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Short Communication

Optimization of *In Vitro* Plant Regeneration from Mature Seeds of Rice (*Oryza sativa* L. cv. Swarna)

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Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide, serving as the primary staple food for more than half of the global population. It plays a crucial role in ensuring food security, particularly in Asia, Africa, and parts of Latin America, where it contributes significantly to daily caloric intake (Abeysekara and Rathnayake, 2024). Rice is the principal staple food crop of India, supporting the dietary needs of more than 50% of the population and contributing significantly to national food security. India is one of the world's largest producers, 150 million tonnes annually (IndiaStat, 2024–2025); however, rapid population growth, shrinking arable land, climate variability, and increasing incidence of biotic and abiotic stresses pose serious challenges to sustaining current production levels Fiyaz *et al.* (2022). Enhancing rice production is therefore essential for ensuring food security and supporting the agrarian economy. Conventional breeding is often constrained by limited genetic diversity and lengthy breeding cycles. In contrast, plant tissue culture and *in vitro* regeneration provide important tools for rapid multiplication of elite genotypes and serve as essential platforms for genetic transformation and genome-editing studies. The development of efficient and reproducible regeneration systems is therefore critical for accelerating rice improvement programmes**.** The success of *in vitro* regeneration depends on several factors, including genotype, explant type, culture conditions, and the concentration and balance of plant growth regulators. Cytokinins such as 6-benzylaminopurine (BAP) and kinetin (Kn) promote cell division and shoot differentiation, whereas auxins such as naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) regulate cell division, differentiation, callus formation, and rooting (Ge *et al.*, 2006; Zhao *et al.*, 2011; Ngomuo *et al.*, 2013; Pasqualetto, 1990; Hasan *et al.*, 2019b). Although MS medium supplemented with auxins and cytokinins has been extensively employed for rice regeneration, the regeneration response to specific hormone concentrations and combinations varies among genotypes. Hence, optimization of the hormonal regime for individual elite cultivars remains important for achieving high and reproducible regeneration efficiency**.**

Swarna is an important indica rice cultivar widely cultivated in India. However, efficient regeneration requires optimization of the hormone concentrations and combinations appropriate to the genetic background of the cultivar. Therefore, the present study evaluated different concentrations of BAP and NAA, with and without kinetin, to identify an effective hormonal regime for *in vitro* regeneration of *O. sativa* cv. Swarna. The optimized protocol is expected to provide a useful basis

for subsequent tissue culture, clonal multiplication, and genetic improvement studies in this cultivar.

Mature, healthy seeds of rice variety, Swarna were dehusked manually under aseptic conditions to remove the outer hull, ensuring optimal exposure of the embryo for subsequent *in vitro* culture. Murashige and Skoog (MS) basal medium was prepared and supplemented with 3% (w/v) sucrose as the primary carbon and energy source, while 0.8% (w/v) agar was used as the gelling agent. The pH of the medium was adjusted to 5.6–5.8 using 1 N NaOH or HCl prior to sterilization. The prepared medium was dispensed into culture vessels and sterilized by autoclaving at 121 °C under a pressure of 15 psi for 20 minutes. After autoclaving, the medium was allowed to cool and solidify under aseptic conditions before use. Dehusked seeds were subjected to surface disinfection to eliminate epiphytic and endophytic contaminants. Initially, seeds were washed with a mild laboratory-grade detergent solution to remove surface debris, followed by immersion in 50% (v/v) sodium hypochlorite solution for 2–3 minutes under gentle agitation. Subsequently, the seeds were treated with 70% (v/v) ethanol for 30 seconds to ensure rapid sterilization. After chemical treatments, the seeds were thoroughly rinsed 3–5 times with sterile distilled water to remove residual sterilants prior to inoculation onto culture media. Seeds were aseptically cultured on Murashige and Skoog (MS) basal medium supplemented with varying concentrations and combinations of the plant growth regulators 6-benzylaminopurine (BAP), naphthaleneacetic acid (NAA), and kinetin (Kn). BAP and NAA were selected based on their reported roles in rice regeneration, while kinetin was included based on its reported effectiveness with NAA in *Oryza sativa* cv. Swarna (Juturu *et al.*, 2016). A BAP: NAA concentration gradient was evaluated to determine the optimum hormonal balance for in vitro regeneration of *Oryza sativa* cv. Swarna, while an additional treatment containing kinetin was included to assess its effect when combined with the optimum BAP:NAA level. The treatments were designed to evaluate the effect of increasing BAP and NAA concentrations and the additional contribution of Kn on seed germination and subsequent morphogenic responses. Six hormone treatments along with a hormone-free control were evaluated as presented in Table 1.

