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ANATOMICAL BASIS FOR UNION COMPATIBILITY AND INCOMPATIBILITY OF BUDDING AND GRAFTING IN SOME ACCESSIONS OF *ANACARDIUM OCCIDENTALE* (ANACARDIACEAE)

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ABSTRACT

The anatomical features of the bud and graft unions of three accessions of Anacardium occidentale (Brazilian Jumbo, Brazilian extra-large, and Indian madras) were studied to determine the compatibility and incompatibility of the unions. Scion, stock, and union diameters of graft and bud were measured and the data obtained were used to discriminate between the compatible and incompatible combinations. The histological features of successful unions revealed a necrotic layer 4 days after propagation. Days 20 and 60 showed the formation of the callus cells, the cambium bridge was also observed 100 days after the propagation. The unsuccessful unions showed a wide gap in the cortex of the unions. Wedge graft and T-bud unions showed 72.92% and 33.33% successes respectively. The results showed that budding and grafting compatibility exists among the three accessions of A. occidentale. The factors that were responsible for the success of the unions were elucidated.

KEY WORDS: *Anacardium occidentale, cashew accessions, vegetative propagation, graft union, T-bud union*

INTRODUCTION

Cashew (*Anacardium occidentale* Linn.) is a tropical tree that belongs to the family Anacardiaceae. The family consists of about 75 genera and 700 species (Nakasone & Paull, 1998). Behrens (1998) described cashew as a tropical tree cultivated in many tropical countries of the world from its center of origin in South and Central America to Africa, Asia, and Tropical Australia. The crop was introduced to Asia and Africa in the 15th and 16th centuries (Ohler 1979). In western Nigeria, the first planting of cashew started in the 16th century at Agege in Lagos (Ventakaramah 1976). The commercial cultivation actually started in the 1950s at Iwo, Eruwa, and upper Ogun in the defunct Western Nigeria Development Corporation (WNDC) (Sanwo *et al.* 1972; Togun 1977). Interestingly, cashew trees are planted not only for the production of nuts but also for afforestation and erosion prevention programs in escarpment areas (Sanwo *et al.* 1972; Togun, 1977). Food and Agriculture Organization of the United Nations reported that the world's production of cashew nuts has increased enormously from 1,932,142 tonnes in 2000 to 4,439,960 tonnes in 2013 due to its demand (FAOSTAT 2013).

Graft compatibility is the ability of two different plants, grafted together, to produce a successful union and to develop satisfactorily into one composite (Santamour 1988). The development of a compatible graft is typically comprised of three major events: adhesion of the rootstock and scion, the proliferation of callus cells at the graft interface or Callus Bridge, and vascular differentiation across the graft interface (Moore, 1982a). On the other hand, graft incompatibility is an interruption in cambial and vascular continuity leading to a smooth break at the point of the graft union, causing graft failure. It is caused by adverse physiological responses between the grafting partners, disease, or anatomical abnormalities.

Although there are many factors that can lead to the success or failure of the union of scion and stock such as physiological and biochemical mechanisms (physiological tolerance or intolerance, respectively, between different cells) (Moore 1984); modification of cells and tissue (inhibition of lignin formation and the establishment of a mutual middle lamella results in weak graft unions) (Buchloh 1960); cell recognition of the grafting partners (Yeoman 1984); and magnetic resonance imaging (MRI). MRI may be useful for detecting graft incompatibilities caused by poor vascular connections (Warmund *et al*. 1993). However, the histo-cytological factor was considered in this work.

The anatomy of the unions (grafts and buds) is essential in determining the factors behind the compatibility and incompatibility of the grafting and budding operations. Earlier, some scientists have found the reasons why some unions are unsuccessive anatomically. For instance, in cherry (*Prunus*) grafts, the number of well-differentiated phloem sieve tubes is much lower at and below the union. This results in greater autolysis of cells, and generally a very low degree of phloem differentiation (Schmid & Feucht 1981). Also, in a union between apricot/plum grafts, some callus differentiation into the cambium and vascular tissue does not occur; however, a large portion of the callus never differentiates (Errea *et al.* 1994). In another situation, Warmund *et al.* (1993) reported that in apple grafts, a vascular discontinuity occurs with xylem interrupted by parenchyma tissue, which disrupts normal xylem function leading to the death of the budded scion.

Propagation of *A. occidentale* for mass production is hitherto faced with problems of appropriate means of production for mass commercial purposes. Cashew is a plant that experiences a high variety of constraints of natural regeneration. However, the propagation of cashew through seeds results in a high level of genotypic and phenotypic variations. This makes conventional breeding, slow and difficult. In view of these challenges, grafting is adopted commercially for the multiplication of varieties of outstanding qualities (Ohler 1979). Degrees of grafting successes throughout cashew-growing countries had been reported (Mahanu *et al.* 2009). Thus, vegetative propagation will be the right option for reviving a balanced proportion of the plant. Studies have shown that graft union success is a key factor in successful graft-take and subsequent performance of grafted seedlings in *A. occidentale* (Mahanu *et al.* 2012). However, research into graft union between accessions of *A. occidentale* is needed for a better understanding of the graft union and causes of graft incompatibility to enhance the development of superior cultivars and increase the ecological range of species for new markets in horticulture and forestry industries. The aim of this study, therefore, is to investigate graft and budding compatibilities and the possible cause of incompatibility of scion and stock in three accessions of *A. occidentale*. The objective is to illustrate the histo-cytological features of a wedge grafting and T-budding of the three accessions.

MATERIALS AND METHODS

The research was carried out at the Botanical Garden of the University of Ilorin, Ilorin, Kwara State, Nigeria. University of Ilorin Botanical Garden lies between latitude 8^0 30'N and longitude 4^0 33'E/ latitude 8.500^0N and longitude 4.550^0E. It is located in the transitional zone between the deciduous woodland of the southern and dry savannah of Northern Nigeria (Jimoh 2003). Three accessions (Brazilian Extra Large, Bel-36; Brazilian Jumbo, BJ-31; Indian Madras, Im-13) of *Anacardium occidentale* (Anacardiaceae) were obtained from the Cocoa Research Institute of Nigeria (CRIN), Ibadan, Oyo State, Nigeria (Figure 1).

