Standardization of Grafting Time of Mandarin (*Citrus reticulata* Blanco) in Central Mid Hill of Nepal

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ABSTRACT

An investigation was carried out to study the performance of mandarin grafts during 2017–18 at Banepa, Kavre to standardize suitable grafting time for two mandarin varieties. The experiment was conducted in two factorial Completely Randomized Design (CRD) with 10 treatments and three replications. The treatments consisted of combinations of five grafting dates (25th November, 10th December, 25th December, 10th January and 25th January) and two mandarin varieties (Dhankuta Local and Ota Ponkan). Scion collected from mother plants of selected varieties were grafted onto a trifoliate orange rootstock by side veneer method in the screen house. The results showed that grafting dates significantly affect the graft success and subsequent growth of grafted sapling of mandarin varieties. The highest graft success (100%) was observed on the grafts prepared in January. The sapling height (47.58 ± 1.93 cm), leaf area (32.74 ± 2.98 cm²), number of primary branches per sapling (3.16 ± 0.20) and increase in diameter above union (17.78 ± 1.47%) were recorded maximum on the grafts prepared on 25th January while the number of leaves per sapling (26.81 ± 1.84) was found superior for 10th January grafted sapling. Most of the important parameters viz., graft success percent, sapling height and leaf area were found non-significant for varieties but the number of leaves per sapling (23.99 ± 1.51), number of primary branches per sapling (2.72 ± 0.21), increase in diameter above union (16.24 ± 0.88%) and at union (13.72 ± 1.54%) was found maximum for Ota Ponkan. Therefore, the study revealed that late January was the most suitable time of grafting for both varieties of mandarin under Kavre, Nepal conditions.

KEYWORDS

Callus; days after grafting; graft success; temperature; sapling

Introduction

Citrus is the world’s major fruit crop with global availability and popularity and is grown all over the world in more than 140 countries covering tropical and subtropical areas of the world (FAOSTAT, 2007; Ramana et al., 1981) with the production of 94 million metric tons of citrus in 2019/20 (USDA, 2021). Citrus is one of the most important fruit crops of Nepal contributing about 23% of total fruit production and total fruit area (MOALD, 2020) having the potentiality to bring economic change in the country due to unique topography, diversified geographical and agro-climatic conditions. APP (1995) prioritized citrus as the main high-value crop for the mid-hill of Nepal. It is grown commercially at different climatic conditions popularly temperate, subtropical and tropical climate mainly in the mid-hill region (750–1400 m) of the country (Shah, 1992). The mid-hill region of Nepal (1000–1500 masl altitude) has a comparative advantage in the production of mandarin. Mandarin (*Citrus reticulata* Blanco) is the highest grown fruit among citrus species in Nepal with the average area, productive area, production and...
productivity of 27951 ha, 17220 ha, 177381 tons and 10.30 t/ha respectively (MOALD, 2020). Mandarin can be propagated by seed, cuttings, layering, grafting or budding. Among the various vegetative methods used to propagate the mandarin, grafting is the most common method practiced in the Nepalese citriculture industry, as it results in the highest success and field establishment (Patel et al., 2010) in which veneer and side grafting are mostly practiced throughout the country (Pandey and Karki, 2019). However, the success of grafting is highly influenced by several factors like temperature, relative humidity, moisture and plant water, the growth stage of scion and rootstock, method of grafting and genetic relationship between the stock and the scion (Hartmann et al., 2002). Weather condition plays a vital role in the success of grafting which ultimately influences graft union formation and subsequent growth of grafted plants (Iqbal et al., 2004). The demand for good quality true to type mandarin sapling is rising annually. The existing citrus orchards are mostly of seedling origin and only 32% of saplings are being produced through grafting and the rest of the demand is being fulfilled by the seedling (MOAC and FAO, 2011). Grafting is generally practiced from November to January at 1000–1500 masl in the Nepalese context. However, it can be done throughout the year under a controlled climatic condition if there is the availability of a suitable scion (Shah, 1992). Few studies conducted in the eastern, western and mid-western regions of Nepal revealed that the winter season is suitable for grafting of mandarin. Poon (1999), Gautam et al. (2001) and Chalise et al. (2013a) reported that November to January is the suitable time of grafting for mandarin in different parts of Nepal. However, commercial mandarin sapling producers of Nepal have been performing grafting haphazardly without considering suitable season and time which resulted in a lower graft success due to unfavorable environmental conditions.

