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The Relationship Between the Histological Features in the Grafting Areas and the Compatibility Degrees of Some Mango Cultivars onto Nucellar Seedlings

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Abstract: These studies were carried out during the period from 2010 to 2011 at the nursery and laboratory of Pomology department, Faculty of Agriculture, Cairo University at Giza. Two experiments with two rootstock ages (21 days and 9 months) were conducted to classify their grafts, according to their compatibility between the scions and the rootstocks (degree of their anatomical connection) as following: The first experiment: Mango Keitt, Alphonso and Ewais were saddle grafted onto 21 days old of Zebda and Sukkary seedlings and top-cleft grafted onto 9 months old of the same rootstocks. Also, each of the rootstock was grafted on the other one. The obtained results of first experiment indicated that, there are two degrees of anatomical connection at both ages of the two rootstocks. 1- The rootstocks of 21 days age: a- High compatibility grafts: These included the grafts of Sukkary/Sukkary (S.), Keitt (K.)/Sukkary, Alphonso (Al.)/Sukkary, Zebda/Zebda (Z.), Keitt/Zebda and Alphonso/Zebda. b- Moderate compatibility grafts: These included the grafts of S./Z., Z./S., E./S. and E./Z.. 2- The rootstocks of 9 months age: a- High compatibility grafts: These included the grafts of S./S., K./S., Al./S., Z./S., E./S.. b- The moderate compatibility grafts: These included the grafts of K./Z., Al./Z., S./Z. and E./Z.. Meanwhile, in the second experiment: Mango Keitt scion was top-cleft grafted onto compound rootstocks Keitt/Sukkary, Alphonso/Sukkary, Keitt/Zebda and Alphonso/Zebda. The results of second experiment indicated that, there are two degrees of anatomical connection at both ages of the ground rootstock: 1- The rootstocks of 21 days old: a- High compatibility grafts: These included the grafts of K./K./S., K./Al./S. and K./K./Z.. b- The moderate compatibility grafts: These included the grafts of K./Z., Al./Z.. 2- The rootstocks of 9 months age: a- High compatibility grafts: These included the grafts of K./K./S., K./K./Z., K./Al./S. and K./Al./Z.. b- The moderate compatibility grafts: This was not noticed in the experimental graft. The histological features of the studies cross section illustrated that necrotic layers, which are scattered and get browning because of enzymatic reactions, characterized all successful graft union regions. Different intensities of an regular disposition uniformly callus tissues were originated from parenchymatic cells of xylem ray of both scion and rootstock, this callus fill the spaces (gaps) between the rootstocks and the scions; and finally, the vascular connection (mechanically strong graft union) form without apparent disruption of the necrotic layer, these new vessels originated by some meristematic cells in the callus. On the contrary, the lower successful grafts exhibited other histological features at the graft union region, where forming the collapsed cells, necrotic layer of dead cells, numerous free callus positions and separation zones overall grafting margins. These bad structures lead to separate the graft partners.

Key words: Mango · Rootstocks · Interstocks · Scions · Histology · Grafting area · Compatibility

INTRODUCTION

Mango (*Mangifera indica,* L.) is an important fruit crop of the tropical and subtropical regions. Also, it is one of the most common and popular fruits since, it considered the king of fruits having delicious taste, captivating flavor with multifarious color and excellent source of nutritive values [1]. Since long, mango is being propagated commercially by grafting. Grafting on an already grafted or budded tree is referred to as double working and double-worked trees therefore consist of three genetically different parts, i.e. the rootstock, interstock and crown [2]. In Florida, old plantings of 'Haden' have been double worked with 'Tommy Atkins' [3].

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Double working can also be used to repair trees affected by disease or cold injury and to develop a strong framework. 'Kalapady' has reportedly been used as an interstock in order to dwarf 'Langra' [4]. In one trial, by Iyer *et al*. [5], 12 interstocks were grafted on two rootstocks for 'Dashehari'; rootstock-interstock combinations significantly affected tree height and vigour. Fruit yield was also influenced by rootstock in all the combinations. 'Kurukkan' interstock on 'ST-9' rootstock yielded more fruit in comparison with 'Ambalvi' on the same rootstock. The maximum yield was obtained with 'Nakkare'/'Chandrakaran' and 'Ambalvi'/'Chandrakaran'; whereas the lowest yield obtained with 'Ambalvi'/'ST-9'. Soule [6] illustrated that there are four stages in formation of the graft union of mangoes: pre callus, callus, cambial bridge and the healed union. Moreover, Moore [7] found that the graft union is initially formed by rapidly dividing callus cells, originating from the scion and rootstock, which later differentiate to form the vascular cambium and associated vascular system.

