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Vegetative Propagation of Cashew (*Anacardium occidentale* L.) by Softwood Grafting in Ghana

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Abstract

An investigation was done to assess the graft success on seedlings and canopy substitution using grafting techniques, retention of basal leaves on the rootstock, age of rootstock and period of grafting in a randomize complete block design with twenty plants per treatment and replicated three times. Young vigorously growing cashew seedlings were used for grafting whilst poor performing cashew trees (in yield and young trees) were stumped to produce shoots for the top working. Significantly ($p < 0.05$) higher grafting success was obtained for softwood grafting technique in the month of July with October recording the lowest for canopy substitution. Grafting on 60 days old seedlings with the retention of four (4) matured basal leaves on the rootstocks significantly ($p < 0.05$) gave high success in graft union whilst the root stocks without basal leaves recorded low graft successes. However, there was significant ($p < 0.05$) off-shoots development below the graft union of the rootstock. Softwood grafting was the best technique for high graft success with the month of July being suitable period for grafting in canopy substitution. Retaining four (4) matured leaves at the base of 60 days old rootstock was the best for achieving high graft success with reduction of infection.

Keywords: vegetative propagation, grafting, cashew (*Anacardium occidentale* L.), rootstock, scion

1. Introduction

Cashew (*Anacardium occidentale* L.) from the anacardiaceae family is one of the world's major edible nut crops and ranks with hazelnuts and almonds in the international trade. It was spread from tropical America to other parts of the tropics by the early Portuguese and Spanish travelers (Ohler, 1979). Cashew is cultivated within the transitional belt and northern region of Ghana which falls within the Guinea savanna belt. It is usually propagated from seeds because it is readily available and easy to germinate and grow, even under adverse soil and weather conditions.

Cashew is easily crossed-pollinated and as a result, variability between trees is wide with respect to vigour of growth, times of flowering, yields and quality of nuts. Vegetative propagation approach may therefore be a viable option to improve quality of cashew planting materials. Cuttings and air-layering propagation were initiated in Ghana with the view of producing clonal materials for the establishment of plantations but the rooting performance was very poor (Cocoa Research Institute of Ghana (CRIG) personal *com*).

Grafting techniques have continuously been developed and improved (Wang, 2011) as a routine method for the agricultural production of many types of plant including fruit trees, vegetables and flowers. Cashew budding and grafting as compared to cutting and air-layering have produced some promising results and are being extensively used (Suassuna et al., 2016). Grafting as observed by Hartmann et al. (2011) hastens reproductive growth, maturity and earlier fruit production. Grafting has enabled plant biologists to study some unique physiological and developmental processes on the circulation of plant sap. Some transmissible factors have been studied using grafting (Asante et al., 2002), flowering (Dole & Wilkins, 1990), and rejuvenation of mature phase plant material (Melnyk, 2017).

Prospects of grafting look promising and grafted seedlings have been tested in Tanzania (Deckel et al., 2001). Bashiru (1998) in his cashew trials observed that for uniformity in planting materials and yields, grafting is the suitable vegetative propagation method. Grafting techniques in cashew have been successful for the following methods: softwood grafting, side grafting and wedge grafting (Martin et al., 2019; Manga et al., 2017).

Success in seedling grafting according to Leopold (1960) and Hartmann et al. (2011) is dependent on the number of retained matured healthy basal leaves since they undergo photosynthesis to provide food to enhance the healing process of the union of the grafted seedlings. Juvenile seedlings (which are mostly the soft wood type) grow rapidly because of rapid hormonal activity that enhance rapid cell division and promote high success in graft union formation (Mina et al., 2018).

Auxins which are natural occurring are found in all plant parts and move from cell to cell causing them to divide to enhance growth in the form of rooting in cuttings, graft union formation, leaf development, flowering, fruiting etc (Haissig 1974). Auxins are translocated with the help of phenols (in the form of conjugation) which prevent the enzyme IAA-oxidase from the destroying and breaking the auxin (Indole Acetic Acid-IAA) down (Hackett, 1970). The phenols aside the conjugation with the auxins, protect the inner parts of the plant from infection (Hackett, 1970). The presence of sugars (simple) result the survival of plants in the form of providing energy. The sugars also like the phenols protect the auxins from being oxidized by the enzyme IAA-oxidase (Haissig, 1974).

Canopy substitution (top working) is the replacement of a canopy of a tree with very promising desirable cultivar. The material to be top worked could be unproductive, or an old cultivar whose fruits are no longer in demand; it could be one with poor growth habits, or possibly one that is susceptible to prevalent diseases or pests (Hartmann et al., 2011). The grafted shoots take advantage of the already existing bulky root system of the old stump to grow and fruit early. The study aims at developing a suitable grafting technique that will facilitate the production of clones for improving the growth and yield of cashew on farmers' farms in Ghana.

