EFFECT OF VARYING CONCENTRATION OF AUXINS AND STEM LENGTH ON GROWTH AND DEVELOPMENT OF *Jatropha curcas* L.

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Abstract

A study was carried out to evaluate the effect of varying hormonal treatments and length of cuttings of clonal materials on the sprouting and rooting abilities of Jatropha curcas L. Two stem cutting lengths: 30cm and 60cm were pre-treated with two types of auxins: Naphthalene Acetic Acid (NAA) and Indole-3-Butyric Acid (IBA) at four concentration levels: 0 (control), 100, 150 and 200mgl^{-1}. The experiment was a factorial laid as randomized complete block design. Results showed that cutting length and hormone concentration had significant effect (P<0.05) on all parameters. The 60cm cuttings performed better than the 30cm cuttings in terms of sprouting, rooting and plant biomass accumulation. The untreated cuttings (control) showed better development than those treated with NAA and IBA. Significant interaction effects were observed between the cutting length and hormone concentration for all parameters (p<0.05). The 60cm cuttings performed better when untreated with hormone while 30cm cuttings performed best when treated with IBA at 200mgl^{-1}. Hence, 60 cm untreated cuttings could probably be used for large scale propagation of J. curcas while 30cm cuttings required hormonal treatment for high performance.

Keywords*: Jatropha, cuttings, auxins, stem length, concentration.*

Introduction

Jatropha curcas L. is a small sized perennial having xerophytic adaptation (Kathiravan*et al*., 2009). It is found growing on uncultivated lands in most parts of Africa. It is a multipurpose plant valued not only for its' medicinal properties and resistance to various stresses but also for its use as an oil seed crop (Heller, 1996; Staubmann, 1999; Openshaw, 2000). It has emerged as the best potential biodiesel crop alternative to petrol diesel (Kou and Chou, 2007; Tint and Mya, 2009; Reinhard, 2007; Belewu*et al.,* 2010). Upon realizing the importance of this crop as source of biodiesel, most countries and states particularly India and most recently, some West and Central Africa countries have adopted the massive cultivation of the crop. The plant is found to incur little or no carbon debt hence it offers immediate and sustained greenhouse advantage and militating against climate change (Becker and Makkar, 2009). Currently Nigerian government has shown great interest in *Jatropha* plant and other biofuel plants. The aim of the government is to gradually reduce the nation's dependence on imported gasoline, reduce environmental pollution as well as create commercially viable industry that can precipitate domestic job (*http://www.jatrophaworld.org;* Federal Government of Nigeria Policy on Bio-fuel, 2008).

The success of the crop and its performance over the years, have been attributed to its method of propagation. In perennials, vegetative propagation is highly preferred for most desirable qualities such as vegetative growth pattern, fruit characteristics, seed quality and oil content (Hartmann and Kester (1983). *Jatropha* can be propagated generatively using seeds and vegetatively using cuttings. While the former leads to genetic variability and makes the plant more prone to diseases, vegetative propagation results in development of true to type and disease free cultivars (Nanda and Kochhar *et al*., 1987; Kochhar*et al*., 2008). In vegetative propagation of *Jatroph acurcas*, there may be the need to look at ways by which optimum growth could be obtained. The performance of

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stem cuttings may depend on the length of cuttings and pre-treatment of such cuttings using growth hormones like indole-3-Acetic Acid (IAA), Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA).

Henning (2003) reported that longer cuttings were amenable for vegetative propagation than shorter cuttings. Pal (1995) also reported that the sprouting behaviours of cuttings depend on factors like the physiological age of the cutting, length and genotype of the mother plant material. Maya *et al*., (2010) showed that varying concentrations of growth hormones applied to *J. curcas* cuttings resulted in corresponding variations in the survival, sprouting and rooting of such cuttings. In spite of the above, there is a dearth of information on the optimum dose and suitable cutting length that best favour vegetative propagation of the Nigerian *J. curcas*. This study therefore aims at evaluating the effect of cutting length and hormone concentration on the growth and development of *J. curcas*. It also aims at determining the optimum stem length and hormone concentration for optimal plant vigor and development.

