Plant Tissue Cult. & Biotech. **34**(2): 153-164, 2024 (December) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v34i2.78830

PTC&B

Optimization of Plant Growth Regulators for Efficient Embryogenic Callus Induction and Subsequent Plant Regeneration in Two Indigenous Aromatic Rice Varieties of Bangladesh

Mimma Afrin[1], Sium Ahmed, Tanvir Ahamed, Mir Md. Mahbubur Rahman, Sayeda Mahfuja Khatun[2], A.K.M. Mohiuddin[1] and Abdullah Mohammad Shohael*

Cell Genetics and Plant Biotechnology Laboratory (CGPBL), Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Key words: Aromatic rice, Callus induction, Kalijira, Somatic embryogenesis, Tissue culture, Tulshimala

Abstract

An efficient regeneration protocol via somatic embryogenesis for two aromatic Indica rice varieties, Kalijira and Tulshimala was developed. Using mature, dehusked seeds, the process involved callus initiation, embryo development and plant regeneration involving MS medium accompanied with specific concentrations of the auxins 2,4-D and NAA, along with the cytokinin BAP. Callus induction was optimized at 2 mg/l 2,4-D, and 1 mg/l NAA, yielding initiation rates of 87% for Kalijira and 95% for Tulshimala. The medium supplemented with 2 mg/l 2,4-D supported robust embryogenic calli development. Mature embryos were successfully regenerated on media comprising 0.1 mg/l NAA and 2.5 mg/l BAP for Kalijira while 2 mg/l BAP for Tulshimala, with 35% of Kalijira calli forming multiple shoots and 65% rooting, on the other hand Tulshimala achieved 70% shoot formation and 100% rooting. Transplanted plantlets adapted well, showing high survival rates.

Introduction

Rice (*Oryza sativa* L.) is an annual grass species from the Poaceae family. Aromatic rice varieties are a unique and limited category of rice as they are regarded as the highest grade because of their aroma, flavor, and texture (Weber et al. 2000). Although aromatic rice is highly priced, its market demand is high (Singh et al. 2000). Aromatic rice is valued

*Author for correspondence: <amshohael@juniv.edu>. [1]Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh. [2]Holding No-55, East Talaimari, Motihar, Rajshahi, Bangladesh.

for its culinary qualities and economic importance in global trade, making it a focus of agricultural research aimed at improving yield, aroma retention, and stress resilience. Ambemohar, Basmati, Chinigura, Gobindobhog, Jasmine, Kali Mooch, Kalijira, Sona Masuri, Texmati, Tulaipanji, Tulshimala, Wehani, and wild pecan rice are some of the popular varieties of aromatic rice (Singh et al. 2000). Some of the well-known local varieties of Bangladesh are Badshabhog, Chiniatab, Chinigura, Kalijira, Kataribhog, Khirshapati, Madhumala, Radhunipagal, Shakhorkora, Tulshimala and Zirabhog (Hossain et al. 2008). Among these, Kalijira, Kataribhog, and Tulshimala rice received Geographical Indication (GI) product tags in Bangladesh (Bangladesh Trade Portal 2024).

The grain production per hectare of most conventional aromatic rice varieties is unsatisfactory. Several efforts have been made to produce high-yielding varieties with superior nutritional quality using classic and mutation breeding, extensive hybridization, omics approaches, genetic engineering, and gene editing (Zafar and Jianlong 2023). Genetic engineering methods are employed to create rice plants with increased yield characteristics and improved stress resistance (Parmar et al. 2017). Usually, tissue culture methods are employed to generate genetically engineered plants, and establishing an efficient tissue culture method is considered a requirement in the development process (Purwantoro et al. 2022). In plant biotechnology, plant tissue culture is an essential method that enables the *in vitro* development of plant cells, tissues, or organs on a nutritional medium. This technique makes it easier to produce genetically uniform plantlets, faster multiplication of disease-free plants, and conserve rare or endangered species (Bhojwani and Dantu 2013).

By manipulating hormonal and environmental conditions, the tissue culture technique can enable somatic embryogenesis, leading to the generation of somatic embryos, which can further result in complete plants with well-formed roots and shoots (Desai et al. 2022). Somatic embryogenesis mimics the phases of zygotic embryogenesis in which somatic cells turn into embryos without fertilization (Desai et al. 2022). This phenomenon can be induced *in vitro* by exposing plant tissues to specific growth regulators under controlled conditions. Somatic embryos can develop into whole plants, making this technique valuable for clonal propagation, genetic engineering, and conservation of genetic resources (Bidabadi and Jain 2020).

