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Short Communication

Study on Time and Method of Grafting on the Graft Success in Grape

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ABSTRACT

The study was conducted to determine the effect of growth regulator, time and method of grafting on the success of graft union and root formation in Flame Seedless grapevines grafted on Dog Ridge rootstock. The grafting operations were performed at different dates i.e. first week of February, Third week of February, first week of August and first week of September. The basal portion of the rootstock cuttings were dipped in IBA at the rate of 2000 ppm for promoting rooting. Following this treatment the cuttings were grafted (tongue and wedge grafting) and were maintained as per experiments and success of graft union was observed. The data revealed that grafts made during third week of February gave the maximum graft take compared to other months, i.e. early February, August and September. Among the two methods of grafting, the maximum graft take was obtained with tongue grafting. Thus, tongue grafting performed on grape vines during the third week of February exhibited higher success in terms of sprouting percentage and other vegetative growth parameters.

Keywords: Grafting method, *Vitis vinifera*, Dog ridge, rootstock, growth regulators

INTRODUCTION

Grape (*Vitis vinifera*) is an important species highly preferred for table purpose as well as for the production of high quality wine. It is native to the Mediterranean region, central Europe, and south western Asia. *Vitis vinifera* contributes to more than 90% of the world's grape production. Grapes are one of the most important fruit crops, accounting for about one quarter of the fruit production of the world. It is grown throughout the temperate regions, especially in warm sunny climates with mild winters and dry periods during fruit ripening. In India, it is being cultivated successfully in both tropical conditions of west and south India and subtropical conditions of north India. Area under grape in India is 123 thousands hectares with an annual production of 2823 thousand MT (Anon 2016). Most of the area of grape cultivation is in Maharashtra, Karnataka, Tamil Nadu, and Andhra Pradesh. In Punjab, it is being cultivated as temperate crop and presently grape occupies an area of 420 hectares with annual production of 28607 MT (Anon 2017). In the traditional viticulture in India, commercial varieties of grapes were grown on their own roots; however, the need for rootstock has been felt during the past couple of decades due to the increasing problem of soil salinity, drought and poor fruitfulness of varieties. The importance of rootstocks in viticulture is well documented. Rootstocks have potential not only for combating the soil problems but can also be a potential tool for manipulating the vine growth and productivity and to alter the shoot vigour thus bringing an equilibrium between growth and yield (Satisha and Adsule, 2008; Walker *et al*, 2000 and Keller *et al* 2001). These effects take place in a more or less indirect manner and consequences of the interactions between environmental factors and the physiology of the scion and rootstock cultivars employed. Dog Ridge rootstock is known for its drought and salt tolerance in major grape growing regions of India and hence is most widely accepted rootstock in India. It is moderately resistant to phylloxera and lime. Manageable growth and quality fruit have been produced from productive

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varieties grafted onto Dog Ridge in porous soils heavily infested with nematodes. However, 'Dog Ridge' cuttings root with great difficulty which hampers unrooted bench graft propagation. Rootstocks vary in their capacity to induce rooting. Hence many practices are followed to obtain good rooting success percentage, which involves use of growth regulators like IBA, cold water treatment to leach out the inhibitors present in the cuttings, bottom heating and girdling (Satisha and Adsule 2008). Currently, indole butyric acid (IBA) is the most widely used auxin to stimulate the rooting process in cuttings owing to its high ability to promote root initiation and its weak toxicity and great stability in comparison to naphthalene acetic acid and indole 3 acetic acid. Pandey *et al* (2011) stated that the application of IBA may have an indirect influence by enhancing the speed of transformation and movement of sugar to the base of cuttings and consequently rooting. Though the technique of grafting is being used to some extent in Maharashtra, however, the information on successful grafting technique for making composite plants of grape under north Indian conditions is still lacking. Keeping this lacuna in view, an attempt was made to standardize grafting in grape cultivars on suitable rootstock under north Indian conditions for possible commercialization in future. Therefore, the present study was planned to compare success in grafting using four different times (First and third week of February, August or September grafting) with two different methods (wedge and tongue) using Flame Seedless as scion and Dog Ridge as rootstock. Season was selected keeping in mind the comparison of graft success in dormant vines and when sap is flowing. Also as prelude to this study, investigation were undertaken to study the effect of IBA on the rooting of stem cuttings of grape rootstock Dog Ridge and dose thus standardized (2000 ppm) was used in this experiment.

MATERIALS AND METHODS

The present investigation was carried out at Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana for two consecutive years (2016-2017). The experimental site is situated at an altitude of 270 m above mean sea level and it geographically lies at the latitude of 23.3° N and longitude of 74.5-76.5° E. Hardwood cuttings of grape rootstock, Dog Ridge with four internodes were used as experimental rootstock material. Flame Seedless, a complex hybrid whose parents include Sultanina, Cardinal, Malaga and Muscat d' Alexandric, was used as the scion variety.

