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# Anatomical Studies on Graft Formation in Cashew (Anacardium occidentale L.)

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#### **ABSTRACT**

The research studied the changes in the graft union with influence on graft success and subsequent performance until the 98th day after initial side grafting of Anacardium occidentale rootstock seedlings. Thirty days after grafting, the necrotic layer and calli cells began enlarging between scion and stock. There was gradual disappearance of the necrotic layer, preceding callus formation and the fusion of the scion and stock at 60 day after grafting. Results at 98-days after grafting showed cambium linkage and a healed union edge. Unsuccessful grafts at 98-days after grafting showed a wide gap at the cortex of scion and stock. Graft union success is a key factor in successful graft-take and subsequent performance of grafted seedlings.

Key words: calli, Linkage. Union, Cortex.

#### INTRODUCTION

Propagation of plants played an important role in mankind since the beginning of civilization when man started to grow crops which were used for himself and his domestic animals some 10,000 years ago. As man progressed in civilization, people started to grow different plants that provided fibres, medication, ornamentals and beauty in addition to food crops. This also brought about several propagation abilities for a variety of plants (Hartmann and Kester, 1983).

Recently, vegetative propagation including grafting has generated interest among tree growers. It has been successful in producing fruit trees of small and manageable sizes which are precocious and productive. The basis of unity (in grafting and budding) between two parts of plants depends on mutual responsibility (Seferoğlu et al., 2004). Apart from tree or plant sizes, grafting produces planting stock that has suitable characteristics such as resistance to pest and diseases. Additionally, it provides an opportunity to clonally propagate selected trees with desirable tee phenotypes.

One of the important crops on the international market that has increasingly gained similar attention is cashew (Anacardium occidentale L.). In Ghana, cashew is an important non-traditional export commodity with raw nut export that increased from 7,212 metric tonnes in 2003 to 16,422 in 2006 (GSC, 2006). Cashew is mainly propagated by seed resulting in genetic variation of the crop (Dolgun et al., 2008). Grafting or budding however results in desirable phenotypes in fruits, seeds, etc. as already mentioned. The anatomical evaluation on graft sections gives the first information about compatibility or incompatibility of unions (Kankaya et al., 1999). The aim of the study was therefore to facilitate an understanding into graft formation process for compatibility /incompatibility of cashew grafts through the changes in the graft union until the 98th day after initial side grafting.

## **MATERIALS AND METHODS**

Cashew seedlings were grafted by using side grafting on rootstocks. Longitudinal and cross sections of 30µm diameter were taken in 30, 60 and 98 days by using microtome for anatomical sectioning and examination. The sections were stained in safranine and later washed three times in distilled water. They were then dehydrated through different concentrations of alcohol starting with 5% 30% 50% 75% and 100%. Tissue paper was used to aid the drying of the specimen and clove oil was added. Subsequently, the sections were mounted onto slides using Canada balsam. The sections were oven dried

at 60°c for 24 hours. Sections were then examined and photographed using photomicroscope fitted with 35 mm camera.

#### **RESULTS AND DISCUSSION**

The transverse section of the cashew seedlings revealed the following anatomical characteristics. The young cashew stem (transverse) was resinous and contained numerous parenchyma cells in the pith and cortex. The section also showed the epidermis at the outermost of the stem. The cambial zone consisted of an unbroken cylinder of cells arranged in radial files in the normal way. To the outside of the cambium were the phloem cells and to the inside of it were the immature and mature xylem cells (plate 1).

Several experiments have confirmed different models of graft union development with the entire process typically divided into stages. Soule (1971) in an earlier experiment indicated four important stages (pre-callus, callus, cambial bridge and the healed union) whiles Dolgun et al., (2008) reported three steps ( callus formation, important differentiation and continuity and vascular tissues formation) in graft formation. The present anatomical study also revealed similar activities of graft union formation between the scion and stock that probably resulted in the graft success or failure observed. The observation, plate 2, showed that prior to callus formation, 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. According to Asante and Barnett (1997), the resin secretion occurring within few minutes of knife wounding subsequently provided the sole cohesive force between the grafting partners in the first few days before a callus bonding is established.

