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Orignal Article

OPTIMIZING BANANA MICROPROPAGATION STRATEGIES THROUGH THE IMPLEMENTATION OF SHOOT TIP CULTURE FOR DISEASE-FREE CULTIVATION OF BANANA CULTIVAR KABRI

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Abstract: ***Tissue culture techniques have revolutionized the field of plant regeneration, with in vitro multiplication through shoot tip culture emerging as a pivotal method for generating pathogen-free planting materials. This technique is of paramount importance in mitigating field inoculum sources and rejuvenating disease-infested areas. This research explores recent developments in shoot tip culture techniques for Musa spp. cultivar Kabri, employing specific formulations of plant growth regulators, including cytokinins (BAP, Kin, 2ip) and auxins (NAA, IAA, IBA). This study investigates key aspects of banana micropropagation, with a focus on sterilization, in vitro shoot tip culture, and root formation and transfer in the field. Surface sterilization, crucial for eliminating contaminants, faces challenges, with ongoing research refining protocols to minimize risks. Evaluating survivability and contamination, the study found optimal results with 70% ethyl alcohol and 0.01% $HgCl_2$ for 25 seconds and 10 minutes. In in vitro shoot tip culture, formulations involving BAP, Kin, 2iP, and Ads were explored. Among the hormones used, the combination of 3.0 mg/l BAP and 2.0 mg/l Ads demonstrated the best results with a 60% success rate for shoot initiation. This formulation resulted in an average of 2.65 ± 0.21 shoots per explant and a shoot length of 2.40 ± 0.42 cm. Root formation experiments revealed that 2.0 mg/l IAA induced an 81% root induction rate, with an average of 4.80 roots/explant and a length of 4.20 cm. Conversely, 2.0 mg/l IBA exhibited a superior 95% root induction rate, producing an average of 5.25 roots/explant with a length of 4.78 cm. Plantlets that had well-developed roots were effectively transferred to the field after undergoing comprehensive acclimatization procedures, achieving a 90% survival rate. These advancements carry significant implications for sustainable banana cultivation practices, providing researchers and practitioners with valuable tools for optimizing banana yields while ensuring pathogen-free crops.***

Keywords*:* Banana cultivation, Shoot tip culture

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INTRODUCTION

Banana (*Musa paradisiaca*, family Musaceae) is a tropical and subtropical fruit crop cultivated on approximately 8.8 million hectares globally [1]. Bangladesh produced 826,151.76 metric tons of bananas in 2020-2021, cultivated across around 122,192.19 acres [2]. The average banana yield in Bangladesh is approximately 16 tons per hectare, considerably lower than the global average of nearly 50 tons per hectare. Despite challenges such as underweight, stunting, and wasting, bananas play a vital role in rural landscapes, contributing to 40.7% of the total fruit production in the country [3]. Bananas are highly nutritious, as highlighted by Sharrock and Lustry [4], and boast easier digestibility compared to various fruits, including apples [1]. Bananas are an abundant source of carbohydrate, with 67 calories per 100g fruit, and are one of the most popular and frequently traded fruits worldwide (5,6). The cultivation of bananas is a year-round practice across the entirety of Bang-ladesh. Numerous studies have been undertaken to assess Bangladesh's banana crop [7-14].

Banana and plantain (*Musa spp*.) cultivation holds significant socio-economic importance in Bangladesh, deeply ingrained in the country's cultural heritage. In North America and Europe, Musa fruits are categorized into 'bananas' and 'plantains' based on their culinary use. These fruits have played pivotal roles throughout human civilization history.

The term 'plantain' now commonly refers to starchy cooking bananas, distinguished by their green or red skins. While sweet bananas are easily digested when consumed as fruit, plantains require boiling, steaming, roasting, or deep frying to become soft and palatable. In Bangladesh, approximately 23 banana cultivars are grown, each with regional preferences [8].

Amritsagar, Champa, Chini Champa, Kabri, Nepali, and Sabri stand as the commercially cultivated varieties in Bangladesh. Particularly celebrated for its outstanding sweetness and resistance to diseases, Kabri emerges as a prominent cultivar extensively cultivated in the nation.