Preparation of Rootstock cuttings

Cuttings of Dog Ridge rootstock and Flame Seedless variety were taken from the Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana. Cuttings were taken from vigorous branches from the previous season's growth, containing healthy, well nourished, and mature wood which were more or less similar in size and appearance. The rootstock (Dog Ridge) cuttings contained 4 internodes from middle portion of cane. The average length and diameter of the Dog Ridge cuttings used for rooting was 18-24 and 0.8cm to 1.4cm, respectively and the cut at the base (lower end) of the cutting was made nearly straight across close below a node while the top is cut at an angle of about 45° and 2 - 2.5cm above a node. The basal 3-4 cm portion of Dog Ridge hard wood cuttings were treated with IBA 2000 ppm by quick dip method for 5 minutes and were allowed to shade dry for 15 minutes.

Preparation of Scion

The Flame Seedless scion sticks were taken from one year old field grown healthy vines grafting. The dormant vines of matching thickness were selected for the grafting experiments. For August and September grafting, the scion shoots were defoliated one week prior to grafting operations. All the cuttings were treated with fungicidal solution containing carbendazim 2 g per litre by dipping them for 10 minutes and then shade dried for 15 minutes. The scion cuttings with two nodes were taken for grafting.

Grafting operations were performed at in first week of February, Third week of February, first week of august and first week of September and two grafting techniques i.e. tongue grafting and wedge grafting were performed. The basal portion of the rootstock cuttings were dipped in IBA of 2000 ppm concentration for promoting rooting. These grafted cuttings were placed in poly bags with potting mixture comprising soil, sand and FYM in 1:1:1 ratio and were maintained in open as per experiments and success of graft was observed.

Out of 30 grafts per treatments five grafts were selected randomly and tagged for recording the observations. These sample plants were used for observing the graft union, vegetative growth, leaf and root characters. Observations on growth parameters were recorded at 60 days interval after grafting. The graft take in different combinations were recorded at end of sprouting days and the percentage graft take was calculated. The number of grafts that survived 90 days after grafting was recorded and percentage survival of grafts was calculated. The number of days required for bud sprouting of scion was recorded by counting the number of days from grafting to initiation of first sprout from nodal region of successful grafts in each replication and treatment and their average was worked out. The girth of the main stem was measured one cm above the graft union (scion girth), at graft union (girth of union) and at one cm below the graft union (stock girth), by the help of Vernier callipers. The girth of the stem was measured one cm below the graft union (stock girth) and at one cm above the graft union (scion girth) with the help of Vernier callipers for five observational plants after 60 days of grafting and the average Stock: scion ratio was calculated. The scion length of five sprouted plants was taken from point of graft union to the tip of the shoot in each replication and treatment and their average were calculated.

Chlorophyll pigments were measured by homogenizing 0.5g fresh leaf sample with 10 ml of 80% chilled acetone and leaving the liquid portion for 15 minutes to centrifuge at 2500 rpm for 15 minutes. The supernatant was collected and the absorbance was determined at 645nm and 665 nm using spectrophotometer as per the equations proposed by Nazar *et al* (2011):

Total chlorophyll (mg g^{-1} fresh weight) = 20.2 (A_{645}) + 8.02(A_{663}) × V/1000×W

(whereA_{645} = absorbance at 645 nm; A_{663} = absorbance at 663 nm; V = total volume of the extract (ml); W = weight (g) of the sample)

The experiment was laid out in factorial randomised block design and data was analysed as per same designs using SAS statistical software.

RESULTS AND DISCUSSION

Graft success

The days of sprouting as influenced by different timing (Fig 1) of grafting clearly revealed that the grafts made in first week of August was earliest to sprout while the grafts made in first week of February took the maximum days to sprout. The grafts made with tongue grafting took fewer days to sprout as compared to wedge grafting. The interaction of two factors was found to be significant. The grafts made in August by tongue grafting took minimum days (15.18) to sprouting while grafts made in first week of February with wedge grafting took maximum days (30.79) to sprout. The variation in grafting success and time of sprouting probably stems from time taken for callus formation which was possibly lesser when vines were in active growing stage. Hussain *et al* (2016) also reported seasonal variation in time taken by grafts to sprout in Italian Olive as they found that olive took minimum days to sprout in June and August. Results corroborate with findings of Yordanov and Tabakov (2009) who reported that tongue grafting gave better results in terms of sprouting in persimmon. The time taken for completion of bud sprouting, that is, when the last scion showed sprouting is presented in Fig1. Time taken by scions for completion of sprouting was influenced by time of grafting and techniques of

grafting, and range varied from 33.29 to 45.4 days among various treatments. When tongue grafting was performed, the grafts planted in third week of February took the maximum days (45.41) for completion of sprouting followed by grafts made in first week of August (38.03). It may be noted here that in both these treatments more number of scions showed sprouting. The wedge grafted plants made in February third week took fewer days (33.29) for completion of sprouting which can be related to the fact that only few scions showed sprouting with this treatment.

