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Histological Development of Grafting in Apple in Cold and High Altitude Conditions

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Authors' contributions

This work was carried out in collaboration between both authors. Author OD designed the study, performed the laboratory works, managed the literature search, wrote the protocol and wrote the first draft of the manuscript. Author HZ carried out the field works, graftings and took samples. Both authors read and approved the final manuscript.

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ABSTRACT

The purpose of this study is to briefly describe graft fusion in relation tissue formation in some apple varieties grafted by chip and T buddings on MM106 in cold climatic and high altitude conditions. Effects of enviromental conditions on plant growing is well known. Plants grow strongly in warm and humid places.But, in cold climate and high altitude conditions grow weak. Therefore, graftings which made in different ecological condition may show different grafting formation time. In such places it is important that the graft fusion takes place rapidly. The short-term fusion will be effective in faster and stronger development of grafted plants.For his reason, it is important to know the most useful grafting method in high and cold places.In this research, it was observed that stocks and scions fits very well and no large gaps and although the callus formation was shorter in chip budding, it soon filled all the voids, and as a result, the cambial continuity and strong vascular connection occurred in short time. For these reasons, chip budding were found more suitable due to less necrotic tissues formation and wounds heal more quiclky compared to classic "T" budding for production of apple sapling in cold climate and high altitude conditions.

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1. INTRODUCING

Effects of enviromental conditions on plant growing is well known.Plants grow strongly in warm and humid places compared with cold climate regions [1-4]. In this relation, also tissues formation in grafting is in relation with ecological conditions and especially temperatures and humidity affect speed of graft formation and scion growth after grafting [5,6]. A good graft formation results high quality saplingsgrowing in short time. But, grafts made in different ecological condition like cold and high regions can show different graft formation time and saplings can show different growth rate and quality. For this reason the establishment of nurseriesin places over 800 meters is not prefered. Nurseries are usually established where the sea level or the maximum height of 300 meters above sea level. However, without the possibility of establishing sea level nursery places, or when required to produce saplings in high places, environmental conditions are effective in sapling production [7]. Therefore, rapid realization of graft fusion is important in such places. Short-term graft fusion will be effective in faster and stronger development of saplings. Therefore, it is important to know the most appropriate grafting method for high and cold places.

On the other hand, histological developments in graft union formation can provide some information about graft success. Major events in graft formation are cohesion of scion and rootsck, proliferation of callus cells, connection of cambiums and formation of vascular continuity. The structural integrity of the graft union not only holds the grafted plant together but it is the reestablishment of anatomical and functional continuity between xylem and phloem that allows for transportation of water and minerals by the xylem, conduction of carbohydrates and other organics by the phloem. The necrotic plates are the layers of crushed and desiccated cell walls at the cut surface of both stock and scion.In addition, necrotic plates serve to seal injured tissue from pathogens and to limit water loss. In most species, callus is not from the vascular cambium itself, but rather from the secondary xylem and phloem cells that were most recently formed from division of the vascular cambium. As the new callus increases in volume, it ruptures the necrotic plate and begins to expand into whatever spaces exist between stock and scion. New vascular cambium differentiates inwards from the vascular cambiums cut ends of the stock and scion. New vascular cambium cells begin to produce to the inside (xylem) and to the outside (phloem). Regeneration and bridging of conducting elements (xylem tracheids and vessels, and phloem sieve tubes) allows for transportaion across stock and scion. Besides, interlocking of new xylem fibers is largely responsible for mechanical strengthening [1,8-16].

The aim of this study is to briefly describegraft union in relation tissue formation insome apple varieties grafted on MM106 in cold climatic and high altitude conditions.

2. MATERIALS AND METHODS

Grafting studies was carried out in the experimental orchard of Bolu Vocational College of Abant İzzet Baysal University. Bolu province is placed in the northern region of Turkey and it has cold climate conditions and high altitude (768m). Graft sections were examined under microscope in Sultanhisar Vocational College, Adnan Menderes University.

In this study, one year old semi-dwarf MM106 was used as rootstock. MM106 is a semi-dwarf apple rootstock that can be easily and cheaply produced when compared to dwarf M9. Because of this feature, it is preferred in recent years. Apple varieties; Golden Delicious, Granny Smith, Mondial Gala and Red Chief were grafted on root stock by using “T” and “Chip” budding techinques at 25 April 2012. 100 plants were budded for each combinations.

