

Full Length Research Paper

Vegetative propagation of cocoa (*Theobroma cacao*) by grafting: Aptitude of grafting on four clones

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The objective of this study is to evaluate the effect of hormones and fertilizers on the aptitude of grafting of four types of clones used in the seed fields for the creation, the regeneration, and the production of pods. The experimental design is a factorial trial (4 × 3 × 2 × 2) with hormone as the main block and other factors completely randomized within the blocks with three replicates. The aptitude of cocoa clones for grafting was assessed from 4320 grafted plants by recording survival rates, budding rates and number of leaves on a weekly basis from the 28th day after application of the hormones. Evaluation results indicated that there is a significant impact of clones, fertilizers, and grafting types on both the survival rate and growth parameters. Notably, the Trinitario ICS40 clone exhibited superior performance with an impressive 82.6% survival rate, a budding rate of 59.3%, and a total leaf count of 404. The different hormones did not have a significant impact on the measured parameters, whereas YaraMilla™ fertilizer demonstrated superior performance in enhancing the growth of grafted plants. Interestingly, side grafting displayed the highest survival rates (83.3, 69.4, 52.7 and 46.9% at 4 weeks, respectively) as well as a budding rate of 56%. However, it did not result in the highest number of leaves. This study represents the first report of grafting cocoa clones at the SODECAO experimental farm, and these findings open up possibilities for the propagation of disease-resistant clones.

Key words: Grafting, cocoa tree, *Theobroma cacao*, clone, hormone, fertilizer.

INTRODUCTION

The West Africa sub-region contributes nearly 70% to the total world production of marketable cocoa beans (Atlas, 2007; Anonymous, 2010). Like many countries in the Gulf

of Guinea, Cameroon derives most of its income from cash crops (cocoa, coffee and banana) and from the sale of hydrocarbons. The presence of oil as the only non-

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renewable resource coveted by all, unfortunately fails to meet the economic needs of the country. The country has been long committed to the diversification of its economy: development of a competitive industrial fabric, a large service market and above all a shift to modern agriculture. The agricultural sector employs about 60% of the working population, mainly on family farms (INS, 2013). Cocoa holds a prominent position in the country's agricultural policy due to the significant economic potential of its beans. It is the second-largest export commodity after petroleum, contributing to approximately 25% of the country's total export value. Cameroon is the fourth largest producer in the world and third in Africa after Ivory Coast and Ghana (FAOSTAT, 2017; Cocoa barometer, 2020). Despite Cameroon's significant contribution to world cocoa production, average cocoa yields remain low, about 300 kg of marketable cocoa per hectare (Babin, 2009). This corresponds to ten times less than the potential yields that can be achieved (3000 kg/ha) when the cocoa tree is cultivated in an adequate cropping system in a favorable environment and under optimal conditions (Toxopeus, 1985).

These low yields are due to a set of constraints such as aging of seed orchards (around 50% of the plantations are above 40 years old, especially in the Centre and South regions), low yields, lack of quality planting materials, the production of disease resistant plants in sufficient quantities, and policies applied by organizations in charge of plant production, to the lack of financial means by structures in charge such as the Ministry of Agriculture and Rural Development (MINADER), Research Institute of Agriculture and Development (IRAD) and Cocoa Development Corporation (SODECAO).

It is clear from the above that planting material is insufficient to meet the growing demand. In recent years, there has been a political will to revive the seed sector through specialized infrastructures to increase cocoa production and improve on economic growth. According to the National Development Strategy (NDS30), the country has set for itself the objective of reaching a milestone of 1,200,000 tons of commercial cocoa per year by the start of 2030. The SODECAO, through its specialized centers play an important role in the production of planting material, and is in a permanent process of finding a solution to reduce production time. It explores the different methods used in cocoa cultivation and explores possibilities for improvement.

The resolution of this problem involves the creation and regeneration of seed orchards through vegetative multiplication of cocoa breeding stocks (highly productive and disease resistant planting materials) using several production techniques.

Grafting has been reported to improve plant resistance, productivity, and fruit quality (Kumar et al., 2017, Krommydas et al., 2018) and has great potential to be explored by SODECAO. Grafting is defined as a

technique that consists of inducing the cambiums of two plants to merge and develop into a single plant. In this union, the portion of the attached selected plant is called a scion while the host plant is called the rootstock. The host assumes the mineral nutrition of the new plant while the scion contains the genetic material with desirable trait to be propagated (Macdonald, 1996; Hartmann et al., 2014). Successful grafting propagation depends on compatibility, the genotype, age, size, type and sources of the rootstock and scion (Page et al., 2012; Munjuga et al., 2013). Environmental conditions also play a major role in grafting success e.g., irrigation, hormones, period of the year for grafting, grafting environment (Copes, 1973; Hibbert-Frey et al., 2010). It is in this context that this study investigates the effects of hormone and fertilizer application on the grafting aptitude of four cocoa clones.

