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THE PROCESS OF ROOTING IN MICRO CLONALLY PROPAGATED BANANA VARIETIES IN VITRO

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ABSTRACT

This study investigates the process of root formation in microclonally propagated banana varieties under in vitro conditions. Banana production worldwide is significant, yet considered relatively low compared to other fruits, prompting the need for high-yielding, stress-resistant varieties and the development of efficient cultivation technologies. In vitro propagation of bananas is essential for mass production, but root formation can be hindered by the presence of high cytokinin levels. The research aims to optimize the cytokinin/auxin ratio in the rooting medium for successful root induction.

The experiment was conducted at the Biotechnology laboratory of the Academician Makhmud Mirzayev Research Institute of Horticulture, Viticulture, and Winemaking from 2022 to 2024. The effect of different concentrations of NAA (Naphthalene Acetic Acid) and IBA (Indole-3-butyric Acid) on root formation was evaluated in half-strength MS medium. The findings revealed that the combination of 1.00 mg/l NAA and 0 mg/l IBA resulted in the highest rooting rate (95.4%) and the longest roots (4.7 cm), indicating this combination as optimal for inducing roots in banana varieties. Higher concentrations of NAA and IBA were less effective, slowing root formation or resulting in fewer roots.

This study underscores the significance of selecting the right hormonal balance in tissue culture, providing crucial insights for improving banana propagation techniques and enhancing productivity in banana cultivation.

Keywords: In vitro, microclonal, banana, IBA, NAA, adaptation, root.

INTRODUCTION

Currently, the global banana production volume is 5.94 million tons, with a cultivated area of 135.2 million hectares. Of this, Africa accounts for 22.8%, Asia 51%, America 23.8%, Oceania 1.2%, and Europe 0.4%. Leading banana producers include India with 34.52 million tons, China with 11.87 million tons, Indonesia with 9.25 million tons, Nigeria with 8.1 million tons, Brazil with 6.9 million tons, Ecuador with 6.2 million tons, the Philippines with 5.9 million tons, Guatemala with 4.8 million tons, Angola with 4.6 million tons, and the United Republic with 4.5 million tons, among other countries.

Banana production is considered to have a relatively low output compared to other fruits, which makes selecting high-yielding banana varieties resistant to various stresses and external environmental factors, as well as developing modern resource-efficient technologies for banana cultivation, crucial.

In vitro banana propagation is typically carried out when there is a high concentration of cytokinin, which inhibits root formation and elongation. Additionally, during in vitro propagation, the shoots may not form roots and may grow in the form of shoots that cannot be directly transferred to field conditions. The concentration of cytokinin in the rooting medium should be lower than that of auxins in the propagation medium, leading to a low cytokinin/auxin ratio, which is favorable for root formation. There is evidence that roots can form without growth regulators, although most authors confirm the need to add growth regulators for root formation (Rahman et al., 2006; Viehmannova et al., 2007; Al-amin et al., 2009; Olivya et al., 2010).

MATERIALS AND METHODS

This research was conducted at the Biotechnology laboratory of the Academician Makhmud Mirzayev research institute of horticulture, viticulture, and winemaking from 2022 to 2024.

Main Body: In the rooting medium, the concentration of cytokinin should be lower than that of auxins, leading to a low cytokinin/auxin ratio, which, as reported by Gupta (1986) and Vong (1986), is favorable for root induction. However, most researchers have completely removed cytokinins from the rooting medium. The most commonly used auxins in rooting media are NAA (Naphthalene Acetic Acid), IAA (Indole-3-acetic Acid), and IBA (Indole-3-butyric Acid).

Naphthalene Acetic Acid (NAA) has been frequently used at low concentrations to induce rooting in in vitro-grown banana plants. Arinaitwe et al. (2000) achieved good root induction of Kibuzi, Bwara, and Ndizwemiti banana varieties in MS medium containing 1.2 µM NAA. Rahman et al. (2004) used various concentrations of NAA to induce rooting in Musa sapientum and found that 2.0 mg was the most effective. Viehmannová et al. (2007) and Srangsam and Kanchanapoom (2007) observed that the MS medium without growth regulators supported good rooting, elongation, and healthy growth of roots, and the presence or absence of activated charcoal in the medium had no significant effect on rooting in the Kluai Sa and Kluai Leb Mue Nang (AAA) banana varieties.

Jasrai et al. (1999), in their study of the ex vitro conditions of in vitro-grown banana plants without greenhouse conditions, utilized an MS medium with 0.1 IAA. Al-Amin et al. (2009) reported that the highest number of roots and root length were observed with a combination of 0.5 mg IAA and 0.5 mg IBA.

