

Graft studies on cashew genotypes

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ABSTRACT

Graft union in cashew (*Anacardium occidentale* L.) was studied using light microscopy. Callus formation was most abundant from regions of the pith and cortex which had been damaged during preparation of the scion and rootstock for grafting. Of particular interest was the observation that the callus cells formed from the pith developed in regular rows resembling vascular cambium or phellogen, rather than in the random manner observed in other species which others have reported. Cambial union was established between 20 and 30 days after grafting by differentiation of callus formed mainly by dedifferentiation of cortical cells. Unlike other species which have been described, no significant amounts of callus were observed to be produced from ray parenchyma of the secondary xylem. The new periderm was established between the surfaces of the graft partners after the cambial union had sealed off the wound edges.

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Introduction

Cashew (*Anacardium occidentale*) 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 travellers (Ohler, 1979). It is usually propagated from seeds because it is readily available and easy to germinate and grow, even under adverse soil and other conditions. The cashew is easily cross-pollinated and as a result, variability between trees is wide with respect to vigour of growth, times of flowering, yields, and quality of

RÉSUMÉ

ASANTE, A. K., BARNETT, J. R. & CALIGARI, P. D.: *Etudes de greffe sur les génotypes d'anacardier*. L'union de greffe de l'anacardier était étudiée en utilisant la microscopie optique. La formation de cal était plus abondante dans les régions de moelle et de cortex qui avaient été endommagés au cours de la préparation de scion et de souche pour le greffage. D'intérêt particulier était l'observation que les cellules de cal formées de la moelle développaient en rang régulier semblable au cambium vasculaire ou phellogène, plutôt qu'en manière aléatoire observée dans d'autres espèces présentées dans la littérature. L'union cambiale était établie entre 20 et 30 jours après le greffage par la différenciation de cal formé surtout par la différenciation des cellules corticales. A la différence des autres espèces qui ont été décrit, aucune quantité considérable n'était observée d'être produit de rayon parenchyme du xylème secondaire. La formation du nouveau périderme entre les surfaces des partenaires de la greffe avait lieu après l'union cambiale avait scellé les bords de la plaie.

nuts. At the social and economic levels, the problem is to ensure effective and rapid propagation of seedling material by resource-poor small-holder populations. Vegetative propagation by grafting has been adopted to ensure uniformity in plantation materials and yields (Bashiru, 1988). However, most work on cashew grafting has provided little information about the biological processes that occur during graft union formation.

Many cashew trees in production are tall, resulting in problems of spraying and other management practices. However, high-yielding dwarf types have been developed in Brazil which

give good yields, precocious flowering and fruiting, and bold nuts. Rootstock type can affect tree size and vigour in apple (Hatton, 1927), citrus (Bitters, 1969), and sweet cherry (Hesse, 1710). McKenzie (1956) observed that rootstock dominance is expressed with growth and anatomical structure. However, it seems that in cashew, the genotype of the scion determines the phenotype.

This work was carried out as part of a study to help determine the most efficient graft protocols by examining the biological processes which take place during graft union formation.

Materials and methods

Seeds of four 'Dwarf' genotypes, namely: B9.3, B6.23, B7.10, and B5.27; and four 'Normal' (Tall) varieties: AC10, AC4, AC28, and ATA19 were sown on 6 November 1997 in the glasshouse at the School of Plant Sciences, The University of Reading. Seeds which germinated did so within 10 days, and seedlings were potted on and placed in the glasshouse where the daily maximum temperature was set at 17 °C. The plants were grafted on 29 January 1998 by using the 'Apical

Side Veneer' grafting technique (Fig. 1). The dwarf genotypes were used as rootstock and the normal (tall) types as scion.

Samples for light microscopic examination were taken 5, 10, 20, and 30 days after grafting. Pieces of graft unions were removed from the rootstock about 2 cm below the base of the union, and scion apices were trimmed off. A single-edged blade was used to remove some of the excess wood on either side of the union, leaving a thin sliver of the graft interface. Specimens were fixed, using vacuum infiltration, with Karnovsky's fixative (Karnovsky, 1965) for 4 h at room temperature. After washing in phosphate buffer (pH 7.0, 0.05), the slivers were cut into smaller pieces about 5 mm in length and post-fixed in 1% aqueous osmium tetroxide for 4 h at room temperature. The specimens were washed three times in sterile distilled water and dehydrated through a graded acetone series, and embedded in 100 % EPON resin. Sections, 4-6 μ m thick, were cut and stained with safranin (1% w/v in alcohol) at room temperature. The sections were examined and photographed with a Reichert Polyvar II photomicroscope.