Graft samples of each rootstock/scion combinations were taken at first 15th day and 30th days intervals until 4 months after grafting. Samples were fixed in ethanol (70%). Transverse sections in 0.2-0.3 mm thickness were cut using a rotary microtome. All sections were examined and photographed with digital microscope Mic-D having colouring property. Five base stages of graft formation; (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 were examined on samples. Meteorogical records of region were given in Fig. 1.

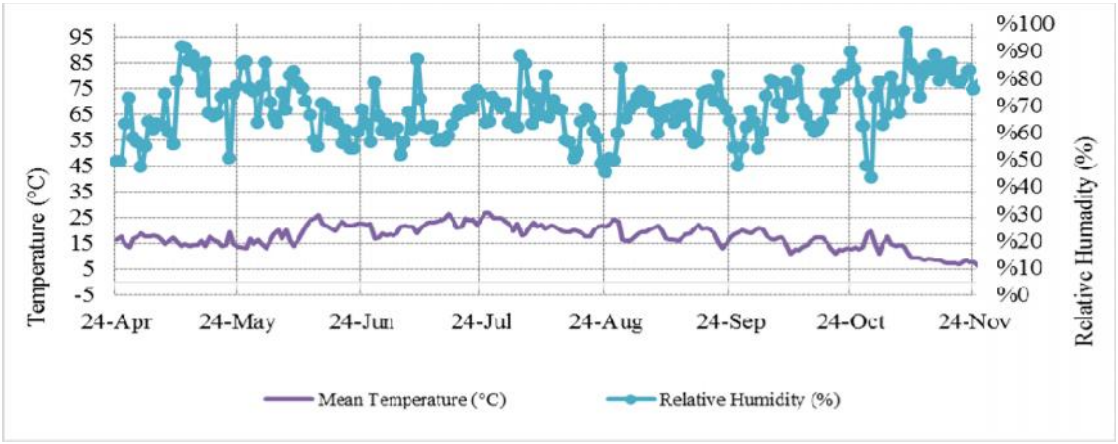

Fig. 1. Meteorogical records in graft union formation period

3. RESULTS

3.1 Histological Observations in "T" Budding Samples

In 15 days samples, There was seen many necrotic layers in all combinations. Necrotic layers formed from dead cells occured as a result of cuts wasclearly visible in the cut sides of scion and especially bark of the stock which cover up scion in all combinations.It was seen low callus formation in all combinations, but necrotic layers were started to fragment by newly formed callus cells. It was seen that callus tissues were produced by cortex of scion and stock.

Sufficient callus formation were observed in 30 days sections of all combinations. Necrotic layers was fragmented mostly by the callus tissue. It was seen in the form of vesiculer callus mass which spread into gaps between stock and scion. There was not observed tissue formation related cambial differentation (Fig. 2).

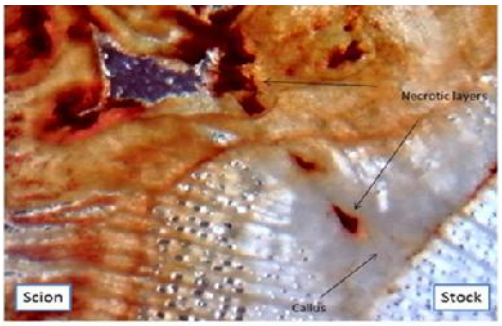

Fig. 2. 30 days section of Golden Delicious " T" budded on MM106

An intensive callus activity and absorbsion of necrotic layers were seen in the graft interface of both scion and stock in 60 days sections. It was seen that the callus continued to proliferate and almost filled all gaps. An indistinct cambium differantiation were seen in callus tissue close places to uninjured cut end sides of graft members in 60 days sections (Fig. 3). Largely eliminated necrotic layers were seen as small points and thin black lines in callus tissue.

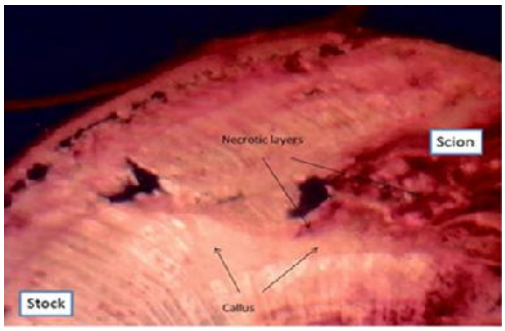

Fig. 3. 60 days section of Granny Smith "T" budded on MM 106

Cambial continuity was clearly seen in 90 days sections of combinations. Cambium bridge is curly form because of the callus mass and necrotic layers. Newly formed xylem and floem were seen as locally strands in callus tissue between scion and stock (Fig. 4). But vascular tissues was not sufficient for strong graft formation.

