International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

Cost Effective Methods of in Vitro Multiple Shoot Production in Two Commercial Diploid Bananas of Kerala

C.P. Sapheera[1], A. Aswini[2], M. Mohammed Billal[3], A. K. Babylatha[4]

[1]Assistant Professor, Fruits Crops Research Station, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala, India.

[2]Assistant Professor, Department of Fruit Science, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India.

[3]PG, Department of Fruit Science, College of Agriculture, Kerala, Agricultural University, Vellanikkara, India.

[4]Professor and Head (Rtd), Fruits Crops Research Station, Vellanikkara, Thrissur, Kerala, India.

Emails: *sapheera.cp@kau.in[1], aswini.a@kau.in[2], mohammed-2021-12-027@student.kau.in[3], babylatha.ak@retd.kau.in [4]*

Abstract

Cost-effective methods for in vitro *propagation of two commercial diploid bananas of Kerala, namely Musa (AB) ' Njalipoovan' and Musa (AA) ' Nivedyakadali' were carried out to reduce unit production cost. Sword sucker, peeper sucker and eye bud explants were found to be equally effective concerning the number of axillary shoots from a single explant in both cultivars. Sucrose 3.0 per cent and table sugar 2.0 per cent produced the maximum number of multiple shoots in Njalipoovan. In the case of Nivedyakadali, table sugar at 2.0 per cent recorded the maximum number of multiple shoots. Half strength of vitamins in full MS medium observed more multiple shoots in cv. Njalipoovan. Half a tablet of vitamin B complex (2.0mgl^{-1}) in the medium resulted in more shoots in the case of cv. Nivedyakadali. Agar 0.7 per cent recorded a significantly higher number of multiple shoots per culture in both cultivars. The treatments involving different types of paper support (filter paper, brown paper and ordinary paper) in liquid media were inferior to agar 0.7 per cent treatment.*

Keywords: *Cost - Effective; Diploid Banana; In Vitro; Micro Propagation; Ms Medium.*

1. Introduction

The commercial micro-propagation industry is considered relevant to agribusiness as tissue culture can break the yield plateau reached by the green revolution [1]. It has several exclusive practical applications leading to crop productivity improvement, which are impossible in conventional science. However, the high production cost of tissue culture plantlets limits the use of this technology. In a state such as Kerala, where productivity is noted to be the lowest due to the polyclonal, homestead system of banana cultivation coupled with the prevalence of sucker-transmitted deadly diseases such as bunching top, bacterial wilt, and nematode infestation, *in vitro* methods are often reliable. The morphological variations in banana cultivars, which consist mainly of triploids and diploids, are vast and complex, with the combination of different degrees of expression of parental species. In Kerela, diploid cultivars have high demand and are getting premium prices in the domestic market. However, scanty tissue culture work has been done in the case of domesticated diploid banana cultivars. Low-cost solutions should reduce manufacturing costs without sacrificing the quality of plants and micropropagules. The plants that are produced must be robust, able to be planted in the field successfully and have a better survival rate [2]. They should also be genetically consistent, virus- and disease-free, and priced comparable to plants made using traditional techniques. Cost-cutting initiatives shouldn't give rise

International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

to high levels of culture contamination or plants with subpar field performance [3-7].

1.1 Carbon Sources

Various viewpoints exist on the advantages of the different carbon sources (sucrose, glucose, and fructose) on the *in vitro* growth of plants. Additionally, it has been noted that glucose can affect how differently plants develop *in vitro*. Smith (1932) wrote on the harmful effects of autoclaved glucose-containing medium, and Hill and Patton (1947) explained that this impact was due to the Maillard reaction, which occurs when an amino acid forms a harmful compound with heat [8]. On the other hand, Ball (1953) noted that the liberation of advantageous amounts of fructose during autoclaving enhanced the nutritional value of sucrose medium for plant tissue. It is evident from the aforementioned literature that different carbon sources have varying effects on the growth of *in vitro* grown plants [9-11].

1.2 Gelling Agents

The physical and chemical characteristics of the culture media are just one of several variables that impact the growth and multiplication of shoots *in vitro* (Israeli *et al*., 1996) [12]. The gelling agent also affects the medium's physical characteristics, such as water potential and nutrient availability, as well as the concentration of mineral nutrients (Singha *et al*., 1985; Kusumoto, 1980). Singha (1982) states that plant species affect how plantlets react to gelling agents *in vitro*. The effects of gelling agents on the chemical makeup of the growth medium with various plant species have been the subject of much research (Stoltz, 1971; Werner and Boe, 1980).

