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Effect of Scion Varieties and Wrapping Materials on the Success of Tongue Grafting on Apple (*Malus* spp.) in Jumla, Nepal

Dharma Prasad Chapai ¹, Sisam Gautam ¹ ■, Madhav Dhital ², Karishma Bhusal ¹, Rabin Nepal ¹, Aayush Rijal ¹

1 Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, 44209, Nepal

2 Department of Horticulture, Agriculture and Forestry University, Rampur, Chitwan, 44209, Nepal

Corresponding email: sisamgautam097@gmail.com

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Abstract This study was conducted at Chandannath municipality, Jumla to study the effect of different scion varieties and wrapping materials on the success of tongue grafting on Apple (*Malus* spp.) from March to July, 2023. For this experiment, four scion varieties of apple i.e. Fuji, Royal Delicious, Vance Delicious, and Red Chief were grafted on Edy Mayal rootstock by using two different wrapping materials (Polyethylene plastic and Grafting tape). The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Royal Delicious was found statistically significant for days to sprout and the number of leaves was on par with Red Chief and Vance Delicious. Royal Delicious showed the minimum days to sprouting (47.3 days) maximum number of leaves (18.50) and minimum sprout length (31.45 cm). In addition, graft success was found to be significant for scion variety at a 10% level of significance. The maximum graft success was observed on both Red Chief and Royal Delicious (96.67%) while was low on the Vance Delicious variety (76.67%). Grafting tape was found to be superior to polyethylene plastic in terms of number of leaves. The graft union tied with grafting tape developed more leaves on scion (18.25) compared to the other tied with polyethylene plastic (16.91) at final observation. No interaction effect was observed at all parameters. Based on the results of this study, Royal Delicious variety is the best scion variety for tongue grafting and grafting tape is the best wrapping material under the climatic conditions of Jumla district.

Keywords Grafting; Graft success; Scion varieties; Wrapping materials

Introduction

Apple (*Malus* spp., 2n=34) is one of the high-value cash crops of Nepal, belonging to the family Rosaceae (Potter et al., 2002). Apples have numerous health benefits due to the phytochemicals present in apples (Boyer and Liu, 2004). The productive area, production, and yield of Apple in Nepal are 6,245 ha, 52,753 Mt, and 8.45 Mt/ha respectively while that of Jumla are 1,454 ha, 13,958 Mt, and 9.6 Mt/ha respectively (MoALD, 2023). The Nepalese government formally introduced apple cultivation in Jumla in 1967 BS, recognizing the region's geographical and climatological suitability and comparative advantage. Apple production in Jumla generates a gross margin of NPR 109,615.48 or approximately USD 1000 per hectare of land, indicating that cultivating apples is a lucrative business in the district (Sapkota et al., 2020). Major apple varieties cultivated in Jumla district are mostly the Delicious varieties. These varieties include Red Delicious, Royal Delicious, and Golden Delicious (Atreya and Kafle, 2016).

Apple propagation can be done through seed or vegetative methods. Propagation through seed does not result in true to type in fruit plants as seeds are heterozygous in nature (Iqbal and Singh, 2020). The major propagation methods of apples include budding, layering, and grafting (Singh et al., 2019). Grafting has the advantage that it is possible to propagate the same genotype and to obtain clones of parent plant (Petri et al., 2019). Different grafting techniques are used in commercial apple sapling production, but tongue and cleft grafting are widely used (Iqbal and Singh, 2020). Tongue grafting is the best-suited method with a high success rate (Vinayak et al., 2023). Grafting means combining and fusing two parts of the plant to allow it to develop as a single plant (Ak et al., 2021). To propagate a scion onto a rooting system, rootstocks are used to gain consistency and precocity in the fruiting part (Ersoy et al., 2010).



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Elasticity of grafting tape helps to prevent the loss of humidity and drying of bud sticks which aids in the healing process by producing wound tissue over the cut surfaces (Zenginbal et al., 2006). Farmers participating in the PMAMP program, specifically the PIU in the Jumla Super Zone, have introduced the Mailing series of rootstock (M111, M9, M6, and M7) in some high-tech nurseries. The rootstocks have been found susceptible to WAA. As Jumla is an organic zone, farmers cannot use chemical pesticides to control the infestation, and botanical pesticides have not been effective in controlling WAA in the mailing series. Currently, normal plastic is the primary wrapping material used at the farmer's level. However, using grafting tape instead of normal plastic would provide a better graft union.

In 2008, the government of Nepal designated Jumla as an organic district (Lewison, 2019). The primary factors that hinder apple production in the Jumla district are various diseases and pests that affect both the trees and fruit such as Apple scab, Nectoria twig blight, Tent caterpillar, Woolly apple aphid, etc. (Subedi and Gautam, 2019). Due to the rise in the demand for organic apples and the susceptibility of Delicious varieties to pests and diseases, the introduction of new varieties, such as Gala, Fuji, and Spur varieties (Vance and Red Chief) was initiated in Jumla. This reflects Jumla's transition phase from subsistence farming to commercial farming, and there is an increasing demand for quality saplings.

