CALLUS INDUCTION AND CHARACTERIZATION IN *ORYZA SATIVA INDICA*

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Abstract

In rice tissue culture, optimizing plant growth regulators (PGRs) is crucial for effective callus induction. The present study investigates the induction of rice callus in Murashige and Skoog (MS) media supplemented with varying concentrations of 6-Benzylaminopurine (BAP) and Indole-3-acetic acid (IAA), namely, in concentrations of 3:1 ratio, 2:1 ratio and 1:1 ratio respectively. The goal was to identify the most effective PGR combination and concentration that promotes optimal callus growth. Callus fresh and dry weight were measured to assess the growth response under different conditions. Highest mean fresh weight of callus was obtained for 3:1 ratio of BAP to IAA (0.55 ± 0.38 gram). The present study provides valuable insights into optimizing rice tissue culture protocols for improved callus induction, offering potential applications in plant propagation and genetic improvement.

Keywords: *Rice tissue culture, Callus induction, Plant growth regulators (PGRs), 6-Benzylaminopurine (BAP), Indole-3-acetic acid (IAA)*

Introduction

Rice is a critical crop for India. It serves as the staple food for most of the population and supports the livelihoods of millions of rural households. India is one of the top five rice exporters globally, the second-largest producer after China, producing 175.58 million tonnes of rice. Asia accounts for 87% of the world's rice consumption (Bhandari Humnath, 2019). India faces significant challenges in maintaining and boosting rice productivity. These challenges include declining land area for cultivation, inefficient use of resources such as fertilizer, water, and labor, and increasing scarcity of water and labor. Other factors such as climate change - manifested through rising temperatures, erratic rainfall, floods, and droughts - further exacerbate these issues, affecting both the quantity and quality of rice production (Thomas & Dobermann, A., 2002). As global rice consumption continues to rise with the increase in world population, India's rice production must evolve to meet both domestic needs and the demand from export markets.

The revenue generated from exporting rice plays a significant role in supporting rural economies and contributing to the national GDP.

Rice is not only an essential source of calories but also provides important nutrients such as magnesium, phosphorus, manganese, selenium, iron, folic acid, thiamin, and niacin, although it is low in fiber and fat (Fukagawa NK, Ziska LH., 2019). It constitutes one-third of the total dietary carbohydrates in many Asian countries and supplies 50 to 80 percent of daily calorie intake for over three billion people worldwide. In India, the average rice consumption is 208 grams per person per day, providing 31% of the recommended energy intake (Fukagawa NK, Ziska LH., 2019).

In India, rice is essential for enhancing farmers' income, as agriculture is the main livelihood for a large part of the population. Understanding the growth and instability of these crops is crucial for ensuring food security and income stability (Khush, G. S. 2005). The economic significance of rice and wheat further underscores the need to focus on sustaining their production. A considerable improvement has been done through traditional rice breeding. Rice breeding has made significant progress towards higher yield, improved quality, greater disease resistance and other important characteristics of agricultural importance in the past and even in the future, it is bound to have an important role (Sun et. al, 1990).

The field of plant biotechnology has seen tremendous progress, particularly in areas such as cell, tissue, and organ culture, over the last decade. In vitro techniques, including callus culture, are essential for improving existing rice cultivars and developing new varieties. By manipulating the ratio of auxin to cytokinin in culture media, it is possible to induce shoot, root, or somatic embryo formation. It should be emphasized that several agronomically desirable rice varieties are resistant to in vitro modification due to poor callus formation, making the existence of an effective in vitro propagation procedure a must. Callus cultures are thus extremely important in plant biotechnology. Several studies have recently identified the role of plant growth regulators for efficient callus induction and plant regeneration (Tariq et. al, 2008; Subramaniam et. al, 2011). The present study aims to identify suitable concentrations of BAP and IAA for induction and characterization of callus in *Oryza Sativa Indica*.

