



## Effects of Different Grafting Techniques and Time of Grafting on Cassava Flowering and Related Characteristics

Abah, S.P., Agu, J.C., Ewa, F. and Egesi, C.N.

National Root Crops Research Institute, Umudike, Nigeria

Corresponding author's email: [abahsp@gmail.com](mailto:abahsp@gmail.com)

### Abstract

By examining factors related to the timing and method of grafting as well as morphological changes during the initial stages of graft development, a field experiment was carried out to identify the grafting approach promoting high compatibility. At the National Root Crops Research Institute's experimental station in Umudike, Nigeria, the study was carried out from May to December 2015. Three components and eight replications made up the factorial randomized complete block design (RCBD) used to set up the experiment. ANOVA was used to compare the differences between data in all tables, and Duncan's Multiple Range Test (DMRT) was used to compare the means of quantitative features. The findings indicated that the grafting success rate increased with rootstock age. The evaluation of the cleft grafting procedure revealed that this technique had a greater grafting success rate than whip grafting. The evaluation of nighttime grafting treatment revealed that this period provided a larger proportion of grafting success than did morning grafting. The increased grafting success rate at night may be attributed to the high levels of humidity, which allow for the callus to form and stabilize the regeneration of the graft union surface between rootstocks and scions. A scion that was grafted at the age of four months produced the most seeds. The cleft grafting technique at night with rootstock between the ages of four and three months produced the optimum grafting compatibility.

**Keywords:** *Grafting time, grafting method, rootstock compatibility, and cassava*

### Introduction

The most extensively consumed root crop in the tropics is cassava (*Manihot esculenta* Crantz) (Nweke *et al.*, 1994; Henry 1995). For more than 600 million people in Africa, Latin America, and Asia, it provides a significant source of food. It is mostly grown for its starchy roots, with a global production of 309 million tons anticipated (FAO, 2021). Vegetable cultivation uses grafting extensively (Lee 2003). Vegetable grafting is regarded as a novel technique and is in increasing demand by farmers in the region of Africa where land use is particularly intensive and continuous cropping is a frequent practice (Khah *et al.* 2006). The capacity of two separate plants to grow into a single composite plant is known as grafting compatibility (Santamour, 1988). Through a high level of compatibility and productivity, the combined plants will show that they have developed more successfully than their original parent tree. The findings from earlier studies on grafting demonstrated that the rootstock and scion grafting combination is not always successful, as demonstrated by the form of incompatibility. Unfavourable environmental factors, pest-disease attacks, and histological incompatibility leading to poor joint ability could all contribute to unity failure. The physiological reactions between graft

partners are unfavourable, and histological abnormalities of the vascular tissue in the callus bridge are the typical causes of incompatibility. According to Schmidt and Feucht (1981), grafting failure can be described in this way as the result of a break in the cambium and vascular continuity that causes a smooth break at the place of the graft union.

A complicated system involving a variety of morphological, physiological, metabolic, and histological connections underlies the reaction to grafting incompatibility. The morphological change involved in the early stages of graft development, as well as the biochemical and histological bases for grafting compatibility, were still poorly understood. Evaluation of grafting compatibility based solely on morphological characteristics is typically time-consuming and subject to significant losses. On the other hand, it is anticipated that evaluating composite plants based on their morphological, histological, and biochemical traits while they are all observed at the same time will save time and money. Additionally, the assessment can be noticed in the grafted plants at an early stage. This study looked at how different grafting techniques and grafting times affected flowering and

related characteristics in cassava.

## Materials and Methods

### *Study site and experimental design:*

The experiment was carried out at the National Root Crops Research Institute's experimental site in Umudike, Nigeria, from May 2015 to December 2015. With three factors and eight replications, the experiment was set up using a factorial in a randomized complete block design (RCBD). The day's grafting time (morning and night) was the first determining factor. Three levels of rootstock age—1 month, 2 months, and 3 months—constituted the second component. The third component was the use of two grafting techniques (cleft and whip grafting). Three grafted plants were planted in each experimental unit, which had a 1 x 3 m plot size.

