COMPATIBILITY EVALUATION AND HISTOLOGICAL OBSERVATION OF GRAFT UNION DEVELOPMENT IN FRUIT SPECIES

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Abstract
Plant grafting is a typical horticultural technique involving combining rootstock and scion to create a new plant. It is among the most common approaches used in agriculture to improve the quality and yield of various crops. Grafting aids in the improvement of plant health, yield, and quality of plant products, hence needs to be studied in details. The growth of callus tissue and the formation of vascular tissues can all be assessed by examining the prepared graft samples. As a result, anatomical evaluation of the compatibility of various rootstock/stock graft combinations is important. On the other hand our understanding of in vitro culture systems has benefited greatly from histological approaches. A good histological investigation based on anatomical and histochemical alterations provides insight into cellular processes and clues that allow for further experimentation to be proposed. In this context, histological analyses of graft sections provide us with preliminary information on the compatibility of combinations in a timely manner. The anatomical technique, on the other hand, is an excellent way to observe the early stages of the grafting process and structural changes at the graft interface, allowing us to observe the formation of fine structures between the scion and rootstock and thus improve our understanding of graft union formation.

Keywords: *Rootstock, Scion, Graft Compatibility, Callus, Histology.*

Introduction

Fruit species have increased more intrigue as of late because of high dietary benefit, phytochemical content and human wellbeing impacts. It has been perceived as a good source of nutrients and minerals, and for their role in preventing nutrient C and nutrient A deficiencies (Kamiloğlu *et al*., 2009; Tosun *et al*., 2009; Zorenc *et al*., 2016)

In many occasions, fruit species fill in as an alternative for nourishment as it promote food security and it likewise improves important nourishment and assorted variety to lessen dullness in human eating regimen. Moreover, these organic products species are edible and are been recognised to contain significant level of nutrient C, proteins, sugar, minerals and so on, therefore assuming a significant role in the nutritional balance of humanity (FAO, 1989).

In addition, these fruits species likewise add to medicinal services conveyance as their barks, natural products, seeds, leaves, roots, and so on could be utilized to fix an assortment of disorder and illnesses (FAO, 1998) and most essentially they additionally add to monetary improvement (Adewusi, 1997). The commitments of these fruits species to human prosperity had been incredibly revealed and should be extraordinarily continued (FAO, 2010). In this manner, Sustainability is fundamental for the consistent stockpile of its assets.

It is important to conserve these hereditary assets in light of the fact of their immediate utilization by man, it likewise assumes a significant role in several ecological functions such as flood and erosion control, maintenance of water quality, and climate amelioration (Jamnadass, *et al*., 2011). For whatever length of time that individuals have lived on the surface of earth, we will even now depend on natural products to support and improve the nature of our lives.

Regularly, these fruit species have various ways where we can raise them either through seeds, or by vegetative propagation. In any case, most time some of these fruit species experience issues in raising them through seeds in light of the fact that their proliferation by seed gives poor outcomes and distinctive seed treatment (scarification and acid treatment) did not essentially improve germination. Might not breed true to type therefore vegetative propagation is sort for.

The aim of this study is to describe, briefly, graft union formation in relation to compatibility/incompatibility growing features among different clonal rootstock and scion

Vegetative propagation

Vegetative recovery is both an artificial and natural procedure (Leakey and Mohammed,, 1985). The artificial procedure is utilized by agriculturist, horticulturists and foresters, to capture and increase individual genotypes, thus producing cultivars and clones (Mudge and Brennan, 1999). It is feasible for multiplication to happen through the arrangement of extrinsic roots and shoots in light of the fact that each cell of the plant contains the hereditary data important to regenerate the entire plant (Amri *et al*., 2010).

