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Short communication

Early identification of graft compatibility through histological studies in mango

G. Nayan Deepak[*], Chandu Singh[**], U. Jeevan[****], H.L. Priyanka[*****] and K. Rama Krishna[***]
Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

Three polyembryonic mango rootstocks namely, Nekkare, Olour and Vellaikolumban grafted with Alphonso were used to investigate grafting success and histological evaluation of graft union. The observations were recorded at 07, 14 and 21 days after grafting (DAG). The results revealed that softwood grafting of Alphonso on to Nekkare rootstock showed significantly higher vegetative growth related parameters for graft union than the rest of rootstocks (Olour and Vellaikolumban). The Olour and Vellaikolumban grafted with Alphonso showed moderate and lesser graft compatibility, respectively. The graft union compatibility was confirmed through histological studies. It was observed on 07th DAG that the tissue was juxtapose, initiated callus formation, merged union in Vellaikolumban, Olour and Nekkare rootstocks with Alphonso, respectively. Similarly, on 14thDAG, continuous proliferation of callus in Alphonso/ Olour, numerous separation areas in Alphonso /Vellaikolumban were observed, while Alphonso//Nekkare had enough callus and which trigger the progressive development of xylem and phloem cells. Furthermore, on 21st DAG, graft union between Alphonso and Nekkare had better connection, vascular bundles differentiation, annual thickenings, xylem formation and xylem strands as compared to Olour and Vellaikolumban rootstocks. Histological observations and growth attributes confirmed that Alphonso grafted on Nekkare rootstock showed high graft compatibility than the rest of rootstocks, hence it can be utilized for the production of more mango saplings.

Key words: *Mangifera indica*, grafting, rootstock, histology.

Mango (*Mangifera indica* L) is an important fruit crops, occupies an area of 2.23 million hectares with annual production of 1.88 m MT (Anonymous, 1). Rootstock plays a crucial role in influencing height o and vigour of plant, size, maturity, colour , storability and yield of mango fruits. The rootstocks provide resistance to biotic as well as abiotic stresses and able to tolerate any hostile weather conditions. The mango rootstock is highly variable in nature, hence selection of appropriate rootstock is prime important to generate uniform seedlings for raising an orchard.. Plants raised on polyembryonic rootstocks showed striking uniformity in growth (Kundan, 4). Mango cultivar Alphonso is superior in quality and is preferred for export purpose. The symptoms of declining of mango trees at the time of commercial bearing period (10-20 years) is due to pests, diseases, environmental variations and also due to graft incompatibility. Incompatibility at the time of formation of graftunion may be due to weak growth of the rootstock/scion. and graft union is supposed to be successful when several functional phloem and xylem connections cross the graft surface. Incompatibility in grafted trees refers to premature senescence caused by physiological and biochemical process. The information on various combinations of cultivars and rootstocks in mango is lacking.

The histological evaluation of graft section will be given the firsthand information regarding compatibility or incompatibility of rootstock and scion combination in a short time (Kankaya *et al*., 3) and also to know the anatomical changes (callus, necrotic layers, cambial differentiation and vascular tissues). In view of above background the present investigation has been framed to know the softwood grafting and histological features in mango.

Three polyembryonic rootstocks, *viz.,* Nekkare, Olour and Vellaikolumban grafted with Alphonso were used to investigate. The rootstocks which attained graftable (pencil) thickness and the scions of one season old were selected from healthy trees of Alphonso. The softwood grafting was performed in april and same has kept in the greenhouse. The observations were recorded *viz*., days to sprouting, number of sprouts, per cent graft union, height of scion (cm), length of sprout (cm), number of leaves on scion and girth of scion (cm) @ 49 DAG. The data was analyzed based on Fischers method and data interpreted as per Panse and Sukhatme (6). For histological studies, the samples were taken at 07, 14 and 21 days old grafts. The fixation, dehydration, infiltration and embedding of samples were carried

*Corresponding author's email: nayan.iari@gmail.com
**Seed Production Unit, ICAR-IARI, New Delhi
***Division of Food Science and Postharvest Technology, ICAR-IARI, New Delhi
****College of Horticulture-Hiriyur 577598, Karnataka
*****Division of Fruit crops, College of Horticulture, UHS Campus, Bengaluru 560065, Karnataka

out according to Omima *et al*., 5. The uniform sections of 8µm thickness were taken using ERMA Rotary Microtome and specimens viewed under a light microscope.