### *Plant materials and grafting implementation:*

TME 419, a high-yielding, non-flowering scion, and TMS 98/0002, a high-flowering rootstock, were the plant materials used for this experiment. Different times were chosen to plant rootstock cuttings between rootstock old treatments. Two months earlier than the treatment with one-month rootstock, the treatment with three months rootstock was planted, followed by the treatment with two months rootstock, which was likewise planted one month earlier. In the experiment field, rootstock plants were propagated using uniform cuttings that were each 25 cm long. Two months after planting each stage, NPK (15:15:15) fertilizer was uniformly applied to all of the stages of the experiment. Scion materials were harvested from mother plantations and produced as shoot cuttings measuring 12 cm in length two months following pruning. The grafting period was conducted simultaneously between the early morning (6–8 am) and grafting time was computed concurrently between rootstock age (2–4 months) and two grafting techniques (cleft and whip). Then, under the field's natural conditions, the grafted treatments were maintained.

### *Plant growth measurement:*

After the grafting, the observations started 1 to 4 months later. The percentage of grafted plants that succeed as well as plant growth (plant height, stem diameter below, above, and at the graft union, number of branches and leaves, number of inflorescences, number of flowers, and number of seeds) are among the characteristics recorded.

### *Statistical Analysis*

ANOVA was used to compare the differences between data in all tables, and Duncan's Multiple Range Test (DMRT) was used to compare the means of observed traits. Figures were drawn with the 2013 version of the Excel program. Plant growth and floral data attained for each grafting combination were used to determine grafting compatibility parameters.

## Results and Discussion

### *Results*

#### *The success of grafting:*

According to the findings of the analysis of variance, the interaction between the rootstocks and scions was what made the graft union successful. The typical success rate for grafting ranged between 68.17% in the morning and 80.17% at night. The results from the morning hours showed that the combinations of grafted plants with 4- and 3-month-old rootstocks and the top cleft grafting method had the highest grafting success rates (82.42 and 78.05%, respectively), while the combinations with 2-month-old rootstocks and the whip grafting method had the lowest grafting success rates (56.44%). The night-time results also showed that the top cleft grafting method and grafted plant combinations with roots that were 4 and 3 months old had higher grafting success rates (94.06 and 85.08%, respectively), while whip-grafted plants with roots that were 2 months old had the lowest success rates (63.14%). The findings demonstrated that the success rate of grafting increased with rootstock age (Fig. 1). The most successful grafts were made from rootstock that was 4 months old, followed by rootstock that was 3 months and 2 months old. It also showed that the evening hours are ideal for effective grafting (Fig. 1).

#### *Grafted plant growth and flowering:*

Growths that were expressed by morphological features would perform better due to the high compatibility of grafted plants. According to the statistical analysis, there was no significant interaction between the grafting period, older rootstock, and grafting technique on plant height, branch height, branch number, and leaf number at 4 months after the graft. The plant height, branch height, number of branches, number of leaves, level of branches, number of male flowers/folk, number of female flowers/folk, and number of seeks/folk, except the number of inflorescences, were all significantly impacted by the rootstock age treatment. Except for branch height and level of branches for grafting procedures, the grafting method and period independently influenced nearly all growth and floral characteristics observed (Tables 1 and 2).

### *Discussion*

#### *The success of grafting:*

The rootstocks' and scions' capacity to form a new union and then develop into a single composite plant determines the grafting's success. Some factors, including the close kinship of the species utilized as a scion or rootstock, the age of the rootstocks, the grafting technique, and the weather conditions at and after the grafting, affect this process. The outcome demonstrated that grafting success rates increased with rootstock age (Fig. 2). It might be because older grafting rootstocks, such as those that were grafted at night and were 4 and 3 months old, had more robust stems than those that were grafted during the day. These rootstocks would be able to enlarge the graft union area and provide support for the vascular tissue regeneration process. Composite plants with a younger rootstock had slower development

and flowering, which was probably caused by the vessel's diameter being smaller. Hydraulic conductivity would decline in response to the reduction in vessel diameter, which would then result in a reduction in stem water potential and stomatal conductance. The evaluation of the cleft grafting procedure revealed that this technique had a greater grafting success rate than whip grafting. The secure linking position and broader graft union surface offered by cleft grafting are likely to be the reasons for its better grafting success rate compared to other grafting techniques. This result is consistent with the findings of Islam *et al.* (2004), who found that the longest cambial layer connection and the ease of wrapping the graft union, which prevent rainwater from entering the plant, may be the reason for the highest percentage of survival of grafted mango plants produced by modified cleft grafting. An extremely low rate of successful union was one of the grafted plants' incompatibility signs. However, rapid grafted plant development and a good graft union were indicators of a successful grafted plant. The results of the evaluation of night-time grafting treatment demonstrated that night-time grafting had a greater success rate than morning grafting. The increased grafting success rate at night may be attributed to the high levels of humidity, which allow for the callus to form and stabilize the regeneration of the graft union surface between rootstocks and scions. Grafting two different plant genotypes together to form one unit would result in growing patterns that are different from those produced by growing each portion independently. According to Martinez-Ballesta *et al.* (2010), the rootstock-scion connection has a very complicated character and varies according to the genotypes utilized as a source for grafting. Histological factors; nutritional and carbohydrate levels; water nutrient absorption and translocation are factors that interact with the scion and rootstock.

