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Research Article

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Effects of different concentrations of IBA and NAA on performance of black mulberry (*Morus nigra* L.) stem cuttings

Othman Hassan Altahir, Elsadig Hassan Elsadig*

Sudan University of Science and Technology, Sudan

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***Corresponding Author:**
Elsadig Hassan Elsadig
E-mail: elsadighassan3000@hotmail.com

ABSTRACT

This study was conducted to evaluate the effects of a range of concentrations of indole-3-butric acid (IBA) and naphthalene acetic acid (NAA) on performance of Black Mulberry cuttings. The concentrations used were IBA (200, 400, 600) and (2000, 4000, 6000), NAA (200, 400, 600) and (2000, 4000, 6000). Two methods of dipping, quick dipping and slow dipping, were adopted. Hard wood cuttings were used. The highest leaf length was obtained by 200 ppm NAA and 600 ppm IBA. NAA 6000 ppm gave the highest number of rooted cuttings. The control gave the largest number of branches. No significant differences in leaf length and root length. As for the number of roots, the best result was given by 6000 ppm IBA.

KEYWORDS: IBA, NAA, Wood cuttings, Mulberry, Moraceae, *Morus*

INTRODUCTION

Black mulberry (*Morus nigra* L.) is a dicotyledonous plant which belongs to the family Moraceae, one of the important fruit trees. It is native to eastern and central China. It is grown under varied climate conditions ranging from temperate to tropical. Mulberry can tolerate wide range of soil types. Mulberry is widely distributed in regions ranging from temperate to sub-tropical, especially in Europe, Asia, North America and Africa (Singh & Bhargava, 2014). Mulberry is economically important and most esteem in China and India. The plants are generally deciduous; sunshine is one of significant factors controlling growth and leaf quality. The optimum soil Ph suitable for mulberry growth is 6.5 to 6.8 (Datta, 2002).

Mulberry is a woody perennial plant that grows rapidly and with extensive root system and simple mutually branches. Leaves can be singular or lobed (Acharya *et al.*, 2022).

Mulberries can be adapted to different enviromental conditions. There are more than 60 species of the genus *Morus* around the world. The varieties of mulberries are of economic importance for feeding silkworms (Acharya *et al.*, 2022).

Mulberry has medical and health importance, especially in eastern countries. Chinese people use juice of mulberry for controlling blood pressure and decreasing cholesterol (Chang *et al.*, 2011).

The plant is vigorously growing, short lived, small and medium in size and may reach ten meters in height (Karthik *et al.*, 2023).

Mulberry leaves are a source of nutrition for *Bomx Spp*. It is important to know the genetic makeup of mulberry to combine morden with traditional breeding techniques to increase manufacture and quality of the silk industry (Karthik *et al.*, 2023).

The famous species of the genus *Morus* which are renowned in Asia are white mulberry (*Morus alba*), black mulberry (*Morus nigra*), and red mulberry (*Morus rubra*) (Jan *et al.*, 2021). Mulberry fruit is sweet and it is eaten when ripe. Edible black mulberry fruits are large and of delicious juice.

Propagation of mulberries by seed is unsuitable because the plants will not be identical to the mother plant, it is heterozygous in nature and cross pollination is the rule in mulberry. Vegetative propagation methods are practiced in most fruit species, it is commercially easier, especially propagation by cuttings which is mostly used. Mulberry is usually propagated through cuttings in tropical and sub-tropical regions. Mulberry trees are used as wind to protect orchards (Datta, 2002).

Chemical hormones are effectively used to promote adventitious root formation in cuttings.

MATERIALS AND METHODS

Site of the Experiments

These experiments were conducted at the nursery of the Department of Horticulture, College of Agricultural Studies, Sudan University of Science Technology, Shambat (latitude 15-39 °N and longitude 32-39 °E).

The objective of the experiments was to study the effect of indole 3- butyric acid (IBA) and naphthalene acetic acid (NAA), at different concentrations, two methods of soaking and one type of stem cuttings (hard wood cutting), on performance of black mulberry stem cuttings.

Experimental Materials

The cuttings were taken from Shambat Research Station Field and the Nursery of the College. Branches growing vigorously, medium to large in size, from 8-years old trees and with spreading branches were used in the study.