2. Materials and Methods

A series of experiments were carried out in 2019 at the Cocoa Research Institute of Ghana substation, Bole located at longitude 02°29′W and latitude 09°01′N within an altitude of 100-600 m with one maximum rainfall (600-1400) mm which occurs from May to September (Hall et al., 1996). The area falls within the Guinea Savanna belt of West Africa with mean annual temperature ranging from 25 to 29 °C. High humidity figures are observed during the rainy period as well as temperatures with low elevation of < 600 m. The common soil type at the area under the study is sandy loam. There are few occurrences of hard pan where cultivation of the crop will not be useful.

Three experiments were conducted over a span of two years at establishing suitable grafting techniques and conditions for improving the cashew grafting in Ghana.

2.1 Procedure for Grafting

Two of the experiments were carried out at the nursery. The soil, which is sandy-loam in nature was taken from a plot located west of the substation and sterilized with hot water (at 100 °C). The hot water was poured on the heaped soil and covered with black polythene sheet for three days before being used for potting and planting of the seeds. The cashew seeds for the experiment were harvested from two desirable trees in the field. The harvested seeds were air-dried for three days ays and immersed in a bucket tree quarters filled with water at room temperature for five minutes with constant stirring. The nuts that remained at the bottom of the container were collected and nursed in potted black polythene bags of dimension, 15 cm × 22 cm packed under a wooden shed covered with green shade net to provide 50% shade. Watering was done on the nursed seeds once every three days depending on the weather. Ten days after seeding, when the plumule had emerged, the net was removed to allow sunlight into young seedlings to avoid etiolation. When the seedlings were between 45 to 56 days old after nursing, shoots identified from identified and tagged cashew trees were pre-cured (cutting the leaves on 10-15cm long shoots and leaving the stub still attached) for four (4) days on the trees and harvested for grafting onto the seedlings. The shoots were collected during overcast days or early morning or later in the afternoon and immediately placed in wet cotton/ newsprint (Akakpo et al., 2014). The procedure for the grafting types, softwood, semi-hardwood and side are as described by Hartmann et al. (2011). After care practice was executed by providing 50% shade for the grafted seedlings immediately after grafting, watering as and when necessary and removal of off- shoots below the grafts union as they appear to prevent the growing apical shoots from dying.

2.2 Experiment One: Period and Type of Grafting Technique on Grafting Success in Canopy Substitution

The experiment aimed at finding the suitable period for grafting and the best grafting technique to be used to achieve a high graft success in canopy substitution. Cashew trees aged seven (7) years were stumped to a height of 50 cm at different periods (April, May, June and July) and fully covered with grass to produce shoots for grafting in July, August, September and October. Harvested pre-cured scions from plus cashew trees (Yeboah and Amoah *pers com*) were grafted on the developed shoots at those periods using the softwood grafting technique (Hartmann et al., 2011). The 4 x 3 factorial experiment gave 12 combinations in a randomized complete block design with ten plants per treatment and replicated three times. All the treatment combinations were repeated in each replicate.

2.3 Experiment Two: Effect of Root Stocks at Different Physiological Ages on Grafting Success

The objective of this experiment was to determine optimum age of the rootstocks before grafting to achieve a high success in a randomized complete block design with 20 plants per treatment in three replicates. Grafting was done on the seedlings at different ages i.e. 60, 80, 100 and 120 days after nursing. Softwood grafting technique was selected because of high success observed in the first experiment and also recommended by Kumar *et al*, (1989). Shade was provided for the grafts with constant watering.

2.4 Experiment Three: Number of Retained Matured Basal Leaves on Root Stocks on Grafting Success

This experiment investigated the required number of leaves to be retained at the base of rootstocks in a randomized complete block design to enhance graft success. The grafting was done with 20 plants per treatment in three replicates using soft wood grafting technique. When the seedlings were 60 days (as recommended in experiment two after nursing) old, three sets of 20 seedlings per set were selected. For the first set, all the leaves on the seedlings were pruned off, whilst the second and third sets had two (2) and four (4) basal matured leaves retained respectively. The three (3) sets were grafted using the softwood grafting technique described by Hartmann et al. (2011) in three replicates.

2.5 Experiment Four: Biochemical Analysis of Rootstock for Grafting

The fourth experiment involved the determination of some plant substances (level of sugars, phenols and auxins) of the rootstocks for grafting.

2.5.1 Determination of Levels of Plant Substances

Sugars were determined by Dubois et al. (1956);

Free phenols were determined by Slinkard and Singleton (1977);

Free auxins by Donate-Correa et al. (2005).

