Influence of Antioxidants andCytokininson *In Vitro*Plantlet Regeneration of Banana cv. Ney Poovan (AB)

ABSTRACT

Aims: Banana is one of the important fruit crop and food source for millionsof people in many countries.In South India, many varieties of banana are cultivated.*In vitro* micropropagation of banana is nowadays pinned towards thedevelopment of disease-free clones. An investigation was carried out in banana cv. Ney Poovan to study theInfluence of Antioxidantsand cytokininson in vitro plantlet regeneration.
Study design: The investigation was carried out in a completely randomized design with 25 treatments replicated thrice.
Place and Duration of Study: Tissue culture laboratory, Bio Centre, Department of Horticulture, Shivamogga 2023-2024.
Methodology: Tocontrol phenolic browning and regenerate multiple plantlets production from the shoot tip, the explants were well sterilized and transferred to culture media containing MS media in combination with antioxidants (Ascorbic acid and Polyvinylpyrrolidone) andcytokinins (6-Benzylaminopurine and Kinetin).
Results: Ascorbic acid 200 mg/l showed less browning across all combinations of BAP and kinetin. On an average the minimum number of days taken for shoot initiation (8.13 days), maximum number of shoots per explant (5.02), maximum shoot length (5.20 cm),and maximum success rate (73.33 %) were recorded in explants treated with (T_{12}) Ascorbic acid 200 mg/l + BAP 2.5 mg/l + Kinetin 2.0 mg/l.
Conclusion: Through this study, it is clear that shoot tip explant treated with MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l + BAP 2.5 mg/l + Kinetin 2.0 mg/lis best for controlling browning and *invitro* shoot regeneration for banana cv. Ney poovan.

Keywords:Micropropagation, Ney Poovan, Ascorbicacid, Polyvinylpyrrolidone (PVP), 6-Benzylaminopurine (BAP), Kinetin

1. INTRODUCTION

Banana (*Musa*spp. L.) is one of the most important cash crops and contributes immensely to global food security. It belongs to the family Musaceae and is distributed all around the world.Banana is considered as fourth most important food in the world after rice, wheat and maize. India occupies the third place in annual production of banana. It originated from the South east Asian region, where the greatest diversity of edible bananas is found [1]. Bananas yield fruit all year long. Of all the fruits, it is the most affordable, obtainableand healthy. Thespecies is typically identified by having a high fiber and carbohydrate content, low protein and no fat. It provides an abundant supply of potassium, as well as vitamins C, B_6 and A. Because of this, bananas and plantains are crucial to food security in the tropics and generate revenue for the farming community through both domestic and international trade.An 100 g unripe cooking banana has 111 kcal energy, 1.2 g fiber, 5.0 mg calcium, 0.5 mg iron, 400 mgpotassium, 116 µg beta-carotene equivalents, 0.04 mg riboflavin, 0.5 mg

niacin, 9.0 mg vitamin C and 0.3 mg vitamin E. In contrast, an 100 g ripe cooking banana has 265 kcal energy, 2.3 g fiber, 6.0 mg calcium, 0.8 mg iron, 610 mg potassium, 149 µg beta-carotene equivalents, 0.02 mg riboflavin, 0.6 mg niacin, 12.0 mg vitamin C and 2.2 mg vitamin E.

Banana and plantains are traditionally propagated through sword suckers. The conventional propagules are not the ideal planting materials because they carry insect pests and disease-causing pathogens. Micropropagation is an alternative and viable technology to produce and supply quality planting materials to the banana growing farmers without any pests and diseases. In terms of yield performance, tissue-cultured plants have been reported to produce 39% higher yield than plants from sword suckers [2].

Different attempts have been made to alleviate thebrowning problem,incorporation of antioxidants into the culture medium is one of them. Antioxidants play a vital role in the standardization of any micropropagation protocol to overcome thebrowning of theexplant.As an antioxidant, potassium citrate-citrate,activated charcoal, ascorbic acid, cystine, polyvinylpyrrolidone, and silver nitrate can be used [3]. Cytokinins comprise a separate class of growth promoters. They stimulate the synthesis of proteins and actively take part in cell cycle control. They are known to overcome apical dominance and proclaim lateral buds from dormant tissues. The cultivars have different proliferation rates which are significantly influenced by cytokinin type, concentration, and ploidy level of the banana cultivars**.** Kinetin (kin) and BAP (6-benzylaminopurine) are major cytokinins used in tissue culture.

