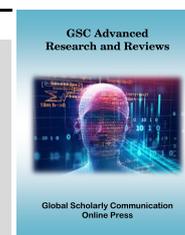


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## Assessment of eight kola trees clones (*Cola nitida* (Vent.) Schott and Endl.) for their ability to cutting from terminal and sub-terminal branch

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### Abstract

The objective of this study was to determine the influence of branch type on the cutting ability of eight kola clones (*Cola nitida*). To this end, the clones were tested in a split-plot experimental design with the factors studied being the clone and the nature of the branch. After seven weeks of experimentation, survival rates were evaluated for each clone and for each type of cutting. The results showed that the clones were significantly different for the survival rates obtained ( $p = 0.00$ ). The terminal branches were more suitable for cutting (70.62% survival) than the sub-terminal branches (33.95%). The age of cutting therefore influenced the success of cutting in the clones studied. At the end this work, we note that clones 305, 323, 330, 903A3 and 911A2 are promising for kola tree cutting from terminal branch and that clone 910A1 cut easily with both terminal and sub-terminal branch.

**Keywords:** Cuttings; Branch; Clone; Sub-terminal

### 1. Introduction

The kola tree (*Cola nitida*) belongs to the family of Malvaceae. It is a tree native to the warm and humid regions of tropical West Africa [1]. To date, 140 species of kola tree have been counted [2] of which two are cultivated for their nuts [3] and their multiple uses [4]. These are *Cola nitida* and *Cola acuminata*. Kola nuts are known for their high caffeine content, between 1.84 and 2.56% [5]. As a result, they are widely used in the food industry for the manufacture of energy drinks [6]. In the pharmaceutical industry, they are used to develop drugs against cardiovascular diseases [7] and are highly valued in traditional pharmacopoeia as a fertility regulator [8] and as a remedy against migraines and indigestion [9]. Other uses of kola nuts include cosmetics and textiles [10]. In addition, the kola nut plays an important role in African society for cultural and customary rituals such as births and weddings [11].

Since 2006, ivorian kola nut production is increasing. Indeed, from 100.000 t/year in 2005 [12], it is now estimated at 260.000 tons of fresh nuts, of which 200.000 tons are exported [13]. This makes Côte d'Ivoire the world's leading producer and exporter of kola nuts. However, the exploitation of the kola tree in Côte d'Ivoire remains close to picking, with the presence of a few trees in the forest zone. In order to improve the production system, homogeneous clonal populations have been established from trees with high yield potential. These trees, sufficiently tested in comparative trials, produce more than 2.5 t/ha, and are being extended to growers [14]. In addition, for more than a decade now, kola has been a popular crop in Côte d'Ivoire with an increasing number of producers [15]. However, the availability of efficient plant material in sufficient quantities remains a major constraint. Faced with this situation, the implementation of techniques for mass production of seedlings through the practice of vegetative propagation constitutes an alternative [16]. Among these techniques, the effectiveness of terminal split grafting was demonstrated by [17]. In this paper, we

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present the response of eight kola tree clones to cuttings from their terminal and sub-terminal twigs. This technique could, in the long term, constitute, in addition to grafting, a boon to the production of mass seedlings for plantation establishment.

## 2. Material and methods

### 2.1. Study site

The study was carried out at the research station of the National Centre of Agronomic Research (CNRA) of Divo (5°48' N, 5°18' W) in Côte d'Ivoire. This station is located in the forest zone, 200 km northwest from Abidjan. The climate is humid tropical with an average annual rainfall of 1249 mm [18]. Temperatures range from 32 °C to 35 °C. Humidity is high, with a humidity level above 80%. Soils are deep, dark brown, sandy-clayey or humusy. The percentage of potassium is low and the pH is acidic [19].

### 2.2. Plant material

The plant material consists of eight kola tree clones. The trees that make up these clones are located in the collection plots. These are two clones originating from Côte d'Ivoire (305 and 323), one clone originating from Nigeria (330) and five clones made up of all-vant (903A3, 910A1, 910A2, 911A2, 913A1). These trees were 51, 36 and 9 years old respectively.