Propagation techniques and experimental designs

The experiment was carried out between May, 2015 and February, 2016. The seeds were soaked for 24 hours (McLaughlin *et al.* 2014). Thereafter the soaked seeds were sown at the depth of about 5cm in black polythene bags filled with topsoil and a planting spacing measured 35cm x 22.5cm. The seedlings were later thinned to one per poly bag after establishment (Mahanu *et al.* 2009). After four months of planting, scions and buds measuring 10 to 12cm, were obtained from the accessions and were grafted and budded against each other respectively. The grafted and budded plants were laid out in a randomized complete block design with 8 replicates and 6 grafts per replication.

Figure 1: Seeds of three accessions of *Anacardium occidentale* obtained from the Cocoa Research Institute of Nigeria (CRIN), Ibadan, Oyo State, Nigeria

Percentage seed germination. The seed germination of each of the accessions was calculated using this formula (Gairola *et al*. 2011):

$$\text{Seed germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

Growth parameters. The following plant parameters of the three accessions were obtained at first, second, third, and fourth months after emergence. The plant height was measured from the soil level to the tip of the plant by using the meter scale. The stem girth was measured with the aid of a vernier caliper. The length of the leaf petiole was measured using a measuring tape. The area of the leaf was determined by using the formula.

$$L \times B \times K$$

Where L = length, B = breadth, K = Franco constant (0.79) (Franco 1939)

Grafting and budding techniques and management. A transverse cut was made on the main stem 15cm above the ground level of the pot. A cleft of 4 to 5cm deep was made in the middle of the decapitated stem of the rootstock by giving a longitudinal cut and a scion was selected with its end cut to a wedge of 4 to 5cm long by chopping the bark and wood from two opposite sides. The scion wedge was inserted into the cleft of the rootstock to ensure that both scion and stock cambial layers are in perfect contact with each other. The graft union was secured firmly by a grafting tape 1.2cm wide and 30cm long (Figure 2a). The scion was then covered with a transparent polythene bag from below the point of union to the tip (Chipojola *et al.* 2013).

A T-shaped cut on the rootstock about 20 to 30cm above the ground was made. The vertical part of the T was about 2.5cm long and the horizontal part was about one-third of the distance around the rootstock. The knife was twisted gently to open the flaps of the bark. On the scion, a selected bud was cut beginning about 1.2cm below the bud and ending about 1.9 to 2.5cm beyond the bud. A horizontal cut about 1.9cm above the bud down through the bark and into the wood was made. The budwood was slipped down into the T-shaped cut under the two flaps of the bark until the horizontal cuts of the bud matched up with the horizontal cut of the T-shape (Figure 2b). After inserting the budwood into the rootstock, the bud and rootstock were wrapped with budding tape (Elam 1997).

Histo-cytological studies of the parent stem, budding, grafted plants. Samples of the grafting and budding union regions were collected at 4, 20, 60, 80 and 100 days after budding and grafting while the parent plant stem was transversely sectioned after four months of planting. Approximately 2cm of the samples were taken and fixed in 70% formalin acetic acid (5% formalin, 5% acetic acid, and 90% ethanol) for a minimum of two weeks and a maximum of two months (Kilany *et al.* 2012). After fixing, the samples were rinsed in distilled water for about an hour to remove acid; thereafter the samples were dehydrated through different concentrations of alcohol (5%, 30%, 50%, 75%, and 100%) for at least 15 minutes to avoid destruction of the cells. The sections were stained with safranin for 10 minutes and later washed 3 times with distilled water. They were again dehydrated through different concentrations of alcohol (Mahanu *et al.* 2012). The sections were permanently mounted onto slides using Canada balsam (Braune *et al.* 1999) and labeled. Sections were then examined under a light microscope and photomicrographed using SONY Cybershot 16.1 megapixels.

Determination of percentage compatibility and incompatibility. After the complete sprouting and successful growth of the scion, the percentage graft-take success was calculated using the formula of Rabi *et al.* (2014):

$$\text{Graft-take success (\%)} = \frac{\text{Number of grafted plants survived}}{\text{Total number of plants grafted}} \times 100$$

$$\text{Graft-take failure (\%)} = \frac{\text{Number of grafted plants failed}}{\text{Total number of plants grafted}} \times 100$$

Statistical analysis. Data obtained from the growth parameters, the grafted and budded plants of the 3 accessions were subjected to one-way analysis of variance (ANOVA) using IBM SPSS version 20.0 computer software package. The histological variables were used to discriminate the compatible from the incompatible combinations (Ermel *et al*. 1995). A probability value of 0.05 was used as a benchmark for significant differences between parameters.

RESULTS AND DISCUSSIONS

Seed germination. Germination of seeds of the 3 accessions of *A. occidentale* occurred within 15 –27 days after sowing. Im-13 seedlings emerged earlier (15 – 18 days), followed by Bel-36 (17 – 23 days) while BJ-31 recorded the longest days of emergence (18–27 days). The number of seeds germinated in the accessions sown differs (Table 1) with BJ-31 recorded the highest (88.75%) germination percentage followed by Bel-36 (81.25%) while Im-13 recorded the lowest (78.75%) germination percentage.

Growth parameters. Mean values of quantitative features of the three accessions of *A. occidentalis* studied in this work are presented in Table 2. Analysis of variance showed that there are significant differences in all the growth parameters among the three (3) accessions ($p = 0.0000 < \alpha = 0.05$). It was further revealed that accession BJ-31 recorded the maximum height throughout the four months after emergence, followed by Bel-36 while Im-13 recorded the minimum height. Accession BJ-31 records the maximum stem girth throughout the four months after emergence, followed by Bel-36, whereas Im-13 recorded the minimum stem girth. The maximum number of leaves was recorded by BJ-31 throughout the four months after emergence, followed by Bel-36, while Im-13 recorded the minimum number of leaves. The maximum length of petiole was observed in BJ-31 throughout the four months after emergence followed by Bel-36, whereas the minimum number of petioles was indicated in Im-13. The maximum number of leaves was observed in BJ-31 throughout the four-month period after emergence followed by Bel-36, whereas Im-13 recorded the minimum leaf area.

