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Invitro Regeneration of Banana by Changing in Hormonal Concentrations I.E. Auxins & Cytokinins

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ABSTRACT: - The study was conducted at the Bochra plant tissue culture laboratory in the Akot district of Akola, Maharashtra. in 2023–2024. Auxins and cytokines' effects on callus induction, shoot, and root regeneration in bananas grown in vitro. One of the major fruit crops and a source of sustenance is the banana. for a million individuals in poor nations. To initiate and multiply shoots, explants (suckers) of banana cultivars were cultivated on Murashige and Skoog (MS) media supplemented with varying doses of BAP and NAA combinations. As people's awareness of bananas' increasing nutritional and therapeutic worth has grown around the world, bananas have become a priority crop. Bananas are traditionally propagated by the vegetative method. However, there are additional issues with this approach, like pests, to look at how varying BAP and NAA concentrations affect the growth and multiplication of shoots in virus-free plants. After 30 days, adventitious plantlets from the culture meristem grew into a glass container in a green bunch of little plantlets. Four BAP level combinations (1.5, 2.0, 2.5, and 3.0) and to examine its impact on shoot and root proliferation, three different doses of NAA (1.5, 2.0, and 2.5) were applied to the media. Among the various concentrations, the largest shoot growth multiplied by around 6–8 clumps was seen at 2.5 ml/l BAP + 1.5 ml/l NAA. The concentration of 1.5 ml/l BAP + 2.5 ml/l NAA produced the longest shoots per plantlet (0.43, 2.24, 2.64 cm).

Keywords: In vitro, Micro-propagation, Sucker, Banana, Auxin, Cytokine

INTRODUCTION: Today, micro-propagated bananas are widely cultivated commercially in several nations; the annual production of these plants is thought to number in the 30 million range. Compared to traditional suckers, micro-propagated banana plants often establish more quickly, grow more quickly, have more consistent production periods, and yield more. Since bananas are among the most blatantly sterile crops in the world, they are propagated vegetative. However, the rate of Musa plant propagation using these techniques is relatively low, as only 4 or 7 plants can be obtained annually from a single sucker, while 1200 plants are needed for a one-acre plantation. Bananas' poor multiplication ratio has prompted extensive research on quick propagation techniques (Lohidas et, al). Suckers are used in traditional breeding processes to multiply banana plants; however, this method has several drawbacks, including the spread of disease, limited availability of high-quality planting material, and a lack of diversity and quantity in production. Tissue culture, which produces an enormous multiplication of banana plantlets at a very quick rate, and sterilized explant (planting material) are two novel approaches that have recently been developed to address these problems. Together with the growing demand for fruit due to the world's population growth, which presents a huge opportunity for material export, these farmers also hope to cultivate bananas on a massive scale using in vitro propagation. This is made possible by the use of significant explants for quick propagation and commercial varieties that are economically significant. (Khaskheli et, al). Because practically all cultivated banana cultivars are triploid, seedless, or seed sterile, bananas belong to a category of crops that are typically propagated through the vegetative components of the plant. For traditional propagation, corms, sword suckers, and large and small suckers are the materials required (Iqbal et, al).. One of the key elements affecting the effectiveness of the micro propagation system is the adventitious buds' rate of multiplication when exposed to benzyl aminopurine (BAP). The most widely used cytokine is benzyl amino purine (BAP), which is used at concentrations between 1 and 3 ml/L. It is frequently used in conjunction with Naphthalene acetic acid (NAA), an auxin, at concentrations between 1 and 2.5 ml/L. The goal of the current study was to determine how quickly banana shoot tips multiply in vitro throughout multiple subcultures utilizing various medium conditions. Auxin, a cytokine, was employed in varying concentrations in MS medium to test the optimal multiple shoot proliferation from the explants and the

formation of roots from shoots. (Wijerathna et, al). Using in vitro micro propagation, several researchers have documented the regeneration of Musa spp. When compared to traditional propagation methods, in vitro banana propagation offers many advantages, such as year-round large-scale production of disease-free planting materials, physiological homogeneity, minimal space requirements for multiplying large numbers of plants, and the availability of disease-free material. Furthermore, as compared to conventionally propagated bananas, micro-propagated banana plants often establish faster, grow faster, have a shorter and more consistent production cycle, and yield more. Despite the abundance of data on banana in vitro propagation, differences in propagation procedures might lead to varying outcomes due to factors including genotype. (Mekonen et, al).

