

**EFFECTS OF STOCK AGE, HORMONE TYPES AND CONCENTRATIONS ON
ROOTING AND EARLY GROWTH OF *VITELLARIA PARADOXA* C.F.GAERTN.
STEM CUTTINGS**

Abstract

This study investigated the effects of stock age, hormones and hormone concentrations on survival and rooting of *Vitellaria paradoxa* stem cuttings with the aim of improving on early maturity of the species. Single node stem cuttings were obtained from 9 and 15 months old seedlings of *V. paradoxa* and treated with Naphthalene Acetic Acid (NAA), Indole Butyric Acid (IBA), coconut water (CW) and distilled water (control) at 100mg/l and 200mg/l concentrations NAA, IBA and 50% and 100% coconut water. Quick dip method was used and the cuttings set in washed and sterilized river sand medium under non-mist propagation in a 2x4x2 factorial experiment laid out in Completely Randomized Design and replicated 3 times. Percentage rooted and percentage die-back were assessed after eight weeks of setting while shoot height (cm), shoot diameter (mm), leaf production and leaf area (cm²) were assessed for three months. The data collected were subjected to descriptive statistics and analysis of variance (ANOVA). Cuttings from both 9 months and 15months old stock recorded higher percentage (90%). The hormone treated cuttings produced the highest rooting at (90%) while control recorded the least (50%). Hormone type also significantly influenced the early growth of the rooted cuttings in term of shoot height, shoot diameter, leaf area and leaf production ($p \leq 0.05$). The highest shoot height, shoot diameter, leaf area and number of leaves were obtained with NAA with mean values of (4.81cm, 3.46mm, 35.08cm² and 5.00) respectively while control had the least (3.80cm, 2.28mm, 27.81cm² and 3.29) respectively. It therefore implies that the use of hormones can improve rooting and early growth of *V. paradoxa* stem cuttings collected from young stock plants.

Keywords: *Vitellaria paradoxa*, Stem cuttings, Growth rt6egulator, IBA, NAA, Coconut water

32 **Introduction**

33 *Vitellaria paradoxa* (Shea butter tree) which is well known for its oil (shea butter) is indigenous
34 to the semi-arid zone of sub-Saharan West Africa and. Shea butter is locally produced from its
35 seeds by rural populations who earn their livelihoods from seed harvesting, processing and sale
36 (Adedokun *et al.*, 2016). Shea butter products became popular as export for West Africa during
37 colonial period (Saul *et al.*, 2003). Apart from shea butter production, this species has
38 multipurpose values in medicinal, confectionery and pharmaceutical industries (Maranz *et al.*,
39 2004; Alander, 2004; Sadiq *et al.*, 2012).

40
41 Principal constraints to fruit production of *V. paradoxa* are long juvenile phase, slow growth,
42 genetic variability and lack of adequate knowledge on cultivation of the species. More
43 importantly, slow growth and late maturation have discouraged the planting of *V. paradoxa*.
44 Various vegetative propagation methods have however been used to raise seedlings because of
45 the advantages of asexual propagation over sexual reproduction through seeds (Hartmann *et al.*,
46 1997; Opeke, 2005). It allows traits of interest in plants to be captured and used for plant species
47 improvement and conservation (Manbir, 2016). The use of these breeding techniques had made it
48 possible to speed up the domestication and commercialization of some highly demanded plants.
49 The presence of necessary genetic information in every plant cell to regenerate the entire plant
50 affords this opportunity (Teiklehaimanot *et al.*, 1996). It is a very useful technique for
51 maintaining and preserving genetic characteristic (Hendromono, 1996). It is useful in the
52 production of cultivars that are seedless, and species which have insufficient supply of seeds due
53 to mammalian predation, pests and disease attack.

