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In vitro Plant Regeneration of an Indigenous Aromatic Rice (*Oryza sativa* L. var. Radhunipagal) through Matured Seed Culture

Md. Taufiqur Rahman Sarkar and S. M. Shahinul Islam*

Plant Biotechnology and Genetic Engineering Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

Key words: Aromatic rice, Callus induction, Genotype, *In vitro* regeneration, Radhunipagal

Abstract

This study aimed to optimize *in vitro* propagation techniques for the indigenous aromatic rice variety called Radhunipagal in Bangladesh, focusing on callus induction, shoot regeneration, and root induction. Mature seeds of the aromatic rice were cultured on MS and N6 media supplemented with different concentrations of 2,4-D, Kn, BAP, NAA, IAA, and IBA. The highest callus induction rate (88.33 ± 0.33%) was achieved with 2.0 mg/l 2,4-D and 0.5 mg/l NAA on MS medium within three weeks of culture. Optimal shoot regeneration (84.00 ± 0.58%) was observed with 2.0 mg/l BAP and 0.5 mg/l NAA, with multiple healthy shoots emerging within three to four weeks on MS medium. Root induction was most successful (100%) on half-strength of MS medium supplemented with 1.0 mg/l IBA, resulting in well-developed roots within three to four weeks of culture initiation. These results demonstrated the effectiveness of *in vitro* propagation techniques for conserving and propagating indigenous rice genotypes. The findings provide valuable insights in optimizing growth regulator concentrations for successful *in vitro* regeneration in an indigenous aromatic rice, supporting conservation, genetic preservation, and advancing biotechnological investigation in the future.

Introduction

Rice (*Oryza sativa* L.) is a fundamental food crop, feeding more than half of the global population (Saskai and Kishitani 2005). It holds significant cultural and economic importance, especially in Bangladesh, where rice is a dietary staple. In Bangladesh, 75.01% of the total cropped area is used for rice production, yielding 37.60 million tons annually from 11.70 million hectares (BBS 2021). The country is also renowned for its indigenous aromatic rice varieties, about thirty six varieties preserved in Bangladesh Rice Research Institute (BRRI) germplasm bank including Radhunipagal, Kalijira, Chinigura,

*Author for correspondence: <shahinul68@gmail.com>.

Modhumala, Tulsimala, Badshahbhog, and Rajbhog (Das and Baqui 2000). These aromatic varieties are highly valued for their distinct fragrance, taste, and quality. In particular, Radhunipagal is a highly aromatic variety, renowned for its fragrance. It offers nutritional benefits, being rich in carbohydrates, vitamins, and minerals while low in fat and cholesterol free, making it ideal for dishes such as pulao and biryani. Its cultivation is also environmentally friendly, requiring fewer fertilizers and less irrigation. Beyond their culinary appeal, aromatic rice varieties are highly prized in Bangladesh's agricultural trade markets (Dutta et al. 1998), with their price being 2-3 times higher than that of coarse rice (Goswami et al. 2022). In Northern Bangladesh, 30% of the rice lands during the Aman season are dedicated to these aromatic varieties (Masud et al. 2023). However, traditional cultivation methods for these rice varieties face several challenges, including low yield potential, susceptibility to pests and diseases, and vulnerability to abiotic stresses like salinity and drought. 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). To overcome these challenges, plant tissue culture techniques offer a promising solution (Siddique et al. 2014). These techniques are critical for the successful application of plant biotechnology and have been employed for varietal development in cereal crops, including rice across many countries.

In vitro techniques also facilitates the introduction of desirable traits, such as increased resistance to biotic and abiotic stresses, improved yield, and adaptability to environmental changes (Wijerathna et al. 2023). By adopting *in vitro* techniques, Bangladesh can conserve its traditional rice germplasm, enhance productivity, and meet the growing demand for premium aromatic rice both locally and globally. *In vitro* propagation methods, such as the cultures of anther, leaf, root, and dehusked seeds are particularly important for exploiting somaclonal variations, *in vitro* selection, and producing new lines via genetic transformation (Islam et al. 2014). Numerous protocols have been developed for *in vitro* regeneration of rice using different explants, including immature seeds (Siddique et al. 2014), mature seeds (Rahman et al. 2021, Afrin et al. 2024), leaf (Karthikeyan et al. 2011), shoot apex (Dey et al. 2012), anther (Khatun et al. 2010, Islam and Tuteja 2012), microspores (Islam et al. 2013), and root (Mandal et al. 2003). Efficient callus induction and plant regeneration are essential steps in this process before implementing any genetic improvement program (Dievart et al. 2016).

