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# Rooting Ability of Cocoa (*Theobroma cacao* L.) Stem Cuttings: Effect of Genotype, Cutting Type, Hormone Concentration and Their Interactions

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author KMDK designed the experiment, carried out the research and wrote the first draft of the manuscript. Author DTT initiated the concept, designed the experiment, performed the statistical analysis and wrote the article. Author RGC wrote the article with literature searches. Authors GYAB and LJB designed the experiment, wrote the protocol and managed the analysis of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** This study aimed to evaluate the rooting ability of stem cuttings of eight-year-old cocoa hybrids disseminated in the 5th agroecological zone of Cameroon.
**Study Design:** The experiment was a 6 x 2 x 3 randomised complete block design with three replications.
**Place and Duration of Study:** The study was conducted at the multipurpose agricultural research station Nkoemvone – Ebolowa from February to September 2017.

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**Methodology:** The studied factors were six genotypes **(**G1 = IMC67 x SNK 64, G2 = SNK 620 x MA 12, G3 = T79/501 x SNK 109, G4 = T79/501 x SNK 64, G5 = SNK 413 x UPA 143 and G6 = UPA 143 x SNK 64), two Cutting types (C1 = Orthotropic and C2 = Plagiotropic) and three rooting hormone concentrations (H1 = 2 tablets per liter of water, H2 = ½ tablet per liter of water and H3 = 1 tablet per liter of water). Rooting ability was evaluated by assessing percentage of rooted cuttings, number of root per rooted cutting and the length of the longest root per rooted cutting.
**Results:** Analysis of variance results indicated that genotype, hormone concentration and most of their interactions were highly significant while cutting type was not significant on the measured traits. The best rooting was obtained with genotype G4 for all the measured parameters. It was shown in this experiment that genotype G4 could be propagated using Rhizopon hormone, with a concentration of ½ tablet per litre of water and plagiotropic cutting type. This is also noticed in their interaction terms, where genotype G4/Hormone concentration 2 and 3 were the best combinations for all parameters.
**Conclusion:** Significant differences among genotypes are an indication that different genotypes may require different conditions for their propagation. As such investigations into the requirement for the propagation of the other genotypes should be considered in the future.

*Keywords: Theobroma cacao; vegetative propagation; hybrid; cutting type; rooting; hormone concentration; 5th agroecological zone.*

## 1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a neotropical perennial plant belonging to the family Malvaceae [1] which develops naturally around the equator between latitudes 20° North and South [2]. The principal objective of cocoa cultivation in all countries is to obtain cocoa beans which constitute a significant raw material for food, pharmaceutical and cosmetic industries [3,4]. Cocoa is cultivated in America, Asia and Africa where it contributes significantly to their economies, especially those of West African countries [5]. The latter sub-region contributes about 70% of global production of cocoa beans [6,7].

Cameroon is the fifth world producer of cocoa beans after Ivory Coast, Ghana, Indonesia, and Nigeria [7]. The cocoa chain represents about 3% of Gross Domestic Product, 6% of the primary GDP and about 30% of the agricultural sub-sector destined for exportation and transformation [8]. Cocoa is cultivated on an area of about 400.000 to 600.000 hectares spread within the humid forest zone [9]. Annual production during the 2014/2015 and 2015/2016 seasons were respectively, 232.530 and 269.495 tons of marketable cocoa beans [10,11]. The monetary value of exported cocoa beans in Cameroon is estimated at 200 billion francs CFA and is a source of sustenance for about 2 million people distributed among about 400.000 producer families [8].

The maintenance of cocoa genetic diversity in Cameroon is done by the establishment of gene banks in which clones from diverse origins and characteristics are planted. These living genebanks are found primarily at the Institute of Agricultural Research and Development (IRAD) station of Nkoemvone in the South Region and maintained by vegetation propagation. Despite the fact that Cameroon makes a considerable contribution to global cocoa production, mean yields (300 kg) per hectare remain low [5]. This corresponds to ten times less its standard potential when cocoa is cultivated under optimum conditions [12]. Factors responsible for the poor yields include:- cocoa brown pod rot disease caused by the fungus (*Phytophthora megakarya)* and mites,*Sahlbergella singularis and Distantiella theobromae* [13,14] *a*geing of cocoa plantations and farmers as well as bad agricultural practices [15-18] have also contributed to reduced production. According to Mahob [19], lack of improved planting material contributes to low cocoa production.