Banana (*Musa spp*.) serves as a staple food crop in tropical and subtropical regions, offering essential nutrition and income. Efficient propagation methods are crucial to meet the increasing demand for this economically significant crop [15].

While sexual reproduction through seeds is natural for many plants, it presents challenges for cultivated banana varieties due to triploid sterility, limited seed viability, and the need for genetic uniformity. Alternative methods, such as tissue culture and vegetative propagation (suckers), are preferred for banana reproduction, allowing for disease-free, genetically uniform, and rapidly multiplying plants [16]. Suckers, as vegetative propagules, can carry diseases from the parent plant to the new generation, posing risks to the entire plantation.

Micropropagation, involving aseptic culture, has emerged as a valuable tool for mass-producing high-quality banana plants. It enables rapid multiplication of elite cultivars, providing disease-free and genetically identical plants [17]. Micropropagation addresses challenges posed by pathogens like Panama disease and Banana Bunchy Top Virus, ensuring disease-free planting material and contributing to sustainable disease management.

Studies, such as those by Tushemereirwe et al. [18] and Daniells et al. [16], have underscored the effectiveness of meristem culture in eliminating viruses, ensuring disease-free planting material. Micropropagation allows for year-round banana plant production, overcoming seasonal limitations. It contributes to the conservation of genetic integrity, preserving desirable traits in cloned plants and facilitating the perpetuation of superior genotypes [19]. Tissue culture ensures genetic uniformity among the propagated plants since they are derived from a single parent plant, beneficial for maintaining desirable traits and characteristics [20].

Efforts are ongoing to refine protocols, minimize explant contamination, and identify effective sterilization techniques in banana micropropagation. Contamination remains a challenge, prompting research into improved sterilization techniques and culture conditions [21,22].

MATERIALS AND METHODS

The experiment was conducted at the Plant Biotechnology and Genetic Engineering Division, Institute of Food & Radiation Biology, Atomic Energy Research Establishment in Savar, Dhaka. Sword suckers were collected from healthy field-

grown plants, washed thoroughly, and then surface-sterilized using liquid detergent, tween-80, ethyl alcohol, and mercuric chloride. Inoculation of explants, specifically the white shoot tips, was performed under aseptic conditions in a laminar airflow cabinet. The inoculated explants were placed on culture media and incubated at optimal conditions of 25 ± 2 ºC, 80-98% humidity, and 60 µE/m^2s^1 light intensity. Subculturing was conducted after two weeks, involving the transfer of greenish shoots to fresh media for further multiplication. Multiple subcultures were necessary to overcome the inhibitory effect of blackish-colored phenolic substances secreted by the shoot tips.

The multiplied shoots were subsequently transferred to rooting media supplemented with half-strength MS [23] media. After a period of 35 to 40 days, the transplants were subjected to a hardening process before being transferred into the soil.

Regenerated plantlets, having developed a robust root system, were carefully removed from culture vessels. The roots were washed, and the plantlets were transferred to polythene bags with a soil-sand-compost mixture. The bags were initially covered to maintain high humidity, gradually perforated over seven days, and then completely removed as the plantlets became established in the soil, eventually being transferred to the experimental field.

Collection of Data: Data were gathered, encompassing parameters and methods such as the percentage of shoot and root response, the count of shoots and roots per explant, and the length of shoots and roots. Each recorded value represents the mean across a sample size of 20 plants.

Data analysis: The data obtained from this study underwent rigorous statistical analysis. One-way analysis of variance (ANOVA) was employed to assess the variation between different treatments. Significance between any two means was determined at a chosen probability level of $p = 0.05$, using the Duncan's Multiple Range Test (DMRT). The statistical software SPSS version 15.0 was utilized for these analysis.

RESULTS AND DISCUSSION

Sterilization: Surface sterilization is a critical step in banana micropropagation, aiming to eliminate contaminants and ensure the establishment of healthy cultures. However, challenges associated with this process have been reported in the literature. Overcoming sterilization challenges in micropropagation involves meticulous attention to various factors, including the choice of sterilizing agents, concentration, and duration of exposure. Several strategies have been proposed in the literature to improve the effectiveness of sterilization protocols.

