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Domestication of *Tetracarpidium conophorum* (Mull. Arg.) Hutch & Dalziel in the Western Highlands of Cameroon: propagation studies

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ABSTRACT
Objective: As a contribution to the domestication of *Tetracarpidium conophorum* (African Walnut), the present study aimed at determining requirements for seed germination and stem cutting.
Methodology and results: Three accessions of seeds, three germination substrates [topsoil, sawdust and mixture of topsoil and sawdust in a 1/1 (v/v) ratio] and different levels of seeds dehydration (consisting of drying seeds at room temperature for 0, 1, 2, 3, 4 and 5 weeks) were tested for their effects on germination parameters. Furthermore, two propagation substrates (river sand and sawdust), five concentrations (0, 0.5, 1, 5 and 10 g/l) of exogenously applied indole-3-butyric acid (IBA) and three positions (apical, median and basal) from which cuttings were taken from the mother plant were tested for their effects on stem cutting parameters. The highest percentage germination combined with the shortest mean germination time was recorded on sawdust substrate. The seed storage behaviour was of intermediate type. *T. conophorum* is amenable to vegetative propagation through the rooting of stem cuttings with or without the use of exogenous growth hormone. The mortality rate of cuttings on sawdust substrate (37 ± 2.4%) was higher than that recorded on river sand (6 ± 1.9%). At eight weeks after planting, cuttings, which were still alive, had rooted at 100%. The highest mean number of roots per rooted cutting (40.2 ± 1.54) was recorded with the combination of fine river sand substrate and 5 g/l IBA.
Conclusion and application of findings: For propagating *T. conophorum* from seeds, it is recommended that fresh mature seeds be sown in sawdust substrate without any pre-germination treatment. For an efficient propagation of *T. conophorum* by stem cutting, fine river sand is recommended as propagation substrate and 5 g/l of exogenous IBA should be applied to cuttings, irrespective of the position from which cuttings are taken from the mother plant. These are valuable information to optimize both the sexual and the asexual propagation protocols for *T. conophorum*. This represents an important step in the domestication process of this valuable plant species that has been exploited in the wild. Researchers and farmers developing nurseries for the domestication of high-valued plant species could benefit from our findings.
Keywords: African walnut, *Tetracarpidium conophorum*, domestication, propagation, seed germination, stem cutting.

INTRODUCTION

Non-timber forest products (NTFPs) contribute to the livelihoods of many people in sub-Saharan Africa (Marshall *et al.*, 2003; Hill *et al.*, 2007 ; Atangana *et al.*, 2014; Ndiaye *et al.*, 2024), and are therefore the focus of particular attention. The domestication of native species producing NTFPs with high socio-economic potential, which involves adopting propagation techniques adapted to the species (Leakey and Simons, 2004; Tchoundjeu *et al*., 2002), is practised in several developing countries in tropical and subtropical regions (Kanmegne *et al*., 2022). Research over the last three decades has led to the development of domestication strategies for several priority species. Nevertheless, some species among which *Tetracarpidium conophorum* have been neglected by research although identified as prime candidates for domestication (Leakey *et al*., 2022). *Tetracarpidium conophorum* (Mull. Arg.) Hutch & Dalziel (commonly known as African walnut), a species of the Euphorbiaceae family (APG III, 2009), is one of the NTFP-producing species extensively used by people in Western and Central Africa (Fig. 1).

Figure 1: *T. conophorum* vine with its bushy foliage crown completely covering a stake plant

The species is a liana measuring 30 to 40 metres (Jiofack, 2012; Gambi *et al*., 2021). It is found in the wild in forested areas of sub-Saharan Africa, including Nigeria and Cameroon, where its seeds are widely used as food (Jiofack, 2012; Ilomuanya *et al*., 2018). In addition to its food use, the plant is also used in traditional pharmacopoeia (Ajaiyeoba and Fadare, 2006; Oladiji *et al*., 2007; Esosa and Ehimwenma, 2017). Indeed, the leaves are used to relieve a number of ailments, including constipation, hiccups, dysentery and syphilis, and as an antidote to snake bites (Oladiji *et al.*, 2007). In West Africa, the leaves are used to solve male fertility problems (Ajaiyeoba and Fadare, 2006; Akomolafe *et al*., 2015). Seeds' consumption helps fight indigestion (Tapsell *et al.*, 2004), increases sexual performance in men (Aladeokin and Umukoro, 2011) and protects against oxidative stress (Nwaoguikpe *et al.*, 2012). Despite the importance of *T. conophorum* in the daily life of people involved in its exploitation, the species has not been domesticated yet. Planting initiatives remain timid. Many farmers confine themselves to protecting wild plants on their farms. The domestication and the cultivation of the species could be a strategy to intensify and diversify smallholder farming systems, thus contributing to the improvement of the well-being of many populations. Research on propagation strategies is an important step in any domestication program. *T. conophorum* is known to propagate naturally by seeds (Jiofack *et al.*, 2012). Nevertheless, the requirements for seed germination as well as the classification of seed storage behaviour have not been documented for the species. Literature on optimal conditions for seed germination is lacking. In addition, literature on efficient method of vegetative propagation of this plant species is almost none-existent. As contribution to the domestication of *T. conophorum*, the present study aimed at 1) determining germination requirements and desiccation tolerance of seeds, and 2) evaluating its amenability to vegetative propagation by stem cutting. This paper examines the influence of seeds' origin, germination substrate and seed dehydration on germination parameters. It also investigates the rooting response of stem cuttings to different propagation substrates, different auxin treatments and different positions from which cutting are taken from the mother plant.