The future of cocoa in Cameroon as inscribed in the cocoa and coffee development plan by 2020 can only be attained through increase of seed banks, replanting of old plantations, establishment of new plantations, which necessitate an increase in demand for improved planting materials. Many structures and projects have been put in place by the government such as project for the selection and diffusion of improved cocoa and coffee planting material (SDMVCC), Fund for the development of cocoa and coffee chains (FODECC) and Cocoa development company (SODECAO). They are responsible for the production and

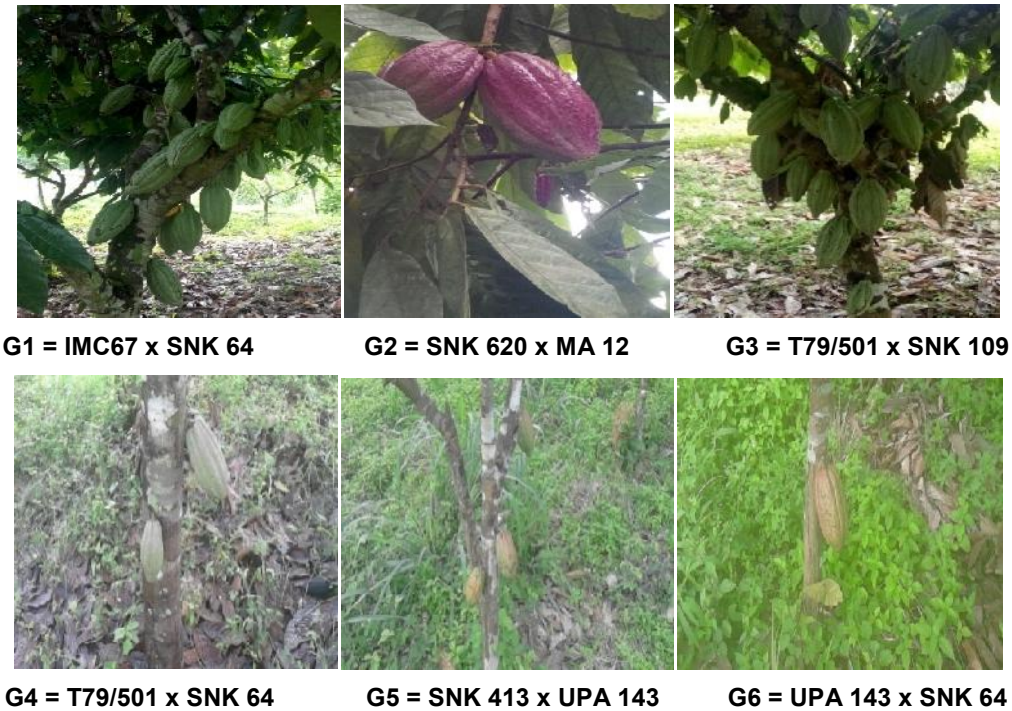
dissemination of disease resistant and improved planting material such as hybrids. This is achieved in IRAD through controlled manual pollination and the establishment of multi-clonal seed orchards from improved local clones belonging to different genetic groups [20]. The first flowering of such hybrids occurs 5 to 7 years after planting [14]. Multi-clonal seed orchards produce hybrids in a heterogenous manner with an uncertainty of individuals' identities since pollination takes place naturally. Studies in multi-clonal seed orchards show that about 97% of cocoa beans obtained results from self-pollination [21,22]. According to the latter authors, manual pollination requires qualified personnel and sophisticated equipment but gives a low rate of success (7 to 29.2 %). The same difficulties encountered in the development of hybrids are sometimes responsible for the high prices charged by producers during sale [23]. There is a necessity for a method of rapid production of these hybrids from improved cocoa planting material. The objective of this study was to evaluate the rooting ability of cuttings of selected cocoa hybrids used in the humid forest zone of Cameroon. Specifically, the study aimed at assessing the effect of genotype, hormone concentration, cutting type and their interactions on rooting of stem cuttings.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site and Establishment of Cuttings