Besides, Huanglongbing also known as citrus greening disease, was reported to decrease the productivity of citrus and a great threat to the Nepalese citrus industry (Regmi and Yadav, 2007). The prevalence of greening disease is higher in lower belts (up to 900 m altitude) as compared to the higher belts (above 1200 m altitude) (MOAC and FAO, 2011). Similarly, Phytophthora root rot is another devastating disease of citrus especially of seedling origin as reported by Regmi et al. (2009). Thus, the grafting of bud wood certified precocious scion of mandarin varieties grafted onto dwarf and disease-resistant trifoliolate orange (Poncirus trifoliata) rootstock in a suitable location and optimum time could be the permanent solution of the citrus decline malady to meet the escalating demand of good quality sapling. Therefore, in the present study, an attempt was made to standardize suitable time of grafting for mandarin varieties for central mid-hills of Nepal.

**Materials and Methods**

The present investigation was carried out under the project ‘Food security improvement through rehabilitation of citrus in Kavre district at Banepa, Kavre during the year 2017–2018 to standardize the suitable time of grafting for mandarin varieties. The site is located at 27.6332° N, 85.5277° E with an elevation of 1500 meters above sea level.

**Design of Experiment**

The experiment was laid out in two factorial Completely Randomized Design (CRD) with 10 treatments. The treatments consisted of combinations of five grafting dates (25th November, 10th December, 25th December, 10th January and 25th January) and two mandarin varieties [Dhankuta Local (Citrus reticulata var. Dhankuta Local) and Ota Ponkan (Citrus reticulata var. Ota Ponkan)] which were replicated thrice (Table 1). There were a total of 30 experimental plots having 8 grafted plants per plot.
Table 1. Treatments combination of mandarin varieties at different dates of grafting

| S.N. | Treatments | Dates of Grafting | Species | Combination |
|------|------------|------------------|---------|-------------|
| 1    | T₁         | November 25, 2017 (D₁) | Citrus reticulata var. Dhankuta Local (V₁) | D₁×V₁ |
| 2    | T₂         | November 25, 2017 (D₁) | Citrus reticulata var. Ota Ponkan (V₂) | D₁×V₂ |
| 3    | T₃         | December 10, 2017 (D₂) | Citrus reticulata var. Dhankuta Local (V₁) | D₂×V₁ |
| 4    | T₄         | December 10, 2017 (D₂) | Citrus reticulata var. Ota Ponkan (V₂) | D₂×V₂ |
| 5    | T₅         | December 25, 2017 (D₃) | Citrus reticulata var. Dhankuta Local (V₁) | D₃×V₁ |
| 6    | T₆         | December 25, 2017 (D₃) | Citrus reticulata var. Ota Ponkan (V₂) | D₃×V₂ |
| 7    | T₇         | January 10, 2018 (D₄)  | Citrus reticulata var. Dhankuta Local (V₁) | D₄×V₁ |
| 8    | T₈         | January 10, 2018 (D₄)  | Citrus reticulata var. Ota Ponkan (V₂) | D₄×V₂ |
| 9    | T₉         | January 25, 2018 (D₅)  | Citrus reticulata var. Dhankuta Local (V₁) | D₅×V₁ |
| 10   | T₁₀        | January 25, 2018 (D₅)  | Citrus reticulata var. Ota Ponkan (V₂) | D₅×V₂ |

**Rootstock Production**

The trifoliate orange (*Poncirus trifoliata*) rootstocks seeds were sown in the nursery bed containing sand, soil and compost in the ratio of 1:1:1 which was maintained inside the screen house in December. About six-month-old rootstocks having 8–10 cm height and 6–8 leaves were transplanted in the polybags containing forest soil: field soil: FYM: sand (1:1:1:1). The rootstocks were then allowed to grow for 1.5 years until all the rootstocks become pencil size thick and height above 25 cm. Weeding, irrigation and fertilization were done regularly for the entire period. The average diameter of rootstock was 7.09 mm at the time of grafting (Table 2).

**Selection and Preparation of Scion**

About 8-9-month-old scion shoots from the terminal shoots of current season growth were collected from the healthy, matured and well-grown mother plants of respective varieties. A shoot containing the number of buds was then separated into a number of scions having two active buds per scion. The average length of scion was 4.2 cm and the diameter was 5.60 mm (Table 2).

