Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 7 (Special Issue): 277-290 (2023) (http://www.wildlife-biodiversity.com/)

Research Article

Effect of cytokinin TDZ and auxin IBA on the succession of plants of the banana plant *Musaa acumanata*, the Grand-Nain hybrid cultivar, using tissue culture technology

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Received: 02 October 2023 / Revised: 17 November 2023 / Accepted: 24 November 2023/ Published online: 28 November 2023.

How to cite: Alrazn, S.M.H., Alkhalifa, A.A.S., Al-Sereh, E.A. (2023). Effect of cytokinin TDZ and auxin IBA on the succession of plants of the banana plant Musaa acumanata, the Grand-Nain hybrid cultivar, using tissue culture technology, Journal of Wildlife and Biodiversity, 7 (Special Issue), 277-290. **DOI**: https://doi.org/10.5281/zenodo.10213198

Abstract

This study was conducted in the Tissue Culture Laboratory of the Agriculture College, Basrah University, Iraq, during the period 9/23/2021 until 8/19/2023 with the aim of studying the effect of different concentrations of cytokinin Tdz and auxin Ibi in the micropropagation of banana plants of the Grand-Nain hybrid variety, by cultivating Both buds and half-buds are in the MS medium. The results showed the concentration of 5.0 mg L^{-1} of TDZ was superior to all treatments, as it gave the highest number of vegetative shoots, the highest number of branch length, the highest number of leaves, and the highest leaf width of 9.33 plant $parts^{-1}$, 6.87 cm, and 4.33 $shoots^{-1}$. 1.867 cm, respectively. The rooting results show a significant superiority to the concentration 3.0 $mg.L^{-1}$ IBA. All treatments in the percentage of root emergence reached 100%, the highest average number of leaves was 4.00 $shoots^{-1}$, the highest plant length was 9.00 cm, and the highest number of roots was 7.67 root $shoots^{-1}$, the highest rate. The root length is 9.17 cm. The acclimatization treatments inside the acclimatization rooms led to the success of plant propagation using sterile soil consisting of peat moss and pearlite in a ratio of 2:1, as it gave the best results after 20 weeks for vegetative growth traits. It is concluded from the study the distinctive role of the cytokinin TDZ in stimulating the vegetative shoots of the Grand-Nain hybrid variety and the possibility of plant differentiation using special treatments to acclimatize the resulting plants.

Keywords: plant tissue culture, banana Grand-Nain, TDZ, IBA

Introduction

The banana plant, *Musaa acumanata* L., is a hybrid Grand Nain cultivar of the commercial banana cultivar currently distinguished by the Chiaquita, which is widely cultivated in Central America and India. Its length ranges between 6 - 8 meters and produces many clusters of fruit. The weight of the bunch may reach 50 - 150 pounds. The Grand Nain hybrid banana plant is considered one of the attractive plants due to its possibility of being used in landscaping and good resistance to winds. It is one of the primary fruit crops for consumers in rural areas in some Asia and African countries and an important source of income (Justine et al., 2022). It is considered a plant. Bananas are giant monocotyledonous herbaceous plants that bear flowers on the panicles in the form of clusters arranged in a spiral. Each cluster of flowers is found in two rows and is called a palm, covered with red scaly leaves (Heslop-Harrison & Schwarzacher, 2007). Bananas are an important crop in many countries. In addition to its high nutritional value and its popularity among many consumers (Kuyu & Tola, 2018). Many researchers were interested in using tissue propagation technology to produce banana seedlings. They used different explant, most of which were the apical and lateral buds, as well as using different combinations of MS media and different plant growth regulators (Strosse et al., 2008; Wong, 1986), and given the size of banana seedlings and the large area Leaves of plants, which is one of the reasons for the death of many seedlings produced by tissue culture. Several protocols, methods and treatments were studied to increase successful plants in the acclimatization process. Various mixtures of growing media were used as well as lighting and humidity rates. These studies were able to reduce the rates of seedling loss and raise the rate of survival and continued success of the acclimatization (Bello-Bello et al., 2019; Hassan et al., 2022; Robinson & Sáuco, 2009), by using the plant tissue culture method, many plants that are free of diseases and pathogens and identical to the mother plant (Previati et al., 2008) can be produced. This technique also leads to the occurrence of many variations in the plants produced. Which can be considered new sources in the process of genetic improvement of plants (Samad et al., 2001).