One of the best diploid banana cultivars is Ney Poovan (AB), which is widely grown commercially as mono cultivation, particularly in Tamil Nadu and Karnataka.Fruits are small, slender, and attractive because of theirivory white pulp and bright yellow peel. Fruits are rich in carbohydrates, minerals, potassium, and other nutritional elements. The cultivar is known for its taste, aroma and long-keeping quality [4]. The plant is highly susceptible to panama wilt and leaf spot diseases [5]. Cultivars also have browning problem under *in vitro* regeneration which is due to theexudation of large amount of phenols Therefore, in the present situation, therequirement of large numbers of quality planting materials for local banana cultivars is needed. Therefore, to find out suitable ways for *in vitro* propagation of Ney Poovan, the objective of this study was to know the effect of different levels of antioxidants on browning of banana explant and to study the influence of Cytokinins on *in vitro* plantlet regeneration of Banana cv. Ney Poovan (AB).

2. EXPERIMENTAL DETAILS

The explants for the present experiment were collected from healthy and vigorously grown sword suckers of cv. 'Ney Poovan (AB group)' of 3 - 4 monthsold and in active growth phase, free from diseases and pests. The sword suckers were collected from afarmer's field, mundre, tarikere taluk, Chikmagalur, Karnataka.The study was conducted at the Tissue Culture laboratory, Bio Centre, Department of Horticulture, shivamogga from September 2023 to April 2024.The experiments were arranged in a completely randomized design (CRD) with 25 treatments each replicated thrice. For each replication, 5 explants were inoculated.

2.1 Sterilization of explant

After removing the outer sheaths of the suckers, they were cut into 5 to 10 cm of pieces. These suckers were kept under running tap water (for 30 mins.). The explants were soaked in Bavistin (2 g/l) solution overnight and then treated with 100 ml/l of sodium hypochlorite solution for 10 minutes and washed with distilled water. These explants were next sterilized under aseptic conditions in the laminar airflow chamber by washing them with sterile water along with two to three drops of Tween 20 emulsifier solution followed by citric acid + Ascorbic acid (100 mg/l + 150 mg/l) respectively for 10 min, also with mercuric

chloride solution (500 mg/l) for 10 min and then with Taxim solution 1 injection/l for 20 min, after each treatment explants were rinsed twice with sterile water so that no remaining chemical residue. After these treatments these explants were trimmed up to 3 to 5 cm before inoculation into MS media.

2.2 Culture media

For shoot initiation and control of explant browning,MS basal media along with activated charcoal 200 mg/l was supplemented with different combinations ofascorbic acid (150 and 200 mg/l) with BAP (2 and 2.5 mg/l) and kinetin (2 and 2.5 mg/l) (T_1-T_{14}) and polyvinylpyrrolidone (0.4 and 0.5 mg/l) with BAP (2 and 2.5 mg/l) and kinetin (2 and 2.5 mg/l) (T_{15}-T_{25}). For this study banana explants were subcultured after every twenty-one days into theinitiation stage (I_1 and I_2), division stage (D_1 and D_2), and multiplication stage (first multiplication cycle and second multiplication cycle).

2.3 Stock Solution and Media Preparation

The media namely MS [6] were prepared by dissolving the appropriate amount of macro and micro nutrients; and organic supplements. Similarly, growth regulators (BAP and Kinetin) stock solutions were prepared using the proportion of 20 mg: 20 ml and stored in the refrigerator at 4^{o}C. The MS culture media were prepared from their respective stock solutions using the appropriate amount of sucrose, plant growth regulators, activated charcoal and agar (7 g/l). Allindividuals and combinations of both growth regulators at two levels of BAP and two levels of kinetin were added separately to the media to study its effect on shoot regeneration. The bottles with media were then dispensed 35 ml each and autoclaved at 121°C for 25 minutes after adjusting the pH to 5.7 with1NNaOH and/or1NHCl.