2.5.2 Parameters Studied

No. of successful grafts (%)

Survival of successful grafts (3-monthly) (%);

Weather data;

% of developed side shoots below the union;

% infection;

% level of auxin, simple sugar and phenol.

Data were collected on the successful and survived grafts as well as the number of shoots developed below the graft union. Analysis of variance (ANOVA) was performed on data collected using the Genstat5 package. The least significant difference (LSD) was used to separate treatment means.

3. Results

The experimental period was characterized by low temperatures with high humidity in June, July and September (Table 1).

Table 1. Mean monthly temperature and humidity of the environment during the grafting period in 2019

Month	Temperature (°C)		Humidity (%)	
	Min.	Max	Min.	Max.
June	24.2	30.5	76	88
July	24.6	29.8	77	88
August	26.0	32.2	71	78
September	23.7	30.7	76	89
October	27.5	32.0	68	78

Minimum temperature significantly affected graft union formation. Relatively low temperature (23-26 °C) levels affected graft formation to a high extent such that 80% (Equation 1) of the variation in the graft success was affected by low temperature.

$$\text{Grafting Success} = 798 - 22.5\ \text{Temp. (Low)} \qquad (R^2 = 80\%; P < 0.001) \qquad (1)$$

Grafting success of rootstocks was not significantly affected by the environmental humidity (low or high).

The treatment interaction showed significant differences ($p < 0.05$) in graft union success with shoots grafted with softwood grafting technique in the month of July giving high success than the other treatments (Table 2). With the exception of September, there was a decreasing order in graft success (for softwood and side grafting) from July to October for all the treatments with October recording low successes.

Table 2. Effect of type of grafting and period of grafting on the success of graft union formation in canopy substitution

Type of grafting	Period of Grafting				Mean
	July	August	September	October	
Softwood	87.1	62.5	72.4	57.1	71.1
Semi-hard wood	65.7	62.5	62.2	43 9	58.5
Side	50.5	43.8	50.8	39.3	46.1
Mean	68.7	56.2	63.5	47.1	

Note. Type of grafting LSD ($P = 0.05$) = 14.6; Period of grafting LSD ($P = 0.05$) = 9.3; Type of grafting × Period of grafting LSD ($P = 0.05$) = 12.4.

Grafting on 60-day-old rootstocks significantly ($p < 0.05$) recorded high percentage graft success (Table 3) than the other days which did not significantly differ from each other. As the rootstocks became matured grafting success became low. This was observed for the 120-day-old rootstocks which gave the lowest union success.

Table 3. Success in graft union formation of cashew root stocks of different ages

Age of rootstock for grafting (days)	Success in graft union formation (%)
60	95.0
80	70.7
100	65.4
120	56.8
LSD ($P = 0.05$)	18.6

Retaining the lower matured basal leaves on the rootstocks played a significant role in graft union success (Table 4). Significantly high grafting success was recorded for retaining four (4) matured leaves at the base of the rootstocks while those without leaves gave the lowest success. The graft success of retaining four (4) leaves on the rootstock was 1.3 times greater than no leaf; whilst the reverse was true for off-shoot development (16.4 times greater for no leaf than for four leaves) (Table 4).

Table 4. Effect of varying the number of leaves at the base of a root stock on grafting success of cashew

Number of leaves retained on rootstocks	Success in graft union formation (%)	Off shoots developed on rootstocks after grafting (%)
No leaf	65.6	62.4
2 leaves	75.1	30.6
4 leaves	88.5	3.8
LSD (*P* = 0.05)	12.6	9.6

Fungal infection was significantly ($p < 0.05$) lower for rootstocks with four (4) retained basal leaves than the other rootstocks. Infection was 10 times greater for the rootstock with all leaves removed than rootstocks with four retained leaves (Table 5).

Table 5. Percent fungal infection of rootstock with retained basal leaves

Leaf Retention on rootstock	Infection (%)
No leaves	50.5
Two (2) leaves	26.7
Four (4) leaves	5.2
LSD (*P* = 0.05)	12.5

Physiologically young rootstocks (60 days old) had ($p < 0.05$) high auxin and sugar levels whilst the old rootstocks (120 days) recorded low levels (Table 6). The reverse was true for the phenol levels.

Table 6. Level of plant substances in rootstocks of different ages for grafting

Age of rootstock (days)	Auxin (%)	Simple sugars (mg/g)	Phenols (µg/g)
60	0.5	245.6	15.7
80	0.2	210.8	21.6
100	0.1	176.7	33.5
120	0.05	150.4	39.7
LSD (*P* = 0.05)	0.1	21.7	12.4

Rootstocks will more leaves significantly ($p < 0.05$) recorded high level of auxins and simple sugars. Whilst there were no significant differences in the phenol levels (Table 7).

