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Effect of Grafting Height on Success and Subsequent Growth of Acid Lime (*Citrus aurantifolia* Swingle) Saplings

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Abstract

A field experiment was conducted at National Citrus Research Program (NCRP), Paripatle, Dhankuta to determine the best grafting height for the highest success of grafting and the maximum growth of saplings during 1st January to 30th December, 2010. Scions were collected from the mother plant 'NCRP-49' grown under screen house and grafted onto one-year-old trifoliate orange seedling rootstocks by shoot-tip method at 4 cm, 8 cm, 12 cm, 16 cm and 20 cm height from the collar region as the treatment. The grafts were planted inside the closed tunnel made from bamboo splits, jute and plastic sheet at 10×8 cm spacing in 64×100 cm experimental plots laid out in randomized complete block design (RCBD) with four replications containing 80 grafts per plot. Treatments were allotted on the experimental plots randomly. The success of grafting was not affected by the height of grafting, however, growth of sapling was found significantly affected by the height of grafting. Observation taken on saplings after one year of grafting revealed that the maximum scion height (42.13 cm), the highest number of leaves per sapling (47.50), the highest growth of scion diameter (55.61%), maximum length of primary branches (31.19 cm), maximum number of secondary branches per sapling ((3.24), the highest length of secondary branches (11.59 cm), the highest canopy volume (15440 cm^3) and the highest graft spread (24.35 cm) were found on the sapling grafted at 16 cm height of the trifoliate orange rootstock. Hence, from the study it is concluded that the most suitable height of grafting acid lime on trifoliate orange rootstock was 16 cm.

Key words: *Citrus aurantifolia*, *Poncirus trifoliata*, shoot-tip, callus, graft success, graft spread and canopy volume

Introduction

Citrus is the most important fruit crop of mid-hill region 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). Acid lime (*Citrus aurantifolia* Swingle) is the second important citrus crop of Nepal after mandarin in terms of area coverage (MOAC 2012). Unlike mandarin and sweet orange, acid lime can be cultivated successfully from Terai to mid-hill region of Nepal. There is enormous scope of acid lime production in Nepal. About 95% of annual market demand of acid lime fruits supplied in the main season and 100% in the off-season in Kathmandu were imported from India (Dhakal *et al*. 2003). Dhakal *et al*. (2002) also reported that 2,110 tones of acid lime worth Rupees 60 millions is being imported annually from India. He also reported that 81% of acid lime saplings are raised from seedling in Nepal.

The production and productivity of acid lime is very low in Nepal due to the use of seedlings for plantation, less care and management of the orchard and plantation of saplings in marginal land. Moreover, the seedling trees are susceptible to *Phytophthora* root rot disease as compared to grafted ones. Saplings prepared by grafting acid lime onto trifoliate orange [*Poncirus trifoliata* (L.) Raf.] are tolerant of *Phytophthora* gummosis, cachexia-xyloporosis and nematodes, especially the *Tylenchulus semipenetrans*. The rootstock is also resistant to the citrus tristeza virus (Aubert & Vullin, 1998). The demand of grafted

sapling is growing day by day within the country. Trifoliate orange seedling has poor growth in open field condition. About two or more years old seedlings of trifoliate are being used for the grafting purpose. Some seedlings are very dwarf to be grafted with the suitable scions. Grafting at too low height can create the problem of rot disease at the point of union of the sapling after plantation. Therefore, a field experiment was carried out to find the suitable height of grafting at the National Citrus Research Program, Paripatle, Dhankuta giving the maximum success of grafting and the optimum growth of the saplings at nursery stage.