Materials and Methods

Stem cuttings from *J. curcas* were collected from the lateral branches of mature plants growing in the wild. These were made into cuttings of two lengths: 30 and 60cm using a meter measuring tape. The cuttings were pre-treated with two types of growth hormone before planting: IBA and NAA at four concentrations which include : 0, 100, 150 and 200 mgl^{-1}. Solutions of NAA and IBA were prepared by dissolving each separately in 10-15ml of ethanol and diluted using distilled water to obtain a crystal clear solution. The experiment was a 2 x 4 factorial laid out as randomized complete block with three replications. Planting was done on the field on thoroughly prepared raised beds enriched with organic manure, at a time when the rain has been well established. Manual weeding was done twice at 3^{rd} and 7^{th} weeks after planting. Plots were irrigated when necessary to supplement rain water.

Data were collected on survival parameters, sprouting behaviour, rooting behaviour and total plant biomass parameters respectively.Survival Parametersincluded the following: survival percentage, percentage termite infestation and percentage stem rot at 30 days after planting. Parameters assessed on sprouting behaviour included number of cuttings that sprouted, mean bud length and mean number of leaves per plant taken at intervals of 2 weeks after planting (WAP). Rooting Behaviourwas assessed 8 weeks after planting which included rooting percentage, mean number of roots/cutting; mean root length per cutting and quality of root formed. Root quality was assessed qualitatively based on the level of root vigour as observed from its appearance. Yield parametersincludedplantfresh weight and plant biomass at 8 weeks after planting. Data obtained were subjected to Analysis of Variance using Genstat 5 (Release 3.2). Significant means were separated using the Least Significant Difference (LSD) Test at a significance level of $p<0.05$**.**Performance ratings for the survival parameters were analyzed and graded as shown below:

Survival Percentage & Stem Rot Percentages	Termite Infestation
1 ---0-20%	1---80-100%
2---20-40%	2---60-80%
3--- 40-60%	3---40-60%
4--- 60-80%	4---20-40%
5--- 80-100%	5---0-20%

Performance ratings for the rooting parameters were graded as shown below:

Percentage of cuttings that rooted	Number of roots & Lengthof roots
1---0-20%	1---0-5
2---20-40%	2---6-10
3---40-60%	3---11-15
4---60-80%	4---16-20
5---80-100%	

Results

Survival Parameters

Table 1 showed the mean values for survival parameters of *J. curcas* at 30 days after planting. Cutting length had significant effect on percentage survival, termite

infestation and stem rot at $p < 0.05$. Survival percentage ranged from 52.7% obtained from 30cm cuttings to 34.5% as found in 60cm cuttings. Cuttings of 30cm length had the highest termite infestation (22.5%) while 60cm cuttings had the least (14.7%). This may probably be due to the high number of survived plants for the 30cm cuttings. The 30cm cuttings had the least stem rot (23.4%) while the 60cm cuttings had the highest stem rot (46.0%). Stem rot is likely to be caused by fungal growth on the stems which appeared more pronounced on longer stem cuttings. Concentration of hormone had significant effect on all survival parameters except survival percentage at $p<0.05$. Cuttings treated with $150mgl^{-1}$hormone had highest termite infestation (30.4%) while the least (11.0%) was observed for the untreated cuttings ($0mgl^{-1}$. This probably indicates that growth hormone does not solely control the survival of *J. curcas* since the untreated cuttings performed best. Percentage stem rot ranged from 9.2 (as found among the control) to 52.7 found among cuttings treated with $150mgl^{-1}$ hormone. The better performance of the untreated cuttings probably showed that vegetative propagation of *J.curcas* is possible without the use of growth hormones as previously reported by Narin and Watna (1983)and supported by Aminul-Islam *et al.* (2010).