MATERIALS AND METHODS

The study was conducted at the nursery of the Cocoa Development Corporation (SODECAO) in Nkoemvone, located in the South Region of Cameroon. Rootstocks consisting of six-month-old cocoa seedlings with mixed genotypes resistant to brown pod disease were obtained from the nursery. Additionally, scions were gathered from genetically improved materials of four cocoa clones (Amazonia Highs: T79/501 - Clone 1 and IMC67 - Clone 2, and Trinitario: SNK16 - Clone 3 and ICS40 - Clone 4) established in the clonal seed orchard of SODECAO Nkoemvone. The characteristics of these clones were previously described by Essola et al. (2017). Two treatments were applied on two grafting types: terminal slot grafting and English or side grafting. The two hormones treatments employed: - the hormone X-CYTE (H1), active ingredient of which is cytokinin, composed of 0.04% cytokinin and 96.96% inert ingredients. - the N-Large hormone (H2) whose active ingredient is gibberellic acid composed of 4% gibberellic acid and 96% inert ingredients and - a control (H0): with no hormones applied. Two fertilizers treatments were also used F1 - The GreenOK® organic fertilizer consisting of NPK in the ratio of Nitrogen (N) 0.05, Phosphorus (P) 0.001%, Potassium (K), Moisture 94.8, organic substances 2.5 % reaction of pH. and F2 - the YaraMilla™ fertilizer which is a complex fertilizer composed of NPK: 20:10:10 + 6SO₃. Total Nitrogen (N): 20% nitric nitrogen (NO₃), 9% ammoniacal nitrogen (NH₄), Total Phosphorus (P₂O₅), Total Potassium (K₂O), Total Sulphur (SO₃). The experimental design was a 4x3x2x2 factorial trial in a randomized incomplete block design with three repetitions. A total of 4320 plants were grafted in this trial with 36 blocks of 120 individuals per hormone x 4 clones x 2 graft types x 2 fertilizers x 3 replications. The design consisted of blocks with hormones as main factor and the others (clone, graft type and fertilizer) as random factors within the hormone blocks. The pots were placed in a shade house constructed with sticks and palm fronds that had a dimension of 12.30 m x 9.5 m or an area of 116.85 m² (Figure 1). The treatments were randomly allocated to the experimental unit.

The rootstocks were then classified in blocks within the shade after cleaning of the nursery environment. Semi-woody twigs which had about 7 to 8 leaves were collected from a seed orchard between 6 and 7 a.m. on orthotropic branches with well-developed buds. To prevent twigs from losing sap moisture, they were immersed in a bucket of water before being transported to the nursery. Using secateurs, each twig was then divided into sections, with each section containing a bud and serving as a scion for the

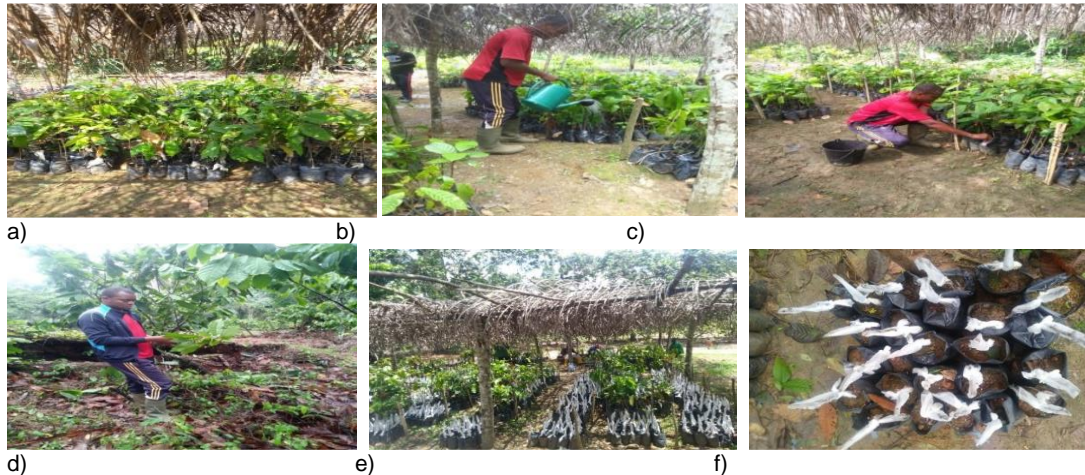


Figure 1. Establishment of the experimental trial (a) rootstocks in experimental blocks (b) irrigation of the rootstocks (c) application of fertilizers (d) collecting scions (e and f) grafted plants.