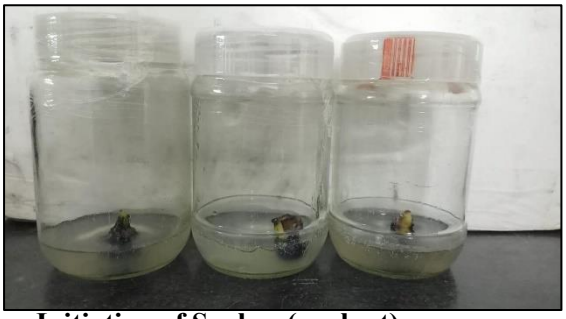
Material & Method: The experiment was carried out at the Bochra, Akola, Plant Tissue Laboratory. Banana sword suckers were gathered from the farm's vicinity. The suckers' outer leaf sheaths were removed to prepare the shoot tips. The explants were carefully cleaned in running tap water for ten to fifteen minutes after being surface sterilized twice with mild detergent and a few drops of Tween 20. After that, the explants were surface sterilized for five minutes using 0.1% mercuric chloride (HgCl2). To get rid of any remaining residues of HgCl2, the sterilized explants were then rinsed five or six times within the clean bench using sterile distilled water. Using fine, sterile forceps and a scalpel, the sterilized explants were aseptically removed from the laminar airflow cabinet. To promote shoot regeneration and multiplication, the removed explants were then inoculated on MS media supplemented with BAP (1.5, 2.0, 2.5, and 3.0 ml/l) both alone and in combination with NAA (1.5, 2.0, and 2.5 ml/l). The medium's pH was brought to 5.8. The cultures were kept at the required 25 C temperature. Every two to four weeks, the established cultures on the shoot induction media were routinely transferred. Days until shoot initiation, the percentage of explants that induced a shoot, and the number of shoots per explant have all been noted after A four-week cultural program. Banana shoots were cultivated in vitro on MS medium supplemented with varying amounts of BAP (1.5, 2.0, 2.5, and 3.0 ml/l) and NAA (1.5, 2.0, and 2.5 ml/l) to initiate root growth. After being left at room temperature for three to four days, the well-rooted plantlets were moved to a plastic pot filled with garden soil to compost, and they were sufficiently moistened to ensure good hardening. (Huq, A. et, al).

RESULT & DISCUSSION: Five distinct BAP and NAA combinations were used to inoculate the meristematic shoot tip explants on MS media. Out of all the therapies, the five combinations listed in Table 2 produced the most successful results. Explants expanded, became green, and started to develop shoots after 14 days. The optimal culture establishment medium was MS + 1.5 ml/l BAP + 2.5 ml/l NAA, and it took an average of 15 to 21 days. The results indicated that BAP + NAA (2.5 + 1.5 ml/l) had the best shoot proliferation, with BAP + NAA (2.0 + 2.0 ml/l) coming in second. Both of the first (2.0+ 1.0 ml/l of BAP + NAA) combinations of the growth regulators showed a poor response in terms of shoot initiation. 90% of the cultures were obtained free of contamination, according to the results. The originally established shoots were subcultured to the liquid medium using the same hormone supplements, resulting in the induction of multiple shoots. A threefold rise in multiplication was observed after 21 days. Increased the number of multiplicities axillary buds by three to four times at each subculture in the same media. The explants were split into smaller clumps after 20 days in MS with BAP (2.5 ml/l) and NAA (1.5 ml/l) medium, after which they reached the stage of robust growth. Each clump of eight to nine shoots was placed in the solid medium for shoot proliferation (Table 2).. Results clearly showed that 2.5 BAP+1.5 NAA media proved to be most productive for shoot proliferation. When these shoots were sub-cultured to the same media, gave rise to a three-to-four-fold increase in proliferating clumps and 3- 4 elongated shoots with root initiation. The elongated shoots were excised when they were subculture in the same conditions. The long branches were removed and cultured separately in the same fresh medium to encourage the formation of long shoots broad leaves and basal roots The highest number of shoots were produced on MS supplemented with 2.5 ml/l BAP +1.5 ml/l NAA and 1.5 ml/l BAP + 2.5 ml/l NAA. Proliferating shoots continued to produce axillary buds on the same fresh media while the greatest number of shoots were produced. While elongated shoots were transferred to root induction media for root development. Four different Concentrations of auxins NAA were used in combinations (0ml/l, 1.0ml/l, 2.0ml/l, 1.5ml/l and 2.5 ml/l) in MS supplemented medium for root induction. The results showed that MS medium supplemented with NAA (2.5ml/l) gave the best results which produced 70% root induction. In the present investigation, shoot meristems were cultured on agar (semisolid) medium