Table 2 showed mean values obtained from the interaction effect of cutting lengths and hormone concentrationson survival parameters of *J.curcas.*Significant interaction effects were observed between cutting length and hormone concentration for survival percentage, percentage termite infestation and stem rot at $p<0.05$. 60cm cuttings untreated with hormone had the best performance while 30cm cuttings treated with hormone at $150mgl^{-1}$ was best in terms of survival and 30cm cutting treated with hormone at $200mgl^{-1}$ was best in terms of stem rot and termite infestation.

Sprouting Parameters

Table 3 showed the mean values of sprouting parameters of *Jatropha curcas* at 75 days after planting. Cutting length had significant effect on number of buds that sprouted /cutting and mean bud length and not on number of leaves /cutting at $p < 0.05$. The 60cm stem cuttings had the highest number of bud sprouts/cutting (10.0) while 30cm cuttings had the least number of bud sprouts (7.0). The highest mean bud length (29.2cm) was found in 30cm cuttings while the least length (25.4cm) was observed from the 60cm cuttings. Highest mean leaf number (64) was observed for 30cm cuttings while 60cm cuttings had 61 though not significantly different. In terms of hormone concentration significant treatment differences was observed only for mean number of buds sprouts /cutting at $p< 0.05$ while others were not. The control (untreated cuttings) had the highest number of sprouted buds/cutting (10.0) while the least number (6.0) was observed from cuttings treated with hormone concentration of $100mgl^{-1}$. Also, the control had the highest mean bud length (30cm). The untreated cuttings (control) had the highest leaf number (69) while cuttings treated with $100mgl^{-1}$ of hormone had the least value (56) though not significant at $p< 0.05$. Interaction effect of length and hormone concentration was shown on table 4. Interaction effect was found to be significant for mean leaf number /cutting at $p < 0.05$. For the mean number of bud sprouts/cutting and mean bud length, no significant interaction effect of length and hormone concentration was observed. The untreated 60cm cuttings (control) produced the highest leaf number/plant (77.0) followed by the 30cm cuttings treated with hormone concentration of $200mgl^{-1}$(69.0) while the least(42.0) was observed from 60cm cuttings treated with $100mgl^{-1}$ hormone.

Rooting Ability

Table 5 presented the mean values of rooting parameters assessed on *J.curcas.*Cutting length and concentration of hormone had significant main effects on some of the parameters assessed on rooting behaviours at $p < 0.05$. The effect of cutting length was significant for percentage cuttings that rooted while hormone concentration was significant for percentage cuttings that rooted and mean root length/ cutting. The 60cm cuttings had the highest percentage of cuttings that rooted (73.8) while the 30cm cuttings had the least (44.2). Root length ranged from 12.9cm to 13.9cm while mean number of roots/cutting was between 14 and 15. No significant difference was observed

between the cuttings of different lengths as regard root length and number of roots per cutting. The untreated cuttings (control) had the highest percentage of cuttings that rooted (83.5) while the least (47.2) was recorded for cuttings treated with150mgl^{-1}hormone. The control also had the highest mean root length (15.2cm) while cuttings treated with 100mgl^{-1} had least (11.4cm).Mean number of roots per cutting ranged from 13 to 15, though no significant treatment difference was observed. Root quality ranged from fair to vigorous. The 60cm cuttings had vigorous root growth while the 30cm cuttings had fair root growth. The untreated cuttings (control) had vigorous root growth while only the 30cm cutting treated with 200mgl^{-1}hormone had vigorous root growth, others had fair root growth.

Table 6 presented mean values obtained from the interaction effect of cutting length and hormone concentration on the rooting behaviours of *J. curcas.* Significant interaction effect was observedfor all rooting parameters assessed at $P < 0.05$.Mean percentage cutting that rooted ranged from 18.3 to 96.3.60cm untreated cutting (control) had the highest rooting percentage (96.3) while the least (18.3) was recorded for 60cm cuttings treated with hormone concentration of 150mgl^{-1}. Number of roots/cutting was between 10.0 and 18. The 60cm untreated cuttings had the highest (18.0) while the least number (10.0) was found among 60cm cuttings treated with hormone concentration of 100mgl^{-1}.Root length ranged from 9.3cm to 17.3cm. The 60cm untreated cuttings produced longer roots (17.3cm) than others treated with hormone.

