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INFLUENCE OF AUXINS ON ROOTING STEM CUTTINGS OF *MORINGA OLEIFERA* LAM IN DRY AND WET SEASONS

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Abstract

The study was designed to evaluate the influence of three types of auxins: Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and, Indole Acetic Acid (IAA) at four concentrations (0mg/l, 100mg/l, 300mg/l and 500mg/l) and two seasons (dry and wet) on rooting mature stem cuttings of *Moringa oleifera.* The experiment was 3×4×2 factorial laid in a completely randomized design with three replicates. The findings of this study show that, *M oleifera* can be propagated vegetatively by rooting the stem cuttings with auxins. The results of the developmental phases of the stem cuttings prior to rooting significantly (P>0.05) showed variations due to auxin concentrations and seasons. Stem cuttings treated with the higher concentration of the three auxins (500ppm of IBA, NAA and IAA) showed that wet season stem cuttings sprouted earlier (18-27 days after planting, DAP) than the dry season stem cuttings (24-30 DAP). The results also showed that auxin application and seasons influenced higher bud development into leaves in the wet than in the dry season (2.0% - 83.4% wet season and, 10% - 56.08% dry season). It was also observed that, untreated (control) stem cuttings and those treated with 100mg/l and 300mg/l did not root in the dry season trial. While lateral roots were observed on stem cuttings across the treatments in the wet season, only those treated with 500mg/l of the auxins produced tap roots with IAA proving most effective. Therefore, rooting stem cuttings of *M. oleifera* with 500mg/l of IAA during the wet season is recommended.

Keywords: *Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), Moringa oleifera, Dry Season and Wet Season.*

INTRODUCTION

Moringa oleifera is a multipotential plant that yields industrial raw materials from all its component parts (leaves, seed, pod, flower, bark and root). Price (2007) reported that *M. oleifera* contains thirty-two (32) chemical substances of nutritional value in its different parts. Of great interest is that, *Moringa* leaves and pods have very high protein (Price, 2007); hence the consumption of these parts of the plant can help combat malnutrition, being a cheap source of protein.

Juicy leaf extracts of *M oleifera,* has also been reported to contain plant growth hormone of the cytokinin group. Price (2007) observed that spraying *Moringa* hormone on the seedlings of maize, bell-pepper, onion, sorghum, coffee and chili-melon significantly increased yield. The same author further reported that a formula composed of 40 - 50% *Moringa* leaves increase milk yield of dairy cow and daily weight gain of cattle by 30%. Rajangam *et al.* (2001) and Fahey (2005) gave a detailed account of the uses of *Moringa* leaves, leaf powder, and the seed powder in herbal medicine. Price (2007) also gave detailed account of water purification and sterilization by *Moringa* leaf, pod and seed powder. He isolated polyelectrolytes from *Moringa* leaves, pod and seed powder and reported that the chemical is the species' active ingredient responsible for water treatment. Rajangam *et al.* (2001) reported the presence of an antibiotic (Pterygospermina) which has powerful antibacterial and antifungal activities. The

chemical was isolated from the flower and bark of *Moringa oleifera* plant. Consequently, due to the high multipotential nature of *Moringa oleifera*, *it* is highly sought after in most countries today. The pods, leaves and seeds are sold raw or canned to generate revenue and for wider distribution and utilization (Rajangam *et al.*, 2001).

Plants propagation is one of man's oldest occupation (Singh, 2004). Hartmann and Kerster (1983) reported that planting materials can be produced in two broad ways: sexually by the seeds and asexually by vegetative parts of plants. In the present day context of conservation of useful plant species, availability of larger quantity of uniform growing seedlings is very important in large scale farm establishment, because the plant products can sustain biotechnological exploitation (Puri,1990). Several reports have shown that seeds of *M oleifera* have no germination problems; that is, the seed germinates readily, but the seedlings take long to attain reproductive phase (Anbarassan *et al.*, 2001; Foidl *et al.*, 2001; Rajangam *et al.*, 2001). Price (2007) reported that the species flowers and fruits once a year; implying that efforts should be intensified on finding other methods of propagating the species when the species fruits and pods are out of season.