1. Given the importance of this plant from an economic standpoint and its lack of culture in Iraq and its scarcity in this region, the study aims to develop an integrated program for the abundant propagation of this plant by multiplying the shoots using the cytokinin TDZ (Thidazuron) and knowing the best concentration, in addition to rooting these shoots. Using auxin (IBA) and finding the best concentration for rooting.

2. Acclimatization of plants resulting from tissue culture of banana plants.

Material and methods

This study was conducted in the Tissue Culture Laboratory of the Agriculture College, Basrah University. In this study, plant parts (explants) were used, represented by the shoot tips of the Grand Nine banana plant . These explants were excised using a sharp scalpel with a length of (1 1.5-) Poison from mothers grown in imported pots, figure (2). Then it was washed with water several times and materials stuck to it, then it was immersed in a glass container containing the Elsa fungicide at a concentration of 500 $mg.l^{-1}$, with the addition of 2-3 drops of liquid soap and stirred continuously for five minutes. These tissues were preserved in a glass beaker containing an antioxidant consisting of citric acid and Ascorbic acid (Abdelghaffar et al., 2023).

It was kept in the refrigerator at a temperature of 4°C for 24 hours until the surface sterilization process was get rid of the effects of harmful phenolic compounds. After that, the surface sterilization process was coundected by transferring it to a laminar flow air cabinet previously sterilized with 70% ethanol and chlorine diluted with distilled water. It was placed directly in a 70% ethyl alcohol solution for five minutes, then washed with distilled and sterile water several times. After that, the lower parts of the plant parts were cut, so their lengths became 0.4-0.5 cm in order to get rid of the sterile material that had entered the plant tissue after sterilizing it with ethyl alcohol, and it was used as a 15 replicates for each type of tissue piece for both experiments. Then the surface sterilization process was carried out as follows. The plant parts were placed directly in a solution of sodium hypochlorite Naocl. The concentration of the active ingredient was 1.05% and at a concentration of 20% v/v with the addition Tween 20 to each. 100 ml of solution, shaking manually from time to time for 30 minutes (Mekonen et al., 2021).

The explants were removed from the sterilization solution and washed with distilled water, then kept in a sterile glass container containing distilled and sterile water until the planting process was conducted to prevent them from drying out. They were grown in MS nutrient medium (Murashige & Skoog, 1962), by taking a weight of 4.33 g. L^{-1} The organic materials are sucrose with a concentration of 30 g. L^{-1} and (PVP) Poly vinyl pyrolidone 500 mg. L^{-1} and a set of vitamins, glycine and proline at 1 mg. L^{-1} Growth regulators were added according to the aim of the experiment.

Heat the nutrient medium to a temperature of 90°C, then distribute it into culture tubes measuring 2.5 x 1.8 cm. The nozzles were blocked with medical cotton, covered with aluminum foil, and then sterilized with an Autoclave sterilizer for a period of 20 minutes and kept in the incubator until planting. The study included the following:

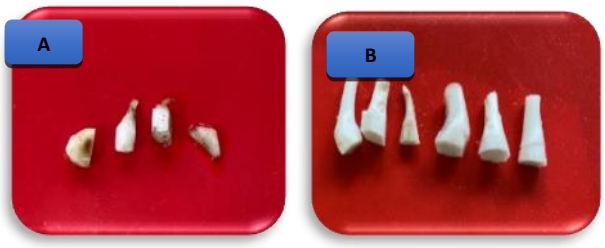

Figure 1. Plant parts taken from a stunted banana plant (A) Whole bud (B) Half buds.

Figure 2. Grand-Nain banana seedlings obtained from private nurseries.

