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Effect of Grafting Methods and Dates on the Graft Take Rate of Persian Walnut in Open Field Condition

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ABSTRACT

A field-based experiment was carried out in Rukum (East), Nepal, from February to June 2020, to assess the effect of different methods and dates of grafting on the graft take rate of Persian walnut (*Juglans regia* L.) in field condition. The experiment comprised ten treatments with combination of two factors. The two factors included the grafting dates including: 13th, 19th, and 26th of February, 4th and 11th of March and grafting methods including: outdoor grafting and bench grafting (tongue grafting in both). The experiment was laid out in a Randomized Complete Block Design with three replications. The findings revealed that bench grafting resulted in significantly better outputs than outdoor method for length of scion (at 30 and 60 days after grafting) and graft take rates. Bench grafting showed 44% graft take followed by outdoor grafting (38% graft take). Plants grafted on 13th and 19th of February showed statistically better results for growth performance over the other dates. Plants grafted on 19th of February showed 67% graft take with 53% saleable plants, but statistically similar to the plants grafted on 13th and 26th of February. The earliest bud burst was observed in plants grafted on 13th and 19th of February in 31 and 33 days, respectively. Bench grafting on 19th February showed significantly higher length of scion (16.4 cm) at 60 days after grafting. However, non-significant results were seen for other parameters due to the interaction. Therefore, bench grafting on 13th,19th and 26th of February led to the best graft take in Persian Walnut.

Abbreviations: CV: Coefficient of Variation, LSD: Least Significant Difference, ha: hectare, mt: Metric tons, PMAMP: Prime Minister Agriculture Modernization Project, RCBD: Randomized Complete Block Design, SEm: Standard Error of Mean.

Introduction

Persian walnut (*Juglans regia* L.) is an important nut tree of the world and belongs to the family Juglandaceae. It has wide adaptability to grow in the temperate regions of the world (Mir & Kumar, 2011). The walnut species growing in Nepal is *Juglans regia* L. (FAO, 2004), which is locally called '*Okhar*'

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(Acharya, 2006). It is grown in the mountain regions of the country having an elevation of 1000- 4000 m (Devkota, 1999) and across the temperate regions as well (Dhakal et al., 2003). Walnut is an important fruit of Hindu rituals, holy festivals and weddings, thus carrying an immense cultural value (Vahdati, 2014). The area under walnut cultivation in Nepal is 2167 ha while the national average production and yield of walnut are 8934 mt and 4.12 mt/ha respectively (MoALD, 2019). Rukum district, on the other hand, has a production of 742 mt (MoALD, 2019).

The propagation methods of walnut trees are divided into vegetative and sexual propagation. In the sexual method of propagation, the seedlings have a long juvenile period and often the varieties are outcrossed, due to which, the seedlings fail to be true to type in nature (Hartman et al., 2001; Ahmed et al., 2012; Wani et al., 2017). Thus, due to heterozygosity, seed propagation does not lead to the inheritance of characters of a definite variety (Gandev, 2018). Therefore, at present, walnut cultivars are preferably propagated by grafting methods (Gandev, 2007). Although true to type seedlings can be produced via grafting, most of the grafting methods are confronted with encounters such as long time for bearing and poor graft take rates (Gandev, 2009). It is due to increased water loss and tissue mortality in the graft union of walnut, which is higher compared to other easily grafted trees (Vahdati, 2003). The low graft take rates of walnut grafting has been of major interest in different countries (Ozkan & Gumus, 2001; Vahdati, 2003; Rezaee and Vahdati, 2008) like Turkey, Iran etc. In Nepal, the area under walnut plantation has extended and the demands for grafted walnut plants have been increasing (FAOSTAT, 2018; MoALD, 2019). Rukum (East) district of Nepal was declared as the 'zone' for walnut production in 2015/16, owing the agro-climatic suitability of the district for walnut plants production and the potential of walnut commercialization as a high value commodity (PMAMP, 2019). However, the production of grafted walnut plants in Nepal is still insufficient to meet the demands within the country (Giri et al., 2019).

