

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

## 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 grows 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. Therefore, it is important to know the most appropriate method for grafting for 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 short time. For these reasons, chip budding were found more suitable due to less necrotic tissues formation and wounds heal more quicky compared classic "T" budding for production of apple sapling in cold climate and high altitude conditions.

**Keywords:** Grafting, Apple, Altitude, Graft Techniques, Histology

## 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 [1,2,3,4]. 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 saplings growing 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 nurseries in places over 800 m is not prefered. Nurseries are usually established where the sea level or the

31 maximum height of 300 meters above sea level. However, without the possibility of  
32 establishing sea level nursery places, or when required to produce saplings in high  
33 places, environmental conditions are effective in sapling production [7]. Therefore,  
34 rapid realization of graft fusion is important in such places. Short-term graft fusion will  
35 be effective in faster and stronger development of saplings. Therefore, it is important  
36 to know the most appropriate grafting method for high and cold places.

37 On the other hand, histological developments in graft union formation can provide  
38 some information about graft success. Major events in graft formation are cohesion  
39 of scion and rootsck, proliferation of callus cells, connection of cambiums and  
40 formation of vascular continuity. The structural integrity of the graft union not only  
41 holds the grafted plant together but it is the reestablishment of anatomical and  
42 functional continuity between xylem and phloem that allows for transportation of  
43 water and minerals by the xylem, conduction of carbohydrates and other organics by  
44 the phloem. The necrotic plates are the layers of crushed and desiccated cell walls  
45 at the cut surface of both stock and scion. In addition, necrotic plates serve to seal  
46 injured tissue from pathogens and to limit water loss. In most species, callus is not  
47 from the vascular cambium itself, but rather from the secondary xylem and phloem  
48 cells that were most recently formed from division of the vascular cambium. As the  
49 new callus increases in volume, it ruptures the necrotic plate and begins to expand  
50 into whatever spaces exist between stock and scion. New vascular cambium  
51 differentiates inwards from the vascular cambiums cut ends of the stock and scion.  
52 New vascular cambium cells begin to produce to the inside (xylem) and to the  
53 outside (phloem). Regeneration and bridging of conducting elements (xylem  
54 tracheids and vessels, and phloem sieve tubes) allows for transportaion across stock  
55 and scion. Besides, interlocking of new xylem fibers is largely responsible for  
56 mechanical strengthening [1,8,9,10,11,12,13,14,15,16].

57 The aim of this study is to briefly describe graft union in relation tissue formation in  
58 some apple varieties grafted on MM106 in cold climatic and high altitude conditions.

## 59 **2. MATERIAL AND METHOD**

60 Grafting studies was carried out in the experimental orchard of Bolu Vocational  
61 College of Abant İzzet Baysal University. Bolu province is placed in the northern  
62 region of Turkey and it has cold climate conditions and high altitude (768m). Graft

63 sections were examined under microscope in Sultanhisar Vocational College, Adnan  
64 Menderes University.

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

71 Graft samples of each rootstock/scion combinations were taken at first 15th day and  
72 30th days intervals until 4 months passed after grafting. Samples were fixed in  
73 ethanol (70%). Transverse sections in 0.2-0.3 mm thickness were cut using a rotary  
74 microtome. All sections were examined and photographed with digital microscope  
75 Mic-D having colouring property. Five base stages of graft formation; (1)  
76 development and positions of necrotic layers, (2) proliferation of callus cells, (3)  
77 formation of callus bridge at the graft interface (4), cambial continuity and (5)  
78 formation of vascular tissues were examined on samples. Meteorological records of  
79 region were given in Table 1.

80 ( Table 1 )

### 81 3. RESULTS

#### 82 3.1 Histological observations in “T” budding samples

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

89 Sufficient callus formation were observed in 30 days sections of all combinations.  
90 Necrotic layers was fragmented mostly by the callus tissue. It was seen in the form  
91 of vesicular callus mass which spread into gaps between stock and scion. There was  
92 not observed tissue formation related cambial differentiation (Fig. 1).