Preparation of Cuttings

Black mulberry hardwood stem cuttings were taken from mature wood of the previous season's growth. At least five nodes were included in each cutting. Length of the cutting was 20 cm and thickness ranged from thick, 1.5 cm and medium, 0.7 cm to thin, 0.3 cm.

Soaking Methods

Slow-dip method (dilute solution)

The basal part (2.5 cm) of the cutting was soaked in dilute solution of IBA or NAA for 24 hours just before insertion into the rooting medium. Concentrations used for soaking the cuttings were: IBA, 200 ppm, 400 ppm, 600 ppm and NNA: 200 ppm, 400 ppm and 600 ppm). Cuttings were placed under shade in the nursery (Table 1).

This slow method is a cumbersome technique that is not commercially popular. Equipments are needed for soaking cuttings for long time, 24 and 48 hours.

Quick-dip method (concentrated solution)

In the quick-dip method, concentrations of solutions were as follows: IBA: 2000 ppm, 4000 ppm, 6000 ppm) and NNA: 2000 ppm, 4000 ppm, 6000 ppm. Duration of soaking or dipping was ten seconds (Table 2).

The basal portion of the cutting dipped in the solution for a short time, 10 seconds.

Table 1: Slow soaking

Concentration, ppm	type of growth hormone	Type of cuttings	Dip period hours
200	IBA	hard woodcutting	24
400	IBA	hard woodcutting	24
600	IBA	hardwood cutting	24
200	NAA	hardwood cutting	24
400	NAA	hardwood cutting	24
600	NAA	hard woodcutting	24

Table 2: Quick soaking

Concentration, ppm	Type of growth hormone	Type of cutting	Dip period second
2000	IBA	hard woodcutting	10
4000	IBA	hard wood cutting	10
6000	IBA	hard wood cutting	10
2000	NAA	hardwood cutting	10
4000	NAA	hard wood cutting	10
6000	NAA	hard wood cutting	10

Preparation of IBA and NAA

The concentrations were prepared as follows:
1 mg/L = 1 ppm
200 ppm = 0.2 gm/L
1 gm = 1000 mg
2000 ppm = 2000 mg/L = 2 gm/L
4000 ppm = 4 g/L

These solutions were prepared at the tissue culture laboratory, Sudan University of Science and Technology, Shambat. A precision balance was used to weigh the material and then dissolved in sodium hydro oxide (NaOH) and made to volume (1000 mL) with potable water.

In the second experiment, the same steps for preparation of indol-3-butyric acid (IBA) concentrations were also followed for naphthalene acetic acid (NAA).

Types of Hormones, Concentrations, Time of Dipping and Type of Cuttings

Duration of experiments

The duration of the experiments was 12 weeks.

Plastic bags

Plastic bags with dimension of about 50-40-30 cm with a number of drainage holes at the bottom were used. They were filled with a soil mixture of sand and silt (1:1). Each plastic bag contained 15 cuttings. The bags were kept under a lath house, 50% shade, and under a covered frame in the nursery.

Irrigation

Water was supplied by putting a hose under the bags, the cutting absorbed water through the holes at the bottom of the bags. The water was applied every other day. The pesticide (Apron star) was added with irrigation water to reduce pathogen infections.

Weeding

Shallow hand weeding was done when required.

Parameters

Experiment parameters measured included number of rooted cuttings, number and width of leaves, number and length of branches, number and length of roots (after three months).

Experimental Design and Statistical Analysis

The experimental units were in complete randomized block design. The data were analyzed using GenStat (computer program) version4 and the means were separated using Duncan's multiple Range Test (DMRT) at $p<0.05$ (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

The results depicted in Table 3 revealed that the concentration NAA 200 ppm gave the highest leaf width followed by IBA 600 ppm, while IBA 200 ppm gave the lowest result. The differences between all treatments were insignificant. Physiological response of application of auxin contributes to growth and development of cuttings. The insignificant result in this experiment might be due to inadequate hormone absorption by basal tissue of the cuttings because of low concentration since the effectiveness of auxin absorption depends on adequate absorption of tissues of cuttings to hormones and this depends on concentration and duration of dipping.

Sokhuma *et al.* (2018), using varying concentrations of NAA and NAA, conducted a study on Himalayan mulberry. They found that IBA 500 ppm gave 3.75 cm leaf width while NAA 500 ppm gave 3.71 leaf width. Their findings are closer in low concentrations to our results.