Vegetative propagation offers consistent with type and availability of superior individuals in a brief timeframe for huge scope of commercial plantation. It is a key apparatus for plant domestication and breeding with numerous focal points which incorporate fast increase of species that is under threat just as offering a one of a kind chance of maintaining a strategic distance from the issues related with seed viability and recalcitrant seeds prevalent in tropical tree species (Leakey, 1990). It is valuable in the creation of cultivars that are seedless, promising species, which have inadequate stockpile of seeds because of mammalian predation, pest and disease attack. A range of approaches can be used for vegetative propagation yet the significant ones are by cuttings, grafting and micro propagation through in vitro culture to multiply ontogenetically mature shoots that are very difficult to propagate (Amri, 2010). Vegetative propagation of plants is basically the multiplication of plant material from vegetative organs so the posterity will contain the specific qualities of the parent plants with regards to genotypes (Macdonald, 1996). This is possible on the grounds that plants have meristematic, undifferentiated cells that can separate to the different organs important to form an entirely different plant. Therefore a bit of plant shoot, root, or leaf can develop to form another plant that contains the specific hereditary data of its source plant. The vegetative propagation methodology can add value in terms of possible conservation, reduction in fruiting age, quick financial returns and subsequently boosting monetary activities and generation of employment both in the rural and urban areas (Jamnadass *et al*., 2011).

Fruits and nut trees are usually propagated by vegetative methods utilizing grafting techniques (Mmng'omba *et al*., 2008). This technique is utilized to make another plant through the union of an appropriate rootstock and an aerial part of another plant of the desired variety, called a scion.

So far nitty gritty degrees of progress have been made on the grafting of some fruits species which include: *Garcinia kola* (orogbo). Yakubu *et al*. (2014) developed up a proper procedure through grafting and budding of *G. kola* and a high level of accomplishment was recorded. Likewise Yakubu *et al*. (2011) examination had a critical achievement in grafting *I. wombolu*. Sanou *et al.,* (2014). Additionally helped out vegetative propagation through grafting on *Vitellaria paradoxa* and recorded a high achievement pace of combination between the root stock and the scion. Before a degree of graft success can be said to be recorded, there is need to affirm the compatibility between the rootstock and the scion through anatomical procedures.

Appropriate rootstocks/scion must be utilized so as to acquire high quality and yield in various climatic and unfriendly soil conditions, to control the growth vigour of trees, to give early resilience to infestation and resistance against diseases and pests. Consequently, there is a need to recognize and comprehend the essential characteristics for development of the fruits species. The principle factor that decides the achievement of grafting is compatibility status between the rootstock and scion.

Fruit tress compatibility

In regard to this the deciding component for the compatibility of the graft union is the capacity of the rootstock and scion cambia layers to bind together. Henceforth, for the improvement of the graft union there must be a cell to cell association between the rootstock and scion as the endo-hormones assists to trigger shoots development in the scion (Yin *et al*., 2012). The external layers uncovered in the cambium area, in both the scion and the rootstock, produce parenchyma cells that blend and join, forming what is commonly called callus tissue. In this callus tissue, cells that align with the intact cambium and graft rootstock differentiate into new foreign cells, which are a pre-requisite for the successful union of vascular plants (Hartmann *et al.,* 2011). After the vascular tissue of the rootstock and scion interfere the activity of xylem and phloem will function to support the plant growth.

On the other hand, frail development of rootstocks may cause incompatibility during the graft formation. Grafting is a troublesome process and waiting for a long time to observe grafting success can cause money, time and effort loss. Considering prepared graft samples, it gives opportunity to evaluate the improvement of callus tissue, the situation of necrotic layers, cambial differentiation, cambial congruity and the advancement of vascular tissues. On the other hand, irregular anatomical developments identified with developing highlights of graft individuals in graft union can cause incompatibility. The incompatibilities that may emerge after the orchard plantation will cause serious economic losses. It has been recommended that irregular anatomical features are the consequences of inconsistency of the tissues of bud and rootstocks, and these variations from the norm emerge either during the development of the graft union as a response to contact between bud and rootstock or during subsequent growth of the composite tree as a result of physiological or biochemical interactions (Simons 1986). In this regard, anatomical evaluations on graft sections give us first information about compatibility or incompatibility of combinations in a short time (Vachun, 1995; Grzyb *et al*., 1998; Kankaya *et al*., 1999).