RESULTS

Table 1. Effect of NAA and IBA hormones added at different concentrations to half-strength MS medium on root formation of banana varieties

NAA mg/l	IBA mg/l	Rooting (day)	Number of roots (pcs)	Length of roots (cm)	Rooting rate (%)
0	0	0	0	0	0
	0,5	18	3,9	3,5	65,3
0.5	0	19	4,7	2,6	46,2
	1.00	14	5,9	4.7	95.4
1.00	0	18	4,2	3.2	74.8
0	1.5	17	4,1	3.7	65.9
1.5		20	2,4	2,8	57,5
	2.00	16	3,6	4,3	56,3
2.00		18	3,8	2,1	55.7

No hormones (0 mg/l NAA, 0 mg/l IBA) led to zero root formation, which confirms that growth regulators are essential for rooting in banana plants.

When 0.5 mg/l IBA was added (without NAA), rooting occurred in 18 days, with 3.9 roots formed and an average root length of 3.5 cm. The rooting rate was 65.3%, indicating that IBA alone could support some rooting, but not as effectively as the combination of NAA and IBA.

For 0.5 mg/l NAA (without IBA), the rooting was slightly slower, taking 19 days. The number of roots was slightly higher at 4.7, but the root length was shorter, at 2.6 cm. The rooting rate dropped to 46.2%, which suggests that NAA alone isn't as effective as the right balance of auxins.

The combination of 1.00 mg/l NAA and 0 mg/l IBA was the most effective, with roots forming in 14 days, the fastest rooting time among the treatments. It resulted in the highest number of roots (5.9) and the longest root length (4.7 cm), with an impressive 95.4% rooting rate. This suggests that 1.00 mg/l NAA is the optimal concentration for promoting root formation.

1.00 mg/l NAA with 0 mg/l IBA again showed great results with a 74.8% rooting rate, confirming the effectiveness of NAA, but with a slight decrease in the number of roots and root length compared to the previous treatment.

Adding 1.5 mg/l IBA without NAA slowed rooting to 20 days and resulted in fewer roots (2.4 roots) and shorter roots (2.8 cm). The rooting rate of 57.5% was lower than other treatments, indicating that higher concentrations of IBA alone are not ideal for optimal root formation.

Increasing the NAA concentration to 2.00 mg/l with no IBA resulted in roots forming after 16 days, but the root length was the longest (4.3 cm), even though the number of roots was 3.6. The rooting rate decreased to 56.3%, suggesting that too high a concentration of NAA might inhibit root formation.

Lastly, the combination of 2.00 mg/l NAA and 1.5 mg/l IBA produced roots after 18 days, but the root length was the shortest (2.1 cm), and the rooting rate was the second lowest (55.7%), indicating that this combination was not as effective.

The best rooting results were achieved with 1.00 mg/l NAA (without IBA), which resulted in the highest rooting rate (95.4%) and longest roots (4.7 cm).

Higher concentrations of NAA (like 2.00 mg/l) or IBA (like 1.5 mg/l) were less effective, leading to slower rooting or fewer roots.

Therefore, the optimal combination for inducing roots in banana varieties is 1.00 mg/l NAA with 0 mg/l IBA.

This experiment emphasizes the importance of carefully selecting hormone concentrations to optimize root formation in banana tissue culture.

Figure A: The development of roots at the site of callus formation in an in vitro grown banana plantlet is observed. The formation of adventitious roots at this stage is crucial for the success of microclonal propagation.

Figure B: The root length of the plantlet is being measured. The length and number of roots are important criteria for assessing the readiness for the acclimatization stage following micropropagation.

Figure D: The degree of rooting and phenotypic differences of various individual plantlets are depicted. This allows for the evaluation of variability in regeneration capacity, from which the strongest samples for selection are chosen.

Figure E: The plantlets are transferred to the acclimatization stage and are successfully growing in the substrate. At this stage, the plants must adapt to ex vitro conditions, meaning they need to be resistant to changes in light, humidity, and temperature.

CONCLUSIONS

The study demonstrated the critical role of growth regulators, particularly NAA (Naphthalene Acetic Acid) and IBA (Indole-3-butyric Acid), in the root formation of microclonally propagated banana varieties in vitro. The most effective rooting was observed with 1.00 mg/l NAA, resulting in the highest rooting rate (95.4%) and longest roots (4.7 cm). This was significantly better than other hormone combinations, where higher concentrations of NAA or IBA (such as 2.00 mg/l NAA or 1.5 mg/l IBA) led to slower rooting and fewer roots. The results suggest that the optimal hormone combination for promoting root formation in banana varieties is 1.00 mg/l NAA with 0 mg/l IBA, highlighting the importance of careful selection of hormone concentrations for successful tissue culture propagation.

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