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## RESEARCH ARTICLE

### APPROPRIATE PLANT GROWTH REGULATORS AND GENOTYPES FOR PROPAGATION OF JOJOBA (*SIMMONDSIA CHINENSIS* L.) CUTTINGS IN SEMI-ARID AREAS OF VOI, KENYA

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#### ABSTRACT

Jojoba is a dioecious desert shrub which produces high quality oil used in cosmetics and lubrication. An experiment was conducted to identify the most appropriate plant growth regulators and genotypes for Jojoba propagation. A 4x4 factorial arrangement laid down in a randomized complete block design was used with 16 treatments replicated 3 times. Treatments comprised of 4 factors of growth regulators and 4 types of genotypes. The experiment was carried out for 5 months in 2013. The variables sampled included roots, leaves and shoot. ANOVA was carried out using SAS statistical package and means were separated using Duncan's Multiple Range Test at  $p \leq 0.05$ . Results showed that Anatone growth regulator gave significantly superior rooting of 24.2% for cuttings compared with the control (11.5%). The male genotypes showed significantly higher rooting of 24.2-37.6% compared with the females (2.2-7.6%). Anatone is recommended for propagation of cuttings in a polythene sheet tunnel since it is also cheap and readily available from agri-veterinary shops. Further research is recommended on screening of a wide range of genotypes especially the females and plant growth regulators for future propagation of Jojoba.

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## INTRODUCTION

Over 80% of Kenya is arid and semi arid land (ASAL) (KARI, 2009) with only a few crops being grown mainly for subsistence purposes. In recent years, there has been considerable interest in using ASALs more productively by promoting crops which can tolerate these conditions such as *Jatropha curcas* (Ngethe, 2007) and Jojoba (*Simmondsia chinensis*) (Thagana et al., 2004). These are multipurpose crops, and have a potential use for rehabilitation as well as provision of income to the poor communities. Jojoba is a high value shrub and a promising cash crop for ASALs (Ahmad, 2001). It produces seeds with 45-55% of their weight as oil which has similar chemical composition to that obtained from Sperm Whale (Hogan and Bemis, 1983), a species threatened with extinction (CITES 2004). The plant is dioecious with male

and female plants in the ratio 1:1 in the field when raised from seed (Undersander et al., 2009). A Jojoba stand can be in production for 100-200 years depending on management (Martin, 1983) and has a deep rooting habit (Forster and Wright, 2002). It is important in cosmetics, lubricant industry, pharmaceuticals as well as electronics and computer industries (Undersander et al., 1990; Amarger and Mercier, 1996; Ward, 2003). Currently, there is low productivity of Jojoba seed, ranging from 2-3 tonnes (t) per hectare (ha) mainly due to high male to female ratio of 1:1 in the existing plantations globally since most of them were raised from seed whereas the recommended ratio is 1:10 for increased productivity (Undersander et al., 1990). The potential for Jojoba yield is 5 t/ per ha/ year in improved and well managed plantations. To overcome this problem, there is need to vegetatively propagate Jojoba through selection of superior genotypes which have high yielding ability, but differ widely in their rooting ability depending on genotype (Bonga and Aderkas, 1992; Bashir et al., 2008; Inoti et al., 2015), cultural factors (Foster et al., 1984) and maturation (Ozel et al., 2006).

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A lot of work on vegetative propagation has been done especially in temperate environments (Lee, 1988; Benzioni, 1997; Zhou, 2002; Bashir *et al.*, 2007, 2008, 2013) but a lot more is needed in tropical areas in order to determine the optimum requirements for Jojoba cuttings in the region. Marino (1982) stated that rooting of cuttings does better when taken during the dormant stage due to high carbohydrate to nitrogen (N) ratio (Berl and Trigiano, 2011).

Jojoba is a difficult- to- root plant and several rooting hormones have been tried with varying success (Bashir *et al.*, 2013). Rooting hormones or plant growth regulators (PGRs) wholly cause a greater percentage of cuttings to root, hasten the formation of roots, induce more roots of cuttings and increase root uniformity (Godfrey *et al.*, 1996; Amri, 2009). Two synthetic auxins namely Indole butyric acid (IBA) and  $\alpha$ -naphthalene acetic acid (NAA) are mostly used, either singly or in a combination (Berl and Trigiano, 2011; Kumlay, 2014). The most naturally occurring auxin in plants is Indole acetic acid (IAA), but breaks down easily, hence less effective (Amri *et al.*, 2010). There is a wide range of PGRs in the market and are available for use by commercial nurseries and in horticultural farms. However, they vary greatly in their composition of active ingredients, performance and price (Gitonga *et al.*, 2010; Ngeno *et al.*, 2013; Kumlay *et al.*, 2014). It is necessary to test different PGRs in order to recommend the best for root initiation in Jojoba cuttings in tropical environment. This will provide a cheaper and more accessible method for vegetative propagation of Jojoba.

