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Effect of Root Trainer on the Success of Grafting and the Survival Rate of Cashew Tree [*Anacardium occidentale* L.(Anacardiaceae)] in Field

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Authors' contributions

This work was carried out in collaboration among all authors. Author CK designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Author JPD managed the analyses of the study. Authors KLK, KKK and IAZB performed the protocol and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Grafting is the most widespread vegetative propagation technique in cashew tree (*Anacardium occidentale* L.). Usually, cashew plant grafting are done on seedlings in plastic bags in nursery. In addition, to the low success rate, grafted plants have a low viability rate in field because main roots were broken during their transfer to the field. This study was carried out to evaluate the grafting success and survival rate of cashew seedlings planted in root trainer with different size (250 cc, 500 cc and 1000 cc). Each type of container was filled with two different growing substrates: coconut fiber and sawdust. Results showed that large size root trainers (1000 cc) have the highest percentage of graft success (96.67%). Rootstocks 45 days old presented the highest percentage of graft success in nursery (94.31%). The longest scions (12 cm) presented the minimum sprouting time (18.21 days). In the field, survival rate 100% of the grafted plants was recorded. In addition to the high survival rate ensured in field, root trainer is eco-friendly reusable and it light nature can prove a profitable technology for raising cashew elite plants regeneration.

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1. INTRODUCTION

Cashew tree *Anacardium occidentale* L. (Anacardiaceae) has excellent soil restoration potential[1,2,3]. It has been introduced in northern Côte d'Ivoire since 1950s to control soil erosion and to increase the forest cover. In Cote d'Ivoire, the climate and soil are ideally suited for cashew cultivation, which is one of the major cash crops [4]. Cashew tree is the third export product after cocoa and rubber [5]. Indeed, the market value and the constantly increasing demand for the nut on the international market have aroused the enthusiasm of Ivorian producers. Today, benefiting from agricultural council, cashew production is booming in the Côte d'Ivoire. In 2017, cashew production was estimated at about 700.000 tonnes. This performance has placed the country at the first rank of producing and exporting countries in the world [6]. Ivorian cashew orchard was estimated in 2017 at 1.350.000 ha, with 350.000 producers [7]. Despite these performances, the average cashew yield per tree is as low as 2.0 kg per year. One of the major reasons for low cashew yields is that most of the plantations were raised from seedlings which usually are genetically heterogeneous and hence variable in quantity and quality of production[5]. In order to increase production, plantation were mostly raised with grafted plants produced in plastic bags in the nursery [8,9]. Unfortunately, there is a high mortality rate in the field after planting. This mortality could be linked to several factors including the poor condition of the tracks connecting the nurseries to the field and the incompatibility of the polystyrene plastic bags and the substrate used. Climate change characterized by the poor distribution of rains could also explain this high mortality rate[10]. Therefore, it is necessary to find an efficient, ecologically and economically technology for the production of plants that can withstand transport and guarantee survival and good plantation. According to [11], the quality of the seedlings in the nursery determines the quality of the trees in the natural environment. Investigations have therefore been carried out in order to optimize the qualitative production of grafted plants in Côte d'Ivoire using root trainers. Root trainer is a cylindrical container made of an opaque material with a wider upper end and another lower end, progressively narrowed and perforated in order to provide favourable conditions for root development. It provides a suitable environment for rapid development of primary, secondary and tertiary roots [12]. It also maintains at an acute angle the tip of secondary and tertiary roots and orientates them in a general movement towards down to form a massive root system consolidated by the substrate[13]. Coconut fiber and sawdust are used as a substrate to optimize the percentage of survival of the plants after planting in the field. The objective of this study was to develop effective strategies that would reduce the mortality of grafted plants transferred to the field.

2. MATERIALS AND METHODS

2.1 Experimental Site

This experiment was carried out in Abidjan at the research station of the University Nangui Abrogoua (05°23´N, 04°00´W). The experimental station is located in the forest zone where the rainfall pattern is bimodal with four distinct seasons, two dry (from December to March and from July to August), and two wet (from April to June and from September to November). The annual average rainfall varied between 1800 and 2000 mm. Mean monthly temperature varies between 27˚C and 30˚C, whereas mean relative humidity ranges between 70% - 84% [14].The soil is ferralitic with a slight clay content at depth. It has a humus horizon, sandy with an organic substance content (2 to 3%).

2.2 Plant Material

Seedlings of 45, 60 and 90 days old in the nursery with a collar diameter ranged from 10-15 mm were used as rootstocks. Seedlings were raised in different sizes (250 cc; 500 cc and 1000 cc) of root trainers (Fig. 1).