The present investigation was revealed, the graft union (Table 1) between Alphonso/Nekkare was highly significant for growth parameters such as minimal number of days taken for sprouting (10.2), maximum graft success (85.8 %), number of sprouts on scion (2.8), height of scion (17.2 cm), length of sprout (5.7 cm), number of leaves (7.9) and girth of scion (1.8 cm). Similarly, the Alphonso/ Olour combination shown moderate graft union. The differential response of scion-rootstocks to softwood grafting may be attributed to varied woody nature of tissues, differential activity, movement of sap and presence of growth promoting/inhibiting factors at the site of graft union. The development of better rootstocks required efficient absorption of moisture, nutrients and more food reserves for the formation of callus at the union region. Thus, early healing of the graft union by faster differentiation of callus into vascular bundles and thereby translocation of moisture and nutrients to the scion portion.

Whereas, the lower graft union was observed in Alphonso/Vellaikolumban *i.e*., the maximum number of days for sprouting (12.6), minimum graft success (60 %), number of sprouts on scion (1.9), height of scion (14.4 cm), length of sprout (3.9 cm), number of leaves (6.4) and girth of scion (1.7 cm) -.The lower percentage of graft success may be attributed to the lack of intimate contact of a cambial region between stock and scion and also due to interference of exudation of latex. The variation may be due to the differences in the quantity of endogenous phenolic compounds in the rootstock or due to the differential capacity of rootstocks in the production of undifferentiated mass of parenchyma cells after grafting. The results are in accordance with Parasana *et al.* (7).

The overview of the graft union on 07 DAG - Transverse section at the graft union zone of Alphonso/Olour was observed that the tissue is juxtaposed; initially some union together is achieved by intercellular adhesion, presence of active dividing cells in scion and it was also observed, enlargement of cells adjoining to the wound surface from living cells and the epidermal cells, vertical resin ducts in the cortex react particularly and quickly, cell division and callus production appears close to the surface in both rootstock and scion. Whereas, the graft union between Alphonso/Vellaikolumban showed similar result to Alphonso/Olour except few observations such as little adhering of scion and rootstock and limited callus formation. Furthermore, the graft union between Alphonso/Nekkare showed that the tissue was juxtaposed, there is a rapid process in callus formation and early callus formation is mainly from the rootstock. An intermingling of callus between rootstock and scion is fair, which in turn increases mechanical strength and eventually fills any gap between graft unions. The group of actively dividing parenchymatus tissues of callus was also noticed. This could be due to the differentiation of callus that involves the developmental process comprising actively dividing cells.

The overview of the graft union on 14 DAG- Transverse section between union of Alphonso/Olour shows an intermingling of callus, ultimately it is the interlocking of xylem fibers (wood) that results in a fairly medium graft union, but still gap was observed between scion and rootstock, which has to be filled with callus and more cellular activity Likewise, the graft union between Alphonso/Vellaikolumban was observed the presence of vascular bundles in minor levels, merging between scion and rootstock is faster, progression of callus formation, but, minor gap were seen in between union margins with few free callusing positions and thin necrotic layer of collapsed cells. The Alphonso/Nekkare combination shows an enhanced development of vascular bundles (xylem and phloem cells), callus cells between graft partners in a satisfactory level and the callus tissue had higher density at the rootstock than at the scion.

The overview of the graft union on 21 DAG- The transverse section of Alphonso/Olour (Fig. 1.i to iii) and there is a progress in graft union, but still difference between rootstock and scion is present. Intermingling of vascular bundles (xylem

Table 1. Growth attributes of Alphonso mango grafted on rootstocks.