The objective of the trial was to identify appropriate plant growth regulators and genotypes for propagation of Jojoba cuttings. The research hypothesized that Jojoba cuttings can be rooted using nonconventional PGRs.

## MATERIALS AND METHODS

### Site description

The research was conducted at Rukinga Wildlife Works Ltd, Maungu, Voi District, Taita Taveta County, Coast Province of Kenya, where Jojoba bushes were planted between 1981 and 1989 on 20 ha of land. It lies between latitudes  $3^{\circ} 23' 60''$  to  $3^{\circ} 24' 26''$  S and Longitudes  $37^{\circ} 40' 60''$  to  $38^{\circ} 35' 25''$  E and at an altitude of 892 meters above the sea level. It is found in semi arid savannahs with a bimodal annual rainfall of 596 mm, temperature average at  $26^{\circ}\text{C}$  and moderate humidity of 59%. Soils are moderately fertile with sandy loam and gravel texture and pH of 5-7 (Thagana *et al.*, 2003; TTDP 2008).

### Experimental design and sampling techniques

The experiment was a 4x4 factorial arrangement laid down in a randomized complete block design (RCBD) with 16 treatments replicated 3 times (Gomez and Gomez, 1984). The treatments comprised 4 factors of PGRs namely: Indole butyric acid (IBA), Roothom, Anatone and the Control as well as 4 types of genotypes (male 1, male 2, female 1, female 2). Roothom and Anatone are non-conventional synthetic PGRs which have not been reported before in Jojoba propagation. Their cost is approximated to be 5 to 10 times lower compared with that of

IBA. Stem cuttings were randomly harvested from each genotype and their combination with the PGRs constituted the 16 treatments per replicate. Each treatment consisted of 10 potted plants. This experiment was carried out from April to August 2013 for a period of 5 months. The stem cuttings were harvested at the dormant stage and each cutting consisted of 5 nodes according to recommendations by Benzioni (1997). IBA was applied at a rate of  $5,000 \text{ mgL}^{-1} + 15.5 \text{ mgL}^{-1}$  boric acid (Singh *et al.*, 2003) and this was put in containers where the freshly cut twigs were quickly dipped for 10 seconds before planting in a sterilized sand container. Roothom (with 0.6% IBA) was applied in powder form which involved dipping the basal freshly cut portion into the powder followed by planting. On the other hand, Anatone was applied at a rate of  $1,000 \text{ mgL}^{-1}$  of IBA. This was placed in a container where the freshly cut twigs were dipped for a period of 5 minutes and then planted inside a polythene sheet tunnel. Both Roothom and Anatone were applied according to manufacturers' recommendations for semi-hardwoods.

### Data collection

Three rooted cuttings were randomly sampled per treatment. The variables scored from rooted cuttings were: plant height, height of new growth, number of shoots, internode length, leaf length, leaf width, number of leaves, single leaf area, total leaf area, root collar diameter, number of roots, root length, root: shoot ratio and fresh total plant biomass. However, survival and rooting percentage were calculated through complete enumeration.

### Data analysis

One-way analysis of variance (ANOVA) model was used to test differences between treatment means using SAS statistical package (SAS, 1996) while the significantly different treatment means were separated using F ratio by Duncan's Multiple Range Test (DMRT) at  $p \leq 0.05$ .

## RESULTS

### Effect of PGRs on survival, rooting and growth of Jojoba cuttings

Anatone showed significantly higher ( $p \leq 0.05$ ) rooting percent (24.2) in relation to the control (11.5) (Table 1). On the other hand, Roothom had the highest number of roots (28.8) as well as the highest root collar diameter (2.3 mm) whereas IBA showed the biggest root length value (19.9 cm) as well as the highest root to shoot ratio (1.3). Among the PGRs tested, IBA showed comparable performance with Anatone and Roothom for survival percent, rooting percent and root growth variables.