### 2.3. Methods

#### 2.3.1. Collection of cuttings

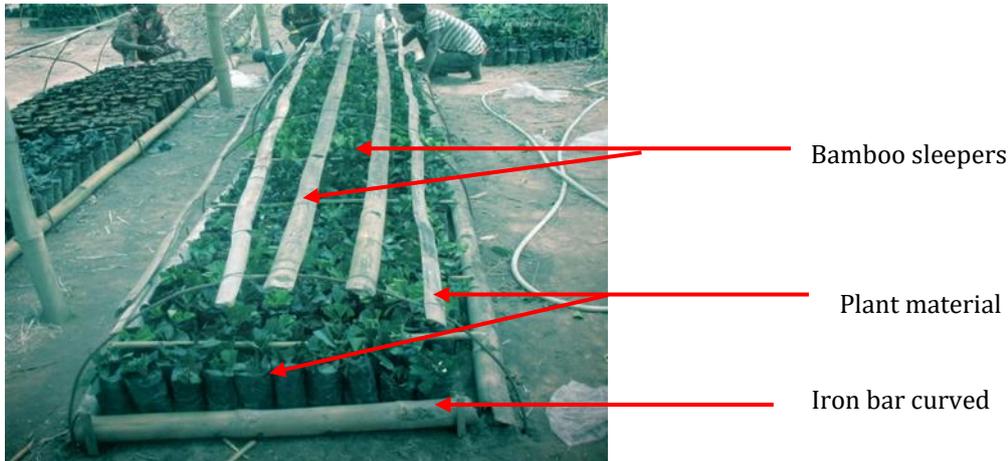
For each clone, cuttings were collected, early in the morning, before sunrise. On the same shoot, two types of cuttings were collected: cuttings from the terminal zone and those from the sub-terminal zone (Figure 1). Each cutting was placed under transplanting conditions by reducing half of its leaf area in order to reduce evapotranspiration. These cuttings were moistened in translucent plastic bags and then transported to the nursery.



**Figure 1** Terminal and sub-terminal branch of the kola tree (*Cola nitida*)

#### 2.3.2. Setting up the test

The test was conducted under tunnels 15 m long and 1 m wide. These tunnels were constructed with curved iron bars arranged along the length film, and placed under a 2.5 m high shade. This system creates a humid environment and a temperature favourable to the good development of cuttings. The bags were filled with potting soil and arranged on 1 m wide, on which bamboo sleepers were mounted (Figure 2). This device is 80 cm high, covered with a translucent plastic e flowerbeds in double rows of 10 pots. The cuttings were then transplanted into the sachets, which had been watered the day before. Immediate watering before covering the tunnels with translucent plastic film followed the transplanting. Regular watering was applied every two days.



**Figure 2** Setting up the experimental system (transplanting of cuttings)

### 2.3.3. Experimental design

The experimental design is a split-plot with three (3) replications. Each tunnel constituted one replication. The factors studied were the plant material (eight (8) clones) and the type of branches: terminal branch (RT) and sub-terminal branch (RST). Each tunnel contained 8 batches of 40 sachets. These 8 batches were randomized in each tunnel. The number of treatments in the trial was 16 (8 clones x 2 branch types). In sum, the trial was conducted with 1920 cuttings (16 treatments x 40 cuttings per clone x 3 replications).

### 2.3.4. Data collection

The observations consisted of existence surveys in each tunnel. These surveys were carried out on a weekly basis for 7 weeks. They consisted of counting live cuttings. The survival rates were then calculated using the following formula:

$$\text{Survival rate} = \frac{\text{Number of live cuttings}}{\text{Number of cuttings}} \times 100$$