The results depicted in Table 4 reflect that the NAA 2000 ppm gave the highest leaf width followed by NAA 4000 ppm and the least result was by NAA 6000 ppm which is significantly different from all treatments and the control. This may indicate that these high concentrations of hormones were ineffective on leaf width and this may be due to the short time of soaking which didn't allow for adequate absorption. These results are comparable with those of (Sokhuma *et al.*, 2018) who used IBA concentrations 1000, 2000, 3000 ppm on Himalayan mulberry. They obtained the best result by IBA 2000 ppm.

The results presented in Table 5 show that the concentration IBA 600 ppm gave taller branches on the cuttings followed by NAA 200 ppm compared with the other concentrations. The differences between all treatments were insignificant.

As depicted in Table 6 the lengths of branches of cuttings were highest due to treatment with IBA 2000 ppm, followed by NAA 2000 ppm. Vasanth *et al.* (2024) used 10 concentrations, five for IBA and five for NAA on *Morus sinesis*. They found that the concentration 5000 ppm resulted in the highest length of the shoot, 22.20 cm, while NAA 5000 ppm recorded 18 cm. These results are in agreement with those of Kako (2011) who stated that IBA significantly increased length of shoot. Sokhuma *et al.* (2018) studied the effect of 4 different concentrations (500, 1000, 2000, and 3000 ppm) of IBA and NAA and recorded that the best result of shoot length of Himalayan mulberry stem cuttings was due to IBA 3000 ppm, while NAA recorded the lowest result.

The results depicted in Table 7 on the number of rooted cuttings show that the concentration which gave the best result was NAA 200 ppm. All results were insignificantly different. This indicates that the low concentrations of these hormones were ineffective in promoting the number of rooted cuttings. These results were in contrast to the findings of Macdonald (1986) who reported that low concentrations of IBA 200 ppm and NAA 300 ppm promoted rooting of apple cuttings taken in early spring. He also stated that many plant growth regulators specifically auxins have the ability to cause an effective impact

Table 3: Effects of low concentrations and slow soaking of IBA and NAA on leaf width

Concentrations	Leaf width	Time of soaking
1-IBA 200ppm	2.71[a]	24 hour
2-IBA 400 ppm	2.48[a]	24 hour
3-IBA 600 ppm	3.06[a]	24 hour
4-NAA 200 ppm	3.12[a]	24 hour
5-NAA 400 ppm	2.87[a]	24 hour
6-NAA600 ppm	2.75[a]	24 hour
7-control	2.93[a]	
C.V%	11.7	

Table 4: Effect of high concentrations of IBA and NAA, quick dipping on width of leaf

Concentration	Width leaf	Time of dipping
1-IBA 2000 ppm	2.99[a]	10 sec
2-IBA 4000 ppm	2.91[a]	10 sec
3-IBA 6000 ppm	2.88[a]	10 sec
4-NAA 2000 ppm	3.07[a]	10 sec
5-NAA 4000 ppm	3.00[a]	10 sec
6-NAA 6000 ppm	1.61[b]	10 sec
Control	2.93[a]	
c.v%	11.7	

Table 5: Effects of low concentrations and slow dipping on length of branches

Concentration	Length of branches	Time of soaking
1-IBA 200 ppm	7.91[a]	24 hour
2-IBA 400 ppm	8.38[a]	24 hour
3-IBA 600 ppm	10.21[a]	24 hour
4-NAA 200 ppm	10.16[a]	24 hour
5-NAA 400 ppm	8.54[a]	24 hour
6-NAA 600 ppm	7.45[a]	24 hour
Control	9.22[a]	
c.v %	16.5	

Table 6: Effects of high concentrations and quick dipping on length of branches

Concentration	Length of branches	Time of soaking
1-IBA 2000 ppm	9.61[a]	10 sec
2-IBA 4000 ppm	9.41[a]	10 sec
3-IBA 6000 ppm	8.41[a]	10 sec
4-NAA 2000 ppm	9.51[a]	10 sec
5-NAA 4000 ppm	9.18[a]	10 sec
6-NAA 6000 ppm	4.89[b]	10 sec
Control	9.22[a]	
CV%	16.5	

Table 7: Effects of low concentrations and slow dipping on number of rooted cuttings