MATERIALS AND METHODS

Characterization of seeds germination

Seed material: Four accessions of seeds that originated from four locations spread in three different agro ecological zones in Cameroon were used in the present study. The four locations were Bafia (belonging to the agro ecological zone of bimodal rainfall humid forest), Fombap (belonging to the agro ecological zone of Western Highland), Manjo and Bale (both belonging to the agro ecological zone of mono modal rainfall humid forest). In each location, a minimum of 400 mature and disease-free follicles (containing one to five seeds each) were collected at the feet of at least 20 randomly selected plants in September 2022 and carried to the Laboratory of Applied Botany of the University of Dschang, Cameroon. In the laboratory, seeds were extracted from follicles and mixed together to have a single seed batch for each location.

Seeds' viability test: Only viable seeds were used in the present study. Seeds' viability was determined using floatation method as follows: after soaking seeds in water for a few minutes, those that floated were considered unviable and were discarded, while those that sank were considered viable and used for further experiments (Ambebe and Achankeng, 2019).

Germination assay: To investigate *T. conophorum* seed germination, viable seeds from each accession were sown 3 cm depth in plastic perforated polythene bags containing germination substrate. The substrate was either topsoil, sawdust or a mixture of topsoil and sawdust in a 1/1 (v/v) ratio. Four accessions and three substrates were thus tested in a 4 x 3 factorial experiment. For each accession of seeds, four replicates each of 50 seeds were sown in each substrate, making a total of 2400 seeds for the assay (4 accessions x 3 substrates x 4 replications x 50 seeds). The seeded polythene bags were then placed in the nursery (23 ± 3 °C) and watered daily using a sprayer. Seeds whose cotyledons emerged above the substrate because of hypocotyls elongation were recorded as having germinated. Germination was recorded daily and the experiment ran for nine months.

Desiccation tolerance test: Viable seeds from a single tree located in Bale were used to assess the desiccation tolerance test. Fresh seeds were spread in a single layer on the top of laboratory bench and left to dry at room temperature. The temperature of the laboratory was 24 ± 2 °C and the relative humidity was 55 ± 5%. At 1-week intervals, seed samples were removed from the laboratory bench top and used for moisture content measurement and germination test. The moisture content was determined by weighing seed sample before and after drying then in the oven at 105 °C for 24 hours. The moisture content was expressed in percentage as follows: MC (%) = [(FM-DM)/FM] x 100, where FM and DM are the mass of samples before and after drying respectively (ISTA, 2004). Each value of moisture content was the mean of four measurements. For the germination test, four replications each of 50 seeds were withdrawn from the bench top at 1-week intervals and left to germinate in sawdust substrate for nine months as described above.

Data analysis: Data were analysed using SPSS 21.0 software package. The dependent variables were germination percentage and mean germination time (MGT), which was calculated according to Bewley and Black (1994), as follows: $MGT = \Sigma(j \times nj)/\Sigma nj$, where MGT is the mean germination time (days); j is the number of days starting from the date of sowing; nj is the number of seeds that germinated at the j^{th} day from sowing. Prior to analysis, percentage data were transformed into arsine square root values (Stroup, 2018). The analysis of variance (ANOVA) was performed using the General Linear Model to determine the significance of the effect of the independent variables (accession, substrate and interaction between both) on germination percentage and mean germination time. Means were compared using Duncan's multiple comparison test (at $p < 0.05$).

Stem cutting assay

Stock plants production: In the present study, cuttings' donor plants were produced from viable seeds, which were extracted from pods, fallen from a total of twelve T. *conophorum* stands in 2022 in the locality of Fombap. These seeds were allowed to germinate at the nursery of the Department of Plant Biology, University of Dschang, in black perforated polythene bags filled with sawdust substrate. The seedlings that emerged from germinated seeds were allowed to grow in the nursery for six months, using raffia bamboos as stakes. The cuttings were then collected from the resulting 6-month-old plants, which measured, between 90 and 120 cm length.

Preparation of cuttings: From the six month-old plants, the top 10 cm of each stem, which represented the most juvenile part, was cut off and discarded. Cuttings were collected from the decapitated shoots, which represented the semi-lignified part of the stem. Three cuttings each measuring 10 to 12 cm length and comprising at least two nodes were taken from each decapitated stock plant; namely one from the apical position (on the first 15 cm from the top), the second from the median position (between the 16^{th} and 30^{th} cm from the top), and the third from the basal position (between the 31^{st} and 45^{th} cm from the top). All the leaves carried by the cuttings were cut off and discarded, except that attached to the upper node. Cuttings were soaked for 10 min in a fungicide solution (Metalaxyl-M 3 g/l) then dried in the shade for 15 minutes before auxin treatment. Auxin treatment consisted of dipping the base of cutting for 10 seconds in one of the five concentrations (0, 0.5, 1, 5 or 10 g/l) of alcoholic indole-3 butyric acid (IBA). After auxin treatment, alcohol was evaporated off using a stream of cold air provided by a portable fan before inserting cuttings in the propagator.

Propagation environment: The propagation environment consisted of non-mist polythene propagators that were constructed following the design by Leakey (2014), with either fine river sand or sawdust as propagation substrate. Within the propagator, relative humidity ranged between 70 and 80%, while temperature was 24 ± 2 °C throughout the experiment period.

Experimental design: The experimental design used in the present study was a split split-plot with three replicates. Within each replicate two substrates (river sand and sawdust), three positions from which cuttings were taken from stock plants (apical, median and basal) and five IBA concentrations (0, 0.5, 1, 5 and 10 g/l) were tested at plot, sub-plot and sub sub-plot levels respectively. Ten cutting were used at each sub sub-plot, making a total of 900 cuttings for the experiment (3 replicates x 2 substrates x 3 positions x 5 IBA concentrations x 10 cuttings). Cuttings were planted by inserting vertically their base 3 cm deep into the substrate. To avoid desiccation, cuttings received a fine spray of water at 2-day intervals.