1.3 Vitamin Sources

Plants need vitamins because they act as catalysts in various metabolic processes. Some vitamins may function as growth *inhibitors when plant cells and tissues are cultured* in vitro [13]. The vitamins nicotinic acid, pyridoxine (B6), myo-inositol, and thiamin (B1) are most frequently found in cell and tissue culture mediums. The one vitamin that virtually all cells need for growth is thiamin. Thiamin is often used in solutions with concentrations between 0.1 and 10.0 mg/litre. Although frequently added to the culture medium, pyridoxine and nicotinic acid are not always necessary for cell development in many species.

2. Method

In vitro propagation through enhanced release of axillary buds was used for the study (Murashige,1974). All aseptic manipulations such as surface sterilization of explants, preparation and inoculation of the explants and subsequent subculturing were carried out under the hood of a clean 'Thermadyne' laminar airflow cabinet. The explants for the study were collected from two popular diploid banana cultivars of Kerala, namely Musa (AB) 'Njalipoovan' and Musa (AA) 'Nivedyakadali' [14-17].

2.1 Plant Materials

To develop a low cost tissue culture technique in *Musa* (AB) 'Njalipoovan' and *Musa* (AA) 'Nivedyakadali', the suckers of this plants were collected from Banana Research Station, Kannara, Kerala Agricultural University, Thrissur. The sword suckers, Peeper suckers and eye buds were used as a source material for *in vitro* studies [18].

2.2 Carbon Sources

The cost of laboratory-grade sucrose, a commonly used carbon source in tissue culture techniques, is very costly and adds to the total unit cost for the production of a single plantlet. Sucrose, the main source of carbon was substituted with various substances to find a low cost alternative to sucrose. In this study, two diploid commercial varieties of Kerala (Njalipoovan and Nivedyakadali) were selected and their comparative performance using different alternate carbon sources was tested. There were four cultures per treatment. Observations on number of multiple shoots formed were recorded after four weeks of culturing [19-21]. The different substitutes used and their levels were as follows

1. Table sugar (1.5, 2.0 and 3.0 %)
2. Glucose (1.5, 2.0 and 3.0 %)
3. Sucrose (1.0, 2.0 and 3.0 %)

2.3 Gelling Agents

Agar, the principal component of gelling agent was substituted with various levels of different gelling substances and supporting materials as given below [22-26].

International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

1. Agar (0.6%)
2. Gelatin (0.3%) + Agar (0.5%)
3. Gelatin (0.7%)
4. Filter paper (Wattman No.1)
5. White paper (Ordinary)
6. Brown paper (Ordinary)
7. Agar (0.7%)

Observations on the number of multiple shoots formed were recorded on four explants per treatment after four weeks of culturing [27].

2.4 Vitamin Sources

In order to reduce cost of vitamins, different strength of vitamins (Nicotinic acid, Pyridoxin HCl and Thiamine HCl) in MS medium was tried [28].

1. Half tablet of vitamin B complex 2.0 mg/ l (Omega -B complex)
2. No vitamins in MS
3. Quarter strength of vitamins in MS
4. Half strength of vitamins in MS
5. Full strength of vitamins in MS (control)

Observations on the number of multiple shoots formed were recorded on four explants per treatment after four weeks of culturing [29].

2.5 Tables

2.5.1 Cost Effective Methods of in Vitro Multiple Shoot Production in Two Commercial Diploid Bananas of Kerala

Table 1 Influence of Different Explants in The Establishment Medium

Explants	Njalipoovan		Nivedyakadali	
	Number of days taken for establishment	Culture established (%)[a]	Number of days taken for establishment	Culture established (%)[a]
Sword sucker	8.00	87.50	10.00	81.25
Peeper sucker	11.00	93.75	10.50	93.75
Eye buds	15.75	87.50	12.75	87.50
CD (0.05)	2.24		0.78	

Table 2 Effect of Different Carbon Sources On in Vitro Multiple Shoot Production of Diplod Banana Cv. Njalipoovan

