Original Research Article

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 (cm^2) were assessed for three months. The data collected were subjected to descriptive statistics and analysis of variance (ANOVA). Cuttings from both 9 months and 15 months 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, 35.08cm^2 and 5.00) respectively while control had the least (3.80cm, 2.28mm, 27.81cm^2 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 mother stock plants.

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

Introduction

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

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

Plant growth-regulating substances or hormones are organic chemical compounds, produced naturally in plants or applied externally, that can affect growth and other plant functions even in very small amounts, on its own or in combination with others (Guney *et al*., 2016). Auxins and gibberellins are the most widely used hormones with usage rates of 20 and 17%, respectively (Kumlay and Eryiğit, 2011). Auxins mostly cause the expansion and growth of cells and initiate cell elongation, tissue growth and root formation, the most common auxin in plants is indole-3-acetic acid (Grunewald *et al*., 2009). Plant growth regulators/ hormones have been successfully employed in many plant species to improve the rootability of stem cuttings (Soundy *et al.*, 2008, Singh *et al*., 2011, Sağlam *et al*., 2014). These include indole-3-acetic acid (IAA), naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) (Adekola and Akpan, 2012, Sardoei *et al*., 2013). There may also be large differences in rooting ability among clones of many plant species and with different types of cuttings (McIvor *et al.*, 2014).

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

Materials and Methods

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

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

Results and Discussion

Results

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

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

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

Shoot Height

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

Shoot Diameter

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

Leaf Area

Stock age, hormone type, hormone concentration and the interactions between hormone types and hormone concentrations has significant effects on the leaf area of *V.paradoxa* rooted cuttings. However, interactions between stock age and hormone types; stock age and hormone concentration and; combined interactions of stock age, hormone type and hormone concentrations of stock age, hormone type and hormone concentrations were not significant (P≤0.05) on the wideness of the leaves of *V.paradoxa* (Table 1). Rooted stock from 9 months old stem cuttings treated with 200mg/l NAA had the widest leaf area (35.08cm^2). this was followed by same stock age cuttings treated with 200mg/l IBA while 15 months old control treatment had the least with 27.81cm^2 (Table 2).

Leaf Production

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

Discussion

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

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

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

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

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

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

Conclusion

Improvement programme is important in promotion of plantation establishment of *Vitellaria paradoxa* and the results of this research study can serve as base line information towards improving the species. The findings from this study showed that rooting, survival and early growth of *V. paradoxa* stem cuttings is influenced by age of plant stock as 9 month old showed better result with the use of NAA at 200mg/L while in the absence of synthetic growth regulator, coconut water can be used as it showed positive effect on rooting of the stem cuttings.

Vegetative propagation methods is suggested to facilitate rapid multiplication of *Vitellaria paradoxa* to meet the increasing demand for planting materials of the species. It is hoped that the use of vegetative propagation would give opportunity for mass propagation of the species for its plantation establishment.

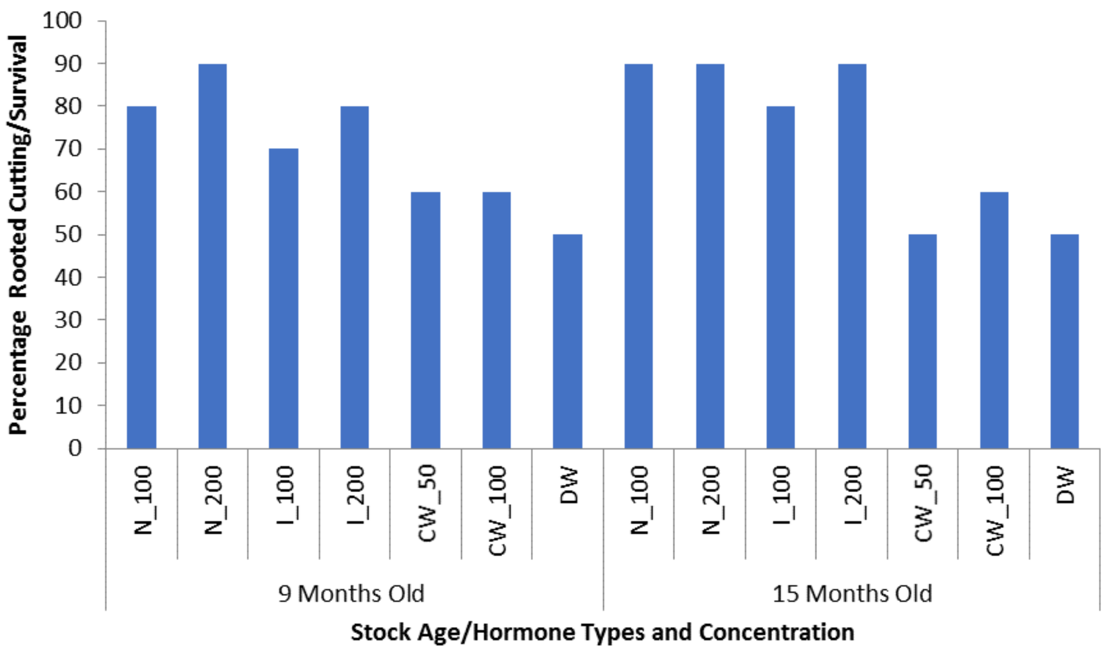

Figure 1: Effect of hormone types and concentrations on percentage survival of *V. paradoxa* stem cuttings from two stock ages

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

Table 1: Analysis of variance for the effect of hormone types and concentrations on growth 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≤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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