Chakrabarty and Sadhu [8] showed that three mango cultivars were splice - grafted onto seedling rootstocks, graft-take was faster for Langra than for Bombai and Himsagar due to more rapid formation of callus and vascular continuity Splice grafting gave earlier graft-take than cleft grafting. Pandey and Singh [9] worked on micro-anatomical of graft joint in mango; the results indicated that initial sprouting and survival of stone-grafted plants was basically due to callus (parenchymatous) tissues, which allowed partial translocation of vital biochemical compounds between rootstock and scion. Moore [10] recorded that lethal cellular has resulted in formation of necrotic layer of collapsed cells that separate the two partners of an incompatible graft. Abd El-Zaher [11] in histological study illustrated that grafting anatomical features of Jackfruit were varied according to the grafting types and the compatibility between scions and the rootstocks that correlated with the grafting success percentage.

The objective of this study was to illustrate histological features of various grafting areas of Keitt scion, using interstocks of Keitt, Ewais, Zebda, Alphonso and Sukkary, with rootstocks of Sukkary and Zebda, in relation to their compatibilities degrees.

MATERIALS AND METHODS

These studies were carried out during the period from 2010 to 2011 at the nursery and laboratory of Pomology department, Faculty of Agriculture, Cairo University at Giza. Sukkary, Zebda mango cultivars were used as rootstocks, meanwhile Keitt and Alphonso cultivars were used as a middle rootstocks (intermediate scion or interstocks) and Keitt cultivar was used as the upper scion. The grafting studies were applied on two experiments as following:

First Experiment: Was carried out by using saddle grafting type of Keitt, Ewais, Zebda, Alphonso and Sukkary grafts onto two ages of the ground rootstocks (Zebda and Sukkary); Viz., 21 days and 9 months. Saddle grafting on Zebda and Sukkary rootstocks of 21 days was applied at September, while that on rootstocks of 9 months was applied at May of each studied seasons.

Second Experiment: W done by using top-cleft grafting method of Keitt graft onto two tested interstocks (Keitt and Alphonso). The top-cleft grafting by Keitt scion was done after 7 months (the next May) for 21 days old rootstocks, or 3 months (the next sep.) for 9 months old rootstocks after success of first graft.

After four weeks of grafting date in both experiments, as the final results of the successful grafts have taken, samples of the grafting union region, which containing approximately 2 cm scion and 2 cm of stock were took, killed and fixed in 70% FAA, stored in 70% ethyl alcohol and softened a minimum of 2 weeks in glycerol-alcohol solution. Transverse sections have taken at the grafting union region at 25 and 50µ using a hand-fed sliding microtome.

The most clearness samples of graft union anatomy were double stained with safranine- fast green, mounted in Canada Balsam according to Johansen [12], microscopically examined and photographed to investigate the histological reasons of the compatibility in the grafts.

RESULTS AND DISCUSSION

Two experiments with two rootstock ages (21 days and 9 months) were conducted to classify their grafts, according to their compatibility between the scions and the rootstocks (degree of their anatomical connection) as following:

The First Experiment: Mango Keitt, Alphonso and Ewais were saddle grafted on 21 days old of Zebda and Sukkary seedlings and top-cleft grafted onto 9 months old of the same rootstocks. Also, each of the rootstock was grafted on the other one.

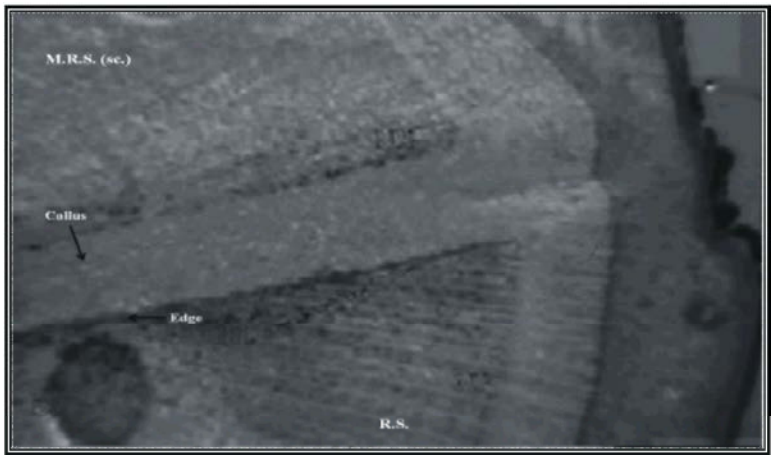

Fig. 1: A light micrograph of cross-section in union zone of mango cv. Sukkary scion saddle grafted onto 21 days old of Sukkary rootstock showing a good formed callus bridge progressed from parenchyma cells of scion and rootstock xylem, sampled 45 days after grafting, X 250

There are two degrees of anatomical connection (high and moderate compatibility grafts) at both ages of the two rootstocks.

