

Effect of Grafting Dates, Methods on Success and Growth of Mandarin (*Citrus reticulata* Blanco) Sapling

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Abstract

A field experiment was conducted at National Citrus Research Program (NCRP), Paripatle, Dhankuta to standardize the time and method of grafting and to assess the success and growth of mandarin saplings. Scions taken from 'Khoku Local' mandarin were grafted onto one-year-old trifoliate orange seedling rootstocks by shoot tip and veneer methods in eight different dates starting from 29th October, 2009 to 12th February, 2010 at 15 days interval. The grafts were planted inside closed tunnel of jute and plastic sheet at 10×10 cm spacing in 50×60 cm² experimental plots in two factorial split-plot design with three replications per treatment, containing 30 grafts per plot. The grafting methods were allotted on main-plots and the dates on sub-plots randomly. The success and growth of sapling was significantly affected by the dates while the methods had no effect. Study results showed that the highest success (96.11 %) was found on 13th January, followed by 28th January (91.11%) and the least (51.67%) on 29th October grafting. The mortality of sprouted grafts was the lowest (0.17%) in 29th December grafting, while it was observed higher in earlier and late season grafting. Observation on growth taken at 180 days after grafting in successful grafts showed that 29th October had the highest number of primary branches (2.2); 14th November had the highest number of leaves (48.47) and secondary branches (2.505); 29th December gave the maximum sapling height (32.86 cm); 13th January produced the lowest number of leaves (21.93) per sapling and 28th January produced the lowest number of primary (1.533) and secondary branches (1.172) per sapling. From the study it was revealed that in Dhankuta, the most appropriate time of grafting was 2nd to 4th week of January.

Key words: citrus, propagation, callus, cambium, mortality, graft success

Introduction

Citrus is the most important fruit crop of Nepal. APP (1995) has envisaged citrus as the number one priority crop for mid-hill region. Citrus is commercially cultivated in 42 mid-hill districts (Regmi *et al.* 2009). Among citrus fruits, mandarin is the most important and popular fruit. It is mainly cultivated throughout the mid-hill regions of Nepal ranging the elevation from 750 m to 1,400 m above mean sea level which stretches from the East to the Far-western region (Shah 1992). Total area, production and productivity of mandarin of Nepal at present is 24,284 ha, 166,612 mt and 10.89 mt/ha respectively (MOAC 2012). Mandarin is the major fruit of Dhankuta which covers

about 718 ha, with total production and productivity of 4,388 mt and 9.20 mt/ha respectively (MOAC 2012).

The productivity of citrus fruit in Nepal is still very low. Several factors are associated in reduced yield. Citrus decline due to diseases and poor nutrient management is a serious problem in Nepal (Baral 2008). *Phytophthora* root rot, huanglongbing (HLB), citrus tristeza virus (CTV), powdery mildew, citrus blight, gummosis etc. are the major diseases of citrus associated with citrus decline in Nepal. Regmi *et al.* (2009) reported seedling trees are highly susceptible to *Phytophthora* root rot disease which is one of the devastating diseases of citrus. Therefore, grafting onto

trifoliolate orange rootstock could be the solution of the citrus decline malady as it is tolerant to *Phytophthora* root rot, resistant to the citrus tristeza virus, less susceptible to blight and extremely suitable for the heavier soil conditions of the country.

The demand of citrus saplings is rising annually. Government of Nepal has put the target to increase citrus areas up to 35,000 ha by 2015. Thus, the area under citrus is to be increased with a rate of more than 1,000 ha per year (Regmi & Shrestha 2001). The annual requirement of citrus saplings is estimated as 300,000 in Nepal. To meet the escalating demand of the saplings, nursery persons should adopt suitable method of grafting at right time.

Success of grafting is highly influenced by the several factors like temperature, relative humidity, moisture and plant water, growth stage of scion and rootstock, air, method of grafting, genetic relationship between the stock and the scion etc. (Hartmann *et al.* 2007). Few studies conducted in citrus in western and mid-western regions of Nepal revealed that winter season is the best time for grafting. In Dhankuta, Paudyal (1999) observed that November is suitable for grafting. Poon (1999) reported in mandarin that 83.75% success with tongue grafting, 85.80% with saddle grafting, 88.73% with shoot tip, 90.35% with cleft and 91.75% with veneer method. For mandarin sapling production, Gautam *et al.* (2001) suggested that grafting from 16th to 31st January was the best time for Lumle condition, who observed the maximum success (87.50%) on 31st January grafting. Similarly, Adhikari (2006) reported the highest graft success (79.73%) in acid lime on 16th January by shoot tip method.

