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EFFICIENT MICROPROPAGATION TECHNIQUES FOR 'GRAND NAINE' BANANAS

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ABSTRACT :

Bananas are famous as the "Apple of Paradise" and form a very vital crop worldwide. The conventionally adopted methods of propagation are linked with problems like slow growth and susceptibility to diseases. The present study determined the optimization of micro-propagation techniques for 'Grand Naine' (G9) banana cultivar. The in vitro study was conducted in Guru Kashi University from 2023-2024. In the present study, meristems were used as explants, and various media formulations were evaluated for improving propagation efficiency. The results indicated that T1 Gamborg B5, T3 Linsmaier & Skoog, T4 Nitsch, T6 Woody Plant, and T7 MS Modified media formulations induced the best response in explants, and the highest response rates obtained in T1, T3, T4, T6, and T7 were 95%, 85%, 78%, 90%, and 88%, respectively. It was also found to bring about shoot and root induction at a faster rate; among them, the fastest induction times were recorded for T1, T3, T4, T6, and T7. This research has consequently opened up the possibility that optimized tissue culture protocols hold for banana production in terms of rapid, disease-free, and genetically uniform planting material.

Keywords:- Banana, Micropropagation, Grand Naine, Tissue Culture, Explant Response

INTRODUCTION :

So-called "Apple of Paradise," the banana is taken as a great delicacy not only for its great taste but also for its nutritional value. This fruit, belonging to the genus Musa in the family of Musaceae, hails from the lush regions of Southeast Asia, specifically the jungles of Malaysia, Indonesia, and the Philippines. Bananas are more than 70 species in the family of Musaceae. The banana fruit is actually a berry with an edible mesocarp and a deliciously soft and seedless pericarp resulting from parthenocarpy and a triploid chromosome level generally represented as 2n=3x=33. Adam's Fig, more popularly known as bananas, are herbaceous plants with a rhizome-like underground corm and an above-ground pseudostem. In fact, banana plants are among the fastest-growing plants.

Banana cultivation is practiced all over the world, spreading over more than 110 countries and producing about 85 million tonnes annually. The country leading in its production is India, followed by Brazil, Ecuador, and China. In India alone, it is grown on approximately 490000 hectares, with an average yield of about 16.9 million tonnes. Maharashtra and Tamil Nadu are among the top states for banana production. Bananas thrive well under agroclimatic conditions characterized by temperatures ranging between 15-35°C and relative humidity of about 80%. They grow at altitudes up to 2000 meters above sea level.

Conventional banana propagation uses suckers, corms, and rhizomes. Among the many setbacks experienced by these techniques are that they are slow-growing, very susceptible to diseases, and offer limited supplies of disease-free planting materials. On the other hand, conventional techniques cannot guarantee genetic uniformity and can be quite labor-intensive. With tissue culture, otherwise called micropropagation, has radically changed the way banana propagation is done by offering a fast, efficient, and scaled way of the same. Tissue culture is a process for growing plant cells, tissues, or organs on artificial medium in an aseptic environment to obtain large quantities of disease-free and genetically uniform planting material within a comparatively short period. According to Loyola-Vargas et al. (2018), the innovation responds to a propagated challenge for improved methods.

Banana tissue culture is important in research because it offers the avenues for the rapid multiplication of planting material that is free of diseases and support for the improvement and breeding programs. This will help to conserve and preserve genetic diversity for maintaining biodiversity and resilience in banana cultivars. Recent advances in tissue culture techniques have brought about quantum jumps in multiplication rate, genetic stability, and plantlet quality. Research has proved tissue culture to be an effective means of banana propagation, with tremendous improvements recorded regarding this aspect. Other studies on the technique have been done on the preservation of endangered banana species and the production of transgenic plants.

MATERIALS AND METHODS :

The present investigation, titled “Efficient Micropropagation Techniques for ‘Grand Naine’ Bananas” was conducted during the years 2023-2024 at the Faculty of Agriculture, Guru Kashi University, Talwandi Sabo. The experiments were carried out in the Biotechnology Lab at the Faculty of Agriculture, Guru Kashi University, situated at 29° 59'18" N latitude and 75°04'43" E longitude. Disease-free, healthy explants of the banana variety "Grand Naine

(G9)" were sourced from the Horticulture experimental field of Guru Kashi University, Talwandi Sabo. Meristems were used as explants for micropropagation. High-purity chemicals, including inorganic salts, vitamins, sucrose, agar, myo-inositol, chelating agents, and growth regulators, were procured from Hi-Media Co. (India) and other companies. Antibiotics and antifungals like streptomycin, streptocyclin, and bavistin were used in the experiments.

The glassware was initially washed with Labolene and a few Milliliters of running tap water. Then, chromic acid solution was used to soak the glassware for 24 hours. After that, it was cleaned thoroughly under running tap water followed by washing with double-distilled water, dried on the draining racks. The test borosilicate glassware is of the Borosil brand. The conical flasks used are of different sizes to study the response of explants against various levels of growth regulators. Other equipment used were Erlenmeyer flasks, beakers, micropipettes, forceps, scalpels, spatulas, sterile blades, jam jars and test tubes.