Rootstocks	Days taken for sprouting	Graft success (%)	Number of sprouts on scion	Height of scion*	Length of sprout*	Number of leaves*	Girth of scion*
Olour	11.47^{b}	74.67^{b}	2.53^{b}	16.43^{b}	5.05^{b}	7.83^{ab}	1.77^{ab}
Vellaikolumban	12.67^{c}	60.00^{c}	1.93^{c}	14.45^{c}	3.98^{c}	6.47^{b}	1.73^{b}
Nekkare	10.20^{a}	85.83^{a}	2.80^{a}	17.27^{a}	5.72^{a}	7.97^{a}	1.80^{a}

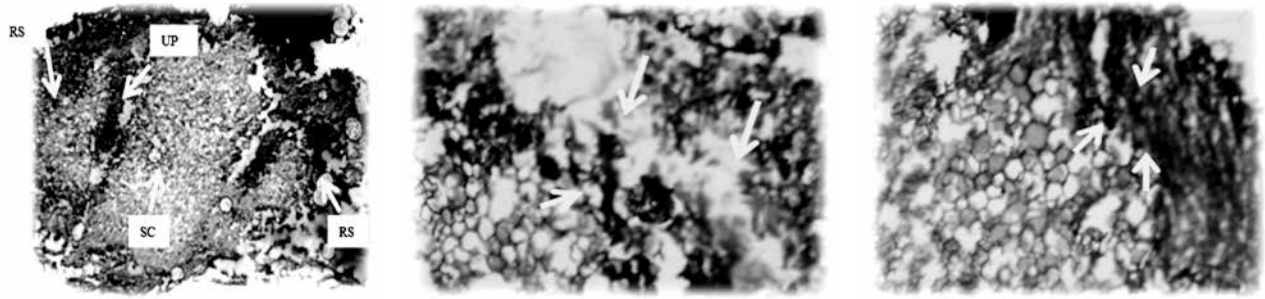
%-Percentage, *-49 days after grafting,

and phloem) was good and comparatively better than Vellaikolumban (moderately compatible nature is there between stock and scion). Further, proliferation of callus internally from the cortex and phloem rays and externally from the pith of the stock united to form a callus cushion between graft components.

In Alphonso/Vellaikolumban (Fig. 2.i to iv), gaps were observed between rootstock and scion. Hence, there is more of parenchymatous cells, instead of vascular bundles (xylem and phloem), which leads to formation of short fusiform initial cells at vascular cambium. Therefore, it produces short xylem fibres, resulting in minimal interlocking at graft union. Eventually the graft union may snap off during high wind or heavy crop load due to brittle wood and relatively low ratio of xylem and phloem formation. Additionally, Alphonso/Nekkare (Fig. 3.i to iii), it was showed the better connection of vascular bundles and the gaps in union region was well filled with callus tissues, and the cambial may be completed or almost completed, which leads to formation of secondary tissues adjacent to the rootstock. It was also observed thepresence of good vascular connection and formed Callus Bridge. Vascular bundles were well differentiated into vessels, having spiral and annual thickenings, xylem formation and xylem strands were seen clearly.

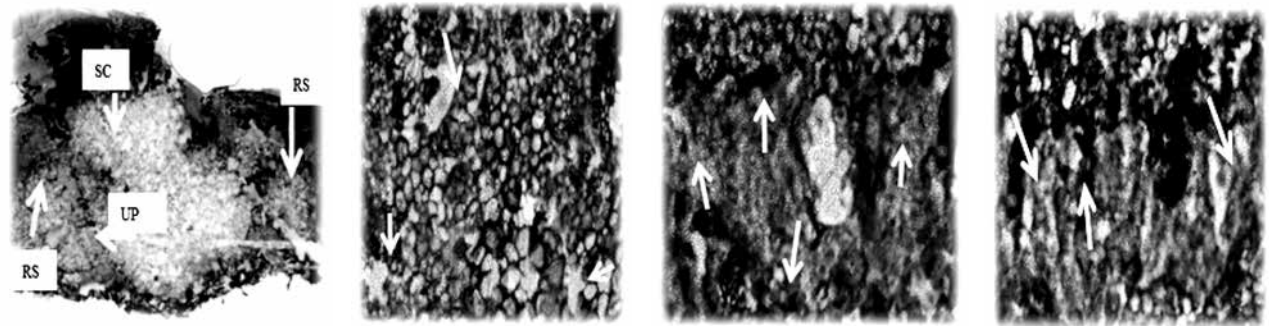
The reason might be due to callus proliferation was more in vigorous and semi-vigorous stock combinations. A successful grafting includes the formation of necrotic layer, callus production, first cohesion of stock and scion by the callus junction, subsequent reduction or elimination of necrotic layer in callus, differentiation of some cells to the cambial cells, bridging of cambium tissues of rootstock and scion, finally formation of strong connection of vascular bundles. Xylem and phloem strands successively bridged the union and necrotic layers were eliminated by the proliferated tissues.

