

EFFECT OF DEFOLIATION TIME OF SCION SHOOTS AND AGE OF ROOTSTOCK ON SUCCESS AND GRAFT DEVELOPMENT IN MANGO (*Mangifera indica* L.) CV. ZEBDA

El-Rouby, Kh A.; S. I. Habashy and A. B. Kamel

Trop. Fruit Division, Horticulture Research Institute, Agric. Res. Center.

ABSTRACT

This investigation was carried out in the Horticulture Research Station, El-Kasasin, Ismailia Governorate during the two successive seasons of 2005 and 2006, to study the effect of defoliation period of scion shoots and age of rootstock on grafting success and subsequently graft growth. Defoliation periods were 0.0, 4.0, 8.0 and 12.0 days prior grafting process and age of rootstocks were 1.2 and 3-years-old. Defoliation was conducted at 20th, 24th, 28th of March and 1st April and grafting process made 1st April. The obtained results showed that, defoliation of scion shoots and age of rootstock significantly affected graft success percentage and graft growth. The best treatment in this concern was defoliation period of 8 days prior detachment and grafting on 1-year-old rootstock which gave the highest success percentage, the shortest time for sprouting, the highest survival percentage, the highest length and diameter values, number of leaves per graft and the highest total leaf area. Healing zone was completely formed after 56 days with samples taken from 8 days defoliation prior grafting process and grafted on 1-year-old rootstock. However, other treatments formed the union zone after 63 days from grafting.

INTRODUCTION

Grafting has many uses among which are three (1) To propagate or assist in propagating plant varieties not otherwise conventionally propagated. (2) To substitute one part of plant for another and (3) To join plant each selected for special properties. In addition, grafting may be used to repair damage, to overcome stock/scion incompatibility, to invigorate weakly plants and to elucidate research problems (Leal, 1970 and Samaddar and Yadov, 1970). Grafting after 10 days from defoliation of scion gave 95% initial success and 75% survival after 6 months (Dhakal and Hoda, 1987). Defoliated scion 8 days earlier gave 70.90% graft take (Kulwall and Toyde, 1989). The age of rootstock did not significantly affect success percentage. Graft survival and subsequent plant growth were improved when 2 different rootstocks were used for single scion. Defoliation of scion shoot was not necessary (Gunjate, 1989). Scion of 2 new mango cultivars were defoliated a week before grafting on 3 different age rootstocks. Percentage of sprouting and survival were higher for Malika than for Amerepali but Amerepali gave more vigorous scion growth and larger leaves. Two years old rootstock gave higher percentage of sprouting and survival and more scion growth with larger leaves than 1 and 3 year old rootstocks (Baipai *et. al.* 1989). Bud wood was defoliated 0,4,8 or 12 days (before detachment) and stored for 3,5 and 7 days before grafting on 1-year-old seedling. The best results were obtained when scion shoots were defoliated 8 days before grafting (Roy and Hoda, 1994). The success of grafting was studied with rootstocks that were 1,2 and 3 year-old and scion that had just been defoliated or had been defoliated 5,10 or 15 days before grafting. Highest grafting success rate and shortest time to sprouting were obtained with scions defoliated 10 days before grafting and 1-

year-old rootstock (Jha and Syamal, 1995). Graft take was greater (90-100%) when scions were cut 5-10 days later and used for grafting. It was only 45% with freshly defoliated scions (Dod *et. al.* 1996). The effect of defoliation (6,8 or 10 days prior to detachment from mother plant) on grafting success were investigated in mango cv. Dashehari. The best treatment for promoting successful grafting was defoliation 8 days prior to detachment. This treatment promoted early sprouting, highest success and highest survival after 6 months (Rajesh *et. al.* 1998 and 1999). The effect of defoliation period, age of scion and age of rootstock on the success of grafting in mango was evaluated. Defoliation of 6 days prior to grafting required the shortest time for sprouting and recorded the highest values for length, diameter of scion, number of leaves per graft, total leaf area, graft survival and grafting success (Jha and Brahmashari, 2002). Concerning union zone formation, 4 stages could be noticed: (1) callus tissues were formed at peripheral union zone between scion and stock after few days from grafting resulted in firm attachment of the components, (2) Callus cells gradually increased and filled the internal air pockets between stock and scion and few secondary phloem and xylem tissue were differentiated, (3) The internal pockets became narrowest which filled with proliferation callus. Differentiation and the appearance of conducting tissues were continued after 49 and 56 days and air pockets were disappeared which revealed that union zone was after formed and (4) The perfect graft union was formed after 56 days from grafting. Simons, 1982; Zagzog, 1988; El-Rouby, 1994 and Walaa, 2007.