Rice research focuses on key agronomic traits such as high grain quality, increased yield, and resistance to diseases, pests, and environmental stress (Rezvi et al. 2023). However, aromatic rice varieties often exhibit drawbacks, including susceptibility to pests and diseases, low productivity, taller growth prone to lodging, and susceptibility to both biotic and abiotic stresses. With the growing global demand for aromatic rice and the current limitations of existing varieties, there is a pressing need to develop improved aromatic rice cultivars that combine superior quality with enhanced resilience and yield potential (Kaewmungkun et al. 2023). Developing indigenous aromatic varieties into high-yielding varieties with enhanced resilience is imperative to support local economy, improve food security, and maintain biodiversity. Therefore, integrating biotechnology

with traditional breeding can accelerate the development of aromatic rice varieties that maintain their characteristic fragrance while exhibiting better performance under diverse agricultural conditions (Prodhan and Qingyao 2020). Establishing a reliable tissue culture method is crucial for supporting the application of modern biotechnology and new breeding techniques in aromatic rice.

In light of these conditions, this study aimed to evaluate *in vitro* callus induction, embryogenesis, multiple shoot formation, root formation, and subsequent regeneration in nutrient media with various amalgamations of growth regulators for two indigenous aromatic rice varieties, Kalijira and Tulshimala. The primary objective was determining the optimal concentrations and combinations of growth regulators needed for effective callus initiation, somatic embryogenesis, and regeneration in these varieties. Ultimately, the goal was to develop an effective regeneration protocol to support future genetic improvement efforts for Kalijira and Tulshimala.

Materials and Methods

The experiment was conducted at the Cell Genetics and Plant Biotechnology Laboratory, Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Dhaka-1342, Bangladesh (23°53'14" N 90°15'56" E). Kalijira and Tulshimala rice seeds were collected from the local farmers of Tangail district, Bangladesh, and used as explants in this study.

The study utilized two auxins, 2,4-Dichlorophenoxyacetic Acid (2,4-D) and α-Naphthalene Acetic Acid (NAA), along with one cytokinin, 6-Benzylaminopurine (BAP). Different concentrations and combinations of these phytohormones were incorporated into the MS medium. For scutellum-derived callus induction, three hormonal treatment groups were employed. The first group contained 2,4-D at concentrations of 1.0, 2.0, 3.0 and 4.0 mg/l. The second group contained NAA at concentrations of 0.5, 1.0, 2.0 and 3.0 mg/l. The third group contained a constant 2.0 mg/l concentration of 2,4-D combined with four different concentrations of NAA (0.5, 1.0, 1.5 and 2.0 mg/l). For embryogenic callus production, varying 2,4-D concentrations of 1.0, 2.0, 3.0 and 4.0 mg/l were employed. For high-frequency plant regeneration, different concentrations of BAP (1.5, 2, 2.5 and 3 mg/l) were paired with a fixed concentration of NAA (0.1 mg/l). A quantity of 30 mg/l (3%) sucrose was used as the carbon source, and 8 mg/l agar (0.8%) was used as a solidifying agent. The pH of the media was maintained at 5.6-5.8 before the addition of solidifying agent. Following this, the medium was sterilized by autoclaving.

For surface sterilization of the explants, the seeds were dehusked and rinsed with distilled water. Then, the seeds were dipped in 70% ethanol for 30 sec followed by thorough rinsing with sterile distilled water three times. A 10% sodium hypochlorite solution was prepared using the commercial bleach Clorox (5.25% sodium hypochlorite) with a few drops of Tween-20. The seeds were treated with this Clorox solution for 25-30

min, after which they were thoroughly rinsed with sterile distilled water 3-5 times. Finally, the seeds were gently kept in sterile tissue paper to remove excess moisture.

All inoculation procedures were conducted in a sterile, aseptic environment using a laminar airflow cabinet, following previously described protocols (Saha et al. 2015 2017). For callus initiation, dehusked sterilized seeds were inoculated. The inoculated explants were incubated in the growth chamber for 10-15 days at 25 ± 2°C in dark conditions. Data on callus initiation frequency and days to callus initiation were recorded. Callus initiation frequency was calculated as follows:

$$\text{Callus initiation frequency (\%)} = \frac{\text{No. of calli}}{\text{Number of inoculated seeds}} \times 100$$

After 15 days, the scutellum-derived calli were transferred to the same medium, producing nodular and compact calli. Selected calli were sub-cultured two times in a new, freshly prepared medium. During subculturing, two types of calli were observed-friable, yellowish, more prominent embryogenic calli and compact, translucent, slimy, non-embryogenic calli. Only the embryogenic sections were sub-cultured after being isolated from the non-embryogenic parts to facilitate embryogenic callus production. Subculturing was conducted twice, with 15-day intervals between each transfer. Data on average callus weight, properties of callus and degree of necrosis were recorded. For high-frequency plant regeneration, fragile and fast-growing embryogenic calli (nodular, white to pale yellow) were moved to the medium. Regeneration frequencies were observed after 20 days. Data on days of shoot initiation, number of shoots per callus, percentage of callus forming multiple shoots, and percentage of callus forming roots were recorded. During the callus induction and regeneration, the culture conditions in the growth chamber were kept at 23-25°C, 50% humidity, and a regulated photoperiod of 16 hrs of light and 8 hrs of darkness.