Table 7 showed values obtained from ranking of the treatments according to the performance rating established for this study. It could be observed that the untreated 60cm cuttings had the best performance as it was scored 27 out of 30 while 30cm cuttings treated with 200mgl^{-1} was scored 25 thereby ranking second in performance. The performance of 30cm cuttings increases with increasing hormone concentration used for the pre-treatment of the cuttings. The reverse is the case for the longer cuttings.

Discussion

The low survival percentage observed for the 60cm cuttings was probably as a result of the high degree of stem rot caused by fungal growth on the stem. The shorter cuttings (30cm) on the hand had higher survival percentage due to low stem rot. The better performance of the untreated cuttings as regards sprouting behaviours suggests that the ability of cuttings to remain established may not necessarily depend on externally applied hormones but other factors. Narin and Watna (1983) and Aminul-Islam *et al.,* (2010) have reported similar findings. 60cm cuttings untreated with hormone was found to produce highest number of buds that sprouted, highest bud length and leaf number. This could be due to the fact that the longer the cuttings, the more the buds on it (CJP, 2010). However, the combined effect of length and concentration showed that 30cm cuttings treated with hormone at 200mgl^{-1} also gave desirable ability to sprout. This is in agreement with findings made by Shrivastava and Barnejee (2008)who reported that treating shorter cuttings with higher concentration of growth hormones produced better results. Growth hormones was found to enhance sprouting ability of shorter cuttings as it stimulates cell growth and shoot formation as reported by Raha and Roy (2001). Shrivastava and Barnejee (2008) had also reported that for shorter cuttings, pre-treatment with higher hormone concentration gave higher sprouting performance.

Longer cuttings (60cm) were found to perform better in terms of all the rooting parameters examined in this study. This could be attributed to the fact that longer cuttings probably have higher food reserves in such cuttings as reported by Aminul-Islam *et al.* (2010). This has been previously reported by Kathiravan *et al.,*(2009) whose findings showed that longer cuttings were more successful in vegetative propagation than shorter cuttings. Similar findings have also been reported by Henning (2003).The better performance of the 60cm untreated cuttings showed that treating cuttings with growth hormones may not necessarily induce rooting better but rather, rooting of cutting may be

influenced more by the availability of other factors such as a rooting media with good aeration and drainage properties as stated by Narin and Watna (1983). Qualitative assessment of the roots showed that cuttings of 60cm length had more vigorous root development than cuttings of 30cm length. Untreated cuttings and cuttings treated with highest hormone concentration ($200mgl^{-1}$) also had vigorous root growth. This can be attributed to the higher food reserves in such cuttings as reported byAminul-Islam *et al.,* (2010).

Conclusion

The present study showed significant variability in the survival, sprouting and rooting behaviour of *Jatrophacurcas* in response to varying cutting lengths and concentration of growth hormones applied.

In terms of sprouting behaviours, the 60cm cuttings were found to perform better than the 30cm cuttings. Significant interaction effect of stem cutting length and hormone concentration was found to affect the sprouting behaviour of the *Jatropha* plant. In most cases, it was observed that the untreated 60cm cuttings performed better than the treated cuttings of the same length. It was also observed that the 30cm cuttings treated with the highest hormone concentration ($200mgl^{-1}$) produced good results.

In terms of rooting behaviours, the 60cm cuttings performed better than the 30cm cuttings. Significant interaction effect of stem cutting length and hormone concentration was found to affect the rooting behaviour of the *Jatropha* plant. In most cases, the untreated 60cm cuttings were found to perform better than the treated cuttings of the same length. It was also observed that the 30cm cuttings treated with hormone at a concentration of $200mgl^{-1}$ produced good results. Based on the performance ratings of parameters assessed in this study, the 60cm untreated cuttings had the best performance. This implied that treating *Jatropha* cuttings with growth hormones may not have any significant effect on the development and rooting of the cuttings provided they are long enough.