Data collection and analysis: Cuttings were inspected for evidence of rooting at 2-week intervals and the experiment ran for two months. At the end of the assay, each cutting was assessed for mortality, rooting and roots count. Data were analysed using IBM SPSS.21 software package. The dependent variables were mortality rate (percentage of dead cuttings), percentage of rooted cuttings and number of roots per rooted cuttings. Percentages were transformed into their arsine square root values before analysis, as described above. Data were submitted to analysis of variance (ANOVA) and means were compared using Duncan's multiple comparison test ($p < 0.05$).

RESULTS

Seeds' germination

Effect of seeds' accession and germination substrate on germination parameters: In the seeded polythene bags, germination was of epigeal type, consisting of the emergence of cotyledon above substrate surface, because of hypocotyls elongation (Fig. 2).

Figure 2: Germinated *T. conophorum* seed showing an epigeal growth

Seeds' accession, germination substrate and combination of both factors had highly significant effects ($p < 0.001$) on both germination rate and mean germination time (Table 1).

Table 1: Results of the analysis of variance (ANOVA) showing the degree of freedom (*df*) and the level of significance (*F* and *p* values) of the effects of accession, substrate and combination of both factors on the parameters of *T. conophorum* seeds' germination

Source of variation	**Germination rate**			**Mean germination time**		
	df	***F***	***p***	***df***	***F***	***p***
Accession	3	52.85	< 0.001	3	125.46	< 0.001
Substrate	2	85.46	< 0.001	2	5.34	0.001
Accession x substrate	6	9.06	< 0.001	6	2.79	0.011

Percentage germination: When considering the four accessions taken all together, the percentage germination recorded on the three substrates were different from each other. It was highest on sawdust (69.7 ± 2.4%), followed by mixture of topsoil and sawdust (53.6 ± 2.6%) and lowest on topsoil (28 ± 2.3%). Likewise, when considering the three substrates taken together, the highest percentage germination was recorded with Bale accession (68.8 ± 2.8%) while the lowest (23.7 ± 2.6%) was recorded with Manjo accession. Percentages germination recorded with Bafia (53.7 ± 3.1%) and Fombap (55.5 ± 3%) were not different from each other but were lower than that obtained with Bale accession and higher than that obtained with Manjo accession. Regarding substrate x accession combinations, the highest percentage germination (83.3 ± 4.1%) was recorded with Bale accession sown on sawdust substrate (Table 2).

Table 2: Germination rate (%) of different *T. conophorum* seed accessions on different substrates

Accessions	**Substrates**			**Means**
	Topsoil	**Sawdust**	**Topsoil + Sawdust**	
Bafia	10.0 ± 3.3^{g}	87.7 ± 3.6^{a}	63.3 ± 5.4^{c}	53.7 ± 3.1^{B}
Bale	46.6 ± 5.6^{d}	$83.3 \pm 4.1a^{b}$	76.6 ± 4.7^{b}	68.8 ± 2.8^{A}
Fombap	45.5 ± 5.6^{d}	71.1 ± 5.1^{b}	50.0 ± 5.58^{d}	55.5 ± 3^{B}
Manjo	10.0 ± 3.3^{g}	36.6 ± 5.3^{e}	24.4 ± 4.8^{f}	23.7 ± 2.6^{C}
Means	$28.0 \pm 2.3^{\gamma}$	$69.7 \pm 2.4^{\alpha}$	$53.6 \pm 2.6^{\beta}$	

Note: In the last column, the means ± SE followed by different capital letters are significantly different; in the last row, the means ± SE followed by different symbols are significantly different; within the table, the means ± SE followed by the same small letter are not significantly different.

Mean germination time: When considering the three substrates taken together, the shortest mean germination time was recorded with Fombap accession (68.46 ± 3.6 days), followed by Manjo (129.65 ± 6.25 days). The longest mean germination times were recorded with Bafia (153. 04 ± 5.43 days) and Bale (158.37 ± 2.27) accessions which were not significantly different from each other. Likewise, when considering the four accessions taken together, the mean germination time obtained in response to the use of sawdust substrate (114.15 ± 5.64 days) was shorter than those obtained with topsoil (134.18 ± 3.41 days) and mixture of topsoil and sawdust (133.80 ± 2.91 days) substrates. These two last substrate were not different from each other for the mean germination time (Table 3)

Table 3: Mean germination times (days) of different accessions of *T. conophorum* seeds on different substrates.

Accessions	Substrates			Means
	Topsoil	Sawdust	Toil + Sawdust	
Bafia	160.24 ± 5.74^{f}	137.33 ± 4.46^{d}	161.54 ± 4.88^{f}	153.04 ± 5.43^{C}
Bale	162.78 ± 5.15^{f}	162.35 ± 6.69^{f}	149.97 ± 5.01^{de}	158.37 ± 3.27^{C}
Fombap	76.33 ± 6.46^{b}	58.70 ± 6.77^{a}	70.34 ± 5.42^{b}	68.46 ± 3.60^{A}
Manjo	137.36 ± 9.25^{d}	98.22 ± 14.46^{c}	153.36 ± 7.55^{ef}	129.65 ± 6.25^{B}
Means	134.18 ± 3.41$^{\beta}$	114.15 ± 5.64$^{\alpha}$	133.80 ± 2.91$^{\beta}$	

Note: In the last column, the means ± SE followed by different capital letters are significantly different; in the last row, the means ± SE followed by different symbols are significantly different; within the table, the means ± SE followed by the same small letter are not significantly different.