Somatic embryogenesis is another important method for plant propagation, supporting agricultural improvement projects (Zaman and Islam 2024). Scutellum-derived callus induction followed by regeneration has been successfully applied in many grasses including wheat (He and Lazzeri 2001), maize (Frame et al. 2011, Morshed et al. 2016), barley (Haque et al. 2015, Islam and Haque 2016), oat (Gless et al. 1998), rye (Eapen et al. 1981), and rice (Afrin et al. 2024). Calli induced from the sub-scutellar tissue of mature seeds are excellent sources for *in vitro* regeneration and transgenic rice production (Wani et al. 2011). While there have been successful reports of plant regeneration from Indica rice tissue cultures, these protocols are not universally

applicable to all rice cultivars (Abubakar et al. 2022). For developing transgenic rice, scientists primarily rely on tissue culture based gene transfer technology (Sikdar et al. 2015, Rakshana et al. 2019). The success of these methods depends on factors such as genotype, explant type, medium composition, plant growth regulators, and the culture environment (Repalli et al. 2019, Pasternak and Steinmacher 2024). By optimizing these *in vitro* techniques, the conservation and propagation of Bangladesh's aromatic rice varieties can be significantly improved, thereby supporting genetic preservation, improved cultivation practices, and sustainable agricultural development. This study focuses on optimizing *in vitro* propagation techniques for Radhunipagal rice specifically aiming to identify the most suitable medium for callus induction using mature seeds and determine the optimal media composition for shoot and root regeneration. By improving the efficiency of plant regeneration, this research support to conserve and propagate indigenous aromatic rice varieties of Bangladesh, ultimately contributing to their genetic preservation and improved cultivation methods.

Materials and Methods

This experiment was conducted using an indigenous aromatic rice (*Oryza sativa* L.) variety Radhunipagal, obtained from Tanore, Rajshahi, Bangladesh. To ensure a sterile environment for *in vitro* propagation, the seeds were dehusked before undergoing sterilization. Firstly, the dehusked seeds were immersed in 70% ethanol for 1 min, followed by three times rinses with double-distilled water to eliminate any ethanol residue. The seeds were then treated with 50% commercial bleach (4.5% sodium hypochlorite) combined with 1-2 drops of Tween-20 for 30 min at 28°C with continuous shaking (180 rpm). After sterilization, the seeds were washed thoroughly several times with double distilled water and dried on sterile filter paper under a laminar airflow cabinet, ready for inoculation.

Callus induction was performed using two different basal media: MS and N6 medium (Chu et al. 1975). The basal media were supplemented with various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) ranging from 1.0 to 3.5 mg/l, either alone or in combination with other plant growth regulators such as NAA and Kn. These combinations aimed to determine the optimal concentration for effective callogenesis and subsequent plant regeneration. The sterilized seeds were placed on the induction medium and incubated in dark at 25 ± 2°C for 3-4 weeks to induce callus formation. After three weeks, the actively growing embryogenic calli were transferred to MS medium supplemented with different concentration and combinations of BAP, NAA, and Kn for shoot regeneration. Various combinations such as BAP alone or BAP + NAA or BAP + Kn or BAP + NAA + Kn were tested. The pH of all media was adjusted to 5.8 before autoclaving. The cultures were incubated at 25 ± 2°C under a light/dark cycle of 14 hrs light (2000-3000 lux) and 10 hrs dark to encourage shoot formation.

Once regenerated shoots reached a height of 5 cm or more, they were transferred to full and half-strength MS medium, which was either rooting hormone free or

supplemented with different rooting hormone auxins like IAA, NAA and IBA to promote root induction. The medium was semi-solidified by adding 0.4% gelrite. Shoots were allowed to develop roots over a period of three to four weeks. Once well-developed roots were formed, the plants were transferred to pots containing soil and acclimatized in a greenhouse. The plants were grown to maturity in the greenhouse, where they continued to develop until seed harvest.