The 60cm untreated cuttings could therefore be preferred for massive cultivation of *Jatropha curcas*. In addition, it is economical to use longer cuttings if available, as the added cost of the chemical will increase production cost. Farmers with low income can make use of 60cm cuttings which do not require pre-treatment with growth hormones.However, where it is difficult to obtain, 30cm cuttings treated with at $200mgl^{-1}$ could be adopted. For large scale cultivation of *Jatropha*, particularly in the Guinea Savanna agro-ecological zone known to be endemic to termite infestation, there may be need for further research on effective ways of protecting stem cuttings against termite attack. The 60cm untreated cuttings were found to be more susceptible to stem rot hence they could be pre-treated with fungicides.

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Table 1 Effect of Cutting Length and Concentration of Hormone on the Survival Parameters of *J. curcas* at 30 days after planting

Treatments		% Survival	% Termite Infes.	% Stem Rot
Cutting Length (cm)	30	52.7^{a}*	22.5^{a}*	23.4^{b}*
	60	34.5^{b}	14.7^{b}	46.0^{a}
	SED	4.8	2.8	4.1
Concentration (mgl^{-1})	0	49.8	11.0^{d}	9.2^{d}
	100	46.2	15.6^{c}	43.6^{b}
	150	45.3	30.4^{a}	52.7^{a}
	200	33.2	17.4^{b}	33.3^{c}
	SED	ns	3.9	5.8

ns – not significantly different at 5% level of probability

* - Means with the same letters within a column of any set of treatments are not significantly different at 0.05 level of probability using the Least Significant Difference (LSD) test.

Table 2 Interaction Effect of Length and Concentration on Survival Parameters of *Jatropha curcas*

Cutting Length (cm)	Hormone Conc. (mgl^{-1})	Survival %	Termite Infest (%)	Stem Rot %
30	0	33.0^{c}*	22.0^{b}	18.3^{d}
	100	63.0^{a}	20.2^{b}	23.8^{c}
	150	66.8^{a}	33.2^{a}	34.8^{c}
	200	48.0^{b}	14.7^{c}	16.5^{d}
60	0	66.7^{a}	0.0^{d}	0.0^{e}
	100	29.3^{c}	11.0^{c}	63.3^{b}
	150	23.8^{d}	27.7^{a}	70.5^{a}
	200	18.3^{d}	20.2^{b}	50.2^{b}
	SED	11.2	9.2	10.2

* - Means with the same letters within a column of any set of treatments are not significantly different at 5% level of probability using the LSD test.

Table 3 Effect of Cutting Length and Hormone Application on sprouting parameters of *Jatropha curcas 75DAP*.

Treatments		Mean Number of buds	Mean Bud length	Mean No. of leaves
Cutting Length (cm)	30	7.0[b]*	29.2[a]	64.0
	60	10.0[a]	25.4[b]	61.0
	SED	0.8	1.9	n.s
Concentration (mgl^{-1})	0	10.0[a]	30.0	69.0
	100	6.0[c]	25.8	56.0
	150	9.0[b]	25.1	63.0
	200	9.0[b]	28.3	61.0
SED		1.2	n.s	n.s
Conc. X length SED		ns	ns	9.9

ns – not significantly different at 5% level of probability.
* Means with the same letters within a column of any set of treatments are not significantly different at 5% level of probability using the LSD test.

