

Genotypic and Plant Growth Regulator interaction on propagation of jojoba (*Simmondsia chinensis* L.) cuttings in semi-arid areas of Voi, Kenya

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Abstract – Asexual propagation is necessary to provide known sex plants in jojoba to boost yields. An experiment was set up to identify the most appropriate Plant Growth Regulator (PGR) and genotype interaction for propagation of cuttings in semi arid areas of Kenya. The experiment was a 42 factorial laid down in a RCBD with 16 treatments replicated 3 times. The treatments consisted of 2 factors namely: PGR and genotype and 4 levels of PGRs which were IBA, Roothom, Anatone and the Control while those of genotypes were M1= male 1, M2= male 2, F1= female 1 and F2= female 2. ANOVA was carried out using SAS package whereas the significantly different treatment means were separated using DMRT at $p < 0.05$. The trial was carried out for 5 months in 2013 at Wildlife works Ltd, Voi. Results showed that Anatone x M2 interaction was the best for rooting (62.7%) whereas IBA x M1 interaction showed the highest performance in most of the variables measured for shoot and foliage growth. This study recommends the use of Anatone x M2 genotype interaction for future propagation of jojoba cuttings. However, further research is recommended on screening of a wide range of PGR x genotype for propagation of jojoba cuttings in semi-arid areas of Kenya.

Keywords: Genotype, Plant Growth Regulator, jojoba, cuttings

1. INTRODUCTION

Jojoba is a high value shrub and a promising cash crop for arid and semi arid lands (ASALs) (Ahmad, 2001; Reddy and Chikara, 2010). The plant produces nuts with 50% of its weight as oil which is similar to that obtained from sperm whale (Hogan and Bemis, 1983). The oil is important in cosmetics, lubricant industry, pharmaceuticals and electronics (Undersander *et al.*, 1990; Ward, 2003).

The success of jojoba cultivation depends upon selection of high-yielding genotypes and their multiplication through vegetative means (Al-Obaidi *et al.*, 2017). Propagating jojoba by direct seeding has genetic heterogeneity, which has raised doubts about the economic feasibility of cultivating jojoba (Kumar *et al.*, 2012). For vegetatively propagated plants, the ability to root is affected by the genotype (Bashir *et al.* 2008; Inoti *et al.*, 2016), cultural factors (Foster *et al.*, 1984) and maturation (Ozel *et al.*, 2006). A lot of work on vegetative propagation in semi-hardwoods has been done especially in temperate environments but a lot more is needed in tropical areas in order to determine the optimum requirement for jojoba cuttings in the latter region (Bashir *et al.*, 2001, 2013). The advantages of using asexual propagules in commercial jojoba plantations are that they provide uniform and predictable plant growth and yield (Lee, 1988; Chaturvedi and Sharma, 1989) and can be sexed earlier before flowering.

There is a wide range of Plant Growth Regulators (PGRs) in the market and are available for use by commercial nurseries and in horticultural farms. However, they vary greatly in their performance when combined with various genotypes (Inoti *et al.*, 2016). It is, therefore, of necessity to test different PGRs such as Anatone, Roothom and others together with specific genotype combination in order to recommend the best for root initiation in jojoba cuttings. This will provide a cheaper and more accessible method for vegetative propagation of jojoba for scaling up the elite plantations.

Rooting hormones in plants stimulate the formation of new root tips in stem cuttings. Two synthetic auxins namely Indole butyric acid (IBA) and α -naphthalene acetic acid (NAA) are mostly used, either singly or in a combination. Rooting hormones or 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; Inoti *et al.*,

2016). These are used in different concentrations and Bashir *et al.* (2009) recommended up to 10 000 mgL⁻¹ for jojoba. High concentrations of auxins promoted cell division which increased the number of roots (Palzkill, 1988).

Bashir *et al.* (2008, 2009) reported effect of jojoba strain x auxin interaction to be significant for all the root parameters. Similarly, Bashir *et al.* (2007) reported significant effect by interactions of jojoba strains and growth regulator combinations on number of shoots and primary root length *in vitro*. Other studies by Owais (2010) reported that rootability of pomegranate is influenced by the interactive effect of cutting age, IBA concentration and variety whereas studies by Rogalski *et al.* (2003) reported significant interaction effects between genotype and IBA concentration in *Prunus* rootstocks for survival.

Bashir *et al.* (2009) reported two-way interaction between jojoba strains and auxins which was significant for all the root parameters as well as for number of leaves per cutting and length and diameter of primary shoot. PKJ-3 strain performed the best compared with PKJ-2 strain which was the least. Interaction between auxin and their concentration significantly affected all the root parameters except the length of the primary root.

