Compatibility studies on cashew-mango graft combinations

A. K. ASANTE
Crops Research Institute, CSIR, P. O. Box 3785, Kumasi, Ghana

ABSTRACT

Studies were conducted on the early stages of graft union formation between cashew (rootstock) and mango (scion) using light microscopy and macroscopy examinations. Resin was secreted as the initial wound response a few minutes after wounding, and this contributed to the cohesion of the rootstock and scion. Callus was formed in all tissues damaged, beginning in the cortex areas of the graft partners. At 30 days, the periderm had formed across the union edges, wound edges had sealed, and the cambial linkage between the stock and scion had been restored.

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RÉSUMÉ

ASANTE, K. A.: *Etudes de compatibilité sur les combinaisons de la greffe d'anacardier-manguier.* Des études se sont déroulées sur les débuts de la formation de l'union de greffe entre l'anacardier (la souche) et le manguier (le scion) utilisant l'évaluation de la microscopie et de la macroscopie optique. La sécrétion de resine était la réaction initiale de la plaie qui se produisait quelques minutes après la blessure et ceci contribuait à la cohésion de la souche et le scion. La formation du cal se produisait dans tous les tissus endommagés, commançant dans les zones du cortex des partenaires de greffe. Après 30 jours, la formation de periderme à travers les bords d' union, le scellage des bords de la plaie et la rétablissement du linkage entre la souche et le scion avaient eu lieu.

Introduction

In the fruit tree industry, rootstocks are selected for vigour difference and other attributes such as adaptation to soil types and wider climatic ranges. However, the problem of incompatibility has assumed an important place in the fruit tree industry as the demand for new rootstocks and varieties has increased. Incompatibility between rootstock and scion is well known; however, the botanical relationship of the components of the graft is an unreliable guide to the behaviour of the graft combinations (Herrero, 1951). According to Beakbane & Roggers (1956), distinct clones used as graft components may differ in their capacities to uptake, synthesis and use of nutrients, in their capacity for transferring nutrients, and in the type and quantity of plant growth regulating substances in their tissues. Toxopeus (1936) attributed the incompatibility in citrus between sweet orange (*Citrus sinensis*) and sour orange (*Citrus aurantium*) to some substance produced by the scion which was toxic to the stock. Nauriyal, Shannon & Frolich (1958) also attributed incompatibility in citrus to environmental factors.

Incompatibility between pear and quince has been attributed to the cyanogenetic glucoside, prusan, in the quince and the phenolic substance called arbutin which is specific to pears (William, 1960). Armstrong & Brison (1947) reported a graft of *Quercus virginiana on Quercus stellata* where breaks were observed in the union after 6 years, and symptoms of ill-health appeared 10 years later. Herber (1927) reported nutrient exchange in natural grafts between trees and lianas in which no vascular connection was established. According to Roach & Thompson (1947), neither vessels nor tracheids seemed to be essential for transference of certain dyes across the union.

According to Herrero (1951), graft incompatibility may be due to any of the following

factors: differences in seasonal periods of growth and vegetative vigour, biochemical differences, mechanical blockade at the union, interface different rates of callusing, and precipitin reactions. Compatibility of the union has been estimated by measuring its resistance to the flow of water (Chang, 1937), by measuring its resistance to forcible breakage (Evans & Hilton, 1957; Lapins, 1959), and by microscopic and macroscopic examination of its anatomical structure (Herrero, 1951; Mosse, 1960). Where these methods have been compared, the last has proved the most satisfactory (Garner, 1948; Lapins, 1959).

Cashew and mango belong to the family Anacardiacea. Both are important commercial crops in the world market. Environmental growth conditions for both crops seem to be similar (Singh, 1960; Ohler, 1979); however, cashew seems more tolerant in drier areas than mango due to its extensive rooting system (Northwood, 1967). Both crops are commercially propagated by grafting, although seedling plants are still in plantations. Grafting mango on cashew rootstock seems laudable for increased mango production in drier areas which are very unsuitable for mango.