MATERIALS AND METHODS

This investigation was carried out in the Horticultural Research Station, El-Kasasin, Ismailia Governorate during the two successive seasons of 2005 and 2006 to study the effect of defoliation period of scion shoots and age of rootstocks on percentage of success and graft development. For this purpose, ten healthy Zebda cv. mango trees were chosen as a source of scions, then, 360 scion shoots similar in diameter (4-5 cm) were divided in 4 groups each of 90 scion shoots and labeled. Leaf defoliation was conducted at 20th, 24th, 28th of March and 1st of April for each group in both studied seasons. Then 120 similar nucleus seedlings of Zebda mango cv., vigorous and free diseases were prepared from each of 1,2 and 3 year-old as rootstocks and a randomized block design of 30 plants (10 plots replicated 3 times) was adapted in each defoliation period of 0.0, 4, 8 and 12 days prior grafting during each season. The grafts were made 25 cm above the soil surface and grafting process was conducted at 1st April during each season.

The treatments were as follows:

- 1- Scions were freshly defoliated (control) and grafted by cleft method on 1, 2,3year-old rootstocks.
- 2- Scions were defoliated 4 days before grafting on 1,2 or 3 –year-old rootstocks.
- 3- Scions were defoliated 8 days before grafting on 1,2 or 3 –year-old rootstocks.

4- Scions were defoliated 12 days before grafting on 1,2 or 3 –year-old rootstocks.

The grafted plants were kept under greenhouse and subjected to normal agricultural practices used in the nursery. The following parameters were calculated as follows:

- 1- Success percentage.
- 2- Graft sprouting (Time from grafting practice to emergence of new growth) (day).
- 3- Survival percentage (retained successful grafts after 6 months from grafting practice)

$$= \frac{\text{No of retain grafts}}{\text{Initial No of successful grafts}} \times 100$$

- 4- Graft length and diameter (cm) were determined after 6 months from grafting.
- 5- Number of leaves per graft and total leaf area (cm²): total area of leaf were determined according to Saidha and Rao(1988) formula:
Leaf area (cm²) = 3/4 (leaf length x leaf width)

Healing zone formation:

Samples of each group in grafts were taken after 2,3,4,5,6,7 and 8 weeks from grafting practice. At the laboratory, samples were cleaned and cut in suitable parts, then killed and fixed in FAA solution (formalin alcohol acetic acid) for at least 36 hours. The dehydration of the samples were performed in increasing concentration of ethylalcohol then clarified in xylol and finally infiltrated with paraffin wax and embedded. Paraffin wax with samples were sectioned by rotary microtome and the sections were stained with safranin then cleared in xylol and mounted in Canada balsam (Johansen, 1940).

Statistical analysis of the obtained data was thoroughly carried out and the individual comparisons were compared by using the new least significant differences (New LSD) according to Waller and Duncan (1969). Interactions studies were carried out as reported by Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

1- Percentage of success:

Data in Table 1 clearly showed that, defoliation 8 days prior detachment gave the highest values of grafting success in average (69.3 and 69.5%) as compared with defoliation 4 days prior grafting (59.1 and 58.7%) or 12 days (59.9 and 59.3%). However the lowest values in average (57.0 and 56 %) were obtained from fresh defoliated scions (0.0 day) in the first and second seasons respectively. Moreover, using 1-year-old rootstock led to superior success percentage (69.3 and 69.9%) as compared with 2 and/or 3-year-old rootstock in descending order. In addition, the highest success percentages (87.3 and 90.2%) were recorded with defoliation 8 days prior grafting practice on 1-year-old rootstock. However, percentage of success

clearly decreased with increasing age of rootstock, this is true in both studied seasons, respectively.