Regenerated shoots with well-developed root systems were removed from the jar and gently washed with sterile distilled water to eliminate any remaining medium. Afterwards, they were moved to pots filled with sterile soil and kept in a growth room at 27°C and 70% relative humidity for 7-10 days. The pots were covered with clear polythene bags to keep the plantlets from drying out, and frequently, water was sprayed on them to maintain adequate humidity levels. The plantlets were relocated to a polyhouse to acclimate to the natural environment. After they were completely established, the plantlets were moved to pots with a 1:1 mixture of autoclaved sand and soil for hardening and further growth.

The experiment was conducted in a completely randomized design (CRD). All data were displayed as the mean ± standard deviation (SD) of three independent biological replications. Significant differences among mean values were compared by Tukey's honestly significant difference (HSD) test at a level of significance of $p \leq 0.05$. Statistical analyses were performed using IBM SPSS Statistics 27.0.

Results and Discussion

Following two weeks of seed inoculation, creamy white swelling (pre-embryogenic masses) close to the root-shoot junction (scutellum area) indicated the onset of callus induction. The optimal 2,4-D and NAA levels for the highest callus initiation were assessed. Tables 1-2 present the findings. The table 1 shows that the callus formation was lowest in the MS medium supplemented with 4 mg/l 2,4-D for Tulshimala, 1 mg/l 2,4-D for Kalijira and also MS medium without any growth regulators (considered as control and data not shown in the table). In contrast, the callusing response of explants was more robust in the MS medium supplemented with 2 mg/l 2,4-D. The percentages of callus formation across different treatment groups for the two varieties were statistically significant.

Table 1. Effects of 2,4-D concentrations on callus initiation from scutellum of mature seeds of Kalijira and Tulshimala.

	Rice varieties			Kalijira				Tulshimala
Concentrations of 2,4-D (mg/l)	1.0	2.0	3.0	4.0	1.0	2.0	3.0	4.0
Days to callus initiation	10 ± 1 a	8 ± 1 a	9 ± 0 a	10 ± 1 a	9 ± 0 ab	7 ± 2 b	10 ± 1 a	11 ± 0 a
Callus formation (%)	52.22 ± 0.2 d	86.66 ± 0.1 a	75 ± 3 b	66 ± 1.5 c	76 ± 1 c	89 ± 2 a	70 ± 1 b	62 ± 2 d

Results are shown as Mean ± SD. Means with same letter within each row are not significantly different according to Tukey's HSD tests (P <0.05).

For NAA, the results clearly showed that callus initiation of explants did not occur at lower NAA values. Callusing and embryogenic responses were found when NAA concentrations were higher. Among these hormonal concentrations, the highest percentage of callus formation was found at 3.0 mg/l NAA for Tulshimala (55%) and Kalijira (45%) (Table 2). Days to callus initiation were varied among three varieties. The percentages of callus formation across different treatment groups for the two varieties were statistically significant.

Table 3 shows the results obtained from the experiments where the callusing responses were observed with four distinct NAA concentrations and a fixed 2,4-D concentration (2 mg/l). From the table, it is apparent that the response was much better when two auxins were used in combination rather than separately. Among these hormonal concentrations, callus induction was maximum for both varieties when the medium was supplemented with 2 mg/l 2,4-D and 1 mg/l NAA. A variation in callus initiation between varieties under identical experimental conditions suggests that callus initiation is genotype-dependent. Previous studies reported the same findings (Alam et al. 2003, Kaur et al. 1999, Summart et al. 2008). There were no significant differences in days to callus initiation in regard to concentrations of growth hormone applications, while significant differences were observed for callus formation.

Table 2. Effects of NAA concentrations on callus initiation from scutellum of mature seeds of Kalijira and Tulshimala.

Rice varieties	Kalijira				Tulshimala			
Concentrations of NAA (mg/l)	0.5	1.0	2.0	3.0	0.5	1.0	2.0	3.0
Days to callus initiation	NCI	NCI	13 ± 1 a	11 ± 0 b	NCI	NCI	10 ± 0 a	11 ± 1 a
Callus formation (%)	N/A	N/A	40 ± 2 b	45 ± 1 a	N/A	N/A	45 ± 3 b	55 ± 2 a

Results are shown as Mean ± SD. Means with same letter within each row are not significantly different according to Tukey's HSD tests (P <0.05). NCI- No callus initiation.

Table 3. Influence of NAA and 2,4-D combination on the percentage of callus formation and the days of callus initiation.