S.No.	Treatments	Number of shoots	Length of longest shoots (cm)	Cultures developing shoots (%)
1	Table sugar 1.5%	2.4	2.6	100.00
2	Table sugar 2.0%	4.1	3.8	100.00
3	Table sugar 3.0%	2.3	2.0	100.00
4	Glucose 1.5%	2.6	3.4	100.00
5	Glucose 2.0%	3.2	4.4	100.00
6	Glucose 3.0%	1.6	3.0	100.00
7	Sucrose 1.5%	2.9	1.7	100.00
8	Sucrose 2.0%	3.1	3.0	100.00
9	Sucrose 3.0% (Control)	4.1	3.1	100.00
CD (0.02)		1.14	1.03	

International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

Table 3 Effect of Different Carbon Sources On in Vitro Multiple Shoot Production of Diplod Banana Cv. Nivedyakadali

Sl no.	Treatments	Number of shoots	Length of longest shoots (cm)	Cultures developing shoots (%)
1	Table sugar 1.5%	2.1	2.0	100.00
2	Table sugar 2.0%	4.9	4.6	100.00
3	Table sugar 3.0%	3.0	2.5	100.00
4	Glucose 1.5%	2.6	1.8	100.00
5	Glucose 2.0%	2.6	3.6	100.00
6	Glucose 3.0%	3.3	2.9	100.00
7	Sucrose 1.5%	2.6	1.8	100.00
8	Sucrose 2.0%	3.6	2.9	100.00
9	Sucrose 3.0% (Control)	4.6	3.6	100.00
CD (0.02)		1.27	0.97	

Table 4 Effect of Different Gelling Agents and Supporting Materials On in Vitro Multiple Shoot Production of Diploid Banana

Sl. No.	Treatments	Njalipoovan			Nivedyakadali		
		Number of shoots	Contamination (%)	Cultures developing shoots (%)	Number of shoots	Contamination (%)	Cultures developing shoots (%)
1	Agar 0.6 %	2.6	0.00	100.00	3.0	0.00	100.00
2	Gelatin 0.3% + Agar 0.5%	1.6	0.00	100.00	2.5	0.00	100.00
3	Gelatin 0.7%	0.0	0.00	000	0.0	0.00	0.00
4	Filter paper	3.0	12.50	87.5	3.5	6.25	93.75
5	Brown paper	2.7	18.75	81.25	2.9	12.50	87.50
6	Ordinary paper	3.1	31.25	68.75	3.1	25.00	75.00
7	Agar 0.7%	4.1	0.00	100.00	4.6	0.00	100.00
CD (0.5)		0.78			1.06		

International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

2.6 Figures

Figure 1 Effect of Different Carbon Sources On in Vitro Multiple Shoot Production of Diplod Banana Cv. Njalipoovan