Differences between treatments and the interaction between the two factors (defoliation period x age of rootstock) were statically significant in both studied seasons. These results coincident the finding of Dhakal and Hoda(1987);Kulwall and Toyd (1989); Baipai *et al.* (1989); Roy and Hoda (1994); Jha and Syamal (1995); Dod *et al.* (1996); Rajesh *et al.*(1998)and (1999) and Jha and Brashmashari, (2002). However, Gunjet (1989) mentioned that, defoliation of scion shoot was not necessary.

Table (1): Effect of defoliation period of scion shoot and age of rootstock on success (take) percentage in Zebda cv. mango during 2005 &2006 seasons.

Rootstock Defoliation	First season				Ave.	Second season			Ave.
	1	2	3	1		2	3		
0 days	61.44	57.3	52.3	57.0	59.7	58.1	50.2	56.0	
4.0 days	65.1	58.0	54.1	59.1	64.3	58.7	53.0	58.7	
8.0 days	87.3	64.5	56.0	69.3	90.2	63.6	54.8	69.5	
12.0 days	63.4	60.1	53.1	58.9	65.3	60.4	52.3	59.3	
Average	69.3	60.0	53.9		69.9	67.0	52.6		

New LSD at 0.05 Defoliation Period = 2.03 Rootstock Age=1.78
Defoliation Period x Rootstock Age= 1.24

2- Graft sprouting:

The obtained results in Table 2 revealed that, with different ages of rootstocks, defoliation 8 days prior grafting gave the lowest number of days required for graft sprouting in average (21.4 and 21.6 days). However, 1-year-old rootstock was superior (21.2 and 21.1 days) than 2-year-old rootstock (24.3 and 24.7 days) and 3-year-old rootstock (25.7 and 26.0 days). Furthermore, the shortest period of sprouting (16.7 and 17.3 days) were obtained from scions defoliated 8 days before grafted on 1-year-old rootstock, while the tallest periods of sprouting (7.1 and 27.7 days) were produced from scions had just been defoliated before grafting (control) and grafted on 3-year-old rootstock. This is clear in both studied seasons, respectively. These results were agree with the findings of Paipai *et al.* (1989); Jha and Syamal (1995); Rajesh *et al.*(1998) and Jha and Brahmashari(2002).

Table (2): Effect of defoliation period of scion shoot and age of rootstock on graft sprouting of Zebda cv. mango during 2005 &2006 seasons.

Rootstock Defoliation	First season				Ave.r	Second season			Ave
	1	2	3	1		2	3		
0 days	24.3	26.2	27.1	25.9	24.6	27.5	27.7	26.6	
4.0 days	23.0	24.1	25.7	24.3	22.3	24.3	26.1	24.2	
8.0 days	16.7	22.9	24.5	21.4	17.3	23.1	24.3	21.6	
12.0 days	20.7	24.0	25.3	23.3	20.1	24.0	25.7	23.3	
Average	21.2	24.3	25.7		21.1	24.7	26.0		

New LSD at 0.05 Defoliation Period= 2.16 Rootstock Age=1.42
Defoliation Period x Rootstock Age= 1.04

3-Survival percentage:

Concerning the effect of defoliation period on survival percentage data in Table 3 show that, the highest average of survival grafts (66.8 and 67.8%) were obtained from defoliation period of 8 days prior detachment. However the lowest percentage of survival grafts (52.7 and 51.1%) produced from 0.0 day period (control) while periods of 4 and 12 days prior detachment gave intermediate values in this concern. In addition, 1-year-old rootstock was superior in average (64.2 and 65.1%) as compared with 2-year-old rootstocks (56.8 and 56.7%) and the inferior 3-year-old ones (52.5 and 50.9%). Thus, the highest percentage of survival grafts (84.3 and 53.3%) were obtained from period of 8 days defoliation of scion shoots prior detachment and grafted on 1-year-old rootstocks in the first and the second seasons, respectively.

The obtained results were in agreement with Dhakal and Hoda, (1987); Paipai *et al.* (1989); Roy and Hoda (1994); Rajesh *et al.* (1998) and (1999) and Jha and Brahmashari (2002).