Rice varieties	Concentrations of 2,4-D and NAA	Days to callus initiation	Callus formation (%)
Kalijira	2 mg/l 2,4-D + 0.5 mg/l NAA	12 ± 1 a	70 ± 2 cd
	2 mg/l 2,4-D + 1 mg/l NAA	10 ± 2 a	87 ± 3 a
	2 mg/l 2,4-D + 1.5 mg/l NAA	11 ± 3 a	75 ± 2 bc
	2 mg/l 2,4-D + 2 mg/l NAA	14 ± 3 a	68 ± 3 d
Tulshimala	2 mg/l 2,4-D + 0.5 mg/l NAA	12 ± 1 a	70 ± 2 cd
	2 mg/l 2,4-D + 1 mg/l NAA	9 ± 2 a	95 ± 2 a
	2 mg/l 2,4-D + 1.5 mg/l NAA	10 ± 2 a	80 ± 3 b
	2 mg/l 2,4-D + 2 mg/l NAA	14 ± 3 a	75 ± 3 bc

Results are shown as Mean ± SD. Means with same letter within each column are not significantly different according to Tukey's HSD tests (P < 0.05).

The generated calli were sub-cultured, leading to multiplication and the development and maturation of somatic embryos in the medium. The development of pre-embryogenic masses following several cultures in auxin-supplemented media provided evidence of an indirect somatic embryogenesis pathway. The results obtained from this experiment are shown in Table 4. The resulting calli were compared structurally based on their external features (Fig. 1) under a microscope. It was noticed that MS media supplemented with 2.0 mg/l 2,4-D produced fragile, yellowish, nodular embryogenic callus for Kalijira and Tulshimala. The texture of the callus was primarily seen as compact at higher hormone doses and friable at lower concentrations. Of the four treatments, MS medium supplemented with 2 mg/l 2,4-D was the most efficient hormonal treatment for maximum proliferation in terms of the average weight of somatic embryogenic callus of both rice varieties. However, there was a lack of statistical significance. Proliferated calli were mostly friable, loose textured, and creamy-yellow, which was an indication of somatic embryos.

Table 4. Impact of varying 2,4-D concentrations on the production of embryogenic calli and determining the physical characteristics of the resulting embryogenic calli.

Rice varieties	Concentrations of 2,4-D (mg/l)	Average callus weight (g)	Properties of callus	Degree of necrotic callus
Kalijira	1.0	0.109 ± 0.03 a	Friable, brown embryogenic callus initiation	+
	2.0	0.143 ± 0.04 a	Fragile, yellowish, nodular embryogenic callus initiation	-
	3.0	0.088 ± 0.03 a	Yellowish-white nodular callus initiation	+ +
	4.0	0.079 ± 0.03 a	Translucent, slimy, and yellowish compact callus initiation	+ +
Tulshimala	1.0	0.128 ± 0.03 ab	Soft, friable, brown color non-embryogenic callus initiation	+
	2.0	0.185 ± 0.04 a	Fragile, yellowish, nodular large embryogenic callus initiation	-
	3.0	0.093 ± 0.02 b	Yellowish-white, translucent, and slimy compact callus initiation	+
	4.0	0.092 ± 0.02 b	Yellowish-brown, compact callus initiation	+ +

+++ = High; ++ = Moderate; + = Low; - = Negative. Results are shown as Mean ± SD. Means with same letter within the column are not significantly different according to Tukey's HSD tests ($P < 0.05$).