Table 2. Diameter of scion and rootstock for grafting dates and species at the time of grafting

| Grafting dates | Diameter of scion (mm) | Diameter of rootstock (mm) |
|----------------|------------------------|----------------------------|
| Nov 25         | 5.26                   | 7.15                       |
| Dec 10         | 5.64                   | 6.95                       |
| Dec 25         | 5.64                   | 7.22                       |
| Jan 10         | 5.64                   | 7.28                       |
| Jan 25         | 5.82                   | 6.85                       |
| Species        |                        |                            |
| DL             | 5.95                   | 7.10                       |
| OP             | 5.25                   | 7.08                       |
| Mean           | 5.60                   | 7.09                       |

**Grafting Technique**

The prepared scions were grafted onto trifoliate rootstock at 15 days interval by side veneer method at 15 cm height from the collar region (Chalise et al., 2013b). A single smooth cut of 3 cm extending downward from the top of the stock was made at 1/3rd of the diameter of the stock with a sharp grafting knife. Another single, slightly downward and inward cut was made on rootstock at 0.5 cm above the lower end of the cut to remove the flip of the rootstock. The side view of the surface
resembled the English letter ‘V’. A slanting cut of 2 cm was made on the scion and then another very short cut from the opposite side was made at an angle of 30°. Scion was gently placed on the cut surface of rootstock so as to fit their cambium layer properly. The joint surface was tightly tied with grafting tape covering some portion of the above and below part of the union. The union was so tied that there was complete airtight condition inside the union. After that, the scion along with some part of rootstock below the union was wrapped completely with parafilm tape to avoid the desiccation of the scion. Intercultural operations such as irrigation, desuckering, weeding, manuring and plant protection activities were carried out regularly. The grafting tape used for joining the scion and rootstock was removed at 120 days after grafting Figure 1.

Data Collection and Analysis

The data regarding graft success percent, sapling height, leaves per sapling, leaf area, number of primary branches and increase in diameter of sapling were recorded at every 30 days interval from 30 days after grafting to 150 days after grafting. The data were taken from randomly selected five sample plants. The meteorological data of the screen house was recorded daily and interpretation and analysis were made based on mean monthly temperature and relative humidity (Table 3). The data were entered into Microsoft Excel 2016 and analysis was carried out by using softwares SPSS 24.0 and R-Studio. Both descriptive and inferential analysis was carried out. Interpretations were made based on results, which were assisted by qualitative and quantitative data/information available from both primary and secondary sources. Graft success percent was calculated by the following formulae:

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\text{Graft success} (\%) = \frac{\text{No of sprouted grafts}}{\text{Total number of grafts}} \times 100
\]  
(Chalise et al., 2013a; Patel et al., 2010).

Figure 1. Procedure of side veneer grafting in mandarin (a. front view of the cut surface of scion and rootstock, b. side view of the cut surface of scion and rootstock, c. placing scion on rootstock and d. tying scion and rootstock together by grafting tape).
Table 3. Mean monthly temperature and relative humidity of screenhouse for the grafts prepared on different dates

| Dates                     | Temperature (°C) | Relative humidity (%) |
|--------------------------|------------------|-----------------------|
| For the grafts prepared on 25th Nov, 25th Dec and 25th Jan |                  |                       |
| 25th November – 25th December | 13.74            | 65.66                 |
| 25th December – 25th January | 12.83            | 67.46                 |
| 25th January – 25th February | 16.42            | 64.63                 |
| 25th February – 25th March | 20.37            | 60.24                 |
| 25th March – 25th April   | 21.97            | 58.95                 |
| 25th April – 25th May     | 23.21            | 71.08                 |
| 25th May – 25th June      | 26.77            | 72.79                 |
| For the grafts prepared on 10th Dec and 10th Jan |                  |                       |
| 10th December – 10th January | 14.77            | 64.08                 |
| 10th January – 10th February | 14.21            | 64.49                 |
| 10th February – 10th March | 18.70            | 63.98                 |
| 10th March – 10th April   | 20.97            | 59.43                 |
| 10th April – 10th May     | 22.94            | 62.51                 |
| 10th May – 10th June      | 24.12            | 74.91                 |

Results

Graft Success

The result showed that graft success percent was significant for dates while it was non-significant for varieties at final observation i.e.150 days after grafting (Table 4). The highest graft success was recorded at grafting performed on 25th January (41.66 ± 2.64%) which was statistically at par with 10th January, 25th December and 25th November while the lowest graft success was observed in grafting performed on 10th December (25.00 ± 6.47%) at 30 days after grafting. The sprouting of scion was continued in succeeding observations and observed up to 120 days after grafting. The 100% graft success was observed on the grafts prepared on 10th and 25th January which were statistically at par with 25th December (97.91 ± 0.09%) grafted sapling while the lowest graft success was observed on the grafts prepared on 10th December (79.16 ± 4.18%) at 150 days after grafting. Similarly, the highest graft success was observed in Dhankuta Local (95.0 ± 2.04%) which was statistically at par with Ota Ponkan (93.5 ± 2.93%) at 150 days after grafting.