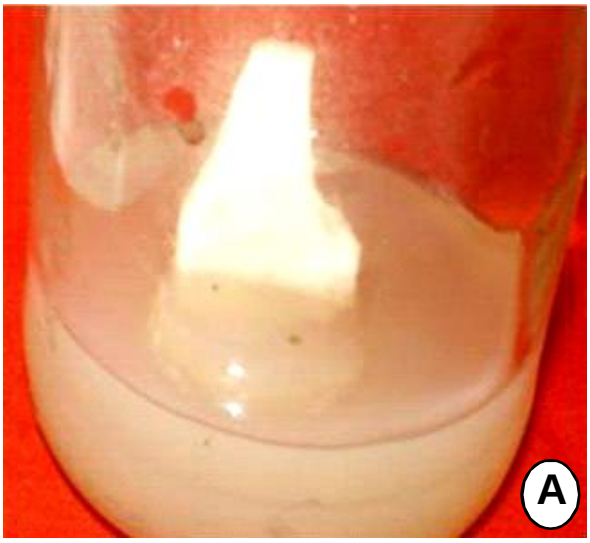
One common issue is the susceptibility of banana explants to contamination despite sterilization efforts. The choice of sterilizing agents, concentration, and duration of exposure can significantly impact the success of the process [24]. Ongoing research focuses on refining sterilization techniques to minimize contamination risks in banana micropropagation [21,22]

The survival rate and contamination levels were evaluated in surface sterilization attempts employing 70% ethyl alcohol followed by 0.01% $HgCl_2$. Optimal survival and minimal contamination were observed at durations of 25 seconds and 10 minutes, and 30 seconds and 10 minutes, respectively (Table-1).

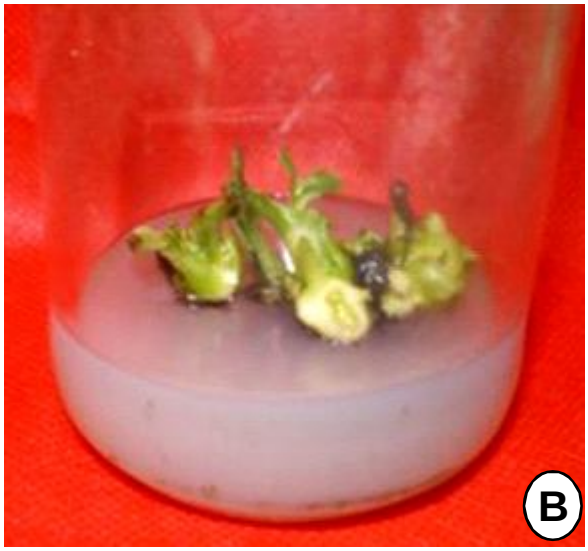
***In vitro* Shoot tip culture:** For establishing *in vitro* regeneration of Musa spp., shoot tip explants were cultured on semisolid MS media. The media, containing various plant growth regulators and additives, facilitated shoot-bud proliferation. Banana tissue culture often faces blackening issues due to oxidation of polyphenolic compounds, but the addition of ascorbic acid alleviates this problem. Subsequent subcultures after 4 to 6 cycles help reduce blackening.

The tissue culture technique involves establishing different plant cell or tissue cultures under suitable conditions for proliferation and plant regeneration. *In vitro* multiplication through shoot tip culture is valuable for producing large quantities of pathogen-free planting materials, reducing field inoculum sources, and rehabilitating infected areas. The shoot tip culture technique for Musa, developed over the last 35 years, is well-established and essential for international Musa breeding programs [25,26].

In the present study the combination of 3.0 mg/l BAP and 0.4 mg/l Kinetin, resulting in a 50% success rate with an average of 1.70 ± 0.34 shoots per explant and a shoot length of 3.10 ± 0.27 cm. In the presence



A: Inoculation of explant



B: Shoot initiation

C: Shoot multiplication

D: Transfer in rooting media

E: Initiation of root

F: Plantlets with root

G: Plants in polybag (Acclimatization)

H: Plants in the field

I: Established plant in the field

of 3.0 mg/l BAP and 0.4 mg/l 2iP, the shoot initiation achieved a 50% success rate, with an average of 1.32 ± 0.33 shoots per explant and a shoot length of 2.55 ± 0.28 cm. Among the hormones used, the combination of 3.0 mg/l BAP and 2.0 mg/l Ads demonstrated the best results with a 60% success rate for shoot initiation. This formulation resulted in an average of 2.65 ± 0.21 shoots per explant and a shoot length of 2.40 ± 0.42 cm (Table 2, Figs. A-C).