Several studies were conducted in the past on citrus propagation at different time with different methods of grafting at different agro-ecological regions of Nepal. However, in Dhankuta, study was limited in pummelo, which is mainly cultivated in Terai regions. Studies and documentation was lacking at Dhankuta in mandarin. In this context, this study was undertaken to standardize the time and method of grafting and assess the subsequent growth of mandarin saplings.

Methodology

The experiment was conducted at National Citrus Research Program, Paripatle, Dhankuta in winter

season. Scions from the mother plant of 'Khoku Local' mandarin were grafted onto one-year-old trifoliolate orange seedling rootstocks by shoot tip and veneer methods on eight different dates starting from 29th October, 2009 to 12th February, 2010 at 15 days intervals. The grafts were planted inside the closed tunnel made from bamboo splits, jute and plastic sheet at 10×10 cm spacing in 50×60 cm² experimental plots in two factorial split-plot design. Treatments were replicated three times. There were a total of 48 experimental plots. Thirty grafts were planted in each experimental plot. The methods of grafting were allotted on the main plots and the dates on the sub-plots randomly. The beds were prepared by digging with spade three times. At final digging, vermi-compost (Nitrogen 1.25-2.50%, Phosphorus 0.75-1.60% and Potash 0.50-1.10%) was mixed thoroughly with soil. Each experimental plot received 5 kg vermi-compost (4kg per plot at final bed preparation and 1kg per plot at top dressing, 4 months after grafting). The tunnel was made by bamboo splits and jute sheet cover from inside and the plastic cover from outside. The height of tunnel at centre was 50 cm from the level of bed. Bamboo splits were used for construction of tunnel by bending and fixing them in a semi-circle shape in the ground. Irrigation, desuckering, weeding, hoeing and topdressing, plant protection, opening and closing of tunnel, removal of plastic laces were done as intercultural operations. First data were taken at 15 days after grafting and continued at every 15 days intervals. Statistical analysis was done by MSTAT-C package and mean separation by DMRT. Graft success and mortality was calculated with the following formulae:

Graft success

Grafts having sprouted scion were recorded in successful graft whereas unsprouted and dead scions were considered as failure ones. In some grafts late sprouting was also observed and finally, sprouting percentage was considered as the graft success.

$$\text{Graft success (\%)} = \frac{\text{Number of sprouted graft}}{\text{Total number of grafts}} \times 100$$

Mortality

All the sprouted scions do not produce successful grafts. Death of sprouted grafts was observed during the study which was recorded in mortality percentage. Mortality percentage was calculated with the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead grafts after sprouting}}{\text{Total number of sprouted grafts}} \times 100$$

Results and Discussion

Graft success

The effect of methods of grafting was observed non significant from initial to final observation. However, the maximum success (82.08%) was given by veneer

method as compared to shot tip method (77.78%). The effect of dates on success and growth of mandarin was found highly significant. At final observation, success percentage is varied from 51.67% to 96.11%. The highest graft success (96.11%) was recorded in sapling prepared on 13th January. Grafting on 28th January gave 91.11% graft success which was statistically at par with 13th January grafting (Table 1).

Table1. Effect of dates of grafting on graft success of mandarin in Paripatle, Dhankuta, 2009/2010

Dates of grafting	Sprouted scion (%)				
	15 DAG	30 DAG	45 DAG	60 DAG	180 DAG
29 th October	21.11 ^a	42.22 ^a	51.11 ^c	51.67 ^d	51.67 ^f
14 th November	11.67 ^b	34.44 ^{bc}	67.78 ^b	71.11 ^c	72.22 ^e
29 th November	5.00 ^c	28.89 ^{cd}	66.11 ^b	72.22 ^c	73.33 ^e
14 th December	3.33 ^c	26.67 ^{de}	68.33 ^b	76.11 ^{bc}	81.67 ^d
29 th December	3.33 ^c	22.78 ^{de}	78.33 ^a	84.44 ^a	88.89 ^{bc}
13 th January	5.00 ^c	21.67 ^e	77.22 ^a	86.67 ^a	96.11 ^a
28 th January	6.67 ^c	26.67 ^{de}	75.00 ^{ab}	86.11 ^a	91.11 ^{ab}
12 th February	15.00 ^b	40.56 ^{ab}	71.11 ^{ab}	80.00 ^{ab}	84.44 ^{cd}
Mean	8.88875	30.4875	69.37375	76.04125	79.93
CV (%)	37.50	17.22	9.96	6.99	6.36
F value	22.48**	13.18**	9.41**	28.34**	46.19**
CD (P<0.05)	3.94	6.21	8.17	6.28	6.01
SEm±	1.36	2.14	2.82	2.17	2.07