The experimental site and propagation procedure have been previously reported by [24]. However, the genotypes used in this experiment consisted of six hybrids (genotypes) G1 = IMC67 x SNK 64, G2 = SNK 620 x MA 12, G3 = T79/501 x SNK 109, G4 = T79/501 x SNK 64, G5 = SNK 413 x UPA 143 and G6 = UPA 143 x SNK 64 of age 8 years old. These genotypes were chosen based on their good performance concerning yield, growth, resistance to pests and diseases and other morphological characteristics (Fig. 1).

The trial was a 6 x 2 x 3 randomized complete block design with three replications. Cuttings were collected early in the morning (before 7 am) from afore mentioned six cocoa hybrids, from C1 = orthotropic (upright stems) and C2 = plagiotropic (side) branches. Cuttings had an average of four leaves and were about 30 cm long. These were quickly dipped into three different concentrations of indolebutyric acid (IBA) (H1: two tablets per litre of water; H2: ½ tablet per litre of water and H3: 1 tablet per litre of water) before setting in, decomposed saw dust disinfected three days before. Alkatene plastic



**G1 = IMC67 x SNK 64** **G2 = SNK 620 x MA 12** **G3 = T79/501 x SNK 109**

**G4 = T79/501 x SNK 64** **G5 = SNK 413 x UPA 143** **G6 = UPA 143 x SNK 64**

**Fig. 1. Observed characteristics of the six genotypes**

pots (24 x 14 cm) containing cuttings were then placed in propagators containing trays filled with disinfected wood chips to provide stability. The experimental unit consisted of 20 cuttings each giving a total of 2160 cuttings (20 cuttings x 6 genotypes x 2 cutting types x 3 hormone concentrations x 3 replications). The trial was watered daily in the morning and evening whereas fallen and dead leaves were removed as reported by [25].

## 2.2 Data Collection and Analyses

Percentage of rooted cutting (rate of survival) was determined by the proportion of life cuttings at the end (after about 3 months) of the experiment to the number of cuttings set. The number of roots per rooted cutting was counted to determine root production, whereas length of the longest root per rooted cutting was measured using a ruler. These data were entered into Microsoft Excel 2013 and analysed using SPSS IBM version 20. Normality test and homogeneity of variance test were conducted to confirm the validity of tests of analysis of variance. Means were separated using Duncan Multiple range test (DMRT). The univariate analysis of variance (ANOVA) with principal factors: genotypes, hormone concentrations, cutting types and interactions: genotype x hormone concentration and genotype x cutting type to test the significance between different parameters at 5 % level using the GLM type III model as follow:

$$Y_{ijkl} = \mu + G_i + H_j + T_k + R_l + G_iH_j + G_iT_k + \varepsilon_{ijkl}$$

Y = Observation for the ijkl$^{th}$ individual, μ = overall mean of the experiment, $G_i$ = the random effect of the i$^{th}$ genotype, $H_j$ = the fixed effect of the j$^{th}$ hormone concentration, $T_k$ = the fixed effect of the k$^{th}$ cutting type, $R_l$ = the random effect of l$^{th}$ replication, $G_iH_j$ = interaction effect of i$^{th}$ genotype x j$^{th}$ hormone concentration, $G_iT_k$ = interaction effect of i$^{th}$ genotype x k$^{th}$ cutting type, $\varepsilon_{ijkl}$ = the sampling error

# 3. RESULTS

## 3.1 Effect of Genotype on the Percentage of Rooted Cuttings, Number of Roots per Rooted Cutting and Length of the Longest Root

The measured parameters varied from 45 to 90% for the percentage of rooted cuttings, 1 to 16 for the number of roots per rooted cutting and 3 to 33.6 cm for the length of the longest root among the genotypes. Results of analysis of variance show that genotype has significant effect on the percentage of rooted cuttings, the number of roots and the length of the longest root at 5% level with P < .000. The DMRT test for mean comparison shows that genotype T79/501 x SNK 64 had highest values in all the measured parameters with 85% ± 5.47 for the percentage of rooted cuttings, 10.05 ± 2.36 for the number of roots per rooted cutting and 13.84 cm ± 4.77 for the length of the longest roots and was significantly different from all the other genotypes used, whereas SNK413 X UPA 143 showed the least percentage of rooted cuttings, number of roots per cutting and length of the longest root with 50.00 ± 6.32%, 3.28 ± 0.87 and 4.76 cm ± 1.10 respectively (Table 1).