Around the globe, along with the work on new methods for the production of inoculating planting material, old and reliable propagation technologies are being improved (Gandev, 2007). Various methods of vegetative propagation in walnut have been reported to give a varying degree of success under different climatic conditions (Ibrahim et al., 1978; Awasthi et al., 1982; Qureshi & Dalal, 1985; Ozkan & Gumus, 2001; Wani et al., 2017). The lower and unstable graft take rate hinders the progression of good varieties, regional consignment and marketability of walnut production in a straight way (Dehghan et al., 2010). Therefore, it is very urgent to discover the main factors affecting the survival of walnut grafting, to standardize the grafting procedure and to resolve the problem of poor survival percentage of grafted seedlings. In fact, it is a work of immense importance to study the uniting process of grafting and the factors affecting graft take rates in walnut (Gandev, 2014). This would assist to meet the international standards for the quality characters of nut and kernel. Hence, the knowledge of an appropriate method and appropriate time of grafting is important to enhance the production, productivity, and area under walnut cultivation (Barut, 2001; Raufi et al., 2017).

The commercial cultivation of deciduous fruits in Nepal is not a traditional practice and the propagation methods haven't evolved throughout the decades (Schnelle, 2012). The classical method of propagation in walnut is tongue grafting (Devkota, 1999). Although tongue grafting is being practiced since decades, the walnut growers are often disappointed with the low graft take rate. So far, there have been limited studies regarding the graft take rate of walnut in Nepal. Ahamad (2020) claim that the poor knowledge regarding the appropriate time and appropriate method of grafting have

constrained the higher graft take rate in walnut. Thus, there is a need to determine the suitable methods and appropriate grafting time of walnut in Nepal. Besides, the high-yielding varieties need to be propagated with a higher graft take rates to make Nepal's walnut industry competitive in the global market (Ahmed et al., 2012).

This implies that a significant improvement in the production of walnut could be achieved via increase in graft take rate of high yielding grafted seedlings. In this limelight, the current study was aimed to determine the appropriate methods and date of grafting in walnut to increase graft take rate in open field conditions.

Materials and Methods

Experimental site

The experiment was carried out in a private nursery, located in Syalapakha, Sisne Rural Municipality-08 under the command area of PM-AMP (Walnut-zone), Rukum (East) district, Nepal (Fig. 2). The research site is located at a latitude of 28.66° North and a longitude of 82.49° East. The altitude of the place is 1200 meters above the mean sea level. The climate of Syalapakha is characterized by three distinct seasons; rainy monsoon (June-October), cool winter (November-February), and mild spring (March-May) (Ahamad, 2020). The temperature of the site over the experimental period (February-June) is shown in Figure 1. The average precipitation was 1.042 mm per day, the total incident solar radiation during the study period was 640.93 kW-hr/ m^2/ day and the average relative humidity was 40.27% (NASA-Power, 2020).

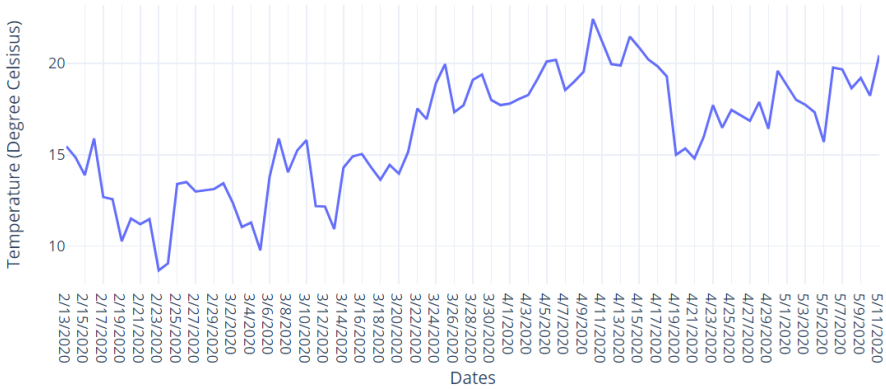

Fig. 1. Temperature of the nursery during research period in Rukum (East) district, Nepal. Source: (NASA-Power, 2020)

Fig. 2. Research site of walnut grafting study located at Syalapakha (Sisne Rural Municipality-08), Rukum (East), Nepal. Source: (Authors archive, 2020)

Experiment details

The experiment comprised of five different dates of grafting; 13th February, 19th February, 26th February, 4th March and 11th March as the first factor and the grafting methods; bench grafting and outdoor grafting method (tongue grafting incorporated in both methods) as the second factor. Thus, the experiment comprised of 10 treatments as shown in Table 1, with three replications of each treatment.