Fig. 1. Seedlings raised in different sizes of root trainers. A: 250 cc; B: 500 cc; C: 1000 cc

2.3 Methods

2.3.1 Root trainer production

In this study, the empty pots of mineral water were used as root trainers. Utilisation of these used plastics could efficiently contribute to environmental control. Root trainers aim to promote a good development of the seedlings root system in order to facilitate their transport and planting in the field.To determine the suitable root trainer size which promote well development of the rootstock, pots were sectioned at different levels, corresponding to different volumes (250 cc, 500 cc and 1000 cc).

2.3.2 Nursery plants

These root trainers were filled with two types of substrates in order to identify the substrate that promotes the formation of a good root-substrate complex. The first type of substrate was coconut fiber, which is a well-drained substrate with low risk of root choking, good water-holding capacity, sufficient aeration, and good structural stability. The second type of substrate is sawdust. This substrate has good water retention capacity and very high porosity. The pre-germinated seeds were sown in these different types of root trainers. After seeds germination, vigorous seedlings with different ages (45, 60 and 90 days) were selected and used as rootstocks for grafting trials. In order to study the role played by the leaves on the success of the grafting, one, two and four leaves were left on the rootstocks. Scion used in this study were collected from productive trees in the field. The collection took place early in the morning and scions were then kept in wet jute bags before transporting in nursery. Different softwood scions length (8, 10 and 12 cm) were selected in order to determine the suitable scion size which promotes grafting success. Grafting method used in this study was the top cleft graft. This method is commonly performed for cashew trees grafting (Fig. 2). It consisted to cut the rootstock at a height where its diameter is similar to those of the scion.

Afterwards, the rootstock was splitted across the center to a depth of about 2 - 3 cm to receive the scion. Then, scion was cutted and two sloping cuts were made about 3-4 cm and inserted into the cleft of the rootstock, taking care to ensure that the cambium layers of the rootstock and scion are in perfect contact with each other and tied with polythene strip [15].Scion was then covered with a transparent film to stimulate its sprouting. The plastic film maintains the humidity inside to protect the scion from drying [16]. Fifty-four treatments were performed (3 types of scions x 3 types of root trainers x 3 number of leaves on the rootstocks x 2 substrates). Each treatment consisted of 30 plants. They were arranged in a complete randomized block with three replicates under a shaded area. Grafted plants were watered every two days. To assess the effects of different factors and their interactions on grafting success, percentage of graft success (percentage of grafted plants with leaf buds), scion sprouting time (days between grafting and leaf bud's formation) and number of suckers formed on the rootstock were evaluated.

2.3.3 Transfer to the field

Thirty days after grafting, successful grafts were transferred to the field for planting. This operation carried out at a density of 7 m x 7 m. A total of 150 plants were planted on an area of 7350 m^2.

2.4 Data Analysis

Means values and standard deviations were calculated for each evaluated parameter with the type of root trainer, substrate, number of leaves and the size of graft. A multiple analysis of variance (MANOVA) performed to evaluate the overall effect of the factors on the measured parameters. An analysis of variance (ANOVA) was then performed when the MANOVA of factor interactions showed a significant difference (P< 0.05). Means compared using Newman-Keuls tests with a 5% probability. Data analysed with the STATISTICA 7.1 software.

Fig. 2. Softwood scion lengths used for the grafting

3. RESULTS

3.1 Effect of Different Factors and their Interactions on Cashew Grafting Success

Multivariate analysis of variance revealed a significant interaction (P< 0.05) between different factors (Table 1). Then, all factors were selected for an analysis of variance (ANOVA). This test shows that substrates, age of the rootstocks (days), number of leaves on rootstocks, graft size (cm) and the interaction substrates* number of leaves on rootstocks influenced significantly grafting success (*P*< 0.05).

3.2 Effect of Substrates on Grafting Success

Substrate exhibited no significant (*P* > 0.05) effect on grafting success and sucker's production of grafted plants (Table 2). However, scion sprouting time (18.72 days) was earliest on coconut fibber than sawdust (19.54 days).

3.3 Effect of Rootstock Age on Grafting Success

Rootstock age doesn't affect significantly (*P* > 0.05) sucker production on grafted plants. However, it has a significance (*P* < 0.05) effect on grafting success and scion sprouting (Table 3). In fact, young rootstocks (45 days old) promote scion sprouting (17.38 days) against 20.71 and 19.71 days for 60 and 90 days old rootstocks, respectively. Young rootstocks had also showed a highest percentage (94.31%) of grafting success.