Table 4. Effect of grafting dates and varieties on graft success of mandarin.

| Dates  | 30 DAG   | 60 DAG   | 90 DAG   | 120 DAG  | 150 DAG  |
|--------|----------|----------|----------|----------|----------|
| Nov 25 | 37.50 ± 3.23 a | 75.00 ± 6.47a | 91.66 ± 2.64b | 91.66 ± 2.64b | 91.66 ± 2.64b |
| Dec 10 | 25.00 ± 6.47 b | 41.66 ± 2.64 c | 50.00 ± 2.99d | 79.16 ± 4.18 c | 79.16 ± 4.18 c |
| Dec 25 | 35.41 ± 2.09 a | 50.00 ± 0.00 c | 77.08 ± 5.03 c | 97.91 ± 2.09 ab | 97.91 ± 2.09 ab |
| Jan 10 | 35.41 ± 2.09 a | 64.58 ± 3.85b | 100 ± 0.00a | 100 ± 0.00a | 100 ± 0.00a |
| Jan 25 | 41.66 ± 2.64 a | 83.33 ± 6.20a | 100 ± 0.00a | 100 ± 0.00a | 100 ± 0.00a |
| LSD    | 10.08*    | 10.17***  | 8.16***  | 7.11***  | 7.11***  |
| SEM±   | 3.30      | 3.83      | 2.13      | 1.72      | 1.72      |

| Species | 30 DAG   | 60 DAG   | 90 DAG   | 120 DAG  | 150 DAG  |
|---------|----------|----------|----------|----------|----------|
| DL      | 31.66 ± 2.95b | 56.66 ± 3.42b | 81.66 ± 5.70a | 95.0 ± 2.04a | 95.0 ± 2.04a |
| OP      | 40.00 ± 1.80a | 69.16 ± 5.57a | 85.83 ± 4.86a | 93.5 ± 2.93a | 93.5 ± 2.93a |
| LSD    | 6.37*    | 6.43***  | 5.16ms   | 4.50ms   | 4.50ms   |
| SEM±   | 2.37      | 2.24      | 2.09      | 2.48      | 2.48      |
| CV     | 23.28  | 13.37  | 8.06  | 6.28  | 6.28  |
| Mean   | 35.83  | 62.91  | 83.75  | 93.75  | 93.75  |

DAG = Days after grafting, CV = Coefficient of variation, LSD = Least significant difference, SEM± = Standard error of mean difference, DL = Dhankuta Local, OP = Ota Ponkan, Means followed by common letter(s) within a column are not significantly different at 5% by DMRT, Significance codes ***at 0.001, **at 0.01, *at 0.05, ns = non-significant
**Height of Sapling**

The effect of grafting dates was found significant for the height of sapling while it was non-significant for varieties (Table 5). The maximum sapling height was observed on 25th January (47.58 ± 1.93 cm) grafted sapling which was statistically at par with 10th January (46.62 ± 3.17 cm) grafted sapling at final observation. The lowest sapling height was recorded on 25th December (33.90 ± 1.40 cm) grafted sapling which was at par with 10th December (35.51 ± 0.88 cm) grafted sapling. Ota Ponkan (41.76 ± 2.06 cm) was found superior but statistically similar to Dhankuta Local (39.67 ± 1.72 cm) for sapling height.

**Number of Leaves per Sapling and Leaf Area**

The result revealed that the effect of grafting dates and varieties on the number of leaves per sapling was statistically significant at 150 days after grafting. The highest number of leaves per sapling were recorded on 10th January (26.81 ± 1.84) grafted sapling which was statistically similar to 25th January (25.30 ± 1.57) grafted sapling. The lowest number of leaves per sapling was observed in grafting performed on 10th December (17.93 ± 1.11) which was at par with 25th December (18.78 ± 1.09) grafts. The maximum leaves number per sapling was observed in Ota Ponkan (23.99 ± 1.51) followed by Dhankuta Local (20.26 ± 1.03) (Table 5).