Table 1. Effect of different concentrations of plant growth regulators on in vitro regeneration of rice variety, Swarna

Treatment	Hormone Combination (mg/L)	Regeneration Frequency (%)	Plant Height (cm)	No. of Leaves	Nature of Roots	No. of Roots	Root Length (cm)	Fresh Weight (g)
Control	MS Basal (No hormones)	52	8.3	4.5	Thin	3	3.8	0.84
T1	BAP 1.0 + NAA 0.1	93	13.4	8.2	Thick	7	6.2	1.42
T2	BAP 2.0 + NAA 0.2	86	12.8	7.6	Thick	6	5.6	1.28
T3	BAP 3.0 + NAA 0.3	74	11.2	6.8	Moderately thick	5	4.9	1.06
T4	BAP 4.0 + NAA 0.4	65	9.8	5.9	Thin	4	4.3	0.95
T5	BAP 5.0 + NAA 0.5	59	8.9	5.1	Thin	3	3.7	0.87
T6	BAP 1.0 + NAA 0.1 + Kn 0.5	81	11.6	7.1	Moderately thick	5	5.3	1.17

Under aseptic conditions in a laminar airflow cabinet, surface-sterilized seeds were placed horizontally on the prepared culture media and the vessels were sealed to maintain sterility. Ten seeds were inoculated for each treatment, with three replicates per treatment. Thus, each treatment consisted of 30 seeds across the three replicates. The complete experiment was independently repeated twice**.** Cultures were incubated at 25 ± 2 °C under a 16

h light/8 h dark photoperiod with cool-white fluorescent light (2000–3000 lux) Hasan *et al.* (2021). The cultures were regularly monitored on callus initiation, explant viability, and any contamination or abnormal growth at regular intervals for up to four weeks after inoculation. Well-elongated shoots were excised and transferred to MS basal medium supplemented with 0.6 mg L^{-1} IBA for root induction. After adequate root development, plantlets were removed, washed to eliminate agar, and transplanted into a sterilized soil–sand mixture. The transplanted plantlets were initially maintained at approximately 85–90% relative humidity for the first week and were gradually acclimatized by reducing the humidity to approximately 70–75% over the following 1–2 weeks. Survival rate during acclimatization was calculated as: Survival rate (%) = (Number of surviving plantlets / Number of plantlets transferred for acclimatization) × 100 (Mosoh *et al.*, 2024). Data was recorded on regeneration frequency (%), plant height (cm), number of leaves, root nature (thin or thick), number of roots, root length (cm), and fresh weight (g) to evaluate the growth and regeneration performance of in vitro regenerated plantlets. Regeneration frequency (%) was calculated as: (Number of seeds producing regenerated shoots / Total number of seeds inoculated) × 100 (Dey *et al.*, 2012). Fresh weight was measured at the end of the culture period, before transfer to the rooting medium, after removing excess agar from the plantlets.

Regeneration efficiency of rice cv. Swarna varied significantly across the different cytokinin–auxin combinations. The highest regeneration frequency (93%) was observed in T1 (1.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA), which also produced the tallest shoots, highest leaf number, greatest fresh weight, and well-developed root systems, indicating optimal morphogenic induction. Treatments T2 (2.0 mg L^{-1} BAP + 0.2 mg L^{-1} NAA) and T6 (1.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA + 0.5 mg L^{-1} Kn) exhibited slightly lower regeneration frequencies of 86% and 81%, respectively, with healthy shoots and moderately developed roots.