Table 1: Germination percentage of the seeds of accessions sown

Accession	Total number of seed sown	Number of germinated seeds	Germination percentage (%)
Im – 13	80	63	78.75
Bel – 36	80	65	81.25
BJ – 31	80	71	88.75

TABLE 2: Growth parameters of the accessions of cashew seedlings

Feature	Accessions	1MAE	2MAE	3MAE	4MAE
Plant height (cm)	Im-13	7.9750^{c}	15.0175^{c}	16.8169^{c}	24.4250^{c}
	Bel-36	11.1313^{b}	23.8125^{b}	30.5581^{b}	40.3269^{b}
	BJ-31	14.6944^{a}	25.3488^{a}	37.1194^{a}	43.8750^{a}
Stem girth	Im-13	0.4469^{c}	0.4856^{c}	0.5219^{c}	0.5819^{c}
	Bel-36	0.5195^{b}	0.5720^{b}	0.6281^{b}	0.9637^{b}
	BJ-31	0.5606^{a}	0.6513^{a}	0.8231^{a}	1.0756^{a}

Number of leaves	Im-13	5.4375[c]	12.0000[c]	15.6250[c]	22.1250[c]
	Bel-36	6.3125[b]	13.6875[b]	21.5625[b]	30.3750[b]
	BJ-31	8.5625[a]	16.4375[a]	28.1250[a]	39.8125[a]
Length of petiole	Im-13	0.2994[c]	0.3875[c]	0.4875[c]	0.6750[c]
	Bel-36	0.3500[b]	0.4625[b]	0.6125[b]	0.8500[b]
	BJ-31	0.3875[a]	0.5062[a]	0.8000[a]	1.2187[a]
Leaf area	Im-13	6.3368[c]	17.8356[c]	35.1831[c]	78.8488[c]
	Bel-36	9.9239[b]	37.3400[b]	69.8219[b]	111.8600[b]
	BJ-31	15.3375[a]	56.9313[a]	120.7100[a]	134.1400[a]

Means in a column not followed by the same letter are significantly different at $P \leq 0.05$
MAE = Month after emergence

Histo-cytological studies

Parent plant stem. It was observed that the stem anatomy of the three accessions was quite similar and typical for woody stem consisting of epidermis, cortex, phloem, xylem, and pith (Figures 3a-c). It was also observed that the cortex and pith contain numerous parenchymatous cells (Figures 3d-e).

Wedge: scion, rootstock and union diameters. The results showed that the scion, rootstock, and union diameters in the compatible unions were not significantly different from the scion diameters in the incompatible unions ($p = 0.640 > \alpha = 0.05$). The results also showed that the scion and rootstock diameter obtained initially after grafting was not significantly different from the scion diameter 100 days after grafting, but the union diameter obtained initially after grafting was significantly different from the scion diameter 100 days after grafting ($p = 0.000 < \alpha = 0.05$) (Table 3).

TABLE 3: Mean values of parameters for compatible and incompatible wedge graft unions and union parameters initially after grafting and 100 days after grafting

Compatibility	Scion and stock union		
Compatibility	Scion	Rootstock	Union
Compatible	0.6500[a]	0.9059[a]	1.0271[a]
Incompatible	0.6214[a]	0.7914[a]	0.9314[a]
Union diameters			
Initially after grafting	0.6500[a]	1.0271[a]	1.0271[b]
100 days after grafting	0.6700[a]	1.0300[a]	1.2882[a]

Means in a column not followed by the same letter are significantly different at $P \leq 0.05$.

T-budding: rootstock and union diameters. Rootstock and union diameters in the compatible unions were not significantly different from the incompatible unions. The rootstock diameter obtained initially after budding was not significantly different from the rootstock diameter 100 days after budding. It was also observed in that the union diameter obtained before budding was significantly different from the union diameter obtained 100 days after budding ($p = 0.000 < \alpha = 0.05$) (Table 4).

Union healing processes. On successfully sprouted and healthy grafts (Figures 2c and d), a light brownish band marked the union between rootstock and scion (Figures 2e and f). However, in the case of wilted graft, a deep dark brown to the black band of tissues was found 90 days after grafting (Figures 2g and h). It was also observed that most of the unsuccessful grafts were recorded during the harmattan period.

Grafting healing processes

Necrotic layer. On the fourth day, a thin necrotic layer was observed on the graft union (Figure 3f and g), but 20 days after grafting showed that prior to callus formation in the cambial region, there was an occurrence of resin secretion onto the wound surface as an initial response of tissue damage during severing of the scion and rootstock (Figures 3j and k). During the first stage of graft formation (necrotic layer formation), no structural differences were observed between compatible and incompatible unions.

Callus formation. The formation of callus was observed from the cells of the cambial region (Figure 4a) on the 20th day. In the entire combinations, callus continued to proliferate and filled the space between rootstock and scion forming a callus bridge. It was also observed at 60 days after grafting that callus has been formed in the pith, xylem ray parenchyma, and cortex. Also, in all the combinations, it was observed that the necrotic layer in the cortex and the cambial region had been significantly reduced (Figures 4b-h). A thick presence of the necrotic layer was observed initially in the pith of all the combinations (Figure 4d). Callus cells were observed in the xylem ray parenchyma in the Bel-36/Im-13 and Bel-36/BJ-31 (Figure 4f). A thin presence of the necrotic layer was recorded in other combinations (Figures 4b, g and h).

Graft-take success in wedge-grafted plants. The values pertaining to graft take success in wedge grafted plants are represented in Figure 4g. The representation showed that more graft-take success (87.5%) was obtained in Bel-36/Im-13 union followed by Im-13/Bel-36 (75%), Im-13/BJ-31 (75%) and Bel-36/BJ-31 (75%). The lowest graft-take success (62.5) was recorded in BJ-31/Im-13 and BJ-31/Bel-36. Figure 4h also showed that Im-13 and BJ-31 have more graft take the success of 75% when used as a rootstock, followed by Bel-36 (68.75). As a scion, Bel-36 recorded the highest graft take success with 81.25%, followed by Im-13 (75) while the lowest was recorded in BJ-31 (62.5).

TABLE 4: Mean value of parameters for compatible and incompatible T-bud unions and union parameters initially after budding and 100 days after budding

Compatibility	Stock union	
Compatibility	Rootstock	Union
Compatible	1.0170[a]	1.1400[a]
Incompatible	1.0090[a]	1.1410[a]
Union diameters		
Initially after budding	1.0117[a]	1.1283[b]
100 days after budding	1.0667[a]	1.3292[a]

Means in a column not followed by the same letter are significantly different at P ≤0.05.