2.4 Data Collection and Analysis

Fiveparameters *viz*., Degree of explant browning, Number of days taken for shoot initiation, Number of shoots per explant, Length of the shoots (cm) and Success rate (%)were recorded after specific intervals of time.Data were statistically analyzed using the analysis of variance (ANOVA) technique applicable to the completely randomized design. The significance of the treatment effect was determined using F-test; the means of the treatments were tested using the critical differences (CD) at the 1% probability level.

3. RESULTS AND DISCUSSION

3.1 Effectofantioxidantson the degree of explant browning

The effect of antioxidants at different concentrations on the degree of explant browning was observedvisually during callus initiation and division stages of banana explant. Minimum phenolic browning was noticed when shoot tip explants were incubated onto (T_6 to T_{13}) MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l across all combinations of BAP and kinetin (score +: minimum browning). Whereas, maximum phenolic browning was obtained when shoot tip explants were inoculated onto (T_{14} to T_{17}) MS basal medium + Activated charcoal 200 mg/l + PVP 0.4 mg/l and MS basal medium +

Activated charcoal 200 mg/l without antioxidants (control) (score + + +: maximum browning of the explants).Ascorbic acid gave better result in preventing browning due to its capacity to convert colorless o-quinones formed as a result of Polyphenol oxidases (PPO) activity back into diphenols.Ascorbic acid also have ability to directly inhibit the enzymatic browning process, scavenge free radicals and support overall cell metabolism makes it a more effective antioxidant in preventing browning in banana tissue culture compared to PVP. Similar results were observed in the findingsof Ndakidemi et al. [7]Nguyen et al. [8]and Strosse et al. [9]

Table.1. The effect of antioxidant and cytokininson browning of explant, shooting parameters and survival percentage in banana *cv.* Ney poovan

Treatments	Treatment details (mg/l)	Degree of explant browning	Days taken for shoot initiation	Number of shoots per explant	Length of the shoots (cm)	Success rate (%)
			Mean	Mean	Mean	
T_1	MS basal medium only (control)	+ + +	13.50	0.23	1.11	6.67 (8.86)
T_2	Ascorbic acid 150 + BAP 2.0 + Kinetin 2.0	+ +	9.99	3.16	3.90	26.67 (30.79)
T_3	Ascorbic acid 150 + BAP 2.0 + Kinetin 2.5	+ +	9.60	3.60	4.29	26.67 (30.79)
T_4	Ascorbic acid 150 + BAP 2.5 + Kinetin 2.0	+ +	9.39	4.66	4.89	33.33 (35.01)
T_5	Ascorbic acid 150 + BAP 2.5 + Kinetin 2.5	+ +	9.98	4.47	4.18	33.33 (35.01)
T_6	Ascorbic acid 200 + BAP 2.0	+	11.93	2.56	3.71	46.67 (43.08)
T_7	Ascorbic acid 200 + BAP 2.5	+	11.82	2.84	3.86	46.67 (42.70)
T_8	Ascorbic acid 200 + Kinetin 2.0	+	12.15	1.89	3.51	46.67 (43.08)
T_9	Ascorbic acid 200 + Kinetin 2.5	+	12.36	1.72	3.40	40.00 (39.23)
T_{10}	Ascorbic acid 200 + BAP 2.0 + Kinetin 2.0	+	8.78	3.94	4.68	46.67 (43.08)
T_{11}	Ascorbic acid 200 + BAP 2.0 + Kinetin 2.5	+	8.66	4.88	5.06	40.00 (39.23)
T_{12}	Ascorbic acid 200 + BAP 2.5 + Kinetin 2.0	+	8.13	5.02	5.20	73.33(59.21)
T_{13}	Ascorbic acid 200 + BAP 2.5 + Kinetin 2.5	+	9.04	4.79	4.92	46.67 (42.70)
T_{14}	PVP 0.4 + BAP 2.0 + Kinetin 2.0	+ + +	10.21	3.32	3.43	26.67 (30.79)
T_{15}	PVP 0.4 + BAP 2.0 + Kinetin 2.5	+ + +	10.00	3.55	3.61	20.00 (26.57)
T_{16}	PVP 0.4 + BAP 2.5 + Kinetin 2.0	+ + +	9.57	3.89	3.93	20.00 (26.57)
T_{17}	PVP 0.4 + BAP 2.5 + Kinetin 2.5	+ + +	10.38	3.92	3.29	26.67 (30.79)
T_{18}	PVP 0.5 + BAP 2.0	+ +	12.16	2.78	3.23	46.67 (43.08)
T_{19}	PVP 0.5 + BAP 2.5	+ +	11.82	2.94	3.35	33.33 (35.01)
T_{20}	PVP 0.5 + Kinetin 2.0	+ +	12.27	1.78	3.00	33.33 (35.01)
T_{21}	PVP 0.5 + Kinetin 2.5	+ +	12.24	1.34	2.93	26.67 (30.79)
T_{22}	PVP 0.5 + BAP 2.0 + Kinetin 2.0	+ +	10.40	3.55	3.73	26.67 (30.79)
T_{23}	PVP 0.5 + BAP 2.0 + Kinetin 2.5	+ +	10.17	3.50	3.81	33.33 (35.01)
T_{24}	PVP 0.5 + BAP 2.5 + Kinetin 2.0	+ +	10.33	3.93	4.37	20.00 (26.57)
T_{25}	PVP 0.5 + BAP 2.5 + Kinetin 2.5	+ +	10.60	3.51	3.75	33.33 (35.01)
	S.Em.±	-	0.17	0.06	0.06	4.47
	C.D. at 1%	-	0.65	0.22	0.21	16.93
	C.V	-	2.80	3.07	2.51	-