Table 1. Experimental design for studying effects of grafting methods and dates on graft take rates of Persian walnut

Grafting method × grafting time	Treatments
Bench Grafting(G1) × 13th February (D1)	T1
Bench Grafting (G1) × 19th February (D2)	T2
Bench Grafting (G1) × 26th February (D3)	T3
Bench Grafting (G1) × 4th March (D4)	T4
Bench Grafting (G1) × 11th March (D5)	T5
Outdoor Grafting (G2) × 13th February (D1)	T6
Outdoor Grafting (G2) × 19th February (D2)	T7
Outdoor Grafting (G2) × 26th February (D3)	T8
Outdoor Grafting (G2) × 4th March (D4)	T9
Outdoor Grafting (G2) × 11th March (D5)	T10

Experimental design

The experiment was carried out as randomized complete block design (RCBD) with three replications for each treatment. The rootstocks (one-year old) were at a spacing of 30×10 cm. Each plot had 20 rootstocks, on which grafting was done. A total of 30 plots were made and there were five rows in a plot with four plants per row. The plot-plot and block-block distance was kept at 50 cm. The grafted seedlings (bench grafted) after callusing were also transplanted at a 30 cm × 10 cm spacing.

Preparation of scions and rootstocks

The scions (6-8 months old) were collected from the trees of 'Hartley' variety, making sure the buds were not damaged. The length of scion sticks was kept at 8-10 cm. The scions having a nearly equal size (pencil-sized) of 0.64 cm with three buds were used for grafting. The scions were kept moist by wrapping in moist gunny bags to avoid desiccation of buds until the time of grafting operation. For the use as rootstock, one-year old wild (hard-shelled) variety seedlings were used having an average diameter of 0.64 cm. Heading back of rootstocks was done at 15 cm above the crown region prior to grafting.

Tongue grafting was done in outdoor conditions, as shown in Figure 3. In case of bench grafting, the rootstocks were pulled out followed by root pruning and tongue grafting similar to that done in the outdoor condition. The bench grafted plants were then kept inside a shade house to induce callusing. The plants inside the shade house were later transplanted to the experimental field after callus formation was noticed. The grafting of all plants was carried out by the same person with same grafting tools.

Field operations

All cultural practices like manuring, hoeing, weeding and irrigation were uniformly carried out during the research study. Fertilizers including 0.113 kg of N-P-K (15-15-15) were applied in every young sapling in two applications, one during late spring and other in the summer as foliage spray. The suckers emerging in the rootstocks were removed every week by rubbing the rootstocks (manually) below the graft union to facilitate easy sprouting of buds.

Fig. 3. Procedure of tongue grafting of Persian walnut in Rukum (East) district, Nepal

Data collection

The biometrical observations were recorded from inner five randomly selected plants of each plot to assess the vegetative traits. Observations from the sample plants were taken at three intervals: 30, 60 and 90 days after grafting. The following parameters were assessed through the data collection:

- Length of scion (cm)

The length of scion from the sample plants was measured using a meter scale.

- Thickness of scion and thickness of graft union (cm)

The thickness of scion of the sample plants was measured at 2.5 cm above the graft union, using a digital caliper and the thickness of graft union was also assessed using the caliper.

- Number of leaves per scion

From the sample plants, the number of leaves per scion were counted.

- Time of bud burst

The data regarding the time of bud burst were calculated by observing the plants on alternate days from the day of planting the grafted plants, and their means were used to calculate the days taken for first bud burst.

- Graft take rate (%)

The number of successful grafts in each treatment was recorded at the end of growing season. The graft having opened leaves from the terminal bud of scion were considered as a successful one. Graft take rate (%) was assessed as:

Graft take rate (%) = (Successful grafts / total number of grafted plants) × 100

- Rate of saleable plants (%)

The number of grafted seedlings that can be sold were estimated at end of the growing season. Saleable plants (%) were assessed as:

Saleable plants (%) = (number of saleable plants/ total numbers of grafted plants) × 100

Data analysis

The data collected were refined and entered in MS-Excel sheet. The data were analyzed to

draw meaningful inferences by using statistical software R Studio Version 4.0. The mean comparison of growth performance, time of bud burst, graft take rates (%), and rate of saleable plants (%) was done using DMRT (Duncan's Multiple Range Test).

Results

Growth performance of grafted plants at 30, 60 and 90 days after grafting

The data on the effect of grafting methods and dates on the length of scion, thickness of graft union, and thickness of scion on 30th day of grafting are shown in Table 2.