Higher cytokinin concentrations (T3–T5) progressively reduced regeneration efficiency. Visual observations indicated increased callus formation and occasional vitrification at higher BAP concentrations, underscoring the importance of a balanced cytokinin–auxin ratio for effective organogenesis. These findings are consistent with previous reports showing that moderate cytokinin–auxin combinations favour shoot regeneration, while elevated cytokinin levels inhibit organized morphogenesis (Noor *et al.*, 2005; Khanna & Raina, 1998; Ge *et al.*, 2006).

Table 2. ANOVA for regeneration and growth parameters under different hormonal treatments

S. No	Characters	Mean sum of squares	
		Treatments (df:06)	Error (df:14)
1.	Regeneration Frequency (%)	315.375**	1.152
2.	Plant Height (cm)	11.199**	0.150
3.	No. of Leaves	5.429**	0.156
4.	No. of Roots	6.714**	1.000
5.	Root Length (cm)	2.657**	0.150
6.	Fresh Weight (g)	0.141**	0.003

*Significant at 5% level; ** Significant at 1 % level

Plantlets in T1 exhibited vigorous shoot elongation, broad leaf expansion, and thick, white roots, indicating an optimal hormonal environment for coordinated shoot and root development. T2 and T6 also produced healthy root systems, although root density and elongation were slightly lower than in T1. In contrast, hormone-free control plantlets had thin, fragile roots and stunted shoots (Table 1), highlighting the essential role of exogenous growth regulators. High cytokinin treatment T5 (5.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA) was associated with visible callus development and delayed shoot regeneration, confirming that supra-optimal cytokinin levels disrupt the auxin–cytokinin balance and hinder organized organogenesis. These results illustrate the antagonistic yet complementary roles of auxin and cytokinin in shoot and root development, where moderate auxin–cytokinin

ratios promote balanced morphogenesis. The observed differences in root and shoot morphology reflect the intertwined roles of auxin and cytokinin in tissue culture, where moderate auxin–cytokinin ratios support balanced organ development, whereas excess cytokinin suppresses root formation and favours unregulated callus proliferation.These findings were on par with the findings of (Kurepa *et al.*, 2022; Bielach *et al.*, 2017)

Shoot height was highest under T1 (13.4 cm) (Fig 1), followed by T2 (12.8 cm) and T6 (11.6 cm), whereas elevated cytokinin treatments (T3–T5) produced shorter, stunted shoots. Similarly, leaf production peaked in T1 (8.2 leaves per plantlet), followed by T2 (7.6) and T6 (7.1). T5 and hormone-free controls had the fewest leaves (4–5 and <4, respectively), indicating that moderate cytokinin combined with low auxin enhances shoot differentiation and foliage expansion.

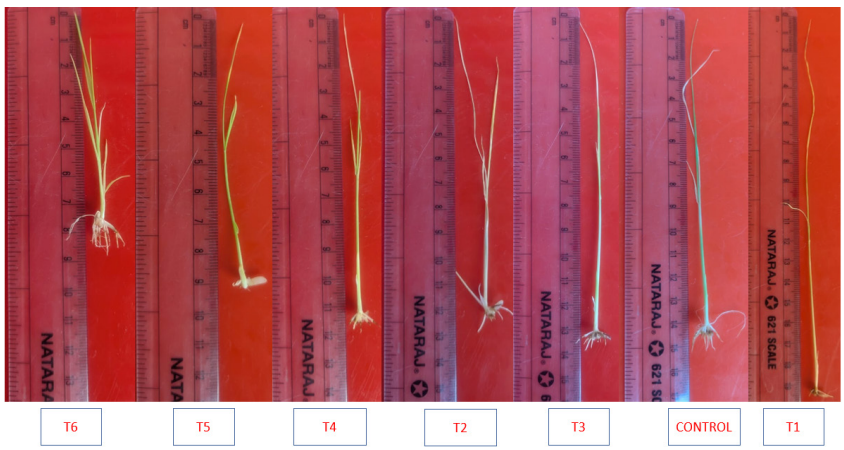

Fig. 1: Plant height of Swarna variety seedlings exposed to T1, T2, T3, T4, T5 and T6 combinations of plant growth regulators along with control.

