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An Efficient *In Vitro* Regeneration Methods for Elite *Indica* Rice Varieties

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ABSTRACT: The findings underscore the importance of selecting appropriate hormonal combinations for maximizing shoot regeneration across different rice varieties. The specific combination of BAP, kinetin, and NAA not only facilitated high regeneration rates but also ensured robust growth and survival of the regenerated shoots. This hormonal synergy is crucial for the efficient propagation of plantlets in vitro, which is a foundational step for various plant breeding and genetic engineering applications. Among the tested varieties, Khandagiri exhibited the highest shoot regeneration and survival rates. This variety showed a remarkable ability to regenerate shoots under the given hormonal conditions, outperforming the other two varieties. The precise shoot regeneration and survival percentages for Khandagiri were at the upper end of the observed range, highlighting its responsiveness to the optimized medium. This superior performance suggests that Khandagiri has a high intrinsic capacity for shoot regeneration when exposed to the combination of BAP, kinetin, and NAA. This makes Khandagiri an excellent candidate for tissue culture applications and genetic transformation efforts, as its high regeneration efficiency can significantly enhance the success rate of these biotechnological processes.

Keywords: Rice, Lalat, MTU-1010, Khandagiri, Callusing, Regeneration.

INTRODUCTION

Plant breeding has been acknowledged as a critical strategy for incorporating beneficial traits into crops, which significantly helps stabilize food supplies essential for the growing global population (Khush, 2013; Mann, 1999; Ansari *et al.,* 2017). In recent years, there has been an increasing demand for a wide range of food products, including vegetables, fruits, and animal-based goods (Fukase & Martin 2020). This demand surge is further complicated by significant changes in global climate patterns and the rising incidence of biotic and abiotic stress factors (Paraschivu *et al.,* 2020). Rice (*Oryza sativa* L.) is particularly crucial in ensuring global food security, serving as a staple food for millions of people worldwide (Khush, 2013; Hiei & Komari 2006).

The development of an effective and reproducible regeneration system, along with a stable genetic transformation, is crucial for advancing rice improvement efforts. According to (Kalhori *et al*., 2017), the ability to reliably regenerate plants from transformed tissues ensures that genetically modified plants can be produced consistently. This is fundamental for implementing various genetic modifications aimed at enhancing desirable traits in rice, such as increased yield, pest resistance, and stress tolerance. The reproducibility of the regeneration system ensures that the genetic modifications can be consistently applied across different batches of plants, which is essential for large-scale agricultural applications and research. Without a reliable system, the genetic improvements cannot be effectively utilized or studied, hindering progress in rice improvement programs. According to Toki *et al.* (2006), the refinement of tissue culture protocols is essential for overcoming these challenges. Specifically, the development of protocols that can efficiently generate embryogenic calli and enable the regeneration of fertile plants from a single cell is crucial. Embryogenic calli are masses of undifferentiated plant cells that have the potential to develop into complete plants. Their formation is a critical step in the tissue culture process, as it provides the material needed for genetic transformation and subsequent plant regeneration.

MATERIALS AND METHODS

Plant Materials. To improve callus induction and regeneration, three elite *indica* rice varieties namely Lalat, MTU-1010, Khandagiri were selected for this experiment. Seeds were collected from breeder seed production farm, RRTTS, BBSR.

Optimization of callus induction. Following the sterilization process, the dried rice seeds were placed on sterile filter papers to ensure a contamination-free environment. These seeds were then cultured on a callus induction medium, specifically Murashige and Skoog medium (MS), as initially described by Murashige and Skoog in 1962. This medium was prepared with ten different treatments, each containing various combinations of growth regulators such as 2,4-Dichlorophenoxyacetic acid (2,4-D), kinetin, and Benzyl aminopurine (BAP). Moreover, each combination of growth regulators was paired with two distinct carbohydrate sources, providing additional variables to optimize callus induction (details provided in Table 1). The cultivation of the seeds was performed in darkness at a controlled temperature of 26°C to facilitate the optimal conditions for callus formation. To ensure the reliability and reproducibility of the results, each experiment included five replications. After an incubation period of 10-12 days, the resulting calli derived from the mature embryos were carefully sub-cultured onto fresh MS medium for an additional 5-7 days. This sub-culturing step is crucial for promoting the further development and differentiation of the calli.

Regeneration of plant. Calli were taken from three different rice varieties (Lalat, MTU-1010, Khandagiri) and placed on six different regeneration media. These cultures were maintained under a 16-hour light/8-hour dark photoperiod at 26 ± 2°C. After successful in vitro regeneration, the plantlets were transferred to culture tubes containing the same MS basal medium to promote shoot elongation. The regeneration process was monitored daily by observing the appearance of green spots, indicating a positive response. Once the plantlets emerged, they were moved to culture tubes with the same MS basal medium to encourage further shoot growth. The number of regenerated shoots was recorded every two weeks. For root proliferation, the in vitro regenerated plantlets were transferred to a half-strength MS basal medium supplemented with seven different concentrations of rooting hormones and sucrose for 10 days.