FIGURE 2: (a) A wedge graft of *Anacardium occidentale* (Bel-36 on Im-13); (b) A T-bud of *A. occidentale* (Im-13 on BJ-31) (c) A successful wedge grafted *A. occidentale* (BJ-31 on Im-13). (d) A successful T-bud *A. occidentale* (Bel-36 on BJ-31) (e) Wedge graft union of 60th day between Im-13 (scion) and BJ-31 (rootstock) (f) Wedge graft union of 70th day between Bel-36 (scion) and Im-13 (rootstock) (g) A failed wedge graft of (Im-13/Bel-36); (h) A failed T-bud (BJ-31/Im-13)

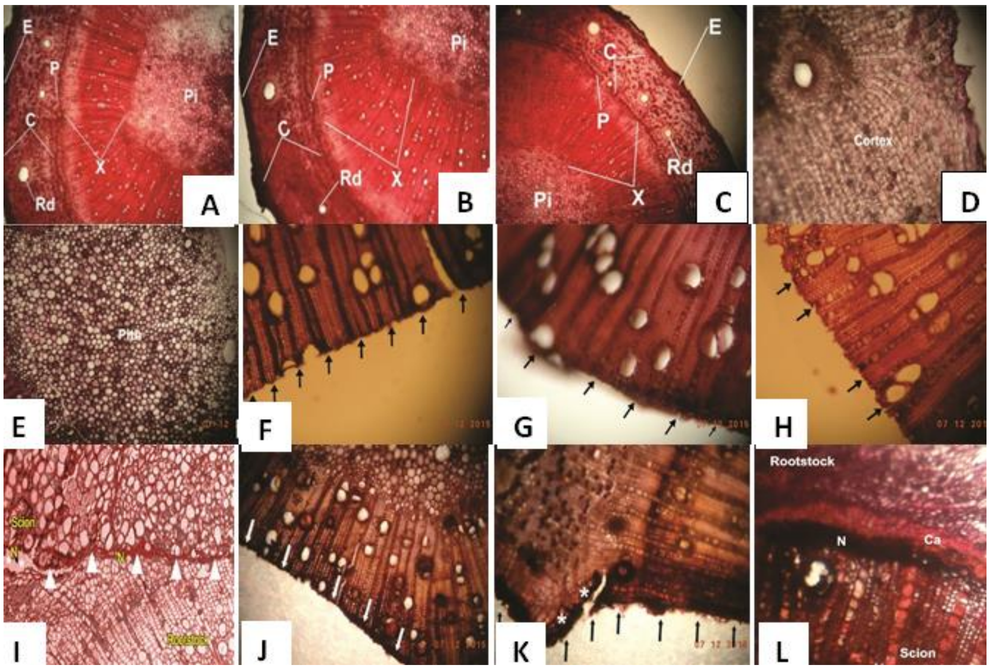

FIGURE 3: light micrograph showing the transverse sections of the stems of **A**- BJ-31; **B**- Bel-36; **C**- Im-13; **D**- BJ-36 with numerous parenchyma cells in the cortex; **E**- Im-13 revealing a large number of parenchyma cells at the pith; **F**- Bel-36 as scion 4 days after grafting (black arrows points to a thin necrotic layer); **G**- Im-13 as a rootstock 4 days after grafting (thin necrotic layer along the black arrows); **H**- Bel-36 (as a bud scion) showing a thin necrotic layer 4 days after budding (along the arrows); **I**- bud union of Bj-31 on Im-13 showing an adhesion in the phloem and cambium region (across the arrow heads) four days after budding; **J**- Bel-36 showing a thick necrotic layer along the white arrow heads and resin deposits 20 days after grafting; **K:** BJ-31 as a rootstock showing a thick necrotic layer, resin (along the black arrows) and callus cells (asterisk) 20 days after grafting; **L**-cross-section of the bud union of Im-13 on Bel-36 showing the emanation of callus cells and necrotic areas 20days after budding. (N-necrotic areas; Ca-callus; X-xylem; E-epidermis; C-cortex; phloem; and Pi- pith; RD- resin duct)

Graft-take success in T-budded plants. The values pertaining to graft take success in T-budding are represented in Figure 4c. The representation reveals more graft take success (50%) in Im-13/Bel-36 and Bel-36/Im-13 followed by Im-13/BJ-31 (37.5%), Bel-36/BJ-31 (37.5%), BJ-31/Bel-36 (37.5%), while the lowest graft-take success was recorded in BJ-31/Im-13 (25%). Figs. 7a and 6b also revealed that Bel-36 had the highest graft take success (43.75%) as rootstock followed by Im-13 (37.5%) and BJ-31 (37.6%). As a scion, Im-13 and Bel-36 recorded the highest graft take success with 43.75%, while the lowest was recorded in BJ-31 (31.25%).