54
55 Plant growth-regulating substances or hormones are organic chemical compounds, produced
56 naturally in plants or applied externally, that can affect growth and other plant functions even in
57 very small amounts, on its own or in combination with others (Guney *et al.*, 2016). Auxins and
58 gibberellins are the most widely used hormones with usage rates of 20 and 17%, respectively
59 (Kumlay and Eryiğit, 2011). Auxins mostly cause the expansion and growth of cells and initiate
60 cell elongation, tissue growth and root formation, the most common auxin in plants is indole-3-
61 acetic acid (Grunewald *et al.*, 2009). Plant growth regulators/ hormones have been successfully
62 employed in many plant species to improve the rootability of stem cuttings (Soundy *et al.*,

63 2008, Singh *et al.*, 2011, Sağlam *et al.*, 2014). These include indole-3-acetic
64 acid (IAA), naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) (Adekola and
65 Akpan, 2012, Sardoei *et al.*, 2013). There may also be large differences in rooting ability among
66 clones of many plant species and with different types of cuttings (McIvor *et al.*, 2014).

67
68 Hormones and rooting media have been reported by various authors to stimulate root formation
69 of plants (Nakasone and Paull, 1999; Hartmann *et al.*, 1990; Awodoyin and Olaniyan, 2000,
70 Buah and Agu-Asare, 2014, Bisht *et al.*,2018). Hartmann and Kester (1983) reported that the
71 response of cutting of many plants is not universal; cuttings of some difficult to root species still
72 root poorly after treatment with auxin. Some vegetative propagation methods include grafting,
73 layering and marcotting. *V. paradoxa* seeds loose viability readily and thus not always available
74 for mass propagation through natural regeneration, it became necessary to investigate vegetative
75 propagation through stem cuttings. The successful rooting of stem cuttings however could be
76 influenced by many other factors like the rooting medium, environmental conditions as well as
77 the physiological status of the stock plant itself (Maile and Nieuwenhuis, 1996). Some trials on
78 the vegetative propagation of *V. paradoxa* by grafting were made by Sanou *et al.* (2004) using
79 five methods of grafting, two methods of pre-treatment of scions and rootstocks and two
80 methods of protection of grafts against desiccation. Success of survival of grafts varied from
81 86.1% to 20.7% with average annual growth rate of 12.6cm and; two grafts produced fruits
82 2years after grafting. Stem cuttings of *V. paradoxa* root with difficulty, producing poor and
83 inconsistent results (Frimpong *et al.*, 1991). Therefore, the study investigated the influence of
84 stock age, hormones and hormone concentrations on survival and rooting of its stem cuttings in
85 order to improve seedlings availability for plantation establishment.

86

87 **Materials and Methods**

88 Single node stem cuttings (~ 10cm in length) were obtained from 9 and 15 months old seedlings
89 of *V.paradoxa* by using sharp (70% Ethanol) sterilized secateurs and were placed in plastic bags
90 containing distilled water (to prevent dehydration of cuttings). Cuttings from the two sources
91 were treated with Naphthalene Acetic Acid (NAA), Indole Butyric Acid (IBA) and coconut
92 water (Olaniyan *et al.*, 2006) while distilled water was used as the control. The hormones were
93 prepared at 100mg/l and 200mg/l concentrations and the coconut water at 50% and 100%

94 concentration. One hundred mg/l concentration was obtained by dissolving 10mg of the
95 powdered hormone in 10mls of ethanol. The solution was then diluted with distilled water to
96 make one litre of the hormone. 50% coconut water concentration was obtained by diluting with
97 50% distilled water. Application was done using quick dip method according to the standard
98 procedure described by Hartmann *et al.*, (1997). 0.5cm basal portion of single node cuttings were
99 dipped into the concentrated solutions of the different hormones for about five seconds and set in
100 washed and sterilized river sand medium under non-mist propagation at West African Hardwood
101 Improvement Project (WAHIP) nursery of the Forestry Research Institute of Nigeria (FRIN),
102 Ibadan in a 2x4x2 factorial in Completely Randomized Design with three replicates. The factors
103 were: 2 stock ages; 4 rooting hormones and 2 concentration levels to have 16 treatment
104 combinations. Percentage rooted and, percentage die-back were assessed after eight weeks of
105 setting while sprout height (cm), diameter (mm), leaf production and leaf area (cm²) were
106 assessed fortnightly for three months. The data collected were subjected to descriptive statistics
107 and analysis of variance (ANOVA) and least significant differences (LSD) at 5% probability
108 level were used to compare the significantly different means.