Table (3): Effect of defoliation period of scion shoot and age of rootstock on percentage of survival grafts of Zebda cv. mango during 2005 &2006 seasons

Rootstock Defoliation	First season				Ave	Second season			Ave
	1	2	3	1		2	3		
0 days	55.4	52.0	50.6	52.7	53.1	51.4	48.7	51.1	
4.0 days	56.8	55.3	52.3	54.8	57.8	54.6	51.4	54.6	
8.0 days	84.3	61.0	55.0	66.8	88.7	61.5	53.3	67.8	
12.0 days	60.1	58.9	52.0	57.0	60.8	59.1	50.2	56.7	
Average	64.2	56.8	52.5		65.1	65.7	50.9		

**New LSD at 0.05 Defoliation Period= 4.51 Rootstock Age=2.76
Defoliation Period x Rootstock Age=**

4-Graft length and diameter:

The effect of defoliation period of scion shoots and age of rootstock on graft length and diameter were presented in Table 4 Data clearly show that, defoliation 8 days prior grafting gave the highest average of graft length (16.4 and 16.3 cm) and diameter (0.9 and 0.8 cm) as compared with 4, 12 or 0 (control) days defoliation periods. Meanwhile, age of rootstock also affected length and diameter of graft which, 1-year-old rootstock gave the highest values of graft length in average (15.4 and 15.3 cm) and diameter (0.7 and 0.7 cm) in comparison with 2 or 3-year-old rootstocks in the first and the second seasons, respectively. In addition, the longest graft (17.3 and 17.9 cm) and the widest (0.8 and 0.9 cm) were obtained when scion shoots were defoliated 8 days prior detachment and grafted on 1-year-old rootstock, while the shortest graft (14.0 and 13.5 cm) and the thinnest (0.5 and 0.5 cm) were recorded with scion that had just been defoliated (control) and grafted on 3-year-old rootstock in the first and the second seasons, respectively. These results were similar with Roy and Hoda(1994), Rajesh *et al.*, (1998) and Jha and Brahmashari (2002).

Table (4): Effect of defoliation period of scion shoot and age of rootstock on graft length and diameter of Zebda cv. mango during 2005 &2006 seasons.

Rootstock Defoliation	First season						Aver		Second season						Aver	
	1-year-old		2-year-old		3-year old		L	D	1-year-old		2-year-old		3-year old		L	D
	L	D	L	D	L	D			L	D	L	D	L	D		
0 days	14.3	0.6	14.1	0.5	14.0	0.5	14.1	0.5	13.9	0.6	13.6	0.5	13.5	0.5	13.7	0.5
4.0 days	15.2	0.7	14.7	0.5	14.3	0.5	14.7	0.6	15.0	0.6	14.5	0.5	14.1	0.5	14.5	0.5
8.0 days	17.3	0.8	16.1	0.7	15.9	0.6	16.4	0.7	17.9	0.9	15.8	0.8	15.3	0.6	16.3	0.8
12.0 days	14.9	0.7	14.2	0.5	14.1	0.5	14.4	0.6	14.5	0.7	14.0	0.6	13.6	0.5	14.0	0.6
Average	15.4	0.7	14.8	0.6	14.6	0.5			15.3	0.7	14.5	0.6	14.1	.05		

New LSD at 0.05 =For length
Defoliation Period = 1.27
Rootstock Age= 1.16
Defoliation Period x Rootstock Age= 2.07
For Diameter
Defoliation P eriod= 0.01
Rootstock Age= NS
Defoliation Period x Rootstock Age= 0.10

5- Number of leaves & total leaf area:

The obtained data in Table (5) showed that, defoliation period of 8 days prior grafting practice gave the highest number of leaves per graft as well as highest leaf area (14.0 and 13.0 leaves) and (131.4 and 135.7 cm²) in comparison with other tested periods. However, 1-year-old rootstock also promoted the average of leaves number per graft (11.2 and 11.4 leaves) and gave the highest average of leaf area (105.1 and 110.2 cm²) as compared with 2-year-old rootstock (10.4 and 10.5 leaves) and (99.8 and 100.4 cm²) and/or 3-year-old rootstock (10.0 and 10.2 leaves) and (93.7 and 98.4 cm²) for number of leaves and graft total leaf area, in the first and second seasons, respectively. In addition, the highest values of leaves number (15.6 and 15.7) and total leaf area (146.1 and 15.4 cm²) were obtained from defoliation of scion shoot 8 days prior detachment and grafted on 1-year-old rootstock. On the other hand, the lowest number of leaves per graft (7.6 and 8.0) and the narrowest area per leaf (72.3 and 77.1 cm²) were found when fresh defoliated scion shoots (control) were grafted on 3-year-old rootstocks, this is clear in both studied seasons, respectively.