## 3.2 Effect of Hormone Concentration on the Percentage of Rooted Cuttings, Number of Roots per Rooted Cutting and Length of the Longest Root

Descriptive statistics of the mean data obtained among the hormone concentrations show that it varies from 61.25 to 73.61% for the percentage of rooted cuttings, 5.17 to 6.53 for the number of root and 7.76 to 9.19 cm for the length of the longest root. Result indicated that the percentage of rooted cuttings was best (73.61% ± 12.30) in hormone concentration H2 (½ tablet per litre of water) which was not significantly different from

**Table 1. Mean values of percentage of rooted cuttings, number of root per rooted cuttings and the length of the longest root per cutting among genotypes**

| Genotype | Number of cuttings | Percentage of rooted cuttings | Number of root per rooted cutting | Length of the longest root |
|---|---|---|---|---|
| G1 (IMC67 x SNK 64) | 360 | $60.00^{e}$ ± 8.94 | $4.30^{d}$ ± 1.47 | $5.57^{e}$ ± 1.50 |
| G2 (SNK 620x MA 12) | 360 | $65.00^{d}$ ± 10.95 | $4.88^{c}$ ± 1.38 | $6.61^{d}$ ± 1.48 |
| G3 (T79/501x SNK 109) | 360 | $80.00^{b}$ ± 10.48 | $6.97^{b}$ ± 2.14 | $11.71^{b}$ ± 2.29 |
| G4 (T79/501x SNK 64) | 360 | $85.00^{a}$ ± 5.47 | $10.05^{a}$ ± 2.36 | $13.84^{a}$ ± 4.77 |
| G5 (SNK 413x UPA 143) | 360 | $50.00^{f}$ ± 6.32 | $3.28^{e}$ ± 0.87 | $4.76^{f}$ ± 1.10 |
| G6 (UPA 143x SNK 64) | 360 | $75.00^{c}$ ± 10.85 | $6.56^{b}$ ± 1.40 | $9.79^{c}$ ± 1.40 |
| *P - value* | | P < .000 | P < .000 | P < .000 |

hormone concentration H3 (1 tablet per litre of water). Results of ANOVA revealed that hormone concentration had a highly significant effect on number of roots with *P* = 0.000. There was no significant difference in root number between ½ and 1 hormone tablet per litre of water, which induced the greatest number of roots with mean values of 6.53 ± 2.83 and 6.33 ± 2.86 respectively. The least number of roots was produced by hormone concentration H1 with 2 tablets per litre of water (Table 2).

ANOVA results reveal that hormone concentration had a significant effect on length of the longest root at the level of 5 % with *P* = 0.000 (Table 2). It was observed that the longest root length was induced by ½ and 1 hormone tablet per litre of water, which showed no significant difference at the 5% level, with mean root lengths of 9.19 ± 4.17cm and 9.18 ± 4.41 cm.

### 3.3 Effect of Cutting Type on the Percentage of Rooted Cuttings, Number of Root and Length of the Longest Root

According to the ANOVA results, cutting type had significant influence on the percentage of rooted cutting at 5 %, with *P* = 0.025 with plagiotrophic cuttings showing the highest (70.37 ± 15.55) percentage of rooted cuttings. Cutting type had no influence on the number of roots per rooted cutting and the length of the longest root with both orthotropic and plagiotrophic cuttings each producing 6 roots per cutting and 8.1 cm for the length of the longest root at 5%, with *P* = 0.87 (Table 3).