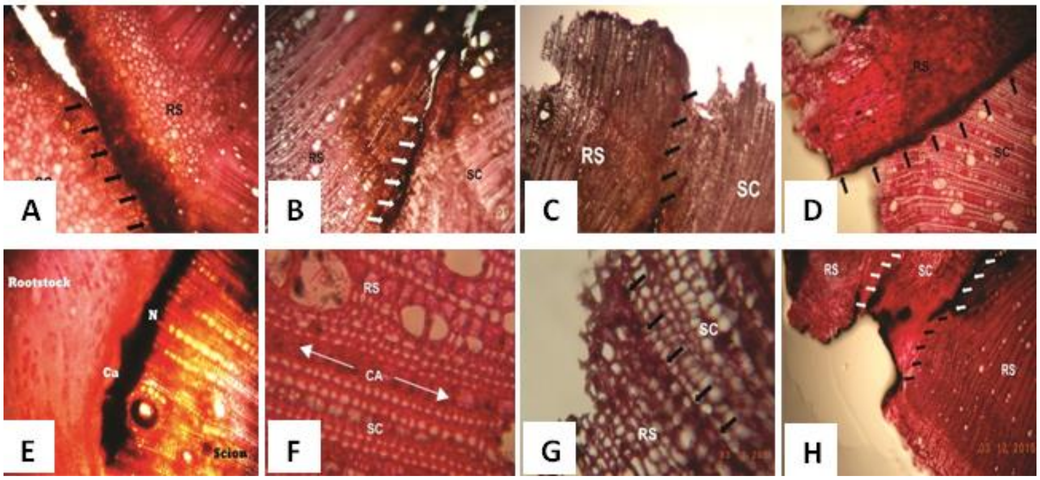

FIGURE 4: The transverse sections of the stems of a 60-day-old graft union of:**A-** BJ-31/Bel-36 showing the formation of callus at the pith (along the black arrows); **B-** Bel-36/BJ-31 showing the formation of callus at the xylem ray parenchyma with the presence of a thick necrotic layer (along the white arrows); **C-** BJ-31/Bel-36 showing the formation of callus at the cambial region towards the xylem (along the black arrows); **D-** Bel-36/Im-13 showing the formation of callus at the xylem ray parenchyma (along the black arrows); **E-** Bel-36 on Im-13 showing a thick presence of the necrotic areas (N). callus (Ca); **F-** Im-13/BJ-31 showing the formation of callus at the xylem ray parenchyma **G**- BJ-31/Im-13 showing the formation of callus at the cambial region towards the xylem (along the black arrows); **H**- Im-13/BJ-31 showing the formation callus at the cortex (along the black arrows). (Pi-pith; X-xylem; P-phloem; C- cortex; Rd- resin duct; E- epidermis, RS- rootstock; N-necrotic, CA- callus; SC-scion and RS-rootstock)

Percentage of wedge grafting and T-budding success. Percentage success and failure in T-budded and wedge grafted (Figure 5-7) plants. It was observed in this experiment that 72.92% of wedge graft plants were successful while only 33.33% of T-bud plants were successful. The successful union was determined by the successful emanation of the cambial cells, while the failed union was determined by the failure of the cambial cells to emanate at the union which eventually leads to the withering of the scion.

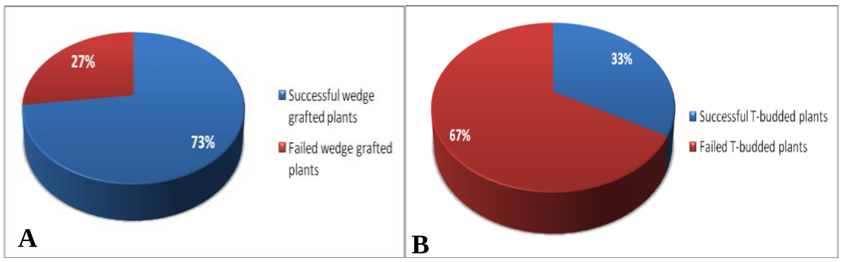

Figure 5: Pie chart showing the percentage of success and failure recorded by (A) wedge grafted plants and (B) T-budded plants

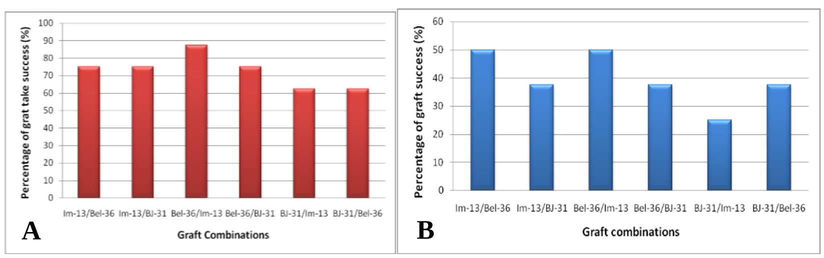

Figure 6: Bar chart showing the graft take success in A: wedge grafted plants B: T-budded plants

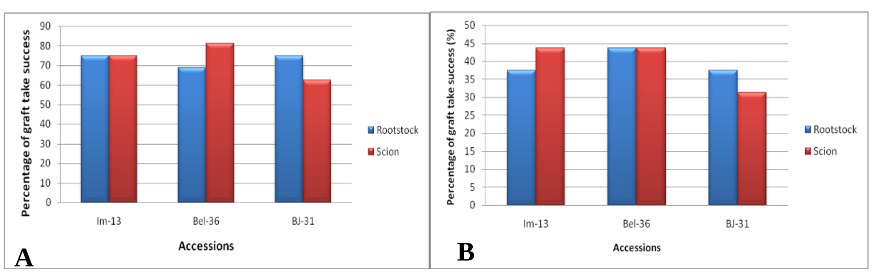

Figure 7: Bar chart showing the percentage success of accessions as A: rootstock and scion in T–budded plants B: rootstock and scion in wedge grafted plants.

Budding healing processes

Adhesion and Formation of necrotic layer. The wounded cells were observed at the interface of the union as a straight and sharply visible necrotic layer. It was also observed that rootstock and scion in all the combinations covered the budding four days after (Figures 3h, i). The adhesion was observed between the tissues of the phloem and cambium. At the wood interface, no adhesion was observed. Also, at the outer bark, no adhesion occurred. There was no observable structural difference between the compatible and incompatible unions during the first stage of graft union formation.

Callus formation. The stock and scion participated equally to callus formation in all the combinations. It was observed that callus was produced by the cambial cells and the young phloem cell layer closest to the wound surface (Figure 3l). The cortex also participated in the formation of callus, but was very rare. Due to the formation of the callus at the interface, the space observed in the first stage of graft formation was filled. As a consequence of the formation of the junction callus, the two partners were tightly joined. The first contact observed between the living cells of the two partners was due to the burst of the necrotic layer (Figures 4e). The xylem ray parenchyma cells also formed callus, but the rate of production decreased with lignification.

Wound vascular cambium and Cambium Bridge. The new cambial cells were observed 100 days after budding transversely and longitudinally (Figs. 8a-p). Necrotic areas and callus cells were also present at the union interface. The cambium bridge shapes of well-matched graft partners were variable: some were slightly curved (Figure 8a), while some were S-shape (Figure 8?). Unsuccessful unions reveal a gap in the cortex of the union. The cambium bridge between rootstock and scion was observed transversely (Figures 8a-c, e, f and l) and longitudinally (Figures 8k-q) 100 days after budding. Though the necrotic layers and callus cells were still much presents at the union interface (Figures 8h and i).