Currently, there is low production of jojoba despite high demand mainly due to high ratio of male to female bushes in the existing plantations since most of them were raised from seed. There is need to vegetatively propagate jojoba through selection of superior genotypes. An experiment was set up with the aim of identifying the most appropriate PGR and genotype interaction for propagation of jojoba cuttings in semi arid areas of Kenya.

2. MATERIALS AND METHODS

2.1 SITE DESCRIPTION

The research was conducted at Rukinga Wildlife Works Ltd, Maungu, Voi, where jojoba bushes have been established. It is located 20 km east of Voi urban centre, Voi District, Taita Taveta County, Coast Province of Kenya. The study site lies in the semi arid savannah which receives an average annual rainfall of 458 mm with a bimodal pattern of distribution. Long rains are received between March and May while the short rains are received between November and December. Temperatures range from 16-37°C with an average of 25°C with moderate relative humidity of 59% and annual number of rainy days being 42.7 (TTDP, 2008). Soils are moderately fertile with sandy loam and gravel texture and pH of 5-7 (Thagana *et al.*, 2003).

2.2 EXPERIMENTAL DESIGN AND SAMPLING TECHNIQUES

The experiment was a 4² factorial laid down in a Randomized complete block design (Gomez and Gomez, 1984) with 16 treatments replicated 3 times. The treatments consisted of 2 factors namely: PGR and genotype at 4 levels each. PGR levels were IBA, Roothom, Anatone and Control while genotypes were male 1, male 2, female 1 and female 2.

A total of 40 stem cuttings were randomly harvested from each genotype and their combination with the PGRs constituted a replicate. The treatment combinations per replicate were as follows: M1I, M1R, M1A, M1C, M2I, M2R, M2A, M2C, F1I, F1R, F1A, F1C, F2I, F2R, F2A, F2C where M1- male 1 genotype, M2- male 2 genotype, F1- female 1 genotype, F2- female 2 genotype. On the other hand, I, R, A, C refer to: IBA, Roothom, Anatone and Control respectively. The treatment combinations were independently and randomly allocated to each replicate. Each treatment consisted of 10 potted plants (Table 1). This trial was carried out from April to August 2013.

The stem cuttings were harvested at the dormant stage and each twig consisted of 5 nodes. IBA was applied at a rate of 5000 mgL⁻¹ + 15.5 boric acid 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.

Table 1: Experimental layout on the effect of PGR \times genotype on macro-propagation of jojoba cuttings

REP 1	REP 2	REP 3
M21	F1A	F1R
M1A	M2A	F2R
F1R	F1C	M2R
F2C	M2R	M1A
F2I	M21	F1A
M1I	F2I	F1R
F1A	M2C	F1C
M2C	M1C	M2C
F1I	F1R	F2A
M2A	M1R	M21
F1C	F1R	F1I
M1R	F2R	M1C
F2A	M1A	M1I
F2R	F1I	F2I
M1C	F2A	M2A
M2R	M1I	M1R

Key: Genotypes consist of male (M1 and M2) and female (F1 and F2) whereas plant growth regulators consist of IBA, Rootbom, Anatone and Control represented by I, R, A and C, respectively. Treatments consist of combinations of both genotype and PGR.

On the other hand, Anatone was applied at a rate of 1000 mgL⁻¹ of water according to manufacturers' recommendations. This was placed in a container where the freshly cut twigs were dipped for a period of 5 minutes and then planted in a polythene sheet tunnel. The cuttings were left to grow for five months (Plate 1).

Three rooted cuttings were randomly sampled per treatment. The variables scored from rooted cuttings were: survival percent, rooting percent, 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, fresh shoot biomass and fresh total plant biomass.



Plate 1: Sprouting jojoba cuttings inside a polythene sheet tunnel

2.3 DATA ANALYSIS

A two-way Analysis of variance (ANOVA) was carried out using SAS statistical package (SAS 1996) whereas the means were separated using Duncan’s multiple range test (DMRT) at $p \leq 0.05$.

3. RESULTS

3.1 EFFECT OF PGR × GENOTYPE INTERACTION ON SURVIVAL AND ROOTING OF JOJOBA CUTTINGS

Results showed significant interaction ($p < 0.05$) between PGRs and genotypes in all the variables measured which included survival percent, rooting percent and root growth (Table 2). The highest survival percentage was shown by Roothom × F2 interaction (93.3%) while the lowest was Roothom × M2 interaction (29.3%). The former was significantly greater ($p < 0.01$) than IBA × F1, IBA × M2, Roothom × F1, Roothom × M2, Anatone × M1, Control × F1, Control × M1 and Control × M2. On the other hand, F2 interactions gave outstanding superior performance in survival percent (87.7-93.3%) compared with the overall mean of 64.9% whereas F1 genotype × PGR interactions showed the lowest rooting percent (0-4.3%) compared with the overall mean of 17.9%.