Effect of desiccation on germination parameters of *T. conophorum* seeds: Figure 3 shows that the fresh *T. conophorum* seeds had an initial moisture content of 39.3 ± 0.25%. As seeds were dried, their moisture content gradually decreased and reached the lowest value (20 ± 0.4%) at four weeks of drying, after which it remained constant. At the same time, the percentage germination slightly increased from 60% at week-0 to 66.6% at week-1, then progressively decreased with decreasing moisture content and reached its lowest value (13.3 ± 0.35%) at week-4 after which it remained constant. The mean germination time of fresh seeds was 46.73 ± 0.23 days. The value of this parameter increased with decreasing moisture and reached the value of 140 ± 2.1 days after five weeks of drying, when the moisture content was 20 ± 0.4%.

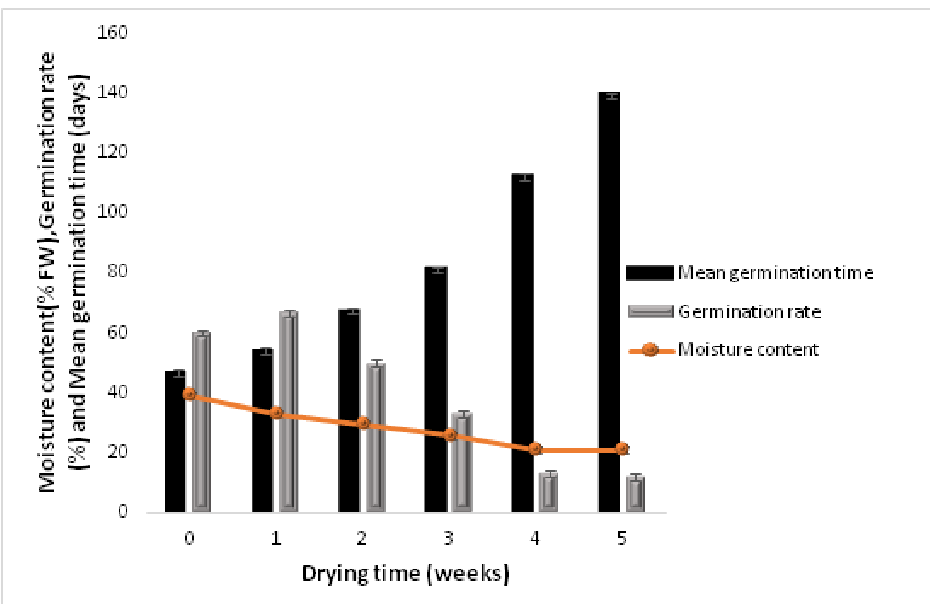

Figure 3: Variation in moisture content, germination rate and mean germination time as a function of drying time of *T. conophorum*

Stem cutting

Morphological differentiation patterns of cuttings: At eight weeks after planting, cuttings, which were still alive, had developed different morphological differentiation patterns. Indeed, 90% of living cuttings had developed roots at their base and a new shoot from the upper axillary bud (Fig. 4a) while 8% had developed only roots at the base (Fig. 4b), and the remaining 2% had lost their leaf and developed roots at their base (Fig. 4c). All the living cuttings had rooted, making 100% of rooted cuttings whatever the substrate, the concentration of exogenously applied IBA or the position from which the cuttings were taken from the mother plant.

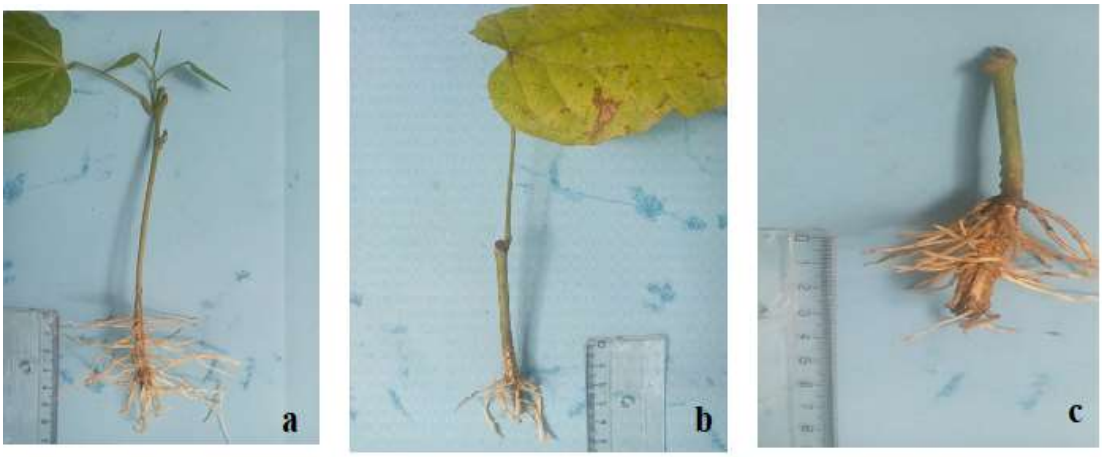

Figure 4: Different differentiation patterns from *T. conophorum* cuttings at eight weeks after planting in propagator. (a) Cutting which had developed both roots and shoot; (b) cutting which had developed exclusively roots; (c) cutting which had lost its leaf and had developed roots

Apart from the percentage of rooted cuttings which did not varied in response to variations of propagation substrate, IBA concentration and position from which the cutting were taken, the other cutting parameters (mortality rate and number of roots per rooted cutting) were variously affected the factors investigated (Table 4).