Methodology

The study was carried out at NCRP, Paripatle, Dhankuta, during 1st January to 30th December, 2010. About 8 months-old scions were taken from the mother plant of acid lime 'NCRP-49' accession grown inside the screen house. Scions were grafted onto one-year-old trifoliate orange seedling rootstocks by shoot-tip method at five different heights (4 cm, 8 cm, 12 cm, 16 cm and 20 cm) from the collar region of the rootstock as the treatments. The grafts were planted inside the closed tunnel made from bamboo splits, jute sheet cover from inside and plastic sheet cover from outside at 10×8 cm spacing in experimental plots laid out in randomized complete block design (RCBD) with four replications. Each 64×100 cm sized experimental plots were supplied with a total of 10 kg vermi-compost (nitrogen 1.25-2.5%, phosphorus 0.75-1.6% and potash 0.5-1.1%) containing 80 grafts. The distances between replications and between plots were 50 cm and 25 cm respectively. Treatments were allotted on the experimental plots randomly. Ten plants were selected from each experimental plot for the study. The regular de-suckering, irrigation, crop protection, hoeing and top-dressing, removal of plastic laces, removal of jute and plastic sheet were done timely in each experimental plot for better growth of the saplings. The recorded data were reduced, arranged in MS-Excel and analyzed by MSTAT-C package. The means were separated by Duncan's Multiple Range Test (DMRT).

The amount of manure was slightly adjusted from the recommendation of Aubert and Vullin (1998), who recommended 80 mt FYM, 0.4 mt TSP (Tripple Super Phosphate (45% P_2O_5) and 0.5 mt of Potassium Sulphate (50% K_2O) for open field production of citrus saplings. Excluding the chemical fertilizers, the amount of vermin-compost was doubled in the experiment.

Results and Discussion

Graft success

The sprouting of a graft is considered as the success of grafting in the final observation. At the initial observation, all the grafts were not sprouted, therefore success was not conformed. Graft success is the major criteria for the selection of a suitable method of grafting, time of grafting and grafting height of the saplings. In the present study, the success of grafting was not found to be significantly affected by the height of grafting. However, at final observation of success at 180 days after grafting, the highest success (99.37%) was given by 16 cm grafting height followed by 20 cm (99.06%) and the lowest (97.81%) by 8 cm. (Figure 1).

Present finding was also supported by Poon (1999) who reported 88.73%, Gautam *et al.* (2001) reported 87.5%, Chalise (2010) reported 77.78% success in mandarin with shoot-tip method whereas Adhikari (2006) reported 79.73% success in acid lime grafted onto trifoliate orange rootstock. The present result was higher than previous findings which may be due to more experienced grafters, more suitable temperature and humidity for callusing and more care of grafts after planting.

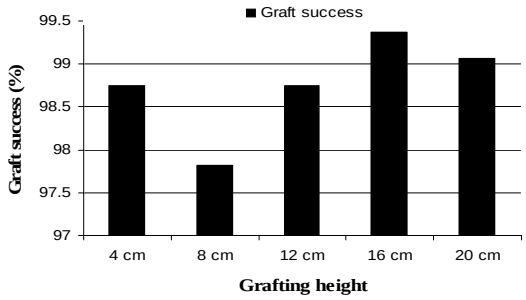

Fig. 1. Effect of grafting height on success of acid lime grafting at 180 days after grafting at Paripatle, Dhankuta, 2010

Growth of scion height

The growth of scion height was significantly affected by the grafting height at 180 and 300 days after grafting while non significant at rest of the observations. At 180 days after grafting, the maximum growth of scion height (27.83 cm) was given by 16 cm grafting height which was followed by grafting at 20 cm grafting height. Similarly, at 300 days after grafting, the highest growth of scion height (39.75 cm) was produced by sapling grafted at 16 cm height followed by 20 cm grafted saplings and the lowest by 4 cm grafted

saplings. At 360 days after grafting the highest growth (42.13 cm) was again produced by 16 cm height grafted saplings and the lowest scion height by 4 cm height grafted saplings (Table 1).