Table 2: Effect of interaction between PGRs and genotypes on the survival and rooting percentages and root growth of jojoba cuttings

PGR × Interaction	G Survival%	Rooting%	Root length (cm)	Number roots	of Root collar diameter (mm)
IBA × F1	46.0cd	0.0f	-	-	-
IBA × F2	91.7a	8.7def	19.2abc	27.3abcd	2.0ab
IBA × M1	63.0abc	37.7b	30.0a	43.0a	3.0a
IBA × M2	50.0cd	37.7b	30.3a	28.9abcd	3.2a
Roothom × F1	58.7bcd	0.0f	-	-	-
Roothom × F2	93.3a	13.0cdef	22.3ab	29.7abc	3.7a
Roothom × M1	66.7abc	25.3bcd	12.7abc	21.8abcd	2.2ab
Roothom × M2	29.3d	21.0bcde	29.8a	39.5ab	3.3a
Anatone × F1	71.0abc	4.3ef	12.9abc	5.0cd	1.3ab
Anatone × F2	92.0a	8.7def	7.2bc	9.7cd	1.0ab
Anatone × M1	50.0cd	21.0bcde	16.8abc	28.5abcd	2.7ab
Anatone × M2	67.0abc	62.7a	28.3ab	29.7abc	2.8a
Control × F1	54.7cd	4.3ef	7.7bc	7.7cd	1.3ab
Control × F2	87.7ab	0.0f	-	-	-
Control × M1	58.7bcd	0.7cdef	9.0abc	12.0bcd	1.8ab
Control × M2	58.3bcd	29.0bc	22.4ab	27.7abcd	3.0a
CV	26	56.6	72.8	77	2.2
Std Dev	24.3	18.9	14.2	18.7	1.7
P value	0.0014	<.0001	0.0073	0.0083	0.0251

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at $p < 0.05$. G= genotype

Rooting percentage was highest for Anatone × M2 interaction (62.7%) which was significantly higher ($p < .0001$) to all the PGR × genotype interactions considered. Rooting did not occur in IBA × F1, Roothom × F1 and also Control × F2, hence excluded from the other growth variables. IBA × M2 interaction showed the highest root length (30.3 cm) which was significantly higher ($p < 0.01$) than IBA × M1, Anatone × F2 and Control × F1 interactions. Similarly, IBA × M1 interaction showed the highest number of roots (43) which were significantly superior ($p < 0.01$) to Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions. On the other

hand, Roothom × F2 interaction showed the highest root collar diameter (3.7 mm) though not significant compared with the other interactions while Anatone × F2 interaction showed the lowest (1.0 mm).

All the PGR × genotype interactions were comparable in performance for survival percent, rooting percent and root growth. However, F2 interactions gave outstanding superior performance in survival percent compared with the other interactions. For root length, IBA × M2 interaction gave the best results, while Anatone × M2 interaction also showed the best rooting percent. For number of roots and root collar diameter, IBA × M1 and Roothom × F2 interactions gave the highest values, respectively.

3.2 EFFECT OF PGRS × GENOTYPE INTERACTIONS ON THE SHOOT GROWTH OF JOJOBA CUTTINGS

Significant interaction ($p \leq 0.01$) was showed by PGRs × genotypes for shoot and foliage growth (Table 3) with the exception of IBA × F1, Roothom × F1 and Control × F2 since they did not root. For all the shoot and foliage variables measured, IBA × M1 interaction showed the highest growth in leaf length, number of leaves, total leaf area, height of new growth and total plant biomass.

Leaf length for IBA × M1 interaction was significantly higher ($p < 0.01$) than Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions. IBA × M1 interaction showed significantly higher number of leaves (21.3) relative to all the other PGR × genotype interactions. IBA × M1 interaction showed significantly greater ($p < 0.01$) total leaf area (78.5 cm²) compared with all the other PGR × genotype interactions except the Control × M2 interaction (49.9 cm²) which was not significant. IBA × M1 interaction showed significantly higher height of new growth in cuttings (13.2cm) compared with all the other PGR × genotype interactions except the Anatone × M2 interaction (8.3 cm) which was not significant. IBA × M1 interaction showed significantly higher ($p < 0.01$) total plant biomass (3.7g) compared with IBA × F2, Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions.