From the perusal of Table 2, when two methods of grafting were appraised together, significant results were observed for length of the scion, while the results were non-significant for thickness of graft union and thickness of scion. Bench grafting of walnut showed significantly higher results over outdoor grafting for the length of the scion (11.76 cm).

On appraising the different dates of grafting, significant results were seen for all the three parameters including length of scion, thickness of graft union, and thickness of scion. The plants grafted on 13th and 19th of February showed statistically similar results with each other for the length of scion (12.2 and 12.37 cm, respectively), thickness of graft union (3.66 and 3.75 cm, respectively) and thickness of scion (1.166 and 1.193 cm, respectively). The results were also significantly higher over rest of the dates for the parameters. The later three dates (26th February, 4th March and 11th March) were statistically similar with each other for the mentioned parameters.

The interaction effect of the methods and date of grafting, on the other hand, exhibited non-significant results for any of the parameters.

The data on the effect of grafting methods and dates on the length of scion, thickness of graft union, thickness of scion and number of

Table 2. Effect of grafting methods and dates on length of scion, thickness of graft union and thickness of scion at 30 days of grafting in Syalapakha, Rukum (East) during 2020

Treatments	Length of scion (cm)	Thickness of graft union (cm)	Thickness of scion (cm)
Grafting methods			
Bench grafting	11.76^{a}	3.297	1.049
Outdoor grafting	10.58^{b}	3.263	1.038
SEm (±)	0.83	0.0238	0.0075
$LSD_{0.05}$	0.74	-	-
CV (%)	8.64	7.28	7.48
F test $_{0.05}$	**	NS	NS
Grafting dates			
13th February	12.20^{a}	3.664^{a}	1.166^{a}
19th February	12.37^{a}	3.748^{a}	1.193^{a}
26th February	10.92^{b}	3.106^{b}	0.989^{b}
4th March	10.22^{b}	3.000^{b}	0.955^{b}
11th March	10.13^{b}	2.880^{b}	0.917^{b}
SEm (±)	1.066	0.398	0.126
$LSD_{0.05}$	1.171	0.289	0.092
CV (%)	8.64	7.28	7.48
F test $_{0.05}$	**	***	***
Grand Mean	11.17	3.28	1.044

*, **, and *** represent significance at 5%, 1% and 0.1% level, respectively. NS = non-significant. Means followed by common letter(s) within column are not significantly different with each other based on DMRT test.

leaves per scion on 60th day of grafting are shown in Table 3.

After evaluating the grafting methods, significant results were seen for length of scion while non-significant results were seen for thickness of graft union, thickness of scion, and the number of leaves per scion. Bench grafting of walnut showed significantly better results in outdoor grafting for length of scion (14.08 cm).

When the grafting dates were assessed, significant results were seen for all the vegetative parameters. Grafting on 19th of February showed statistically similar results with 13th of February for length of scion (14.81 cm and 13.80 cm respectively), and both dates showed significantly higher results over other dates of grafting for the length of scion. Grafting on the 13th and 19th of February also showed statistically similar results with each other for the thickness of graft union (3.76 cm and 3.90 cm respectively) and thickness of scion (1.19 cm and 1.27 cm respectively). Both the dates showed significantly higher results over the plants grafted on 26th February, 4th March, and 11th of March for the thickness of scion and thickness of graft union. The latter three dates were again, statistically similar with each other for the thickness of graft union and the thickness of scion. Grafting of the plants on 19th of February showed significantly higher results for the number of leaves per scion (4.71) over other dates of grafting. Grafting on 13th of February, 26th February and 4th March showed statistically similar results with each other for the number of leaves while the lowest number of leaves were recorded when grafting was done on the 11th of March (1.70).

The interaction effect of the methods and dates of grafting, on the other hand, exhibited significant results for length of scion as shown in Figure 4. Significantly higher length of scion (16.4 cm) was observed in bench grafted plants on the 19th of February while outdoor grafting on the 11th of March showed the lowest length of scion (10.50 cm). However, the results (thickness of graft union, thickness of scion, and the number of leaves per scion) were seen non-significant considering the interactions.