SEM± = Standard error of mean difference, CV = Coefficient of variation, CD (Pd^{0.05}) = Critical difference at probability value 0.05, Treatment means followed by common letter(s) within a column are not significantly different at 5% by DMRT, DAG=Days after grafting

The maximum success of grafting (96.11%) was recorded on 13th January in Paripatle, Dhankuta at 25.69°C average tunnel temperature and 90±5% relative humidity. At the same time, average air temperature and relative humidity was recorded as 12.52°C and 63.07% respectively. The temperature and relative humidity inside the tunnel was observed about 2-times and 1.5-times higher respectively as compared to air temperature and relative humidity which was achieved

by closing all the edges of plastic sheet immediately after the irrigation of grafts and soaking of jute sheet. Due to the effect of temperature on callus activity, the percentage of success of grafting was observed minimum (51.67%) on 29th October at 36.76°C of average tunnel temperature, then started rising up to the 13th January (96.11%) at 25.69°C and declining started from 28th January (91.11%) at 27.60°C and onwards (Figure 1)

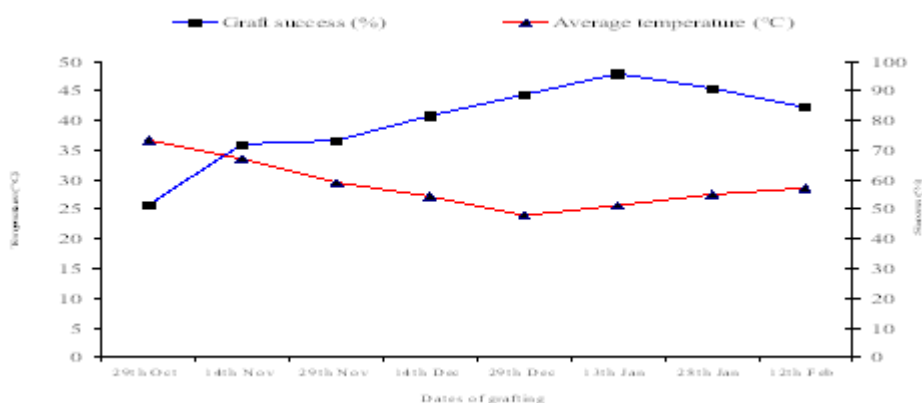


Fig.1. Relation of average tunnel temperature with graft success in different dates of grafting in Paripatle, Dhankuta, 2009/2010

Present finding was also supported by Poon (1999). He reported 88.73% success with shoot tip and 91.75% success with veneer method in mandarin at Dailekh. Gautam *et al.* (2001) reported that 31st January grafting gave the highest (87.50%) and 16th January produced 85.00% graft success in mandarin at Lumle, Kaski. Similarly, Adhikari (2006) reported highest graft success (79.73%) in acid lime grafted onto trifoliolate orange rootstock on 16th January by shoot tip method at Rampur, Chitwan. Present result was better in comparison to the past researches in citrus. This may be due to more favorable environmental condition of Dhankuta.

Mortality of sprouted grafts

The effect of grafting methods on mortality was found statistically non significant. However, the higher percentage of mortality (1.58%) was recorded in shoot tip method as compared to veneer method (1.12%). The effect of dates of grafting on mortality was registered statistically highly significant. The mortality of graft as affected by dates varied from 0.17% to 3.70%. The highest graft mortality (3.70%) was recorded in the grafts prepared on 12th February while the lowest on 29th December (Table 2).