grafting exercise. Six-month-old rootstocks were prepared by removing all their leaves with the use of a grafting knife, and a slit was cut into each of them. A scion was then cut into bevel shape and inserted into the slit and tied up to prevent air or water infiltration and the plant covered with a polythene plastic bag. Ten mg of the granulated fertilizer ((YaraMilla™) was then introduced into each pot, whereas 30 ml of the mineral fertilizer (GreenOK®) was initially dissolved in 15 l of water before used. Hormone application was carried out 21 days after grafting. The hormones gibberellic acid and cytokinin were applied to leaf surfaces using a sprayer at a dose of 10 ml/15 L of water. Manual weeding was carried out in the nursery and insecticide/fungicide applications carried out fortnightly. The insecticide used was Actara 25 WG, containing thiamethoxam as the active ingredient at a concentration of 250 g/kg. It was applied at a rate of 4 g/15 L of water. The fungicide used was Ridomil Gold Plus WP, consisting of 60 g of mefenoxam and 600 g of copper as active ingredients, and it was applied at a dose of 50 g/15 L of water for a total of 4320 plants. The plants were watered every other day, following the recommendations of Kebe et al. (2009). Survival rates were assessed weekly starting from the 28th day after the application of hormones, with evaluations continuing for a total of 49 days after grafting.

The budding rate was determined by counting the number of scions having emitted buds and the number of leaves was determined by counting the number of leaves only at the first week. The raw data were inputted and formatted with Microsoft Excel and then imported into SPSS statistical package for analysis. Descriptive statistics and histograms were carried out with Excel. The statistical tests for analysis of variance (ANOVA) and DMRT was used for the comparison of means.

RESULTS AND DISCUSSION

Effect of clone on survival rate measured weekly for four weeks, budding rate and number of leaves at the first week

The analysis of variance of survival rate showed a highly significant difference between clones for weeks 2, 3, 4

and budding rate while the number of leaves showed no significant difference at the 5% threshold. The clone 4 showed the highest mean survival rate while the clone 1 showed the lowest mean survival rate (Table 1). The higher mean observed in 4 clone may be as a result of its geographic origin and genetic constitution. This clone 4 is very vigorous despite the fact that it is sensitive to brown rot disease. Moreover, it is precocious as it produces within a short time, unlike Clone 1 which is of the Amazonian origin. These findings line up with those of Essola et al. (2017) who reported a mean survival of 45 and 52.57% for cuttings of SNK16 and T79/501 respectively. Similarly, Kamga et al. (2018) obtained a 60% survival rate with hybrid cocoa cuttings, whereas Séry et al. (2019) reported a 41.7% survival rate with *Cola nitida* cuttings. From the 4320 grafted plants established in this experiment, 4039 grafted plants were recorded after data cleaning and 3285 (81%) survived at the first week of measurement and then decreased to 1794 (44.4%) at the fourth week data collection. Similarly, Barrera-Ramírez et al. (2021) observed a decrease in survival from 100 to 50% at 40 days after grafting and 27.9% at 90 days after grafting of *Pinus pseudostrobus*. According to Barnett and Weatherhead (1989), graft failure could result from the scions' inability to receive water from the rootstocks before the formation of the graft union, although water stress could not have been a contributing factor in the current trial. However, Hibbert-Frey et al. (2010) pointed out that water availability is important but not a primary factor for graft success, specifying that the physiological condition of both the scions and rootstocks could also affect graft success. Dadzie et al. (2014) observed optimum grafting success in *Cola nitida* with six months old rootstock, with a decreasing rate of success as the rootstock became older. Apart from rootstock age, the latter authors also indicated that success depends on the compatibility of

Table 1. Variation in survival rate (%), budding rate (%) and number of leaves among clones of *T. cacao* grafts.