Materials and Methods

Rice grains were obtained from International Rice Research Institute, Hyderabad. The variety sourced was Improved Samba Masoori. The seeds were sterilized with 70% ethanol for 1 minute, followed by 10% sodium hypochlorite for 15 minutes, and washed 6 to 7 times with distilled water. The seeds were cultured on basal MS medium (Murashige et. al, 1962) without growth hormones for callus induction. They were kept in the dark at a temperature of 25±1°C for 2 weeks. The callus was subcultured into test tubes containing MS medium with plant growth regulators BAP and IAA in the ratios of 3:1, 2:1 and 1:1 in replicates of 10 each. They were incubated at 25±1°C with a photoperiod of 16 hours, for four weeks. The callus obtained was weighed to calculate the fresh weight and was dried in a hot air oven for 48 hours at 45°C to obtain dry weight. An analytical balance was used to maintain accuracy of the weight measurements.

The following parameters were calculated:

1. Callus Induction Frequency

Callus induction frequency is the percentage of seeds that develop callus after inoculation. It varies depending on the plant variety, the type of media, and the concentration of plant growth regulators (PGRs) (Tariq et. al, 2008).

Callus Induction Frequency = [No. of explants producing callus/No. of explants plated]×100

2. Callus Growth Efficiency

This efficiency measure considers the biomass produced relative to the input (e.g., medium volume or growth regulators).

These calculations can provide insight into the physiological response of the rice calli to different ratios of BAP and IAA.

Formula = DW/Medium Volume or Growth Regulator Amount

3. Tissue Culture Efficiency

This measure evaluates the overall performance of the culture system based on growth parameters such as fresh weight and dry weight. It can be used to assess how well the callus is regenerating in response to the treatments.

This could be related to the volume of medium or the total amount of plant growth regulators used.

Formula = Total DW Produced/Total Media or Resource Input

4. Growth Index

The growth index can be calculated by combining both the fresh and dry weights to give a comprehensive view of the overall growth.

This gives an overall sense of the callus tissue's growth by considering both water and dry matter content.

Formula = [FW+DW]/2

5. **Growth Efficiency Index**

The growth efficiency index is another way to assess how efficiently the plant converts available resources into dry matter. It compares both the fresh and dry weights over time. This measures the dry matter production relative to the fresh weight in the final culture, giving an insight into the callus's efficiency in converting resources. This gives the percentage of the total fresh weight that has been converted into dry matter. Higher growth efficiency values indicate that a greater proportion of the fresh weight is due to biomass rather than water content.

Formula = [DW/FW]*100

Results and Discussion

Table 1 depicts fresh and dry weight of calli in different ratios of BAP: IAA and their callus induction frequency. The highest mean fresh weight was observed in the 3:1 ratio of BAP: IAA (0.55±0.38) while the highest mean dry weight was observed in the 2:1 ratio of BAP: IAA (0.06±0.06). Tariq et. al, (2008), reported the highest mean weight of callus with 2, 4 - D to be 0.27 grams (Tariq et. al, 2008). However, the present study reveals a 3:1 ratio of BAP: IAA as a better combination for callus induction. This may be because BAP (6-Benzylaminopurine) is a synthetic cytokinin that promotes cell division and shoot induction, while IAA (Indole-3-Acetic Acid) is a natural auxin that stimulates cell elongation and dedifferentiation, which is essential for callus formation. Together, they regulate cell proliferation and maintain an optimal callus structure for further subculturing. These results may suggest the role of growth hormones for callus induction and tissue differentiation. Table 1 also depicts the callus induction frequency, which was found to be the same in 3:1 and 2:1 (80%) while (70%) in 1:1 ratio of BAP : IAA. The highest callus induction frequency was observed at 3:1 and 2:1 ratio, with an 80% success rate (Subramaniam et al., 2011). Subramaniam et al., 2011 and Rashid et. al, 2003, obtained varying degrees of callus induction in rice, while our results are more uniform (Rashid et. al, 2003). This could be because they worked with different varieties while our study focussed on a single variety and its response to different concentrations of BAP in combination with IAA.