Acclimatization. The acclimatization process is a critical phase for the successful transition of plantlets derived from somatic embryos from an in vitro environment to natural growing conditions. This process ensures that the young plantlets can adapt to soil conditions and continue their growth and development.

Transfer to Soil Mixture. The plantlets derived from somatic embryos were transferred to a carefully prepared soil mixture consisting of sand, soil, and cow dung in a ratio of 1:2:1. This specific combination was chosen to provide an optimal balance of drainage, aeration, and nutrients. Sand ensures proper drainage and prevents waterlogging, soil provides essential minerals and a medium for root growth, and cow dung adds organic matter and nutrients, promoting healthy growth.

Greenhouse Conditions. Once transplanted, the plantlets were placed in a greenhouse where the relative humidity was maintained at 85%. This controlled environment is crucial for acclimatization, as it protects the delicate plantlets from external stressors such as harsh weather conditions, pests, and diseases. The high humidity helps reduce transpiration and prevents the young plants from drying out, facilitating their adjustment to the new growing medium.

RESULTS AND DISCUSSION

The highest frequency of callus induction was achieved using Murashige and Skoog (MS) medium supplemented with specific concentrations of growth regulators. The optimal combination was found to be 3.0 mg/l of 2, 4-Dichlorophenoxyacetic acid (2,4-D) and 0.25 mg/l of Benzylaminopurine (BAP). This combination proved to be highly effective in promoting the initiation and proliferation of callus tissue from the explants. Across the three tested indica rice varieties, the callus induction percentage varied, ranging from 38.15% to 82.45%. This indicates that the effectiveness of the hormonal treatment can differ based on the genetic background of the variety. Among the tested varieties, MTU1010 exhibited the highest callus induction rate at 82.45%. This high percentage suggests that MTU1010 is particularly responsive to the chosen hormonal combination, making it an excellent candidate for tissue culture and subsequent genetic transformation studies. Following MTU1010, the variety Khandagiri showed a substantial callus induction rate of 72.68%. This result also indicates a good response to the 2,4-D and BAP supplementation, though slightly less pronounced than MTU1010. Among various exogenously applied hormones, auxins are particularly effective in promoting the conversion of somatic cells to embryogenic cells. Studies by Cooke et al. (1993), (Khalequzzaman *et al.,* 2005; Sridevi *et al.,* 2005; Tyagi *et al.,* 2007; Hoque *et al.,*2013) have demonstrated that 2,4-D is one of the most effective growth regulators for achieving this conversion (Fig. 1).

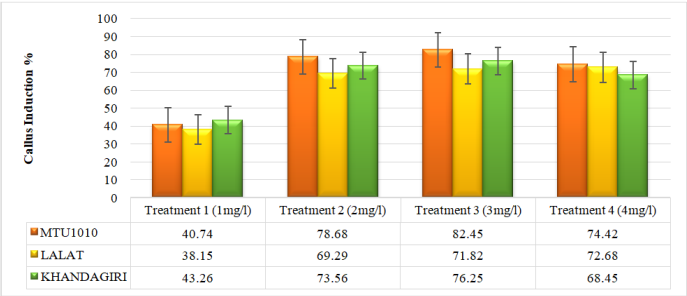

Fig. 1. Effect of 2,4 D on callusing of MTU-1010, Lalat and Khandagiri.

The highest frequency of shoot regeneration was achieved using Murashige and Skoog (MS) medium supplemented with a specific combination of growth regulators. The optimal concentrations identified were 1.5 mg/l of Benzylaminopurine (BAP), 1 mg/l of kinetin, and 0.1 mg/l of Naphthaleneacetic acid (NAA). This precise blend of hormones was found to be the most effective in promoting shoot induction and subsequent growth. (fig2). The percentage of shoot regeneration varied significantly across the tested indica rice varieties, ranging from 39.28% to 69.66% (detailed in Table 1). This variation indicates that the hormonal treatment's effectiveness can differ based on the specific genetic makeup of each variety. Among the tested varieties, Khandagiri exhibited the highest shoot regeneration and survival rates. This variety showed a remarkable ability to regenerate shoots under the given hormonal conditions, outperforming the other two varieties. The precise shoot regeneration and survival percentages for Khandagiri were at the upper end of the observed range, highlighting its responsiveness to the optimized medium.