Note: MS basal medium + Activated charcoal 200 mg/l is common in all treatments, The values given in parenthesis are arc sine transformed values, BAP- 6- Benzylaminopurine, PVP- Polyvinylpyrrolidone

+ - minimum browning, + + - medium browning, + + + - maximum browning

3.2 Effectofantioxidants and cytokininsonshooting parameters of banana cv. Ney Poovan

Days taken for shoot initiation

After completion of thesecond multiplication stage, on an average, the days taken for shoot initiation varied significantly (Table1). The minimum average number of days for shoot initiation (8.13 days) was recorded in (T_{12}) MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l + BAP 2.5 mg/l + Kinetin 2.0 mg/l, which was on par with (T_{11}) MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l + BAP 2.0 mg/l + Kinetin 2.5 mg/l (8.66 days) and (T_{10}) MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l + BAP 2.0 mg/l + Kinetin 2.0 mg/l (8.78 days). The various combinations of BAP and kinetin were helpful for theinitiation and regeneration of plants, but the difference in the results may be due to the endogenic levels of cytokinin. These results also support the findings of Zaffari et al. [10] showed that BAP used in combination with kinetin, the rate of shoot multiplication recorded higher, Shukla et al. [11] recorded highest number of shoots/explant (5.2 shoots) in Cavendish dwarf variety when inoculated on MS medium fortified with combination of BAP and kinetin at a concentration of 2mg/l and 1mg/l and Azam et al. [12] reported that the highest number of shoots (5.2 per explants) in cultivar 'BARI-1'was found on the MS medium supplied with combination of 2 mg/l BAP with 1mg/l kinetin.

The number of shoots per explant

The number of shoots per explant differed significantly among different treatments. On an average, after second multiplication stage the maximum number of shoots per explant (5.02) was observed in (T_{12}) MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l + BAP 2.5 mg/l + Kinetin 2.0 mg/l, followed by (T_{11}) MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l + BAP 2.0 mg/l + Kinetin 2.5 mg/l (4.88). The combination of BAP and kinetin gave better result in shoot proliferation than individual effect of both BAP and kinetin. BAP and kinetin in combination are generally known to reduce the apical dominance and induce both axillary and adventitious shoot formation from shoot tip explants in banana system Jafari et al. [13].