93 An intensive callus activity and absorbsion of necrotic layers were seen in the graft  
94 interface of both scion and stock in 60 days sections. It was seen that the callus  
95 continued to proliferate and almost filled all gaps. An indistinct cambium  
96 differantiation were seen in callus tissue close places to uninjured cut end sides of  
97 graft members in 60 days sections (Fig. 2). Largely eliminated necrotic layers were  
98 seen as small points and thin black lines in callus tissue.

99 Cambial continuity was clearly seen in 90 days sections of combinations. Cambium  
100 bridge is curly form because of the callus mass and necrotic layers. Newly formed  
101 xylem and floem were seen as locally strands in callus tissue between scion and  
102 stock ( Fig. 3). But vascular tissues was not sufficient for strong graft formation.

103 In the sections taken on 120th days samples, callus was almost fill all gaps and  
104 vascular bridge were established between scion and stock. It was not seen  
105 incompatibility related histologically development (Fig. 4).

106 ( Figure 1-4 )

### 107 **3.2 Histological observations in “Chip” budding samples**

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

113 In 30 days sections, A strong callus formation were seen in all combinations.  
114 Necrotic layers formed soon after due to cuts was clearly seen in cut sides of stock  
115 and scion. Necrotic layers was broken by the newly formed callus tissues. There was  
116 not seen a clear evident related cambial differentiation (Fig. 5).

117 Clear cambial continuity was observed in 60 days samples of all combinations An  
118 intensive celluar activity was seen in all combinations. Cellular activity was not only  
119 for callus proliferation but also absorbsion of necrotic layers.. Necrotic layers were  
120 largely elimineted by callus. They were seen in cortex region close to bark as thin  
121 black lines and small black points in callus (Fig. 6). 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. 7).

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 successfully 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 histological or cellular development (Fig. 8).

( Figure 5-8 )

#### 4. DISCUSSIONS

Usually, a successful grafting includes formation of necrotic layers, callus proliferation, elimination of necrotic layers by proliferated callus, differentiation 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 an independent event. In subsequent days, cortex tissue starts to produce callus. In time, callus tissue continues to proliferation and spread into gaps placed between stock and scion. In the mean time, necrotic layers have been eliminated by callus. Callus absorbs most of the necrotic layers [17,18,19,20]. Also in this study, all grafts showed a similar 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 known that dead cells due to wounding are transformed to black necrotic strands or points. As a result of this, it was seen necrotic strands and points in callus and cut surface even 90 days samples. Necrotic layers were not completely absorbed by callus which proliferated from cortex. This result is in agreement with previous researches which indicate wounding is much in "T" buddings compared chip budding. On the other hand, Chip budding samples showed that wounding is a very small amount. As a result of small wounding, necrotic layers were a small amount and generally placed in cut sides of cortex. It was clearly that callus cells proliferated from cortex parenchyma of stock and scion. In the course of time, callus which spreads into gaps filled all gaps between scion and stock and necrotic layers were absorbed. Results are in agreement with previous researches [1,17,20].

In present reserach, 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 period samples of all combinations were seen smiliar observations. Results is agreement with previous reseraches [10,22,23]. According to [1] T budding is generally used in propagation of fruit trees and chip budding is not preffered due to it is not simplier and faster. But, in contrast [22] reported that chip budding gives higher graft success in apple. Researchers reported that chip budding was more effective than T budding [24]. Smiliar results were also reported by [25,26,27].

Researchers who studied on apple and pear sapling production by using “T”budding in Van ecological condition which have 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 between them in chip budding. 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.