1 Materials and Methods

1.1 Experimental site

The experiment was conducted in an Apple nursery located at Chandannath municipality of Jumla district. The site was located in the high hills of Nepal at an altitude of 2,510 m. The experimental site comprises of temperate and dry climate with snow in winter.

1.2 Experimental materials

One year-old disease-free rootstocks were taken and four varieties of scion, 10 cm long and having 2 buds each were collected from the Horticulture Research Station, Jumla. Tongue grafting was performed on the 8th of March, 2023, and tied with two different types of wrapping materials. The grafts were left in the nursery for union success and observed at every 15-day interval.

1.3 Experimental design

The experiment was conducted in two factorial Completely Randomized Block Design (RCBD) comprising 8 treatments and 3 replications. As a result, there were 24 plots with individual areas of 50 cm * 30 cm (1,500 cm sq.) which were designed for homogenous field conditions as present in our field. Each plot contained 10 saplings. Among these 5 plants were selected randomly for taking the measurement.

1.4 Treatment details

The experiment was organized around two primary factors: scion variety and wrapping materials (Table 1).

1.4.1 Factor one-Scion varieties

Four different varieties of apple scions which are common in Jumla district were selected. These varieties include:

- (1) Fuji (V1): The Fuji variety is known for its distinctive attributes like its sweet flavor, crisp texture, and appealing coloring making it a popular choice.
- (2) Royal Delicious (V2): Distinctive features of the Royal Delicious variety are its large conical shape with yellow skin covered with red strips. Sweet flavor and attractive colors make it a popular choice in the market.
- (3) Red Chief (V3): Red Chiefs are known for their delicious taste with cream-colored flesh. The fruit is large and conical in shape having red strips which is favored in retail markets.
- (4) Vance Delicious (V4): Vance Delicious has distinguishing features like deep red coloration with a sweet flavor and whitish flesh.

1.4.2 Factor two-Wrapping materials

Two types of wrapping materials were selected and used to wrap the graft union which is necessary to prevent drying. These include:



- (1) Polyethylene plastic (W1)
- (2) Grafting tape (W2)

Table 1 Treatment details used in the study

S.N	Name of treatment	Symbol	Treatment	
1	Fuji + Polyethylene plastic	V1*W1	T_1	
2	Fuji + Grafting tape	V1*W2	T_2	
3	Royal Delicious + Polyethylene plastic	V2*W1	T_3	
4	Royal Delicious + Grafting tape	V2*W2	T_4	
5	Red chief + Polyethylene plastic	V3*W1	T ₅	
6	Red chief + Grafting tape	V3*W2	T_6	
7	Vance Delicious + Polyethylene plastic	V4*W1	T ₇	
8	Vance Delicious + Grafting tape	V4*W2	T_8	

1.5 Data collection and measurement methodology

Five experimental saplings per plot were selected and tagged for taking the measurement leaving saplings from all sites of the experiment unit as border plants. The growth and success of the grafts were precisely monitored and recorded at regular intervals, following a structured approach to ensure accuracy and consistency in data collection.

1.5.1 Graft sprouting time

The time taken for each graft to sprout was observed and documented. This data was recorded at 15-day intervals starting from the date of grafting, providing insights into the initial growth response of the grafts.

1.5.2 Sprout length increment

The growth in length of the first sprout was a critical parameter. Measurements were taken at 60 and 75 days after grafting (DAG) using a scale. This information helped assess the rate of growth and vigor of the sprouts.

1.5.3 Sprout girth increment

The growth in girth of the first sprout was observed and measurements were taken at 60 and 75 days after grafting (DAG). This information helped assess the graft compatibility and the success of graft union.

1.5.4 Number of fully opened leaves

Measurements of the total number of fully opened leaves were taken at 60 and 75 days after grafting (DAG). This information assisted in evaluating the healthy vascular connection and the successful vegetative growth.

1.5.5 Graft success

The total number of successfully sprouted scions and the total number of dried scions were recorded.

The graft success rate was then calculated using the formula:

1.6 Statistical analysis

The obtained data were entered and processed in Microsoft Excel. Rstudio software was used to analyze the data obtained. Duncan's Multiple Range Test (DMRT) was used for mean separation at a 5% level of significance.

2 Results and Analysis

2.1 Graft sprouting time

The result of ANOVA showed graft sprouting time was found to be significantly influenced by scion variety at 75DAG, on par with Red Chief i.e. 49.67, and Vance Delicious i.e. 54.83. Royal Delicious showed the minimum days to sprouting i.e. 47.3 days and variety Fuji showed the maximum days to sprouting i.e. 57.3 days. The effect of the wrapping materials on graft sprouting time was statistically non-significant. However, at 75DAG the graft sprouting time with polyethylene plastic and grafting tape were found to be 53.5 days and 51.08 days respectively. The interaction between scion and wrapping material was found to be non-significant at 75 DAG.