Effect of different levels of TDZ on the multiplication of shoots of the Grand Nine banana plant

Cytokinin TDZ was used at different concentrations (0.0,2.5,5.0,7.5) mg L-1 with the presence of NAA at a fixed concentration of 0.2 mg L^{-1} in the process of uncovering the vegetative shoots of the Grand Nine banana plant. The explants were planted after sterilization (as mentioned previously) vertically in the nutrient medium supplied with salts. MS, at a rate of one plant part in each test tube, then the crops were incubated with light for a period of (8) weeks, and the following measurements were taken:

2- Number of shoots for each explants.

3 - Length of vegetative shoot. cm.

4-The number of leaves per vegetative shoot

5- leaf width.cm.

Effect of different levels of IBA on the rooting of banana plant shoots resulting from the multiplying stage

The vegetative shoots resulting from the vegetative multiplication stage were sectioned using a sharp, sterile scalpel and planted in the rooting medium provided with concentrations of (0, 2, 3, 4) mg L^{-1} IBA, with TDZ at a fixed concentration of 0.5 mg L^{-1}. The seedlings were incubated with the same incubation conditions as before for a period of time. (8) weeks and the following measurements were taken.

$$\text{Percentage of rooting}=\frac{Number\ of\ rooted\ vegetative\ Shoots}{Number\ of\ vegetative\ shoots}\times 100$$

- Number of roots
- Length of shoot. cm
- Number of leaves per plant
- Notes

Acclimatization of the resulting plants

This process was conducted in the culture room under the stratified air flow table, then the stunted banana plants were placed in a glass container containing sterile distilled water covering half the height of the plants, and a glass cover was placed over the mouth of the container, for a period of 8-10 days, with change of water every two days. The plantlets were transferred to pots with dimensions of 15 x 15 cm, containing of sterile soil, they were covered with plastic containers and left in the planting room. After 7-10 days, these covers were gradually removed for three hours daily, taking into account The plants were watered with distilled and sterile water every 3-5 days, and after 4 weeks, the covers were completely removed, and the following measurements were taken:

Vegetative growth indicators: Measurements were taken once a month for 20 weeks.

- Average plant height (cm).
- Average leaf length (mm).
- The average number of leaves is a vegetative shoot -¹.
- 4 – Average leaf width (cm).

Statistical analysis

1 - The study experiments were designed according to a completely randomized design (R.L.S.D.), and data analyzed using analysis of variance, and the means were compared according to the (R.L.S.D.), with a probability level of 0.01%.

2 - Use the ready-made statistical analysis program Genestat V.12 to analyze the results.

Results and discussion

The effect of different levels of TDZ on shoot multiplication of banana plant variety Grand– Nain

The results in Table (1) indicate that TDZ has increasing the number of multiplied shoots of banana plants in the nutrient medium prepared with MS salts and at different levels of TDZ 0.0, 2.5, 5.0, 7.5 mg L-¹ with the presence of NAA at 0.2 mg L^{-1} after eight weeks of culture in the light, the MS nutrient medium with level 5.0 mg L^{-1} was significantly excelled of TDZ by giving it the highest number of shoots, amounting to 9.33 shoots per explant, and with a significant difference from the other concentrations, as the control gave the lowest number of shoots. The shoots amounted to 2.33 shoots per explant -¹, and the results showed that the level of 5.0 mg L^{-1} gave the highest length of the vegetative shoot, the number of leaves, and the leaf width, as it reached 6.87 cm, 4.33 leaves, and 1.86 cm, respectively, while it was in the control treatment. It reached 1.00 cm, 2.00 leaves, and 0.96 cm. The reason for the higher concentration of 5.0 mg L-¹ of TDZ may be because it has a higher efficiency than other cytokanins by stimulating the accumulation of cytokanins in explant tissues (Victor et al., 1999). as well as This concentration is considered appropriate in the process of stimulating the process of vegetative replication, meaning that there is a fact that the cytokinin TDZ causes the breakage of the apical dominance, and as a result, the increase in the area of vegetative replication and the elongation of cells during the changes that accompany plant growth (Anjum & Abbasi, 2016), and there is also a relationship for TDZ in Obstructing the action of cytokinin-oxidizing enzymes, as these enzymes reduce divisions in the meristematic region (Hare, 1998). There is a role for cytokinin in building ribonucleic acid (RNA), proteins, and enzymes inside cells, which encourages the process of cell division and shoot multiplication (Ibrahim et al., 2013; Lazar, 2003).