Materials and methods

Cashew seedlings were raised from seeds obtained from Tanzania, and scion wood was obtained from a monoembryonic variety of mango called Alphonso plant. Before grafting, the cashew and mango were grown in a glasshouse maintained at 22 °C. The anatomical study was carried out with light microscope at the School of Plant Sciences, University of Reading, between Sep 97 and Mar 98. The field study was carried out at the Crops Research Institute, Kumasi, Ghana, between Jun and Dec 98.

Plants were grafted when seedlings were 6 weeks old after germination by using the apical side veneer technique (Garner, 1948). The leaves on the scion wood were trimmed off with a sharp knife, leaving about 2-cm short petioles on the stem. Sixty grafted materials each were used for the anatomical and field studies. Samples for microscopic examination were taken at 5-day intervals, beginning 5 days after grafting till 30 days. The graft union was harvested by cutting the rootstock about 3 cm below the union.

A single-edged razor blade was used to trim excess wood on either side of the union, leaving a long, narrow strip including the interface. This was fixed in Karnovsky's fixative (Karnovsky, 1965) under vacuum infiltration for 4 h at room temperature. The specimen was washed in 0.05 *M* phosphate buffer at *p*H 7.0, divided transversely into 5-mm sections, and post-fixed in osmium tetroxide (1 % w/v in water). Subsequently, the specimens were washed in distilled water, dehydrated through a graded acetone series, and embedded in EPON resin. Sections 4-6 µm thick were cut with a Reichert 'Ultracut' ultramicrotome and stained with safranine (1 % w/v in alcohol). The sections were then examined and photographed with a Reichert Polyvar 11 microscope.

Field observations were made and data collected on bud sprouting, height of graft shoot, number of shoot leaves, and shoot dry matter yield till plants were 3 months old (Table 1). Six of the grafted plants were used for destructive sampling each on 5, 10, 20 and 30 days, leaving 30 plants for success rate. The 'strip' method described by Priestley (1993) was used to study the union formation at regular intervals, starting 1 month after grafting till 6 months. The bark was carefully peeled off at the graft union to facilitate the study of the graft-union anatomy.

TABLE 1

Graft Shoot Growth

Parameters	*Days after grafting*					
	20	*25*	*30*	*40*	*60*	*90*
No. of leaves	2.0	2.5	2.5	4.0	6.5	13.2
Shoot height (cm)	3.6	5.0	6.5	7.6	7.8	9.6
Shoot dry matter (g)	0.06	0.08	0.12	0.19	0.30	2.5

Results

The initial wound response of both graft partners was resin secretion a few minutes after wounding. Resin together with cytoplasmic debris from wounded cells formed a necrotic layer. This layer remained at the interface until callus was formed. Apart from a few ray cells that had enlarged in the scion by the 5th day after grafting, no remarkable cellular activity was observed in the graft partners (Fig. 1 and 2).

More cellular activity in both graft partners was observed 10 days after grafting. The pith cells of the rootstock had enlarged and divided (Fig. 3), while the cortex areas of the graft union were filled with callus from both graft partners, forming a callus bridge (Fig. 4). The infilling callus from the cortex areas had also begun to proliferate towards the pith zone (Fig. 5).

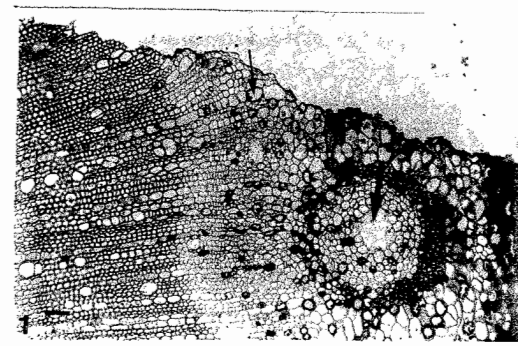

Fig. 3. Transverse section of a 10-day-old graft union showing enlargement and division (arrow) of pith cells. Scale bar = 100 μm