### 2.3.5. Statistical analysis

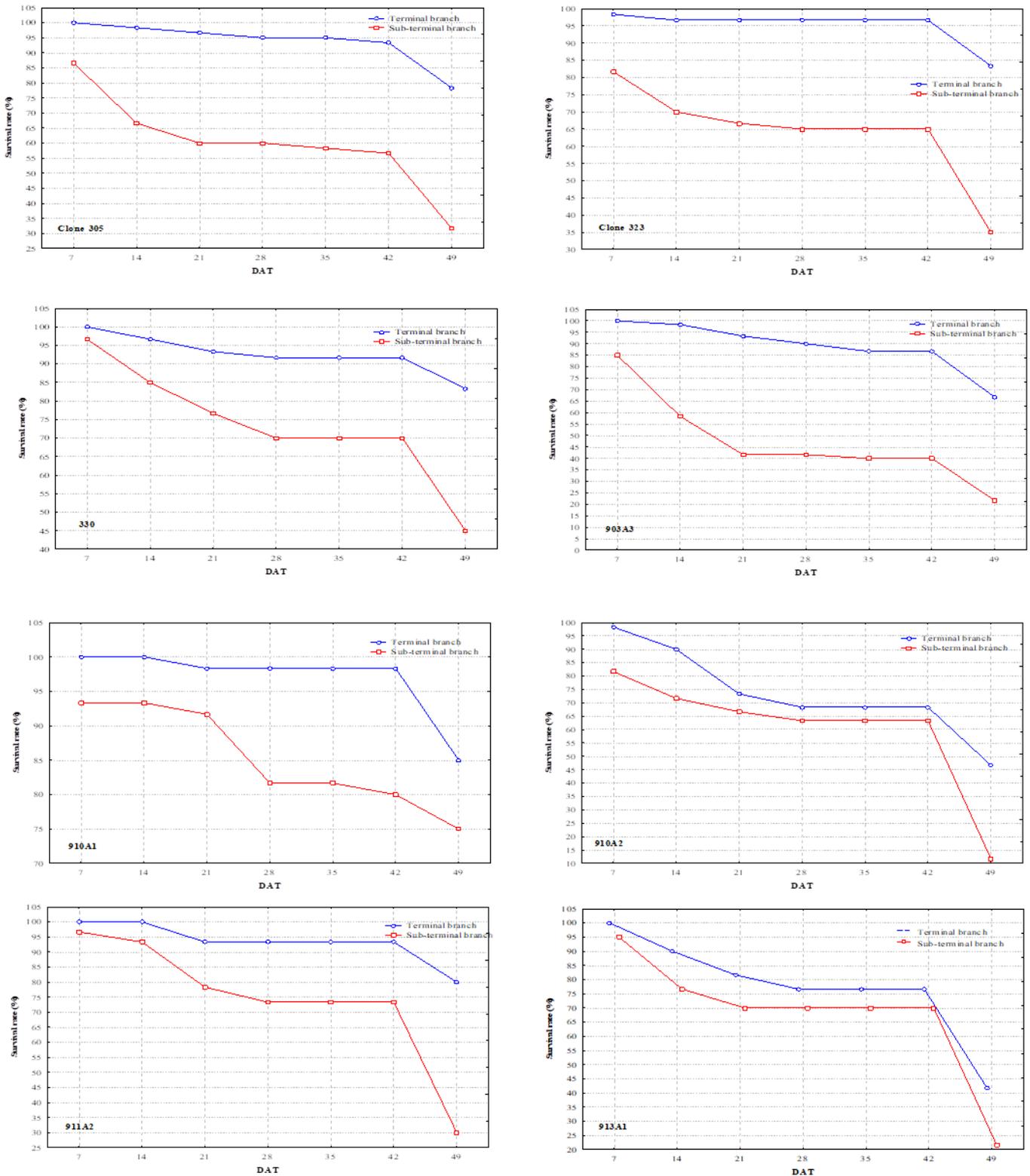
The collected data was firstly, recorded on an EXCEL sheet. The mean survival rates were calculated, and, compared using an analysis of variance with two classification criteria. Then the means were compared by Duncan's test at the 5% cut-off. This test was performed after an angular transformation of the arc sine square root data by  $p/100$  [20], where  $p$  represents a percentage proportion. The purpose of this transformation was to make the variables asymptotically normal. In addition, for each clone and for each type of twig, survival rate curves were constructed (Statistica 7.1).

## 3. Results

Throughout the trial, cuttings from terminal twigs showed higher survival rates than those from sub-terminal branch (Figure 3).