Concentration	Number of rooted cuttings	Time of soaking
1-IBA 200 ppm	2.65[ab]	24 hours
2-IBA 400 ppm	2.57[ab]	24 hours
3-IBA 600 ppm	2.56[ab]	24 hours
4-NAA 200 ppm	2.82[a]	24 hours
5-NAA 400 ppm	2.17[ab]	24 hours
6-NAA 600 ppm	2.16[ab]	24 hours
Control	2.72[ab]	
C.V%	17.3	

on adventitious root formation at different concentrations. The best result is affected by the types of cutting, auxin and species. Wounding materials accumulate at the base of the stem cutting and stimulate rooting giving more opportunity for the success of the rooting process. The activity at this period becomes more efficient depending on wounding linked by bio material and auxin level.

As depicted in Table 8, the number of rooted cuttings was higher due to the treatment IBA 6000 ppm. This finding is in conformity with Kalyoncu *et al.* (2009) who found, in their study on the effects of humidity level and IBA application on soft wood cuttings of black mulberry, that IBA 5000 ppm gave the best result and other studies indicated that highest rooting ratio was by IBA 6000 ppm. There is a relationship between capacity of rooting and sclerenchyma tissue. Beakbane (1961) found that sclerenchyma tissue in the phloem of some plant cuttings might *restrict* root initials formation on cuttings.

The results shown in Table 9 revealed significant differences between low concentrations and slow dipping of IBA and NAA treatments on one side and the control on the other side, all lower than the control. No significant differences between the treatments. The best results were NAA 200, 400 ppm and the control.

Table 10 depicted the effects of high concentrations of hormones on the number of branches. The best result obtained by the control followed by treatment with IBA 2000 ppm. This indicated that IBA had no significant differences from the control while NAA had significant negative effects on number of branches. This finding is in conformity with Pandey and Bisen (2010) who reported that there are some factors, which control branching, play, perhaps, a major role in growth response in cuttings.These factors include endogenous auxin, phenols, carbohydrates and other materials that promote cell division and growth.

Table 8: Effects of high concentrations and quick dipping on number of rooted cuttings

Concentration	Number of rooted cuttings	Time of soaking
1-IBA 2000 ppm	2.46[ab]	10 sec
2-IBA 4000 ppm	2.37[ab]	10 sec
3-IBA 6000 ppm	2.65[a]	10 sec
4-NAA 2000 ppm	1.98[b]	10 sec
5-NAA 4000 ppm	2.39[ab]	10 sec
6-NAA 6000 ppm	1.27[c]	10 sec
Control	2.72	
C.V%	17.3	

Table 9: Effects of low concentrations and slow dipping on number of branches

Concentration	Number of branches	Time of soaking
1-IBA 200 ppm	2.93[abc]	24 hour
2-IBA 400 ppm	2.68[abc]	24 hour
3-IBA 600 ppm	2.74[abc]	24 hour
4-NAA 200 ppm	3.26[ab]	24 hour
5-NAA 400 ppm	3.26[ab]	24 hour
6-NAA 600 ppm	2.65[abc]	24 hour
Control	3.40[a]	
C.V%	20.5	

Table 10: Effects of high concentrations and quick dipping on number of branches

Concentration	Number of branches	Time of soaking
1-IBA 2000 ppm	3.25[ab]	10 sec
2-IBA 4000 ppm	2.67[abc]	10 sec
3-IBA 6000 ppm	3.04[abc]	10 sec
4-NAA 2000 ppm	2.08[cd]	10 sec
5-NAA 4000 ppm	3.01[cd]	10 sec
6-NAA 6000 ppm	1.60[d]	10 sec
Control	3.40[a]	
C.V%	20.5	

The data depicted in Table 11 revealed that the best result in this experiment was obtained by the control followed by treatment with NAA 400 ppm. This indicated that NAA 400 ppm gave the highest number of leaves but all treatments had no significant differences from the control and hence low concentrations of hormones did not affect the of number of leaves.

The results shown in Table 12 indicated that the highest number of leaves on cutting was due to the treatment IBA 2000 ppm. This finding is in conformity with Regimental *et al.* (2022) who obtained highest number of leaves on *Morus alba* when treated with IBA 2000 ppm compared with varying concentrations of IBA. In this study all treatments had no significant differences from the control.

Sokhuma *et al.* (2018), studying the effects of IBA and NAA on the number of leaves on Himalayan mulberry stem cuttings, found that the concentration IBA 3000 ppm was superior, while NAA was lower than the control.