Auxins, a group of growth regulating substances are recognized as phyto-hormones. These play very important role in co-ordinating many growth and developmental processes in plant life cycles (Griffith, 1940). IAA, IBA and NAA are popularly used auxins in vegetative propagation of plant by rooting their stem cuttings or marcorting. Therefore, in order to ensure availability of seedlings, especially when the seeds are lacking or out of season as commonly observed in the dry seasons, this research focused on assessing the influence of IAA, IBA and NAA on rooting stem cuttings of *M. oleifera* in both dry and wet seasons.

MATERIALS AND METHODS

Experimental Site and Collection of Planting Materials

The study was conducted under shade in the Botanical Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka (UNN) during December 2011 to April 2012 (dry season trial) and May to September 2012 (wet season trial). Branches (current years' growth) of the specie were obtained from the trees growing in the staff quarters of UNN in the first week of each season.

Experimental Design

The experiment, a 3x4x2 factorial, in Completely Randomized Design (CRD) was made up of four main treatments; (auxins) and no auxin (control), three sub-effects/auxin concentrations (0mg/l, 100mg/l, 300mg/l, 500mg/l) and two seasons (wet and dry).

Preparation of Plant Materials for Planting

The branches of *M. oleifera* were defoliated, after which 720 stem cuttings (26-28cm long) bearing 4-5 buds were obtained. The stem cuttings were divided into four batches. The first batch of 180 was planted without auxin treatment (the control). The other three batches (180 each) were allocated to three auxins concentrations (IBA, NAA and IAA at 100mg/l, 300mg/l and 500 mg/l each) under investigation. Each batch of stem cuttings allocated for auxins treatment were subdivided into three lots of 60 stem cuttings; after which each stem cutting lot was tied with a twine, before introduction into its appropriate auxin concentration (soaking) for 24 hours in an airy laboratory to allow the woody stem cutting to sufficiently absorb the auxins.

Data Analysis

The data obtained on days to bud sprouting, number of buds that sprouted per stem cutting, root parameters (percentage and mean root length) were

subjected to the analysis of variance (ANOVA) and the means were separated using New Duncan Multiple Range Test (NDMRT) (Gomez and 1984).

RESULTS AND DISCUSSION

Table 1 showed that, across treatments in the dry season, the period of initial buds sprouting are within 24 to 35 days after planting (DAP). The results also showed that, in both seasons (dry and wet), stem cuttings treated with the highest concentration (500mg/l) of auxins: IBA, NAA and IAA, respectively sprouted buds within 24 to 30 Days After Planting (DAP); earlier than stem cuttings treated with lower concentrations (100mg/l and 300mg/l) of the same auxins (28 – 33 DAP). Untreated stem cuttings (control), took the longest time to sprout buds (28 – 35 DAP) (Table 1). It is however notable that, across treatments, bud sprouting occurred much earlier in the wet season than in the dry season. Again, the mean performances shows that stem cuttings treated with 500mg/l of IBA and IAA were significantly (P>0.05) different from the other treatments in initiating bud sprouting, even though, 500mg/l of IAA was most outstanding. However, in the wet season, during the final bud sprouting at all auxin concentrations, time of sprouting was statistically at par except when IAA was applied at the highest rate.

Development of the buds that sprouted on the stem cuttings of *M. oleifera* into leaves was observed within 6 – 7 days after sprouting. Table 2 shows that, in the dry season, the percentage of buds that developed into leaves ranged from 10.50% to 56.08%. The result also shows that stem cuttings treated with 500mg/l of IBA and IAA were highly and significantly (P>0.05) different from the other treatments in inducing development of buds into photosynthesizing leaves. Even though, higher percentage of leaves developed from buds in the wet season, a similar trend was observed with that of the dry season as stem cuttings treated with 500mg/l of the auxins performed better. Similarly, bud development into leaves on the untreated (control) stem cuttings was higher in the wet season than in the dry season (Table 2).