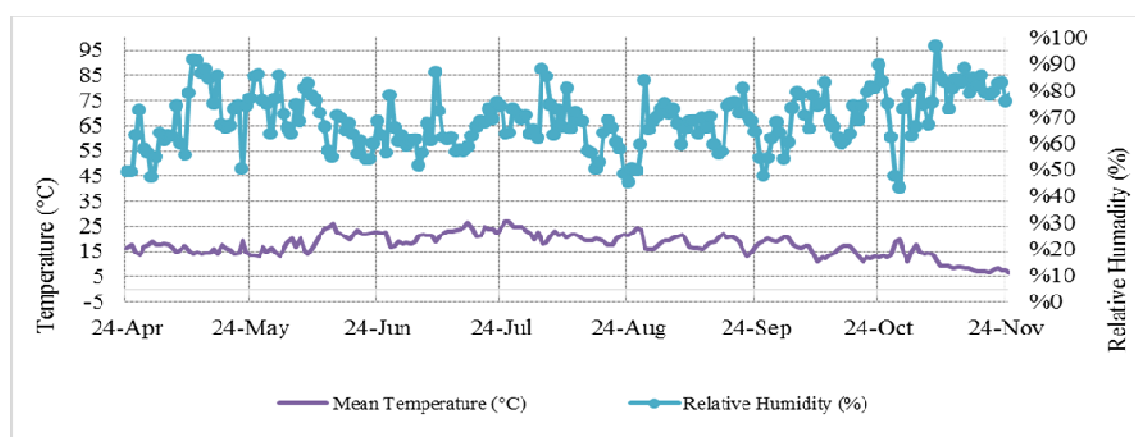
# REFERENCES

1. Hartmann H.T, Kester DE, Davies FT, Geneve LR. Plant propagation. principles and practices. seventh edition. regents / prentice hall international editions. Englewood cliffs, New jersey. USA, 2002.
2. Duran C and Gulek H.. Effects of the ecological factors on vegetation in river asins of northern part of mersin city (south of Turkey). Biological Diversity and Conservation. 2010;3(3): 137-152
3. Kose B. The Role and Importance of The Light and Temperature in Viticulture. Turk J Agric Res. 2004;1: 203-212
4. Kaya B and Aladad C. Precipitation, temperature and vegetation relations in the conditions of Konya. Selcuk University Journal of Social Sciences institute. 2009.;22: 465-478
5. Demirsoy H, Bilginer S. Anatomically Investigation of Graft Union in Some Compatible and Incompatible Peach/Plum Grafts Combinations. J. of Fac. of Agric. OMU. 2006;21(1):89-94
6. Ozkaynak E and Samancı B. Recent advances in environmental control in micropropagation, Batı Akdeniz Agricultural Research Institute, Derim. 2003;20 (1):450-462
7. Kadan H. and Yarılgac T.. Studies on propagation by dormant t-budding of apples and pears under van ecological conditions, Yuzuncu Yil University, Agricultural Faculty. Journal of Agricultural Sciences (J. Agric. Sci.). 2005;15(2): 167-176
8. Atkinson C.J, Else M.A, Taylor L. and Dover CJ. Root and Stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill). Journal of Experimental Botany. 2003;54 (385):1221-1229
9. Moore R. A Model for graft compatibility–incompatibility in higher plants, Amer.J.Bot., 1984;71(5):752-758
10. Simons RK. Graft union characteristics as related to dwarfing in apple (*Malus domestica* Borkh). Acta Hort. 1986;160: 57-66
11. Soumelidou K, Battey NH, John P, Barnett JR. The anatomy of the developing bud union and its relationship to dwarfing apple. Annals of Bot. 1994;74:605-611
12. Vachun Z. Rootstock for Apricot. The current situation and main problems. Acta Horticulturae, 1995:384:459-465.