Table 4: Results of the analysis of variance showing the degree of freedom (*df*) and the levels of significance (*F* and *p* values) of the effects of different factors (substrate, position, IBA concentrations and their interactions) on the variables investigated (mortality rate, percentage of rooted cuttings and mean number of roots per rooted cutting)

Source of variation	**Mortality rate**			**Percentage of rooted cuttings**			**Mean roots count**		
	df	***F***	***p***	***df***	***F***	***p***	***df***	***F***	***p***
Substrate	1	86.54	< 0.001	1	-	-	1	31.97	< 0.001
Position	2	0.45	0.95	2	-	-	2	2.38	0.093
[IBA]	4	1.40	0.23	4	-	-	4	38.87	< 0.001
Substrate x Position	2	0.91	0.23	2	-	-	2	3.80	0.052
Substrate x [IBA]	4	5.26	< 0.001	4	-	-	4	7.75	0.001
Position x [IBA]	8	1.08	0.09	8	-	-	8	2.08	0.036
Substrate x Position x [IBA]	4	0.92	< 0.001	4	-	-	4	0.56	0.688

Mortality rate of cuttings: The analysis of variances (Table 4) showed that the substrate and the combination of substrate and IBA concentration had a significant influence ($p < 0.001$) on the mortality rate. However, IBA concentration and position had no individual influence on the mortality rate of *T. conophorum* cuttings. When considering the IBA concentrations and the positions taken all together, the mortality rate obtained on the sand substrate (6 ± 1.9%) was significantly lower than that obtained with sawdust (37 ± 2.4%) (Fig. 5). Regarding the combination between the substrate and the concentration of IBA, the mortality rate on the sand substrate ranged between 0 and 10% whatever the concentration of IBA, whereas on the sawdust substrate, the mortality rate was between 40% and 50% whatever the concentration of IBA (Fig. 6).

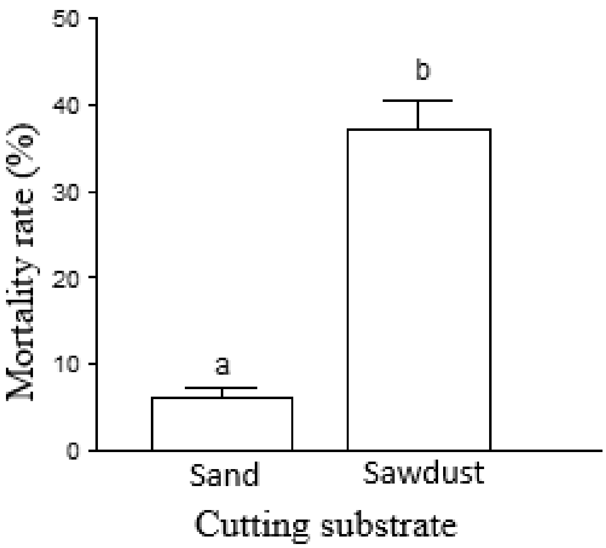

Figure 5: Mortality rate of *T. conophorum* cuttings on different substrates

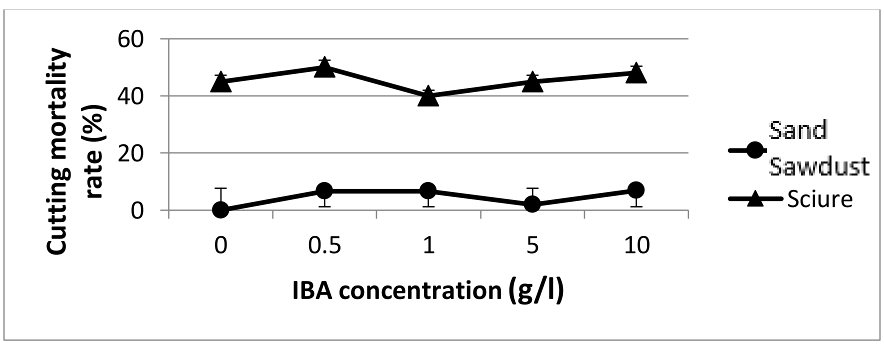

Figure 6: Variation of the mortality rate of *T. conophor*um cuttings as a function of the concentration of exogenous IBA on different propagation substrates.

Number of roots per rooted cutting: The results of the analysis of variance (Table 4) indicate that both substrate and IBA concentration had highly significant effect on the mean number of roots per rooted cutting, whereas the effect of position was not significant. The combinations between substrates and IBA concentrations, as well as the combination between positions and IBA concentrations also had significant effects on mean roots count. Whatever was the substrate, the number of roots per rooted cutting increased with increasing IBA concentration and reached its highest value when IBA concentration was 5 g/l, after which the mean roots count decreased with increasing IBA concentration. At each IBA concentration, the number of roots per rooted cutting recorded on sand substrate was significantly higher than that recorded on sawdust. The best substrate x [IBA] combination for inducing prolific rooting was thus sand x 5 g/l IBA with which a mean of 40.2 ± 1.54 roots per rooted cutting was recorded (Fig. 7).With regard to the combinations between the position of the cuttings and the concentration of IBA, table 5 indicates that, when considering the IBA concentrations taken all together, the numbers of roots per rooted cutting that were recorded with the three different cutting positions were not significantly different from each other. However, the mean roots count increased with increasing IBA concentration and reached its highest value (32.3 ± 1.4) at 5 g/l IBA after which the mean roots count decreased with increasing auxin concentration. Cuttings taken from the base position and treated with either 5 g/l or 10 g/l IBA resulted in highest mean roots counts (i.e. 33.4 ± 2.6 and 34 ± 3.2 respectively, with no significant difference among each other).