Fig. 1. Transverse section of a 5-day-old scion (mango) showing enlargement of ray cells (small arrow). Resin canal which was severed during graft incision is empty (big arrow). Scale bar = 100 μm

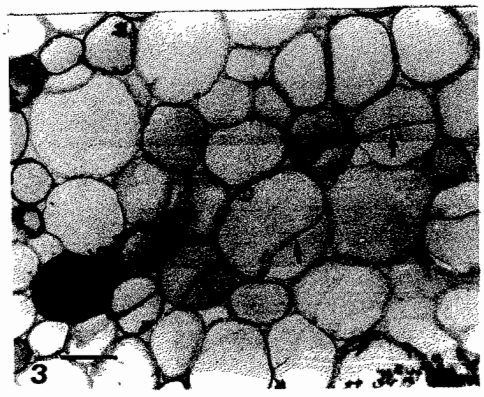

Fig. 2. Transverse section of a 5-day-old rootstock cashew showing enlargement of cortical cells (big arrow). Scale bar = 100 μm

By the 20th day, callus proliferation had greatly increased and the graft interface had been filled (Fig. 6 and 7). Callus cells were produced in files perpendicular to the interface. However, the necrotic layers continued to exist as a thin layer at the interface. By 25 days, the interface was fully filled with callus, the necrotic layer had been absorbed as a result of callus proliferation (Fig. 8), and the callus cells had bridged the two graft partners.

At 30 days, the original cut made during preparation of graft partners was obliterated by mixed callus and there was no trace of the necrotic layer. The cambium continuity was restored by 30 days and callus cells outside the cambium zone had undergone several divisions,

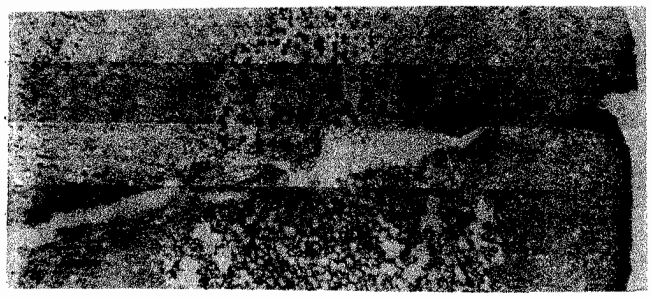

Fig. 4. Transverse section of a 10-day-old graft union showing establishment of callus bridge in the cortical region. The other part of the union remains separated. Scion at the top, stock at the bottom. Scale bar = 100 μm

Fig. 5. Transverse section of a 10-day-old graft union. The graft partners have been bridged in the cortical region by the developing callus. Scion at the top, stock at the bottom. Scale bar = 100 μm

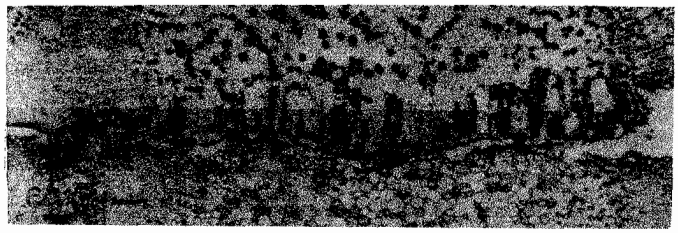

Fig. 6. Transverse section of a 20-day-old graft union showing callus development at the interface. Scion at the top, stock at the bottom. The callus cells are in rows. Scale bar = 200 μm

resulting in undifferentiated cells at the edge of the union (Fig. 9). This mass of callus cells pushed the dead cells and remnants of the original vascular cambium outwards, leaving the living cells inside the interface. The wound phellogen had started to develop beneath the closing layer to seal off the union edge.