In this study of union compatibility among three accessions of *A. occidentale*, a chronological sequence of events similar to that previously reported for other woody and/or non-woody plants were observed (Ada & Ertan 2013; Kilany *et al.* 2012; Mahanu *et al.* 2012). In the propagation of the plants through seeds, it was observed that the sizes of the cashew nuts influenced the germination percentage and the duration of the emergence of the seedlings. Adebisi *et al*. (2015) reported that the large cashew seed nuts had the highest seedling emergence above the medium and small size with a marginal increase, meanwhile small nuts emerged earlier than others. Hammed *et al*. (2011) findings on growth and nutrient uptake of cashew seedlings as affected by nut-size in the nursery revealed that seedlings raised from jumbo nut-size have higher quantitative plant vigor followed by seedlings raised from medium size while those from madras nut-size recorded lowest plant vigor. This was also the case in the present study because in all the quantitative characters recorded, Brazilian jumbo had the maximum plant vigor while the Indian madras had the minimum.

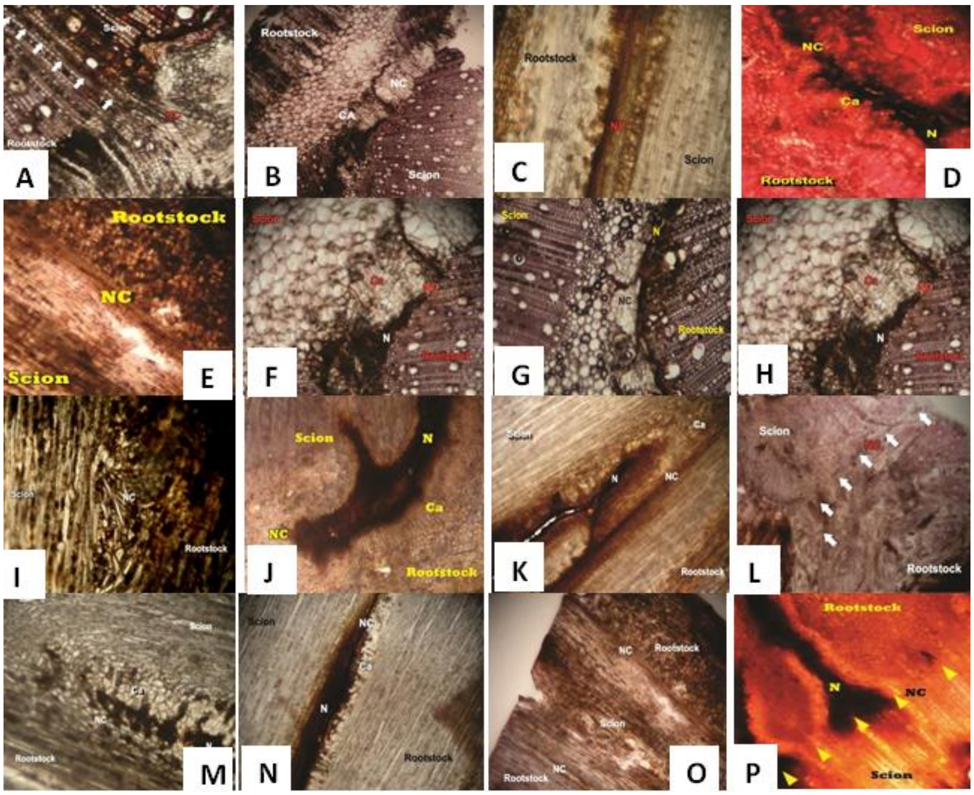

FIGURE 8: Light micrograph of 100 days after grafting **A**- new cambium in Im-13/Bj-31 union; **B**- Bj-31 grafted onto Im-13. Revealing the presence of (CA) and (NC); **C**- Im-13 grafted onto BJ-31, (NC); **D**- bud scion of Im-13 on Bj-31 showing the emanation of the (NC) after budding.; **E**- longitudinal section of the bud union of Im-13 on Bj-31 showing the emanation of the (NC); **F**- Bel-36 grafted onto BJ-31. Showing a heavy presence of the (N) and (NC); **G**- Bel-36 grafted onto BJ-31. Showing the presence of (N), (CA) and (NC); **H**- Bel-36 grafted onto BJ-31 shows the emanation of the (NC) and (N); **I**- longitudinal section in union area of Bel-36 grafted onto BJ-31 (NC); **J**- longitudinal section in union area of Bel-36 grafted onto BJ-31 section showing a heavy presence of the (N), (Ca) and (NC); **K**- Bej-36 on Bj-31 showing the emanation of the (NC), N and Ca; **L**- Im-13 grafted onto Bel-36 showing NC (arrows); **M**- between Im-13 and bel-36; **N**- longitudinal section showing necrotic layer in the union between Bel-36 and im-13. NC, N and Ca; **O**- a longitudinal section showing tissues in graft union between Im-13 and bel-36. (NC); **P**- Cross-section of the bud union of Bel-36 on Im-13 showing the emanation of the NC (arrow heads) after budding x1200 (NC- New cambium; Ca- Callus; N-Necrotic area)

The transverse sections of the stem of the accessions of *A. occidentale* were similar, so the tissues were properly matched. It is known that success in grafting of higher plants, mainly depends on two essential factors; the physiological compatibility between rootstock and scion, and proper matching of the different tissues of graft partners (Mahanu *et al.* 2012). The presence of numerous parenchyma cells at this stage is for effective conduction of water and nutrients and for the repair of damaged or broken cells, and this allows the plant to heal itself in most cases against injury (Hacke *et al*. 2001). The significant presence of the parenchymatous cells in the cortex and pith may also have contributed to the success of the unions.

The parameters obtained from scion, rootstock, and union diameters from the grafted plants in the compatible grafts were not important enough to be differentiated from the incompatible grafts. Also, the parameters obtained from rootstock and union diameters from the compatible budded plants were not significantly different from the incompatible budded plants. So, in this study, the parameters listed were not measured for successful or failed unions. There was an increase in stem diameter at the graft and bud unions compared with the scions and stocks. Results agreed with the findings of Tshokoeva & Tsonev (1995), who reported marginal differences between scion and stock diameters in grafted apricot trees, but a significant increase in diameter at the union which could be attributed to accumulation of metabolites (presumably phenols and carbohydrates) as a result of partial cambium continuity of the union (Mng'omba *et al.* 2007).