109

110 **Results and Discussion**

111 **Results**

112

113 **Effects of Stock Age, Hormone Types and Concentrations on rooting of *V. paradoxa* stem** 114 **cuttings**

115

116 Trials of growth hormones of varied concentrations on stem cuttings from two different aged
117 planting stocks of *V. paradoxa* significantly improved the rooting of the species Fig. 1. Rooting
118 varied with the stock age, hormone types and concentrations. NAA treated stem cuttings had
119 higher survival than other hormones with 90% rooting success recorded for 100mg/l and
120 200mg/l in 15 months old cuttings and also for 200mg/l treated 9 months old cuttings. This was
121 followed by IBA with 80% while coconut water had the least of 50% (Fig 1).

122

123

124 **Effects of Stock Age, Hormone Types and Concentrations on early growth of rooted *V.***
125 ***paradoxa* stem cuttings**

126

127 **Shoot Height**

128 Analysis of variance on effects of stock age, hormone types and concentrations showed
129 significant differences ($P \leq 0.05$) on subsequent shoot growth of the rooted stem cuttings of
130 *V.paradoxa*. However, the interactions between stock age and hormones, stock age and hormone
131 concentrations and; the interactions of the three factors had no significant effect ($P \leq 0.05$) on the
132 shoot growth of the rooted cuttings of *V.paradoxa* (Table 1). The highest shoot height growth
133 was recorded in 9 months old and 15 months old rooted cuttings treated with 200mg/l NAA with
134 4.81cm and 4.71cm respectively. This was followed by 15 months old cuttings treated with
135 100% coconut water while control for 9 and 15 months old cuttings had the least with 3.88cm
136 and 3.80cm respectively (Table 2).

137 **Shoot Diameter**

138 The shoot diameter of rooted *V.paradoxa* stem cuttings in terms of stock age, hormone types,
139 hormone concentration and the interaction between hormone type and concentration showed
140 significant effects at ($P \leq 0.05$); while interactions between stock age and hormone types; stock
141 types and hormone concentration levels and; interactions of stock age, hormone types and
142 hormone concentrations were not significant on the shoot diameter growth of *V.paradoxa* (Table
143 1). 9 and 15 month old rooted cuttings treated with 200mg/l NAA had the widest shoot diameter
144 (3.46mm and 3.40mm) respectively and 100mg/l NAA performed next with 3.37mm and
145 3.30mm. However, the least shoot diameter was recorded for 15 months old rooted stem cuttings
146 in the control treatment with 2.28mm (Table 2).

147

148 **Leaf Area**

149 Stock age, hormone type, hormone concentration and the interactions between hormone types
150 and hormone concentrations has significant effects on the leaf area of *V.paradoxa* rooted
151 cuttings. However, interactions between stock age and hormone types; stock age and hormone
152 concentration and; combined interactions of stock age, hormone type and hormone
153 concentrations of stock age, hormone type and hormone concentrations were not significant
154 ($P \leq 0.05$) on the wideness of the leaves of *V.paradoxa* (Table 1). Rooted stock from 9 months old

155 stem cuttings treated with 200mg/l NAA had the widest leaf area (35.08cm²). this was followed
156 by same stock age cuttings treated with 200mg/l IBA while 15 months old control treatment had
157 the least with 27.81cm² (Table 2).

158

159 **Leaf Production**

160 Analysis of variance for the effects of hormone type and concentration on two stock ages showed
161 that there were significant differences ($P \leq 0.05$) in stock age, hormone types, hormone
162 concentrations, interaction between stock age and hormone type and; hormone types and
163 concentrations. Interactions between stock age and hormone concentrations and; combined
164 interactions among stock age, hormone types and hormone concentrations were not significant
165 ($P \leq 0.05$). 200mg/l NAA and 200mg/l IBA applied on 9 months old stem cuttings produced the
166 highest number of leaves with 5.0 and 4.56 respectively. These were followed by cuttings from
167 100% coconut water (4.0) (Table 2).