Data were collected at various stages: 3-4 weeks for callus induction, 3-4 weeks for plantlet regeneration, and 3 weeks for root induction. Parameters recorded including the percentage of callus induction, percentage of plantlet regeneration, number of plantlets per callus, average plantlet height, number of roots per plantlet, and average root length. Then collected data were subjected to statistical analysis using a completely randomized design (CRD). All the experiments carried out by three replicate to minimize error. For data analysis Microsoft Excel and IBM SPSS (version-25) were used.

Results and Discussion

Callus induction from mature, dehusked seeds of the rice variety, Radhunipagal was evaluated using different basal media. Dehusked rice seeds have been considered good explants for seed germination and callus formation (Bano et al. 2005, Abubakar et al. 2022). Explants were cultured in MS and N6 media supplemented with different concentrations of 2,4-D singly and in combination with NAA or Kn. Percentages of explants induced calli were recorded after 3 weeks of inoculation of the explants (Table 1). Among them the highest 88.33 and 75% of induced callus in the medium supplemented with 2.0 mg/l 2,4-D and 0.5 mg/l NAA for MS and N6 media respectively (Table 1). Results showed that callus initiation occurred within one week and by the third week, the calli were compact, light yellow, and globular, typical of embryogenic callus (Fig. 1a-c). This outcome is consistent with previous studies that reported similar callus morphology in rice (Khalequzzaman et al. 2005, Abiri et al. 2017). In MS medium the callus induction ranged from 41.67 to 88.33% while in N6 medium ranged from 40 to 75% (Table 1). This superiority of MS over N6 medium agrees with earlier studies (Pandey et al. 1994, Islam et al. 2013) which also reported higher callus induction in rice. 2,4-D was the most influential growth regulator for callus induction, with an optimal concentration of 2.0 mg/l leading to a 76.67% induction rate.

Above this concentration, the callus induction rate declined, which concurs with the findings of Mosavi et al. (2001), and Islam et al. (2013) also reported that similar declines at higher auxin concentrations. So, the MS medium containing 2,4-D with 2.0 mg/l concentration was the best one for callus induction that supported earlier reports (Roly et al. 2014, Kumari et al. 2020, Chitphet et al. 2025). This highlights the critical role of maintaining moderate auxin levels to avoid inhibitory effects on callus formation. Furthermore, the addition of NAA enhanced the quality and robustness of the callus, a synergistic effect also noted in earlier studies (Din et al. 2016). In contrast, combining 2,4-D with Kn led to a lower callus induction rate, confirming that cytokinins like Kn

tend to promote organogenesis over callus formation when used in combining with auxins. The present findings, with a 70% induction rate on MS medium supplemented with Kn, mirror the results, which highlighted the disruptive potential of excess cytokinins on callus development (Mendoza and Kaeppler 2002).

Table 1. Effects of various concentrations, combinations of auxins and cytokinins on callus induction using MS and N6 media

Growth regulators (mg/l)	Nutrient media	Response of explants (%) induced callus (± S.E.)	Degree of callus formation
2,4-D			
1.0	MS	45.00 ± 0.58^{p}	+
	N6	41.67 ± 0.33^{q}	+
1.5	MS	63.33 ± 0.88^{h}	++
	N6	58.33 ± 0.33^{j}	++
2.0	MS	76.67 ± 0.88^{b}	+++
	N6	73.33 ± 0.33^{d}	+++
2.5	MS	66.67 ± 0.88^{f}	++
	N6	63.33 ± 0.88^{h}	++
3.0	MS	53.33 ± 0.33^{m}	++
	N6	50.00 ± 0.58^{n}	+
2,4-D + NAA			
1.0 + 0.5	MS	46.67 ± 0.33^{o}	+
	N6	45.00 ± 0.58^{p}	+
1.5 + 0.5	MS	66.67 ± 0.33^{f}	++
	N6	61.67 ± 0.33^{i}	++
2.0 + 0.5	MS	88.33 ± 0.33^{a}	+++
	N6	75.00 ± 0.58^{c}	+++
2.5 + 0.5	MS	70.00 ± 0.00^{e}	+++
	N6	66.67 ± 0.33^{f}	++
3.0 + 0.5	MS	55.00 ± 0.58^{l}	++
	N6	46.67 ± 0.33^{o}	+
2,4-D + Kn			
1.0 + 0.5	MS	41.67 ± 0.33^{q}	+
	N6	40.00 ± 0.00^{r}	+
1.5 + 0.5	MS	56.67 ± 0.67^{k}	++
	N6	53.33 ± 0.33^{m}	++
2.0 + 0.5	MS	70.00 ± 0.58^{e}	+++
	N6	66.67 ± 0.33^{f}	++
2.5 + 0.5	MS	65.00 ± 0.58^{g}	++
	N6	58.33 ± 0.33^{j}	++
3.0 + 0.5	MS	50.00 ± 0.58^{n}	++
	N6	45.00 ± 0.58^{p}	+