Several authors (Puri, 1990; Awoleye, 1991; Bassil *et al.,* 1991 and Nzekwe, 2002) have reported that prior to rooting, bud sprouting on stem cuttings occur first. Bud sprouting on stem cuttings is a sign of resumption of metabolic activities on stem cuttings. The results presented in Tables 1 and 2 shows that bud sprouting and bud development into leaves on stem cuttings of *M. oleifera* was more prolific in the wet season than in the dry season. The observed variation agrees with earlier reports presented by Araya *et al.,* 2007 and Puffy *et al.,* 2008. This variation can also be attributed to the wet season providing better enabling environment (most likely, sufficient moisture and adequate temperature) for bud sprouting and subsequently leaves development. The possibility of water stress in the dry season may have on the other hand been responsible for limited bud sprouting in the dry season.

In the dry season, stem cuttings treated with lower concentration of the auxins (100mg/l and 300mg/l) did not survive and hence did not root (Tables 3 and 4). The result however showed that high concentration (500mg/l) of the auxins resulted in low percentage rooting of the stem cuttings ranging from 11% to 15% with IAA performing most and NAA, least (Table 3). In the wet season however, the control and the low concentrations of the three auxins (100mg/l and 300mg/l) induced rooting on stem cuttings ranging from 5% to 42% with IAA also proving more effective than NAA and IBA (Table 3). Comparatively, the results showed that rooting of stem cuttings of *M. oleifera* was better in the wet than in the dry season; in that in the wet season, percentage rooting of the stem cuttings were higher than that of the dry season, even at low auxin

concentrations (100mg/l and 30mg/l) and the control.

In the dry season; the control, 100mg/l and 300mg/l of the three auxins respectively, did not induce the production of either lateral or tap root systems on the stem cuttings (Tables 3 and 4). However, 500mg/l of the auxins produced lateral roots only which ranged from 4.5cm to 7.0cm with that produced by IAA (averaging 7.0cm) being significantly ($P<0.05$) different from those produced by IBA and NAA (Table 4). On the other hand, in the wet season, lateral roots were produced on stem cuttings irrespective of treatments. The mean lateral root length ranged from 3.0cm to 8.0cm with the longest (8.0cm) recorded on stem cuttings treated with 500mg/l of IAA. The results also showed that, in the wet season, no tap root was produced on control and stem cuttings treated with 100mg/l and 300mg/l of the three auxins used. However, tap roots were were produced on stem cuttings treated with 500mg/l of the auxins with the longest (17.0cm) on IAA treated roots and the least (10.0cm) on NAA treated stem cuttings.

The prolific rooting of the stem cuttings of *M. oleifera* in the wet season as observed in this research is in line with the findings of Bassil *et al.,* 1991 and Nzekwe, 2002. This can be attributed to a better enabling environment in the wet season. The observation of higher rooting on stem cuttings treated with the highest concentration (500mg/l) of the three auxins used in this research is in line with earlier reports that suggests that, stem cuttings of woody plants, root when adequate auxin concentration are applied (Hartman and Kerster, 1983; Erez, 1984; Ofori *et al.,* 1996; Klein *et al.,* 2000; Araya *et al*., 2007 and Puffy *et al.,* 2008). This could also be attributed to the more number of leaves formed that might have helped in photosynthesis that have assisted in early and proper root development.

The results also showed that within the auxins (IBA, NAA and IAA), the highest percentage rooting response (75%) was achieved when the stem cuttings were treated with 500mg/l of IAA, with NAA being the least (48%). This implies that, IAA was more active in influencing rooting on stem cuttings of *M. oleifera* than IBA and NAA in that order. While some workers, in variance with the present findings, have reported that IBA is more effective than NAA and IAA (Hartman and Kerster, 1983; Proebsting, 1984; Araya *et al*., 2007), the findings in this research agrees with that of Griffith (1940) that showed that IAA was more effective than IBA and NAA in rooting stem cuttings of Douglas fir. Since the seeds of the species have been reported to have no germination problem, vegetatively propagating the species by rooting the stem cuttings could spare the seeds for industrial exploitation, while large number of uniform seedlings needed for the conservation of the species can be produced by rooting the stem cuttings.