The Rootstocks of 21 Days Age

High Compatibility Grafts: These included the grafts of Sukkary/Sukkary (S.), Keitt (K.)/Sukkary, Alphonso (Al.)/Sukkary, Zebda/Zebda (Z.), Keitt/Zebda and Alphonso/Zebda. This type of grafts seems clearly in graft pattern of Sukkary/Sukkary (Fig. 1) where the anatomical features of the cross section characterized by a brown necrotic layer that limit and thin on the whole edges of the grafting regions, forming regular condense uniformly callus that fill the spaces between the scion (middle rootstock) and the ground rootstock (callus bridge), a good connection by callus bridge was cleared without new vessels formation, it may be a juvenile phase of ground rootstock affect on the vessels differentiation by reducing.

Moderate Compatibility Grafts: These included the grafts of S./Z., Z./S., E./S. and E./Z.. The graft pattern of Ewais/Zebda (Fig. 2) have a determined anatomical features; Viz., numerous separation areas along the graft union margins, numerous free callus positions and thin necrotic layer of collapsed cells that may be lead to separate the two graft partners. Some callus bridges formed that cause the scion still alive.

The Rootstocks of 9 Months Age

High Compatibility Grafts: These included the grafts of S./S., K./S., Al./S., Z./S., E./S. and Z./Z.. Ideal pattern of this type is a graft of Sukkary/Sukkary (Fig. 3) that characterized by clear anatomical features; Viz., different intensities callus cells originated by xylem ray parenchyma cells of both the scion and the rootstock, so there were two intensities callus types, a clear brown necrotic layer between the two callus types and new vessels initiated inside the scion's callus, that reflected a good vascular connection between the rootstock and the scion and consequently highly compatibility. Also, it could be noticed that the formed callus fill the gaps between the rootstock and the scion in the graft union region.

The Moderate Compatibility Grafts: These included the grafts of K./Z., Al./Z., S./Z. and E./Z.. The best pattern of this type is cleared in Ewais/Zebda (Fig. 4) graft union that characterized by a few scattered callus groups initiated from xylem ray cells of the ground rootstock, separation area, collapsed and dead cells and necrotic layers of dead cells. These anatomical featured in the graft union region indicated that the separation may be occur.

It worth to mention here that it is better to graft Ewais scion by top-cleft technique on 9 months old of Sukkary rootstock rather than Saddle grafting on 21 days old seedlings of the Sukkary rootstock.

On the opposite, either of Keitt or Alphonso was less successful on 9 months old of Zebda Seedlings compared with that on 21 days old.

The Second Experiment: Mango Keitt scion was top-cleft grafted onto compound rootstocks Keitt/Sukkary, Alphonso/Sukkary, Keitt/Zebda and Alphonso/Zebda.

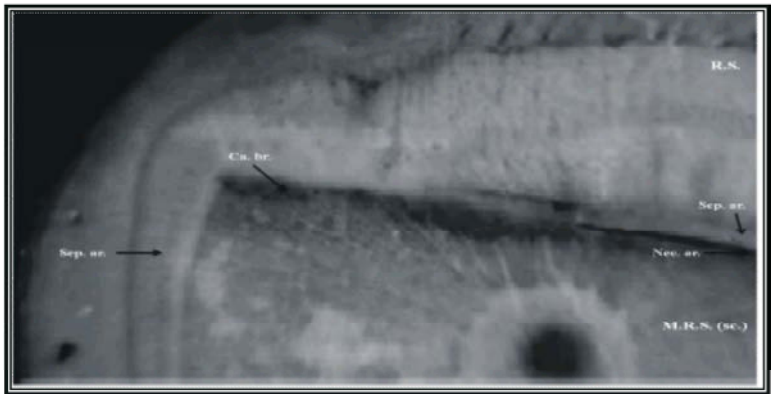

Fig. 2: A light micrograph of cross-section in union zone of mango cv. Ewais scion saddle grafted onto 21 days old of Zebda rootstock showing separation areas, some callus bridge and necrotic layer, sampled 45 days after grafting, X 250