Table 1. Effect of grafting height on growth of scion height of acid lime saplings at Paripatle, Dhankuta, 2010

Treatments (Grafting height)	Scion height (cm)					
	60 DAG	120 DAG	180 DAG	240 DAG	300 DAG	360 DAG
Grafting at 4 cm	7.23	15.61	17.76^{c}	24.13	30.35^{b}	32.07
Grafting at 8 cm	8.12	17.30	20.51^{bc}	25.66	30.39^{b}	33.64
Grafting at 12 cm	8.60	18.44	22.94^{abc}	27.01	32.90^{ab}	35.09
Grafting at 16 cm	7.40	19.18	27.83^{a}	31.24	39.75^{a}	42.13
Grafting at 20 cm	6.94	17.56	23.69^{ab}	28.35	35.45^{ab}	38.11
F value	0.86^{ns}	1.31^{ns}	5.08*	2.49^{ns}	3.35*	3.17^{ns}
CV (%)	19.28	13.36	14.77	12.60	12.78	12.37
CD (P≤0.05)	2.27	3.63	5.13	5.29	6.65	6.90
SEm±	0.74	1.18	1.67	1.72	2.16	2.24

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

Present findings were also supported by Dubey and Singh (2003). They reported 29.53 cm scion height at 11 months after grafting Darjeeling mandarin grafted onto rough lemon rootstock. Scion height of 21.23 cm was reported by Adhikari (2006) in acid lime grafted onto trifoliate orange rootstock at 4 months after grafting. Similarly, Chalise (2010) reported 17.86 cm height of mandarin at 6 months after grafting onto trifoliate orange rootstock. However, the present result was higher than past findings.

Number of leaves per sapling

The number of leaves per sapling prepared by grafting at different height on the rootstock was found significant at 300 days after grafting while non significant at the rest of the observations. At 300 days after grafting, the significantly higher number of leaves per sapling (53.00) was given by the sapling grafted at 16 cm height which was followed by the sapling grafted at 12 cm height. Statistically, 12 cm and 16 cm grafting heights were at par. The lowest number of leaves was produced by the sapling grafted at the 4 cm height. At 360 days after grafting, all the grafting heights were not significantly different statistically, however, the maximum leaf number (47.50) was given by 16 cm height grafting (Table 2). This may be due to fast healing of the wounds of the grafts at this height.

Table 2. Effect of grafting height on number of leaves per sapling of acid lime at Paripatle, Dhankuta, 2010

Treatments (Grafting height)	Number of leaves					
	60 DAG	120 DAG	180 DAG	240 DAG	300 DAG	360 DAG
Grafting at 4 cm	5.60	19.66	23.15	29.85	38.85^{b}	39.75
Grafting at 8 cm	7.00	21.50	23.13	32.22	43.05^{b}	42.58
Grafting at 12 cm	8.55	25.18	26.90	35.05	45.83^{ab}	44.65
Grafting at 16 cm	7.45	24.53	25.80	31.95	53.00^{a}	47.50
Grafting at 20 cm	7.85	23.38	25.20	30.85	42.42^{b}	42.88
F value	2.59^{ns}	2.19^{ns}	1.22^{ns}	0.92^{ns}	3.47*	0.89^{ns}
CV (%)	18.77	13.39	12.13	12.70	12.74	13.89
CD (P≤0.05)	2.11	4.71	4.64	6.26	8.76	9.30
SEm±	0.68	1.53	1.51	2.03	2.84	3.02

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

Present findings were also supported by Dubey and Singh (2003). They observed 47 leaves per sapling in Darjeeling mandarin grafted onto rough lemon at 330 days after grafting. In another study, Adhikari (2006) reported the highest number of leaves (47) per plant at 135 days after grafting in acid lime in Chitwan. Similarly,

Chalise (2010) reported 48.47 leaves of mandarin sapling at 180 days after grafting.

Growth of diameter

The growth of diameter of different parts of saplings was studied during the research periods. The growth of collar diameter, below the union diameter and at the union diameter were found statistically non significant. However, the growth of scion diameter was found significant at 360 days after grafting. The highest growth (104%) of collar region was given by 16 cm grafting height and the lowest (69.11%) by 4 cm grafting height. Below the union diameter was maximum (67.46%) in 8 cm grafting height and the lowest (54.14%) in 20 cm height grafting. Similarly, the highest growth of union diameter (79.24%) was given by 16 cm grafting height and the lowest (62.34%) by 20 cm grafting height. The scion diameter growth was recorded maximum (55.61%) in 16 cm grafting height and the minimum (28.06%) in 8 cm grafting height. Among the four different parts of sapling the collar diameter growth was found maximum followed by union diameter and below the union diameter and the least growth on scion diameter (Figure 2).

