

MACROPROPAGATION OF AN ENDANGERED MEDICINAL PLANT, *ENANTIA CHLORANTHA* OLIV.

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Abstract. The effects of auxins and leaf size on the rooting of juvenile, single-node leafy stem cuttings of *Enantia chlorantha* Oliv. were investigated. Percentage survival of the cuttings differed among the auxins, their levels, and cutting leaf sizes. Half-leaf cuttings had the highest mean percentage survival of 70% under both naphthalene acetic acid (NAA) and the control followed by indole-3-butyric acid (IBA) (60%) and the combination of IBA/NAA with 40% survival. Among full-leaf cuttings, the control treatment and the combination of IBA/NAA had a mean percentage survival of 100%, followed by IBA with 80% and NAA with 40%. Callusing of cutting was also affected by the different auxin treatments. Among the auxins and under full-leaf cuttings, the combination of IBA/NAA had the highest mean percentage of callused cuttings of 100%, followed by the control with 90%, while NAA had 40%. Callusing among the half-leaf cuttings was highest under the control and NAA (70%), while the lowest mean value of 30% was obtained under NAA. Rooting among the cuttings was affected by the IBA treatment only. Full-leaf cuttings treated with IBA at 50 ppm and the control treatment gave the highest percentage of rooted cuttings of 10% each. Auxin type, auxin concentration, leaf size, interaction between auxin type and concentration, interaction between auxin type and leaf size, and interaction between auxin concentration and leaf size had no significant effect on the percentage rooting of cuttings. Better survival and rooting rates under the control treatment, compared to those treated with rooting hormones at higher concentrations, imply that *E. chlorantha* can be propagated vegetatively at reduced cost, thus ensuring that this technology can be adopted with minimum capital to yield expected results.

Key Words. *Enantia chlorantha*; half-leaf cuttings; full-leaf cuttings; auxins.

Since medieval times, the forest has provided food and herbs to maintain good health for humans (Gbadamosi 2002). Medicinal plants are widely used by all sections of the population, either directly as folk remedies or indirectly in the preparation of modern pharmaceuticals.

Enantia chlorantha is a medicinal tree species used for the treatment of malaria and other ailments of the human body. Gill and Akinwunmi (1986) reported the use of infusion of the plant bark for the treatment of cough and wounds. Wafo et al. (1999) noted the antiviral activity of extracts from the dried stem bark. However, in Nigeria, its most prominent use has been for the treatment of malaria. Malaria has a direct impact on the socioeconomic development of people, especially in the tropical world. The World

Health Organization (WHO) reported that an average of 3,000 people are infected by malaria every minute and approximately one million die of the disease yearly.

Due to the prevalence of malaria in tropical Africa, plants that demonstrate potency against the disease are usually overexploited. Prance (1994) observed that overexploitation can lead to severe reduction of a species range and subsequent extinction of species, particularly tropical species. With the alarming exploitation and destruction of natural forests, the future of trees is on farms. There is, therefore, the need to domesticate and introduce these useful forest trees species to agroecosystems in order to prevent their extinction.

Leakey and Simons (1998) noted that the development of vegetative propagation techniques represent the first step in the process of domestication of a tree species. Vegetative propagation offers a unique opportunity of avoiding the problem of recalcitrant seeds predominant in tropical tree species. It also facilitates the transference of the genetic potential as well as the nonadditive variance of the parent to the new plant (Puri and Khara 1992). However, the rooting media, auxin concentration, and leaf area of cuttings are known to influence the rooting ability of juvenile stem cuttings (Mudge and Brennan 1999).

This study was carried out to determine the possibility of raising *E. chlorantha* through cuttings with a view to making it available for planting by farmers.

MATERIALS AND METHODS

Matured seed of *Enantia chlorantha* were obtained from Lafe near Ore, Nigeria (latitude 6° 44' N, longitude 4° 52' E) and raised in the nursery of the Department of Forest Resources Management, University of Ibadan. At 9 months, a total of 200 single-node stem cuttings were obtained from the seedlings. One hundred stem cuttings had their leaves reduced by 50% of the original sizes, while the remaining 100 cuttings remained as whole leaves.