CONCLUSION

The findings of this study showed that, *Moringa oleifera* can be propagated vegetatively by rooting the stem cuttings. It also shows that 500mg/l of three applied auxins was adequate in influencing rooting on the stem cuttings of *M. oleifera*. However, while it is recommended that other concentrations of the auxins could be assessed in further studies, the overall performance of 500mg/l of IAA as observed in this research, suggest that it can be used in propagating the stem cuttings of *M. oleifera*.

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Table 1: Effect of auxin concentrations on time of bud sprouting from stem cuttings of *M. oleifera* in dry and wet seasons

	Dry Season (December 2011 to April 2012)		Wet Season (May to September 2012)	
Treatments	Initial Bud Sprouting (DAP)	Final Bud Sprouting (DAP)	Initial Bud Sprouting (DAP)	Final Bud Sprouting (DAP)
IBA (mg/l)				
0	34a	36ab	28a	30a
100	32ab	35bc	25ab	28a
300	30bc	33b	23bc	27a
500	27d	31b	18cd	21ab
NAA (mg/l)				
0	35a	38a	28a	31a
100	33a	34b	27a	30a
300	32ab	33b	25ab	28a
500	30bc	32b	23bc	27a
IAA (mg/l)				
0	34a	37a	28a	30a
100	30bc	33b	24bc	27a
300	28c	30bc	20c	24ab
500	24d	28c	16d	18b
Mean	30.07	33.33	23.75	22.28
SE(±)	3.11	2.78	3.82	9.38
CV(%)	0.10	0.08	0.16	0.02

Figures followed by the same letter(s) along the vertical column are not significantly different ($P<0.05$)

DAP – Days after Planting.

Table 2: Effects of auxin concentrations on bud development into leaves on the stem cuttings of *M. oleifera* in two seasons

Treatments	Development of Leaves from Buds (%) Dry Season	Development of Leaves from Buds (%) Wet Season
IBA (mg/l)		
0	13.20d	16.50d
100	14.20d	24.50d
300	32.20b	42.70cd
500	50.70a	78.50ab
NAA (mg/l)		
0	10.00d	12.00d
100	13.50d	18.30d
300	27.80b	27.90d
500	42.50ab	52.60bc
IAA (mg/l)		
0	10.50d	18.00d
100	16.30cd	25.30d
300	23.40bc	62.08bc
500	56.08a	83.40a
Mean	25.90	38.53
SE(±)	15.62	24.00

Figures followed by the same letter(s) along the vertical column are not significantly different (P > 0.05).

Table 3: Effect of Auxins and Seasons on Percentage Rooting of M. oleifera Stem Cuttings

Treatments	Rooting of Stem Cuttings (%) Dry Season	Wet Season
IBA (mg/l)		
0	00	05
100	00	16
300	00	28
500	13	65
NAA (mg/l)	00	00
0	00	06
100	00	14
300	00	25
500	11	48
IAA (mg/l)		
0	00	05
100	00	20
300	00	42
500	15	75
Mean	3.25	29.03
SE(±)	1.24	1.57

Table 4: Effect of auxins on types and length of roots developed

	Dry Season		Wet Season	
Treatments	Mean of Tap Root Length (cm)	Mean Lateral Root Length (cm)	Mean of Tap Root Length (cm)	Mean Lateral Root Length (cm)
IBA (mg/l)				
0	-	-	-	3.2c
100	-	-	-	4.5bc
300	-	-	-	6.7ab
500	-	5.2b	15.5a	7.2a
NAA (mg/l)				
0	-	-	-	3.0c
100	-	-	-	4.0c
300	-	-	-	6.0b
500	-	4.5b	10.0b	7.0ab
IAA (mg/l)				
0	-	-	-	3.0c
100	-	-	-	5.2bc
300	-	-	-	6.8ab
500	-	7.0a	17.0a	8.0a
Mean		5.5	14.17	5.38
SE(±)		1.08	3.00	1.73

Figures followed by the same letter(s) along the vertical column are not significantly different (P>0.05)