Various studies have highlighted the significance of specific cytokinin and auxin combinations in promoting shoot initiation in banana micropropagation [27-29]. Similar to the observed variances in responsiveness among banana cultivars in your study, previous research has noted cultivar-specific differences in tissue culture responses [30]. Researchers have found that adjuvants (Ads) play a crucial role in promoting shoot multiplication in banana genotypes, emphasizing the importance of media composition [31].

Shoot tip culture techniques for Musa, utilizing specific growth regulator combinations, offer a reliable method for *in vitro* proliferation, shoot bud induction, contributing to the production of pathogen-free planting materials [30,32-35].

Root formation: The application of 2.0 mg/l IAA resulted in an 81% root induction rate, with an average of 4.80 roots per explant and an average root length of 4.20 cm. Conversely, the use of 2.0 mg/l IBA demonstrated a superior root induction rate of 95%, accompanied by an increased average of 5.25 roots per explant. The average length of roots in this case was 4.78 cm. The treatment with 1.5 mg/L NAA yielded an 82% root induction rate, with an average of 3.88 roots per explant and an average root length of 3.70 cm. Comparatively, the treatment employing 2.0 mg/l IBA showcased the highest root induction rate (95%) and the greatest average number of roots (5.25 cm), while the 1.5 mg/L NAA

Figs. A to I are different phases of *in vitro* shoot tip culture of Banana Cultivar Kabri

Table-1: The survival rate of explants and the occurrence of contamination during sterilization using 70% Ethyl alcohol followed by 0.1% $HgCl_2$.

Sterilizing agent	Duration	Survival %	Contamination%
70% Ethyl alcohol followed by 0.1% $HgCl_2$	15 seconds and 05 minutes	80	85
	20 seconds and 05 minutes	76	80
	25 seconds and 05 minutes	68	75
	30 seconds and 05 minutes	63	70
	15 seconds and 10 minutes	60	62
	20 seconds and 10 minutes	55	50
	25 seconds and 10 minutes	52	35
	30 seconds and 10 minutes	50	25
	25 seconds and 15 minutes	40	15
	30 seconds and 15 minutes	25	05

Table 2: Effect of different combinations and concentrations of hormones with MS media on *in vitro* shoot tip culture of banana cultivar Kabri (AAB) at 40 days after inoculation.

Hormone combination	Concentrations of BAP + Kin (mg/l)	% shoot tip responded to shoot induction	No. of shoots/explant Mean ± SE	Length of Shoots (cm) Mean ± SE
BAP and Kin	1.5 + 0.1	0	0	0
	2.0 + 0.2	25±0.43[a]	1.42 ± 0.02[a]	1.61 ± 0.03[a]
	2.5 + 0.3	38±0.86[b]	1.65 ± 0.06[c]	1.72 ± 0.05[b]
	3.0 + 0.4	50±1.02[c]	1.70 ± 0.08[d]	3.10 ± 0.07[d]
	3.5 + 0.5	48±1.24[c]	1.57 ± 0.12[b]	2.10 ± 0.10[c]
BAP and 2iP				
	1.5 + 0.1	0	0	0
	2.0 + 0.2	25±0.52[a]	1.00 ± 0.03[a]	1.40 ± 0.07[a]
	2.5 + 0.3	38±0.78[b]	1.21 ± 0.07[b]	1.43 ± 0.05[a]
	3.0 + 0.4	50±0.63[c]	1.32 ± 0.06[c]	2.55 ± 0.08[c]
	3.5 + 0.5	48±0.86[c]	1.24 ± 0.09[b]	2.14 ± 0.11[b]
	1.5 + 50	32±0.67[a]	1.10 ± 0.03[a]	1.62 ± 0.04[a]
BAP and Ads	2.0 + 100	40±0.91[b]	2.48 ± 0.09[b]	1.75 ± 0.07[ab]
	2.5 + 150	54±0.69[c]	2.60 ± 0.10[c]	1.88 ± 0.09[ab]
	3.0 + 200	60±1.23[d]	2.65 ± 0.14[c]	2.40 ± 0.12c
	3.5 + 250	65±1.06[e]	5.66 ± 0.17[d]	3.43 ± 0.16d