Two types of rooting hormones (auxins), namely, indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) and their combinations IBA/NAA (1:1), all at four levels of 0, 50, 100, and 150 ppm, were used in treating the cuttings. Hormone application was by quick-dip method (Oni 1987). Sixty cuttings were treated with each type of auxin and their combination; thus, 10 cuttings were allocated to each treatment level and leaf size, while 20 cuttings were under

the control treatment, with 10 cuttings allotted to each leaf size (Table 1). After auxin treatment, the cuttings were set in germination trays containing washed and sterilized river sand at the rate of 10 cuttings per tray. Thereafter, the trays were placed under a high-humidity propagator and watered in the morning and evening with a fine-meshed sprayer. The complete block design was used in setting the cuttings.

Assessment was done after 60 days, for the following parameters:

- percentage survival of cuttings
- percentage of callused cuttings
- percentage of rooted cuttings
- number of roots per cutting
- length of longest root per cutting

The data obtained were subjected to analysis of variance using the SAS package. All percentile data were arc sine transformed before analysis.

Table 1. Experimental design and allocation of treatment to cuttings.

Cutting	IBA	NAA	IBA/NAA	Control	Total
50% leaf	30	30	30	10	100
100% leaf	30	30	30	10	100
Total	60	60	60	20	200

RESULTS

Percentage Survival of Cuttings

Table 2 gives the summary of percentage survival of cuttings under the different auxins and their concentrations. Percentage survival differed among the auxins and their levels. Also, leaf size influenced percentage survival of the cuttings under the different auxin treatments. Half-leaf cuttings had the highest mean percentage survival of 70% with NAA and the control followed by IBA with a value of 60%, while the lowest mean value of 40% was obtained with the combination of IBA/NAA. Among the full-leaf cuttings, the control and the combination of IBA/NAA had a mean percentage survival of 100%, followed by IBA with 80% and NAA with 40%.

Mortality also differed among the auxins and leaf sizes. The highest mean percentage mortality of 60% was recorded among half-leaf cuttings receiving NAA treatments and the combination of IBA/NAA, while the lowest mean value of 30% was obtained with the control and NAA. Mortality among the full-leaf cuttings was highest for NAA and IBA/NAA (60%), while the control had 0%. Percentage survival for the different auxin levels also differed. IBA at 50 ppm had 80% survival for full-leaf cuttings, while NAA at 50 ppm had 70% survival for full-leaf cuttings. The combination of IBA/NAA at 50 ppm had 100% survival for full-leaf cuttings. IBA at 150 ppm had 60% survival for half-leaf

cuttings, while NAA at 150 ppm had 70% survival for half-leaf cuttings. The combination of IBA/NAA at 100 ppm and 150 ppm had 50% survival each for half-leaf cuttings. Auxin type, concentration of auxin, and leaf area of cuttings, as well as interaction between auxin and leaf size and between auxin concentration and leaf size, had significant effect on the survival of the cuttings. (Table 3)

Percentage of Callused Cuttings

Callusing of cuttings was also affected by the different auxin treatments (Table 2). Among the auxin treatments used on full-leaf cuttings, the combination of IBA/NAA had the highest mean percentage of callused cuttings (100%), followed by the control with 90%; the least mean value of 40% was obtained with NAA. Callusing among the half-leaf cuttings was highest for the control and NAA (70%), and the lowest mean value of 30% was obtained with NAA. Among the auxin levels, NAA at 150 ppm produced the highest mean percentage of callused cuttings (70%) among half-leaf cuttings, followed by IBA at 150 ppm, with 60%; the lowest mean value of 30% was obtained with NAA at 50 ppm. Among the full-leaf cuttings, the highest percentage of callused cuttings (100%) was observed under the combination of IBA/NAA at 50 ppm followed by the control at 90%, while the lowest value of 40% was observed under NAA at 150 ppm and IBA/NAA at 100 ppm.

Percentage of Rooted Cuttings

The effect of auxin type, auxin concentration, leaf size, interaction between auxin type and concentration, interaction between auxin type and leaf size, and interaction between auxin concentration and leaf size failed to show any significant effect on percentage rooting of the cuttings (Table 3). The percentage of rooted cuttings was affected by the IBA treatment only. Full-leaf cuttings treated with IBA at 50 ppm and the control showed the highest percentage of rooted cuttings of 10% each. Rooting was not observed for any half-leaf cutting nor for any auxin treatment or auxin level.