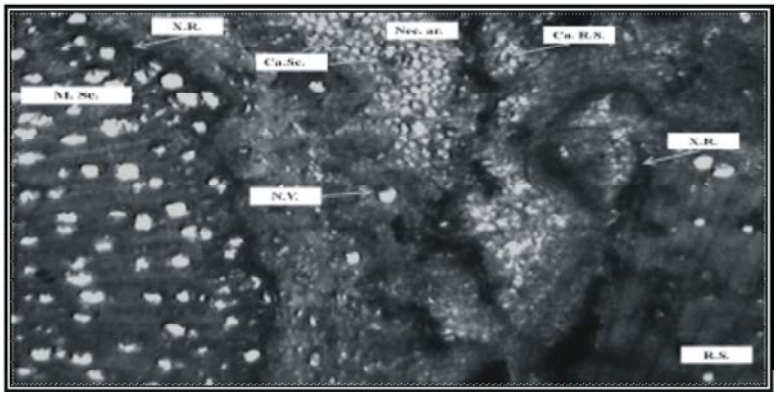

Fig. 3: A light micrograph of cross-section in union zone of mango cv. Sukkary scion top-cleft grafted onto 9 months old of Sukkary rootstock showing two types of callus originated from xylem rays cells of scion and rootstock and distinguished by a thin necrotic layer; and new vessels initiated in many positions of callus. Sampled 45 days after grafting, X 250

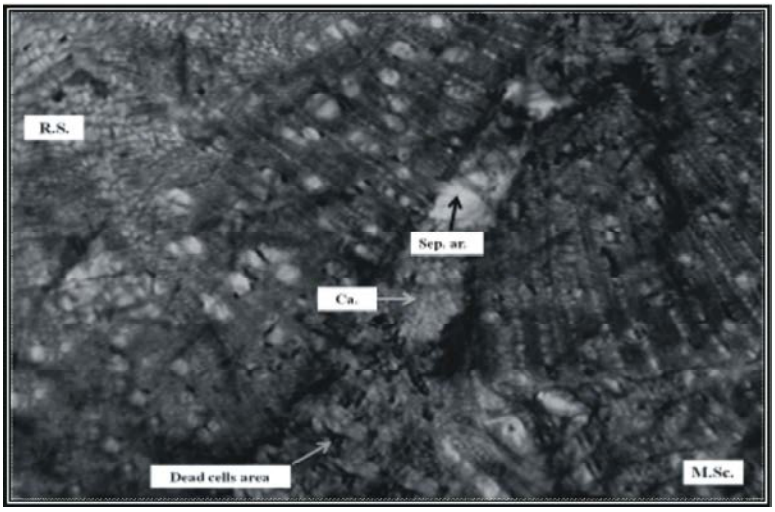

Fig. 4: A light micrograph of cross-section in union zone of mango cv. Ewais scion top-cleft grafted onto 9 months old of Zebda rootstock showing dead cells area, some scattered callus and separation zone. Sampled 45 days after grafting, X 250

Fig. 5: A light micrograph of cross-section in union area of mango cv. Keitt scion top-cleft grafted onto 21 days old of compound rootstock namely Keitt/Sukkary showing a good callus bridge formation. Sampled 45 days after grafting, X 250

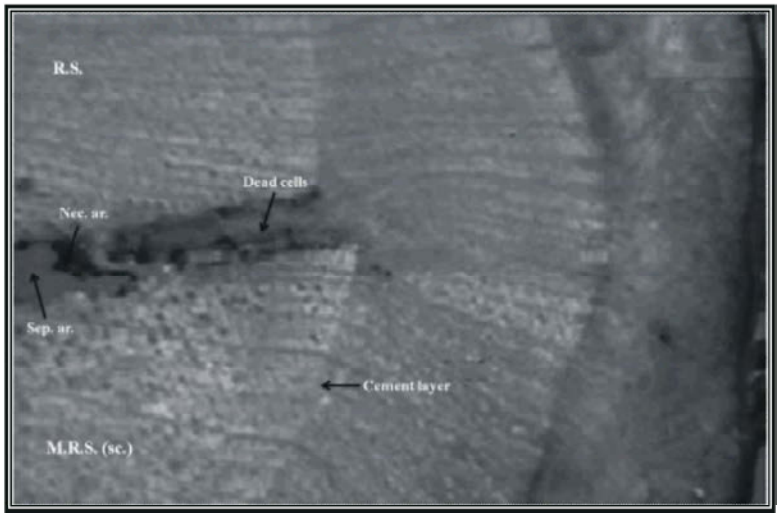

Fig. 6: A light micrograph of cross-section in union area of mango cv. Keitt scion top-cleft grafted onto 21 days old of compound rootstock namely Alphonso/Zebda showing separation zone, dead cells and necrotic area. Sampled 45 days after grafting, X 250