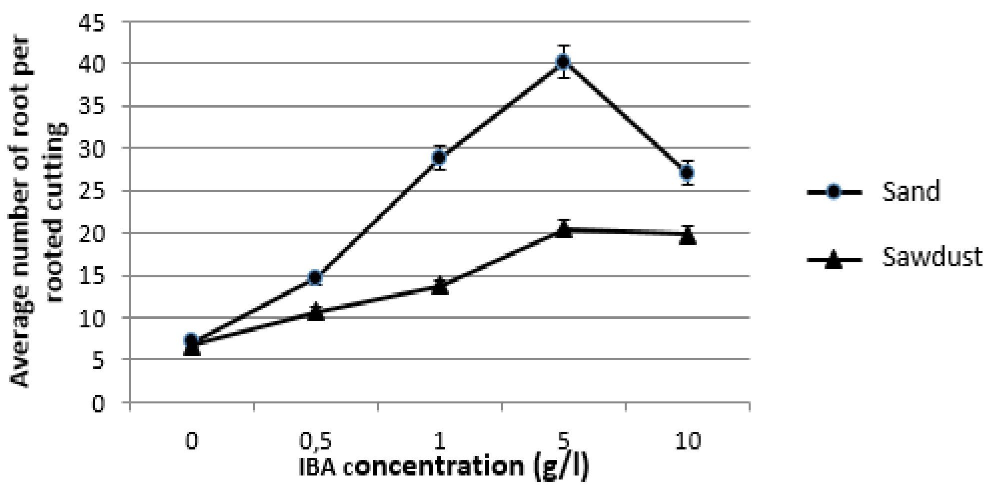

Figure 7: Variation in the average number of roots per rooted cutting of *T. conophorum* as a function of the concentration of exogenous IBA and on different cutting substrates.

Table 5: Average number of roots per rooted cutting under the effects of different concentrations of exogenous IBA and different sampling positions of *T. conophorum* cuttings.

[AIB] (g/l)	**Position**			**Mean**
	Apical	Median	Basal	
0	5.73 ± 2.1^{e}	7.55 ± 2.3^{de}	8.20 ± 2.6^{de}	6.9 ± 1.3^{D}
0,5	12.36 ± 2.7^{d}	10.94 ± 2.1^{d}	18.90 ± 2.6^{c}	14.2 ± 1.5^{C}
1	22.42 ± 2.1^{c}	19.36 ± 2.0^{c}	20.40 ± 2.6^{c}	23.4 ± 1.3^{B}
5	32.17 ± 2.0^{a}	29.88 ± 2.7^{ab}	33.40 ± 2.6^{a}	32.3 ± 1.4^{A}
10	18.31 ± 2.0^{c}	25.15 ± 2.1^{bc}	34.00 ± 3.2^{a}	24.7 ± 1.4^{B}
Mean	19.50 ± 1.0^{ns}	19.37 ± 1.0^{ns}	22,94 ± 1.2^{ns}	

Note: In the last column, the means ± SE followed by different capital letters are significantly different; within the table, the means ± SE followed by different small letters are significantly different; ns: no significant effect of the position of the cuttings on the mean number of roots per rooted cutting.

DISCUSSION

Seed provenance significantly affected both the germination rate and the mean germination time of *T. conophorum* seeds. This was not surprising, since many previous studies have established that within the same species, germination characteristics could vary between and within populations, and even within individuals (Baskin and Baskin, 2001; Bischoff *et al.*, 2006; Kanmegne *et al.*, 2016). Some of these variations have been attributed to either the genotype or the environmental conditions in which the seed matured on the parent plant (Donohue *et al.*, 1998; Baskin and Baskin, 2001). Nevertheless, variations in germination data as reported in this study could not be linked to the geographical origin of the seeds, since Manjo and Bale accessions, which originated from the same agro-ecological zone were different for their germination rates as well as for their mean germination time. These variations may be attributed to differential germination responses, which is common in seed batches. The substrate may indirectly influence the germination process, through its impact on the availability of water and oxygen (Justice, 1972). As seeds were watered daily, there was no problem of water availability. The differences among the different substrates for their effect on germination parameters may thus be attributed to different levels of aeration. The best seed germinability (highest germination percentage and shortest mean germination time) recorded on sawdust substrate in this study is an indication that this substrate had the most adequate aeration needed for seed germination. Seeds of a plant species may display orthodox, intermediate or recalcitrant storage behaviour (Daws *et al.*, 2006). In the present study, seeds, which were dried to a water content of 20% fresh mass, still germinated, although at a rate lower than that recorded with fresh (undried) seeds. This indicates that *T. conophorum* seeds can be classified as intermediate, since dehydration reduced the germinability of seed without suppressing it. The position from which cutting were taken on the mother plant did not affect cuttings' mortality rate, percentage of rooted cuttings or mean number of roots per rooted cutting. These results contrast with those reported with many other plant species (Tchoundjeu and Leakey, 2001; Kanmegne *et al.*, 2015) where the rooting success of stem cuttings drastically varied with the position from which they were collected on the mother plant. These variations have been associated with the physico-chemical gradients existing along the stem, including the gradient in the level of tissue lignification (Leakey, 2004; Mapongmetsem *et al*., 2012). It seems that these gradients were not enough pronounced in the climbing plant used in this study, resulting in cuttings' responses not influenced by their position. The best response of stem cutting (lowest mortality rate, highest percentage of rooted cuttings and highest mean number of roots per rooted cutting) was recorded with fine river sand substrate, compared to sawdust substrate. The determinism of these different responses of cuttings to different substrates remains to be elucidated, but these results provide evidence for practical recommendation of sand as propagation substrate for *T. conophorum* stem cutting. The significant role of auxin in root initiation is abundantly documented (Leakey, 2004; 2014). Application of exogenous indole butyric acid (IBA) has been used to induce root formation from stem cuttings of a wide range of plant species (Leakey *et al.*, 2022). In the present study, although exogenous IBA enhanced mean roots count with 5 g/l being the optimal concentration. *T. conophorum* cuttings rooted at 100% even without exogenous auxin application. Similar results were previously reported with many other species including *Bucholzia coriacea* (Akinyele, 2010), *Balanites aegyptiaca* (Mukhtar, 2019) and *Canarium schweifurthii* (Kanmegne *et al.*, 2022), and could be due to the amount of endogenous auxin already present in the plant tissues at the moment of severance. This indicates that *T. conophorum* can be successfully propagated by stem cuttings with or without growth hormone. Nevertheless exogenously applied IBA would have a beneficial effect on the number of roots per rooted cutting, with 5 g/l IBA being the optimal concentration for prolific rooting of stem cuttings.