168

169 **Discussion**

170

171 Regeneration of forest and savanna trees must be seen as a process which combines the socio
172 economic and silvicultural aspects with an optimal use of available technology. Various
173 vegetative propagation methods have been attempted to raise tree seedlings because of the
174 advantages of asexual propagation over sexual reproduction through seeds especially when seeds
175 are recalcitrant in nature. According to Oni (2000), vegetative propagation techniques have
176 gained grounds for mass propagation of improved genetic materials. Improvement in stem
177 cutting propagation methods had facilitated significantly the management of many indigenous
178 tree species in the natural forests and plantations (Luukkanen, 1998). Mehrabani *et al.*, (2016)
179 also reported that the immediate formation and the subsequent growth of roots are the most
180 influential factors affecting the survival of cuttings.

181

182 The findings of this study revealed that NAA, IBA and coconut water were effective in the
183 rooting of *V. paradoxa* stem cuttings. This is in agreement with the findings of Ofori *et al.*
184 (1997) who worked on the effect of stock plant age, coppicing, stem cutting length and nodal
185 position on the rooting ability of leafy stem cuttings of *Milicia excelsa* treated with IBA.

186 Cuttings from younger seedlings (1-2 years) rooted more appreciably than those from old plants.
187 In this study however, older cuttings (15months old) rooted better under the influence of
188 hormones (Fig.1), the differences in species under consideration could be responsible for the
189 disparities as each plant species respond differently to the same conditions. Plant growth
190 regulators such as IBA, IAA and NAA are known to accelerate the rate of rooting and increase
191 final rooting percentage and number of roots on cuttings (Gehlot *et al.*2014; Ibrahim *et al.* 2015)
192 Similarly, Chakraborty *et al.*, (1992) investigated stem cuttings in two *Terminalia species* using
193 varied concentrations of IBA. They reported total failure in *Terminalia bellirica* irrespective of
194 plant portion, hormone concentration and month of planting while *T.chebula* treated with
195 4000ppm IBA produced encouraging results in all the cases. Ameyaw (2009) found that growth
196 regulator enhanced the rooting of *Lippia multiflora* Moldenke in Ghana. Trials at IRBET/CTFT
197 in Burkina Faso, using 0.5% indol-3- butyric acid (IBA) and 0.5% indol-3 acetic acid (IAA),
198 produced callous tissue but no roots (Picasso, 1984). Lack of rooting from the research may have
199 been as a result of the application of an insufficient concentration of hormone as research at
200 Cocoa Research Institute of Ghana (CRIG), Ghana, indicated that cuttings rooted best at higher
201 hormone concentrations. Rooting was most successful (22%) when a medium of pure black soil
202 was used, and cuttings were treated with 1.5% IBA (Adomako *et al.*, 1985) using sand-rice husk
203 as growth medium (1:1) gave similar results (Frimpong *et al.*, 1991). This stressed that the
204 response of different plant species vary to growth regulators and different concentrations.

205

206 In this study, it was also observed that rooting success increased with increase in hormone
207 concentrations. This agrees with the work of Sato and Sano (1999) on possibility of vegetative
208 propagation of *Diospyros lotus* L. using leafy 2 year old stem cuttings. Also from this study, the
209 highest rooting rate was obtained with the highest NAA concentration (Fig.1). The implication of
210 this result is that high concentrations of NAA will be appropriate for stem rooting in *V.*
211 *paradoxa*. A study by Kipkemoi *et al.*, (2013) showed that stem cuttings of *Strychnos heningsii*
212 treated with IBA and Seradix 2 powder produced more and longer roots and had higher rooting
213 % than those treated with IAA and NAA. Also, the mean number of root and rooting % of
214 cuttings increased with increasing concentration (up to 0.015%) with IBA, NAA and IAA
215 hormones.