### 3.4 Interaction Effect between Genotype/Hormone Concentration on the Percentage of Rooted Cuttings, the Number of Roots and Length of the Longest Root

Significant interaction effect was found in genotype/hormone concentration on the number of rooted cuttings (*P* = 0.012) and the length of the longest root (*P* = 0.00). There was no significant effect on the percentage of rooted cutting and the results could not be reported further. The highest mean number of root per rooted cutting was obtained from the interactions between genotype 4 and hormone concentrations 2 and 3, which showed mean root per cutting as 10.7 and 10.5 respectively (Fig. 2, 3). This was followed by genotype 4/ two tablets per litre of water (8.8 mean roots). For the mean number of roots genotype, 4 was highest in all the concentration applied. Genotype 5/hormone concentration 1 had the lowest mean number of roots with 2.9. It was shown that hormone concentration 1 recorded the lowest mean number of roots for all the genotypes.

**Table 2. Mean values of percentage of rooted cuttings, number of root per rooted cuttings and the length of the longest root per cutting among hormone concentrations**

| Hormone concentration | Number of cuttings | percentage (%) of rooted cuttings | Number of roots per rooted cutting | Length of the longest root (cm) |
|---|---|---|---|---|
| 2 tablets per liter of water | 720 | $61.25^{b}$ ± 12.67 | $5.17^{b}$ ± 2.39 | $7.76^{b}$ ± 3.27 |
| ½ tablet per liter of water | 720 | $73.61^{a}$ ± 12.30 | $6.53^{a}$ ± 2.83 | $9.19^{a}$ ± 4.17 |
| 1 tablet per liter of water | 720 | $72.63^{a}$ ± 15.20 | $6.33^{a}$ ± 2.86 | $9.18^{a}$ ± 4.41 |
| *P - value* | | *P* <.000 | *P* <.000 | *P* <.000 |

**Table 3. Mean values of percentage of rooted cuttings, number of root per rooted cuttings and the length of the longest root per cutting among cutting types**

| Cutting type | Number of cuttings | percentage of rooted cuttings | Number of root per rooted cutting | Length of the longest root |
|---|---|---|---|---|
| Orthotropic | 1080 | 67.963 b ± 14.16 | 6.006 a ± 2.73 | 8.731 a ± 4.06 |
| Plagiotropic | 1080 | 70.370 a ± 15.55 | 6.020 a ± 2.8 | 8.700 a ± 4.01 |
| *P* - value | | *P* = .025 | *P*= .873 | *P* = .817 |

The longest roots were induced by interactions between genotype 4/concentration 2 and 3, with mean root lengths of 14.6 and 14.8 cm respectively (Fig. 4, 5). Other interesting results came from interactions between genotype 3/concentration 3 (12.4 cm), genotype 3/concentration 2 (12.2 cm), genotype 4/concentration 1(12cm). On their part, interactions between genotype 6/concentrations 3, 2 and genotype 3/concentration 1 each produced longest roots of about 10 cm.

## 4. DISCUSSION

The results obtained on the genetic potential give us information on the effectiveness of the differences in the rooting of the cuttings from the difference in the genetic potential of the hybrids. Current literature on cocoa propagation does not provide information on the rooting of cuttings taken from hybrids, as cuttings are often collected mostly from clones. Similar results were obtained by Hall [26]. Toxopeus [27], and Wood and Lass [3], reported that differences in cocoa genotypes lead to differences in their rooting abilities. The fact of having obtained the best rooting parameters with hybrid T79 / 501 x SNK64, T79 / 501 x SNK 109, UPA 143 x SNK 64, could be explained by the fact that in these hybridizations the African clones are involved (SNK : Cameroonian clone, and T79 / 501: Ghanaian clone), thus corroborating with the observations of [26], who reported that Amazonian high clones such as UPA 143, and those belonging to the Trinitario group such as T79 / 501 and the SNK, are more efficient during cuttings, and therefore root better. For the effect of hormone concentration, it was also observed that the higher hormone concentration had a negative impact on the percentage of rooted cuttings whereby hormone concentration H1 (2 tablet per litre of water) had the lowest (61.25 ± 12.67) mean percentage of rooted cuttings. In the test of the effect of concentration on the rooting of cuttings, the results showed that the concentration of the rhizogenic hormone solution