Degree of callus formation: + indicates slight growth, ++ indicates average growth, +++indicates profuse growth. The same letter in the column did not differ significantly at the 5% level of probability (DMRT by SPSS. 25).

After three weeks of callus induction, the calli were transferred to MS medium supplemented with varying combinations of BAP, NAA, and Kn to assess shoot regeneration. Among them, 2.0 mg/l BAP with 0.5 mg/l NAA was found to be the best combination where 84% of calli regenerated into shoots. The highest number of shoots regenerated per culture was 7.89 ± 0.31. The lowest percent (18.67%) regenerated plantlet was medium supplemented with 0.5 mg/l BAP (Table 2 and Fig. 1d-f). The combination of 2.0 mg/l BAP and 0.5 mg/l NAA yielded the highest shoot regeneration rate at 84%, producing an average of 7.89 ± 0.31 shoots per culture with plantlets reaching an average height of 5.38 ± 0.07 cm (Table 2). These results agreed well with the findings of Ramesh et al. (2009) and Karthikeyan et al. (2009) also similarly reported that BAP and NAA are an optimal combination for shoot regeneration in rice. BAP, as a cytokinins, is essential for activating meristematic tissue and stimulating shoot formation (Sarkar and Alam 2022).

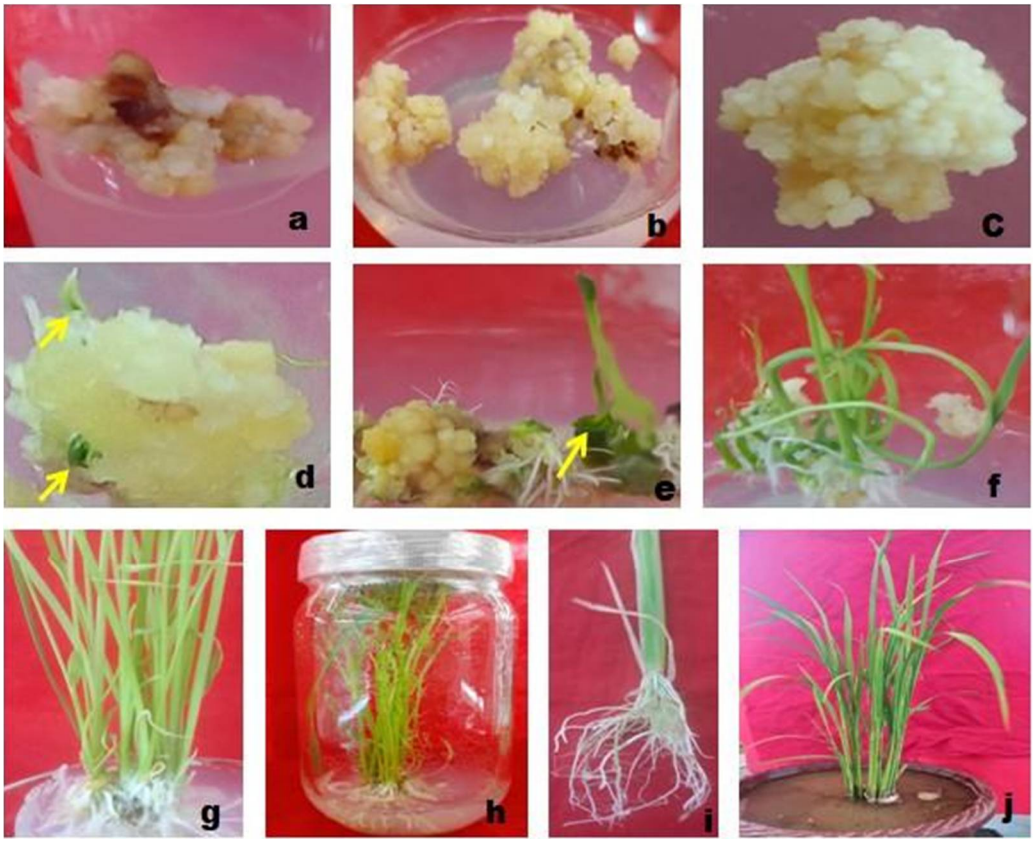

Fig. 1(a-j). Different developmental stages of *in vitro* regeneration from callus in endangered rice variety Radhunipagal: (a) scutellar-derived callus with seed, (b-c) 3 weeks old calli transferred to regeneration medium showing initiation of greenish callus, (d) seed derived calli started to regenerate (arrow), (e-f) green shoots (arrow) from embryogenic calli in shooting medium, (g-h) healthy green plantlets with good root system, (i) a complete plantlet showing roots, and (j) acclimatized plantlets in pots.