Hence, anatomical assessment of the concurrence of various rootstock/stock graft combinations is a rescuer approach. Fruit trees are formed by a combination of the rootstock that gives root framework and the scion that produces the crop. All together for that combination to be effective, an acceptable relationship among scion and rootstock is essential However, graft incompatibility happens as often as possible in combinations (Errea *et al*., 2001). Grafting is a fundamental practice in fruit species tree management to guarantee high plantation yield productivity and high return quality. But, the issue of incapability among rootstock and scion in fruit culture has existed since the time grafting and budding were first utilized (Sitarek, 2006). The term incompatibility with respect to grafted fruit trees is characterized as a premature senescence brought about by physiological and biochemical procedure (Feucht, 1988). A graft union is considered as effective when several functional phloem and xylem connects across the graft surface (Schoning and Kollman, 1997). However, incompatible grafts can grow several years with no outside manifestation of incompatibility, showing presence of functional vascular connection (Mosse, 1962; Errea *et al*., 1994). Some anomalous developments in the structure of the union are found in some anatomical examinations on graft union. These variations such as, poor vascular connections, phloem degeneration and cambial or vascular discontinuity in the association zone can cause mechanical weakness and resulting breakdown of the union (Hartmann *et al*., 1997; Errea *et al*., 2001). Likewise vascular tissues that developed between the rootstock and scion were organized in a whirling pattern, and became necrotic during subsequent development of the plant (Simons and Chu, 1984; Soumelidou *et al*., 1994).

In this manner graft compatibility situations between various rootstocks/stock ought to be assessed by understanding the plant's anatomy systems which will assist breeders with developing a procedure for its further enhancements. Along these lines, rootstocks and scion ought to be controlled by the anatomical perceptions sections prepared from graft zone.
More or less, the point of this exploration is to look at the early biological processes that occur in phases of graft union development in relation to compatibility/through anatomical assessment of the different rootstock/scion combinations.
As part of the investigation concerning the fundamental reasons for limited compatibility and incompatibility, we report the procedure of a histological investigation of various grafts undertaken so as to acquire more data about the sequential arrangement of graft development in compatible and incompatible combinations.

Histology

Plant histology can be characterized essentially the study of the microscopic structure or attributes of cells and their assembly and arrangement into tissue and organs. Histological techniques are broadly utilized in numerous areas of research. Basic investigation is a significant initial phase in the investigation of the association and changes in the plant body, and it is an extremely valuable methodology in the investigation of plant morphogenesis. The development of the tissues is dynamic and changes from moment to moment, investigating the slides give the data with respect to the progressions that occurs during development process. Most histological samples need preparation before microscopic observation; these methods depend on the specimen and method of observation.
This system utilizes microscopic study of plant cell and tissues through staining, sectioning and examing at under a magnifying instrument (electron or light magnifying instrument) (Shostak, 2013). There are different techniques used to study tissue characteristics and microscopic structures of the cells. Histological procedure is a progression of technique undertaken in the readiness of test tissues by utilizing histological stains to aid in the microscope study (Anderson, 2011). This procedure takes five key stages which include; fixation, processing, embedding, sectioning and staining (Titford, 2009).

Fixation

Fixation in histology: Fixation alludes to the utilization of chemicals to preserve the natural tissue structure and maintain the cell structure from degradation. Mostly, 10% neutral buffered formalin or NBF (4% formaldehyde in phosphate buffered saline). Ross and Pawlina 2016). Generally, samples of graft combination of rootstock/scion taken at intervals depending on the type of plant are used for this procedure. The samples are immersed in fixative for 24 hrs. After 24 hours the materials will thoroughly be washed and put away in 70% alcohol. Mostly used when a light microscope is to be used to conduct the study Fixatives enhance the preservation of tissues and cells through an irreversible procedure cross-connecting protein. The fixation stage retains the chemical composition of the tissues, hardens the cells or tissues for sectioning and delays degradation (Titford, 2009). In addition, fixatives changes tissue penetration and influence antigen exposures which might be beneficial or detrimental (Iyiola and Avwioro, 2011). There are various fixatives being used, yet the formaldehyde fixatives are the most usually utilized (Black, 2012). The neutral buffered formalin (NBF) stabilizes amino acids in proteins and offers great tissues and cell structure preservation. Formalin fixation usually prompts degradation of mRNA, miRNA, and DNA as well as denaturation and alteration of proteins in tissues. In any case, extraction and investigation of nucleic acids and proteins from formalin-fixed, paraffin- embedded tissues is possible utilizing appropriate protocols (Weiss *et al.*,2011, Bennike *et al*., 2016).