With discussing the growth of sapling diameter, Adhikari (2006) reported the highest growth (67.88%) of the scion diameter, while Chalise (2010) recorded the highest growth (60.33%) of collar diameter over the initial growth among collar diameter, below the union diameter union diameter and scion diameter.

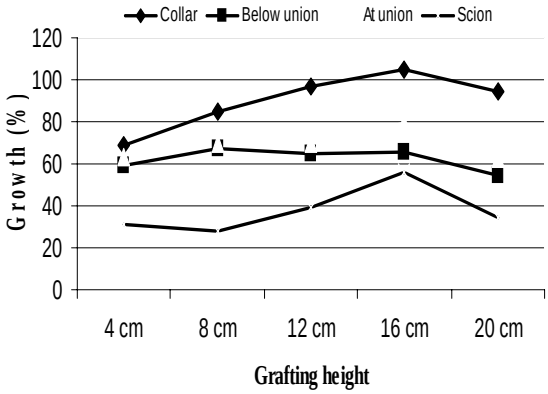

Fig. 2. Effect of grafting height on growth of diameter of different part of acid lime sapling over initial growth at 360 days after grafting in Paripatle, Dhankuta, 2010

Number of primary branches per sapling

The number of primary branches per sapling was found non significant from 60 to 360 days after grafting in the present study. However, at 360 days after grafting the highest number of primary branches per sapling (2.425) was produced by the sapling grafted at 12 cm height which was followed by 4 cm grafting height and the lowest number of primary branches was recorded in sapling grafted at 16 cm height (Table 3).

Table 3. Effect of grafting height on number of primary branches per sapling of acid lime at Paripatle, Dhankuta, 2010

Treatments (Grafting height)	Number of primary branches					
	60 DAG	120 DAG	180 DAG	240 DAG	300 DAG	360 DAG
Grafting at 4 cm	1.35	1.77	1.87	1.92	2.07	2.28
Grafting at 8 cm	1.42	1.72	1.75	1.77	1.86	2.12
Grafting at 12 cm	1.50	1.97	2.05	2.10	2.27	2.42
Grafting at 16 cm	1.55	1.65	1.65	1.74	1.84	2.09
Grafting at 20 cm	1.40	1.77	1.85	1.90	1.97	2.15
F value	0.78^{ns}	0.65^{ns}	0.97^{ns}	0.93^{ns}	2.04^{ns}	0.68^{ns}
CV (%)	12.54	16.76	16.38	15.68	12.39	15.04
CD ($P \leq 0.05$)	0.28	0.46	0.46	0.46	0.38	0.51
SEm±	0.09	0.15	0.15	0.15	0.12	0.17

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

Length of primary branches

The length of primary branches was found significant at 180, 300 and 360 days after grafting while non significant at the rest of the observations. At 180 days after grafting the highest length of primary branches (18.51 cm) was recorded in 16 cm height grafted sapling

with which 12 cm and 20 cm were at par statistically and the lowest length (11.70cm) was given 4 cm grafting height. At 300 days after grafting, maximum height (27.92 cm) was again given by 16 cm and the lowest (20.55 cm) by 4 cm height of grafting. At 360 days after grafting, the highest length (31.19 cm) was recorded in 16 cm height of grafting and the lowest (21.86 cm) in 4 cm grafting height (Table 4).