These results agreed with (Lee, 2001; Shirani et al., 2009; Smitha et al., 2014) when studying the multiplication of banana plant shoots, showed that the best medium for multiplying vegetative shoots is 5.0 mg L-¹ TDZ.

Table 1. The effect of different levels of cytokinin TDZ on the multiplication of vegetative shoots of the banana plant, the Grand-Nain variety, after eight weeks of culture in the light

leaf width (cm(	Number of leaves .plant^{-1}	shoots length (cm(	Number of shoots.explant -^{1}	treatments TDZmg L^{-1}
0.96	2.00	1.00	2.33	0.0
1.30	4.00	3.67	3.67	2.5
1.86	4.33	6.87	9.33	5.0
1.56	3.67	4.30	4.67	7.5
0.54	1.11	1.61	2.09	RLSD p≥ 0.01

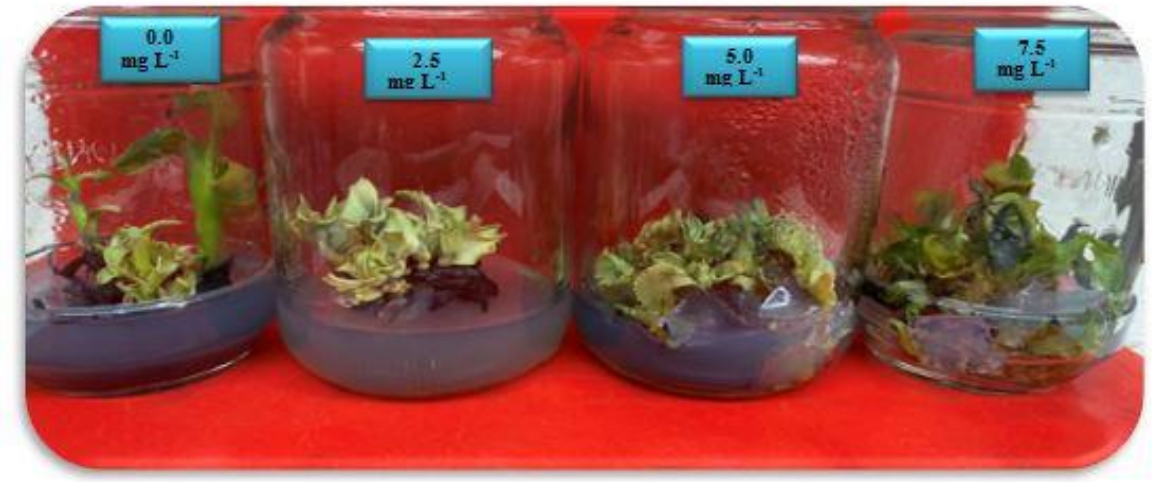

Figure 3. The effect of different levels of TDZ on the multiplication of banana vegetative shoots variety Grand - Nain eight weeks after the beginning of cultivation in the light.

Effect of different levels of IBA on the rooting of shoots of banana plants

Table (2) showed the effect of different levels of auxin IBA(0.0, 2.0, 3.0, and 4.0) mg L^{-1} with the 0.5 mg L^{-1} TDZ at a fixed concentration on the rooting of the vegetative shoots of banana plants resulting from... Multiplication experiments after eight weeks in the light, figure (4), where a varying response was observed in the percentage of different auxin concentrations, as the 3.0 mg L^{-1} IBA treatment achieved the highest percentage of rooting of the shoots, reaching 100% in the(2.0, 3.0, and 4.0) mg. L$^{-1,}$ while the control treatment gave the lowest average rooting percentage, amounting to 59.0%. The results also indicate that level 3.0 mg L-¹ was significantly excelled on all auxin treatments in giving the highest average root length and number, shoot length and number. The leaves reached 9.17 cm, 7.67 root shoots -¹, and 9.00 cm and 4.00 shoot leaves -¹, respectively. The reason for the superiority of the 3.0 mg L-¹ IBA treatment may be due to the role that auxin plays in stimulating the formation of roots on the