Embedding

The stored materials in 70% alcohol will then are dehydrated by passing them in ethanol-xylene series.. Samples are transferred through series of progressively more concentrated ethanol baths, up to 100% ethanol to remove remaining traces of water. (Ross and Pawlina 2016). And keeping in mind that lack of hydration, the infiltration will be done by adding paraffin wax to the ethanol-xylene mixture by this procedure the paraffin accumulate into the callus. After saturation the callus were embedded in paraffin wax and blocks will be prepared. These blocks ill at that point be fixed to rider for microtome sectioning. This tissue embedded in paraffin wax assists with providing a mode of support and to permit the cutting of the thin tissue slices (Ross and Pawlina 2016)
In this step, the aim is to expel water from the selected tissues to set them and encourage the cutting of slender segments of slides, more thinly for use in light microscopes and thick for the electron microscope. In general, water should initially be expelled from tissues (dehydration) and replaced with a medium that either hardens directly, or with an intermediary fluid (clearing) that is miscible with the embedding media. For light microscopy, paraffin wax is most frequently utilized embedding material. (Mark, 2019). The procedure is repeated through a hydrophobic clearing substance, for example, xylene to expel the alcohol (Titford, 2009). Once penetrated in paraffin, tissues are situated in molds which are loaded up with wax; once situated, the wax is cooled, it hardening the block and tissue.

Segmenting

The solidified and castened blocks will be utilized for microtomic segments. The blocks are subjected to 15-20 microns segmenting or relying upon the necessary microns. The segments are prepared in ethanol-xylene series and finally stained with toxyline stain. The stained sections were made permanent utilising DPX mountant
For light microscopy, a blade mounted in a microtome is utilized to cut tissue sections which are mounted on a glass microscope slide. (Ross and Pawlina 2016)] In histology, sectioning alludes to the preparation of 'ribbon' like microtomes of a tissue to mount it on a microscope slide for examination (Cai *et al*., 2014). In this case, arrangement of thin sections of tissues of required thickness are sliced and arranged through the paraffin method. After the histological procedures, five basic phases of the graft formation will be analyzed on the samples: ((1) development and positions of necrotic layers, (2) proliferation of callus cells, (3) formation of callus bridge at the graft interface, (4) cambial continuity and (5) formation of vascular tissues.

To this end, some degree of accomplishment had been obtained from grafting through histology procedures and this include and not limited to discoveries of Yildirim *et al*., (2010) who detailed a histological examination on histological investigation on the graft formation in some spur and vigorous apple varieties grafted on Ottawa 3 rootstock and found out that there was a good union formation in the sections prepared from subsequent samples of graft combinations. The grafted seedlings necrotic layers were eliminated in the callus and were transformed to bark. Callus filled the gaps between interface of the stock and the scion. Cambial continuity occurred successfully and vascular tissues were connected. Thus, no evidences for tissue incompatibility were found in the study. Histological observations showed a compatible graft union between the graft partners which have different growing characteristics in relation to tissue formation.

Likewise, in apple rootstocks/scion combinations Dolgun *et al*., (2009) observed a successful level of compatibility between the graftage when observed through a microscopic study. Histological observations showed a compatible graft union in relation to tissue formation between graft members which have different growing features.