Table 1: The effect of different concentration (mg/L) of BAP (6-benzylaminopurine) + Kinetin + 0.5 NAA in MS medium on shoot regeneration

Treatment Name	Treatment (mg/l)			Plantlet Regeneration (%)		
	BAP	NAA	Kinetin	MTU-1010	LALAT	KHANDAGIRI
RT1	2.0	0.5	0.5	58.25	45.58	59.62
RT2	2.5	0.5	0.5	53.44	42.14	57.23
RT3	2.0	0.25	0.5	63.78	49.25	63.86
RT4	1.5	0.1	1	67.21	57.60	69.66
RT5	2.5	0.5	1	48.42	39.28	47.83
RT6	2.0	0.5	1	52.28	43.45	54. 15

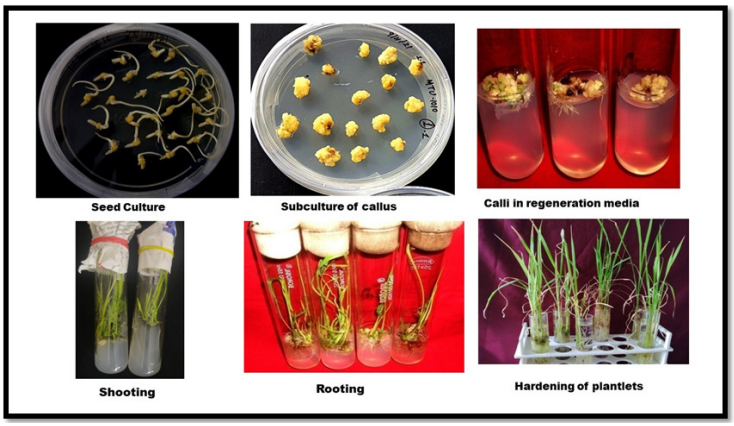

Fig. 2. Callus Induction and plant regeneration through somatic embryogenesis of four *Oryza sativa indica* rice.

CONCLUSIONS

The findings underscore the importance of selecting appropriate hormonal combinations for maximizing shoot regeneration across different rice varieties. The specific combination of BAP, kinetin, and NAA not only facilitated high regeneration rates but also ensured robust growth and survival of the regenerated shoots. This hormonal synergy is crucial for the efficient propagation of plantlets in vitro, which is a foundational step for various plant breeding and genetic engineering applications. Among the tested varieties, Khandagiri exhibited the highest shoot regeneration and survival rates. This variety showed a remarkable ability to regenerate shoots under the given hormonal conditions, outperforming the other two varieties. The precise shoot regeneration and survival percentages for Khandagiri were at the upper end of the observed range, highlighting its responsiveness to the optimized medium. This superior performance suggests that Khandagiri has a high intrinsic capacity for shoot regeneration when exposed to the combination of BAP, kinetin, and NAA. This makes Khandagiri an excellent candidate for tissue culture applications and genetic transformation efforts, as its high regeneration efficiency can significantly enhance the success rate of these biotechnological processes.

REFFERENCES

Ansari, A., Wang, C., Wang, J., Wang, F., Liu, P., Gao, Y., & Zhao, K. (2017). Engineered dwarf male-sterile rice: a promising genetic tool for facilitating recurrent selection in rice. *Frontiers in Plant Science*, *8*, 2132.

Fukase, E., & Martin, W. (2020). Economic growth, convergence, and world food demand and supply. *World Development*, *132*, 104954.

Hiei, Y., & Komari, T. (2006). Improved protocols for transformation of indica rice mediated by Agrobacterium tumefaciens. *Plant Cell, Tissue and Organ Culture*, *85*, 271-283.

Hoque, K. M. A., Azdi, Z. A., & Prodhan, S. H. (2013). Development of callus initiation and regeneration system of different indigenous indica rice varieties. *J. Biol*, *1*, 46-51.

Kalhori, N., Nulit, R., Go, R., Zulkifly, S., Azizi, P., & Abiri, R. (2017). Selection, characterizations and somatic embryogenesis of Malaysian salt-tolerant rice (*Oryza sativa* cv. MR219) through callogenesis. *International Journal of Agriculture and Biology*, *19*(1), 157-163.

Khalequzzaman, M., Haq, N., Hoque, M. E., & Aditya, T. L. (2005). Regeneration efficiency and genotypic effect

of 15 Indica type Bangladeshi rice (*Oryza sativa* L.) landraces. *Plant Tissue Cult*, *15*(1), 33-42.

Khush, G. S. (2013). Strategies for increasing the yield potential of cereals: case of rice as an example. *Plant Breeding*, *132*(5), 433-436.

Mann, C. C. (1999). Crop scientists seek a new revolution. American Association for the Advancement of Science.

Paraschivu, M., Cotuna, O., Paraschivu, M., & Olaru, A. L. (2020). Effects of interaction between abiotic stress and pathogens in cereals in the context of climate change: an overview. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, *49*(2), 413-424.

Sridevi, G., Dhandapani, M., & Veluthambi, K. (2005). Agrobacterium-mediated transformation of White Ponni, a non-basmati variety of indica rice (*Oryza sativa* L.). *Current Science*, 128-132.

Toki, S., Hara, N., Ono, K., Onodera, H., Tagiri, A., Oka, S., & Tanaka, H. (2006). Early infection of scutellum tissue with Agrobacterium allows high-speed transformation of rice. *The Plant Journal*, *47*(6), 969-976.

Tyagi, H., Rajasubramaniam, S., & Dasgupta, I. (2007). Regeneration and Agrobacterium-mediated transformation of a popular indica rice variety, ADT39. *Current Science*, *93*, 678-683.

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