216 In the absence of the synthetic hormones, unripe coconut water can be a good alternative as it has
217 positive influence on its root development. Koyejo *et al.*, (2006) in a study on the propagation of
218 *Massularia acuminata* (G. Don) Bullock ex Hoyle also found out that the stem cuttings treated
219 with coconut water had better callus formation and prolific rooting. Olaniyan *et al.*, (2006) also
220 reported the effects of varieties and local rooting hormones on air layering of sweet orange using
221 coconut water and de-ionized water. It was observed that coconut water medium and distilled
222 water treatments played little role in boosting root development in marcotting sweet orange
223 varieties. The influence of coconut water was not as pronounced as the synthetic hormones (IBA
224 and NAA) in this study, even though it can serve as an alternative and a good source of natural
225 hormones. According to Dunsin *et al.*, (2016) in an experiment conducted on alternative
226 hormone on rootability of *Parkia biglobosa*, coconut water supported higher rooting percentage
227 of the species over other plant extracts. Ogati, (2015) also successfully used coconut water as
228 root setting medium for *Rhizopora stylosa* hypocotyl propagation.

229
230 The growth parameters of *V. paradoxa* were significantly positively influenced by the growth
231 hormones, Table 1 and Table 2. According to Vlabu *et al.*, (2000), plant hormones had the
232 ability to increase plant chlorophyll and adequate application aided growth in plants. In their
233 experiment, Kinetin was found to induce more sprouting than other treatments. The highest
234 values in stem height and stem diameter values were obtained with 5000 ppm IBA treatment in
235 an experiment on the effect of some plant growth regulators (hormones) on germination and
236 certain morphological traits of *L. artvinense* seeds (Guney *et al.*, 2016). In another study on the
237 same species, the stem number of 0.43 was increased to 0.92, the stem height of 1.53 mm was
238 increased to 6.55 mm and the stem diameter of 0.97 mm was increased to 4.3 mm with the
239 application of hormones (Sevik and Cetin, 2016). In the studies by Usman and Akinyele (2015)
240 on the effects of growth hormones on the sprouting and rooting ability of *Massularia acuminata* ,
241 IBA at 1000ppm had the highest shoot length and number of leaves was not affected by growth
242 hormone.

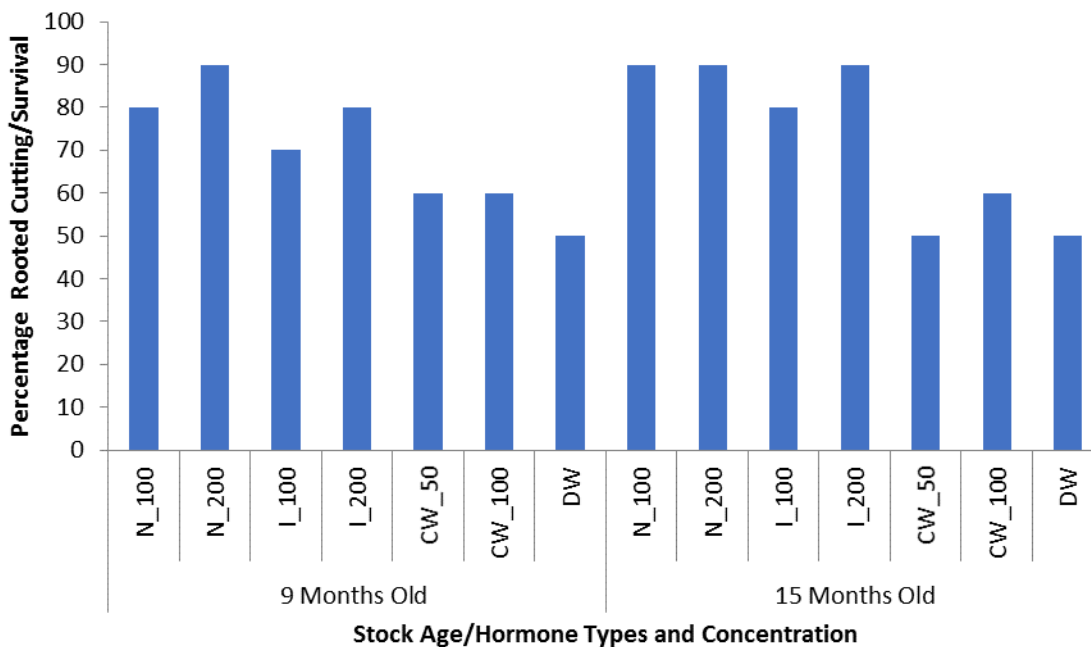
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244 **Conclusion**