The observed variation in shoot height and leaf number across treatments reflects the critical role of the auxin–cytokinin balance in regulating in vitro growth. Moderate cytokinin levels, as in T1, promoted optimal shoot elongation and higher leaf production, whereas elevated cytokinin or suboptimal auxin concentrations reduced shoot vigour and leaf number Sosnowski *et al.* (2023). These findings are consistent with established reports showing that balanced cytokinin–auxin ratios enhance shoot proliferation and leaf development, while excessive cytokinin often retards organogenesis and limits adventitious shoot formation.

T1 produced the most vigorous root systems, with thick (Figure 2C), white roots averaging 7 (Figure 2A) per plantlet and a mean length of 6.2 cm (Figure 3A). T2 and T6 supported substantial root formation (6 and 5 roots per plantlet; lengths 5.6 cm and 5.3 cm, respectively), while T3–T5 and control plantlets had thin, fragile, and shorter roots. These observations are consistent with the known antagonistic effects of cytokinin and auxin, where excessive cytokinin suppresses root initiation, and auxin promotes adventitious root development. Lakehal *et al.* (2019).

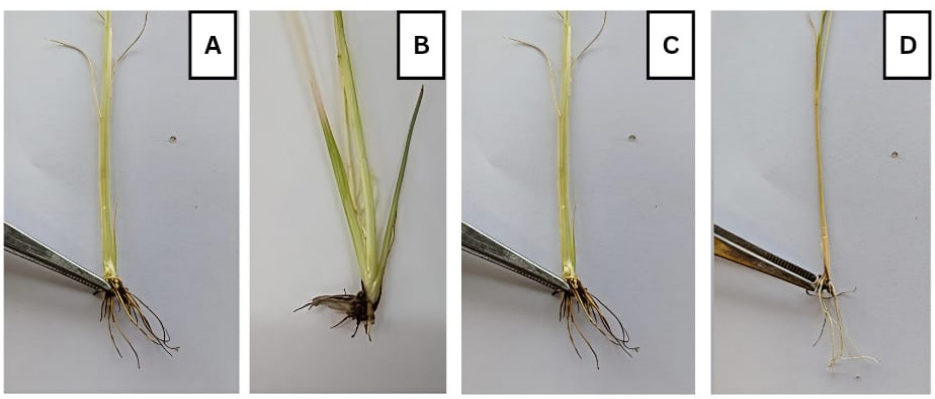

Fig. 2: Root number and Nature of roots observed for T1(1 mg/L BAP + 0.1 mg/L NAA)and control. (A) No. of roots Swarna seedling exposed to T1 (1 mg/L BAP + 0.1 mg/L NAA). (B)No of roots produced by the control (C) Nature of roots of T1 (1 mg/L BAP + 0.1 mg/L NAA) (D) Nature of roots of control.

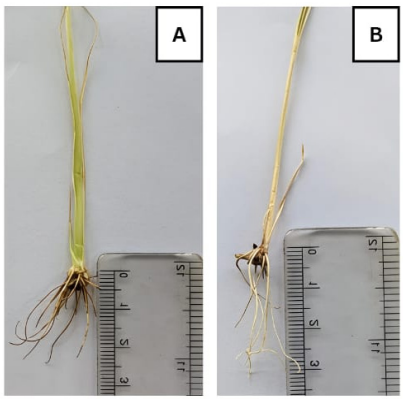

Fig. 3: (A) Root length of seedling of T1(1 mg/L BAP + 0.1 mg/L NAA) treatment (B) Root length of seedling in control.