At the sub-terminal branch level, from day 7 to day 21 after transplanting, the survival rate of cuttings progressively decreased. From the third week (day 21), this rate tended to stabilize until the forty-second day. However, in clones 910A1, 911A2, 910A2 and 330, stable survival was initiated 7 days after the other clones, either on day 28 (Figure 3). For terminal branch, similar trends to those obtained for sub-terminal twigs observed. In clones 305 and 910A1, survival of cuttings dropped from day 7 to day 21 and was stable until day 42. With clones 330, 910A2, 911A2 and 913A2, the decline in survival of cuttings was prolonged until day 28, followed by stability until day 42 after transplantation. Clone 323 marked the longest time of stable survival of cuttings (14-42 days after transplantation) and clone 903A3 marked the longest phase of decreasing survival (7-35 days after transplantation). Nevertheless, for all

clones and all branch types combined (Figure 3), a sharp drop in survival was observed during the last week of observation (42 - 49 days).



**Figure 3** Survival curves of cuttings from terminal and sub-terminal twigs in 8 kola trees clones

Analysis of table 1 shows a highly significant effect of the clone on the survival rate of cuttings. In addition, branch type also showed a strong significant effect. Therefore, the interaction (clones x twigs) strongly influenced the survival rate of cuttings ( $F = 5.90$ ;  $p = 0.0002$ ), whether terminal or sub-terminal in nature.

**Table 1** Analysis of variance for the study of clone and branch effects and their interactions on the survival of cuttings.

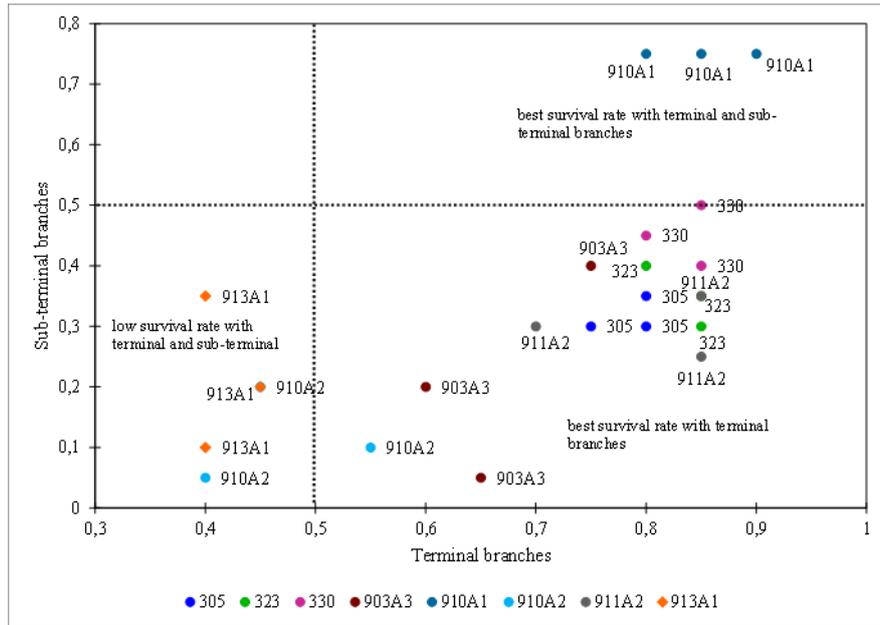
Source of variation	DF	SC	MC	F	p
Clones	7	1.19	0.17	31.91	0.0000
Branch	1	1.61	1.61	300.74	0.0000
Clones*branch	7	0.22	0.03	5.90	0.0002
Error	32	0.17	0.00		
Total	47	3.20			

The survival rate of cuttings varied between clones (Table 2). These inter-clonal variations are observed both at the terminal (CV = 24.36%) and sub-terminal (CV = 58.55%) branches. They ranged from 41.67 to 85% for terminal branch and 11.67 to 45% for sub-terminals. In general, terminal branch recorded the best survival rates (average 70.62%), about twice as high as those of sub-terminal branch (33.95%). Clone rankings indicate that with terminal twigs, clones 910A1, 911A2, 323, 330 and 305 had the highest survival rates, with order-of-magnitude averages ranging from 66.67 to 85%. On the other hand, with the sub-terminal branch, only clone 910A1 showed a relatively high survival rate (75%) compared to survival rates below 50% for the other clones.