The result revealed that the effect of grafting dates was found significant for leaf area while it was non-significant for varieties (Table 5). The highest leaf area was observed on 25th January (32.74 ± 2.98 cm²) grafted sapling which was similar to 10th January (29.29 ± 2.02 cm²) grafted sapling. The lowest leaf area was observed on 25th December (22.92 ± 2.11 cm²) grafted sapling which was statistically at par with grafts prepared on 25th November (24.16 ± 0.91 cm²) and 10th December (24.26 ± 2.36 cm²). The maximum leaf area was recorded in Dhankuta Local (27.61 ± 1.34 cm²) which was statistically at par with Ota Ponkan (25.73 ± 1.88 cm²).

**Number of Primary Branches per Sapling**

The effect of grafting dates and varieties on the number of primary branches per sapling was significant at 150 days after grafting (Table 5). The highest number of primary branches per sapling was recorded on 25th January (3.16 ± 0.20) grafted sapling followed 10th January (3.00 ± 0.37) grafted sapling while the lowest number of primary branches per sapling was recorded on 10th December (1.63 ± 0.11) grafted sapling which was similar with 25th December (2.10 ± 0.18) grafted sapling. The highest number of primary branches per sapling was observed in Ota Ponkan (2.72 ± 0.21) and the lowest was recorded in Dhankuta Local (2.26 ± 0.19).

| Table 5. Effect of grafting dates and varieties on sapling height, leaf number, leaf area and number of primary branches at 150 days after grafting |
|---------------------------------|-----------------|-----------------|-----------------|
| Dates                           | Height of sapling (cm) | Number of leaves | Leaf area (cm²) |
| Nov 25                          | 39.98±2.01b       | 21.80±2.31bc     | 24.16±0.91b     |
| Dec 10                          | 35.51±0.88bc      | 17.93±1.11d      | 24.26±2.36b     |
| Dec 25                          | 33.90±1.40c       | 18.78±1.09cd     | 22.92±2.11b     |
| Jan 10                          | 46.62±3.17a       | 26.81±1.84a      | 29.29±2.02ab    |
| Jan 25                          | 47.58±1.93a       | 25.30±1.57ab     | 32.74±2.98a     |
| LSD                              | 4.76***           | 3.73***          | 6.66*           |
| SEm(±)                          | 1.87             | 1.58            | 2.07            |

| Species                        |                  |                  |
|--------------------------------|-----------------|-----------------|
| DL                             | 39.67±1.72a     | 20.26±1.03b     |
| OP                             | 41.76±2.06a     | 23.99±1.51a     |
| LSD                            | 3.01*           | 2.36***         |
| SEm(±)                         | 1.89           | 1.27            |
| CV                             | 9.68            | 13.96           |
| Mean                           | 40.72           | 22.13           |

CV = Coefficient of variation, LSD = Least significant difference, SEm(±) = Standard error of mean difference, DL = Dhankuta Local, OP = Ota Ponkan, Means followed by common letter(s) within a column are not significantly different at 5% by DMRT, Significance codes ***at 0.001, **at 0.01, *at 0.05, ns = non-significant.
**Increase in Diameter**

The result revealed that there was no significant difference observed in the increase in diameter below union for both dates and varieties. The highest increase in diameter below union was observed for 25th January (5.18 ± 0.72%) grafted sapling with the superior performance of Ota Ponkan (5.07 ± 0.39%) grafts. The increase in diameter above union was found significant for both date and varieties. The highest increase in diameter above union was reported on 25th January (17.78 ± 1.47%) grafted sapling which was at par with 10th January (16.62 ± 1.11%) grafted sapling while the lowest increase in diameter above union was observed on 10th December (13.30 ± 0.74%) grafted sapling. Ota Ponkan (16.24 ± 0.88%) had the highest increase in diameter above the union. The diameter at union was found significant for varieties and non-significant for grafting dates. The increase in diameter at union was recorded higher for the grafts prepared on 25th January (12.22 ± 3.29%) while the least increase in diameter at union was recorded on 25th December (8.75 ± 0.15%) grafted sapling. Ota Ponkan (13.72 ± 1.54%) grafts showed the highest increase in diameter at union (Table 6).