shoots (Mahood, 2021). Using MS salts with auxin in stimulating the formation of roots on the shoots, in addition to using it MS, and this causes a reduction in the salt levels of the nutrient medium, which leads to a reduction in the level of nitrogen in the nutrient medium, and as a result, it leads to an increase in the proportion of carbohydrates to Nitrogen (C/N) stimulates the formation of root cells and their number (Gubišová et al., 2013). As for the increase in root length in the 3.0 mg L^{-1} IBA treatment, this may be due to the occurrence of the phenomenon of nutrient tropism and competition for nutrients when the concentration of MS salts is reduced by half in the nutrient medium, as it encouraged the roots to stimulate the roots. To spread over greater distances in the nutrient medium in order to compensate for the decrease in the amount of nutrients (Khan et al., 2015), or its cause may be due to the positive effect of auxins added to the nutrient medium in stimulating cell division of the cambium-generating layer, which in turn increases the formation of roots and their roots (Kose et al., 2021), and the effect of auxin IBA in increasing the average number of vegetative shoots, their lengths, and the average number of leaves is due to the role of auxins in building proteins and enzymes for the process of cell division and expansion, thus increasing cell elongation and improving vegetative growth by increasing the root mass in the shoots. Vegetative growth, and thus an increase in the absorption of nutrients that are transported upward and lead to the growth and opening of buds, which leads to the production of good vegetative growth (Sosnowski et al., 2023).

The results are consistent with the findings of (Tan et al., 2018) regarding the effective of hormone in stimulating the vegetative growth traits of plants propagated using plant tissue culture technology.

Table 2. The effect of different concentrations of auxin IBA on the rooting of vegetative shoots of the Grand-Nain banana plant after eight weeks of incubation in the light.

Concentration of IBA mg L^{-1}	Rooting percentage %	Number of main roots	Root length (cm)	Shoot length (cm)	Number of leaves
0.0	59.0	2.00	5.67	5.60	3.33
2.0	100.0	3.33	5.83	6.43	2.33
3.0	100.0	7.67	9.17	9.00	4.00
4.0	100.0	6.33	7.00	5.83	2.33
RLSD $p \geq 0.01$	68.81	1.93	4.05	1.99	1.37

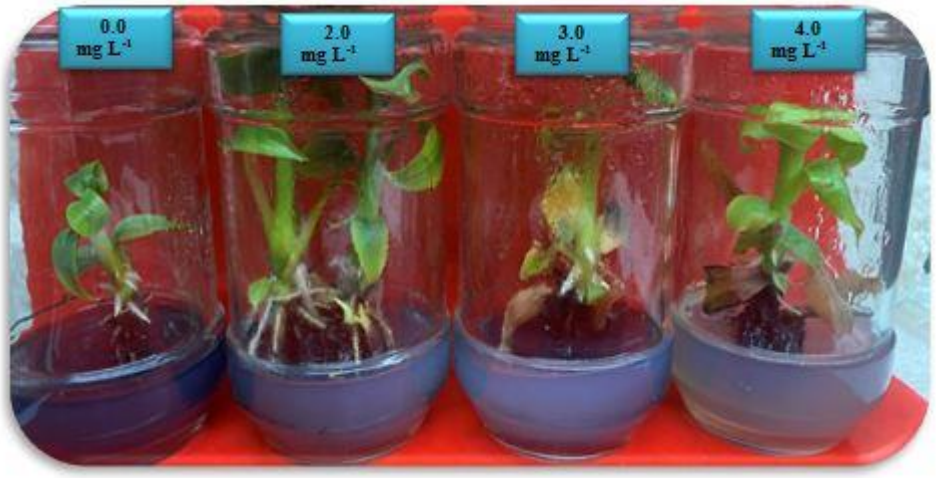

Figure 4. Rooting of the vegetative shoots of the Grand Nain banana plant in different levels of IBA after eight weeks in the light.