Biomass accumulation, as indicated by fresh weight, varied notably with the different hormonal treatments, reflecting overall growth performance under each PGR regime. The highest mean fresh weight (1.42 g) was recorded in T1, followed by T2 (1.28 g) and T6 (1.17 g), indicating that moderate cytokinin coupled with low auxin levels supported more robust shoot and root development. In contrast, treatments with elevated BAP concentrations (particularly T4 and T5) exhibited reduced fresh weight, which is likely attributable to a shift toward excessive callus proliferation at the expense of organized shoot and root formation, thereby limiting biomass accumulation. These responses are consistent with the findings of Bielach *et al.* (2017), broader observations in plant tissue culture that balanced auxin–cytokinin interactions favour constructive organogenesis and growth, whereas hormone imbalances, especially higher cytokinins, can lead to diminished fresh weight and altered morphogenic patterns.

Excised shoots were effectively rooted on MS medium containing 0.6 mg L^{-1} IBA, producing well-developed roots essential for successful ex vitro establishment. A total of 20 rooted plantlets were transferred to the sterilized soil–sand mixture, of which 18 survived acclimatization, resulting in a survival rate of approximately 90%. Following transplantation, plantlets were acclimatized under high-humidity greenhouse conditions, followed by gradual reduction of humidity to facilitate establishment.

Fig. 4: Seedlings maintained at greenhouse with elevated humidity for acclimatization

Overall, T1 (1.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA) was the most effective treatment, producing the highest regeneration frequency, tallest shoots, most leaves, robust roots, and maximum biomass. T2 and T6 were also favourable, supporting healthy shoot and root development, with low kinetin in T6 enhancing shoot growth without excessive callus proliferation. Higher cytokinin treatments consistently reduced regeneration efficiency and inhibited rooting, emphasizing the importance of a low cytokinin–auxin ratio for coordinated morphogenesis in indica rice.

Conclusion

The present study established a robust and reproducible in vitro regeneration system for rice variety, Swarna, highlighting the pivotal role of hormone balance in determining morphogenic outcomes. The comparative evaluation of different BAP concentrations, together with an additional kinetin treatment, identified T1 (1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA) as the most effective treatment under the conditions evaluated. Among the treatments evaluated, 1 mg L^{-1} BAP combined with 0.1 mg L^{-1} NAA (T1) consistently produced the highest regeneration frequency, enhanced shoot elongation, increased leaf number, greater biomass, and well-developed, thick root systems. These results confirm that balanced auxin–cytokinin interactions are essential for coordinated shoot and root development, consistent with observations in cereal tissue culture studies.

Moderate cytokinin–auxin combinations, such as T2 (2 mg L^{-1} BAP + 0.2 mg L^{-1} NAA) and T6 (1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA + 0.5 mg L^{-1} kinetin), also supported substantial regeneration and healthy plantlet morphology, indicating that low kinetin supplementation can enhance shoot growth without excessive callus proliferation. In contrast, higher cytokinin concentrations (T3–T5) reduced morphogenic efficiency, likely due to excessive callus formation and impaired organ differentiation, a phenomenon commonly reported in rice tissue culture.

Rooting was effectively induced on MS medium supplemented with 0.6 mg L^{-1} IBA, producing strong root systems, and plantlets exhibited a high survival rate (~90%) during acclimatization, demonstrating the practical reliability of this regeneration protocol. Overall, the present study provides an optimized and comparatively evaluated regeneration protocol for rice cv. Swarna that can serve as a useful baseline for further studies involving rice tissue culture and crop improvement.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Darmagaru Shivani: Compilation and writing original draft; R Dakshina Raj: Literature survey and drafting themanuscript; A. Kavitha Reddy: Data curation and tabulation; G. Madhuri: Literature survey and figures; Abdul Fiyaz R: Editing; Darmagaru Shivani: Conceptualization, drafting and editing the final draft; G. Madhuri: Editing; A. Kavitha Reddy Editing and supervision

Ethical Approval

The article doesn't contain any data involving ethical approvals.

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No

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