Table 7. Level of plant substances in rootstocks with number of leaves retained at the base

Number of leaves retained at the base	Auxin (%)	Simple sugars (mg/g)	Phenols (µg/g)
No leaf	0.04	138.6	20.1
Two (2) leaves	0.3	160.8	16.6
Four (4) leaves	0.6	211.3	12.8
LSD (*P* = 0.05)	0.1	21.7	Ns

4. Discussion

The results obtained in the grafting techniques showed softwood grafting giving high graft success comparable to that of the other grafting techniques. The side grafting on the hand recorded the least graft success. Normally the shoot tip of the rootstock (softwood) is highly meristematic and enhances growth and development through rapid cell division (callus formation and wound healing) (Warschefsky et al., 2016). Grafting therefore at the tip of the shoot (softwood portion) of the stock for canopy substitution will promote faster union than the side and the semi-hardwood grafting on the shoot which are physiologically mature and slows down the union formation than the soft wood. The high success may also be due to low temperatures with moderately high cloud cover during the experimental period. Temperatures and humidities as reported by Muneer et al. (2016) and Wei et al. (2016) are some of the environmental factors that influence the healing of graft union and further promote callus formation. These factors also reduce evapotranspiration and desiccation of the scions to promote a high grafting success. This could be the probable cause of the high success for soft wood grafting technique in July and September which recorded moderate temperatures with high humidity figures. Temperatures recorded for October were very high and might have contributed to the low graft successes since they reduce/slow down cell division (cell injury) and auxin production (Nanda & Melynk, 2018). Akakpo et al. (2014) working on the shea tree observed that young plants especially with soft succulent growth have high level of some plant substances eg simple sugars and auxins responsible for rooting of stem cuttings.

The age of the rootstocks had significant effect on the success of the graft union formation. Ghamari et al. (2016) working on the cashew plant observed a decrease in grafting success with old seedlings. High levels of growth inhibitors increase in old seedlings (ageing) which negatively affect cell processes resulting in the slowing down of the healing process. This was recorded in the biochemical analysis where phenol levels in the 120-days old rootstocks were high, supporting the observation made by Ghamari et al. (2016). Sala et al. (2012) made similar observation in cutting propagation and stated that juvenile materials grow faster due to the presence of high levels of carbohydrates and auxins which are translocated to the rooting zone and this can also occur at the scion and root stock (interface) zone.

The role of leaves had already been described (Dirr & Hensser, 2005) as providing certain nutritive materials beneficial for cell division as well as their innate abilities to produce endogenous plant growth substances for callus formation to enhance the healing process of the graft union. The dependence of rooting on the carbohydrate supply has been amply demonstrated (Sala et al., 2012) and other materials such as vitamins and nitrogenous materials are supplied by the leaves as well.

The result of the biochemical analysis showed high levels of auxin and sugar which supported the observations made. The presence of leaves according to Jules et al. (1981) on the rootstock exerts a powerful influence through cell division. Leaves are also responsible for transporting co-factors which complement both carbohydrates and auxins to perpetuate the rooting of cuttings and healing process of graft unions. The rootstocks with all the leaves removed significantly produced a lot of side shoots below the union and affected the grafting success and may be due to the competition of nutrients by the off-shoots at the expense of the sprouted apical bud. Photosynthetic activity and auxin production will also reduce when there are no leaves on the root stock to provide food for the tissues and could also affect the grafting success (Jules et al., 1981). The phenols at low concentration played an active role in preventing infection (Hackett, 1970). It also functions as a protector of the auxins which is in the form of conjugation (forming a complex which cannot be easily be broken or oxidized) (Hackett, 1970). The high graft success of the rootstock with four retained basal leaves could be explained by its lower observed fungal infection as compared to the rootstock without basal leaves where fungal infection on the wounds (leaves removed) can interrupt the movement of sap and other plant nutrients (by blocking their pathway) which play a major role in graft formation (Abo Rehab et al., 2013).

5. Conclusion

The outcome of this study suggests that, soft wood grafting technique is the best for the multiplication of clones for the establishment of plantations and canopy substitution with July being the suitable period for grafting to achieve success in canopy substitution. Grafting on a root stock with retained four (4) matured leaves at base enhanced a high grafting success and reduced the incidence of multiple off-shoots whilst grafting on a 60-day old root stock gave a high graft success. Cashew rootstocks with all the matured basal leaves stripped off developed a lot of side shoots which retarded the healing process and significantly reduced the grafting success. Also rate of infection was high due to the creation of wounds at the site of the removed leaves.

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