Results for the foliage and shoot (Table 2) showed that out of the nine variables tested, there were no significant differences among the treatments. On the other hand, the control showed the lowest values for all the variables tested. However, IBA was highest in 5 variables namely: leaf length, leaf width, number of leaves, total leaf area and height of new growth which were higher than the control by 27%, 33%, 39%, 43% and 47% respectively. Roothom had the highest height, total

fresh plant biomass and internode length which were higher than the control by 38.8%, 38.9% and 39.2% respectively. Similarly, Anatone showed the highest single leaf area which was higher than the control by 37.5%.

( $p \leq 0.05$ ) percentage (37.6%) compared with M1, F2 and F1 genotypes which showed 24.2%, 7.6% and 2.2% respectively. M1 genotype was also significantly higher than F2 and F1 genotypes.

**Table 1. Effect of different PGRs on the survival and rooting percentages and root growth of Jojoba cuttings under Polythene sheet tunnel**

PGR type	Survival %	Rooting %	Root length (cm)	Number of roots	Root collar diameter (mm)	Root to shoot ratio
IBA	62.7a	21.0ab	19.9a	24.8a	2.1a	1.3a
Anatone	70.0a	24.2a	16.3a	18.2a	2.0a	1.2a
Roothom	62.0a	14.8ab	16.2a	28.8a	2.3a	1.1a
Control	64.8a	11.5b	9.8a	11.8a	1.5a	0.8a
CV	26.9	70.1	75.8	79.4	76.1	74.9
Std Dev	24.3	18.9	14.2	18.7	1.7	1.1

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at  $p \leq 0.05$

**Table 2. Effect of different PGRs on the foliage and shoot growth of Jojoba cuttings under Polythene sheet tunnel**

PGR type	Leaf length (mm)	Leaf width (mm)	Number of leaves	Single leaf area (cm <sup>2</sup> )	Total leaf area (cm <sup>2</sup> )	Height (cm)	Height of new growth (cm)	Internode length (mm)	Total fresh plant biomass (g)
IBA	23.0	9.3	8.4	2.1	28.8	8.7	6.0	15.9	1.7
Anatone	21.4	8.8	5.9	2.4	22.8	9.1	4.8	14.7	1.6
Roothom	21.6	9.2	6.7	2.3	23.8	9.8	4.6	16.8	1.8
Control	15.5	6.8	5.1	1.5	16.5	6.0	3.2	10.2	1.1
CV	68.3	65.9	90.5	66.0	93	80.5	74.3	74.2	89.3
Std Dev	18.1	7.7	6.9	2.0	26.9	7.5	4.4	12.8	1.5
$p \leq 0.05$	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = No significant differences among the means in each column according to DMRT at  $p \leq 0.05$

**Table 3. Effect of different genotypes on the survival and rooting percentages and root growth of Jojoba cuttings under Polythene sheet tunnel**

Genotype	Survival%	Rooting%	Root collar diameter (mm)	Number of roots	Root length (cm)	Root to shoot ratio
F1	57.6b	2.2c	0.7c	3.2c	5.2c	0.4c
F2	91.2a	7.6c	1.7bc	16.7b	12.2bc	0.6bc
M1	59.6b	24.2b	2.4ab	26.3ab	17.1b	1.1b
M2	51.2b	37.6a	3.1a	31.4a	27.7a	2.3a
CV	26.7	70.1	76.1	79.4	75.8	74.9
Std Dev	24.3	18.9	1.7	19.7	14.2	1.1

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at  $p \leq 0.05$

**Table 4. Effect of different genotypes on the shoot and foliage growth of Jojoba cuttings under Polythene sheet tunnel**

Genotype	Height (cm)	Height of new growth (cm)	Internode length (mm)	Total fresh plant biomass (g)	Leaf length (mm)	Leaf width (mm)	Number of leaves	Single leaf area (cm <sup>2</sup> )	Total leaf area (cm <sup>2</sup> )
F1	2.5b	1.2b	4.2c	0.5c	5.0b	2.0c	1.6b	0.5c	4.7b
F2	7.4ab	2.5b	10.3bc	1.2bc	12.3b	4.8c	4.0b	0.9c	6.6b
M1	11.5a	7.6a	18.7ab	2.0ab	27.4a	10.5b	11.2a	2.6b	39.7a
M2	12.1a	7.2a	24.3a	2.6a	36.7a	16.7a	9.5a	4.3a	40.8a
CV	80.5	74.3	74.2	89.3	68.3	65.9	90.5	66	93
Std Dev	7.5	4.4	12.8	1.5	18.1	7.7	6.9	20	26.9

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at  $p \leq 0.05$

### Effect of genotypes on survival, rooting and growth of Jojoba cuttings

Results on propagation of genotypes (Table 3) showed significant differences ( $p \leq 0.05$ ) among all the genotypes in survival percent, rooting percent and root growth variables. F2 genotype showed significantly the highest ( $p \leq 0.05$ ) survival (91.2%) compared with the other genotypes; M1, F1 and M2 which showed 59.6%, 57.6% and 51.2% respectively. For rooting percent, M2 genotype showed significantly higher

For root collar diameter and number of roots, M2 genotype showed significantly higher values ( $p \leq 0.05$ ) compared with F1 and F2 genotypes. Similarly, for root length and root to shoot ratio, M2 genotype showed significantly greater performance compared with M1, F2 and F1 genotypes.