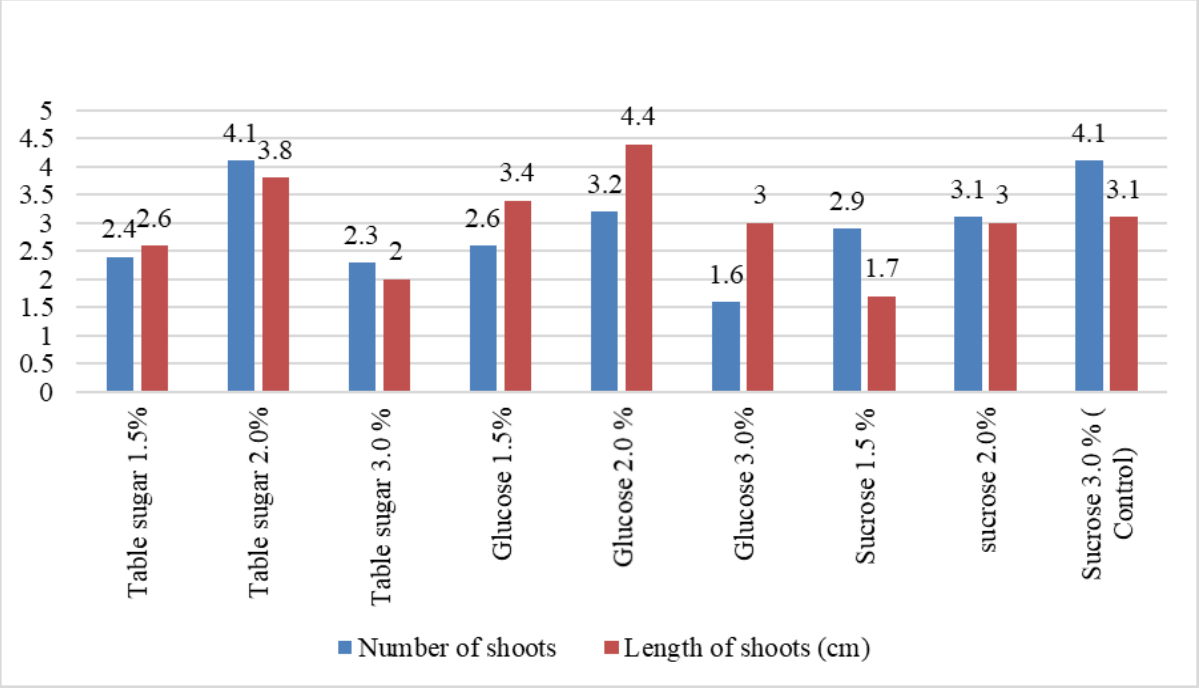
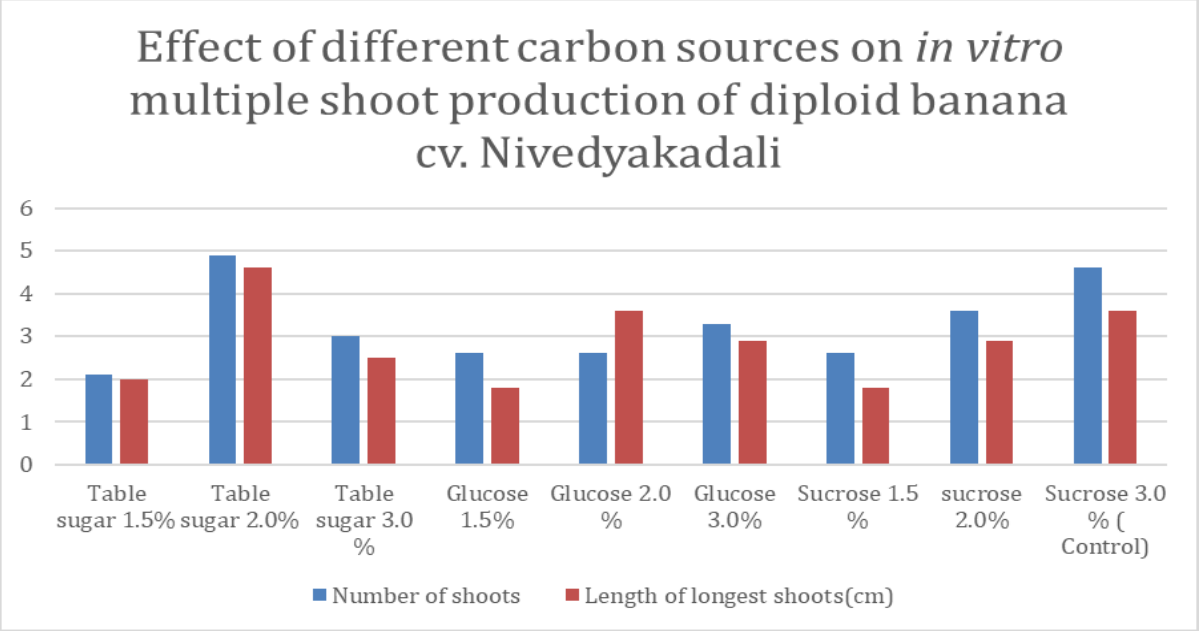


Figure 2 Effect of Different Carbon Sources On in Vitro Multiple Shoot Production of `Diplod Banana Cv. Nivedyakadali