Table2. Effect of dates of grafting on mortality of sprouted grafts at 180 days after grafting in Paripatle, Dhankuta, 2009/2010

Dates of grafting	Mortality % 180 DAG
29 th October	1.90 ^b
14 th November	1.60 ^b
29 th November	1.40 ^b
14 th December	0.31 ^c
29 th December	0.17 ^c
13 th January	0.29 ^c
28 th January	1.40 ^b
12 th February	3.70 ^a
Mean	1.35
CV%	42.29
F value	24.99**
CD (P<0.05)	0.78
SEm±	0.238

SEm± = Standard error of mean difference, CV = Coefficient of variation, CD (Pd^{0.05}) = Critical difference at probability value 0.05, Treatment means followed by common letter(s) within a column are not significantly different at 5% by DMRT, DAG=Days after grafting

The higher mortality of sprouted graft prepared on 12th February was thus due to the lack of moisture needed to meet transpiration loss from the newly

expanded leaves because union formation competed only at the end of March when there was higher temperature. Randhawa and Bajwa (1958) reported that normal union between the scion (Blood Red) and rootstock (Karna Khatta) was completed in about six weeks after grafting. Moreover, the transpiration loss from the tender leaves at higher temperature was high because lack of well developed cuticle layer outside the epidermis and higher number of stomata on the leaves. Thus lack of sufficient water was the principal cause of the death of early sprouted scion of the graft.

Number of leaves per sapling

The effect of methods of grafting on number of leaves per sapling was found non significant at 180 days after grafting. Shoot tip and veneer grafted saplings produced about 30 leaves per sapling (29.89 in shoot tip and 29.71 in veneer). The effect of dates of grafting on number of leaves per sapling was found highly significant at 180 days after grafting. The number of leaves per sapling was varied from 21.93 to 48.47 with mean value of 29.79. The highest number of leaves per sapling (48.47) was recorded on 14th November grafted sapling, whereas the lowest (21.93) on 13th January grafted sapling (Table 3).

There was intimate contact of two graft components, the scion and the rootstock; callus cells also became fully differentiated to xylem and phloem ensuring the free movement of water and mineral nutrients to the leaf through xylem and photosynthate through phloem to the other parts of sapling. Early season grafted saplings also had well developed root system to absorb water and nutrient, the rate of photosynthesis was also higher because of easy availability of raw materials for photosynthesis. Greater the food availability, there would be more growth of whole plant. Therefore, this may be one of the reasons that October and November grafted saplings produced higher number of leaves. Present finding was also supported by Dubey and Singh (2003). They observed 47 leaves per sapling in Darjeeling mandarin grafted onto rough lemon at 11 months after grafting. Similarly, in another study, Adhikari (2006) reported the highest number of leaves per plant (47) at 135 days after grafting in acid lime in Chitwan.

Sapling height

Effect of methods of grafting on sapling height was found statistically non significant at 180 days after

grafting. Sapling height was recorded as 31 cm in shoot tip grafted sapling and 31.88 cm in veneer grafted sapling. However, the effect of dates was found significant at 180 days after grafting. The height of sapling varied from 29.03 cm to 32.86 cm with mean

value 31.44 cm. Maximum height of sapling was registered on 29th December and the minimum on 29th October. Except 29th October grafted sapling, rest dates were statistically at par (Table 3).

Table 3. Effect of dates of grafting on growth of different parameters of mandarin sapling at 180 days after grafting in Paripatle, Dhankuta, 2009/2010

Dates of grafting	Sapling growth			
	Number of leaves/sapling	Sapling height (cm)	Number of primary branches/sapling	Number of secondary branches/sapling
29 th October	34.38 ^b	29.03 ^b	2.20 ^a	2.34 ^{ab}
14 th November	48.47 ^a	31.91 ^a	2.15 ^{ab}	2.50 ^a
29 th November	33.23 ^b	31.97 ^a	2.02 ^{ab}	2.15 ^{ab}
14 th December	24.73 ^c	30.39 ^{ab}	1.88 ^{ab}	1.81 ^{bcd}
29 th December	26.93 ^{bc}	32.86 ^a	1.80 ^{bc}	2.03 ^{abc}
13 th January	21.93 ^c	30.43 ^{ab}	1.87 ^{abc}	1.33 ^{de}
28 th January	24.19 ^c	32.74 ^a	1.53 ^c	1.17 ^e
12 th February	24.53 ^c	32.17 ^a	1.92 ^{ab}	1.44 ^{cde}
Mean	29.79	31.44	1.92	1.85
CV (%)	20.31	6.79	14.08	26.15
F value	12.55**	2.38*	3.61**	6.19**
CD (P≤0.05)	7.16	2.53	0.32	0.57
SEm±	2.47	0.87	0.11	0.19