**Table 2** Mean survival rates of cuttings from terminal and sub-terminal branch in 8 clones of kola tree

Clones	Terminal branches	Sub-terminal branches
305	78.33 a	31.66 bc
323	83.33 a	35.00 bc
330	83.33 a	45.00 b
903A3	66.67 b	21.66 cd
910A1	85.00 a	75.00 a
910A2	46.67 c	11.67 d
911A2	80.00 a	30.00 bc
913A1	41.67 c	21.67 cd
Means (%)	70.62	33.95
CV (%)	24.36	58.55
F <sub>(7,16)</sub>	28.87	14.81
p	0.000	0.000

The point cloud of cuttings survival rates (Figure 4), revealed three sets of clones. We recognize that the success rate is important when it reaches at least 50%. Thus, the first set consists of a single clone: 910A1, whose survival rates are well above 50%, both for terminals (85%) and sub-terminals (75%). The second set consisted of five clones: 330, 305, 323, 911A2 and 903A3. These clones showed the best survival rates with the terminal twigs (55-83%), while with the sub-terminals the values were below 50%. Two clones represent the third set: 913A1 and 910A2. With these clones, cuttings survival rates were low for both terminal and sub-terminal twigs.



**Figure 4** Scatterplot of survival rates of cuttings from terminal and sub-terminal branch

#### 4. Discussion

The differences observed between clones in survival rates were significant. Clones 330, 305, 323, 911A2 and 903A3 showed the best survival rates with terminal twigs. 910A1, showed the best rates with both types of twigs. In other words, these clones showed a good aptitude for direct cutting under tunnel. Clones 910A2 and 913A1, showed a poorer aptitude. Thus, it can be noted that in *Cola nitida*, some trees respond better to cuttings, while in others, obtaining plants from cuttings is more complex. Similar results were obtained by [21] on the same species. According to [22], the success of *Cola nitida* cuttings may be genetic in origin, and thus influenced by genotype. Survival of cuttings was higher with terminal twigs (70.62%) than with sub-terminal twigs (33.95%). This difference in behaviour reveals the importance of the nature of the cuttings on survival rates. It is thought to be due a priori to the age of the branch types (August or not) as indicated by [23]. In fact, in *Cola nitida*, branch growth is discontinuous, and occurs in waves of two vegetative shoots during one year: from March to April and from September to October [24]. Each outbreak is thus followed by a 4-month latency period. The alternation of shoots and stops is manifested by changes in the structure of the branches. Thus, branches from a recent flush (terminal branch) are very chlorophyllous, without any sign of hardening, with a degree of firmness that makes them more suitable for cutting [25]. On the other hand, sub-terminal branches, which are less chlorophyllous, show signs of hardening and lose their leaves easily from the first few weeks during cutting, with mortality rates reaching 59% [26], either 41% survival. Thus, the advanced age of cuttings has a negative influence on the success of cuttings in cola trees, as indicated by the work of [23]. This explains the low survival rate of cuttings from sub-terminal branch in our trial.

#### 5. Conclusion

This study compared eight cola clones and two types of twigs in terms of their suitability for tunnel cutting. The results showed that survival rates varied from one clone to another. In addition, terminal twigs were suitable for cutting than sub-terminal twigs. At this stage of our study, it appears that clones 330; 305; 323; 911A2 and 903A3 have a good ability to tunnel cut from the terminal twigs. These clones could therefore constitute a basic material potential for cola tree cutting. However, it would be interesting to evaluate their behaviour in the field after tunnel survival. As for clones 910A2 and 913A1, their aptitude for cutting was less good. However, it is possible to improve this trait by playing on certain factors such as the frequency of watering or the nature of the substrate. Clone 910A1 is easily cut with both terminal and sub-terminal shoots. This exceptional character suggests that this clone should be studied in a special and in-depth way.

## Compliance with ethical standards

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### Disclosure of conflict of interest

There is no conflict of interest concerning this article. The authors have obtained all institutional requirements and authorizations for publication. The article has not been previously published in the present form anywhere else.

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