CONCLUSION AND APPLICATION OF RESULTS

The results of the present study revealed that *T. conophorum* seeds could germinate without any pre-germination treatment. The seeds' accession, the germination substrate and the level of seeds dehydration significantly affected the germination percentage and the mean germination time. *T. conophorum* seeds exhibited intermediate seed-storage behaviour. For an efficient germination of *T. conophorum* seeds, sowing fresh (undried) mature seeds on sawdust substrate is recommended. *T. conophorum* is amenable to vegetative propagation by stem cutting with or without exogenous growth hormone application, in non-mist polythene propagators. Application of 5 g/l IBA to the cuttings, and the use of fine river sand as propagation substrate resulted in the lowest mortality rate of cuttings (0%), the highest percentage of rooted cuttings (100%) and the highest mean number of roots per rooted cutting (32.3). This provides evidence for practical recommendation of these propagation conditions (sand substrate x 5 g/l IBA) for *T. conophorum* stem cutting. This study provides valuable information to optimize both the sexual and the asexual propagation protocols for *T. conophorum*. This represents an important step in the domestication process of this valuable plant species that has been exploited in the wild in many parts of tropical Africa. Researchers and farmers developing nurseries for the domestication of high-valued plant species indigenous to the tropical Africa could benefit from our findings.

REFERENCES

Ajaiyeoba EO and Fadare DA, 2006**.** Antimicrobial potential of extracts and fractions of the African walnut—*Tetracarpidium conophorum*. African Journal of Biotechnology 5(22): 2322–2325.

Akinyele AO, 2010. Effects of growth hormones, rooting media and leaf size on juvenile stem cuttings of *Buchholzia coriacea* Engler. Annals of Forest Research 53(2): 127-133.

Akomolafe SF, Oboh G, Akindahunsi AA, Afolayan AJ, 2015. *Tetracarpidium conophorum* Mull. Arg. Hutch and Daziel inibits FeSO4- induced lipid peroxydation in rats genitals. BMC Complementary and Alternative Medicine 15(57): 1-8. https://doi.org/1186/s12906-015-0547-1.

Aladeokin AC, Umukoro S, 2011. Physiopharmacological properties of an aqueous extract of *Tetracarpidium conophorum* Hutzh and Dalziel in mice. Journal of Natural Medicine 65(3-4): 411-416.

Ambebe FT and Achankeng N, 2019. Influence of pre-sowing treatment and substrate on germination and seedling vigor of *Acacia angustissima* (Mill) Kuntze, *Entandrophragma angolense* (Welw.) C.DC. and *Newtonia camerunensis* Villiers. Research

Journal of Agriculture and Forestry Science *7*(2): 25-35.

APG III (The Angiosperm Phylogeny Group III), 2009. An update of the Angiosperm Phylogeny Group: classification for the orders and families of flowering plant: APG III. Bot J Linn Soc. 161(2): 105-121.

Atangana A, Khasa D, Chang S, Degrande A, 2014. Tropical agroforestry. Springer, Berlin. https://doi.org/10.1007/978-94- 007-7723-1.

Baskin CC, Baskin JM, 2001. Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego, Diego, San Francisco, New York, Boston, London, Sydney, Tokyo.

Bewley JD and Black M, 1994. Seeds: physiology of development and germination. Plenum Press, New York.

Bischoff A, Vonlanthen B, Steiner T, MullerScharer H, 2006. Seed provenance matters – Effects on germination of four plant species used for ecological restoration. Basic and Applied Ecology 7: 347-359.

Daws MI, Garwood NC, Pritchard HW, 2006. Prediction of desiccation sensitivity in seeds of woody species: a probabilistic model based on two seed traits and 104 species. Annals of Botany 97: 667-674. DOI: https://doi.org/10.1093/aob/mcl022

Donohue K and Schmitt J, 1998. Maternal environment effects in plants: adaptive plasticity. In: Mousseau TA and Fox CW (eds) Maternal Effects as Adaptation. University Press, Oxford, UK.

Esosa SU, Ehimwenma SO, 2017. Antioxidant Properties Associated with the Biochemical Changes in the Development of African Walnut (*Tetracarpidium conophorum*) Fruit. The Saudi Journal of Life Sciences 2: 217-229

Gambi KG, Moganga BB, Mweru JPM, 2021**.** Contribution à l'étude de la filière « Kasu » *Tetracarpidium conophorum* (Mull. Arg) Hutch & Dalz. (Euphorbiaceae) : un des produits forestiers non ligneux de Kisangani et ses environs (R.D. Congo). Journal of Business management 7 (1): 2455-6661

Hill T, Ouedraogo Y, Conditamde L, 2007. L'entreprise villageoise d'exploitation des arbres au Burkina Faso : appuyer le développement des petites entreprises dont l'activité est fondée sur les produits forestiers non ligneux. Unasylva-La foresterie à petite échelle 58 (228). https://www.fao.org/tempref/docrep/fao/010/a1346f/a1346f00.pdf

Ilomuanya MO, Amenaghawon NA, Odimegwu J, Okubanjo OO, Aghaizu C, Oluwatobiloba A, Akimien T, Ajayi T, 2018. Formulation and optimization of gentamicin hydrogel infused with *Tetracarpidium conophorum* extract via a Central Composite Design for topical delivery. Turk Journal of Pharmaceutical Science 15(3): 319-327.