Table 4: Interaction Effect of Length and Hormone Concentration on the Number of leaves/plant of *J. curcas*

Cutting Length (cm)	Hormone Conc. (mgl^{-1})	15DAP	30DAP	45DAP	60DAP	75DAP	Mean
30	0	3.0[c]*	24.0[a]	33.0[c]	58.0[b]	60.0[b]	35.6
	100	7.0[a]	24.0[a]	37.0[b]	64.0[a]	68.0[a]	40.0
	150	5.0[b]	25.0[a]	39.0[b]	57.0[b]	57.0[b]	36.6
	200	9.0[a]	26.0[a]	40.0[b]	64.0[a]	69.0[a]	41.6
60	0	6.0[a]	31.0[a]	57.0[a]	74.0[a]	77.0[a]	49.0
	100	2.0[c]	16.0[b]	29.0[c]	39.0[c]	42.0[c]	25.6
	150	3.0[c]	17.0[b]	30.0[c]	54.0[b]	58.0[b]	32.4
	200	2.0[c]	25.0[a]	44.0[a]	66.0[a]	66.0[a]	40.6
SED		1.9	3.7	5.5	9.3	9.9	

* -- means with same letters within a column of any set of treatments are not significantly different at 5% level of probability using the LSD test.

Table 5 Effect of Cutting Length and Hormone Application on the Rooting behaviours of *Jatropha curcas* at 8 weeks after planting.

Treatments		Percentage of cuttings that rooted (%)	Mean No. of roots /cutting	Mean Length of roots / cutting (cm)	Quality of roots formed
Cutting Length (cm)	30.0	44.2[b]*	14.0	12.9	Fair
	60.0	73.8[a]	15.0	13.9	Vigorous
	SED	3.6	ns	ns	
Concentration (mgl^{-1})	0	83.5[a]	15.0	15.2[a]	Vigorous
	100	49.1[c]	13.0	11.4[b]	Fair
	150	47.2[c]	14.0	12.8[b]	Fair
	200	56.4[b]	15.0	14.3[a]	Vigorous
	SED	5.0	ns	1.1	

ns – not significantly different at 0.05 level of probability.

* -- Means with the same letters within a column of any set of treatments are not significantly different at 5% level of probability using LSD test.

Table 6: Interaction Effect of Length and Concentration on the Rooting behaviours of *Jatropha curcas*.

Cutting Length (cm)	Hormone Conc. (mgl^{-1})	Mean % of cutting that rooted	Mean No. of roots/ cutting	Mean Root length/ cutting (cm)
30	0	70.7[c]*	12.0[c]*	11.3[c]*
	100	70.7[c]	14.0[b]	13.5[b]
	150	76.0[b]	14.0[b]	14.7[b]
	200	78.0[b]	15.0[b]	16.2[a]
60	0	96.3[a]	18.0[a]	17.3[a]
	100	27.5[d]	10.0[c]	9.3[d]
	150	18.3[e]	15.0[b]	11.0[c]
	200	34.8[d]	17.0[a]	14.2[b]
	SED	7.1	2.1	1.6

*- Means with same letters within a column of any set of treatments are not significantly different at 5% level of probability using the LSD test.

Table 7: Table of Performance Ratings for the Interaction Effect of Length and Concentration on the Sprouting and Rooting behaviours of *J. curcas*

Cutting Length (cm)	Hormone Conc. (mgl^{-1})	SP Index	TIF Index	SRP Index	PCR Index	NRC Index	LRC Index	Scoring
30	0	2	4	5	4	3	3	21
	100	4	4	4	4	3	3	22
	150	4	4	4	4	3	3	22
	200	3	5	5	4	3	4	24
60	0	4	5	5	5	4	4	27
	100	2	5	5	2	2	2	18
	150	2	4	2	1	3	3	15
	200	1	4	3	2	4	3	17

Keys : Survival Percentage (SP) :-**1**=0-20%, **2**=20-40%, **3**=40-60%, **4**=60-80%, **5**=80-100%. Termite Infestation (TIF) & Stem Rot Percentage (SRP) : **1**=80-100%, **2**=60-80%, **3**=40-60%, **4**=20-40%, **5**=0-20%. Percentage of Cuttings that Rooted (PCR):-**1**=0-20%, **2**=20-40%, **3**=40-60%, **4**=60-80%, **5**=80-100%.

Number of Roots/Cuttings (NRC) & Length of Roots/Cuttings (LRC):-**1**=0-5, **2**=6-10, **3**=11-15, **4**=16-20.