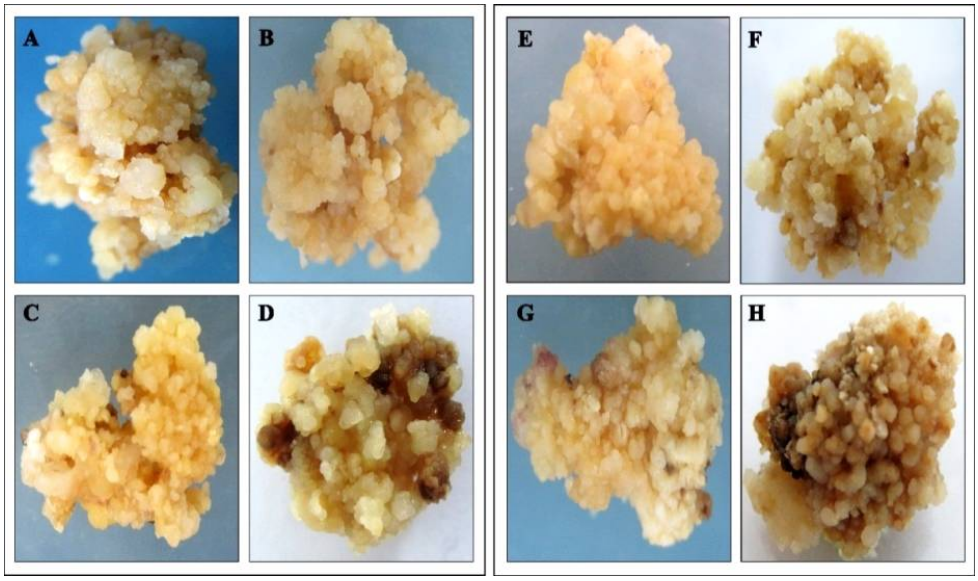

Fig. 1. Initiation and maturation of embryogenic calli of Kalijira and Tulshimala at different concentrations of 2,4-D. (A) Somatic embryogenesis at 1 mg/l 2,4-D for Kalijira rice seeds; (B) Somatic embryogenesis at 2 mg/l 2,4-D for Kalijira rice seeds; (C) Somatic embryogenesis at 3 mg/l 2,4-D for Kalijira rice seeds; (D) Somatic embryogenesis at 4 mg/l 2,4-D for Kalijira rice seeds; (E) Somatic embryogenesis at 1 mg/l 2,4-D for Tulshimala rice seeds; (F) Somatic embryogenesis at 2 mg/l 2,4-D for Tulshimala rice seeds; (G) Somatic embryogenesis at 3 mg/l 2,4-D for Tulshimala rice seeds; (H) Somatic embryogenesis at 4 mg/l 2,4-D for Tulshimala rice seeds.

Embryogenic calli showed high responsiveness to regeneration (Seraj et al. 1997). In addition, scutellum explants have the benefit of rapid and straightforward callus production, and no seedling germination is required to obtain explants (He and Lazzeri 2001, Joyia and Khan 2013). Although all somatic plant cells are theoretically totipotent, the success of callus induction and regeneration in rice is strongly influenced by factors such as explant origin, media composition, and genetic background (Hartke and Lörz 1989, Long et al. 2022, Pasternak and Steinmacher 2024).

Regeneration was performed with four different hormonal combinations of NAA and BAP supplemented in MS medium to find the appropriate concentrations for maximum regeneration of shoots. Data was documented across 6 weeks of inoculation and given in Table 5. The table shows that the regeneration responses significantly varied with the varying combinations of auxin and cytokinin. It was also evident from the results that the two varieties differed slightly in regeneration performance; Tulshimala had the highest regeneration efficiency, followed by Kalijira. While the embryogenic callus was put into MS media supplemented with 0.1 mg/l NAA + 2 mg/l BAP, 70% of the embryogenic callus produced multiple shoots in Tulshimala.

Table 5. Impact of regeneration media supplemented with varying quantities of BAP and NAA on multiple shoots and root development parameters.

Rice varieties	Concentrations of NAA and BAP	Days to shoot initiation	No. of shoots per callus	Callus forming multiple shoots (%)	Callus forming roots (%)
Kalijira	0.1 mg/l NAA + 1.5 mg/l BAP	23 ± 1.73 a	1 ± 0.00 d	15 ± 1 c	50 ± 2 c
	0.1 mg/l NAA + 2 mg/l BAP	21 ± 1 a	3 ± 0.58 b	20 ± 2 b	55 ± 2 bc
	0.1 mg/l NAA + 2.5 mg/l BAP	21 ± 1 a	4 ± 0.18 a	35 ± 1 a	65 ± 3 a
	0.1 mg/l NAA + 3 mg/l BAP	25 ± 3 a	2 ± 0.25 c	15 ± 2 c	60 ± 2 ab
Tulshimala	0.1 mg/l NAA + 1.5 mg/l BAP	22.3 ± 2.52 a	5 ± 0.00 bc	45 ± 5 bc	100 ± 0 a
	0.1 mg/l NAA + 2 mg/l BAP	20 ± 2 a	7 ± 0.58 a	70 ± 1 a	100 ± 0 a
	0.1 mg/l NAA + 2.5 mg/l BAP	21 ± 1 a	5 ± 0.18 b	50 ± 3 b	100 ± 0 a
	0.1 mg/l NAA + 3 mg/l BAP	24.6 ± 3.1 a	3 ± 0.25 c	40 ± 4 c	100 ± 0 a

Results are shown as Mean ± SD. Means with same letter within each column are not significantly different according to Tukey's HSD tests ($P < 0.05$).

There were seven shoots on average per regenerated callus. All calli were found to have roots. In Kalijira, 35% of the embryogenic callus formed multiple shoots when the embryogenic calli were transferred to MS medium supplemented with 0.1 mg/l NAA and 2.5 mg/l BAP. There were four shoots on average per regenerated callus. On the other hand, roots were noticed in 65% of calli, which was much lower than Tulshimala. These results indicated that Tulshimala had the maximum regeneration capacity, followed by

Kalijira. Fig. 2 shows the progression of callus initiation, callus proliferation, somatic embryogenesis induction, somatic embryo regeneration, and acclimatization of regenerated plantlets of Kalijira and Tulshimala. Comparative Regeneration responses for two different aromatic rice varieties are presented in the graph shown in Fig. 3.