Similar results is agreement with Asante (2) and he observed the resin was secreted as the initial wound response a few minutes after wounding and this contributed to the cohesion of the rootstock and scion. Yogananda (8) reported there were four main stages in the formation of graft union *viz.,* precallus, callus stage, cambial bridge stage and healed union stage. On the contrary, the lower success of graft exhibited formation of the collapsed cells, necrotic layer of dead cells, numerous free callus positions and separation zones along grafting margins. If the



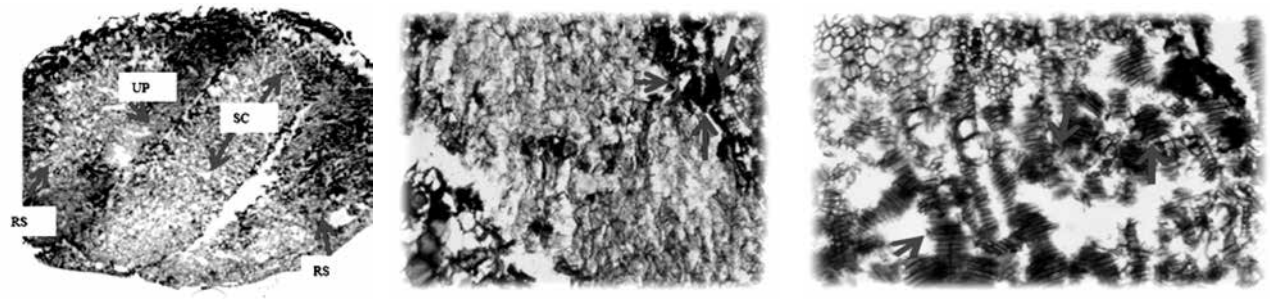
i. Overview of scion and stock ii. Improved, still distinguishing of stock and scion iii. Merging, Xylem formation and xylem strands were fairly seen

Fig. 1. Transverse Section of Alphonso/ Nekkare graft union at 21 days after grafting.



i. Overview of scion and stock ii. Numerous gaps present iii. Intermingling of callus between stock and scion iv. parenchyma cells

Fig. 2. Transverse Section of Alphonso/ Vellaikolumban graft union at 21 days after grafting.



i. Overview of scion and stock

ii. Good connection between scion and stock

iii. well differentiated vascular bundles exhibit vessels, having spiral and annual thickenings

Fig. 3. Transverse Section of Alphonso/ Nekkare graft union at 21 days after grafting.

rootstock is of a slow growing race, the scion will grow faster than the stock and swelling is formed above the graft union which weakens and is easily broken by the wind. Too rapid to growth of the stock may cause expulsion of the scion. Likewise, Omima *et al.* (5) observed the secretion of resin at the graft interface was a defensive response attributable to wounding during preparation of scion and stock for grafting. The overall effect of these factors was the production of a necrotic layer on the cut surface of the graft partners. The necrotic layer created a barrier to prevent contact between the scion and stock, numerous free callus positions and excessive accumulation of this layer could contribute to the mechanical failure of unions.

In this research, variation from moderate to high graft success between scion and rootstocks were observed, which was later confirmed by histological studies. Anatomical studies in the formation of graft union revealed that there was a moderate to high compatibility among the rootstocks and scion. Among the rootstocks, scion cv. Alphonso grafted on rootstock Nekkare was highly compatible compared to others..

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