and on liquid medium for shoot initiation. In liquid media, the shoot buds showed vigorous multiplication in the form of clumps. The multiplying shoot tips on liquid media were incubated. Better results were shown in liquid media at the shaker. After culture establishment, shoot lets were transferred to, proliferating shoots continued to form axillary buds on the same fresh medium. For root induction, four distinct auxin concentrations (NAA) were combined (0 ml/l, 1.0 ml/l, 2.0 ml/l, 1.5 ml/l, and 2.5 ml/l) in MS-enriched media. The results showed that the greatest results, resulting in 70% root induction, were obtained using MS media supplemented with NAA (2.5 ml/l). In the current study, shoot meristems were cultivated for shoot initiation on both liquid and agar (semisolid) media. The shoot buds displayed clump-forming, aggressive proliferation in liquid media. On liquid media, the multiplying shoot tips were nurtured. In the shaker with liquid media, better outcomes were observed. Shoot lets were moved to an agarose-based solid medium for shoot proliferation. This multiple shoot formation by meristem slices on agar medium. Five different combinations with various concentrations of BAP and NAA were used in this study to analyze the creation of the culture. On an agar media, meristem slices generate numerous shoots. To analyze the shoot initiation and shoot multiplication and resultantly MS medium of 2.5 ml/l of BAP and 1.5 ml/l of NAA showed good results both for shoot initiation and multiplication. The findings of the present study showed that the shoot subculturing in the same hormonal-supplemented medium gave rise to a three-to-four-fold increase in shoot proliferation which resultantly developed healthy rooted plants from each clump in root induction media. The invitro raised plantlets were successfully established in the polythene pots containing cocopeat+ clay+ garden soil in a 1:1:1 ratio. Successful rooting was attained within 25-30 days while Vessey & Rivera (1981) reported root formation occurred 50 days after shoot development. Berg and Bustamante (1974) noted that root formation needed 2-3 months. proliferation in the 1:1:1 ratio of cocopeat, clay, and garden soil in the polythene containers, the in vitro-grown plantlets were successfully established. shoot growth, successful rooting was achieved in 25–30 days. According to Berg and Bustamante (1974), the development of roots requires two to three months. proliferation in root induction media, which resulted in the development of healthy rooted plants from each clump in root induction media. The invitro raised plantlets were successfully established in the polythene pots containing cocopeat+ clay+ garden soil in a 1:1:1 ratio. Successful rooting was attained within 25-30 days while Vessey & Rivera (1981) reported root formation occurred 50 days after shoot development. Berg and Bustamante (1974) noted that root formation needed 2-3 months. (Iqbal, et, al).

Table I: Effect of concentration of plant growth regulator BAP and NAA on in vitro shoot proliferation from shoot tip explants of banana

Treatment	Hormonal combination	No. of culture bottles	Observation
1	MS+NAA(0.5mg/l) +BAP(1.5mg/l)	10	Culture initiation start to Induce Shoot and root culture



Initiation of Sucker (explant)

AFTER 14 DAYS

AFTER 21 DAYS

Table II: Effect of hormones on multiplication of shoots in different concentrations

Sr no.	Media	Hormonal combination (Concentration)			No. of culture bottles	No. of shoot	Observation
		NAA		BAP			
1)							
I.	MS	-	+	2ml/lit	10	2-3	Stunted growth
II.	MS	-	+	3ml/lit	10	5-6	Minimum multiplication of shoot growth
III	MS	2ml/lit	+	2ml/lit	10	6-8	Maximum multiplication of shoot growth
2)							
I.	MS	1.5ml/lit	+	2.5ml/lit	10	8-9	Maximum multiplication of shoot growth
II.	MS	2.5ml/lit	+	1.5ml/lit	10	5-6	Maximum multiplication of growth

I) MS+BAP (2ml/l) **II) MS+BAP (3ml/l)** **III) MS+BAP (2ml/l) +NAA (2ml/l)**

I) MS+BAP (2.5ml/l) +NAA (1.5ml/l) **II) MS+BAP (2.5ml/l) +NAA (1.5ml/l)**

RESULTS AFTER 21 DAYS

1)

I) MS+BAP (2ml/l) **II) MS+BAP (3ml/l)** **III) MS+BAP (2ml/l) +NAA (2ml/l)**

2)

I) MS+BAP (2.5ml/l) +NAA (1.5ml/l) **II) MS+BAP (2.5ml/l) +NAA (1.5ml/l)**

CONCLUSION: - In the current investigation, we discovered that the optimal concentrations for in vitro shoot and root regeneration were 2.5 mg/L BAP and 1.5 mg/L NAA. The highest results for banana regeneration in vitro were obtained when 2.5 mg/L BAP ± 1.5 mg/L NAA and 1.5 mg/L BAP ± 2.5 mg/L NAA were combined. As a result, an appropriate methodology that could be used for the quick in vitro production of banana plantlets was developed. Further studies have been carried out in the future to evaluate field performance for the ultimate enhancement of bananas under certain circumstances.

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