Number and Lengths of Roots per Cutting

The effects of auxin type, auxin concentration, and leaf size, as well as the interaction between the auxin type and concentration, the auxin type and leaf size, and the auxin concentration and leaf size were not significant on the number and length of roots per cutting of *E. chlorantha* (Table 3). The highest number of roots (3) and largest length (3 cm [1.2 in.]) of roots were obtained among full-leaf cuttings under the control treatment followed by full-leaf cuttings treated with IBA at 50 ppm, with values of 1 and 0.6 cm [0.24 in.] for root number and length of root, respectively. Rooting was not obtained with the other treatments.

Table 2. Summary of percentage survival, mortality, callusing, and rooting among juvenile stem cuttings of *Enantia chlorantha* treated with two auxin types and their combination.

Assessment parameter	Level									
	Control	IBA			NAA			IBA/NAA		
	0 ppm	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm
Mortality (50% leaf)	30	50	50	40	60	50	30	60	50	50
Mortality (100% leaf)	0	20	50	30	30	50	60	0	60	30
Survival (50% leaf)	70	50	50	60	40	50	70	40	50	50
Survival (100% leaf)	100	80	50	70	70	50	40	100	40	70
Callusing (50% leaf)	70	50	50	60	30	40	70	40	50	50
Callusing (100% leaf)	90	60	50	60	60	50	40	100	40	70
Rooting (50% leaf)	0	0	0	0	0	0	0	0	0	0
Rooting (100% leaf)	10	10	0	0	0	0	0	0	0	0

Table 3. Summary of analysis of variance for percentage survival, percentage of callusing, percentage of rooting, and number and length of roots per cutting of *Enantia chlorantha*.

SV	DF	% survival		% callusing		% rooting		No. of roots	Root length
		MS	F value	MS	F value	MS	F value	F value	F value
Hormone (H)	3	0.27	5.57*	0.39	1.14 ^{ns}	0.00	0.00 ^{ns}	1.28 ^{ns}	2.23 ^{ns}
Concentration (C)	3	0.31	6.31*	0.23	0.75 ^{ns}	0.00	0.00 ^{ns}	1.28 ^{ns}	1.2 ^{ns}
Leaf Size (L)	1	0.41	8.45*	0.002	0.00 ^{ns}	0.00	0.00 ^{ns}	0.8 ^{ns}	
H × C	3	0.13	2.61 ^{ns}	0.17	0.51	0.00	0.00 ^{ns}	0.00 ^{ns}	
H × L	3	0.27	5.52*	0.35	1.02 ^{ns}	0.00	0.00 ^{ns}	1.28 ^{ns}	
C × L	3	0.23	5.23*	0.28	0.82 ^{ns}	0.00	0.00 ^{ns}	1.28 ^{ns}	
Error	3	0.05		0.34		0.00			

* = Significant at 5%; ns = not significant at 5% level of probability.

DISCUSSION

The development of propagation method is the first step in any domestication effort (Leakey and Simons 1998). The possibility of rooting single-node cuttings of *E. chlorantha* is crucial to its domestication strategy. Usually, to enhance the rooting potential of nodal cuttings of plant species, these cuttings are treated with rooting hormones (auxins). Nanda (1975) noted that auxins are associated with the division and elongation of meristematic cells, and the differentiation of reserve food material, because auxins increase the activity of hydrolyzing enzymes. Also, concentrations of these hormones can be varied to ascertain the optimum level.

In this study, cuttings of *E. chlorantha* did not produce roots when treated with high concentrations of hormones; IBA at 50 ppm gave the highest percentage rooting of cuttings. This finding agreed with that of Puri and Verma (1995), who reported that lower concentrations of IBA and NAA triggered rooting in *Dalbergia sissoo*, while higher concentrations inhibited it. Also, Rana et al. (1987) noted that higher doses of auxins led to a supraoptimal level of rooting, which had inhibitory effects.