Table 2 shows Callus Growth Efficiency, Growth Index, Tissue Culture Efficiency and Growth Efficiency Index observed in 3:1, 2:1, 1:1 ratios of BAP: IAA, and the combined mean. Callus Growth Efficiency (0.125g), Tissue Culture Efficiency (0.3g/L) and Growth Efficiency Index (15.625%) was found to be highest in 2:1 BAP : IAA (Fig. 2) ratio when compared with other combinations. The Growth Index does not show much variation across the different combinations (Fig. 1).

Conclusion

The 3:1 ratio proves to be optimal for callus induction and characterization, providing the largest mean fresh weight. This combination may be useful for callogenesis in Indica varieties. However, in terms of callus induction frequency and tissue culture efficiency, the 2:1 ratio of BAP : IAA indicates considerably higher Callus Growth Efficiency and Tissue Culture Efficiency values, making it a useful protocol for resource - limited applications. Overall, these findings contribute valuable insights into the development of improved plant tissue culture methodologies.

Table No. 1: Fresh and Dry Weight of Calli in Different Ratios of BAP to IAA with their Callus Induction Frequency

Growth Hormone Ratio	BAP/IAA = 3:1		BAP/IAA = 2:1		BAP/IAA = 1:1	
	FW(g)	DW(g)	FW(g)	DW(g)	FW(g)	DW(g)
Tube 1	1.27	0.11	0.92	0.2	0.41	0.05
Tube 2	0.78	0.05	0.5	0.1	0.83	0.07
Tube 3	0.48	0.04	0.47	0.03	0.5	0.02
Tube 4	0.31	0.05	0.36	0.12	0.47	0.01
Tube 5	0.4	0.02	0.28	0.03	0.98	0.11
Tube 6	0.78	0.08	0.37	0.02	0.42	0.01
Tube 7	0.94	0.05	0.48	0.06	0.47	0.05
Tube 8	0.51	0.04	0.46	0.04	-	-
Tube 9	-	-	-	-	-	-
Tube 10	-	-	-	-	-	-
Mean+SD	0.55±0.38	0.04±0.03	0.38±0.25	0.06±0.06	0.41±0.32	0.03±0.03
Callus Induction Frequency	80%		80%		70%	

Table No. 2 : Callus Growth Efficiency, Growth Index, Tissue Culture Efficiency and Growth Efficiency Index Observed in 3:1, 2:1, 1:1 and Combined Mean of BAP/IAA

Growth Hormone Ratio	BAP/IAA 3:1	BAP/IAA2:1	BAP/IAA 1:1	Combined Mean
Callus Growth Efficiency (g/ml)	0.069	0.125	0.100	0.097
Growth Index (g)	0.296	0.222	0.220	0.246
Tissue Culture Efficiency (g/l)	0.220	0.300	0.160	0.227
Growth Efficiency Index (%)	8.044	15.625	7.843	10.504

Figure 1: Growth Index and Growth Efficiency Index vs. BAP: IAA

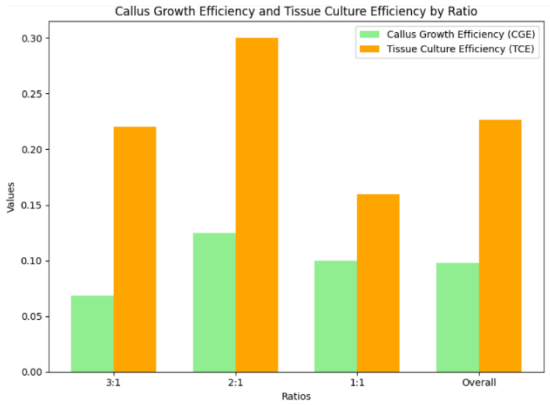

Figure 2: Tissue Culture Efficiency and Callus Growth E vs. BAP: IAA

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