Values with identical letters within the same column indicate no significant differences at a p ? 0.05 according to the Duncan's Multiple Range Test (DMRT).

treatment exhibited a slightly lower root induction rate (82%) and fewer roots (3.88 cm). The specific selection of a plant growth regulator emerges as a critical factor influencing root development in banana tissue culture (Table 3, Figs. D-F).

The highest percentage (100 %) of root induction was recorded by Rahman et al. [28] in 1.0 mg/l NAA. Raut and Lokhande [36], Khanam et al. [37] and Dore Swamy et al. [38] utilized MS medium with varying concentrations of IBA to enhance banana rooting. Conversely, Bhaskar et al. [39] identified optimal *in vitro* rooting on Knudson's medium with a 5 mg/l NAA supplement. The outcomes related to the impact of NAA in this study align with the observations of Raut and Lokhande [36] and Habib [40]. Some investigators used IBA and IAA for root induction in banana and got satisfactory results. Habiba et al. [41] regenerated roots on half strength MS media having 1 and 2 mg/l IBA respectively. According to all investigators and the results of this study.

Transplantation: Established plantlets with well-developed roots were successfully transferred to the field following thorough acclimatization procedures. All *in vitro* propagated plantlets exhibited 90%

Table-3: Effect of different combinations and concentrations of hormones with ½ strength MS media on *in vitro* root initiation, no. of root per explants and length of root of banana cultivar Kabri (AAB) at 35 days after inoculation. Values with identical letters within the same column indicate no significant differences at a p Ã 0.05 according to the Duncan's Multiple Range Test (DMRT).

Name of Hormones	Concentrations of hormones (mg/l)	% shoot responded to root induction	No. of root/ explants Mean ± SE	Length of root (cm) Mean ± SE
	0.5	51± 0.43^{a}	3.50 ± 0.03^{a}	2.67 ± 0.09^{a}
	1.0	60± 0.64^{b}	3.89 ± 0.07^{b}	2.81 ± 0.18^{b}
IAA	1.5	68± 0.77^{c}	4.52 ± 0.09^{c}	3.12 ± 0.17^{c}
	2.0	81± 1.24^{d}	4.80 ± 0.16^{d}	4.20 ± 0.13^{d}
	2.5	71± 0.94^{c}	3.54 ± 0.12a	2.98 ± 0.09^{b}
	0.5	77± 0.63^{a}	3.15 ± 0.08^{a}	3.27 ± 0.04^{a}
	1.0	84± 0.88^{b}	3.60 ± 0.06^{b}	3.52 ± 0.06^{b}
IBA	1.5	90± 1.14^{c}	4.27 ± 0.10^{c}	3.96 ± 0.10^{c}
	2.0	95± 1.10^{c}	5.25 ± 0.19^{e}	4.78 ± 0.16^{e}
	2.5	83± 0.98^{b}	4.88 ± 0.16^{d}	4.44 ± 0.09^{d}
	0.5	59± 0.60^{a}	2.60 ± 0.02^{a}	2.82 ± 0.07^{a}
	1.0	65± 1.02^{b}	3.10 ± 0.05^{b}	2.98 ± 0.06^{a}
NAA	1.5	82± 1.06^{c}	3.88 ± 0.09^{d}	3.70 ± 0.09^{c}
	2.0	80± 0.98^{c}	3.60 ± 0.10^{c}	3.52 ± 0.10^{c}
	2.5	78± 0.83^{c}	3.25 ± 0.09^{b}	3.23 ± 0.98^{b}

survival when transferred from pots to the field. These findings align with the results reported by Cronauer and Krikorian [27], who successfully established *in vitro* rooted banana shoots in pots (Figs. G-I).

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