Table 3. Effect of grafting methods and dates on length of scion, thickness of graft union, thickness of scion and number of leaves per scion at 60 days of grafting in Syalapakha, Rukum (East) during 2020

Treatments	Length of scion (cm)	Thickness of graft union (cm)	Thickness of scion (cm)	Number of leaves per scion
Grafting methods				
Bench grafting	14.080[a]	3.480	1.115	3.20
Outdoor grafting	12.101[b]	3.458	1.107	3.06
SEm (±)	1.399	0.015	0.0052	0.0994
$LSD_{0.05}$	0.915	-	-	-
CV (%)	9.11	6.97	8.01	11.59
F test $_{0.05}$	***	NS	NS	NS
Grafting dates				
13th February	13.800[ab]	3.758[a]	1.196[a]	3.380[b]
19th February	14.811[a]	3.900[a]	1.277[a]	4.711[a]
26th February	12.875[bc]	3.308[b]	1.053[b]	3.343[b]
4th March	12.250[c]	3.283[b]	1.045[b]	2.533[bc]
11th March	11.716[c]	3.095[b]	0.985[b]	1.700[c]
SEm (±)	1.235	0.3424	0.1208	1.119
$LSD_{0.05}$	1.447	0.293	0.108	1.201
CV (%)	9.11	6.97	8.01	11.59
F test $_{0.05}$	**	***	***	***
Grand Mean	13.09	3.47	1.11	3.13

*, **, and *** represent significance at 5%, 1% and 0.1% level, respectively. NS = non-significant. Means followed by common letter(s) within column are not significantly different with each other based on DMRT test.

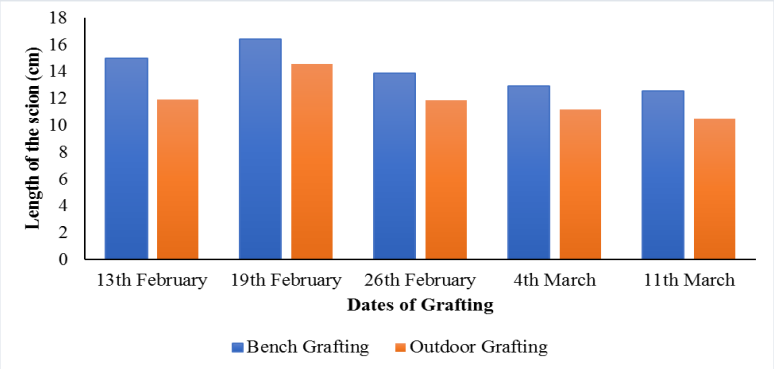

Fig. 4. Interaction effect of methods and dates of grafting of Persian walnut on length of scion at 60 days of grafting in Syalapakha, Rukum (East) during 2020

The data on the effect of grafting methods and grafting date on the length of scion, thickness of graft union, thickness of scion and number of leaves per scion on 90th day of grafting is shown in Table 4.

On appraising the grafting methods, the results were non-significant for any of the parameters, while significant results were seen for all the parameters as a result of the grafting dates. The plants grafted on the 19th of February showed the highest length of scion (31.48 cm) at 90 days of grafting and the result was significantly higher over the rest of the grafting dates for the parameter while the lowest length of scion was reported in plants grafted on 11th of March. On the other hand, grafting on 19th of February showed the highest thickness of graft union (6.07 cm), thickness of scion (1.93 cm), and the number of leaves per scion (11) but the results were statistically at par with the plants grafted on 13th of February. However, both the dates showed significantly higher graft take over the other dates of grafting for the parameters. Grafting on 26th of February, 4th of March, and 11th of March showed statistically similar results for the parameters.

The interaction of grafting methods and dates, on the other hand, showed non-significant results for the parameters.

Table 4. Effect of grafting methods and dates on length of scion, thickness of graft union, thickness of scion and number of leaves per scion at 90 days of grafting in Syalapakha, Rukum (East) during 2020

Treatments	Length of scion (cm)	Thickness of graft union (cm)	Thickness of scion (cm)	Number of leaves per scion
Grafting methods				
Bench grafting	24.43	5.147	1.638	8.75
Outdoor grafting	23.18	5.133	1.634	8.32
SEm (±)	0.887	0.009	0.003	0.30
$LSD_{0.05}$	-	-	-	-
CV (%)	13.33	9.61	9.41	11.51
F test $_{0.05}$	NS	NS	NS	NS
Grafting dates				
13th February	24.77^{b}	5.55^{a}	1.76^{a}	9.835^{a}
19th February	31.48^{a}	6.07^{a}	1.93^{a}	11.33^{a}
26th February	21.42^{bc}	4.85^{b}	1.54^{b}	7.587^{b}
4th March	20.89^{bc}	4.75^{b}	1.51^{b}	7.173^{b}
11th March	20.47^{c}	4.48^{b}	1.42^{b}	6.776^{b}
SEm (±)	4.61	0.65	0.207	1.95
$LSD_{0.05}$	3.85	0.586	0.186	2.22
CV (%)	13.33	9.61	9.41	11.51
F test $_{0.05}$	***	***	***	**
Grand Mean	23.81	5.14	1.64	8.54