The importance of leaf area on the rooting ability of cuttings of tropical species has been documented (Leakey and Coutts 1989). Survival, callusing, and rooting of cuttings of *E. chlorantha* were better when a whole leaf was retained on each cutting compared to when the leaves were reduced to half size; this finding is in line with the observation of Badji et al. (1991), who noted that the presence of leaves promoted rooting and significantly improved survival of *Acacia senegal* cuttings. Leakey and Simons (1998) cautioned, however, that retention of too many leaves on cuttings might cause increased water loss, which can eventually lead to death of the cutting.

Observation of natural stands of *E. chlorantha* revealed inadequate natural regeneration. Thus, the possibility of vegetative propagation of this species may solve the problem of nonavailability of seeds as well as the dormancy associated with the seeds. This will facilitate the availability of planting stock and subsequent integration of the species into the agroecosystem.

A better result for survival and rooting for the control compared to those treated with higher concentrations of rooting hormones implies that *E. chlorantha* can be propa-

gated vegetatively at reduced cost, thus ensuring that this technology can be adopted with minimum capital to yield expected result.

In conclusion, *E. chlorantha* can be regenerated via single-node cuttings carrying the whole leaf, either without hormone treatment, or with even better results using low concentrations of IBA or combination IBA/NAA hormones.

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Résumé. Les effets des auxines et de la dimension des feuilles sur l'enracinement de boutures juvéniles à un œil de *Enantia chlorantha* Oliv ont été étudiés. Le pourcentage de survie des boutures différait selon les auxines, leur taux et les dimensions des feuilles. Les boutures avec une demie feuille ont enregistré le pourcentage moyen de survie le plus élevé, soit de 70%, à la fois avec l'acide acétique de naphthalène (AAN) et le groupe-témoin, suivies de celles avec l'acide indole-3 butyrique (AIB) avec un taux de survie de 60%, et enfin celles avec une combinaison de AAN et de AIB avec un taux de survie de 40%. Parmi les boutures avec une pleine feuille, le groupe-témoin et le groupe avec une combinaison de AAN et de AIB ont enregistré un pourcentage de survie de 100%, suivies du groupe avec l'AIB avec un taux de 80%, et du groupe avec l'AAN avec un taux de 40%. La formation de cal chez les boutures était aussi affectée par les différents traitements d'auxines. Parmi les auxines et les boutures comportant une pleine feuille, la combinaison de AAN-AIB a enregistré la moyenne la plus élevée en terme de pourcentage au niveau de la formation d'un cal avec un taux de 100%, suivi du groupe-témoin avec un taux de 90% et du groupe avec l'AAN avec un taux de 40%. La formation d'un cal parmi les boutures à demie feuille a été la plus élevée au sein du groupe-témoin et du groupe avec l'AAN avec un taux de 70%, alors que le taux le plus faible a été obtenu avec le groupe de l'AAN avec un pourcentage de 30%. L'enracinement parmi les divers types de boutures n'a été affecté uniquement qu'avec celles traitées avec l'AIB. Les boutures à pleine feuille traitées avec l'AIB à 50 ppm, ainsi que celles appartenant au groupe-témoin, ont enregistré le pourcentage le plus élevé d'enracinement avec un taux de 10% pour chacun des groupes. Le type d'auxine, la concentration, la dimension foliaire, l'interaction entre les auxines et leur concentration, l'auxine et la dimension foliaire, tout comme la concentration et la dimension foliaire n'ont eu aucune influence significative par rapport au pourcentage d'enracinement des boutures. Les meilleurs résultats de survie et d'enracinement des boutures traitées avec des hormones d'enracinement à des concentrations élevées, et ce par rapport aux boutures du groupe-témoin, peuvent être obtenues si le *E. chlorantha* est propagé végétativement à de faibles coûts, ce qui diminue les coûts et assure de ce fait que cette technologie puisse être adoptée avec des capitaux minimum par rapport aux résultats escomptés.