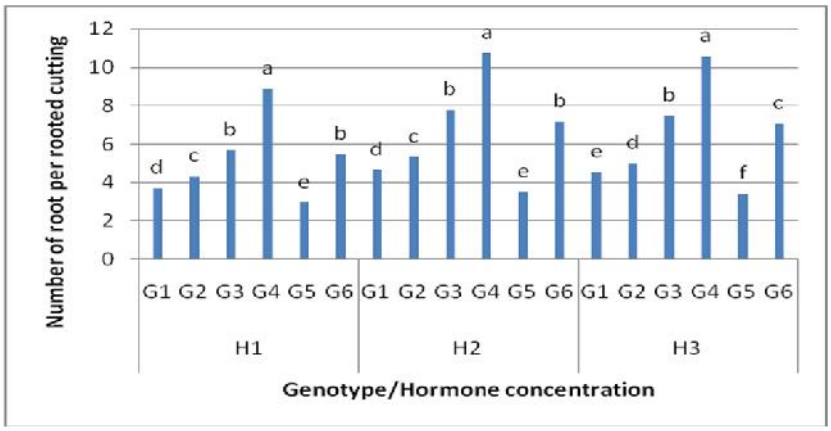



**Fig. 2. An interaction effect between genotype/Hormone concentrations on number of roots**

a b c

**Fig. 3. Age of rooted cuttings, a) Genotype 3/Two Rhizopon tablets per litre of water b) Genotype 3/½ Rhizopon tablet per litre of water c) Genotype 4/1Rhizopon tablet per litre of water**

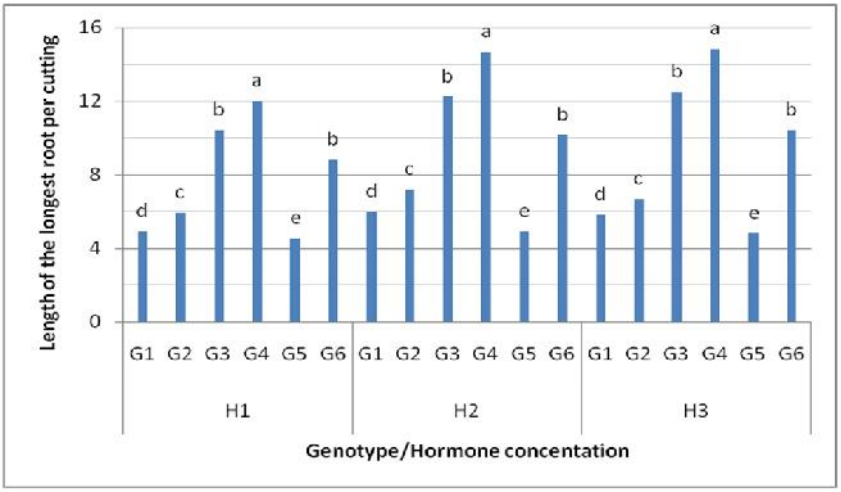
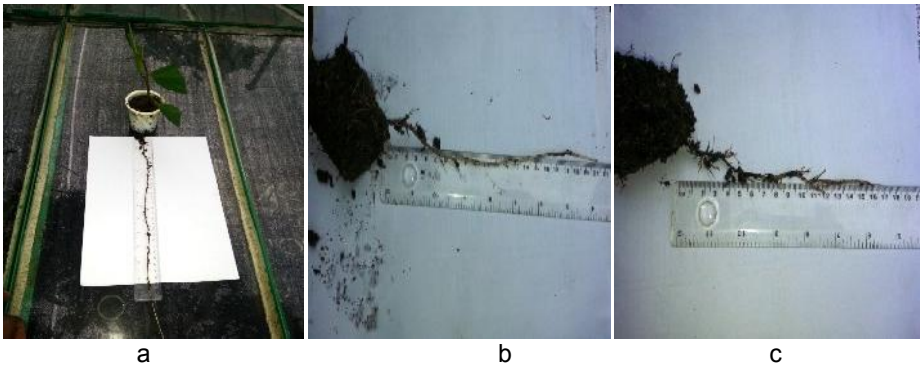