Furthermore, a similar kind of result by Farsi *et al*. (2017) was obtained from histological study of grafted seedlings of *Juglans regia* L. cv. Chandler scions (walnut). Cross and longitudinal sections of the graft union were taken for examining different stages of grafting process. The first callus cells were initiated from cambium layer of rootstock thus making a weak connection between the two parts. New vascular connections between rootstock and scion were later observed while vascular connections were increased in central parts of the graft union but later the grafted seedlings were tightly connected to each other and necrotic layer disappeared in most of cross and longitudinal sections. Although there was a weak connection between two parts at the initial stage of development of graft process which after some days the two parts were strongly connected by xylem vessels

Ermel *et al*. (1996) studied the comparative histological study of compatible and incompatible pear/pear and pear/quince grafts. And it was observed there was an adhesion between the two partners along the inner bark tissues (phloem and cambium), while a necrotic layer appeared at the graft interface. The first cell divisions were observed in the inner bark interface and this led to the formation of a junction callus, inside which new cambial strands (neocambia) appeared, starting from the cut edges of the scion and stock cambia, and joining later when they began to produce both phloem and xylem derivatives

Dolgun *et al*. (2009) reported from his histological study conducted in order to observe the graft formation of nectarine varieties Armking, Cherokee, Stark Red Gold and Independence, which were grafted on pixy rootstock. In every combination, callus formation, new cambium differentiating, cambial continuity and vascular tissues formation were noted successfully. All combinations were found compatible in histological observations.

In a study conducted by Özdemir *et al*. (2018), graft compatibility between almond cultivar rootstocks /scion was histologically investigated. The histological analysis of scion/ rootstock combinations was performed and at 30 days after grafting, the callus cells developed but cambial continuity has not occurred between the rootstock and scion tissues in all scion/rootstock combinations. 12 months after grafting, cambial relation was established, vascular differentiation was observed, regular parenchymatic tissue properties and scleroid cells and sclerenchyma bundles were seen in the graft union. There was no problem in terms of rootstock-scion compatibility

Histological studies in herbaceous plants are reported. Take for instance Fan *et al*. (2015) reported in a study of the structural development of graft union formation in tomato plants was examined using Scanning Electron Microscopy (SEM) and a paraffin sectioning technique. The images of the transection revealed that parenchymal cells from the graft union divided and proliferated in most combinations, and these cells could be detected at various stages of development. The SEM images of the scion and rootstock longitudinal sections showed that many interconnecting structures appeared and were followed by a vascular rearrangement that did not connect the scion and rootstock. But as time goes on, vascular bundle bridges appeared connecting the scion and rootstock. The connection of graft union between rootstock and scion is influenced by the plant growth condition.

Graft incompatibility in solanaceous plants by Kawaguchi *et al*., (2008) was investigated using representative cultivars of tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), and pepper (*Capsicum annuum* L.). Evaluations of the extent of graft (in) compatibility were made by examining survival percentages, fruit yields, and fruit quality in grafted plants. Tomato/pepper (scion/rootstock) and pepper/tomato grafts were considered severely incompatible, and the tomato/eggplant and eggplant/tomato grafts were considered moderately incompatible. The anatomy of the graft unions was also observed. Growth inhibition and high mortality in tomato/pepper and pepper/tomato grafts (that is,., severe graft incompatibility) was observed due to discontinuities in the vascular bundles at the graft union, which prevented the translocation of assimilates, mineral nutrients, and water between scions and rootstocks. Plant responses to graft incompatibility varied in solanaceous plants depending on the scion and rootstock combination.

The grafting of two cucumber scions (*Cucumis sativus* L.)—Taiko hybrid and Tsuyoi hybrid— onto a Tropical hybrid pumpkin rootstock (*Cucurbita moschata* L.) was studied by Baron *et al*., (2018). The initial establishment of grafting through anatomical cuts on the region of union graft was evaluated

The results of the anatomical study uncovered that the Taiko cucumber scion grafted onto pumpkin rootstock showed earlier establishment of the graft compared to the Tsuyoi cucumber scion grafted onto pumpkin rootstock. The filling of spaces by parenchyma cells (callus tissue) was more effective on Taiko cucumber scions grafted onto pumpkin rootstock.

Conclusion

To buttress the points about grafting and anatomical studies, there is need to understand fully well that generally, 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 stock and scion, and finally formation and strong connection of vascular tissues. However, the anatomical technique is an excellent way of approach to observe the early stages of the grafting process and the structural changes at the graft interface, which ensured observation of the generation of the fine structures between the scion and rootstock, thus increasing our understanding of the graft union formation.

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