Macroscopic examination of the graft union showed that the degree of union formation depended on the relative diameters of stock and scion. It was observed that the healed union was smooth, and bridges of perfectly connected wood elements of stock and scion had established. Where discontinuity in bark was observed, distortion in the wood at the union was also noticed.

In successful graft, the scion retained its normal green colour with axillary buds remaining plump. The petioles left attached to the scion were indicative of success in union healing. In successful graft, the attached petioles gradually yellowed and dropped off cleanly if touched by the 10th day. In failed graft, the petioles shrivelled, became black, and adhered tightly to the blackening scion.

Perceptible swelling of successful grafts started after the 10th day, and such buds started to show small, tender leaves that produced shoots by 20 days.

In the earlier study, the success rate was 73.3 per cent, while 83.3 per cent was scored in the last study.

Discussion

Light electron microscopy showed that union formation in cashew-mango graft combination involved initial adhesion of stock and scion, callus formation, and cambial bridging of the graft partners as observed in other woody species such as citrus (Mendel, 1936), peaches (Scaramuzzi, 1952), pears on quince (Thiel, 1954), poplar (Braun, 1958), apples (Mosse & Labern, 1960), sitka spruce (Miller & Barnett, 1993), and mango (Asante & Barnett, 1997). The

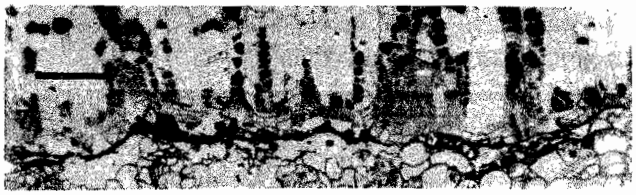

Fig 7 Transverse section of a 20-day-old graft union showing the interface fully filled with callus cells in definite rows. Scion at the top, stock at the bottom. Scale bar = 100 μm

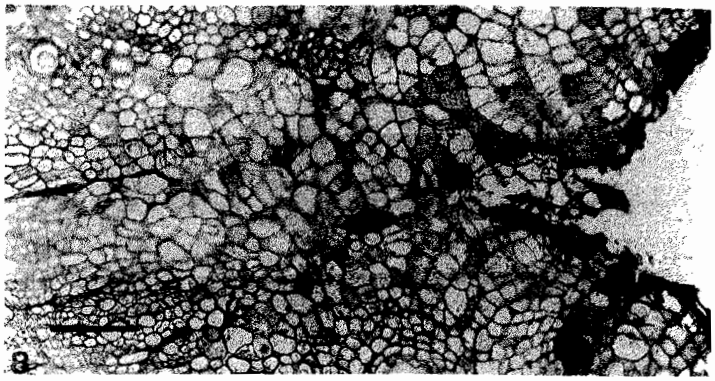

Fig. 8 Transverse section of a 25-day-old graft union showing the interface fully filled with mixed callus The wound edge has been sealed with the proliferating callus. Scion at the top, stock at the bottom. Scale bar = 100 μm

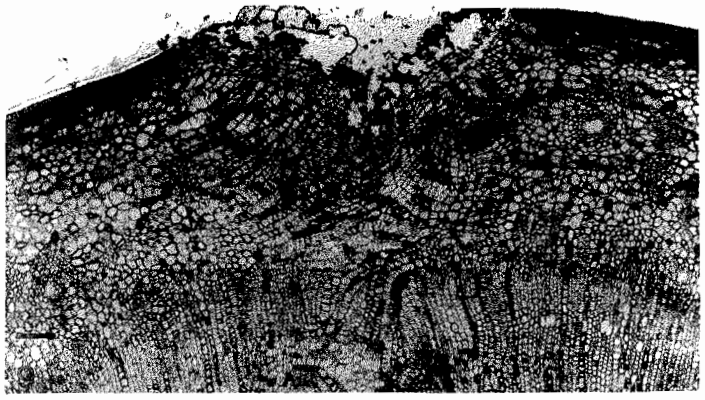

Fig. 9. Transverse section of a 30-day-old graft union showing the interface fully filled with callus. The wound edge is sealed and periderm formation has started. Scion at the left, stock at the right. Scale bar = 100 μm

process of union formation was continuous and clearly related to the compatibility of the graft combinations.