The addition of NAA helped facilitate the transition from callus to organized shoot structures, reinforcing the importance of an auxin-cytokinin balance in shoot regeneration (Lee and Huang 2014, Haque and Islam 2019). The similarity effect of BAP in combination with NAA has been reported to facilitate regeneration in rice callus cultures (Wani et al. 2011). The effectiveness of this hormonal balance is underscored by the significantly lower regeneration rate (73.33%) observed when Kn was added to the medium, a result also noted by Wu and Chen (1987), who observed cytokinin interference with shoot elongation.

Table 2. Effects of various concentrations and combinations of BAP, NAA, and Kn on shoot regeneration from embryogenic callus in MS medium

Growth regulators combination (mg/l)			Frequency of regeneration (%)	No. of shoots regenerated per culture	Average plantlet height
BAP	NAA	Kn	(± S.E.)	(± S.E.)	(cm)
0.5	–	–	18.67 ± 0.33^{m}	2.60 ± 0.76^{g}	3.14 ± 0.06^{j}
0.5	+ 0.5	–	26.67 ± 0.67^{k}	3.00 ± 0.08^{g}	3.32 ± 0.07^{i}
0.5	+ 0.5	+ 0.5	22.67 ± 0.67^{l}	3.20 ± 0.05^{g}	3.12 ± 0.02^{j}
1.0	–	–	41.33 ± 0.88^{j}	2.92 ± 0.25^{g}	3.28 ± 0.05^{i}
1.0	+ 0.5	–	45.33 ± 0.88^{i}	3.33 ± 0.16^{g}	3.48 ± 0.05^{h}
1.0	+ 0.5	+ 0.5	40.00 ± 0.58^{j}	3.11 ± 0.16^{g}	3.32 ± 0.02^{i}
1.5	–	–	49.33 ± 0.33^{h}	4.17 ± 0.22^{f}	3.84 ± 0.02^{g}
1.5	+ 0.5	–	62.67 ± 0.33^{d}	5.63 ± 0.29^{de}	4.08 ± 0.05^{f}
1.5	+ 0.5	+ 0.5	57.33 ± 0.33^{f}	5.00 ± 0.25^{ef}	3.86 ± 0.02^{g}
2.0	–	–	72.00 ± 0.58^{b}	6.11 ± 0.33^{cd}	5.12 ± 0.07^{b}
2.0	+ 0.5	–	84.00 ± 0.58^{a}	7.89 ± 0.31^{a}	5.38 ± 0.07^{a}
2.0	+ 0.5	+ 0.5	73.33 ± 0.67^{b}	7.06 ± 0.28^{b}	4.82 ± 0.05^{c}
2.5	–	–	61.33 ± 0.88^{de}	5.36 ± 0.24^{de}	4.84 ± 0.02^{c}
2.5	+ 0.5	–	66.67 ± 0.88^{c}	6.82 ± 0.28^{bc}	5.04 ± 0.02^{b}
2.5	+ 0.5	+ 0.5	62.67 ± 0.33^{d}	5.67 ± 0.26^{de}	4.66 ± 0.02^{d}
3.0	–	–	56.00 ± 0.58^{f}	4.92 ± 0.22^{ef}	4.44 ± 0.02^{e}
3.0	+ 0.5	–	60.00 ± 0.58^{e}	5.71 ± 0.23^{de}	4.64 ± 0.02^{d}
3.0	+ 0.5	+ 0.5	52.00 ± 0.58^{g}	5.08 ± 0.19^{e}	3.96 ± 0.02^{g}

The same letter in the column did not differ significantly at the 5% level of probability (DMRT by SPSS. 25).