*, **, and *** represent significance at 5%, 1% and 0.1% level, respectively. NS = non-significant. Means followed by common letter(s) within column are not significantly different with each other based on DMRT test.

Time of bud burst, graft take rate (%) and rate of saleable plants (%)

The data on the effect of grafting method and grafting date on time of bud burst, graft take rate (%) and rate of saleable plants (%) are shown below in Table 5.

Significant differences were observed in graft take rate due to different grafting methods while non-significant results were seen for the time of bud burst and rate of saleable plants. On appraising the grafting methods, the data regarding the graft take rate revealed that bench grafting method showed a higher graft take rate (44%), which was significantly higher than the outdoor grafting method (38%).

On appraising the grafting dates, significant differences were seen among grafting dates for time of bud burst, graft take rate, and rate of saleable plants. The evaluation of grafting dates showed that minimum time of bud burst (31 days) were recorded when grafting was carried out on 13th of February which was statistically similar to the time of bud burst for plants grafted on the 19th of February (33 days). Time of bud burst in plants grafted on 11th of March were significantly higher (52 days) followed by the plants grafted on 4th of March, 26th of February, 19th of February and 13th of February, respectively. Plants grafted on 13th, 19th, and 26th of February showed statistically similar results for graft take rates (63%, 58% and 53%, respectively), and the results were significantly higher over the plants grafted on 4th and 11th of March for the graft take rates.

The highest saleable plants (%) were recorded in plants grafted on 19th of February (57%) which was significantly higher than the plants grafted on 26th of February for percentage of saleable plants. The grafting dates, 13th and 19th of February were statistically at par with each other for the rate of saleable plants. The lowest rate of saleable plants was recorded in the plants grafted on the 4th and 11th of March, which were statistically similar to each other.

However, the interaction effect of grafting methods and dates revealed non-significant results for any of the parameters.

Table 5. Effect of grafting methods and dates on time of bud burst, graft take rate (%) and rate of saleable plants (%) in Syalapakha, Rukum (East) during 2020

Treatments	Time of bud burst (days)	Graft take rate (%)	Rate of saleable plants (%)
Grafting methods			
Bench grafting	39.73	44.44^{a}	37.11
Outdoor grafting	39.80	38.32^{b}	33.70
SEm (±)	0.047	4.325	2.40
$LSD_{0.05}$	-	6.04	-
CV (%)	5.24	19.03	23.48
F test $_{0.05}$	NS	*	NS
Grafting dates			
13th February	31.33^{d}	57.50^{a}	48.33^{ab}
19th February	32.83^{d}	62.72^{a}	56.77^{a}
26th February	36.17^{c}	52.83^{a}	46.05^{b}
4th March	47.00^{b}	15.76^{b}	12.21^{c}
11th March	51.50^{a}	18.05^{b}	13.67^{c}
SEm (±)	8.97	22.62	20.90
$LSD_{0.05}$	2.526	9.55	10.08
CV (%)	5.24	19.03	23.48
F test $_{0.05}$	***	***	***
Grand Mean	39.77	41.38	35.41

*, **, and *** represent significance at 5%, 1% and 0.1% level, respectively. NS = non-significant. Means followed by common letter(s) within column are not significantly different with each other based on DMRT test.

Discussion

Effect of different methods and date of grafting on growth performance of scion

The current study revealed that grafting on 13th and 19th of February resulted in a higher length of scion, thickness of scion and thickness of graft union while grafting on 19th of February led to the higher number of leaves per scion. Mir and Kumar (2011), in their experiment in India, reported the highest length of scion and highest number of leaves when grafting was done on the 3rd week of February which is in line with the findings of the present study. Similarly, Ahmed et al. (2012) also reported higher number of leaves per scion in the plants in which tongue grafting was done. The higher length of scion, thickness of scion, thickness of graft union and a greater number of leaves could be attributed to better uptake of nutrients from rootstock to the scion and uniform growth as a result of formation of proper bridge between scion and rootstocks (Skene et al., 1983; Ozkan and Gumus, 2001), when grafting was performed during 3rd week of February.