The secretion of resin at the graft interface was a defensive response attributable to wounding during preparation of scion and rootstock for grafting. The physical pressure exerted by the binding tape used to hold the graft partners together compressed the damaged and partially differentiated cells which distorted and displaced softer tissues. The overall effect of these factors was the production of a necrotic layer on the cut surfaces of the graft partners. The necrotic layer created a barrier to prevent contact between the scion and rootstock, and excessive accumulation of this layer could contribute to the mechanical failure of unions (Buck, 1937; Fletcher, 1964); it also limited desiccation and, therefore, dying back of the deeper tissues (Noel, 1968). In most dicotyledonous grafts, it is the production of callus on the cut surfaces that leads to the establishment of scion-stock contact (Stoddard, Frederick & McCulley, 1980). However, it was evident that visible cellular connection across the union was lacking until 5 days after grafting to account for any cohesion. The necrotic layer may, therefore, be assumed to have some significance in attaching scion to rootstock as observed by Wright (1893).

The onset of callus development fragmented the necrotic layer which was eventually absorbed. According to Thiel (1954), the degree of compatibility of two plants depends on their ability to reabsorb the primary isolating layer (necrotic layer) formed between stock and scion. It is

probable that the dark staining material that enclosed many of the callus cells as the union developed may arise during the absorption of the necrotic layer, with the enzymes responsible for the breakdown of the necrotic layer material also affecting the walls of the living cells. Callus development varied according to the tissues removed. Callus production was observed in the cortex areas 5 days after grafting, and it was considered that this initial establishment of callus bonding between the graft components was important in the development of successful graft. Callus production in the cortex areas encouraged the union of cambia and the production of a continuous xylem and phloem network between the graft components. The cumulative effect of these bondings was the early supply of water and nutrients to the scion, leading to early shoot growth from the scion buds. Callus bonding between scion and stock in the cortex areas was necessary in protecting the cambial region of the interface from pathogen invasion, desiccation, and other external factors (Miller & Barnett, 1993).

According to Moore & Walker (1981), callus proliferation during grafting probably serves three main functions: providing cells at the graft interface through which vascular tissue may subsequently be formed, fragmentation of the necrotic layer to create direct contact of the living cells, and contributing to increased tensile strength of the graft union.

The rows of callus cells were significant in establishing early callus bridge across the union between scion and rootstock. These rows of cells appeared peculiar in the study, since callus tissues in graft unions of many woody plants appear as a random mass of parenchyma cells. The rows of cells observed in this study confirm the observation by Asante & Barnett (1997) on mango graft union formation that rows of callus produced appeared as a striking feature.

It was evident that callus formation in the scion preceded that in the rootstock during the first 20 days after grafting, possibly because the two graft components are different plant species and that mango might be more responsive than cashew. Kostoff (1928) found that one member of a graft union may predominate in the production of callus, and this may be a function of the interspecific or intergeneric differences implicit in the rootstock and scion. Various factors that contribute to earlier callus production in graft partners include sufficient water supply (Kostoff, 1928) and high levels of starch and sugars (Kuester, 1925). After the restoration of cambium and vascular elements, the structure and configuration of the union then resembles in structure, the configuration of the stem tissue before grafting.

The observations on graft shoot growth point to features directly associated with compatibility of graft partners. Herrero (1951) observed that growth can be affected by incompatibility in the reduction of shoot elongation of the scion and of the radial growth of the stock and scion. However, growth does not indicate either compatibility or incompatibility, since an interruption in cambial and vascular continuity can lead to a smooth break at the point of union of old grafted plants (Mosse, 1962).

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