- 218 13. Gryzb Z.S, Sitarek M. Growth and cropping of plums grafted on pixy rootstock  
219 and planted in differentiated density. VI International Symposium on Plum and  
220 Prune Genetics, Breeding, Pomology. 1998;478:14-16.
- 221 14. Kankaya A, Ozyigit S, Tekintas FE, Seferoglu G. Compatibility of Some Plum  
222 and Apricot Cultivars on Pixy Rootstock. Third National Horticulture Congress,  
223 Ankara, 1999;1: 295-299.
- 224 15. Errea P, Garay J, Marin A. 2001. Early detection of graft incompatibility in apricot  
225 (*Prunus armeniaca* L ) using in vitro techniques. *Physiologia Plantarum*.  
226 2001;112:135-141
- 227 16. Ikeuchi M, Sugimoto K and Iwase A. Plant callus: mechanisms of induction and  
228 repression, *Plant Cell*. 2013;.25(9): 3159–3173.
- 229 17. Stoddart FL, McCully ME. Effects of stocks and scion organs on the formation of  
230 graft union in coleus, a histological study, *Botanical Gazette* 1980;141(4): 401-  
231 412
- 232 18. Estrada-Luna A.A, Lopez-Peralta C, Cerdanes-Soriano E.. In vitro micrografting  
233 and the histology of graft union formation of selected species prickly pear cactus  
234 (*opuntia* spp). *Sci. Hort*. 2002;92:317-327
- 235 19. Sitarek M. Incompatibility problems in sweet cherry trees on dwarfing rootstock,  
236 *Latvian J. Of Agro*. 2006;9:140-145
- 237 20. Pina A and Errea P.. Influence of graft incompatibility on gene expression and  
238 enzymatic activity of UDP glucoze pyrophosphorylase. *Plant Sci*. 2008;174: 502-  
239 509
- 240 21. Dolgun O, Tekintas FE, Ertan E.. A histological investigation on graft formation of  
241 some nectarine cultivars grafted on pixy rootstock. *World Journal of Agricultural*  
242 *Sciences*. 2008;4(5): 565-568.
- 243 22. Kviklis AM. Rationalization of budding methods. *Horticultural Abstracts*,  
244 1986;56::8511
- 245 23. Tekintas FE, Dolgun O. An investigation on compatibility in some peach and  
246 nectarin cultivars grafted on almond seedlings. *Yuzuncu Yıl Univ. Journal of*  
247 *Agric. Faculty*. 1996; 6(1):51-54.
- 248 24. Skene D.S, Shepherd HR, Howard BH. Characteristic anatomy of union  
249 formation in T and chip-budded fruit and ornamental trees. *Journal of Horticultural*  
250 *Science*, 1983;58(13): 295-299.

25. Howard B.H, Skene DD, Coles JS. The effects of different grafting methods upon the development of one-year-old nursery apple trees. Journal of American Social and Horticultural Sciences. 1974;49(3): 287-295.
26. Stoyan I. The application of chip budding to fruit tree propagation. Horticultural Abstract. 1984;54-5098
27. Czarneck B. Comparative study of two methods of apple budding. Horticultural Abstract. 1990;60(2):3108.

Table 1. . Meteorogial records in graft union formation period



(T Budding sections)

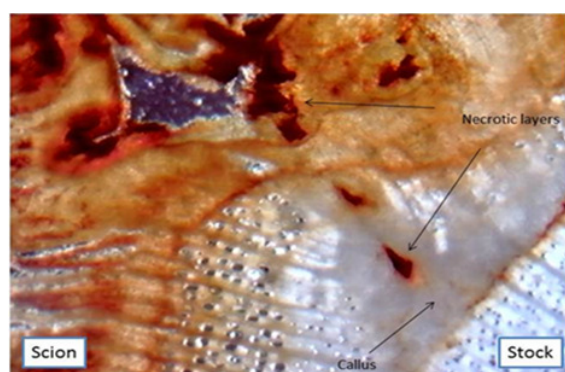
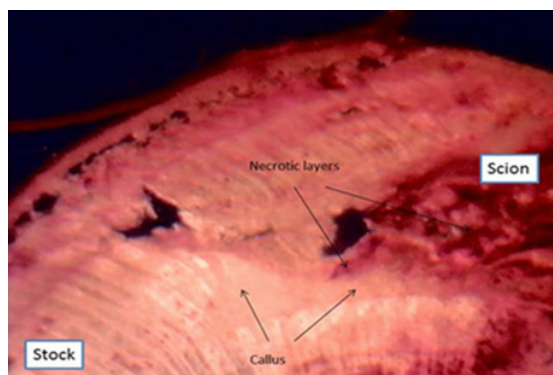
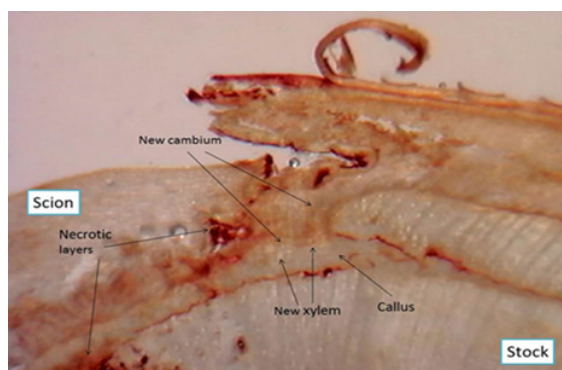