Zusammenfassung. Die Effekte von Auxin und Blattgröße auf das Wurzeln von jungen Stecklingen von *Enantia chlorantha* mit einer Node wurden untersucht. Die Überlebensrate differierte zwischen den Auxinen, ihrem Grad und der Blattgröße. Die Stecklinge mit einem halben Blatt verzeichneten die höchste durchschnittliche Überlebensrate von 70% unter NAA-Behandlung und der Kontrolle, gefolgt von 60% unter IBA und 40% Überlebensrate unter der Kombination von NAA/IBA. Unter den Stecklingen mit einem ganzen Blatt hatte die Kontrollbehandlung und die Kombination von NAA/IBA eine durchschnittliche Überlebensrate von 100%, gefolgt von 80% mit IBA und 40% mit NAA. Die Kallusbildung der Stecklinge wurde auch beeinflusst durch die unterschiedlichen Auxinbehandlungen. Unter den mit

Auxin behandelten Stecklinge mit ganzem Blatt hatte die Kombination von NAA/IBA den höchsten durchschnittlichen Prozentsatz von Kallusbildung (100%), gefolgt von der Kontrolle mit 90% und NAA mit 40%. Die Kallusbildung von Stecklingen mit einem halben Blatt war am größten unter der Kontrolle und NAA bei 70%, während der niedrigste durchschnittliche Wert von 30% unter NAA beobachtet wurde. Das Wurzeln der Stecklinge wurde nur durch IBA beeinflusst. Die Stecklinge mit dem ganzen Blatt mit der IBA-Behandlung von 50ppm und die Kontrollbehandlung gab den höchsten durchschnittlichen Anteil an bewurzelten Stecklingen von je 10%. Auxintyp, Konzentration, Blattgröße, die Interaktion zwischen Auxin und Konzentration, Auxin und Blattgröße sowie auch die Konzentration und Blattgröße hatte keinen signifikanten Effekt auf den Anteil an bewurzelten Stecklingen. Ein besseres Ergebnis bei der Überlebensrate und der Bewurzelung unter der Kontrolle verglichen mit diesen unter der Behandlung mit Phytohormonen in höheren Konzentrationen impliziert, dass *E. chlorantha* vegetativ zu reduzierten Kosten vermehrt werden kann, unter der Annahme, dass diese Technologie mit einem Minimum an Kapital verwendet werden kann um die erwarteten Resultate zu erzielen.

Resumen. Se investigó los efectos de las auxinas y tamaño de las hojas en el enraizamiento de un corte de tallo de *Enantia chlorantha* Oliv. El porcentaje de supervivencia de los tallos fue diferente entre las auxinas, sus niveles de follaje y tamaño de las hojas. Los tallos con la mitad de follaje registraron el porcentaje medio más alto de supervivencia, de 70%, bajo ácido naftaleno acético (NAA) y el control, seguido por ácido bórico (IBA) (60%) y la combinación de IBA/NAA con 40% de supervivencia. Entre los tallos con follaje completo, el tratamiento de control y la combinación de IBA/NAA tuvieron un porcentaje promedio de supervivencia de 100 seguido por IBA con 80% y NAA con 40%. La formación de callosidad en los tallos estuvo siempre afectada por la aplicación de los diferentes tratamientos de auxinas. Entre las auxinas y en los tallos con follaje completo, la combinación de IBA/NAA tuvo el porcentaje más alto de callosidad del 100% seguido por el control con 90%, mientras NAA tuvo 40%. La callosidad entre los tallos con la mitad de follaje fue más alta bajo control y NAA (70%), mientras el valor más bajo de 30% fue obtenido bajo IBA. El enraizamiento entre los cortes estuvo afectado solamente por el tratamiento IBA. Los tallos con follaje completo tratados con IBA a 50 ppm y los tratamiento de control dieron los porcentajes más altos de enraizamiento de 10% cada uno. El tipo de auxina, la concentración, el tamaño de la hoja, la interacción entre auxinas y la concentración, el tamaño de la hoja y auxina, así como la concentración y tamaño de la hoja no tuvieron efecto significativo en el porcentaje de enraizamiento de los tallos. Un mejor resultado de supervivencia y enraizamiento bajo el control, comparado con los tratamientos con hormonas de enraizamiento a altas concentraciones, implica que *E. chlorantha* puede ser propagado vegetativamente y reducir de esta manera los costos, asegurando que esta tecnología puede ser adoptada con mínimo capital y buen rendimiento.