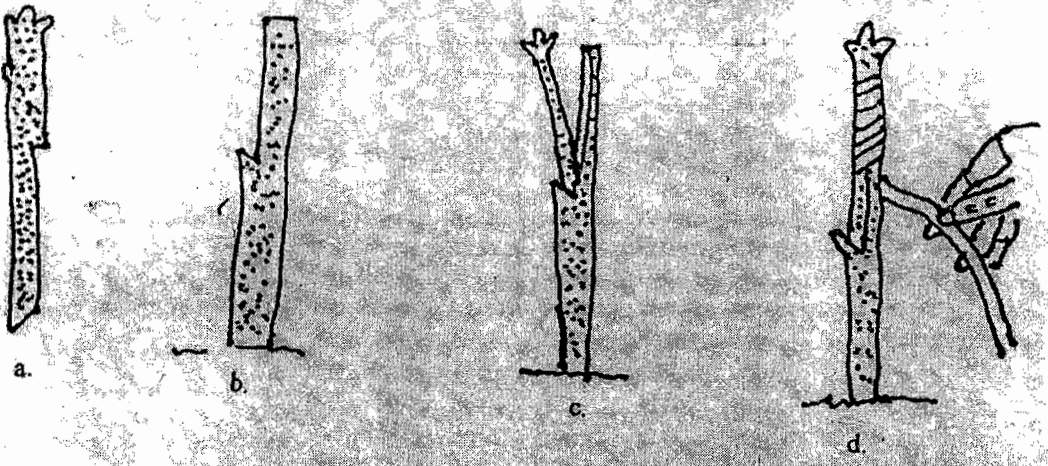


Fig. 1. Diagrammatic representation of apical side-veneer graft assembly in cashew.

- a. prepared scionwood ready for use
- b. prepared rootstock ready to receive scionwood
- c. introducing the scion to the stock
- d. binding the scion and the rootstock together

Results

The results showed that graft union formation in cashew genotypes followed the basic pattern described in other species. The initial wound response was the secretion of sticky resin from resin canals onto the wound surfaces of the stock and scion within a few minutes of knife wounding. The resin secreted, together with cell wall fragments, formed a necrotic layer on the cut surfaces of the graft components (Plate 1). The resin was inadequate to bond the stock and scion during fixation, and they fell apart after fixation of samples in which callus had not yet been formed.

The first cell divisions and enlargement, as a result of wounding, were observed 5 days after grafting in the cortical parenchyma and pith cells (Plates 1 and 2). The new division planes were predominantly parallel to the cut surface of the graft component. By 10 days, callus formed between the cortical regions of the graft partners had merged (Plate 3), disrupting the necrotic layer. A gap persisted between tissues at the centre of the graft, although it was not clear whether this was a consequence of resin which was removed during the dehydration stage of specimen preparation, or because callus formation from the cortical regions had forced the partners apart.

Cell divisions in the pith had produced files of callus cells arranged perpendicularly to the cut surface (Plates 3 and 4). The cells of these files were quite different in appearance from the pith cells which caused their production. Instead, they resembled the vascular cambium or phellogen of woody stems in appearance. Two types of cells were noted: the first were rectangular in transverse section, highly vacuolated, with a thin primary wall; the second were filled with osmiophilic material resembling the tannins or phenolic compounds found in cortical parenchyma. Repeated cell divisions resulted in the proliferation of callus cells at the graft interface, and by 20 days, the stock and scion had produced enough callus of this type to fill the gap between their piths (Plates 5 and 6). Callus cells that were produced in the region between the xylems of the

scion and rootstock were smaller, regular in shape, and intermingled to the extent that it was difficult to determine whether they originated from the scion or rootstock (Plate 6). There was also evidence of cambial development in this callus. Although the necrotic layer was mostly absorbed by this stage, fragments of it were still visible.

By 30 days, cambial union had been established and secondary tissues had been produced, although vessels were noticeably absent in xylem which was produced from the linking cambium (Plate 7). Even where the rows of callus cells that were produced by the pith had been forced into close contact, there was little evidence of intermingling of the tissues (Plate 8). In the regions of the graft where the xylems of the scion and rootstock were in direct contact, there was little evidence of callus formation even after 30 days (Plate 9). The necrotic layer persisted. However, there was evidence of deposition of osmiophilic compounds in cells of the rays.