Results on the genotype effect on shoot and foliage (Table 4) showed significant differences ( $p \leq 0.05$ ) among the genotypes in all the shoot and foliage variables. M2 genotype was significantly higher ( $p \leq 0.05$ ) than M1, F2 and F1 genotypes

for leaf width and single leaf area. M2 genotype also showed significantly higher performance in leaf length, number of leaves, internode length, total leaf area, total plant biomass and height of new growth compared with F2 and F1 genotypes. On the other hand, M1 genotype was also significantly higher ( $p \leq 0.05$ ) than F2 and F1 genotypes in leaf length, leaf width, single leaf area, number of leaves, total leaf area and height of new growth. M2 genotype gave the best growth followed by M1, F2 and F1 genotypes in decreasing order for root, shoot and foliage variables tested (Table 3, 4).

## DISCUSSION

### Effect of PGRs on survival, rooting and growth of Jojoba cuttings

Successful rooting of Jojoba cuttings can be achieved by the use of different auxins but their performance varies greatly among them. Work by Kebede *et al.* (2013) indicates that *Prunus africana* and *Syzygium guineense* rooted successfully without hormone application in the control treatment. Successful rooting without auxin application has been reported in a number of tropical tree species such as *Nauclea* and *Vochysia* (Leakey and Coutts, 1989). These findings partially agree with the present study since IBA and Roothom were not significantly different relative to the control in rooting percent. This could be explained by the presence of inherent IAA in the plant tissues. The results of the present study showed that the effect of IBA was similar to Anatone and Roothom in propagation of Jojoba cuttings. However, earlier findings have reported IBA use for rooting of cuttings to be superior to NAA (Tchoundjeu *et al.*, 2002; Berl and Trigiano, 2011; Ngeno *et al.*, 2013). The use of Anatone was also reported by Gitonga *et al.* (2010) to be comparable to NAA in initiating the rooting of in vitro bananas but recommended that further research should be conducted to analyze the active ingredients of Anatone and other non conventional PGRs. Several studies have reported varying rooting percent depending on auxin type and concentration, humidity and temperature. Creating an atmosphere of 100% humidity contributed to high degree of rooting (Brown and Campbell, 1985). Thomson (1982) reported 30-70% rooting with 4,000 mgL<sup>-1</sup> of IBA under intermittent misting after every 4 minutes. About 56% and 61% rooting in Jojoba was reported by Bashir *et al.* (2001) by using 1,500 and 4,000 mgL<sup>-1</sup> of IBA respectively in polyethylene sheet having 90-95% humidity and temperature of 15 to 30°C. Very high rooting rates of cuttings treated with IBA, NAA and IAA (100 mgL<sup>-1</sup> each) of 82%, 80% and 76%, respectively were reported by Zhou (2002) who used young semi-lignified shoots of Jojoba under appropriate temperature and humidity control.

This study showed rooting percent which ranged from 11.5 to 24.2% with IBA (21%) and was more comparable to a recent work by Eed and Burgoyne (2015) using Jojoba cuttings treated with IBA, IAA and NAA at 4,000mgL<sup>-1</sup> and culturing under plastic tunnel conditions showed a range between 5 to 66% in various potting media. These results were considered good when no modern tools such as intermittent mist propagation system are available in some countries. The present study was conducted under temperatures of 23 to 28°C

and 80-95% humidity. The optimum temperature for rooting is between 25 and 32°C (Berl and Trigiano, 2011). All these conditions applied in this study are within the range used previously by other authors.