Fig 1.30 days section of Golden Delicious T budded on MM106



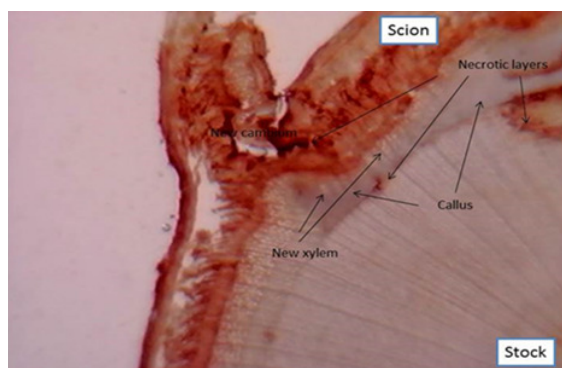
263

264 Fig 2. 60 days section of Granny Smith T budded on MM 106



265

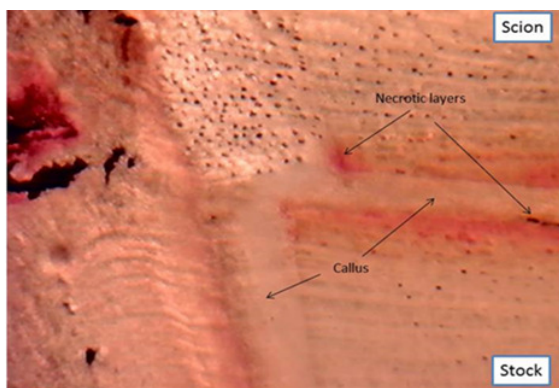
266 Fig 3. 90 days section of Mondial Gala T budded on MM106



267

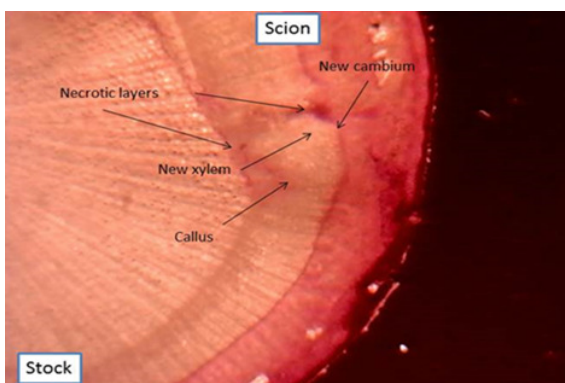
268 Fig 4. 120 days section of Red Chief T budded on MM106

269 (Chip Budding sections)



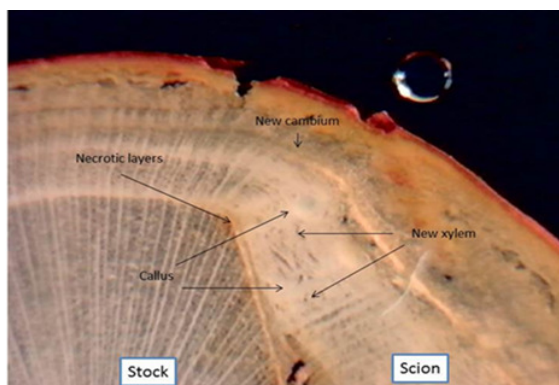
270

271 Fig 5.30 days section of chip budded Mondial Gala on MM106



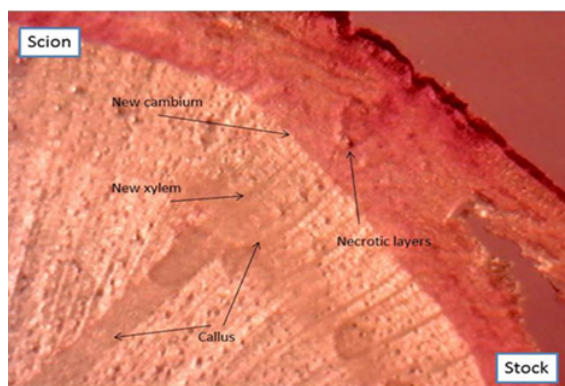
272

273 Fig 6. 60 days section of Red Chief chip budded on MM106



274

275 Fig 7.90 days section of Golden Delicious chip budded on MM106



276

277 Fig 8. 90 days section of Granny Smith T budded on MM 106