In contrast with our experiment, Mehta et al. (2018), in their experiment in Jammu, reported higher length of scion, higher thickness of scion and higher number of leaves when tongue grafting was done during the first week of March. Similarly, Wani et al. (2017) in their experiment in Kashmir, reported a higher length of scion, number of leaves per scion and thickness of scion when grafting was done on 20th and 30th of January which was in contrast with the results of the present study. This may be due to the suitable periods and methods of walnut grafting differing from one ecological area to another (Achim and Botu, 2001).

Effect of different methods and date of grafting on time of bud burst

Earlier bud burst in our experiment was observed in plants grafted on 13th of February followed by those grafted on 19th of February. Similar results were also reported by Singh et al. (2019), in their experiment in India, when grafting was done during earlier weeks of February. Similarly, Mir and Kumar (2011) also reported earlier bud burst (29 and 32 days) when plants were grafted on 3rd and 4th week of February which is in accordance with our findings. In contrary, Mehta et al. (2018) reported the minimum number of days (25 days) to bud burst in tongue grafting when performed during 1st week of March which was followed by the same method when performed during 2nd week of March (30 days).

The earlier bud burst in plants grafted during 13th and 19th of February could be attributed to early stimulation of the division of phloem ray cells, xylem ray, xylem parenchymal cells and parenchymal cells between periderm and phloem, which resulted in earlier callus formation (Soleimani et al., 2010). This led to the earlier flow of sap and nutrients from rootstock to the buds which led to early bud burst. The late bud burst in plants grafted on last week of February and earlier weeks of March could be attributed to poor temperature and humidity, thereby delaying the callus formation period (Hartman et al., 2001; Karadeniz, 2005; Vahdati and Zarei, 2006).

Effect of grafting methods on the graft take rate (%) and rate of saleable plants (%)

In our experiment, we observed 44% graft take when bench grafting was performed. Similar results were also reported by Giri et al. (2019) in their experiment at Rajikot, Jumla, where they found the success rate of bench grafting in walnut vary from 44 to 57% in case of Hartley variety. However, higher success (63%) was observed in our experiment when grafting was done on 19th of February, which is in contrast with their results, where they observed a higher graft take in plants grafted during 2nd week of March. It could be attributed to variation in geography and climate of our experimental sites (Ebrahimi et al., 2009). Similarly, Ahmad et al. (2018) recommended that for higher graft take rate in

walnut, bench grafting should be done 15 cm above ground level during mid-February which is in accordance with our findings. Our results are also in agreement with the previous reports (Ozkan and Gumus, 2001; Ozkan et al., 2001; Vahdati and Zarei, 2006; Dehghan et al., 2010), who reported that method of grafting is very important in grafting success.

In contrast with our experiment, Sadeghi Majd et al. (2019), in their experiment in Iran, reported a graft take rate of 62% with tongue grafting method which was only 44% in our case. It can be attributed to the use of different graft cover material in their experiment. Wani et al. (2017), in their experiment in India, reported a very low success rate of grafting in open field condition in their experiment when tongue grafting was carried out from 10th January to 20th of February in two different years 2013/14, which is in contrast with our experiment as we obtained a better success rate when tongue grafting was done on 13th,19th and 26th of February. This may be due to the suitable periods and methods of walnut grafting differing from one ecological area to another (Achim and Botu, 2001).

The better graft take rate in bench grafted plants in our field experiment could be due to appropriate graft union. The appropriate temperature and humidity might have been met inside the shade house, which was conducive for appropriate graft union (Vahdati and Zarei, 2006). On the other hand, the outdoor grafted plants were exposed to high intensity sunlight, high daytime temperatures, and low humidity in the field condition which might have resulted in poor callusing (Hartman et al., 2001) and thereby low graft take and lower rate of saleable plants. It may also be due to the active growth of scion tissues and loss of tolerance to injury during the time period (Ebrahimi et al., 2009). The better graft take rate in our experiment, even in the open field condition, may be due to the use of a sharp grafting knife, use of fresh scion wood and turgidity in the cells due to proper irrigation before grafting and after one week of grafting. However, some of the scions in our experimental field failed to continue growth and did not survive the three months after grafting. This failure may be a result of warm and dry summer or an inadequate rootstock/scion connection that produces vessels (Sadeghi Majd et al., 2019).