#### ***Grafted plant growth and flowering:***

The morphological characteristics of the rootstock look better the older it is. This was shown by the rootstocks grafted at 4 months, followed by 3 and 2 months, and then by a wider shoot diameter at the position of below, above, and the graft union, as well as the number of seeds/foik formed that were more successful at the night hours (Tables 1 and 2). However, neither the age of the rootstock nor the grafting technique had an impact on the branch height or branch levels. Scion that was grafted at the age of 4 months produced the most seeds. A graft between rootstocks and scions with the same stem diameter made the graft union's surface easier to adhere to one another, ensuring that the meristem tissue between the grafted areas would be perfectly attached. The rapid formation of the continuous cambium and vascular tissue will then lead to a typical cycle of plant metabolism. Furthermore, the meristem tissue between the cutting and the contacting area would not adhere correctly due to the graft union between rootstocks and scions that had varying stem diameters. Water, nutrients, and assimilation of delivered photosynthetic product would all be blocked as the vessel tissues only coincide

on one side, preventing regeneration and continuity of vascular tissue. Incompatibility between rootstocks and scions is a sign that the grafting procedure was failed and inappropriate. The observation of grafted plants revealed that the rate of increase in stem diameter at the graft union was greater than that at the graft unions above and below levels. This result was consistent with Tshokoeva and Tsonev's (1995) findings, according to which the diameter of the stem grew greatly at the graft union location but there was little variation between the diameter of the scion and rootstock in grafted plants. The increased stem diameter at the grafted union may have been caused by metabolites (which may have included phenols and carbohydrates) that accumulated due to the partial cambium continuity in the grafted union. According to Errea (1998), a grafted plant's scion and rootstock portions' gap of assimilated translocation causes certain chemicals to accumulate at the grafted union. Additionally, a lot of calluses developed into parenchyma cells that were still undifferentiated, which led to the large grafted union. Physical analysis from the prior study also revealed that the difference in growth rate and callus production between the two portions was due to the rootstock's stem bark being substantially thicker than that of the scion ones. The identification of stress- and pathogen-resistant rootstocks, the compatibility of the graft union in terms of the rapid formation of the vascular connections between the rootstock and the scion, and the rapid renewal of root and canopy growth, are the main factors that determine the success of grafting (Cohen *et al.*, 2007). This diversity in the grafting abilities of different plant species and cultivars is presumably due to how well they can distinguish a circulatory system over the callus bridge and produce callus parenchyma. Based on the observation of morphological characteristics, treatment of rootstocks that were four and three months old performed better than rootstocks that were just two months old. As a result, the four-month-old rootstock was chosen as the ideal grafting time. However, the three-month-old rootstock can also be chosen because it reduced time and money and showed only a small or insignificant difference when compared to the four-month-old rootstock. In comparison to the whip grafting approach, the cleft grafting method performed better across every factor. In comparison to the morning grafting session, the night grafting performed better across all metrics.