Discussion

This study has shown that the formation of the graft union between dwarf and normal cashew genotypes follows a similar trend to that observed in other fruit trees. However, the process seems, in some ways, to differ in detail from that described for other species, e. g. citrus (Mendel, 1936), *Pinus silvestris* (Dormling, 1963), *Pseudotsuga menziesii* (Copes, 1969), *Malus* and *Prunus* (Robitaille & Carison, 1970), *Picea sitchensis* (Barnett & Weatherhead 1988; Miller & Barnett, 1993), *Malus domestica* (Soumelidou *et al.*, 1994), and quince/pea grafts (Ermel, Catesson & Poessel, 1993).

Of particular interest is the observation of rows of cells produced from the piths of the scion and rootstock, where the pith had been severed during preparation of the graft partners. These cells differ from those of normal callus in their regular shape, their thin walls, and in the division planes leading to their production that are almost entirely parallel to the cut surface of the pith. The lack of mingling of the callus produced by the piths of the scion and stock implies that this tissue has no functional

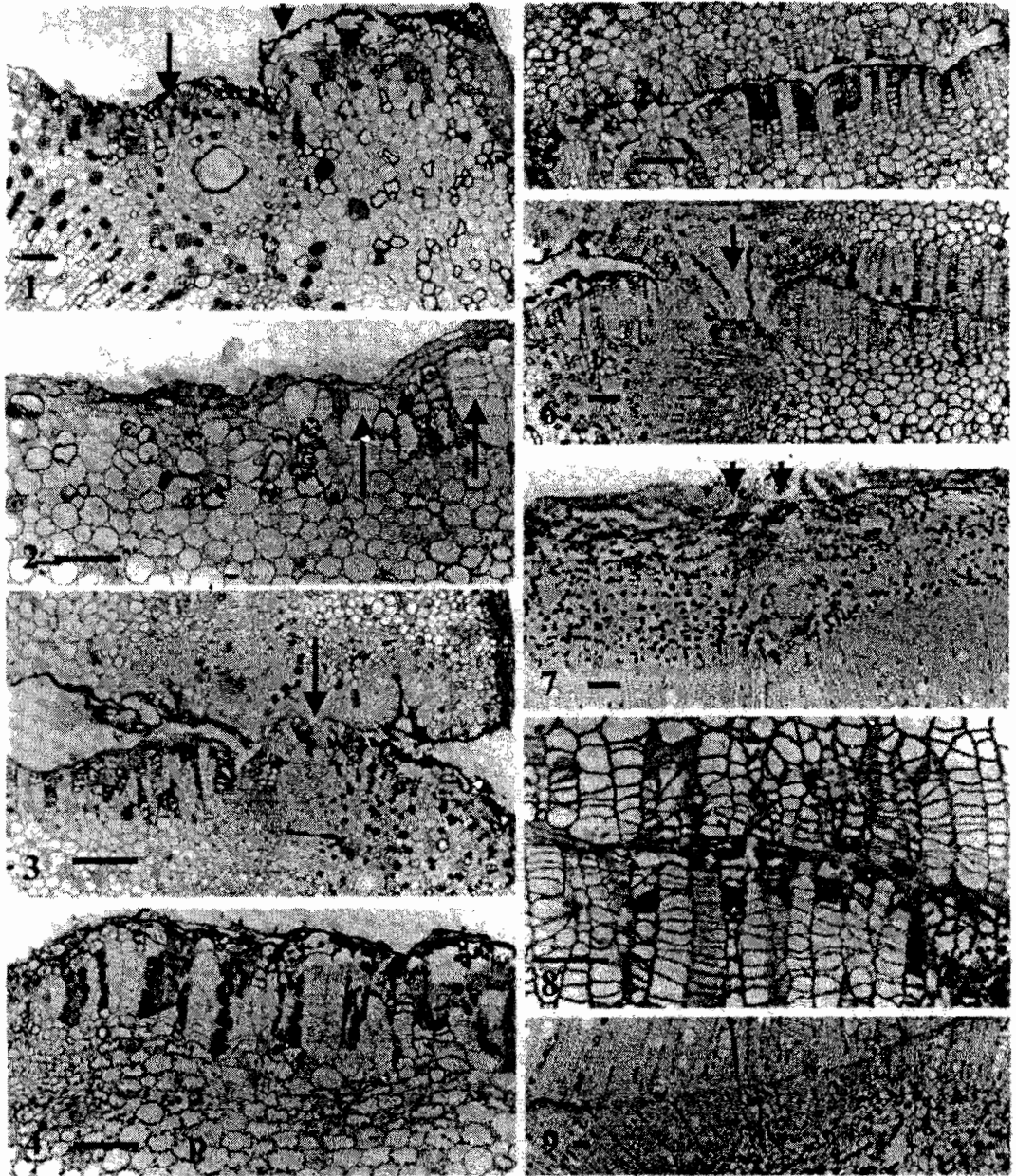


Plate 1. Transverse section through the rootstock of a 5-day-old graft. A necrotic layer has formed along the cut surface of the stem (arrows). Some cell division has taken place below this layer, with the new cell walls predominantly parallel to the cut surface. Scale bar = 100 μ m

Plate 2. Transverse section through the cut pith of a 5-day-old rootstock showing re-differentiation of pith cells and divisions more or less parallel to the cut surface (arrows). Scale bar = 100 μ m.