2.2 Number of fully opened leaves

The number of fully opened leaves was found to be significantly influenced by scion variety and wrapping materials at both 60DAG and 75DAG (Table 2). Royal Delicious was found statistically significant on par with other varieties. The maximum number of leaves was found in variety Royal Delicious i.e. 10.5 in 60DAG and 18.5 in 75DAG. Whereas, the minimum number of leaves was found in variety Fuji i.e. 8.83 in 60DAG and 16.33 in 75DAG. The effect of wrapping materials on the number of fully opened leaves was also found to be significantly different. The grafting tape-tied scion showed a higher number of leaves i.e. 10.42 in 60DAG and 18.25 in 75DAG compared to polyethylene plastic-tied scion i.e. 9.25 in 60DAG and 16.91 in 75DAG.

The interaction between scion and wrapping material was found to be non-significant at both 60DAG and 75DAG.

Table 2 Effect of scion variety and wrapping materials on the number of days to sprouting of graft and number of leaves on tongue grafting of apple in Jumla, 2023

Treatments	Days to sprout	Number of leave	Number of leaves	
Factor A (Scion varieties)	75DAG	60DAG	75DAG	
Fuji	57.3 ^a	8.83 ^b	16.33 ^b	
Royal Delicious	47.3°	10.50^{a}	18.50 ^a	
Red Chief	49.67 ^{bc}	10.00^{a}	18.00 ^a	
Vance Delicious	54.83 ^{ab}	10.00^{a}	17.50 ^{ab}	
SEm (±)	2.00	0.36	0.49	
LSD	6.089*	1.11*	1.48*	
Factor	-	-	-	
B (Wrapping material)	-	-	-	
Polyethylene plastic	53.5	9.25 ^b	16.91 ^b	
Grafting tape	51.08	10.42^{a}	18.25 ^a	
SEm (±)	1.42	0.26	0.35	
LSD	ns	0.78**	1.046*	
Grand mean	52.29	9.83	17.58	
CV (%)	9.40	9.12	6.79	

Note: Means with the same letter within a column do not differ significantly at p=0.05 by DMRT. *= Significant at 5% ($p\le0.05$), **= Significant at 1% ($p\le0.01$), ***=Significant at 0.1% ($p\le0.001$), ns= non-significant, SEm= Standard error of the mean, LSD= Least Significant Difference, CV= Coefficient of variance, DAG= Days after grafting

2.3 Sprout length increment

The effect of scion variety on sprout length was found to be significant at both 60DAG and 75DAG. The maximum sprout length was found to be 12.68 cm in Vance Delicious at 60DAG and 37.78 cm in Fuji variety at 75DAG. Fuji variety was found statistically significant with Vance Delicious i.e. 12.68 cm. Variety Royal Delicious showed minimum sprout length at both 60DAG and 75DAG i.e. 9.65 cm and 31.45 cm respectively.

The result of ANOVA also showed the effect of wrapping materials on sprout length to be statistically non-significant. However, at 60DAG and 75DAG the sprout length was found to be 10.45 cm and 33.29 cm in graft tied with polyethylene plastic, and the sprout length of 11.62 cm and 33.79 cm in graft tied with grafting tape.

The interaction between scion and wrapping material was found to be non-significant at both 60DAG and 75DAG.

2.4 Sprout girth increment

The effect of scion variety and wrapping materials on sprout girth increment was found to be statistically non-significant in both observations i.e. 60DAG and 75DAG (Table 3). At 60DAG, the maximum sprout girth was found to be 1.36 cm on the Vance Delicious variety and the minimum sprout girth was found to be 1.25 cm on Red Chief. At 75DAG, the maximum sprout girth was found to be 1.59 cm on the Fuji variety and the minimum sprout girth was found to be 1.44 cm on the Vance Delicious variety. All the scion varieties were found to be statistically similar for sprout girth.



The interaction between scion and wrapping material was found to be non-significant at both 60DAG and 75DAG.