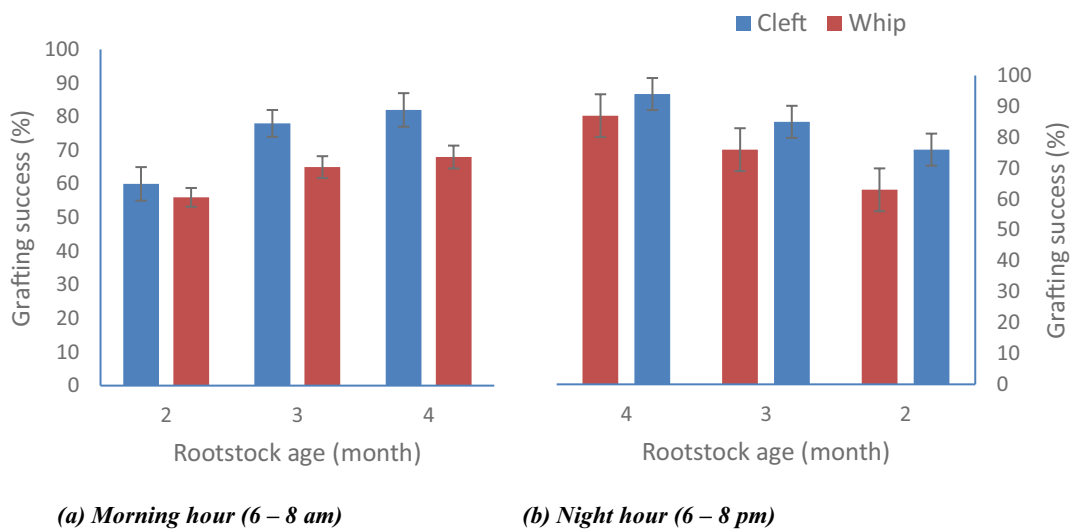
#### **Conclusion**

Consequently, we can conclude that the optimum cassava grafting compatibility and flowering were provided by grafting combinations between four-month-old rootstock at night utilizing the cleft grafting method. Good agricultural techniques should be applied to maximize the yield of grafted plants.

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**Fig. 1: Grafting success rate at 3 levels of rootstock age (2, 3, 4 months old), period of grafting (morning and night hour) and 2 grafting methods used (cleft grafting and whip grafting) on grafted plants**

**Table 1: Effects of period of grafting, rootstock age and grafting method used on growth parameters of grafted plants 4 months after grafting**

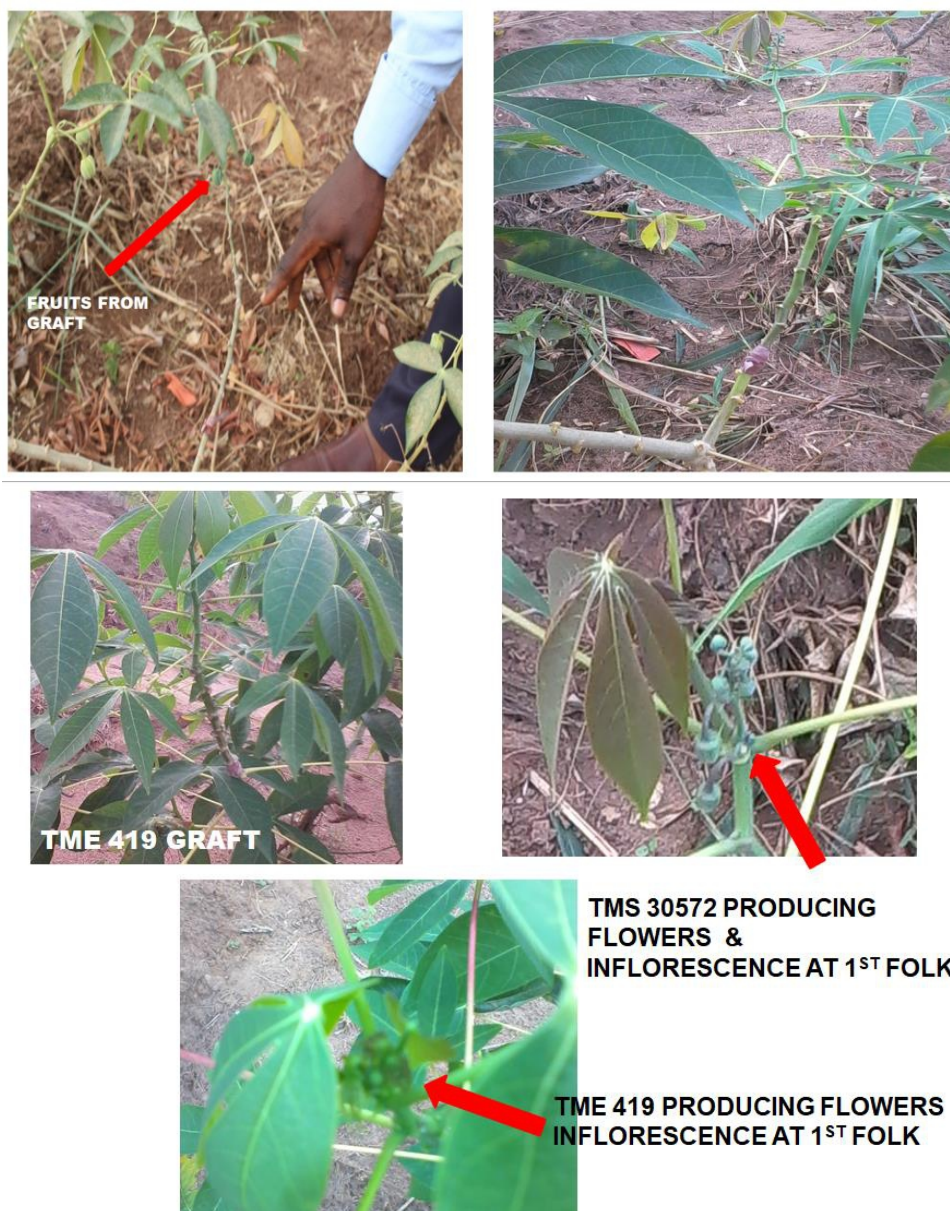
Treatments		Plant height (cm)	Branch height (cm)	Number of branches	No of leaves	Stem diameter		
						Below (cm)	At graft union(cm)	Above (cm)
Rootstock age	2 months	64.12 <sup>b</sup>	21.64 <sup>b</sup>	2.14 <sup>b</sup>	27.16 <sup>b</sup>	2.59 <sup>b</sup>	2.57 <sup>b</sup>	2.44 <sup>b</sup>
	3 months	84.86 <sup>a</sup>	36.83 <sup>a</sup>	2.42 <sup>b</sup>	29.60 <sup>b</sup>	3.28 <sup>ab</sup>	2.95 <sup>b</sup>	2.69 <sup>ab</sup>
	4 months	89.57 <sup>a</sup>	39.42 <sup>a</sup>	3.68 <sup>a</sup>	38.41 <sup>a</sup>	3.71 <sup>a</sup>	3.22 <sup>a</sup>	3.18 <sup>a</sup>
Grafting methods	Cleft	83.34 <sup>a</sup>	34.33 <sup>a</sup>	3.58 <sup>a</sup>	33.73 <sup>a</sup>	3.95 <sup>a</sup>	3.75 <sup>a</sup>	3.71 <sup>a</sup>
	Whip	71.59 <sup>b</sup>	33.67 <sup>a</sup>	2.46 <sup>b</sup>	24.64 <sup>b</sup>	2.28 <sup>b</sup>	2.23 <sup>b</sup>	2.08 <sup>b</sup>
Period of grating	Morning	71.82 <sup>b</sup>	31.50 <sup>a</sup>	3.85 <sup>a</sup>	21.45 <sup>b</sup>	2.25 <sup>b</sup>	2.18 <sup>b</sup>	2.17 <sup>b</sup>
	Night	82.14 <sup>b</sup>	34.56 <sup>a</sup>	2.73 <sup>b</sup>	35.40 <sup>a</sup>	3.67 <sup>a</sup>	3.47 <sup>a</sup>	3.56 <sup>a</sup>