245 Improvement programme is important in promotion of plantation establishment of *Vitellaria*
246 *paradoxa* and the results of this study can serve as base line information towards improving the

247 species. The findings from this study showed that rooting, survival and early growth of *V.*
 248 *paradoxa* stem cuttings is influenced by age of plant stock as 9 month old showed better result
 249 with the use of NAA at 200mg/L while in the absence of synthetic growth regulator, coconut
 250 water can be used as it showed positive effect on rooting of the stem cuttings. Vegetative
 251 propagation methods is suggested to facilitate rapid multiplication of *Vitellaria paradoxa* to meet
 252 the increasing demand for planting materials of the species. It is hoped that the use of vegetative
 253 propagation would give opportunity for mass propagation of the species for its plantation
 254 establishment.

255



256

257 **Figure 1: Effect of hormone types and concentrations on percentage survival of *V.***
 258 ***paradoxa* stem cuttings from two stock ages**

259 *N-NAA 100mg/l, NAA 200mg/l, I-IBA 100mg/l, IBA 200mg/l, CW-Coconut Water 50%, Coconut Water 100%, DW-*
 260 *Distill Water (Control)*

261

262 **Table 1: Analysis of variance for the effect of hormone types and concentrations on growth**
 263 **of *V. paradoxa* stem cuttings from two stock ages**

Source of variation	Shoot Height	Shoot Diameter	Leaf Area	Leaf Production
Stock Age (SA)	35.22*	232.60*	66.36*	27.46*

Hormone (HO)	264.58*	3475.90*	83.22*	12.24*
Concentration (CO)	178.47*	56.73*	62.37*	51.55*
SA*HO	1.94ns	1.21ns	1.99ns	9.39*
SA*CO	1.79ns	0.09ns	0.25ns	2.42ns
HO*CO	22.19ns	5.23*	10.40*	4.95*
SA*HO*CO	1.34ns	0.07ns	1.68ns	0.27ns

*significant ($P \leq 0.05$); ns-not significant ($P > 0.05$)

Table 2: Mean table for the effect of hormone types and concentrations on growth of *V. paradoxa* stem cuttings from two stock ages

Stock Age	Hormone Types	Con. Level (100mg/l)	Shoot Height (cm)	Shoot Diameter (mm)	Leaf Area (cm ²)	Leaf Production	
9 Months Old	NAA	100	4.59±0.03c	3.37±0.02d	33.59±0.11d	3.92±0.27d	
		200	4.81±0.06d	3.46±0.03e	35.08±0.34f	5.00±0.38g	
	IBA	100	4.22±0.05b	2.41±0.02ab	30.87±0.71b	3.83±0.42c	
		200	4.56±0.15c	2.48±0.03b	34.14±0.24e	4.56±0.31f	
	Coconut Water	50%	4.18±0.11b	2.82±0.03c	32.30±0.36c	3.25±0.29b	
		100%	4.55±0.19c	2.90±0.05c	33.56±0.69d	4.00±0.49e	
	Control		3.88±0.04a	2.37±0.04a	29.94±1.03a	3.29±0.25a	
	15 Months Old	NAA	100	4.47±0.08c	3.30±0.07e	31.10±2.82c	3.29±0.37b
			200	4.71±0.06e	3.40±0.03f	34.09±0.69f	4.00±0.24f
		IBA	100	4.13±0.04b	2.37±0.02b	30.15±0.69b	3.12±0.56a
200			4.50±0.13c	2.44±0.04b	33.00±1.09e	3.79±0.44d	
Coconut Water		50%	4.10±0.11b	2.76±0.03c	31.80±0.32c	3.29±0.28b	
		100%	4.69±0.11d	2.85±0.03d	32.01±1.55d	3.75±0.40d	
Control			3.80±0.06a	2.28±0.04a	27.81±0.54a	3.63±0.48c	

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