For root induction, shoots (5-7 cm) were excised and cultured to hormone free as well as medium containing various concentrations of auxins like IBA, IAA, or NAA medicated full MS and half strength MS (MSS1) medium. Half strength MS medium produced the highest percentage of roots in almost every plant growth regulators (PGRs) concentrations, while 1.0 mg/l IBA showed the maximum number of roots 16.10 ± 0.64 and 13.50 ± 0.62 in half and full strength MS medium respectively (Table 3 and Fig. 1g-i). The highest root induction rate of 100% was achieved in MSS1 medium supplemented with 1.0 mg/l IBA, which produced an average of 16.10 ± 0.64 roots per shoot.

Table 3. Effects of different concentrations of auxins on adventitious root formation from *in vitro* grown micro-shoots on full (MS) and half-strength MS (MSS1) media

Auxin type	Concentration (mg/l)	Medium strength	Response of micro shoots rooted (%)	Number of roots per micro shoot (± S.E.)	Average root length (cm) (± S.E.)
IBA	0.1	MSS1	82.22 ± 0.33^{i}	9.90 ± 0.77^{hij}	2.13 ± 0.19^{hijk}
		MS	80.00 ± 0.58^{j}	7.30 ± 0.26^{lm}	2.10 ± 0.15^{ijk}
	0.2	MSS1	86.67 ± 0.58^{g}	10.50 ± 0.43^{fghi}	2.43 ± 0.22^{defghijk}
		MS	84.44 ± 0.33^{h}	9.30 ± 0.63^{hijk}	2.33 ± 0.33^{ghijk}
	0.5	MSS1	95.56 ± 0.33^{c}	10.90 ± 0.59^{efgh}	2.50 ± 0.29^{cdefghijk}
		MS	91.11 ± 0.33^{e}	10.30 ± 0.88^{ghi}	2.60 ± 0.20^{abcdefghi}
	1.0	MSS1	100.00 ± 0.0^{a}	16.10 ± 0.64^{a}	3.10 ± 0.06^{a}
		MS	97.78 ± 0.33^{b}	13.50 ± 0.62^{bc}	2.93 ± 0.03^{abcd}
	1.5	MSS1	97.78 ± 0.33^{b}	14.10 ± 0.42^{b}	2.87 ± 0.09^{abcdef}
		MS	97.78 ± 0.33^{b}	11.60 ± 0.34^{defg}	2.77 ± 0.12^{abcdefg}
	2.0	MSS1	97.78 ± 0.33^{b}	12.00 ± 0.37^{cdef}	2.63 ± 0.13^{abcdefgh}
		MS	95.56 ± 0.33^{c}	10.90 ± 0.33^{efgh}	2.40 ± 0.23^{efghijk}
NAA	0.1	MSS1	80.00 ± 0.58^{j}	9.00 ± 0.45^{ijk}	2.10 ± 0.06^{ijk}
		MS	77.78 ± 0.33^{k}	6.90 ± 0.43^{m}	2.07 ± 0.03^{cdefghijk}
	0.2	MSS1	88.89 ± 0.33^{f}	9.90 ± 0.36^{hij}	2.40 ± 0.10^{efghijk}
		MS	86.67 ± 0.58^{g}	8.10 ± 0.43^{klm}	2.27 ± 0.13^{jk}
	0.5	MSS1	95.56 ± 0.33^{c}	10.30 ± 0.68^{ghi}	2.63 ± 0.09^{abcdefgh}
		MS	93.33 ± 0.58^{d}	9.20 ± 0.42^{ijk}	2.57 ± 0.22^{bcdefghij}
	1.0	MSS1	97.78 ± 0.33^{b}	14.30 ± 0.54^{b}	2.97 ± 0.03^{abc}
		MS	95.56 ± 0.33^{c}	12.60 ± 0.67^{cd}	2.90 ± 0.10^{abcde}
	1.5	MSS1	93.33 ± 0.58^{d}	12.00 ± 0.33^{cdef}	2.67 ± 0.17^{abcdefg}
		MS	93.33 ± 0.58^{d}	10.50 ± 0.43^{fghi}	2.70 ± 0.15^{abcdefg}
	2.0	MSS1	93.33 ± 0.58^{d}	9.30 ± 0.50^{hijk}	2.50 ± 0.25^{cdefghijk}
		MS	93.33 ± 0.58^{d}	8.20 ± 0.29^{klm}	2.37 ± 0.19^{fghijk}
IAA	0.1	MSS1	82.22 ± 0.33^{i}	9.20 ± 0.33^{ijk}	2.07 ± 0.03^{jk}
		MS	80.00 ± 0.58^{j}	6.90 ± 0.18^{m}	2.03 ± 0.03^{k}
	0.2	MSS1	84.44 ± 0.33^{h}	9.80 ± 0.33^{hij}	2.47 ± 0.09^{cdefghijk}
		MS	82.22 ± 0.33^{i}	8.40 ± 0.34^{jkl}	2.30 ± 0.10^{ghijk}
	0.5	MSS1	86.67 ± 0.58^{g}	10.60 ± 0.33^{ghi}	2.70 ± 0.06^{abcdefg}
		MS	84.44 ± 0.33^{h}	9.30 ± 0.45^{hijk}	2.60 ± 0.21^{abcdefghi}
	1.0	MSS1	97.78 ± 0.33^{b}	14.30 ± 0.37^{b}	3.03 ± 0.09^{ab}
		MS	95.56 ± 0.33^{c}	12.40 ± 0.22^{cde}	2.93 ± 0.12^{abcd}
	1.5	MSS1	93.33 ± 0.58^{d}	13.30 ± 0.26^{bc}	2.73 ± 0.13^{abcdefg}
		MS	91.11 ± 0.67^{e}	10.30 ± 0.47^{ghi}	2.67 ± 0.18^{abcdefg}
	2.0	MSS1	91.11 ± 0.33^{e}	9.80 ± 0.33^{hij}	2.53 ± 0.22^{bcdefghijk}
		MS	88.89 ± 0.33^{f}	8.60 ± 0.34^{jkl}	2.43 ± 0.12^{defghijk}