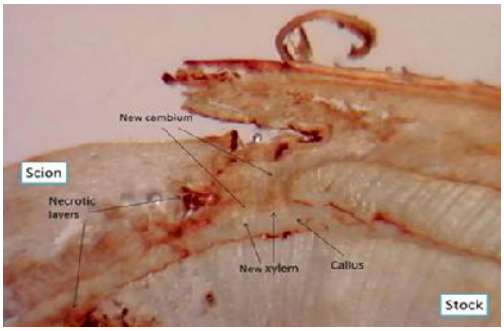

Fig. 4. 90 days section of Mondial Gala "T" budded on MM106

In the sections taken on 120th days samples,callus was almost fill all gaps and vascular bridge were established between scion and stock. It was not seen incompability related histologically development (Fig. 5).

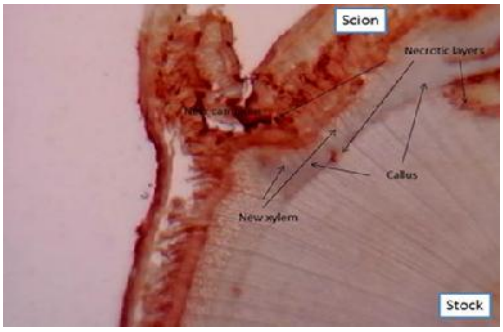

Fig. 5. 120 days section of Red Chief "T" budded on MM106

3.2 Histological Observations in "Chip" Budding Samples

In 15 days sections, It was seen clear but small amount necrotic layers placed close to cut interface of scion and stock. Callus production was low but sufficient. There are thin gaps between scion and stock especially xylem tissues of both stock and scion. Newly formed callus was produced from cortex tissues close to cutsides of cambiums of stock and scion.

In 30 days sections, A strong callus formation were seen in all combinations. Necrotic layers formed soon after dueto cuts was clearly seen in cut sides of stock and scion. Necrotic layers was broken by the newly formed callus tissues. There was not seen a clear evident related cambial differentiation (Fig. 6).

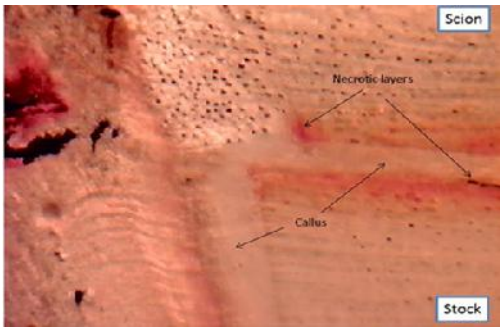

Fig. 6. 30 days section of chip budded Mondial Gala on MM106

Clear cambial continuity was observed in 60 days samples of all combinations An intensive celluar activity was seen in all combinations. Cellular activity was not only for callus proliferation but also absorbsion of necrotic layers. Necrotic layers were largely elimineted by callus. They were seen in cortex region close to bark as thin black lines and small black points in callus (Fig. 7).

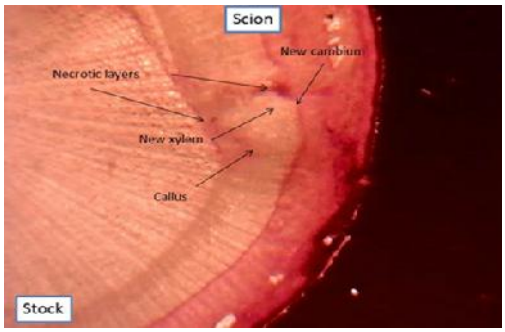

Fig. 7. 60 days section of Red Chief chip budded on MM106

New cambium derived from callus cells started to produce vascular elements. Especially, new xylem cell strands were observed in callus along graft line close to bark (Fig. 8).