Table 1. Multivariate analysis of variance of different factors and their interactions on cashew grafting success

Factors	F-*value*	*p-value*
{1}Substrates	4.22	*0.007*
{2} Age of rootstocks (days)	20.08	*<0.001*
{3}Number of leave on rootstocks	3.21	*0.005*
{4} Graft size (cm)	8.23	*<0.001*
Substrates*Age of rootstocks (days)	1.30	*0.258*
Substrates* Leaf number on rootstocks	3.58	*0.002*
Age of rootstock (days)* Leaf number on rootstocks	1.08	*0.377*
Substrate* Graft size (cm)	0.39	*0.888*
Age of rootstocks (days)* Graft size (cm)	1.11	*0.353*
Number of leave on rootstocks* Graft size (cm)	1.33	*0.201*
Substrates* Age of rootstocks (days)* Leaf number on rootstocks	1.42	*0.154*
Substrates* Age of rootstocks (days)* Graft size (cm)	0.32	*0.985*
Substrates* Number of leave on rootstocks* Graft size (cm)	0.61	*0.833*
Age of rootstocks (days)* Number of leave on rootstocks* Graft size (cm)	1.11	*0.334*
1*2*3*4	1.06	*0.385*

Table 2. Effect of the substrate type on grafting success

Factors	Evaluated parameters		
	Percentage of graft success (%)	**Scion sprouting time (days)**	**Mean number of suckers per scion**
CF	91.17±1.05a	18.72±0.23b	0.29±0.06a
S	88.95±1.15a	19.54±0.29a	0.2±0.05a
F-value	2.83	9.52	0.99
P-value	*0.96*	*0.002*	*0.32*

Mean values in a colon followed by the same letter are statistically equal (Newman Keuls test at α=0.05%). S: Sawdust; CF: Coconut fiber

Table 3. Influence of rootstock age on evaluated parameters

Factors	Evaluated parameters		
	Mean percentage of graft success (%)	**Scion sprouting time (days)**	**Mean number of suckers per scion**
D1	94.31±1.01a	17.38±0.18 a	0.2±0.05 a
D2	89.72±1.5 b	20.71±0.35 b	0.36±0.08a
D3	86.14±1.2 b	19.71±0.26 b	0.19±0.06a
F-*value*	12.79	52.94	1.73
P-value	*<0.001*	*<0.001*	*0.18*

Mean values in a colon followed by the same letter are statistically equal (Newman Keuls test at α=0.05%). D1: 45 days; D2: 60 days D3: 90 days

3.4 Influence of Leafy Rootstocks on Grafting Success

Scion sprouting and sucker formation were not influenced by the presence of leaves on rootstock (Table 4). However, the number of leaves on rootstocks favours significantly ($P < 0.05$) graft success ($P < 0.05$). Indeed, rootstocks with four leaves induced the highest percentage of graft success (93.05%) contrary to those of one and two leaves.

3.5 Effect of Graft Size on Grafting Success

The percentage of graft success and scion sprouting time were significantly influenced by graft sizes ($P < 0.05$). Scion length 3 (12 cm) took fewer days (18.21 days) to sprout while lengths 2 (10 cm) and 1 (8 cm) took longer to sprout (19.74 and 19.45 days respectively). The highest percentage of graft success (94.62) was obtained with Scion length 3.The lowest percentages of graft success (86.67 and 88.89) were obtained with scion length 2 (10 cm) and length 1 (8 cm), respectively.

3.6 Combined Effect of Substrates and leaf Number on Root stocks on Grafting Success

Results of Table 6 shows that these different factors combination influence significantly Scion sprouting time ($P< 0.05$). No significant effect observed for sucker production on rootstocks and the percentage of graft success ($P> 0.05$). The earliest sprouting times of the scion (18.48 days)was obtained with rootstocks, grown on the sawdust substrate and bearing four leaves (SNL3). The longest sprouting times (19.93 and 20.22 days) of scion were observed with rootstocks bearing one and two leaves cultivated on sawdust (SNL1 and SNL2).

3.7 Multivariate Analysis of the Effect of Root Trainer Volumes on Grafting Success

Multivariate analysis of variance revealed a significant influence ($P < 0.05$) of root trainer on grafting success (Table 7). Substrates volumes and appearance of the rootstock were selected for an analysis of variance.

3.8 Effect of Root Trainers Volume son Grafting Success

The effect of root trainer volumes on graft success is presented in table 8. The mean number of suckers produced was not significantly influenced by root trainer size ($p > 0.05$). Therefore, the mean percentage of graft success and scion sprouting time were significantly influenced ($p < 0.05$). Indeed, the highest percentage of graft success (96.67) was obtained with V3 (1000 cc). While the lowest percentage (80.83 and 85.42) were obtained with V1(250 cc) and V2 (500 cc) respectively. Scion from V3 took fewer days (17.28 days) to sprout while those from V1 and V2 took longer (20.73 and 20.16 days) to sprout.