Clone	Survival 1	Survival 2	Survival 3	Survival 4	Budding rate	Number of leaves
Clone 1	80.9 ^a	64 ^b	50.6 ^b	41.2 ^b	51.9 ^c	395 ^a
Clone 2	79.7 ^a	67.4 ^{ab}	51.4 ^b	46.5 ^a	56.7 ^b	381 ^a
Clone 3	82.6 ^a	67.7 ^{ab}	53.7 ^{ab}	46.2 ^b	61.4 ^a	422 ^a
Clone 4	82.2 ^a	70.7 ^a	56.3 ^a	43.7 ^{ab}	59.3 ^{ab}	404 ^a
Mean	81.3	67.4	52.9	44.4	57.3	400.5
P-value	0.315	0.016	0.048	0.051	0,000	0.512
Sig.	NS	***	***	***	***	***

the scion genotype and the rootstock. Moore (1983) specifies that the developmental stage for compatible grafting in herbaceous plants involves the formation of isolation layer, adhesion of scion and rootstock and reconnection of vascular bridge between scion and rootstock. Barrera-Ramírez et al. (2021), pointed out that grafting success involves morphological, anatomical, physiological and biochemical aspects of a plant, whereas Gaspar et al. (2017), indicated that grafting season influences the percentage of grafting success and survival.

Although clone 3 had the highest value for the budding rate and number of leaves (Figure 2), it was not significantly different from clone 4 (Table 1). Clone 4 was the highest in survival rate and among the highest in the budding rate is a vigorous, early maturing and high-quality plant material used as scions. These findings are similar to those of Essola et al. (2017) who observed a flushing rate of 77.5% for ICS40, 35 % for T79/501 and 7 % for SNK16 in a cocoa cutting rooting trial.

The results of this study corroborates with those of N'zi et al. (2019) and Almeida and Valle (2007) who respectively reported differences between clones in terms of the growth and development parameters of cocoa after one year of field transplantation of ten grafted cocoa clones, and that the growth period is characterized by the expansion of leaves and the elongation of shoots.

Effect of graft types on survival rate measured weekly for four weeks, budding rate and number of leaves measured only at week one

ANOVA results showed significant difference in survival rate, budding rate and number of leaves between graft types. T2 showed significantly higher survival rates during the weekly measurement than T1 and a continuous decrease in survival rates was observed within and between graft types from week 1 to week 4 (Table 2). Similar results were reported by Séry et al. (2020) who observed a survival rate of 81.67% with terminal slot grafts in *Cola nitida* and Takoutsing et al. (2014) who reported a 100% success rate with Top grafting in *Garcinia lucida*, but differ from those of Asaah

et al. (2012) who observed that side tongue grafting is the most appropriate grafting method for *Allanblackia floribunda*.

Significantly higher budding rates were observed for T2 (83.3%) than T1 (79.4%) during week 1 of measurement contrast to the report of Kouassi et al. (2018) who observed that top grafting induced a significantly higher number of buds. Barrera-Ramírez et al. (2021) observed a higher shoot growth and grafting success with side-veneer grafting in *P. pseudostrobis*, unlike in the present study.

The latter authors attributed this to the fact that terminal tip/insertion, the leader bud was replaced and grafted at a higher height, thus,

The rootstock could reduce the flow of water and nutrients required for growth.

The number of leaves according to the two grafting types showed that Type 1 (863) induced a significantly higher number of leaves than Type 2 (739) in line with the findings of Kouassi et al. (2018).

who observed a significantly higher number of leaves with terminally grafted cocoa seedlings from the first to the fourth week. The possible reasons could be due to differences in nutrient uptake resulting from differences in carbohydrate stock in the seedlings (Tchoundjeu et al., 2010).

Effects of hormones on the survival rate, budding rate and number of leaves in *Theobroma cacao* grafts

ANOVA revealed significantly different in weeks 2, 3, and 4 in survival rate and no significant difference at week one, budding rate and number of leaves at 5 % level with respect to the different hormones applied (Table 3).

Hormone treatments gibberellic acid (Hormone 2) showed significantly higher mean survival rates than the control (Hormone 0) in most of the weeks of measurements (Table 3). However, Rama and Dingse (2014) observed that a plant growth regulator (triacontanol) had a significant effect on leaf number and other growth parameters on cacao seedlings after the age of 14 weeks



Figure 2. Grafted plants with leaves of *T. cacao* grafts.

Table 2. Variation in survival rate (%), budding rate (%) and number of leaves of *T. cacao* grafts on different grafting types.

Graft type	Survival 1	Survival 2	Survival 3	Survival 4	Budding rate	Number of leaves
Graft type 1	79.4 ^a	65.7 ^a	53.2 ^a	42 ^b	41.3 ^b	863 ^a
Graft type 2	83.3 ^a	69.4 ^a	52.7 ^a	46.9 ^a	56 ^a	739 ^b
Mean	81.3	67.4	52.9	44.4	57.3	801
P-value	0.64	0.19	0.127	0.024	0.001	0.012
Sig	NS	NS	NS	***	***	***

Table 3. Variation in survival rate (%), budding rate (%) and number of leaves of *T. cacao* grafts treated with hormones.