The resin provided a temporary bond (cementing medium) between the two graft partners (Tiedeman, 1989) and by itself did not contribute to union formation. It eventually appeared as a dark staining or necrotic layer scattered between the grafted components (Dolgun et al., 2008) that could pose either as a "barrier" or "insulating layer" (Mendel, 1936). The excessive accumulation of necrotic layer could result to mechanical failure of union (Tiedeman, 1989; whereas Noel (1968), Cline and Neely (1983) mentioned that the presence of the necrotic layer at the wounded surfaces limited desiccation and death of the deeper tissues and reduced the entry of pathogens. Observation also indicated that the necrotic layer remained at the interface until callus was subsequently formed and completely filled the

graft interface by 30<sup>th</sup> day after grafting (plate 3). Most of the callus cells enlarged and divided mostly in the stock (Dolgun *et al.*, 2008). Asante and Barnett (1997) mentioned that callus formation is crucial to the survival of the scion and the rapid physical contact of opposing callus cells intermingling and inferlocking, filling up the spaces between the scion and rootstock, provides a bridge for the transport of water and nutrients (Miller, 1991). Copes (1969) stated that this callus bridge develop most often in the cortex and non-functional phloem area where contact layers were continuous with the ray and phloem parenchyma being the chief contributors to its growth.

However, failure of grafts will occur due to water stress when scions are unable to receive water from the rootstocks before the graft union is formed (Barnett and weatherhead, 1989; Mergen, 1954). The phenomenon indicates the response of the scion to corresponding increases in transpiration and temperature (Beeson and Proebsting, 1988). At this stage the continuous formation and intermingling callus caused the gradual disappearance of the necrotic layer between the two graft components. A greater proportion of the necrotic layer by the 60th day had been eliminated and the grafting line became less distinct (Dolgun et al; 2008); (plate 4). Dolgun et al. (2008) and Tekintas and Dolgun (1996) also reported that between 45 – 60 days after grafting callus tissues had filled all spaces and cambial continuity were observed clearly.

Alongside were also observations of vascular tissues produced by newly formed cambium. However, the establishment of the cambial continuity is considered vital for the production of vascular tissues in ensuring a successful completion of the grafting process. By the 98<sup>th</sup> day, the union process appeared transformed as a common cambium ring was formed between the rootstock and scion (plate 5) but this study could not confirm clearly the complete formation of vascular tissues. This is due to the fact that reports concerning vascular tissue connections vary. Seferoğlu et al. (2004), for example indicated that a period of 6 - 12 months was necessary to observe completion of vascular connection between the scion/bud and rootstock. Other reports noted vascular connection in 6 - 8 weeks after grafting (Soule, 1971; Moore, 1981; 1982 and 1993). In citrus, Tekintas (1991) reported that cambial continuity occurred in 45 (forty five) days after grafting. Several literature reports indicate that vascular differentiation begin after establishment of cambial continuity and

the strong connection occur in a short time in a compatible graft. This occurrence (Continuity or Connectivity) is considered the last stage of the successful grafting with an established and functional vascular connection that provides water and nutrient flow from rootstock to scion/bud (Turkoglu, 1990; Unal and Ozcagiran, 1986; Moore, 1983, 1984; Tekintas, 1988; Watanabe and Nakazatomi 1990; Unal, 1992; Asante and Barnett, 1997). Hartmann et al. (1997), reported that for a scion to successfully resume its growth, the vascular connection has to be established and in addition the scion must have a meristematic region (bud) to resume shoot growth and eventually to supply photosynthates to the root system. Unsuccessful grafts showed gaps at the periphery or cortex of the graft components (plate 6). Several factors and conditions have been attributed to failure of graft formation. Amongst others, the change of the temperature and relative moisture especially during and after grafting directly affects the development of a good graft union (Balta, et al., 1996; Tekintas, 1988) and high day temperatures with warm nights are stressful for successful union (Hinesley, 1981). Shade improved the success of grafts during high day temperatures and decreased ambient air temperatures, and thus improved graft success (Frey, 2009). Apart from the fact that some species form large amounts of callus than others the species that form callus quickly are likely to unite if properly grafted and those that form callus slowly are less likely to unite. However, it is important to make a definite effort to place scion on the stock, to have the two cambium layers match at least on one side to prevent a delay of ultimate union (Dolgun et al.; 2008).

## **CONCLUSION:**

Results and observation from the experiment indicate adequate and early callus formation is important for initial scion survival and subsequent formation of the cambium and vascular tissues. Again the proper matching of the two components contributes significantly to graft union success.

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