For leaf width, the Control × M2 interaction gave the highest width (18 mm) which was significantly larger than IBA × F2, Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions. Single leaf area was highest for Anatone × M2 interaction (5.2 cm²) and was significantly higher ($p < .0001$) than all the other PGR × genotype interactions except IBA × M1, IBA × M2, Roothom × M1, Roothom × M2 and Control × M2 interactions.

Height was highest for Roothom × F2 interaction (17.6 cm) which was significantly superior ($p \leq 0.01$) compared with Anatone × F1, Anatone × F2 and Control × F1 interactions. Similarly, internode length was highest for Anatone × M2 interaction (27.8 mm) which was significantly higher than Anatone × F2 interaction (6 mm).

Table 3: Effect of PGRs and genotype interactions on the shoot growth of jojoba cuttings

PGR×G Interaction	Height (cm)	Height of growth (cm)	new Internode (mm)	length Total fresh plant biomass (g)
IBA × F1	-	-	-	-
IBA × F2	7.4abc	3.5bcd	13.0abc	0.7bc
IBA × M1	16.3ab	13.2a	27.7a	3.7a
IBA × M2	11.3abc	7.1bc	22.9ab	2.6ab
Roothom × F1	-	-	-	-
Roothom × F2	17.6a	5.4bcd	22.3ab	3.3a
Roothom× M1	8.1abc	6.1bcd	17.3abc	1.5abc
Roothom× M2	13.6ab	6.9bc	27.3a	2.3ab
Anatone × F1	4.7bc	3.0bcd	8.0abc	1.0bc
Anatone × F2	4.7bc	1.1cd	6.0bc	0.7bc
Anatone × M1	14.6ab	6.6bc	16.8abc	2.0abc
Anatone × M2	12.3ab	8.3ab	27.8a	2.7ab
Control × F1	5.3bc	1.7cd	8.7abc	1.0bc

Control × F2	-	-	-	-
Control × M1	7.3abc	4.5bcd	12.8abc	0.7bc
Control × M2	11.4abc	6.7bc	9.2abc	2.7ab
CV	72.6	67.8	71.8	75.3
Std Dev	77.5	4.4	12.8	1.5
P value	0.0108	0.0006	0.0028	0.0028

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at $p \leq 0.01$. G= genotype

IBA × M1 interaction showed the highest performance in most of the variables measured for shoot and foliage growth. Male genotypes especially M1 showed the best performance in shoot and foliage growth compared with the female genotypes. This was clearly demonstrated in total fresh plant biomass, leaf width, single leaf area and internode length. IBA also showed the highest performance compared with the other PGRs in the shoot and foliage growth.

3.3 EFFECT OF PGRS × GENOTYPE INTERACTIONS ON THE FOLIAGE GROWTH OF JOJOBA CUTTINGS

Significant interaction ($p \leq 0.01$) was showed by PGRs × genotypes foliage growth (Table 4) with the exception of IBA × F1, Roothom × F1 and Control × F2 since they did not root. For all the foliage variables measured, IBA × M1 interaction showed the highest growth in leaf length, number of leaves and total leaf area.

Leaf length for IBA × M1 interaction was significantly higher ($p < 0.01$) than Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions. IBA × M1 interaction showed significantly higher number of leaves (21.3) relative to all the other PGR × genotype interactions. IBA × M1 interaction showed significantly greater ($p < 0.01$) total leaf area (78.5 cm²) compared with all the other PGR × genotype interactions except the Control × M2 interaction (49.9 cm²) which was not significant.

For leaf width, the Control × M2 interaction gave the highest width (18 mm) which was significantly larger than IBA × F2, Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions. Single leaf area was highest for Anatone × M2 interaction (5.2 cm²) and was significantly higher ($p < 0.0001$) than all the other PGR × genotype interactions except IBA × M1, IBA × M2, Roothom × M1, Roothom × M2 and Control × M2 interactions.