**Fig. 4. An interaction effect between genotype/Hormone concentration on length of the longest roots**



a b c

**Fig. 5. Length of the longest root a) Genotype 4/ hormone concentration 2 b) Genotype 4/ hormone concentration 3 c) Genotype 4/ hormone concentration 1**

has indeed a consequence on the rooting; these results corroborate those from the [28, 27]. Wood and Lass [3] and Essola et al. [24] experiments, which reported that hormones are one of the important factors influencing the rooting of cuttings; having obtained better rooting results at the lower concentrations of 1 and 0.5 tablets per liter rather than the highest concentration of 2 tablets per liter, may be due to the fact that the hormone used is based on auxin, slowing the activity of stimulation of rhizogenesis, due to an excessive concentration of auxin, as underlined by Charvet-Candela [29]. However, the non-significance of the rooting results recorded for hormone solution concentrations of ½ tablet per liter and 1 tablet per liter both gave better results, it would be sensible and recommendable for users of Rhizopon, opting for the lowest concentration, ½ tablet, which could not only be economically important, but also reduce plant production failures that sometimes occur when the hormone is missing or being not easily accessible.

The observation of the non-significance of the rooting differences of cuttings according to whether they are orthotropic or plagiotropic, is in line with the results obtained by Murray [30] cited by Lockwood [31], who reported that the root system of cocoa plants grown from rooted plagiotropic cuttings is similar to that of seedlings grown from seeds. Several authors [32, 33, 8], report that the plants resulting from the rooting of plagiotropic cuttings adopt a bulky canopy in culture and have a low resistance to lodging and water stress, this being explained in that these plants do not develop a taproot, unlike plants resulting from the cutting of orthotropic branches;

in the case of this study, these pivotal roots have not been observed on orthotropic cuttings that have been experimented; this could be explained by the stage of root development, perhaps not advanced enough to be able to distinguish between lateral roots and pivoting roots. These same authors as well as Lee [34] emphasized that this lack of taproot is a disadvantage for cultivation, especially during periods when rainfall is not sufficient; However, Boulay [35] considers that this lack of root actually affects little the mineral nutrition of the plant, because the cocoa tree is a humicole species, which have nearly 85% of its roots grouped in the surface horizon in the first 25 cm approximately. Interaction effects have never been studied in propagation techniques in cocoa and comparison can only be done using some tropical forest and fruit trees. Significant interaction effect between genotype and hormone (NAA) in rooting ability of *P. santalinoides* was reported by Dembele et al. [36].

## 5. CONCLUSION

The present study focused on the evaluation of the rooting ability of hybrids of cacao in the wet forest zone with bimodal rainfall in Cameroon, where it was necessary to test the influence of the genetic potential, the concentration of Rhizogen hormone solution (Rhizopon) and cutting types on the rooting of the cuttings. Testing these effects showed that all the cocoa hybrids that have been the subject of this experiment can indeed be propagated vegetatively by stem cuttings. However, more specifically, the number of buds emitted, the percentage of rooted cuttings, the number of roots emitted and the length of the longest root emitted vary greatly depending on the hybrid. Hybrids T79 / 501 X SNK 64, T79 / 501 X SNK 109 and UPA 143 X SNK 64 gave better results, unlike the hybrids SNK 413 X UPA 143, which gave unsatisfactory results. The concentration of the solution of the hormone used has an effect on the rooting of the cuttings with the best rooting obtained for concentration 0.5 tablet of Rhizopon per liter. It was noticed that among the concentration used, a uniform trend could be found, the lowest concentration having the highest values for all the measured parameters. Thus, indicating higher concentrations may be detrimental for the propagation of these hybrids. However, the conclusion will need more experimental procedures with higher concentrations. There was an interaction effect between genotype and hormone concentration for all the measured parameters, while no significant effect between genotype and cutting type. With the increasing demand of planting materials in this zone of Cameroon it is recommended on the basis of this work carried out to evaluate the cutting ability of cocoa hybrids to clone the cocoa hybrids by cuttings. This will not only reduce the time it takes for stock plants to flower but also reduce the problems with sophisticated equipment required by manual pollination.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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