The first event that emerged at the interface of the graft and bud unions was the formation of a necrotic layer. The necrotic layers are composed of cells injured when budding or grafting (Kilany *et* al. 2012, Hartman *et al*. 2010). The presence of the necrotic layer at the wounded surfaces limited desiccation and death of the deeper tissues and reduces the entry of pathogens into the plant body (Cline & Neely 1983); whereas the excessive accumulation of the necrotic layer could result in mechanical failure of the union (Tiedeman 1989). In the present study, no excessive accumulation of the necrotic layer was observed both in graft and budding unions.

The resin observed in the necrotic region provided a temporary bond (cementing medium) between the two graft partners (Kilany, 2012) and by itself did not contribute to the union formation. The resin secretion occurring within a few minutes of knife wounding but more pronounced during callus formation subsequently provided the sole cohesive force between the grafting partners in the first few days before a callus bonding is established (Asante & Barnett 1997). Resin appears as a dark staining or necrotic layer scattered between the grafted components (Dolgun *et al.* 2008). No structural differences were observed between compatible and incompatible unions during the first stage of graft formation. Adhesion observed in the budding combinations was due to the deposition of wall precursors at the graft interface and their subsequent polymerization (Moore & Walker 1981; Moore, 1982b).

According to Pina and Erra (2005), callus formation is independent of other events in graft development. Asante & Barnett (1997) mentioned that callus formation is crucial to the initial sprouting and the survival of the scion. The rapid physical contact of opposing callus cells intermingling and interlocking, filling up the space between the scion and rootstock, provides a bridge for the transport of water and nutrients (Miller 1991). Callus can be formed from the cambial region, cortex, pith, and xylem ray parenchyma (Hartman *et al*. 2010). The formation of callus was observed from the above-listed regions as observed in the experiment of grafting and budding of the three accessions of the cashew plant. It is a primary response to wounding in compatible and incompatible.

There is a gradual disappearance of the necrotic layer between the two graft components caused by the intermingling callus. Despite the fact that callus formation occurs as a wound reaction and is found in compatible and incompatible grafts, the content and nature of the cells involved in the sequence of graft formation can also play an important role in triggering the responses that lead to the formation of a strong and successful union (Pina and Errea 2005; Ada & Ertan 2013). However, unsuccessful (or failed) grafts will occur due to water stress when scions are unable to receive water from the rootstock before the graft union is formed; this ultimately leads to corresponding increases in transpiration and temperature (Barnett & Weatherhead 1989; Beeson & Proebsting 1988). Unsuccessful grafts revealed parting at the cortex of the graft components; this was also reported by Mahanu *et al.* (2012). Shade improved the success of grafting during high day temperatures and decreased ambient (surrounding) air temperatures, and thus improving graft success (Frey 2009).

Unsuccessful grafting and budding unions in this experiment could be a result of the dust-bearing land-wind (harmattan) period that brings about a reduction in the relative humidity. The basic requirement for a successful graft union is the formation of vascular connections (Wang & Kollmann 1996) and in addition, the scion must have a meristematic region (bud) to resume shoot growth and eventually supply photosynthates to the root system (Hartman *et al.* 2010). These new vessels, however, originated from some meristematic cells in the callus. The wound cambium bridge shape can be slightly curved or S-shaped if the graft partners were well matched. According to Soumelidou *et al*. (1994), this shape suggests the existence of a high degree of coordinated activity between the cut edges of the pre-existing cambia. This bridge was noticed in the cambia region of this study. Ada & Ertan (2013) reported that the fact that the new vascular connections could not be well differentiated or weakly established has been the main reason for incompatibility in woody plants. In this present study, a few necrotic areas were observed at the graft union, but the cambial connection was observed in all longitudinal and transverse sections. Also, the vascular connection was established successfully between rootstock and scion 100 days after grafting and budding. In this experiment, wedge grafting recorded the highest success rate compared to T-budding.

CONCLUSIONS

Grafting and budding unions' compatibility exists in all the combinations among the three accessions of *A. occidentale*. Though, the success rate of the wedge-grafted plants supersedes the T-budded plants due to adequate contact between the scion and rootstock. The results obtained show that the success of the graft and the bud union depends on the following factors: the choice of grafting methods; high relative humidity; proper matching of the components; early callus formation and formation of the cambium and vascular tissues.

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REFERENCES

- Ada, S. & Ertan, E. (2013). Histocytological study of the graft union of the chestnut (*Castanea sativa* Mill)/oak (*Quercus vulcanica* Boiss). Agriculture, Forestry and Fisheries 2(2):110-115.
- Adebisi, M. A., Kehinde, T. O., Abdul-Rafiu, A. M., Amira, J. O., Oyewumi, A. A., Oni, O. D. & Onyeka, C. V. (2015). Influence of nut size, hydro priming duration and storage period on seedling emergence and early seedling vigor characters in cashew (*Anacardium occidentale* L.). Journal of Tropical Agricultural Science 38(4):465-480
- Asante, A. K. & Barnett, J. R. (1997). Graft union formation in mango (*Mangifera indica* L.). Journal of Horticultural Science 72:781-790.
- Barnett, J. R. & Weatherhead, I. (1989). The effect of scion water potential on graft success in sitka spruce (*Picea sitchensis*). Annals of Botany 64:9-12.
- Beeson, R. C. & Proebsting W. M. (1988). Relationship between transpiration and water potential in grafted scions of *Picea*. Physiologia Plantarum 74:481-486.
- Behrens, R. (1998). About the spacing of cashew nut trees. Proceedings of International Cashew and Coconut Conference Dar-Salaam. pp. 48-52.