| **Table 6. Increase in diameter of mandarin grafts at 150 days after grafting** |
|---------------------------------|----------|----------|
| **Increase in diameter (%)**    | Diameter below union | Diameter above union | Diameter at union |
| **Dates**                       |           |           |                   |
| Nov 25                          | 3.95±0.37a | 14.35±0.65bc | 11.47±1.46a       |
| Dec 10                          | 3.87±0.40a | 13.30±0.74c  | 11.97±1.70a       |
| Dec 25                          | 3.58±0.79a | 13.76±0.89c  | 8.75±0.15a        |
| Jan 10                          | 4.50±0.81a | 16.62±1.11ab | 11.56±2.52a       |
| Jan 25                          | 5.18±0.72a | 17.78±1.47a  | 12.22±3.29a       |
| **LSD**                         | 2.09**    | 2.55**     | 5.62***           |
| **SEm(±)**                      | 0.61      | 0.92       | 1.82              |
| **Species**                     |           |           |                   |
| DL                              | 4.75±0.48a | 14.08±0.45b | 8.67±0.30b        |
| OP                              | 5.07±0.39a | 16.24±0.88a | 13.72±1.54a       |
| **LSD**                         | 1.32**    | 1.61***    | 3.55***           |
| **SEm(±)**                      | 0.43      | 0.66       | 0.92              |
| CV                              | 41.12     | 13.94      | 41.52             |
| Mean                            | 4.22      | 15.16      | 11.20             |

CV = Coefficient of variation, LSD = Least significant difference, SEm(±) = Standard error of mean difference, DL = Dhankuta Local, OP = Ota Ponkan, Means followed by common letter (s) within a column are not significantly different at 5% by DMRT, Significance codes: ***at 0.001, **at 0.01, *at 0.0, ns = non-significant

**Discussion**

**Effect of Grafting Time on Graft Success**

The success and establishment of grafts were significantly affected by the prevailing temperature and relative humidity of the propagation environment under where grafting is performed (Singh and Singh, 2006). The temperature and relative humidity affect the graft union by influencing callus formation. Meteorological data showed that late grafted sapling i.e. grafting performed in January received higher temperatures compared to other dates at the time of graft union formation (Table 3). The low temperature and lower relative humidity are unsuitable for the callus activity, as the callus cells are very delicate and need optimum temperature and high relative humidity during graft union formation (Dhakal, 1979). The rapid formation of callus tissue resulted in faster wound healing and accumulation of resinous material along with lucid vascular continuity which might be the main reason for slightly higher graft success in late grafted sapling compared to the early grafted sapling (Kilany et al., 2012). Similarly, a very low amount of GA within the scion taken in severe winter may be one of the reasons for lower sprouting on 10th December grafted sapling, as GA determines the breaking of dormant bud. The present finding was supported by Gautam et al. (2001), as they reported...
the highest graft success percent from 16th January (85%) to 31st January (87.5%) in mandarin at Lumle, Kaski. The present finding was also in line with Chalise et al. (2013a). They reported the maximum graft success on 13th January (96.11%) which was statistically at par grafting carried out on 28th January (91.11%) in mandarin at Paripatle, Dhankuta.

Linear regression analysis was done to study the effect of temperature and relative humidity on graft success percent. The analysis showed that the model was significant for temperature for all grafting dates except 25th November while the model was non-significant for relative humidity for all dates. However, the regression coefficient of humidity for grafts prepared on 10th January and 25th January was found to be positive. The optimum temperature and positive influence of humidity might be one of the main reasons for the highest graft success in late grafted saplings compared to early dates (Table 7).

Table 7. Linear regression equation showing the effect of temperature and humidity on the graft success percent.

| Grafting dates | Equation       | SE   | R²  | P value |
|----------------|----------------|------|-----|---------|
| Temperature    |                |      |     |         |
| Nov 25         | Y = 3.89X+11.04| 20.26| 0.44| .22ns   |
| Dec 10         | Y = 5.77X-50.81| 10.56| 0.85| .02*    |
| Dec 25         | Y = 6.50X-51.58| 5.77 | 0.96| .002**  |
| Jan 10         | Y = 6.71X-54.82| 9.77 | 0.90| .01**   |
| Jan 25         | Y = 5.78X-40.83| 14.43| 0.75| 0.05*   |
| Relative humidity|                |      |     |         |
| Nov 25         | Y = -3.60X+306.27| 22.50| 0.31| .32ns   |
| Dec 10         | Y = -8.98X-620.16| 17.08| 0.61| .11ns   |
| Dec 25         | Y = -1.05X+139.41| 32.03| 0.03| .76ns   |
| Jan 10         | Y = 0.53X+46.05 | 31.77| 0.01| .85ns   |
| Jan 25         | Y = 0.90X+25.48 | 28.44| 0.05| .71ns   |