The rooting chamber environment was manually controlled in the present study which could explain the average performance in rooting. Seasonal effects on ortet and rooting chamber environment affects rooting (Marino, 1982; Bashir *et al.*, 2013). The use of polyethylene sheet tunnel for Jojoba cuttings propagation is a successful cheaper technique compared with greenhouse or mist propagation chambers (Garrity, 2004; Bashir *et al.*, 2008; Amri, 2010). The results of this study are consistent with those of other studies using nonconventional rooting hormones such as 150 mgL<sup>-1</sup> of Seradix 2 which was reported by Ngeno *et al.* (2013) to show significantly higher mean rooting percent in *Strychnos heeingsii* cuttings compared with NAA and IAA but was similar to IBA. Similarly, Araya (2005) also reported Seradix 2 to increase root numbers in *Athrixia phyllicoides* (Bush tea) in South Africa. Seradix 2 was reported to have 0.3% IBA, Nicotinamide adenine dinucleotide (NAD) carrier and Thiram fungicide which could have contributed to its good rooting performance (Ngeno *et al.*, 2013). Semi-hardwood cuttings require 0.1-0.5% of rooting hormone (Amri, 2009).

Bashir *et al.* (2009) reported that the highest level of IBA (10,000 mgL<sup>-1</sup>) was the most effective and therefore, could be applied to Jojoba cuttings for mass multiplication from a selected strain/clone/plant. El-Deen *et al.* (2014) reported studies on Carob propagation and noted that IBA at 8,000 mgL<sup>-1</sup> + NAA at 200 mgL<sup>-1</sup> gave the highest values of the parameters measured followed by IBA at 6,000 mgL<sup>-1</sup> + NAA at 200 mgL<sup>-1</sup>. Gehlot *et al.* (2014) reported 80% rooting in *Azadarichta indica* using 250 mgL<sup>-1</sup> IBA with sand media.

### Effect of genotypes on survival, rooting and growth of Jojoba cuttings

Jojoba genotypes differed significantly from each other in their effect on survival percent, rooting percent and growth variables in this study. Although results show high survival for F2 genotype, it will eventually die if roots do not form, hence the importance of rooting in propagation of cuttings (Berl and Trigiano, 2011). Other similar results (Mohamed *et al.*, 2013; Mousa and Bakhshwain, 2014) reported that Jojoba genotypes/clones differed in their response to rooting and growth when cultured in vitro. Rogalski *et al.* (2003) reported that genotype differed significantly for survival in *Prunus* sp. which was being acclimatized. Several authors have reported that ability to root is affected by the genotype (Kesari *et al.*, 2010; Hassanpour and Ali Shiri, 2014). Bashir *et al.* (2008) reported that the Jojoba cuttings of PKJ-3 strain were the most responsive to the various levels of auxins, followed by those of PKJ-6. Hence, both strains are the most suitable for multiplication through cuttings. Similar findings are reported by the present study where genotype M2 was the best followed by M1 in their response to various PGRs. Rooting of cuttings ranged from 2.2 to 37.6% in this study which were within the findings of other authors who had reported results of between 5 to 95% depending on clone type, soil type and the season

(Greenwood *et al.*, 1980; Sadhu, 1989; Eed and Burgoyne, 2015). However, season has been seen to have the greatest effect (Bashir *et al.*, 2009). The mother plants used in this study were above 25 years old and the ability of cuttings to form roots tends to decrease with the age of the mother plant (Paton *et al.*, 1970; Zhou, 2002; Mourao, 2008; Awang *et al.*, 2011) due to increase in rooting inhibitors such as essential oils and phenolic compounds (Kibbler *et al.*, 2002). This can be reversed by de-topping of the mature plant to encourage new sprouts near the base assuming juvenile characteristics (Sadhu, 1989). Some of the differences noticed in this study could be attributed to differences among the genotypes, variation of the concentration of the PGRs used and the infrastructure (Polythene sheet tunnel) which was manually controlled. These findings are consistent with earlier findings by Bashir *et al.* (2008) who reported that environmental factors such as shade and high humidity inside the polysheet can even be more important than the auxin concentration. Partial shading was provided by *Delonix elata* tree which gave 50-70% shade in the present study.

Multiplication of superior genotypes through rooted cuttings will help to scale up planting of favourable male to female ratio of 1: 10 in the field hence increasing the yields per unit area in future plantations. The results of this study will be used by farmers, researchers and policy makers in economic utilization and reclamation of ASALs. In Conclusion, Anatone was superior in rooting Jojoba cuttings since it resulted into significantly higher rooting compared to the control under a polythene sheet tunnel. Propagation results of various genotypes tested showed significant differences within and between sexes in rooting. The study recommended that Anatone and the male genotypes (M1 and M2) be used in the propagation of Jojoba cuttings in future. Further research is necessary to test a wide variety of cheaper PGRs in terms of their performance, active ingredients and their concentrations using various superior female genotypes of Jojoba cuttings.

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