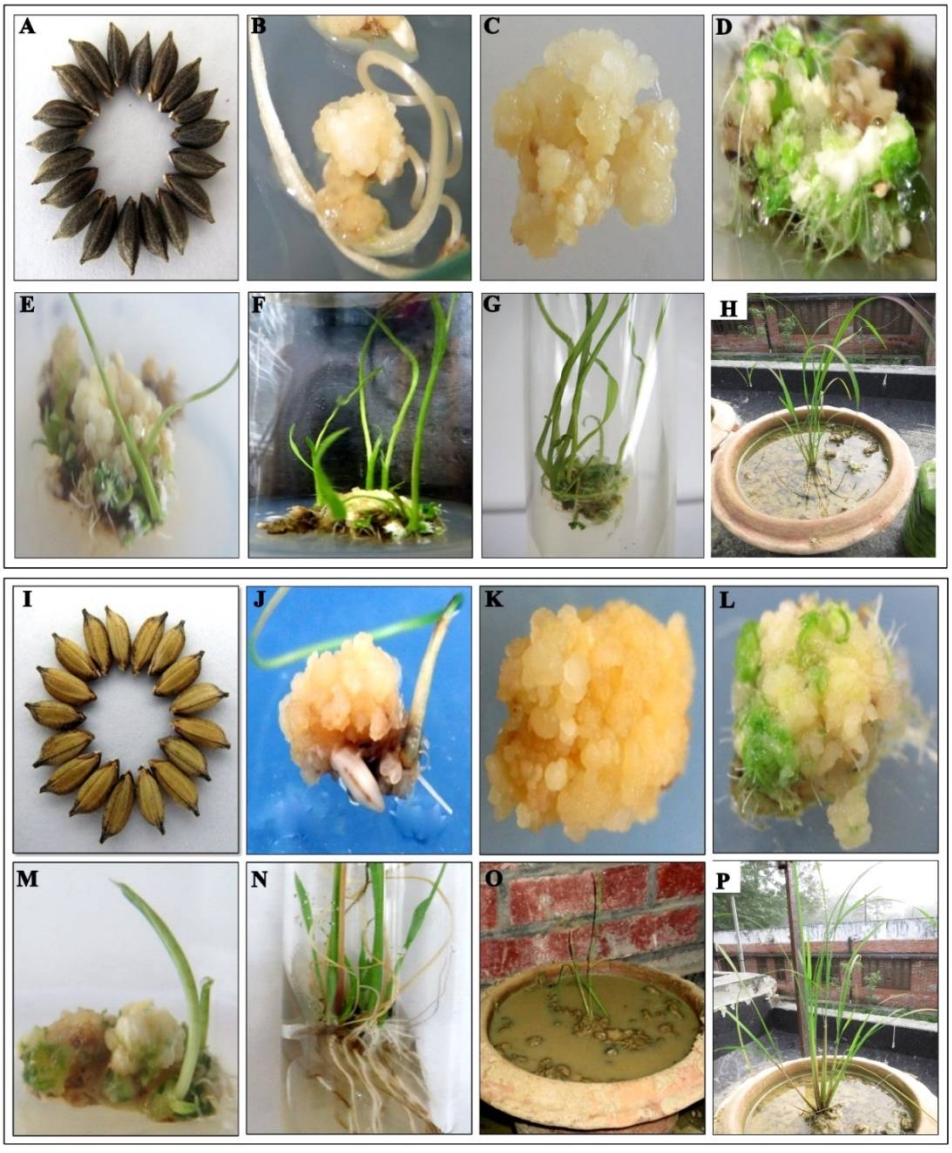

Fig. 2. (A-H) Callus initiation, callus proliferation, induction of somatic embryogenesis, regeneration of somatic embryos, and acclimatization of regenerated plantlets of Kalijira; (I-P) Callus initiation, callus proliferation, induction of somatic embryogenesis, regeneration of somatic embryos, and acclimatization of regenerated plantlets of Tulshimala.