Effect of grafting date on the graft take rate (%) and rate of saleable plants (%)

In our experiment, we observed 63% graft take and 57% saleable plants when grafted on 19th of February and 18% graft take with 12% and 13% saleable plants, when grafting was done on 4th and 11th of March, respectively. Similar results were reported by Mir and Kumar (2011) in their experiment in India, where they reported a graft take of 67% and 57% of saleable plants when tongue graft on 3rd week of February and 10-20% graft take with 10-13% saleable plants in plants grafted on 1st and 2nd week of March. Similarly, Aminzadeh et al. (2013), in their experiment in Iran, stated that time of grafting has significant effect on graft take and grafting survival. The authors determined that the most suitable time for minigrafting is February with 83% grafting success compared to grafting performed at other dates (early January, March and early May) which is in accordance with our study. Moreover, Sayed et al. (2000), in their experiment in Egypt, also recommended that best time of grafting in walnut is the dormant season, particularly February, when grafting is found to be quite successful as both the scion and rootstocks are in dormant conditions.

In contrary, Wani et al. (2017), in their experiment in India, reported a very low graft take rate in open field condition in their experiment when tongue grafting was carried out from 10th January to 20th of February in two different years 2013/14. The higher root pressure of walnut trees in early-Spring, correlated with increased mineral uptake and soil temperatures (Ewers et al., 2001), xylem

bleeding, and the accumulation of sap under the graft cover (wax or plastic tape), could have resulted in a disappointing rate of graft take in their experiment. Likewise, Ahmed et al. (2012), in their experiment in Jammu and Kashmir, reported the highest graft take rate when grafted on 15th of March in case of tongue grafting, which is also in contrast with our results. This may be due to the suitable periods and methods of walnut grafting differing from one ecological area to another (Achim & Botu, 2001).

The combined effect of suitable temperature, humidity and active state of scion and rootstock tissues during 13th,19th and 26th of February might have permitted maximum regeneration of parenchyma cells in cambium region (Ahmed et al., 2012) leading to a higher graft take and higher rate of saleable plants in our experiment. The temperature after grafting in walnut should be maintained at about 27° C for better callus formation and increased graft take rate while the night temperature shall be near about 12° C (Rongting and Pinghai, 1993; Vahdati and Zarei, 2006). In case of plants grafted on the 13th,19th and 26th of February, earlier callus formation was observed (31, 33 and 36 days respectively) and the appropriate temperature and humidity was met, which was conducive for appropriate graft union. Similarly, the total phenolics contents in plant parts of walnut were reported to be low in early-Spring and late-summer, with a peak at the beginning of summer (Solar et al., 2006), implying the suitability of early-spring for walnut grafting. The lower graft take rate in plants in which grafting was done during early March in our experiment may be due to high intensity sunlight, high daytime temperatures, and low humidity during late spring and early summer (Kuden and Kaska, 1997; Hartman et al., 2001) which caused poor callusing.

The different values attributed to grafting success in our experiment and the overall poor success rate of grafting in walnut could be attributed to the physiological status of scion. The shortness of ferulic acid in walnut scions and the presence of coumaric acid and catechin result in an increased content of IAA oxidase and thereby leading to poor callus formation (Rongting & Pinghai, 1993). Juglone (5-hydroxyl-1,4-naphthoquinone), a well-known phenol, is found specifically in scion tissues of walnut as well as in xylem and phloem sap. It is chemically reactive leading to the formation of free radicals (Solar et al., 2006). The formation of such highly reactive free radicals during grafting is associated with several oxidative stresses such as hypoxia, extreme temperature, and wounding that could play a role in death-inducing signals leading to the irreversible damage of affected tissues through necrosis, apoptosis and cell death (Jones, 2001; Breusegem and Dat, 2006; Rezaee et al., 2008), responsible for a relatively poor success in walnut grafting.

Conclusion

The current study aimed to determine the appropriate grafting method and date for better graft take rate in walnut. It can be concluded from the current findings that the dates, 13th,19th and 26th of February are preferred for grafting and the bench grafting method can be practiced for a better graft take rate. The results of this study can be modified for different climates and regions to be useful for walnut vaccine studies, PM-AMP, Walnut Zone, Rukum, future walnut growers or researchers as well as the concerned stakeholders.

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Conflict of interest

The authors indicate no conflict of interest for this work.

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