Table 3 Effect of scion variety and wrapping materials on the average length and girth of sprouts on tongue grafting of apple in Jumla, 2023

Treatments	Sprout length (cm)		Sprout girth (cm)	Sprout girth (cm)	
Factor A (Scion varieties)	60DAG	75DAG	60DAG	75DAG	
Fuji	11.85 ^{ab}	37.78 ^a	1.30	1.59	
Royal Delicious	9.65 ^b	31.45 ^b	1.29	1.52	
Red Chief	9.95 ^b	33.05^{b}	1.25	1.57	
Vance Delicious	12.68a	31.88 ^b	1.36	1.44	
SEm (±)	0.79	1.37	0.04	0.09	
LSD	2.41*	4.15*	ns	ns	
Factor B (Wrapping materials)	-	-	-	-	
Polyethylene plastic	-	-	-	-	
Grafting tape	10.45	33.29	1.28	1.53	
	11.62	33.79	1.32	1.53	
SEm (±)	0.56	0.96	0.027	0.06	
LSD	ns	ns	ns	ns	
Grand mean	11.03	33.54	1.3045	1.53	
CV (%)	17.61	10.00	7.24	14.46	

Note: Means with the same letter within a column do not differ significantly at p= 0.05 by DMRT. *= Significant at 5% (p \le 0.05), **= Significant at 1% (p \le 0.01), ***=Significant at 0.1% (p \le 0.001), ns= non-significant, SEm= Standard error of the mean, LSD= Least Significant Difference, CV= Coefficient of variance, DAG= Days after grafting

2.5 Graft success

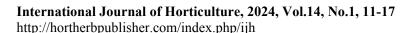
The result of ANOVA showed graft success was found to be statistically significant at 10%. Royal Delicious and Red Chief were found statistically significant on par with Fuji i.e. 80.00%. Results showed the maximum graft success was found on Red Chief and Royal Delicious i.e. 96.67% on both varieties and minimum graft success on Vance Delicious variety i.e. 76.67% (Table 4). The effect of wrapping material on graft success was found to be non-significant. Graft tied with polyethylene plastic showed 85% graft success. Graft tied with grafting tape showed 90% graft success.

The interaction between scion and wrapping material was found to be non-significant.

Table 4 Effect of scion variety and wrapping materials on the graft success (%) on tongue grafting of apple in Jumla, 2023

Treatment	Graft success (%)		
Factor A (Scion varieties)			
Fuji	80.00^{ab}		
Royal Delicious	96.67 ^a		
Red Chief	96.67 ^a		
Vance Delicious	76.67 ^b		
SEm (±)	5.87		
LSD	17.82		
Factor B (Wrapping material)	-		
Polyethylene plastic	85.00		
Grafting tape	90.00		
SEm (±)	4.15		
LSD	ns		
Grand mean	87.50		
CV (%)	16.45		

Note: Means with the same letter within a column do not differ significantly at p=0.05 by DMRT. *= Significant at 5% ($p\le0.05$), **= Significant at 1% ($p\le0.01$), ***=Significant at 0.1% ($p\le0.001$), ns= non-significant, SEm= Standard error of the mean, LSD= Least Significant Difference, CV= Coefficient of variance, DAG=Days after grafting





3 Discussion

During the research period, the parameters under study were carefully measured and documented. Data analysis was done by using reliable software. Graft sprouting time was found to be statistically significant for scion varieties. Fuji variety showed the maximum days to sprouting and Royal Delicious showed the minimum days to sprouting. The observed result shows that the difference in graft sprouting time might be due to varietal characteristics. Fuji apple variety is a late maturing variety and has high chilling hours (Parkes et al., 2020). For bud breaking, variation in different varieties might be due to translocation of food reserves and cambial activity (Bose et al., 2019).

Number of fully opened leaves was found to be maximum in Royal Delicious variety and minimum in Fuji variety of apple. Similarly, grafting tape was found to be a more suitable wrapping material for vegetative growth such as the number of leaves as observed in the result obtained. Findings observed are also following (Devkota et al., 2020) reported that grafts tied with grafting tape-recorded the maximum number of leaves on the Royal Delicious variety among the rootstocks grafted on 11th March at both 60DAG and 90DAG. It may be due to the elasticity of grafting tape that helps to prevent the drying of bud sticks and also allows for the production of wound tissue over cut surfaces as stated in (Zenginbal et al., 2006).

Sprout length increment was found to be statistically significant for scion varieties. The observed result shows that the changes in the length of the sprouts of different scion varieties are due to varietal characteristics and genetic makeup. Similar findings were observed that reported a significant effect on plant height (Iqbal et al., 2016). Graft success was found to be maximum on Red Chief and Royal Delicious varieties. Sprout girth increment was found statistically non-significant for both scion varieties and wrapping materials. No interaction was observed between scion variety and wrapping material on any of the parameters under consideration.

4 Conclusion

Royal Delicious variety was the best scion variety as devised from the study. From the research above Royal Delicious variety has shown better response than other varieties, thus the respective variety is suggested. Grafting tape has shown a higher number of leaves compared to polyethylene plastic, thus grafting tape is recommended as wrapping material.

Authors' contributions

DPC and SG were involved in conducting the experiments, data analysis and interpretation, and drafting of the manuscript. RN and AR assisted during the layout and data collection. KB and MD were involved in the revision and final shape of the manuscript. All authors read and approved the final manuscript.

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