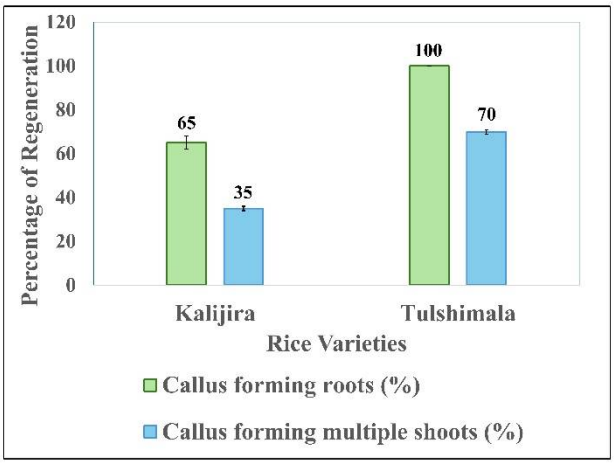

Fig. 3. Percentage of callus-forming roots and multiple shoots. Results are shown as Mean ± SD.

Indirect plant regeneration involves the sequential developmental steps of callus induction and proliferation, embryogenic callus development, and plant regeneration (Gandonou et al. 2005). To regenerate complete plants, the totipotent cells in the calli followed distinct developmental routes. Plant development and morphogenesis are regulated mainly by varying dosages of growth regulators. In general, shoot morphogenesis is induced in a medium with a high concentration of cytokinin and a low quantity of auxin. The induction of root primordia requires auxin either by itself or in combination with low levels of cytokinin (Evans et al. 1981).

In the present study, calli grew in the regeneration medium with low concentrations of NAA in combination with varying concentrations of BAP to develop the shoots. It has previously been documented that the stimulatory action of BAP and NAA together promotes regeneration in Indica rice callus cultures (Mandal et al. 2003, Ramesh et al. 2009). Jubair et al. (2008) employed the same hormone combinations in MS media to regenerate the rice variety Topa, where they observed 20% regeneration frequency with an average of 2 shoots per explant at 0.5 mg/l NAA + 3 mg/l BAP (Jubair et al. 2008). Goswami et al. (2022) observed the regeneration responses of the aromatic rice variety Doairgura, where 100% regeneration response and 15.3 shoots per callus were found at 1 mg/l NAA + 2.0 mg/l BAP combination (Goswami et al. 2022).

In vitro regenerated plants survived after being transferred to pots, indicating good adaptability of somaclones in ambient environmental conditions. Additionally, all plantlets regenerated from mature embryo-derived calli developed good root systems, which was a significant aspect of the current study.

In conclusion, this study establishes an efficient and reliable somatic embryogenesis and regeneration protocol for the Bangladeshi indigenous aromatic rice varieties Kalijira and Tulshimala. This protocol holds promise for enhancing crop improvement efforts through biotechnological applications. However, improving aromatic rice remains

challenging due to its unique genetic complexity and sensitivity to environmental conditions. In this context, advanced genetic transformation or genome editing techniques offer exciting opportunities for precise trait enhancement, potentially overcoming these limitations while preserving the distinctive qualities of aromatic rice. Notably, Tulshimala displayed a superior tissue culture response compared to Kalijira, suggesting it may be a particularly promising candidate. Nonetheless, both varieties demonstrated potential for use in genetic modification studies, underscoring the value of further research on these aromatic rice genotypes. By advancing a streamlined tissue culture and regeneration system, this work paves the way for more effective breeding and transformation efforts to unlock the genetic potential of aromatic rice varieties.

References

Alam M F, Khatun SM, Khandakar IA, Khalekuzzaman M, Shohael AM and **Parvez S** (2003) Genetic parameter on callus induction and plant regeneration using three explants in four rice cultivars of Bangladesh. Bangladesh j. genet. Biotechnol. **4**: 59-62.

Bangladesh Trade Portal (2024) Website: https://wwwbangladeshtradeportalgovbd/kcfinder/upload/files/Announcement_1724566233pdf [accessed 5 November 2024]

Bhojwani SS and **Dantu PK** (2013) Plant tissue culture: an introductory text. Vol. 318. India: Springer

Bidabadi SS and **Jain SM** (2020) Cellular, molecular, and physiological aspects of *in vitro* plant regeneration. Plants. **9**: 702.

Desai P, **Desai S**, **Rafaliya R** and **Patil G** (2022) Plant tissue culture: Somatic embryogenesis and organogenesis. Adv. Plant Tissue Cult. 109-130.

Evans DA, **Sharp WR** and **Flick CE** (1981) Growth and behavior of cell cultures: embryogenesis and organogenesis. Plant Tissue Culture: Methods and Applications in Agriculture: 45-113.

Gandonou CH, **Errabii T**, **Abrini J**, **Idaomar M**, **Chibi F** and **Senhaji S** (2005) Effect of genotype on callus induction and plant regeneration from leaf explants of sugarcane (Saccharum sp). African J. Biotech. **4**: 11.

Goswami B, **Banu TA**, **Akter S**, **Afrin S**, **Habib A** and **Khan S** (2022) *In vitro* regeneration of aromatic rice (*Oryza sativa* L. var. *doairgura*). Bangladesh J. Bot. **51**: 677-682.