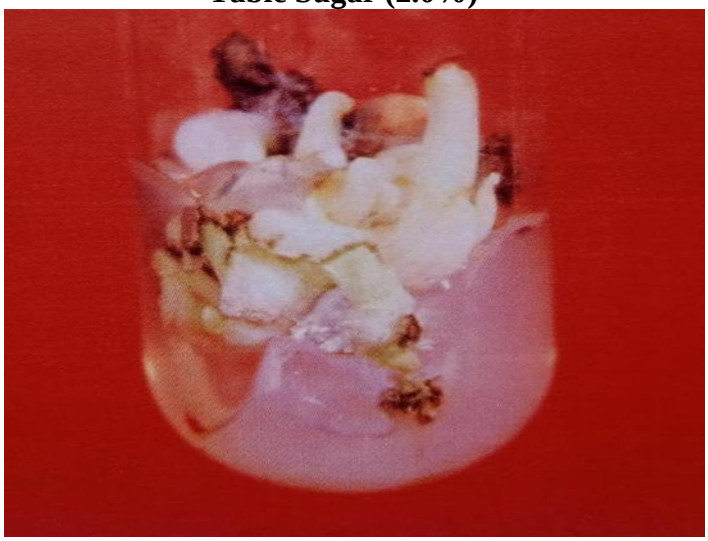


International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

2.7 Plates

Plate 1 In vitro Multiple Shoot Production of Diploid Banana cv. Njalipoovan in full MS+table Sugar (2.0%)

Plate 2 In Vitro Multiple Shoot Production of Diploid Banana Cv. Nivedyakadali in Full MS + Table Sugar (2.0%)



3. Results

3.1.Results

The experimental results evaluating the influence of different explants on cv. Njalipoovan and cv. Nivedyakadali presented in Table 1, indicated notable variations in both the percentage of established cultures and the number of days required for culture establishment. For Njalipoovan, sword sucker explants established cultures in the shortest time (8.00 days) with an 87.50% success rate. Peeper sucker explants, while taking longer to establish (11 days), achieved a higher establishment rate of 93.75%. Eye bud explants required more time for establishment (15.75 days) with 87.50% success rate. A similar pattern was observed for cv. Nivedyakadali concerning the time required for culture establishment. Peeper sucker explants had the highest establishment rate (93.75%), followed by eye bud explants (87.50%). Data on the number of multiple shoots produced, the length of multiple shoots, and the percentage of crops developing shoots when cv. Njalipoovan is exposed to different carbon sources are presented in Table 2, Figure 1, and Plate 1. The treatments with 3.0% sucrose and 2.0% table sugar, as highlighted in Plate 1, resulted in the highest number of shoots (4.1). This was comparable to the number of shoots produced by 2.0% glucose and 2.0% sucrose treatments (3.1 each). The longest shoot length was observed with the 2.0% glucose treatment (4.4 cm), which was similar to the results for the 2.0% table sugar (3.8 cm) and 1.5% glucose (3.4 cm) treatments. In the diploid banana cultivar Nivedyakadali, the highest number of multiple shoots (4.9) and the longest shoots per culture (4.6 cm) were produced in a medium containing 2.0% household sugar, as shown in Table 3, Figure 2, and Plate 2. The number of shoots produced with 3.0% sucrose (4.6) and 2.0% sucrose (3.6) were comparable to those produced with 2.0% household sugar. Similarly, the length of shoots per culture in the 2.0% household sugar treatment was comparable to those produced with 3.0% sucrose and 2.0% glucose (3.6 cm). The data on the number of shoots produced and the percentage survival rate for cv. Njalipoovan and cv. Nivedyakadali in media containing different gelling agents and supporting materials are presented in Table 4. For cv. Njalipoovan, the highest number of multiple shoots (4.1) was produced in the medium containing 0.7% agar. Similarly, in cv. Nivedyakadali, the highest number of multiple shoots (4.6) was also produced in the 0.7% agar medium, with a 100% shoot development rate. Other effective treatments included plain paper, brown paper, and

International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

0.6% agar. Explants in the 0.7% gelatin treatment did not survive, as the medium failed to solidify. Table 4 provides information on the number of shoots each culture of the Njalipoovan and Nivedyakadali cultivars generated at various vitamin concentration strengths. Maximum number of multiple shoots were observed in the cv. Njalipoovan at half strength of vitamins in full MS (5.3), which was comparable to half a vitamin B complex pill in medium (4.4). For the cv. Nivedyakadali, a half tablet of vitamin B complex in medium produced the greatest number of shoots (4.8), which was comparable to the control treatment using full strength vitamins in MS (4.6) and half strength vitamins in MS medium (4.8).