The same letter in the column did not differ significantly at the 5% level of probability (DMRT by SPSS. 25).

This aligns with the use of half-strength MS medium to promote root formation by minimizing nutrient competition between roots and shoots (Abubakar et al. 2022). IBA's stability and prolonged action in stimulating cell elongation and division at the shoot base made it the most effective auxin for root induction, a finding consistent with multiple studies (Karthikeyan et al. 2009, Wang et al. 2020). While both NAA and IAA also resulted in good root induction rates (97.78%), IBA was superior in terms of root

number and uniformity. Earlier research by Yusnita et al. (2017) supports these observations, confirming that IBA consistently induces more robust root systems compared to other auxins. IBA, an important PGR, promotes *in vitro* rooting in rice (Rahman et al. 2021). The success of root induction in Radhunipagal through this protocol provides a reliable method for ensuring the survival and establishment of regenerated plantlets during acclimatization. Transfer of regenerated rice plants into sterile soil after hardening has also been reported earlier (Karthikeyan et al. 2009). The transferred plants got established into soil and exhibited normal growth (Fig. 1j).

This study confirms that MS medium supplemented with 2.0 mg/l 2,4-D and 0.5 mg/l NAA is highly effective for callus induction in the Radhunipagal rice variety. For shoot regeneration, the combination of 2.0 mg/l BAP and 0.5 mg/l NAA was found to be the most effective, while root induction was best achieved with 1.0 mg/l IBA in half-strength MS medium. These results not only confirm existing findings but also provide specific optimizations for the Radhunipagal variety, establishing a clear protocol for efficient *in vitro* propagation. These findings are significant for the genetic conservation of this indigenous aromatic rice variety and lay a foundation for future research focused on enhancing tissue culture techniques for other indigenous and commercially valuable rice genotypes use to crop improvement efforts.

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