Table 4. Effect of grafting height on length of primary branches of acid lime sapling at Paripatle, Dhankuta, 2010

Treatments (Grafting height)	Length of primary branches (cm)					
	60 DAG	120 DAG	180 DAG	240 DAG	300 DAG	360 DAG
Grafting at 4 cm	4.54	10.20	11.70^{b}	16.54	20.55^{b}	21.86^{b}
Grafting at 8 cm	5.24	10.93	13.85^{b}	18.06	22.21^{b}	23.02^{b}
Grafting at 12 cm	5.77	11.49	14.65^{ab}	17.09	22.08^{b}	23.74^{b}
Grafting at 16 cm	4.97	12.37	18.51^{a}	22.83	27.92^{a}	31.19^{a}
Grafting at 20 cm	4.86	11.27	14.75^{ab}	19.07	25.20^{ab}	27.40$^{a\,b}$
F value	0.66^{ns}	0.81^{ns}	3.39*	2.41^{ns}	3.61*	4.28*
CV(%)	22.32	15.70	18.19	17.13	13.16	14.54
CD (P≤0.05)	1.75	2.72	4.12	4.94	4.78	5.70
SEm±	0.57	0.88	1.34	1.60	1.55	1.85

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

Number of secondary branches per sapling

The secondary branches of sapling were recorded only after 4 months after grafting. The number of secondary branches were found non significant at 120 days to 360 days after grafting. However, at 360 days after grafting, the highest number of secondary branches (3.24) was produced by the sapling prepared by the grafting at 16 cm height which was followed by 12 cm height grafted sapling (3.158) and the lowest number (2.438) was produced by sapling grafted at 4 cm height (Table 5).

Table 5. Effect of grafting height on number of secondary branches per sapling of acid lime in Paripatle, Dhankuta, 2010

Treatments (Grafting height)	Number of secondary branches				
	120 DAG	180 DAG	240 DAG	300 DAG	360 DAG
Grafting at 4 cm	0.62	1.15	1.49	2.12	2.44
Grafting at 8 cm	1.19	1.50	1.70	2.58	2.90
Grafting at 12 cm	1.30	1.86	2.03	2.71	3.16
Grafting at 16 cm	0.87	1.45	1.82	2.78	3.24
Grafting at 20 cm	1.32	1.54	1.66	2.26	2.74
F value	1.21^{ns}	1.47^{ns}	0.62^{ns}	0.92^{ns}	1.07^{ns}
CV (%)	51.80	27.74	29.41	24.03	21.70
CD (P≤0.05)	0.85	0.64	0.79	0.92	0.97
SEm±	0.27	0.21	0.25	0.30	0.31

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

Length of secondary branches

The length of secondary branches was found significant at 180 and 360 days after grafting and non significant at the rest of observations. At 180 days after grafting, the highest length (7.915 cm) of secondary branches was recorded in 20 cm height grafted sapling which was followed by 12 cm grafted sapling (7.445 cm) and the lowest length (5.425 cm) by 4 cm height grafted sapling. At 360 days after grafting, the highest length (11.59 cm) of secondary branches was given by 16 cm height grafted sapling followed by 20 cm grafted sapling (10.20 cm) and the lowest (9.215 cm) by 8 cm height grafted sapling (Table 6).

Table 6. Effect of grafting height on length of secondary branches of acid lime sapling at Paripatle, Dhankuta, 2010

Treatments	Length of secondary branches (cm)				
(Grafting height)	120 DAG	180 DAG	240 DAG	300 DAG	360 DAG
Grafting at 4 cm	3.00	5.42^{b}	7.44	8.92	9.47^{b}
Grafting at 8 cm	5.22	6.64^{ab}	7.73	8.64	9.21^{b}
Grafting at 12 cm	5.50	7.44^{a}	8.30	9.49	9.79^{b}
Grafting at 16 cm	4.07	7.15^{a}	9.74	10.81	11.59^{a}
Grafting at 20 cm	6.92	7.91^{a}	8.97	9.58	10.20^{ab}
F value	1.79^{ns}	3.96*	3.24^{ns}	2.96^{ns}	3.65*
CV (%)	44.86	13.84	12.28	10.24	9.71
CD (P≤0.05)	3.41	1.47	1.60	1.50	1.50
SEm±	1.11	0.48	0.52	0.48	0.49