These results are in agreement with the findings of Roy and Hoda, (1994); Jha and Syamal (1995) and Jha and Brahmashari (2002).

Healing zone:

Samples were taken after grafting or those taken up to 14 and 21 days failed to give normal sections which the scions/stock union zone was separated through the sectioning (Fig1). From the microscopic examination of cross sections in samples taken after 28 days from grafting callus tissues were formed in union zone between scion and stock tissues. The callus formation was proliferated from tissues mainly near cambium and seemed in a good connection between stock and scion (Fig 2). Callus formation was greater in the rootstock side compared with the scion. These observations are occurred with scions of 8 days defoliation prior grafting practice treatments with 1,2 and 3 year-old rootstocks.

Sections from samples taken after 35 days from grafting showed that, callus cells gradually increased and filled the internal air pockets between stock and scion and few tissues from secondary phloem and xylem were differentiated from the cambial layer due to apparently contact with the callus tissues with the cambium of stock and scion. (Fig 3). From the microscope examination of cross sections in grafting taken after 42 days from grafting, the internal air pockets became narrowest with filled with proliferation callus cells within in the two healed parts. Most vascular tissues initiated and formed via the stock and scion from the callus tissue (Fig 4). After 49 days from grafting, a narrowest air pockets between parts were formed which revealed that union zone was often complete in view but not perfect (Fig 5). With the time progressed from grafting and after 56 days, graft union became perfect and the new tissues appeared homogenous. The air pockets were disappeared and the vascular tissues were progressed into the parentally mother cells formed with stock and scion a good complete connection (healing zone Fig 6). These stages were completely with samples taken from 8 days defoliation prior grafting practice and grafted on 1-year-old rootstock. However, other treatments formed the union zone after 63 days from grafting. These results were in agreement with the finding of Simons (1982); Walaa (2007) on mango; Zagzog (1988) on guava and El-Rouby (1994) on Annona.

It can be concluded that, defoliation of scion shoots 8 days prior grafting and using 1-year-old rootstock gave the highest success percentage, early graft sprouting, the highest survival percentage, the best subsequent graft growth and highest number of leaves as well as total leaf area per graft, in addition to shortest time for healing zone formation.

Removing of leaves caused accumulation of nutrients in buds and increased auxin amount in apical and terminal buds, whereas, a rapid and long term responses occur for morphogenesis. Auxin increased cell division activity and enhanced callus cells formation. Auxin also increased respiration rate and a positive relation were found between plant growth and auxin concentration (Rubinstein and Leopold, 1963 and Rubery, 1981).

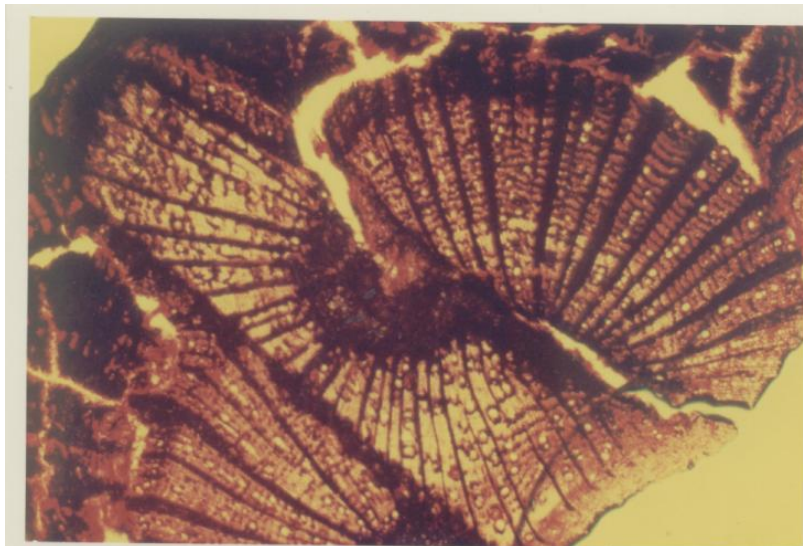


Fig.(1): C.S. in union zone after 28 days X 250
Notice: Sections were separated .