Culture vessels and instrument sterilization methods included autoclaving appliances, which comprised cotton-plugged test tubes, flasks, culture bottles, jam jars, forceps, and Petri dishes at 15 psi pressure and 121°C for 20 minutes, followed by drying in a hot air oven at 80-100°C for 2-4 hours. Sterilization of instruments included dipping in ethanol, flaming, and exposure to UV in a laminar flow hood. The ethanol-sterilized working surface and aseptic manipulations under laminar airflow were set up. The media were prepared by mixing the required amount of solutions in 500 ml of distilled water with continuous stirring. Sucrose was added at 3% and agar at 0.8%. The pH was adjusted to 5.8 using 1N NaOH and 1N HCl on a pH meter. Such media were thoroughly boiled, stirred to avoid agar clumps, cooled briefly, and then poured into sterilized bottles. Sterilization of media in an autoclave was done at 121°C and 15 psi for 20 minutes. The media were stored at room temperature for use within a week.

Explants were collected from healthy plants in the experimental field of the Department of Agriculture, Guru Kashi University, Talwandi Sabo. Meristems were extracted from the mother plant and placed in jars filled with distilled water to minimize contamination. Explants were washed under running tap water, leaves were removed with a scalpel, and uniform-sized meristems (1-1.5 cm) were prepared. Surface sterilization of explants involved testing various sterilizing agents—ethanol, Bavistin, streptomycin, mercuric chloride ($HgCl_2$), and citric acid—at different concentrations and exposure times to deactivate surface microbes and reduce contamination rates.

Inoculation and incubation of culture were conducted under aseptic conditions in a laminar airflow cabinet. The cabinet was thoroughly cleaned with ethanol, and equipment was sterilized by immersion in 70% ethanol and flaming. Surface-sterilized explants were transferred from beakers to sterile petri dishes using sterilized forceps, then inoculated into vessels containing the medium. After inoculation, culture jars and test tubes were placed in a culture room maintained at 25±2°C for germination, with a photoperiod of 16 hours of light (2000-3000 lux) followed by 8 hours of darkness.

Different media used in the micropropagation process included Gamborg (B5), White, Linsmaier & Skoog (LS), Nitsch, CHU n6, Woody Plant, MS Modified, and Schenk & Hildebrandt (SH) Media, each with specific compositions to support various stages of banana plant development. Observations recorded included the percentage survival of explants, the response of explants to culture media, days to shoot initiation, the number of leaves per explant, shoot length, days to root induction, number of roots per explant, and root length. This comprehensive methodology aimed to optimize micropropagation techniques and hardening strategies for banana cultivar 'Grand Naine (G9),' ultimately contributing to advancements in banana cultivation and agricultural sustainability.

Treatments

Original Media Name	Assigned Treatment Name
Gamborg (B5) Media	T1
White Media	T2
Linsmaier & Skoog (LS) Media	T3
Nitsch Media	T4
CHU n6 Media	T5
Woody Plant Media	T6
MS Modified Media	T7
Schenk & Hildebrandt (SH) Media	T8

RESULTS AND DISCUSSIONS :

The response of banana explants to different treatments, including the number of days taken for shoot and root induction, provides valuable insights into the efficiency and effectiveness of tissue culture protocols for the Cultivar G9 banana. Here is a summary of the results and discussion based on the experiment data.

Explant Response

The explant response varied among different treatments:

- Highest Response Rates: T1 (95%), T6 (90%), and T3 (85%). These media formulations may provide optimal conditions for the initiation and proliferation of banana explants.
- Moderate Response Rates: T7 (88%), T4 (78%), T5 (75%), and T8 (70%), suggesting moderate success in promoting explant response.
- Lowest Response Rate: T2 (60%), indicating that its composition or nutrient content may be less favorable for explant growth and development.

Days Taken for Shoot Induction

The number of days taken for shoot induction differed among the treatments, ranging from 3 to 7 days:

- Fastest Shoot Induction: T1, T3, T6, and T7 required only 3 days.

- Moderate Shoot Induction: T4 and T8 required 4 days.
- Slower Shoot Induction: T5 required 6 days, while T2 took the longest at 7 days.

Days Taken for Root Induction

The number of days taken for root induction also varied, with durations ranging from 5 to 10 days:

- Fastest Root Induction: T4 and T6 required 5 days, followed closely by T1, T3, and T7 (5 days).
- Moderate Root Induction: T8 required 7 days.
- Slowest Root Induction: T5 required 8 days, while T2 took the longest at 10 days.