3.2.Discussion

The experiment reveals that sword sucker and peeper sucker explants exhibit similar performance characteristics, requiring less time for culture establishment compared to eye bud explants. This disparity in performance can be attributed to differences in endogenous phytohormone levels, nutrient availability, metabolite composition, and interactions among growth factors. Previous studies by Cronauer and Krikorian (1984a and b), Vuylsteke and De Langhe (1985), Wong (1986), and Bhaskar (1991) support these findings, emphasizing the variability in banana tissue culture response based on explant type and genotype. In the present study, Njalipooven produced the maximum multiple shoots at a sucrose concentration of 3.0%, which was comparable to the concentrations of table sugar (2.0%). Similarly, cv. Nivedyakadali exhibited a maximum number of multiple shoots in a medium containing 2.0% table sugar. These results agree with previous findings indicating positive effects of various carbon sources other than sucrose (Babylatha, 1993; Okuna, 1996). Sunderasu (2003) also reported positive results for cost-effective *in vitro* multiple shoot production of bananas using table sugar at 2.0% concentration. Table sugar is an effective substitute for sucrose as the primary carbon source in tissue culture media. The results revealed that the diploid cultivars responded more or less similarly to the different gelling agents and supporting materials. The treatments involving different types of paper supports (filter paper, brown paper and ordinary paper) in liquid media were also satisfactory even though inferior to agar by 0.7 per cent. The beneficial effects of a liquid medium with filter paper and other supporting materials have been pointed out by different workers (Babylatha, 1993; Bhagyalakshmi and Singh, 1995 and Teng, 1997). The experiment conducted I this work highlights that Njalipoovan exhibited the highest number of multiple shoots when cultured in full MS medium at half the normal strength of vitamins, which was comparable to the effect of half a tablet of vitamin B complex (2 mgl^{-1}). Similarly, in the case of Nivedyakadali, the use of half a tablet of vitamin B complex resulted in the highest number of multiple shoots, comparable to both full and half-strength vitamin concentrations in full MS media. However, shoot formation was insufficient when vitamins were diluted to a quarter strength. According to Magneye and Escobido (1996), traditional banana propagation methods could be made more cost-effective by excluding the expensive organic medium component myo-inositol. Sundarasu (2003) further supported this by demonstrating that reducing the vitamin B complex level by half could lead to a 32.22% reduction in vitamin costs. Additionally, Sundarasu noted that in the Nendran (AAB) variety, halving the vitamin concentration resulted in a substantial increase in multiple shoot production, while quartering the vitamin concentration led to poor shoot development.

Conclusion

This study highlights the critical factors influencing banana tissue culture, including the type of explants, carbon sources, vitamin concentrations and gelling agents. Diploid banans viz, Njalipoovan (AB) and Nivedyakadali (AA) cultivars responded optimally to half a tablet of vitamin B complex for multiple shoot formation, demonstrating comparable effectiveness to full MS medium at half the normal strength of vitamins. These findings underscore the potential for cost reduction and efficiency improvement in banana tissue culture protocols by fine-tuning these variables. By incorporating alternative nutrient sources like table sugar and adjusting vitamin levels, the study provides a framework for more economical and effective banana propagation strategies, essential for sustainable agriculture and genetic improvement

International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

efforts in banana cultivation.

Acknowledgements

The paper forms a part of the P G thesis submitted by the first author to Kerala Agricultural University, Vellanikkara, Thrissur Kerala.

References

[1]. Babylatha A.K. 1993. *In vitro* propagation studies on banana and papaya, Ph.D (Hort.) thesis, Gujarat Agricultural University, Navasari, 204 p.

[2]. Ball, E. 1953. Hydrolysis of sucrose by autoclaving media: a neglected aspect in the technique of culture of plant tissue. Bull. Torrey Bot. Club 80: 409-411

[3]. Rajasekar, R., et al. "Development of compatibilized SBR and EPR nanocomposites containing dual filler system." Materials & Design 35 (2012): 878-885.

[4]. Velu Kaliyannan, Gobinath, et al. "Influence of ultrathin gahnite anti-reflection coating on the power conversion efficiency of polycrystalline silicon solar cell." Journal of Materials Science: Materials in Electronics 31 (2020): 2308-2319.

[5]. Rajasekar, R., et al. "Investigation of Drilling Process Parameters of Palmyra Based Composite." (2021).

[6]. Moganapriya, C., et al. "Achieving machining effectiveness for AISI 1015 structural steel through coated inserts and grey-fuzzy coupled Taguchi optimization approach." Structural and Multidisciplinary Optimization 63 (2021): 1169-1186.

[7]. Sachin, S. Raj, T. Kandasamy Kannan, and Rathanasamy Rajasekar. "Effect of wood particulate size on the mechanical properties of PLA biocomposite." Pigment & Resin Technology 49.6 (2020): 465-472.