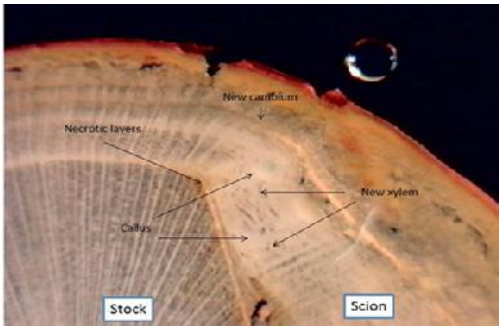

Fig. 8. 90 days section of Golden Delicious chip budded on MM106

In the sections taken on 90th day and following days, it was seen that callus completely filled all gaps between stock and scion. Xylem and floem tissues were succesfully bridged scion and stock. Necrotic layers were absorbed by proliferated callus. There were seen a few necrotic layers as small points. It was not seen an abnormal histolgical or cellular development (Fig. 9).

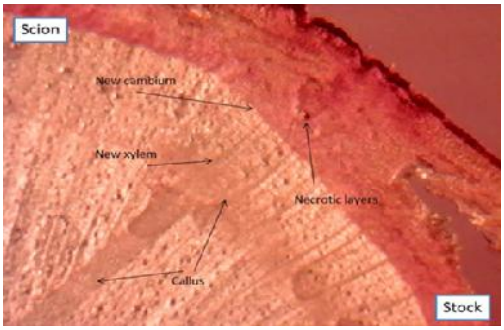

Fig. 9. 90 days section of Granny Smith chip budded on MM 106

4. DISCUSSION

Usually, a succesfull grafting includes formation of necrotic layers, callus propliferataion, elimination of necrotic layers by proliferatred callus, differentition of some cells to cambium cells, establishing of cambial continuity and bridging of vascular tissues finally. Necrotic layer formation as a result of wounding is a defensive mechanism against to invasion of pathogens and it is a independent event. In subsequent days, cortex tissue starts to pruduce callus. In time, callus tissue continue to preliferation and spread into gaps placed between stock and scion. In the mean time, necrotic layers has been eleminated by callus. Callus absorbs most of the necrotic layers [17-20]. Also In this study, all grafts showed a similiar necrotic layer formation and elimination. Especially in the "T" graft samples a high amount of necrotic layer formation was observed.It is a result of excessively cuts and woundings especially in stock. It is well know that dead cells due to wounding are transformed to black necrotic strands or points. As result of this, it was seen necrotic strands and points in callus and cut surface even 90 days samples. Necrotic layers was not completely absorbed by callus which proliferated from cortex. This result is in agreement with previous researches which indicated wounding is much in “T” buddings compared chip budding. On the other hand,Chip budding samples showed that wounding is very small amount. As a result of small wounding, necrotic layers was small amount and generally placed in cut sides of cortex.It was clearly that callus cells proliferated from cortex paranchyma of stock and scion. In the course of time, callus which spreaded in gaps were fill all gaps between scion and stock and necrotic layers were absorbed. Thus, our results are in agreement with prevous researches [1,17,20,21].

In the present research, most clear difference was seen in establishing cambial continuity. Cambial continuity was clearly seen in 90 days samples of “T” budding, but in “chip” budding samples, cambial continuity were seen in 60 days samples. This situation may be a result of the excessive wounding and long filling time of the callus for gaps in “T” grafts. Because, it was seen that much necrotic layers and large gaps between scion and stock.

But, in “chip” budding samples, it was seen small amount necrotic layers. Also, it was seen small gaps between xylem and cortex zones of scion and stock due to close cuts surfaces of graft members. Gaps between scion and stock were filled in short time by callus and a strong cambial continuity established in short time. In 90th and other periodsof samples of all combinations, smiliar observations were seen. Results is agreement with previous reseraches [10,22,23]. According to Hartmann et al. [1] “T” budding is generally used in propagation of fruit trees and chip budding is not preffered because it is not simplier and faster. But, in contrast Kviklis [22] reported that chip budding gives higher graft success in apple.Researchers reported that chip budding was more effective than “T” budding [24]. Smilar results were also reported by many authors [25,26,27].

Kadan and Yarılgac who studied apple and pear sapling production by using “T” budding in Van ecological condition which has cold climate and high altitude (1727 m) reported that it couldn’t be obtained any first or second class saplings [7].

5. CONCLUSION

Chip budding were found more suitable than classic “T” budding for production of apple sapling in cold climate and high altitude conditions due to less wounding, less necrotic layer formation and wounds heal more quickly. In addition,it was observed that stock and scions fits very well and there is no large gaps betwen

them in chipbudding. Callus formation is less and and fills all gaps in short time. As a result of this,clear cambial continuty and strong vascular connection occured in short time. Histological observations have shown that the chip budding is relatively more suitable due to quick and strong fusion of tissues in the shorter time in cold and high places.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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