Table 4: Effect of PGRs and genotype interactions on the foliage growth of jojoba cuttings

PGR×G Interaction	Leaf length (mm)	Leaf width (mm)	Number of leaves	Single leaf area (cm ²)	Total leaf area (cm ²)
IBA × F1	-	-	-	-	-
IBA × F2	20.7abcd	7.0bcd	3.7bc	1.2cdef	3.5def
IBA × M1	42.5a	15.5abc	21.3a	3.8ab	78.5a
IBA × M2	28.7abc	14.6abc	9.6bc	3.6abc	33.2bcdef
Roothom × F1	-	-	-	-	-
Roothom × F2	23.7abcd	10.3abcd	9.7bc	2.0cdef	20.1bcdef
Roothom × M1	25.3abc	10.2abcd	7.3bc	3.1abcd	33.8bcdef
Roothom × M2	37.3ab	16.3ab	9.8bc	4.2ab	41.4bc
Anatone × F1	11.3cd	5.7cd	3.3bc	1.3cdef	12.7cdef
Anatone × F2	5.0cd	2.0d	2.7bc	0.3ef	2.7ef
Anatone × M1	28.3abc	9.5abcd	10.0bc	2.6bcde	37.0bcde
Anatone × M2	40.7a	17.9a	7.4bc	5.2a	38.7bcd
Control × F1	8.7cd	2.3d	3.0bc	0.7def	6.3cdef
Control × F2	-	-	-	-	-

Control × M1	13.3bcd	6.7bcd	6.0bc	1.1cdef	9.6cdef
Control × M2	39.8a	18.0a	11.3b	4.2ab	49.9ab
CV	62.4	63.1	79.6	63.7	80
Std Dev	18.1	7.7	6.9	2	26.9
P value	0.0003	0.0002	0.0023	<.0001	0.0002

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at $p \leq 0.01$. G= genotype

IBA × M1 interaction showed the highest performance in most of the variables measured for foliage growth. Male genotypes especially M1 showed the best performance in foliage growth compared with the female genotypes. This was clearly demonstrated in leaf width and single leaf area. IBA also showed the highest performance compared with the other PGRs in the foliage growth.

4. DISCUSSION

Anatone × M2 interaction was the best for rooting (62.7%) and was significantly higher relative to all the interactions. All the PGR × genotype interactions showed significant differences relative to the control in all the variables measured with the exception of root collar diameter and internode length. However, there are limited studies on auxin × genotype interactions in jojoba and other semi-hardwoods in the tropics.

This study is consistent with the work by Bashir *et al.* (2008) who reported effect of jojoba strain × auxin interaction to be significant for all the root parameters as well as for number of leaves, length and diameter of primary shoot. Strain × auxin concentration was also significantly different for diameter of primary root, number of leaves and shoot length. Similarly, Bashir *et al.* (2007) reported significant effect by interactions of jojoba strains and growth regulator combinations on number of shoots and primary root length *in vitro*.

Other studies by Owais (2010) reported that rootability of pomegranate is influenced by the interactive effect of cutting age, IBA concentration and variety. Significant interaction effect was observed in rooting percent, number of roots and weight of roots. Further work by Ansari (2013) and Sarrou *et al.* (2014) reported significant interaction effect between time of cutting collection, media, auxin and cutting thickness on rooting characteristics in pomegranate. Sarrou *et al.* (2014) observed that melatonin can be substituted for IBA to produce rooting. Studies by Rogalski *et al.* (2003) reported significant interaction effects between genotype and IBA concentration in *Prunus* rootstocks for survival which corroborates with the present study. Khattab *et al.* (2014) showed significant effect on rooting due to interaction between auxin, cutting date and wounding in jojoba cuttings which was consistent with a study reported by Hegazi *et al.* (2010) on olive cultivars. Further research by Bashir *et al.* (2013) reported significant differences between jojoba genotypes when combined with IBA which is in agreement with this study.

Hasanuzzaman *et al.* (2007) noted significant effect between genotypes and synthetic hormones (Milstim and litosen) interaction in *Capsicum annum* for number of leaves which is consistent with the findings of this study. However, they found that height and number of branches were not significant which was contradictory to the current findings. Similarly, work by Kesari *et al.* (2010) contradicted this study by stating that interaction among auxins, genotypes and month of collection had no significant effect on root induction and differentiation in *Pongamia pinnata*. Some bacteria such as those belonging to the genus *Agrobacterium* and rhizobia release auxin and can have positive effect on rooting of cuttings (Sezai *et al.*, 2003). Dodd *et al.* (2010) reported interaction between bacteria isolates and apple rootstock genotype which resulted in elongation of roots. Similar results were reported by Gosal *et al.* (2010).

5. CONCLUSION

Anatone × M2 interaction was the best for rooting (62.7%) and was significantly higher relative to all the interactions. Similarly, all the PGRs (IBA, Anatone and Roothom) × genotype interaction showed superior performance relative to the control in all the variables measured with the exception of root collar diameter and internode length.

6. RECOMMENDATIONS

Propagation of jojoba cuttings using Anatone × M2 interaction is recommended by this study. However, there are more prospects for further interaction studies between various PGRs and female genotypes in order to get the best combination for scaling up production in ASALs.

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