Hormone	Survival 1	Survival 2	Survival 3	Survival 4	Budding rate	Number of leaves
Hormone 0	81.96 ^a	67.53 ^{ab}	53.2 ^a	43.33 ^{ab}	57.65 ^a	421 ^a
Hormone 1	80.29 ^a	65.14 ^b	49.18 ^b	42.63 ^b	55.59 ^a	601 ^a
Hormone 2	82.68 ^a	69.63 ^a	56.66 ^a	47.07 ^a	58.66 ^a	580 ^a
Mean	81.33	67.42	53.00	44.42	57.27	534
P-value	0.236	0.035	0,000	0.038	0.235	0.496
Sig	NS	***	***	***	NS	NS

Effects of different fertilizers on the survival, brate and number of leaves of *Theobroma cacao* grafts

ANOVA revealed highly significant difference in survival rate at week one, budding rate and number of leaves of *T. cacao* grafts with different fertilizer applications. F2 (mineral fertilizer) were higher than for those treated with organic fertilizer (F1) for all the measured parameters (Table 4).

The above result is possibly because F2 has a higher concentration of nitrogen as indicated by the chemical formula N: P: K in the ratio of 20:10:10 + SO₃, than F1 which consists of NPK in the ratio 0.05: 0.01: 1.2. The results are similar to that of Kouassi (2012) who reported that nitrogen had a positive effect on the growth of cocoa seedlings in the nursery. Souza and Carmello (2008) also observed a significant increase in plant height following nitrogen fertilizer application. Nitrogen is an essential element in the growth of cocoa plants, especially in soils with low N-content.

This can also probably be because fertilizer treatment F2 got dissolved gradually through daily watering of plants. Thus, a gradual release of mineral elements may

be required especially for phosphorus uptake. Phosphorus has been identified as very important for the growth and development of the cocoa tree (Ouattara et al., 2019). The slow growth at the beginning of the experiment is possibly because the fertilizer was still in granules and not available to the plant roots. GreenOK™, being in liquid form and given as foliar application could only promote significant development if it was applied several times.

The result of this trial shows that fertilizer application is not necessary at the very early growth stage as the effects were not visible at only 21 days after grafting. But as recommended by Kebe et al. (2009), young plants should be observed for the effect of fertilizer application for a period of about 6 to 8 months.

This will allow for the identification of the optimum age for fertilizer application. Barrera-Ramírez et al. (2021) observed a consistent increase in plant height with grafted *P. pseudostrobis* whose rootstock received 3, 5 and 8 kg per m³ of multicote fertilization and micronutrients, with respective plant heights of 136, 143.5 and 148 cm, showing that fertilizer application could have a positive effect on the growth of grafted plants

Table 4. Variation in survival rate (%), budding rate (%) and number of leaves of *T. cacao* grafts with different fertilizers application.

Fertilizer	Survival 1	Survival 2	Survival 3	Survival 4	Budding rate	Number of leaves
Fertilizer 1	79.1 ^b	67.7 ^a	52.4 ^a	44.9 ^a	55.1 ^b	730 ^b
Fertilizer 2	83.5 ^a	67.1 ^a	53.5 ^a	43.9 ^a	59.3 ^a	872 ^a
Mean	81.3	67.4	53.0	44.42	57.27	801
P-value	0.000	0.713	0.488	0.509	0.007	0.025
Sig	***	NS	NS	NS	***	***

Conclusion

The aim of this study was to update the data available to SODECAO Nkoemvone in Cameroon for use in the multiplication of large numbers of clones for the creation and regeneration of seed orchards.

It was noticed that clones, grafting types and fertilizers were significantly different for the treatment parameters studied. Clones 4 were the highest for survival rates and among the highest for the budding rate and the mean number of leaves. Although graft type 2 had the highest survival rate and budding rate it did not produce highest number of leaves. Fertilizer 2 was the best fertilizer treatment used in this clone grafting study while Hormone 2 was the highest among the hormone types though not significantly different from the other hormones tested. Vegetative propagation by grafting has been neglected in Cameroon in favor of sexual or generative reproduction. However, it should be noted that grafting has the advantages of producing disease free plants when resistance rootstocks are grafted with highly productive scions. These results lead us to conclude that the grafting of cocoa trees is a technique that may contribute to solving the problems of cocoa cultivation in the Cameroonian, in terms of production of clones intended for the seed fields. However, attention to the experimental conditions (the grafting environment, temperature, clones to be used) still need to be handled carefully.

For future perspectives, we suggest the conduct of larger-scale studies aimed at achieving grafting ability with all clones in the seed fields of SODECAO of Nkoemvone with a view to further updating data to support a large multiplication by grafting of cocoa tree stock.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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