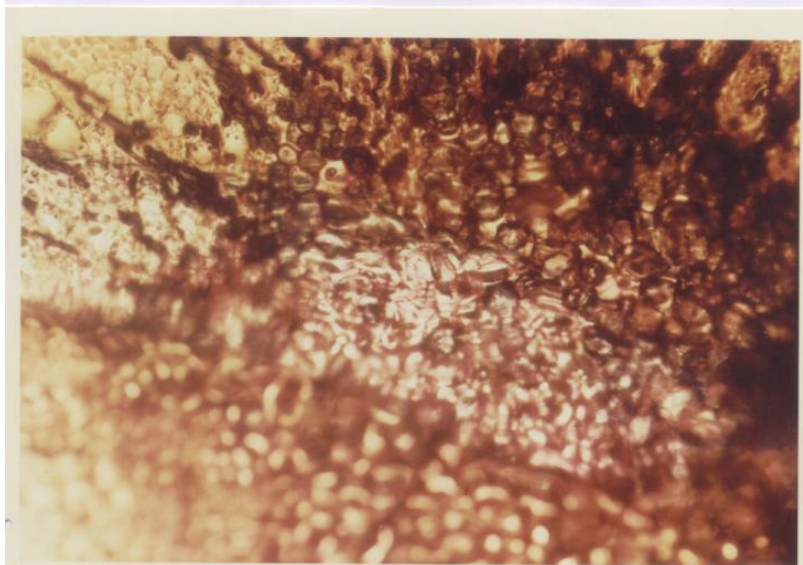


Fig.(2) : C.S. in union zone after 35 days X 250
Callus tissues were formed at the peripheral of union zone
between scion and rootstock (initial stage of healing) .

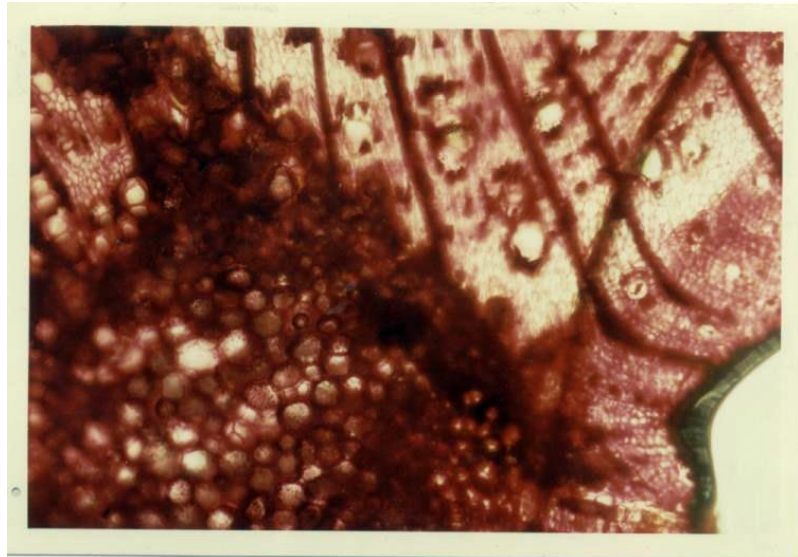


Fig. (3) :C.S. in union zone after 42 days X 250
Callus cells gradually increased and filled
the internal air pockets between stock and scion .

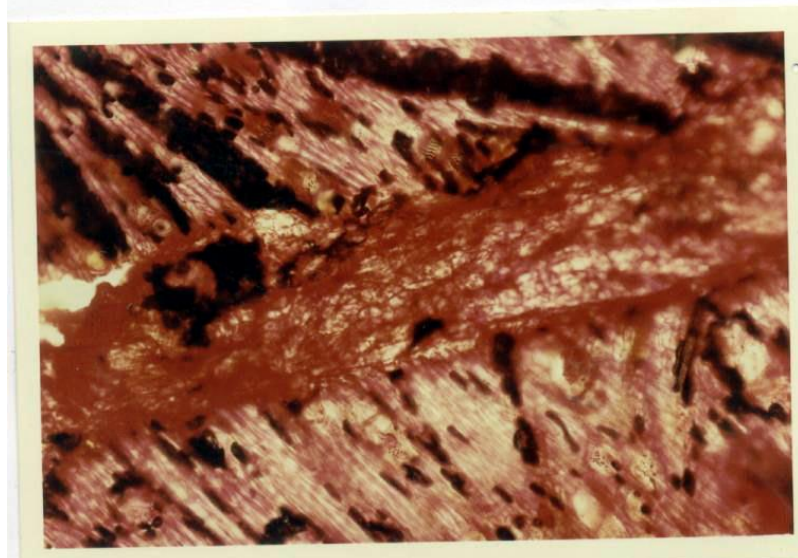


Fig. (4): C.S. in union zone after 49 days X 250
Few tissues from secondary phloem and xylem
differentiated from the cambial layers in callus.