There are two degrees of anatomical connection (high and moderate compatibility grafts) at both ages of the ground rootstock.

The Rootstocks of 21 Days Old

High Compatibility Grafts: These included the grafts of K./K./S., K./Al./S. and K./K./Z.. This type of grafts seems clearly in graft pattern of K./K./S. (Fig. 5), where the anatomical features of the cross section illustrated that forming regular condensed uniformly callus that fill the spaces between the upper scion (Keitt) and the middle or compound rootstock (Keitt/Sukkary), that reflected a good connection or forming a good graft union region by callus bridge, so the upper scion still alive, in spite of no forming a new vessels that is expected form in a latter time in a callus area. Also, no clear necrotic layer was noticed, which may be caused by a speed callus formation by both graft partners as shown in this cross section.

The Moderate Compatibility Grafts: These included the graft of K/Al./Z.. In this type of grafts (Fig. 6) graft union characterized by separation zone, dead cells and

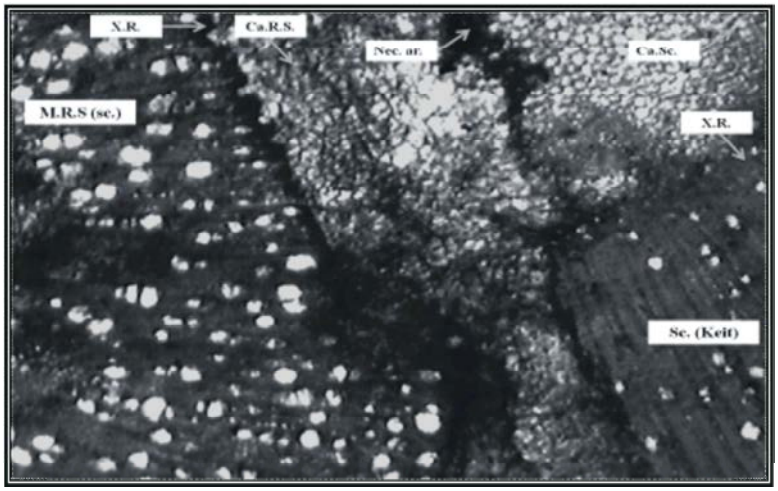

Fig. 7: A light micrograph of cross-section in union area of mango cv. Keitt scion top-cleft grafted onto 9 months old of compound rootstock namely Keitt/Sukkary showing two types of callus originated from xylem rays cells of scion and middle rootstock and differentiated by a thin necrotic layer. Sampled 45 days after grafting, X 250

condense brown necrotic layer, which may be lead to separate the two graft partners, as the graft partners in this case are still adhere by a "cement" or adhesive material of some resinous materials.

The Rootstocks of 9 Months Age

High Compatibility Grafts: These included the grafts of K./K./S., K./K./Z., K./Al./S. and K./Al./Z.. Ideal pattern of this type is a graft of K./K./S. (Fig. 7), that characterized by a limit and thin necrotic layer is surrounded the whole margins of the graft union area; also, different intensities callus cells originated by xylem ray parenchymatic cells of both the upper scion (Keitt) and the middle or intermediate scion (Keitt) that previously grafted onto the ground rootstock (Sukkary). So, there were two intensities callus types, as they could be distinguished and differentiate between them by a clear brown necrotic layer. The formed callus with its two types filled the gaps and spaces in the graft union region between the two graft partners, that lead to a good connection and consequently the water and nutrients could be pass through the callus area towards the upper scion "Keitt" for feeding it and still alive.

The Moderate Compatibility Grafts: This was not noticed in the experimental graft.