ISTA, 2004. International rules for seed testing. Rules 2004. International Seed Testing Association, Zurich, Germany.

Jiofack TBR, 2012. Gestion des populations d'un produit forestier non ligneux à usage multiple : (*Tetracarpidium conophorum* (Mull. Arg.) Hutch. & Dalz. (Euphorbiaceae) dans les systèmes d'aménagement forestier au Cameroun. Thèse, Université de Kinshasa, 286 pages.

Jiofack TBR, Lejoly J, Tchoundjeu Z, Guedje MN, 2012**.** Agroforestry and socioeconomic potential of a non-conventional liana: *Tetracarpidium conophorum* (Müll. Arg.) Hutch. & Dalz. in Cameroon. Bois et Forêts des Tropiques 313(3) : 35-45.

Justice OL, 1972. Essentials of seed testing. In: Kozlowski TT (ed) Seed biology Vol. 3, Academic Press, New York and London, USA and UK.

Kanmegne G, Anoumaa M, Fotso, Mbouobda HD, Omokolo ND, 2016. Germination and sensitivity to desiccation of *Cola anomala* (K. Schum.) seeds. Fruits 71(2): 87-92.

Kanmegne G, Anoumaa M, Mbibong DA, Mbouobda HD, Fotso, Omokolo DN, 2015. Domestication of *Cola anomala* K. Schum. (Sterculiaceae): amenability to vegetative propagation. Forests, Trees and Livelihoods 24(1): 18-26

Kanmegne G, Atchioutchoua SR, Tsopmbeng NGR, 2022. Propagation of agro-forestry tree species by air-layering: a case study of *Canarium schweinfurthii* Engl. in the Western Highlands of Cameroon. Journal of Applied Biosciences 174: 18103-18112.

Leakey RRB, 2004. Physiology of vegetative reproduction. In: Burley J, Evans E, Younquist JA (eds.) Encyclopaedia of forest sciences. Academic Press, London, pp: 1655-1668.

Leakey RRB, 2014. Plant cloning: Macropropagation. In: van Alfen N et al. (eds) Encyclopedia of agricultural and food systems Vol. 4. Elsevier Publishers, San Diego, pp: 349-359.

Leakey RRB, Tientcheu Avana M-L, Awazi NP, Assogbadjo AE, Mabhaudhi T, Hendre PS, Degrande A, Hlahla S, Manda L, 2022. The Future of Food: Domestication and commercialization of indigenous food crops in Africa over the third decade (2012–2021). Sustainability 14, 2355. https://dx.doi.org/10.3390/su14042355

Leakey RRB, Simons AJ, 2004. Tree domestication in agroforestry: New visistas in agroforestry. Agroforestry Systems 61(1): 167-181.

Mapongmetsem PM, Djoumessi MC, Yemele MT, Fawa G, Doumara DG, Noubissie TJB, Avana TML, Bellefontaine R, 2012. Domestication de *Vitex doniana* Sweet. (Verbenaceae): influence du type de substrat, de la stimulation hormonale, de la surface foliaire et de la position du nœud sur l'enracinement des boutures uninodales. Journal of Agriculture and Environment for International Development – JAEID 106(1): 23-45.

Marshall E, Newton AC, & Schreckenberg K, 2003. Commercialization of non-timber forest products: First steps in analysing the factors influencing success. Inter For Rev 5 (2):128-137.

Mukhtar RB, 2019. Effects of media and hormone concentrations on vegetative propagation of *Balanites aegyptiaca*. Journal of Forestry Research 30: 73-76.

Ndiaye S, Djighaly IP, Ndiaye S, Dramé AF, 2024. Dynamique de l'exploitation des produits forestiers non ligneux commercialisés dans le Département de Goudomp, Sénégal. European Scientific Journal 20(9): 47-61. https://doi.org/10.19044/esj.2024.v20n9p147.

Nwaoguikpe RN,Ujowundu CO, Wesley B, 2012. Phytochemical and biochemical compositions of African walnut *Tetracarpidium conophorum*. Journal of Pharmaceutical and Biomedical Science 20(9): 1-5.

Oladiji AT, Abodunrin TP, Yakubu M, 2007. Some physicochemical characteristics of the oil from *Tetracarpidium conophorum* (Mull. Arg.) Hutch. Journal of Biochemistry and Molecular Biology 22(2): 93-98.

Stroup WW, 2018. Analysis of non-Gaussian data. In: Glaz B and Yeater M (Eds.) Applied statistics in agricultural, biological and environmental sciences. ASA, CSSA and SSSA Books, Madison, USA, ISBN: 9780891183600, pp: 449-509. https://doi.org/10.2134/appliedstatistics.2015.0081.c16

Tapsell LC, Gillen IJ, Patch CS, 2004. Including walnuts in a low-fat/modified-fat diet improves HDL cholesterol-to-total cholesterol ratios in patients with type 2 diabete. Diabetes Care 27(12):2777-83.

Tchoundjeu Z, Avana ML, Leakey RRB, Simons AJ, Asaah E, Duguma B, and Bell JM, 2002. Vegetative propagation of *Prunus africana*: effects of rooting medium, auxin concentration and leaf area. Agroforestry Systems 54:183-192

Tchoundjeu Z, Leakey RRB, 2001. Vegetative propagation of *Lovoa trichilioides*: Effects of provenance, substrate, auxins and leaf area. Journal of Tropical Forest Science 13(1): 116-129.