Plate 3. Transverse section through a 10-day-old graft union. Callus produced from the cortices of the scion and rootstock had merged (arrow), disrupting and absorbing the necrotic layer. Rows of cells may be seen which have been produced by re-differentiation of pith. Scale bar = 200 μm

Plate 4. Transverse section through a 10-day-old rootstock showing the rows of cambium-like cells produced by re-differentiation of pith cells (P) and subsequent divisions parallel to the wounded tissue surface. Scale bar = 100 μm

Plate 5. Transverse section through a 20-day-old graft showing the rows of callus produced by scion and rootstock almost filling the space between them. Scale bar = 200 μm

Plate 6. Transverse section of a 20-day-old graft. By this time, the tissues produced by the cortical regions of the scion and rootstock have merged to the point where it is

difficult to identify with certainty the origin of some of the tissues (arrow). Note that the callus produced by the piths of scion and rootstock has not merged. Scale bar = 200 μm

Plate 7. In this 30-day-old graft, cambial fusion has occurred. The joint between the scion and rootstock is marked by the absence of vessel elements from the xylem. New periderm tissues have been produced to seal the edges of the wound (arrows). Scale bar = 200 μm

Plate 8. Callus produced by the pith and rootstock is still separated after 30 days. No obvious mingling or fusion of tissues has occurred. Scale bar = 100 μm .

Plate 9. Transverse section through a 30-day-old graft union showing a region where the cut xylems of the partners were brought into contact during graft preparation. There is no evidence of callus formation along the interface. Scale bar = 200 μm .

role in the formation of the graft union. The apparent lack of callus formation from any parenchyma where the secondary xylems were in contact is also surprising, because of observations on other species (Barnett & Weatherhead, 1988; Soumelidou *et al.*, 1994; Asante & Barnett, 1997). In these cases, ray cells close to the cut surfaces of secondary xylem produced callus, which eventually proliferated to fill the space created between the graft partners as callus formed by the cortex forced them apart against the resistance of the binding tape.

Thus, in contrast with other species which others have reported, the graft union appears to rely almost entirely on the activity of the cortical parenchyma in producing callus to bridge the partners and eventually differentiate to form cambium.

The resin which is secreted after graft incision may be interpreted as a defensive wound response, which is a result of the preparation of the graft partners and the physical pressure exerted by the graft tape to hold the components together. The layer of dead cells that formed between the scion and the stock reduced the physical contact between the graft partners; and where this layer was thick, callus formation was delayed (Fletcher, 1964). The layer is known to protect the underlying cells from desiccation (Noel, 1968).

This layer of crushed cells has been referred to as callus line (Kostoff, 1928), barrier zone (Sass, 1932), insulating layer (Mendel, 1936), necrotic layer (Stoddard & McCully, 1979), and contact layer (Copes, 1969). The disappearance of the necrotic layer is probably due to its absorption by the proliferating callus cells (Stoddard & McCully, 1979). Thiel (1954) suggested that the degree of compatibility of two plants depended on their ability to reabsorb the primary isolating layer formed between stock and scion.

Callus formation is important for a successful union formation in providing a ground tissue for the differentiation of the cambial and vascular elements, fragmentation of the necrotic layer to create direct contact of the living cells, and contributing to increased tensile strength of the union (Sass, 1932; Crafts, 1934; Moore & Walker, 1981a, b).

Callus bonding between scion and stock in cortical areas is necessary for early transference of water between the graft partners. Callus connection in this area also protects the graft union against the entry of external pathogens into the interface, desiccation, and other factors that are detrimental to the success of the graft union (Miller & Barnett, 1993).

The rows of cells produced by the pith indicate that some factor controls the preferred plane of division parallel to the surface of the cut tissue.

Pressure has been suggested as a factor (Barnett & Asante, in press). However, here, the cells were dividing into what seemed an empty space. One possibility is that the space was filled with resin before processing the tissue for microscopy, and that this would provide the necessary pressure. There was no evidence that the rows of cells were involved in establishing early callus bridges across the union between stock and scion.

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