3): Nitrogen is a component of protein, nucleic acid, chlorophyll, and some coenzymes and is required in greatest amounts.
3. Potassium phosphate monobasic (KH_2PO_4): It is an essential component of nucleic acid, phospholipids and energy-rich compounds.
4. Calcium chloride ($CaCl_2.2H_2O$): It has functions like the synthesis of the cell wall, membrane function, cell signalling
5. Magnesium sulphate ($MgSO_47H_2O$): It is a vital component of amino acids, vitamins, cofactors, iron sulphur protein (electron carrier),

Micro-elements

1. Manganese (II) sulphate monohydrate ($MnSO_4.4H_2O$): Manganese is found in metalloproteins.
2. Boric acid (H_3BO_3): It is a component of the cell wall required for cell division of optical meristem.
3. Zinc sulphate hyptahydrate ($ZnSO_4.7H_2O$): It is required for the activity of various enzymes.
4. Potassium iodide (KI): It improves the growth of roots and callus.
5. Molybdic acid ($Na_2MoO_4.2H_2O$). It is an enzyme cofactor:
6. Copper sulphate pentahydrate ($CuSO_4.5H_2O$): It serves as an enzyme cofactor and functions in electron reactions.
7. Cobalt chloride hexahydrate ($CoCl_2.6H_2O$): It is a component of some vitamins.

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a. Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$): It is required by the plant for chlorophyll synthesis as a constituent of cytochrome and Fe-S protein.
b. Ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2-EDTA): EDTA is usually used in conjunction with iron sulphate. This complex with iron allows slow and continuous release of iron into the medium.

Organic nutrients/vitamins

1. Nicotinic Acid: It is required by plants for synthesizing the amino acids.
2. Pyridoxine HCI: It stimulates the growth of the cells in some cases.
3. HCI: It is an essential one for many plant cells.
4. Inositol: It is a growth factor and is thought to be important in membrane and cell wall development.
5. Agar: Agar has long been used to solidify media for plant tissue culture. The type of agar or gelling agent used can influence the growth of the tissue in culture,
6. Sucrose: Sugars serve as an energy source for plant culture. Sucrose is the most commonly used sugar in plant culture media.

Amino acid

1. Glycine: It serves as a source of amino acid.

Appendix II

Detail medium components used in various treatments:

For callus initiation

1. T_1 (PT-100G)
2. MS powder 4.5 mgL^{-1}, Sucrose 30 gL^{-1}, pH 5.8, Gelrite 1,6 g/250 mL, 24-D (2 mgL^{-1}) T_2 (PT-0115)
3. MS powder 4.5 mgL^{-1}, Maltose 30 gL^{-1}, pH 5.8, Gelrite 1.6 g/250 mL, 2,4-D (2 mgL^{-1}) T_3 (PT-100)
4. MS powder 4.5 mgL^{-1}, Sucrose 30 gL^{-1}, Proline (0.5 gL^{-1}), Glutamine (0.5 gL^{-1}), Ph 5.8, Gelrite 1.6 g/250 mL, 2,4-D (2 m gL^{-1}).

For shoot differentiation

- MS powder 1.20 gL^{-1}, Sucrose 7.5 gL^{-1}, pH 5.8, Gelrite 1.40 g/250 mL, NAA (1.5 m gL^{-1}), BAP (2.5 mgL^{-1})

For root induction

- MS powder 2.25 gL^{-1}, Sucrose 15 gL^{-1}, pH 5.8, Agar 2 g/250 mL, IBA (0.5 m gL^{-1})

Appendix III

ANOVA and CRD data analysis for NSP, NCI and NEC

For data analysis here single factor CRD has been followed.

Here,

>Trt=as. factor (r$ Treatment)

The results of the ANOVA table for NSP, NCI and NEC

The ANOVA table for NSP, NCI and NEC are presented in Tables 8,9, and 10

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Table 8. ANOVA for NSP of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	3.6351e-27	1.8175e-27		1 0.3966
Residuals	12	2.1810e-26	1.8175e-27		

Appendix IV

Table 9. ANOVA for NCI of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	938.53	469.27	3.145	0.07975
Residuals	12	1790.40	149.20		

Appendix V

Table 10. ANOVA for NEC of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	202.8	101.400	4.5134	0.03455 *
Residuals	12	269.6	22.467		

P Value ≤ 0.05 (*)

P Value ≤ 0.01 (**)

P Value ≤ 0.001 (***)

Note: NSP (Number of Seed Plating), NCI (Number of Callus Induction), NEC (Number of Embryogenic Callus)

Appendix VI

Table 11. Treatment-wise table for NSP mean, CV and LSD of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Statistics	Mean	CV	LSD
Treatment	120	3.552714e-14	5.874777e-14

Table 12. Treatment-wise table for NCI mean, CV and LSD of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Statistics	Mean	CV	LSD
Treatment	38.73	31.54	16.83

Table 13. Treatment-wise table for NEC mean, CV and LSD of local rice cultivar (Kataribhog) in MS medium supplemented with 2,4-D

Statistics	Mean	CV	LSD
Treatment	16.2	29.26	6.53