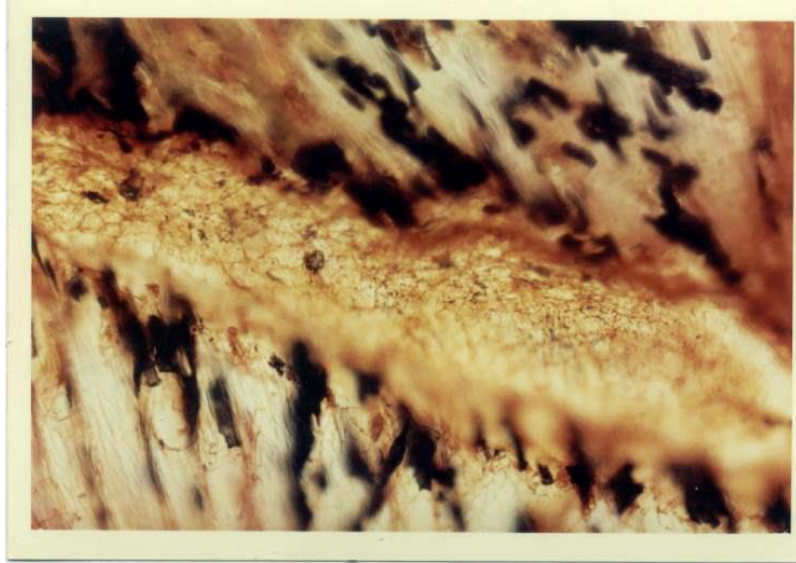


Fig. (5); C.S. in union zone after 49 days X 250
The internal air pockets became narrowest and filled with proliferation callus.

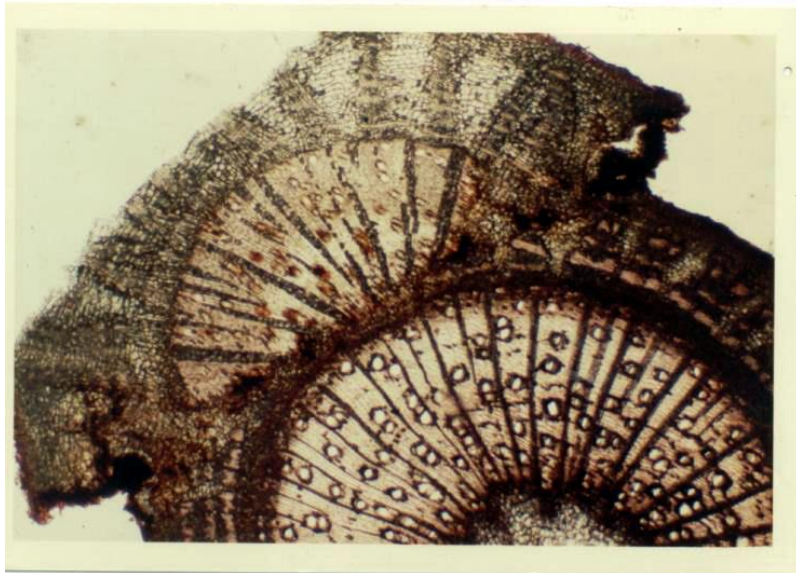


Fig. (6): C.S in union zone after 56 days X 250
Perfect graft union was formed.