Table 1: Explant response, days taken for shoot initiation and root initiation

Treatments	Explant response	No. Of days taken for shoot induction	No. Of days taken for root induction
T1	95	3±1	5±1
T2	60	7±1	10±1
T3	85	4±1	6±1
T4	78	4±1	5±1
T5	75	6±1	8±1
T6	90	3±1	5±1
T7	88	3±1	5±1
T8	70	4±1	7±1

Number of Leaves per Explant at 7th, 14th, and 21st Day

The evaluation of banana explant growth, including the number of leaves per explant at different time points, revealed the following:

- At 7 Days: Higher leaf numbers were observed in T1, T3, T4, and T6 (2 to 3 leaves), while T2, T5, T7, and T8 had fewer leaves (1 to 2 leaves).
- At 14 Days: All treatments showed an increase, with T1, T3, T4, T6, and T7 averaging 4 to 5 leaves. T2, T5, and T8 averaged 2 to 3 leaves.
- At 21 Days: Further increases were seen, with T1, T3, T4, T6, and T7 averaging 5 to 6 leaves. T2, T5, and T8 averaged 3 leaves.

Table 2: Average No. Of leaves per explant at 7th, 14th, and 21st day.

Treatments	At 7 days	14 days	21 days
T1	3 ±1	5 ±1	6 ±1
T2	1 ±1	2 ±1	2 ±1
T3	2 ±1	4 ±1	5 ±1
T4	2 ±1	4 ±1	4 ±1
T5	1 ±1	3 ±1	3 ±1
T6	3 ±1	4 ±1	5 ±1
T7	3 ±1	4 ±1	5 ±1
T8	2 ±1	3 ±1	3 ±1

Length of Shoots per Explant at 7th, 14th, and 21st Day

The length of shoots per explant varied across treatments:

- At 7 Days: T1, T3, T4, and T6 exhibited longer shoots (2.5 to 4.5 cm). T7 had the longest shoots (4 cm).
- At 14 Days: T1, T3, T4, T6, and T7 maintained longer shoots (4 to 6 cm).
- At 21 Days: T1, T3, T4, T6, and T7 continued to show longer shoots (5 to 6.5 cm).

Table 3: Average length of shoots per explant at 7th, 14th, and 21st day.

Treatments	At 7 days	14 days	21 days
T1	4.5	6	6.5
T2	1.5	3	4
T3	2.5	5	6
T4	2	4	5.5
T5	2	4	5
T6	3	4.5	5.3
T7	4	5.5	6.5
T8	1.8	2.5	4

Number of Roots per Explant at 7th, 14th, and 21st Day

The number of roots per explant varied significantly:

- At 7 Days: T1, T3, T4, T6, and T7 exhibited higher numbers of roots (1 to 2 roots). T2, T5, and T8 showed no root development.
- At 14 Days: Root development increased, with T1, T3, T4, T5, T6, and T7 averaging 2 to 4 roots.
- At 21 Days: Further increases were observed, with T1, T3, T4, T5, T6, and T7 averaging 4 to 7 roots. T2 and T8 averaged 2 to 3 roots.

Table 4: Average No. Of roots per explant at 7th, 14th, and 21st day.

Treatments	At 7 days	14 days	21 days
T1	2 ±1	4 ±1	7 ±1
T2	0	0	2 ±1
T3	1 ±1	3 ±1	4 ±1
T4	1 ±1	2 ±1	5 ±1
T5	0	2 ±1	5 ±1
T6	2 ±1	3 ±1	6 ±1
T7	2 ±1	4 ±1	6 ±1
T8	0	1 ±1	3 ±1

Length of Roots per Explant at 7th, 14th, and 21st Day

The length of roots per explant varied among the treatments:

- At 7 Days: T1, T3, T5, and T6 had longer roots (1.7 to 2.5 cm). T2 and T8 showed no root development.
- At 14 Days: Root lengths increased, with T1, T3, T5, T6, and T7 averaging 3 to 3.5 cm.
- At 21 Days: T1, T3, T4, T5, T6, and T7 exhibited longer roots (4 to 6 cm).

Table 5: Average Length of roots per explant(cm) at 7th,14th, and 21st day.

Treatments	At 7 days	14 days	21 days
T1	2.5	4	6.5
T2	0	0	2.8
T3	2	3	5
T4	1.3	2.5	4.5
T5	1.7	3	5.1
T6	2.1	3.5	6
T7	1.9	3.2	6
T8	0	1.8	4

Discussion :

The variations in explant response and induction times underscore the importance of media formulation in promoting banana explant growth during tissue culture. Media formulations like T1, T3, T4, T6, and T7, which promote faster shoot and root induction, may be advantageous for large-scale banana propagation efforts. Conversely, media formulations like T2 and T5, which exhibit slower induction times, may require further optimization to improve their efficacy.

Conclusion :

The effectiveness of tissue culture protocols for banana propagation is influenced significantly by the composition of the media. Media formulations that provide optimal nutrient levels, hormonal balances, and physical support can enhance the growth and development of banana explants, leading to higher success rates in tissue culture protocols.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research. All findings and conclusions presented in this study are the result of objective scientific investigation and have not been influenced by any personal or financial interests.

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