The histological features of the studies cross section illustrated that necrotic layers, which are scattered and get browning because of enzymatic reactions, characterized all successful graft union regions, this layer was formed as a result of wounding and act as a defensive mechanism to invasion of pathogens; different intensities of an regular disposition uniformly callus tissues were originated from parenchymatic cells of xylem ray of both scion and rootstock, this callus fill the spaces (gaps) between the rootstocks and the scions; and finally, the vascular connection (mechanically strong graft union) form without apparent disruption of the necrotic layer, these new vessels originated by some meristematic cells in the callus. In addition, adhesive material "cement", which believed that it is a resinous material and secreted by the secratorycanals of the rootstocks and scions, was noticed at the outer margins of the graft union area. On the contrary, the lower successful grafts exhibited other histological features at the graft union region, where

Table 1: List of abbreviations

K.	Keitt
Al.	Alphonso
E.	Ewais
Z.	Zebda
S.	Sukkary
M.R.S. (Sc.)	Middle rootstock used as a scion
R.S.	Rootstock
Ca.br.	Callus bridge
Sep.ar.	Separation area
Nec.ar.	Necrotic area
Ca.Sc.	Callus of the scion
Ca.R.S.	Callus of the rootstocks
X.R.	Xylem rays
N.V.	New vessels
M.Sc.	Middle scion
Ca.	Callus
M.R.S.	Middle rootstock

forming the collapsed cells, necrotic layer of dead cells, numerous free callus positions and separation zones overall grafting margins. These bad structures lead to separate the graft partners.

In agreement with these results, Pandey and Singh [9] worked on micro-anatomical of graft joint in mango, takes samples up to observe the status of graft union between stock and scion tissues of successfully sprouted mango stone grafts of Amrapali cultivar both in healthy and in partially wilted plants at 2- and 4- month after grafting. Graft joints were collected, preserved in hydrofluoric acid solution, transferred to 70, 80, 90, 95 and 100% ethyl alcohol and kept for 72, 48, 24, 12 and 6 h, respectively and specimens were embedded and stained. Even on successfully sprouted and healthy grafts, a light brownish band incompletely marked the union between rootstock and scion. However, in the case of partially wilted grafts, a deep dark brown to black band of tissues was found at 2 months after grafting. At 4 months after grafting, however, the union between rootstock and scion in healthy grafts was complete. Whereas, in partially wilted grafts, the complete union between rootstock and scion failed to take place, marked by prominent deep brownish-black band of dead tissues. The results indicated that initial sprouting and survival of stone-grafted plants was basically due to callus (parenchymatous) tissues, which allowed partial translocation of vital biochemical compounds between rootstock and scion.

Additionally, Moore [10] recorded that lethal cellular has resulted in formation of necrotic layer of collapsed cells that separate the two partners of an incompatible graft. Meanwhile, the compatible grafts and the new parenchyma cells are produced adjacent and internal to the necrotic layer, soon they intermingle and interlock, filling up the spaces between scion and rootstock and development in the healing of a graft union. The cambial activity in the callus has resulted in the production of secondary tissues that have joined the vascular tissues of the stock and scion.

The previous results was similar to those of Abd El-Zaher [11] who found that the histological studies illustrated that grafting anatomical features of Jackfruit were varied according to the grafting types and the compatibility between scions and the rootstocks that correlated with the grafting success percentage. In addition, the latex resinous materials secreted by the secretary canals of the scion and the rootstock, were acted as an adhesive material “cement” between the scion and the rootstocks.

In this trend, Soule [6] illustrated that there are four stages in formation of the graft union of mangoes: 1-pre callus (4 days after budding): where only a wound periderm was present. 2- Callus (8 days after budding); where proliferation from tissues mainly near the cambium resulted in firm attachment of the components. 3- Cambial bridge (12 days after budding); where cambial layers from stock and scion formed a bridge and then the vascular tissues could be differentiated within 36-48 days. 4-The heald union (6-8 months after budding: where several sylinders of new tissues were present and the lateral shift of the scion to align with the stock had begun. Moreover, Moore [7] found that the graft union is initially formed by rapidly dividing callus cells, originating from the scion and rootstock, which later differentiate to form the vascular cambium and associated vascular system. The development of a compatible graft is typically comprised of three major events: adhesion of the rootstock and scion, proliferation of callus cells at the graft interface or callus bridge; and vascular differentiation across the graft interface.

In addition, Chakrabarty and Sadhu [8] showed that three mango cultivars were splice - grafted onto seedling rootstocks. Graft-take was faster for Langra than for Bombai and Himsagar due to more rapid formation of callus and vascular continuity. Splice grafting gave earlier graft-take than cleft grafting, possibly due to a greater formation of wound periderm and accumulation of resinous material in the latter method.

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