Hartke S and **Lörz H** (1989) Somatic embryogenesis and plant regeneration from various indica rice (*Oryza sativa* L) genotypes. 205-214.

He GY and **Lazzeri PA** (2001) Improvement of somatic embryogenesis and plant regeneration from durum wheat (*Triticum turgidum* var. *durum* Desf) *scutellum* and inflorescence cultures. Euphytica. **119**: 369-376.

Hossain MF, **Islam MS**, **Rahman MM**, **Faruk MO** and **Ershad MG** (2008) Yield and quality performance of some aromatic rice varieties of Bangladesh. J. Agrofor. Environ. **2**: 155-158.

Joyia FA and **Khan MS** (2013) Scutellum-derived callus-based efficient and reproducible regeneration system for elite Indica rice varieties in Pakistan. Int. J. Agric. Biol. **15**: 27-33.

Jubair TA, **Salma U**, **Haque N**, **Akter F**, **Mukti IJ**, **Haque A** and **Ali MR** (2008) Callus induction and regeneration of local rice (*Oryza sativa* L.) variety Topa. Asian J. Plant Sci. **7**: 514-517

Kaewmungkun K, **Tongmark K**, **Chakhonkaen S**, **Sangarwut N**, **Wasinanon T**, **Panyawut N** and **Muangprom A** (2023) Development of new aromatic rice lines with high eating and cooking qualities. J. Integr. Agric. **22**: 679-690.

Kaur N, **Gill MS**, **Gill R**, **Bharaj TS** and **Gosal SS** (1999) Factors enhancing somatic embryogenesis and high frequency plant regeneration in rice. Crop Improv. **26**: 23-27.

Long Y, **Yang Y**, **Pan G** and **Shen Y** (2022) New insights into tissue culture plant-regeneration mechanisms. Front. Plant Sci. **13**: 926752.

Mandal AB, **Maiti A** and **Biswas A** (2003) Somatic embryogenesis in root derived callus of indica rice. Plant Tiss. Cult. **13**: 125-133.

Parmar N, **Singh KH**, **Sharma D**, **Singh L**, **Kumar P**, **Nanjundan J** and **Khan YJ** (2017) Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. 3 Biotech. **7**: 1-35.

Pasternak TP and **Steinmacher D** (2024) Plant growth regulation in cell and tissue culture *in vitro*. Plants. **13**: 327.

Prodhan ZH and **Qingyao SHU** (2020) Rice aroma: A natural gift comes with price and the way forward. Rice Sci. **27**: 86-100.

Purwantoro A, **Purwestri YA**, **Lawrie MD** and **Semiarti E** (2022) Genetic transformation via plant tissue culture techniques: current and future approaches. Adv. Plant Tiss. Cult. 131-156.

Ramesh M, **Murugiah V** and **Gupta AK** (2009) Efficient *in vitro* plant regeneration via leaf base segments of indica rice (*Oryza sativa* L). Indian J. Exp. Biol. **47**: 68-74.

Rezvi HUA, **Arif MTU**, **Azim MA**, **Tumpa TA**, **Tipu MMH**, **Najnine F** and **Dawood MFA** (2023) Rice and food security: climate change implications and the future prospects for nutritional security. Food Energy Secur. 12: e430.

Saha P, **Afrin M**, **Mohiuddin AKM** and **Shohael AM** (2015) *In vitro* regeneration of grass pea (*Lathyrus sativus* L). Jahangirnagar Univ. J. Biol. Sci. **4**: 1-8.

Saha P, **Afrin M**, **Mohiuddin AKM** and **Shohael AM** (2017) Development of an effective *in vitro* regeneration protocol for BARI Mash 2 (*Vigna mungo* L) an important legume crop in Bangladesh. Jahangirnagar University J. Biol. Sci. **6**: 23-33.

Seraj ZI, **Islam Z**, **Faruque MO**, **Devi T** and **Ahmed S** (1997) Identification of the regeneration potential of embryo derived calluses from various indica rice varieties. Plant Cell Tiss. Org. Cult. **48**: 9-13.

Singh RK, **Singh US** and **Khush GS** (2000) Aromatic rices. Int. Rice Res. Inst.

Summart J, **Panichajakul S**, **Prathepha P** and **Thanonkeo P** (2008) Callus induction and influence of culture condition and culture medium on growth of Thai aromatic rice, Khao Dawk Mali 105, cell culture. World Appl. Sci. J. **5**: 246-251.

Weber DJ, **Rohilla R** and **Singh US** (2000) Chemistry and biochemistry of aroma in scented rice In Aromatic Rices. Oxford & IBH Publ. 29-46.

Zafar S and **Jianlong X** (2023) Recent advances to enhance nutritional quality of rice. Rice Sci. **30**: 523-536.

(Manuscript received on 08 November, 2024; revised on 30 November, 2024)