- Braune, W., Leman, A. & Taubert, H. (1999). Plant Anatomic Laboratory, Band II. Spectrum Heidelberg, Berlin. p. 368.
- Buchloh, G. (1960). The Lignification in Stock-scion Junctions and its Relation to Compatibility. In Pridham, J. B. ed. Phenolics in Plants in Health and Disease. Pergamon Press, Long Island City, New York.
- Chipojola, F. M., Mwase, W. F., Kwapata, M. B., Njoloma, J. P., Bokosi, J. M. & Maliro, M. F. (2013). Effect of tree age, scion source and grafting period on the grafting success of cashew nut (*Anacardium occidentale* L.). African Journal of Agricultural Research 8(46):5785-5790
- Dolgun, O., Tekintas, F. E. & Ertan, E. (2008). A histological diagnosis of apricot/peach almond graft incompatibility: Statistical analysis of data from 5-month-old grafts. Acta Hort. 384:497-503.
- Elam, P. (1997). Budding and grafting *Citrus* and avocados in the home garden. University of California, Division of Agriculture and Natural Resources 8001:1-5.
- Ermel, F. F., Catesson, A. M. & Poessel, J. L. (1995). Early histological diagnosis of apricot/peach ·almond graft incompatibility: Statistical analysis of data from 5-month-old grafts. Acta Hort. 384: 497–503.
- Errea, P., Felipe, A. & Herrero, H. (1994). Graft establishment between compatible and incompatible *Prunus* spp. Journal of Experimental Botany 45:393–401.
- FAOSTAT. (2013). Food and Agricultural Organization Statistics. http://faostat.fao.org/default.aspx. Retrieved January 2, 2015.
- Franco, C. (1939). Relation between chromosome number and stomata in *Coffea*. *Bot. Gaz*. 100: 817–818.
- Frey, H. H. (2009). Factors affecting graft success and early growth of Fraser fir (*Abies fraseri*) M.Sc. Thesis. Forestry Raleigh, North Carolina. p. 20
- Gairola, K. C., Nautiyal, A. R. & Dwivedi, A. K. (2011). Effect of temperatures and germination media on seed germination of *Jatropha curcas* Linn. Advances of Bioresearch 2(2): 66 – 71.
- Hacke, U. G., Stiller, V., Sperry, I. S., Pitterman, I. & McCulloli, K. A. (2001). Cavition, fatique, embolism and refilling cycles can weaken the cavitation resistance of xylem. Plant Physiology 125: 770-786.
- Hammed, L. A., Lawal, B. A. & Kolapo, K. A. (2011). Growth and nutrient uptake of cashew (*Anacardium occidentale* L.) seedlings as affected by nut-size in the nursery. African Journal of Agricultural Research 6(17):3962-3971.
- Hartman, H. T., Kester, D. E., Davies, F. T. & Geneve, R. L. (2010). Plant Propagation: Principles and Practices. Eighth Edition. Prince Hall, New Jersey. pp. 415-457.
- Hinesley, L. E. (1981). Initial growth of Fraser fir seedlings at different day/night temperature. For. Sci. 27:545-550.
- Jimoh, H. I. (2003). Erosion tolerance range of land use surface: implication on land resource use and management techniques in Ilorin, Nigeria. International Journal on Environmental Studies 60(5): 445-452.
- Kilany, O. A., Adb El-Zaher, M. H. & Hamed, H. H. (2012). The relationship between the histological features in the grafting areas and the compatibility degrees of some mango cultivars onto nucellar seedlings. Journal of Horticultural Science and Ornamental Plants 4(1): 58-65.

- Mahanu, G. K., Adjei, P. Y. & Asante, A. K. (2009). Comparative studies on ages of rootstock seedlings and grafting methods in cashew (*Anacardium occidentale* L.). Ghana Journal of Horticulture 7:11-17.
- Mahanu, G. K., Adjei, P. Y. & Asante, A. K. (2012). Anatomical studies on graft formation in cashew (*Anacardium* occidentale L.). Agriculture and Biology Journal of North America 3(4):150-153.
- Miller, H. (1991). The effects of heat on graft union formation in Dormant sitka spruce (*Picea sitchensis* (Bong, Carr.) PhD. Thesis, University of Reading.
- Mng'omba, S. A., Du Toit, E. S., Akinnifesi, F. K. & Venter, H. M. (2007). Histological evaluation of early graft compatibility in *Uapaca kirkiana* muell Arg. scion/stock combinations. HortScience 42(3): 1-5.
- Moore, R. (1982a). Graft formation in *Kalanchoe blossfeldiana*. Journal of Experimental Botany 33: 533–40.
- Moore, R. (1982b). Studies of vegetative compatibility-incompatibility in higher plants. V. A morphometric analysis of the development of a compatible and an incompatible graft. Canadian Journal of Botany 60: 2780–87.
- Moore, R. (1984). A model for graft compatibility-incompatibility in higher plants. American Journal of Botany 71:752–58.
- Moore, R. & Walker, D. B. (1981). Graft compatibility-incompatibility in plants. BioScience 31: 389–91.
- Nakasone, H. Y. & Paull, R. E. (1998). *Tropical Fruits*. CAB International, U.K. p.432.
- Ohler J. (G. 1979); *Cashew*. Royal Tropical Institute, Tropen, Amsterdam. p.260.
- Pina, A. & Errea, P. (2005). A review of new advances in mechanisms of graftcompatibility incompatibility. Scientia Horticulturae 106:1-11
- Santamour, F. S. (1988). Graft compatibility in woody plants: an expanded perspective. Journal of Environmental Horticulture 6: 27–32.
- Sanwo, J. O., Kuti, B. O. & Osundolire, M. (1972). Cashew germplasm collections. *The* Annual Report of Cocoa Research Institute of Nigeria, p. 100-110.
- Schmid, P. P. & Feucht, W. (1981). Differentiation of sieve tubes in compatible and incompatible Prunus Graftings Scientia Horticulturae 15: 349–54.
- Soumelidou, K., Battey, N. Y., John, P. & Barnett, J. R. (1994). The anatomy of the developing bud union and its relationship to dwarfing in apple. Annals of Botany 74: 605-611.
- Togun, A. (1977). A review of the prospect of cashew in Ibadan (Southwestern Nigeria).Agricultural Industry, p. 39.
- Tshokoeva, M. D. & Tsonev, R. V. (1995). Compatibility of rootstock and scions in apricots trees. Acta Hort. 384: 471–476.
- Ventakaramah, T. M. (1976). Cashew nut production and processing: Nigeria agronomic aspect of cashew Nut production. Cocoa Research Institute of Nigeria, p.39
- Wang, Y. & Kollmann, R. (1996). Vascular differentiation in the graft union of invitro grafts with different compatibility: Structural and functional aspects. Journal of Plant Physiology 147: 521–533.
- Warmund, M. R., Barritt, B. H., Brown, J. M., Schaffer, K. L. & Jeong, B. R. (1993). Detection of vascular discontinuity in bud unions of 'Jonagold' apple on mark rootstock with magnetic resonance imaging. Journal of the American Society for Horticultural Science 118: 92–6.

- Yeoman, M. M. (1984). *Cellular Recognition Systems in Grafting. Encyclopedia of Plant Physiology*. Springer Verlag, Heidelberg. pp. 453–7