[8]. John, Agnes Aruna, et al. "Folic acid decorated chitosan nanoparticles and its derivatives for the delivery of drugs and genes to cancer cells." Current Science (2017): 1530-1542.

[9]. Bhaghyalakshmi, R. and Singh, S.N. 1995. Role of liquid versus agar – gelled media in mass propagation and ex vitro survival in bananas. Pl. Cell Tiss. Org. Cult. 41: 71-7

[10]. Bhaskar, J. 1991. Standardisation of in vitro propagation technique in banana. M.Sc (Hort.) thesis, Kerala Agricultural University, Trichur, 88 p.

[11]. Cronauer, S.S. and Krikorian, A.D. 1984 a. Multiplication of Musa from excised stem tips. Ann Bot. 53:321 – 328

[12]. Cronauer, S.S. and Krikorian, A.D. 1984b. Rapid multiplication of bananas and plantains by in vitro shoot tip culture. Hortscience 19:234-235

[13]. Gamborg, O. L. and shyluck, J. P. 1981. Nurtrition, media and characterization of plant cell and tissue culture. plant cell tissue culture: methods and applications in Agriculture (eds. Thorpe, T.Z) Academic press, New york, pp 21-44

[14]. Gawel, N.J. and Robacker, C. D. 1990. Somatic embryogenesis in two Gossypium hirsutum genotypes on semi – solid versus liquid proliceration media. pl. Cell. Tiss. Org. Cult. 23:201-204

[15]. Hill, E. G. and Patton, A. R. 194 7. The millard reaction in microbial assay. Science, N.Y. 105: 481-482.

[16]. Israeli, Y., Lahav, E. and Reuveni, O. 1996. In vitro culture of bananas and plantains. In S. Gowen ed., Bananas and Plantains. Chapman and Hall, London. 1-612.

[17]. Kusumoto, M. 1980. Effects of coconut milk, agar and sucrose concentrations and media pH on the proliferation of Cymbidium protocorm-like bodies cultured in vitro. J. Soc. Hort. Sci. 48: 503-509.

[18]. Magnaye, L.V. and Escobido, E.O. 1996. Commercial micropropagation of popular local banana cultivars. Philipp J. Crop. Sci 19:40-41.

[19]. Murashige, T. and skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures pl. physiol. 15:473.

[20]. Okuna, K. 1996. A trial of improvement of micropropagation of Brassica campestris cv. Kukitochina by cost stimulation. J. Soc. High Technol. Agric. 8(4): 253-263

International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2024.0302
e ISSN: 2584-2854
Volume: 02
Issue: 06 June 2024
Page No: 2054-2062

[21]. Singha, S. 1982. Influence of agar concentration on in vitro shoot proliferation of Malus spp. 'Almey' and Pyrus communis 'Seckel'. Am. Soc. Hort. Sci. 107: 657-660.
[22]. Singha, S., Townsend, E. C. and Oberly, G. H. 1985. Mineral nutrient status of crab apple and pear shoots cultured in vitro on varying commercial agars. J. Am. Soc. Hort. Sci. 110: 407-411.
[23]. Smith, M. L. 1932. The effect of heat on sugar solutions used for culture media. J. Biochem. 26: 1467-1472.
[24]. Stoltz, L. P. 1971. Agar restriction on the growth of excised mature iris embryos. J. Am. Soc. Hort. Sci. 96: 681-684.
[25]. Sundarasu, N. 2003. Studies on in vitro shoot tip culture of banana for the development of cost effective small scale production system. M.Sc.(Hort.) thesis, Kerala Agricultural University, Thrissur. 87p.
[26]. Teng, W. L. 1997. Regeneration of Anthurium adventitious shoots using liquid or raft culture. Pl. Cell Tiss. Org. Cult. 49: 153-156
[27]. Vuylsteke, D and De Langhe E. 1985 Feasibility of in Vitro propagation of bananas and plantains. Trop. Agric. 62:323-328.
[28]. Werner, E. M. and Boe, A. A. 1980. In vitro propagation of malling 7 apple root stock. HortScience. 15: 509-510.
[29]. Wong, W.C. 1986. In vitro propagation of banana (Musa spp.): Initiation, proliferation and development of shoot – tip cultures on defined media. pl. Cell Tiss. Org. Cult. 6:159-166.