Length of the shoots (cm)

Results on average length of the shoots varied significantly among different treatments. The maximum shoot length (5.20 cm) was observed in (T_{12}) MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l + BAP 2.5 mg/l + Kinetin 2.0 mg/l, which was on par with (T_{11}) MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200 mg/l + BAP 2.0 mg/l + Kinetin 2.5 mg/l (5.06 cm). Adding ascorbic acid 200 mg/l to the culture media not only helped to reduce polyphenolic oxidation but also greatly increased the number of shoots produced and shoot length. Ascorbic acid can enhance the activity of cytokinins by maintaining a favorable redox state, which may improve the signaling pathways involved in shoot induction and multiplication Smirnoff [14]. Correspondingly positive outcomes were noted in the studies of Habiba et al. [15] and Sunitha [16].

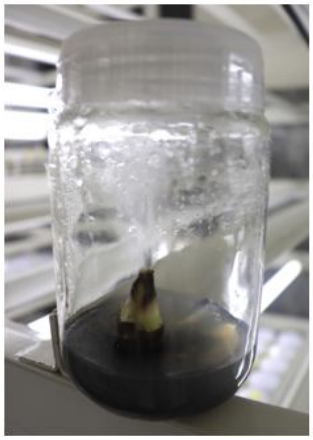
Success rate (%)

From the study, we observed that significantly maximum success rate in micropropagation of banana cv. Ney Poovan (73.33 %) was recorded in T_{12} where explants were treated with MS basal medium + Activated charcoal 200 mg/l + Ascorbic acid 200

mg/l + BAP 2.5 mg/l + Kinetin 2.0 mg/l. This is because of overall reduction in contamination and browning of explants observed in this treatment.

Fig. 1. Effect of antioxidants on explant browning of banana cv. Ney Poovan

T_{12} - Minimum browning



T_{19} - Medium browning

T_1 - Maximum browning

Fig. 2. Multiple shoots produced from explant cultured on MS medium supplemented with Ascorbic acid 200 mg/l + BAP 2.5 mg/l + Kinetin 2.0 mg/l

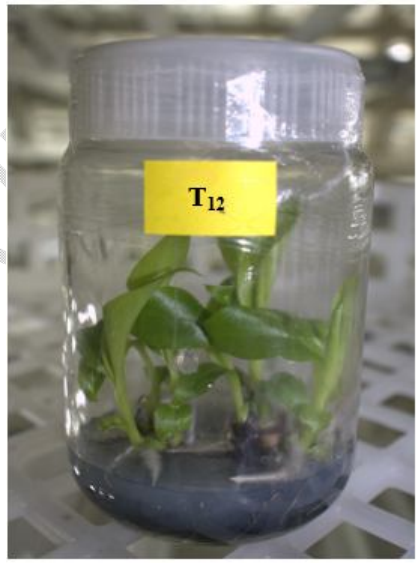
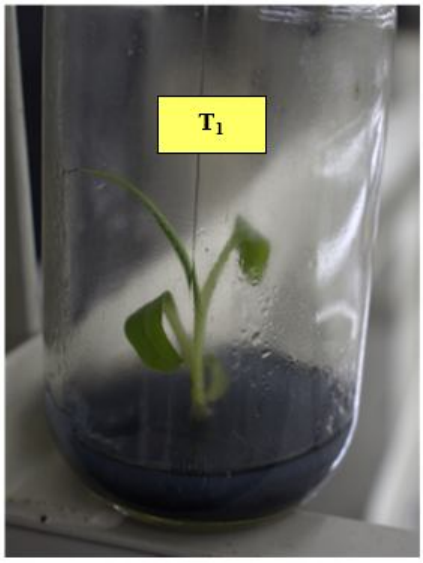


Fig. 3. Shoots produced from explant cultured on MS medium supplemented without any antioxidant and cytokinin



4. CONCLUSION

This study concludes that *in vitro* culture is the best method for the propagation of banana. Through, this study it is confirmedthat basal medium (MS) supplementedwith activated charcoal 200mg/l + Ascorbic acid 200 mg/l + BAP 2.5 mg/l + kinetin 2.0 mg/lis best for phenolic browning reduction, multiple shoot generation and growth. Thus, this researchrevealed important information related to producingdisease-free plantlets by *In-vitro* micropropagation of bananacultivar Ney Poovan which is in demand.

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Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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