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

Graft spread

The average graft spread of sapling was found highly significant at 180, 300 and 360 days after grafting, significant at 240 days after grafting and non significant at the rest of the observations. At 180 days after grafting, the maximum graft spread (12.43 cm) was observed on 16 cm height grafted sapling and the minimum (9.62 cm) in 4 cm grafted sapling. Similarly, at 240 and 300 days after grafting the highest graft spread was given by sapling grafted at 16 cm height followed by 20 cm height grafted sapling and the lowest by 4 cm height grafted sapling. Again at 360 days after grafting, the extra graft spread (24.35 cm) was recorded in 16 cm height followed by 20 cm and the lowest in 4 cm height grafted sapling (Figure 3).

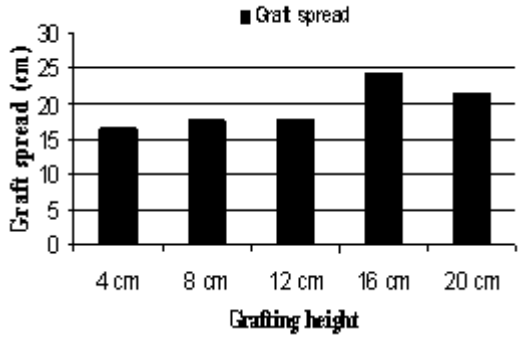

Fig. 3. Effect of grafting height on spread of acid lime sapling at 360 days after grafting at Paripatle, Dhankuta, 2010

Canopy volume

Canopy volume of sapling was calculated by the formula ð.D^2.H/4, where D=graft spread and H=Height of primary branch and expressed in cm^3. A slight change in the graft spread and height can make much difference. The canopy volume of sapling was found significantly affected by the grafting height at 180 and 240 days after grafting and highly significantly affected at 300 and 360 days after grafting. From 180 to 360 days after grafting, the highest volume of canopy was recorded in sapling grafted at 16 cm height followed by 20 cm grafted ones and the lowest in 4 cm grafted sapling. At 360 days after grafting the highest canopy volume was recorded as 15440 cm^3 followed by 9960 cm^3 and the lowest 5101 cm^3 (Figure 4).

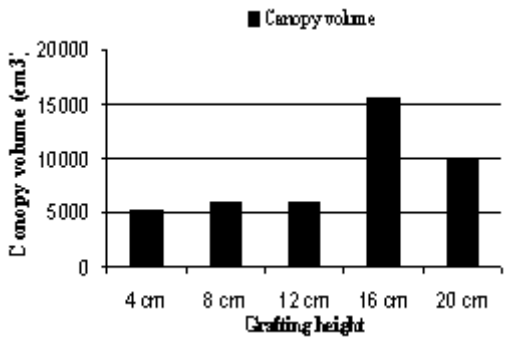

Fig. 4. Effect of grafting height on canopy volume of acid lime sapling at 360 days after grafting in Paripatle, Dhankuta, 2010

The recommended height of sapling in citrus species for plantation is 45 cm to 60 cm (Shah 1992). To attain this height, the age of the sapling should be one to one and half year for open field condition. Most of the citrus saplings are produced by grafting the desirable species/varieties onto the trifoliate orange rootstock. About one and half year is taken by the trifoliate orange to attain the graftable size which compels the nursery owners grafting at much lower height even at 2.5 cm or

less above the collar region. The lower grafting results the infection of the orchard tree at graft union by soil-borne fulgal diseases when the union buried into the soil surface. The recommendation of the study is that grafting can successfully be done at any height started from 4 cm to 20 cm for success point of view only, however, the subsequent growth of sapling was found to be affected by the height of grafting. At shorter height, the growth of sapling was found slower and at higher grafting height the growth was found higher up to 16 cm only. Beyond this height sapling growth was again found retarded in the field condition. Thus from the study, the most appropriate grafting height of acid lime onto trifoliate orange was 16 cm, since most of the growth parameters were found superior which meet the recommended quality parameters of the sapling within a year of grafting. Higher grafting also minimizes the possible attack of diseases at union in main field condition.

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