SEM± = Standard error of mean difference, CV = Coefficient of variation, CD (Pd^{0.05}) = Critical difference at probability value 0.05, Treatment means followed by common letter(s) within a column are not significantly different at 5% by DMRT

Higher number of leaves per sapling produced more photosynthate and plant growth regulators, especially auxins, cytokinins and gibberellins, resulting faster growth of shoot as well as root. Auxins have apical dominance property of main shoot over the side branches resulting into increased sapling height. The increased number of primary and secondary branches also produced the higher number of leaves which ultimately resulted the increased sapling height (Hartmann *et al.* 2007). Present finding was also supported by several researchers. Paudyal (1999) found somewhat similar height of pummele grafted onto the trifoliolate orange rootstock in November. Gautam *et al.* (2001) reported the highest sapling height grafted on 20th November and 7th December and lowest on 3rd March grafting. In another study, Dubey and Singh (2003) reported that 11 months after grafting, Darjeeling mandarin grafted onto rough lemon rootstock produced 29.53 cm scion height. Similarly, the scion height of 21.23 cm was reported by Adhikari (2006) in acid lime grafted onto trifoliolate orange rootstock at 4 months after grafting. In present study, 6 months after grafting, mandarin onto trifoliolate orange rootstock produced much lower scion height (17.86 cm) this was may be due to the use of dwarfing

rootstock and difference in species taken under study and difference in climatic condition of the research sites.

Number of primary branches

Effect of methods of grafting on number of primary branches per sapling was non significant. Sapling propagated with shoot tip and veneer grafted sapling produced 1.95 and 1.89 primary branches per sapling. The effect of dates of grafting on number of primary branches per sapling was found statistically highly significant at 180 days after grafting. Sapling prepared on 29th October produced the highest number of primary branches (2.20) and 28th January produced the minimum (1.53) branches per sapling (Table 3).

Highly significant positive correlation was observed between the number of primary branches per sapling and the number of leaves (0.62), sapling height (0.57) and the number of secondary branches per sapling (0.61) (Table 4). The ultimate growth of sapling was dependent primarily on the number of leaves per sapling which was affected by the number of primary and secondary branches on the plant. Adhikari (2006)

reported highest number of primary branches (3.11) per sapling in acid lime grafted on 16th January at 135 days after grafting in Chitwan, which was higher than

the present results. This may be due to the difference in species taken under the study and climatic condition of the research sites.

Table 4. Coefficient of correlation (r) between different parameters of mandarin sapling in Paripatle, Dhankuta, 2009/2010

	Number of leaf/sapling	sapling height (cm)	Number of primary branches/sapling	Number of secondary branches/sapling
Number of leaves/sapling	1.00	0.86**	0.62**	0.84**
sapling height (cm)		1.00	0.57**	0.79**
Number of primary branches/sapling			1.00	0.61**
Number of secondary branches/sapling				1.00

** Correlation is highly significant at the 0.01 level (2-tailed)

Number of secondary branches

Effect of methods of grafting on number of secondary branches per sapling was found non significant. Veneer and shoot tip grafted sapling produced 1.90 and 1.79 secondary branches per sapling respectively. However, the effect of dates of grafting was observed highly significant on number of secondary branches per sapling. The highest number of secondary branches (2.50) was recorded on 14th November grafted sapling while the lowest number of branches (1.17) on 28th January grafted sapling (Table 3).

Highly significant positive correlation was found between the number of secondary branches and the number of leaves (0.84), sapling height (0.79) and the number of primary branches (0.61) (Table 4). Higher number of leaves per sapling accumulated more photosynthate and phytohormone which contributed for higher growth of sapling. Greater sapling height, more number of primary branches gave the higher number of secondary branch per sapling in mandarin. The number of secondary branches per sapling in present study was much lesser than reported by Adhikari (2006). He observed 5.20 secondary branches per sapling on 31st January grafted sapling of acid lime in Chitwan. This may be due to the difference in species taken under the study and climatic condition of the research sites.

The present study showed that mandarin fruits can be propagated successfully through grafting. Either shoot tip or veneer method can best be used with higher rate of success, because shoot tip and veneer method did

not differ significantly in their effect on success and growth of sapling. The most appropriate time of grafting at Dhankuta is identified as 2nd week of January to the end of January.

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