3.9 Planting

Vigorous 30-day-old grafted plants were transplanted to the field (Table 9).These vigorous plants are characterized by the presence of at least two buds on the stem and a well-developed root system; forming a good, finely attached root-substrate complex (Fig. 3A and 3B).In the field, 100% survival rate of the grafted plants was recorded two months after planting (Fig. 3C).

Table 4. Effect of the number of leaves on rootstocks and grafting success

Factors	Evaluated parameters		
	Mean percentage of graft success (%)	Scion sprouting time (days)	Mean number of suckers per scion
NL1	88.20±1.24 b	19.28±0.31 a	0.23±0.06 a
NL2	88.92±1.44 b	19.37±0.35 a	0.17±0.05 a
NL3	93.05±1.32a	18.75±0.31a	0.34±0.08 a
F-*value*	5.23	2.1	1.38
P-*value*	*0.006*	*0.13*	*0.25*

Mean values in a colon followed by the same letter are statistically equal (Newman Keuls test at α=0.05%).NL1 : one leaf, NL2 : Two leaves, NL3 : Four leaves.

Table 5. Effect of graft size on grafting success

Factors	Evaluated parameters		
	Mean percentage of graft success (%)	**Scion sprouting time (days)**	**Mean number of suckers per scion**
SL1	86.67±1.41 b	19.45±0.39 b	0.25±0.07 a
SL2	88.89±1.33b	19.74±0.32 b	0.27±0.08 a
SL3	94.62±1.1a	18.21±0.31a	0.23±0.05 a
F-*value*	12.88	12.55	0.08
P-*value*	*<0.001*	*<0.001*	*0.92*

Mean values in a column followed by the different letters are significantly different at P < 0.05 (Newman-keuls - test). Scion length 1 (SL1=8 cm), Scion length 2 (SL2=10 cm) and Scion length 3 (SL3=12cm).

Table 6. Combined effect of substrates and leaf number on rootstocks on grafting success

Factors	Evaluated parameters		
	Percentage of graft	**Scion sprouting time**	**Number of suckers**
CFNL1	86.59±1.85 a	18.63±0.35 ab	0.3±0.1 a
CFNL2	89.7±1.97 a	18.51±0.36 ab	0.27±0.09 a
CFNL3	90.56±2.18 a	19.03±0.51 ab	0.31±0.1 a
SNL1	89.81±1.61 a	19.93±0.5a	0.15±0.06 a
SNL2	88.14±2.13 a	20.22±0.6 a	0.09±0.04 a
SNL3	95.56±1.4 a	18.48±0.38 b	0.38±0.13 a
F-value	2.19	6.89	0.92
P-value	*0.12*	*0.002*	*0.4*

Mean values in a column followed by the different letters are significantly different at P < 0.05 (Newman-keuls - test).S: Sawdust; CF: Coconut fiber NL1: one leaf, NL2: Two leaves, NL3: Four leaves

Table 7. Multivariate analysis of the effect of root trainer volumes on grafting success

Factors	**F-*value***	***p-value***
Substrates	1.03	*0.40*
substrates volumes (cc)	2.68	*0.03*
Appearance of the rootstock	4.61	*0.01*
Substrates* substrate volume (cc)	0.42	*0.86*
Substrates*Appearance of the rootstock	0.04	*0.99*
Volume of the substrate (cc) *Appearance of the rootstock	0.87	*0.52*
Substrates*Volume of the substrate (cc) *Appearance of the rootstock	0.44	*0.85*

Table 8. Effect of root trainer volumes on grafting success

Factors	Evaluated parameters		
	Mean percentage of graft success (%)	**Scion sprouting time (days)**	**Mean number of suckers per grafted plant**
V1	80.83±5.14b	20.73±0.62 b	0.42±0.17 a
V2	85.42±3.45 b	20.16±0.75b	0.29±0.19 a
V3	96.67±1.42 a	17.28±0.41a	0.19±0.11 a
F-*value*	5.71	6.97	0.59
P-*value*	*0.009*	*0.004*	*0.56*

Means in a column with the same letters are statistically equal (Newman Keuls test at α=0.05%). V1: 250 cc; V2: 500 cc; V3: 1000 cc

Table 9. Grafted plants transplanted in the field

Factors	Evaluated parameters	
	Percentage survival of grafted plants(%)	Scion sprouting time (days)
V1	100±00	14±00
V2	100±00	14±00
V3	100±00	14±00