Table 11: Effects of low concentrations and slow dipping on number of leaves

Concentration	Number of leaves	Time of soaking
1-IBA 200 ppm	2.91[ab]	24 hour
2-IBA 400 ppm	2.63[ab]	24 hour
3-IBA 600 ppm	2.82[ab]	24 hour
4-NAA 200 ppm	2.80[ab]	24 hour
5-NAA 400 ppm	3.11[ab]	24 hour
6-NAA 600 ppm	2.11[ab]	24 hour
Control	3.01[ab]	
C.V%	19.9	

Table I2: Effects of high concentrations and quick dipping on number of leaves

Concentration	Number of leaves	Time of soaking
1-IBA 2000 ppm	3.48[a]	10 sec
2-IBA 4000 ppm	3.19[ab]	10 sec
3-IBA 6000p pm	2.48[ab]	10 sec
4- NAA 2000 ppm	3.27[a]	10 sec
5-NAA 4000 ppm	3.04[ab]	10 sec
6-NAA 6000 ppm	2.11[b]	10 sec
Control	3.01[ab]	
C.V%	19.9	

Table 13: Effects of low concentrations and slow dipping on length of roots

Concentration	Length of roots	Time of soaking
1-IBA 200 ppm	4.94[a]	24 hour
2-IBA 400 ppm	5.24[a]	24 hour
3-IBA 600 ppm	5.07[a]	24 hour
4-NAA 200 ppm	5.04[a]	24 hour
5-NAA 400 ppm	4.88[a]	24 hour
6-NAA 600 ppm	5.79[a]	24 hour
Control	5.13[a]	
CV%	17.3	

As depicted in Table 13, the highest length of roots was obtained by NAA 600 ppm followed by IBA 400 ppm. There were no significant differences between all treatments. This showed that the low concentrations and slow dipping had no significant effect on length of roots.

The results shown in table (14) indicated no significant differences in root length between all concentrations except NAA 6000ppm which gave significantly lower result than all treatments.

The results presented in Table 15 indicated that the highest number of roots was obtained by NAA 400 ppm followed by IBA 600 ppm. There were no significant differences between the treatments. This shows that the low concentrations did not enhance number of roots of the cuttings because other factors were involved.

The results depicted in Table 16 revealed significant differences between IBA and NAA treatments. IBA 6000ppm gave the highest number of roots. This result is in agreement with Salah El den *et al.* (2023) who reported that the highest rooting of evaluated cultivar mulberry was due to treatment with IBA 6000 ppm.

Table 14: Effects of high concentrations of hormones and quick dipping on length of roots

Concentration	Length of root	Time of soaking
1-IBA 2000 ppm	5.00[a]	10 sec
2-IBA 4000 ppm	4.68[a]	10 sec
3-IBA 6000 ppm	4.89[a]	10 sec
4-NAA 2000 ppm	5.10[a]	10 sec
5-NAA 4000 ppm	5.10[a]	10 sec
6-NAA 6000 ppm	3.00[b]	10 sec
Control	5.13[a]	
CV%	17.3	

Table 15: Effects of low concentrations of hormones and slow dipping on number of roots

Concentration	Number of roots	Time of soaking
1-IBA 200 ppm	3.08[c]	24 hour
2-IBA 400 ppm	3.79[abc]	24 hour
3-IBA 600 ppm	4.49[abc]	24 hour
4-NAA 200 ppm	3.80[abc]	24 hour
5-NAA 400 ppm	4.57[abc]	24 hour
6-NAA 600 ppm	3.44[c]	24 hour
Control	3.21[c]	
CV%	25.4	

Table 16: Effects high concentrations of hormones and quick dipping on number of roots

Concentration	Number of roots	Time of soaking
1-IBA 2000 ppm	4.53[abc]	10 sec
2-IBA 4000 ppm	5.69[a]	10 sec
3-IBA 6000 ppm	5.84[a]	10 sec
4-NAA 2000 ppm	3.56[bc]	10 sec
5-NAA 4000 ppm	3.11[c]	10 sec
6-NAA 6000 ppm	2.20[c]	10 sec
Control	3.21[c]	
CV%	25.4	

Endogenous factors in hard wood cuttings together with external factors overcome barriers and promote growth of cuttings.

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