* refers to significant at 0.05, ** refers to significant at 0.01 and ns refers to non-significant

Effect of Grafting Time on Growth

The highest height of late grafted sapling i.e. 10th and 25th January grafted sapling might be due to more favorable environmental conditions from the time of graft union formation up to the vegetative growth phase. The favorable environmental conditions promote the callus formation and differentiation of callus cells to xylem and phloem that ensure the better movement of water and nutrient toward different parts of the plant, ultimately resulting in the formation of more photosynthetically active leaves. Higher the photosynthetically active leaves, the higher will be the production and translocation of photosynthates and growth regulators especially auxin from leaves which resulted in higher plant height in January grafted sapling. Auxin is also involved in the wound response, as the auxin is transported basipetally from leaves and buds and triggers vascular regeneration across the graft junction (Matsuoka et al., 2016; Mazur et al., 2016; Melnyk et al., 2015). The present finding was supported by Chalise et al. (2013a) in mandarin and Adhikari (2006) in acid lime.

The variation in the number of leaves during different months was due to exposure of grafts to different environmental conditions. The favorable environmental conditions primarily accelerate the early bud breaking and secondarily influence the maximum leaf flushing as well as the maximum number of leaves due to early healing and graft union formation. The early grafted sapling had the lowest number of leaves per sapling which might be due to low temperature and relative humidity at the time of graft union formation and leaves emergence. The low temperature and unfavorable relative humidity hindered the differentiation of callus cells that ultimately reduced the transport and movement of water and mineral nutrients through xylem and photosynthate from other parts of the sapling. The present result was in contrast with Chalise et al. (2013a), as they reported a higher number of leaves for early dates as compared to the present results but the number of leaves for January grafted sapling was similar. The present finding was in agreement with Adhikari (2006) who reported the
highest number of leaves on acid lime grafts prepared in January. The highest leaf area of January grafted sapling might be due to favorable environmental conditions at the time of leaves formation and growth.

The growth of saplings is dependent primarily on the leaf area and the number of leaves per sapling, which ultimately determines the number of primary and secondary branches on the plant. The highest number of primary branches in January grafted sapling might be due to the highest sapling height, higher leaf area, a greater number of leaves and favorable weather conditions. The present finding was supported by Adhikari (2006). Chalise et al. (2013a) reported the lower number of primary branches on January grafted mandarin sapling as compared to the present result which might be due to the difference in species and climatic conditions of the research area. The number of primary branches in Ota Ponkan was higher due to higher sapling height and maximum leaf number. The present result was supported by Dubey and Singh (2003) and Seletsu et al. (2011), as they reported significant variation in the number of branches in different citrus species.

The growth of shoot is primarily determined by the production of photosynthates and their subsequent translocation to different parts of plants. The highest increase in diameter above, below and at the union in January grafted sapling might be due to faster growth resulted from higher leaf area and a maximum number of leaves per sapling, as the production of photosynthates primarily depends upon the leaf area and a number of leaves per sapling. The increase in diameter was less in present findings as compared to findings of Chalise et al. (2013a) in mandarin which might be due to differences in age and physiological conditions of scion and rootstock and prevailing environmental conditions. The variation in diameter for Dhankuta Local and Ota Ponkan might be due to differences in the number of leaves per sapling and leaf area. Dubey and Singh (2003) reported a significant difference in scion and rootstock diameter for different mandarin varieties.

**Conclusion**

Based on the findings of this study, it can be concluded that January was a suitable time for grafting of both mandarin varieties (Dhankuta Local and Ota Ponkan). The grafting carried out on 25th January grafted sapling showed superiority with respect to most of the economically important parameters viz; the height of sapling, leaf area, number of primary branches and diameter of rootstock and scion shoot. So, farmers and commercial nurseries have recommended to perform grafting in the last week of January for both mandarin varieties in the central mid-hill of Nepal. Moreover, a multi-location trial at different agro-climatic conditions is recommended to identify suitable grafting time for different mandarin varieties to obtain reliable results.

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