V1: 250 cc; V2: 500 cc; V3 : 1000 cc

A B C

Fig. 3. Transplanting process of grafted plant; A: Successful grafted plant from root trainer; B : Grafted plant in transplanting ; C : Grafted plant two months old in field

4. DISCUSSION

Compared to the classical cutting technique, grafting is an efficient technique of vegetative propagation and plant varietal improvement. It has the advantage of considerably reducing the flowering time, especially in perennial plants [17,18] and avoids the thorny rooting problem encountered in many plant species. Grafting is one of the techniques used for propagation in many plants, including cashew trees. Its success depends on several factors including the rootstock substrate, the age of the rootstock, the length of the graft, the diameter of the graft and the rootstock, the presence of leaves on the rootstock and the appearance of the graft [19,20] but also the grafting season and the genotype [21,22].

Coconut fiber optimized the percentage of grafting success (91.17%) compared to sawdust (88.72%). Hydrated coconut fibre provided more gap space and better water and nutrient retention capacity in the root zone promoting an increased rooting. In addition, coconut fiber would contain no plant parasitic fungi, insects or nematodes to prevent proper plant growth. On the other hand, sawdust would contain tannins, phenols, resins or terpenes, which would be toxic for plants (Give reference). Also, plants would suffer from nitrogen deficiency because bacteria responsible for decomposing organic matter would be eliminated by the heat it accumulates [23].Rootstocks of 45 days old used in this study promoted a rapid scion sprouting time (17.38 days) and increasing the percentage of grafting success (94.31%) compared to rootstocks of 60 and 90 days old. This early recovery could also be due to the juvenility of the rootstocks and scions. Indeed, juvenile plants show a high meristematic activity responsible for the healing of the weld between the rootstock and scion [24]. Grafting resumption results physiologically in the fusion of the cambium of the graft and that of the rootstock. According to [25], cambium fusion would take place between the 20th and 30th day after cashew grafting. Furthermore, [26] showed that in most fruit trees, grafting was resumed three to four weeks after grafting. While our results showed a relatively short time (17.77

days). We can also deduce that the fusion of the two cambiums would start as early as the second week after grafting. Our results confirm those of [27] on African plum trees. The work of [21] showed that the juvenility of rootstocks would be important in grafting success. They also showed that 30 days old mango rootstocks produced buds early (15.78 days) compared to 40 days old rootstocks, whose graft recovery time was 17.13 days after grafting.

Results reveals that higher percentage of grafting success (94.62 %) and rapid scion sprouting time (18.21days) was obtained on case of 12 cm long scions and lower percentage of success were observed in case of 8 and 10 cm. According to [28], the success of longer scions may be due to more mobilization of reserve food material and release of the same at the time of initiation of new growth. But [29] observed higher success of grafts with 10 cm than 5 cm long scions in mango. Indeed, the small scion length could allow the nutrients drawn by the rootstock to reach the graft quickly before it deteriorates. [30] have also shown that small (8 cm) mango grafts have a higher success percentage than large grafts. Grafting success may also depend on the quantity and quality of the rootstock substrate. Indeed, in the present study, large root trainers (highest capacity) favoured grafting success. Large capacity of root trainers would favour a good development of root system of rootstock and consequently a better supply of nutritive resources such as water and mineral salts to the graft [13]. In addition, a large size supposes an important quantity of organic matter necessary for the mineralization activities of microorganisms, guaranteeing the production of mineral elements necessary for plant growth and development. The relatively high capacity of root trainers would favour a better aeration of the substrate and consequently an increase in microbial activities [31]. Large capacity of the root trainers could promote the development of rootstocks root system. The percentage of viability (100%) obtained with the root trainer, in this work, is higher than that obtained (72.5%) by [4] with polyethylene plastic bags. Indeed, this device would promote a good development of root system of plant's. It makes it possible to have a rather coherent root and substrate complex. During the plantation, grafted plants remain finely attached to the substrate thanks to this complex. Plants are therefore less stressed and suffer less injuries related to root breakage, especially of the main root, during transport and planting in the field.

5. CONCLUSION

The major difficulty encountered with grafted plants is the low viability rate recorded after planting. Root trainers have optimized the scion sprouting time and percentage of grafting success. Coconut fibber and sawdust substrates, leafy rootstocks favour better grafting success. However, an important quantity of substrate is necessary for the development of an adequate root system of the rootstock. Root trainers can contribute efficiently to the production of vigorous grafted plants and ensure to them a high percentage of viability in field. This technology can be used to boost cashew production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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