REFERENCES

- Baipai, N. P.; A. R. Singh; V. Yati and O. P. Chaturvedi (1989): Effect of cultivars and age of rootstock on the performance of veneer grafting in mango. *Acta Hort.*, 231, 259-262.
- Dhakal, B. R. and Hoda, M. N. (1987): Effect of defoliation period and storage of scion shoots on success of veneer grafting in mango. *Bihar Agric. College Sobour, India, Progressive Hort.*, (19):3-4, 167-170.
- Dod, V. N.; S. G. Bharad and P. C. Pogar (1996): Vigour of in-stun soft wood mango grafts in relation to defoliation period and storage of scion shoots. *Commercial fruit nursery, Akola, India, J. Soils and Crops*, (6):202-203.
- El-Rouby, Kh. A. (1994): Studies on the propagation of *Annona*. MSc Thesis Fac Agric Zagazig Univ. Egypt.
- Gunjate R. T. (1989): Standardization of stone grafting for the Konkan region. *Acta Hort.*, 231:164-167.
- Jha, U. N. and V. S. Brahmashari (2002): Success in stone grafting of mango as influenced by the defoliation of scion and age of rootstock and scion shoots. *India Hort. J.*, 15(1):9-17.
- Jha, B. K. and M. M. Syamal (1995): Effect of defoliation of scion and age of rootstock for veneer grafting in mango (*Mangifera indica*) cv. Amrapali. *India Hort. J.*, 12(1):109-112.
- Johansen, D. A. (1940): *Plant Microtechnique*. McGraw Hill Book Company New York and London.
- Kulwall, L. V. and G. S. Toyde (1989): Studies on propagation of mango varieties by soft wood grafting under Akola condition. *Acta Hort.*, 231:256-258.
- Leal, F. J. (1970): Notes on guanabana (*Annona muricata*) in Vinzuela. *Proc. Trop. Amer.Soc. Hort. Sci.*, 14:118-121.
- Rajesh, K.; B. P. Jain and R. Kumar (1998): Effect of bud wood defoliation and storage on the success of soft wood grafting in mango (*Mangifera indica*) cv. Dashehari. *India Hort. J.* 11(1):49-54.
- Rajesh, K.; C. Singh and R. Kumar (1999): Stone grafting in mango and effect of defoliation and storage of bud wood. *India J. Research* 11(1):75-76.
- Roy, S. and M. N. Hoda (1994): Effect of period of defoliation and storage of bud-wood on veneer grafting in mango. *India J. Appl. Biology* 4(1-2):30-32.
- Rubery, P. H. (1981) : Auxin receptors. *Ann. Rev. Plant Physiol.* 32:569-596.
- Rubinstein, B. and A. C. Leopold (1963) : Analysis of the auxin control of bean leaf abscission. *Plant Physiol.* 38: 262.
- Saidha, T. and V. N. Rao (1988): A rapid method for leaf area measurement in mango. *India J. Hort.*, 42(1-2):71-73.
- Samaddar, H. N. and P. S. Yadov (1970): A note in the propagation of cashewnut, avocado, jackfruit and custard apple. *South Indian Hort.*, 18:47-49 (*Hort. Abst.*, 42:2947).

Table (5): Effect of defoliation period of scion shoot and age of rootstock on number of leaves per graft and total leaf area of Zebda cv. mango during 2005 & 2006 seasons.

Rootstock a Defoliation	First season						Aver		Second season						Aver	
	1year-old		2-year-old		3-year old		No	Area	1year-old		2-year-old		3-year old		No	Area
	No	Area	No	Area	No	Area			No	Area	No	Area	No	Area		
0 days	8.0	74.9	8.0	73.1	7.6	72.3	7.9	73.4	8.3	80.0	8.0	77.1	8.0	77.1	8.1	78.1
4.0 days	9.3	87.1	8.4	80.0	8.4	78.7	8.7	81.9	9.60	92.6	8.3	78.7	8.1	78.1	8.7	83.1
8.0 days	15.6	146.1	13.7	130.2	12.6	118.0	14.0	131.	15.7	151	13.5	130	13.0	125	14.1	135.7
12. days	12.0	112.4	11.4	115.7	11.3	105.8	11.6	111.	12.1	116.	12.0	115	11.7	112	11.9	115.1
Average	11.2	105.1	10.4	99.8	10.0	93.7			11.4	110.	10.5	100.	10.2	98.4		

New LSD at 0.05 = For
 Defoliation Period = 2.24
 Rootstock Age= 2.54
 Defoliation Period x Rootstock Age= 2.04
 For Area
 Defoliation Period = 8.71
 Rootstock Age= 7.31
 Defoliation Period x Rootstock Age= 7.62