Acclimatization of the resulting plants

The results in figure (5) showed the process of hardening the seedlings resulting from the rooting experiments. The plantlets were removed from the nutrient medium and immersed in the fungicide Elsa at 500 mg L^{-1}. The seedlings were then placed in glass bottles containing distilled and sterile water that covered the root system of the seedlings with nozzles covered. The bottles were covered with their own covers and then left for 8-10 days, taking into account changing the water in the bottles every two or three days and removing the glass covers gradually. The reason for performing this step may be due to its significant effect in reducing the substances that the plant absorbed from the MS nutrient medium during its growth in it. This helps to dilute the cellular juice of the tissues, as water flows to the root cells according to the laws of diffusion (Taiz & Zeiger, 2006). It also contributes to the growth of the plant roots by making room for the plant roots to expand and for the transition from heterotrophic nutrition to autotrophic nutrition, that is, food manufacturing and maintaining the internal humidity of plants (Lakho et al., 2023; Sehrawat et al., 2016).

Figure 5. shows the process of hardening the plants by extracting them from the MS nutrient medium, washing them with distilled water (A), placing them in a solution containing the fungicide Elsa (B), and then placing them in a glass container containing distilled water (C).

The results in fig (6) show growing plantlets in plastic pots with dimensions of 15 x 15 cm . The plantlets were watered with sterile distilled water every 3-5 days while providing appropriate lighting. And the appropriate temperature and maintaining the required humidity in the atmosphere surrounding the plants by covering them with transparent plastic covers and leaving them in the acclimatization room. After 7-10 days, the covers were partially removed for three hours a day to gradually reduce the humidity around the plants, where this is considered important in increasing the efficiency of the stomatal system by working properly. Naturally, this increase in the efficiency of the roots in absorbing nutrients from the agricultural medium, which has a positive effect on the growth of the vegetative system. After 4 weeks, the covers were completely removed, as shown in Fig (7). The reason for this is due to providing the above appropriate conditions to increase the capacity of The plant relies on itself in manufacturing its own food to increase its bitterness Metabolic suppression in the formation of carbohydrates and proteins and increased cell division, and this in turn led to an increase in the general growth of acclimatized plants, as the plants at this stage become prepared for transfer to external conditions (Chandra et al., 2010), and these results are consistent with what was indicated by many studies (da Silva et al., 2017; Kadleček et al., 2001).

Figure 6. shows banana plants grown in plastic pots with a diameter of 10 cm and covered with plastic covers.

Figure 7. Completely removing the plastic covers from the plants

The results in Table (3) show the growth of banana plants in the stands containing sterilized soil composed of peatmoss and sand after 4-20 weeks of planting inside the acclimatization rooms. New leaves formed on the plant after 20 weeks and were significantly excelled on the other periods. 4, 8, 12, and 16 weeks. The number of leaves was 7.00 leaves $^{-1}$, the plant height was 53.50 cm, the average leaf width was 14.40 cm, and the leaf length was 25.57. The lowest average was obtained for the number of leaves, a leaf $^{-1}$, the plant height was cm, the leaf diameter and the leaf length were cm. During the period of 4 weeks of the acclimatization process, it reached 15.40, 2.67, 5.93, and 13.23, respectively. The reason for the increase in vegetative growth indicators of the banana plant may be due to the longer the incubation period, the greater the ability of the plant to rely on itself in manufacturing foodstuffs due to the increase in the root system. The plant adapted to the agricultural medium, which helped in the formation of new growth, as well as an increase of cell division, which led to height of the plant with an increase in the time period (Alzate Acevedo et al., 2021).

Table 3. shows some changes in the vegetative traits of banana plants after 20 weeks of acclimatization

Time period	Vegetative shoots height (cm)	leaf length (mm)	Number of leaves vegetative shoots-1	Sheet width (cm)
4weeks	15.40	13.23	.267	5.93
8weeks	23.73	14.07	3.00	9.37
12weeks	43.20	15.73	4.67	10.93
16weeks	50.37	20.30	6.33	11.40
20weeks	53.50	25.57	7.00	14.40

R-LSD P≥ 0.01	9.06	2.88	1.63	3.02

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