Values in the same row and the same column followed by the same letters were not significantly different at the 5% level based on the DMRT test

**Table 2: Effects of period of grafting, rootstock age and grafting method used on floral parameters of grafted plants 4 months after grafting**

Treatments		No of Inflorescence/ Folk	Level of branches	No Male Flowers/folk	No female flowers/folk	No seeds/folk
Rootstock age	2 months	3.54 <sup>a</sup>	3.43 <sup>b</sup>	56.70 <sup>c</sup>	6.53 <sup>b</sup>	16.84 <sup>c</sup>
	3 months	3.35 <sup>a</sup>	3.67 <sup>b</sup>	61.90 <sup>b</sup>	8.92 <sup>ab</sup>	25.62 <sup>b</sup>
	4 months	3.71 <sup>a</sup>	4.48 <sup>a</sup>	80.78 <sup>a</sup>	11.17 <sup>a</sup>	33.05 <sup>a</sup>
Grafting methods	Cleft	3.85 <sup>a</sup>	3.69 <sup>a</sup>	80.64 <sup>a</sup>	10.35 <sup>a</sup>	30.41 <sup>a</sup>
	Whip	2.63 <sup>b</sup>	3.16 <sup>a</sup>	65.48 <sup>b</sup>	7.43 <sup>b</sup>	18.67 <sup>b</sup>
Period of grating	Morning	2.78 <sup>b</sup>	3.76 <sup>b</sup>	71.34 <sup>b</sup>	7.84 <sup>b</sup>	19.72 <sup>b</sup>
	Night	3.17 <sup>a</sup>	4.18 <sup>a</sup>	79.38 <sup>a</sup>	11.04 <sup>a</sup>	32.85 <sup>a</sup>

Values in the same row and the same column followed by the same letters were not significantly different at the 5% level based on the DMRT test.



**Plate 1: The pictorial view of the grafting experiment**