Graft take/Sprouting percentage

The graft take / sprouting percentage as influenced by different grafting times and grafting techniques were recorded at end of sprouting days (Fig 2). Among the different timings for grafting, the grafts made in February third week showed highest sprouting percentage followed by grafts made in first week of August. Out of the two techniques, the tongue grafting showed better results in terms of sprouting of grafts when practiced in third week of February as evident from graft take data but in months of August and September, the success rate of grafting was better with wedge grafting. The interaction between grafting technique and time of grafting showed that grafts made in third week of February using technique of tongue grafting gave highest graft take (61.67 %). On the other hand, the grafts made in first week of February using wedge grafting showed lowest graft take (14.0%). A significantly higher percentage of sprouting in February third week might be due to active growing meristematic stage exhibited by both the rootstock and scion, which facilitates callus formation and thereby enhances grafting success (Stino *et al*, 2011). Higher percentage of sprouting and bud take success with tongue grafting has been observed in apricot (Dwivedi *et al*, 2000), in apple (Kumar and Ananda, 2004) while Bellini (2002) and Chauhan *et al* (2007) reported that tongue grafting produced higher success in terms of graft take in persimmon. The data on percentage survival of grafts 60 days after grafting (Fig 2) distinctly showed that tongue grafting showed superiority in survival percentage of grafts over wedge grafting. However, the option of graft techniques is significantly influenced by the time of grafting. The tongue grafting resulted in higher survival percentage of grafts made in February while wedge grafting gave better results in months of August and September. It is commonly observed that scions which fail to sprout showed wilting and withering very early. In other words, if their senescence could be avoided or at least delayed graft take can be improved. The two way interaction between grafting technique and time of grafting revealed that the maximum survival percentage (48.5%) was noted for tongue grafting in third week of February. The lowest survival percentage (7.3%) was recorded in grafts made in first week of February using wedge grafting. The higher success rate might be because of favourable temperature and relative humidity prevailing during the period following grafting and rapid sap flow in rootstock and scion which might have favoured the healing process and established the continuity of cambial and vascular tissues for graft take. Similar results were also observed by Ghojage *et al* (2011) who recorded maximum grafting success in the month of February (81.66%) which were at par with October (80.00%).

Vegetative growth of grafts

The diameter of rootstock just below the grafted point was measured at 60 days after grafting and the values are presented in the Table 1. Stock diameter was highest in grafts made in August followed by that of grafts made in September irrespective of the method of grafting. The average stock diameter was significantly higher in tongue grafted plants than wedge grafted plants at this stage. The stock diameter was the highest (5.23mm) in August planted grafts made with tongue grafting whereas the lowest stock diameter (4.05 mm) is recorded in wedge grafted plants made in September. The scion diameter was highest in August grafted plants 60 days after grafting. The effect of grafting techniques

Table 1. Effect of method of grafting and season on vegetative parameters of grafts 60 days after grafting

Grafting Time	Stock diameter (mm)		Scion diameter (mm)	
	Tongue grafting	Wedge grafting	Tongue grafting	Wedge grafting
GT1	4.29	4.05	3.45	3.89
GT2	4.90	4.12	3.58	3.52
GT3	5.23	4.95	4.42	3.80
GT4	4.57	4.03	3.35	3.32
CD ($P < 0.05$)	GT= 0.41, Method= 0.22 , GT x Method=0.15		GT=0.45, Method= 0.24, GT x Method=0.11	
	Shoot length (cm)		Scion: Stock ratio	
	Tongue grafting	Wedge grafting	Tongue grafting	Wedge grafting
GT1	11.45	12.42	0.81	0.96
GT2	13.72	14.52	0.73	0.86
GT3	12.66	15.43	0.85	0.77
GT4	12.47	12.72	0.74	0.83
CD ($P < 0.05$)	GT= 0.93, Method= 0.97, GT x Method=0.72		GT=0.05, Method=0.07, GT x Method=0.11	
	Leaf number		Total chlorophyll content (mg/g)	
	Tongue grafting	Wedge grafting	Tongue grafting	Wedge grafting
GT1	12.3	10.3	1.42	1.36
GT2	17.8	14.7	1.32	1.29
GT3	12.4	11.4	1.34	1.33
GT4	10.4	9.5	1.23	1.26
CD ($P < 0.05$)	GT=0.20, Method=0.22, GT x Method=0.12		GT=0.11, Method= NS, GT x Method=NS	

GT1= 1st week of February, GT2= 3rd week of February, GT3=1st week of August and GT4=1st week of September.

was also significant ($P < 0.05$). The highest value was obtained tongue grafting at 60 DAG while it was low for wedge grafted plants in all the grafting times. The scion diameter is the highest in August planted grafts made with tongue grafting whereas lowest scion diameter was recorded in wedge grafted plants which were grafted in September. The higher increase in diameter observed in August might be due to climatic and environmental factors variation. These findings are in accordance with the results reported by Singh *et al* (2005) in guava. Stock scion ratio is one of the most important factors to in stock-

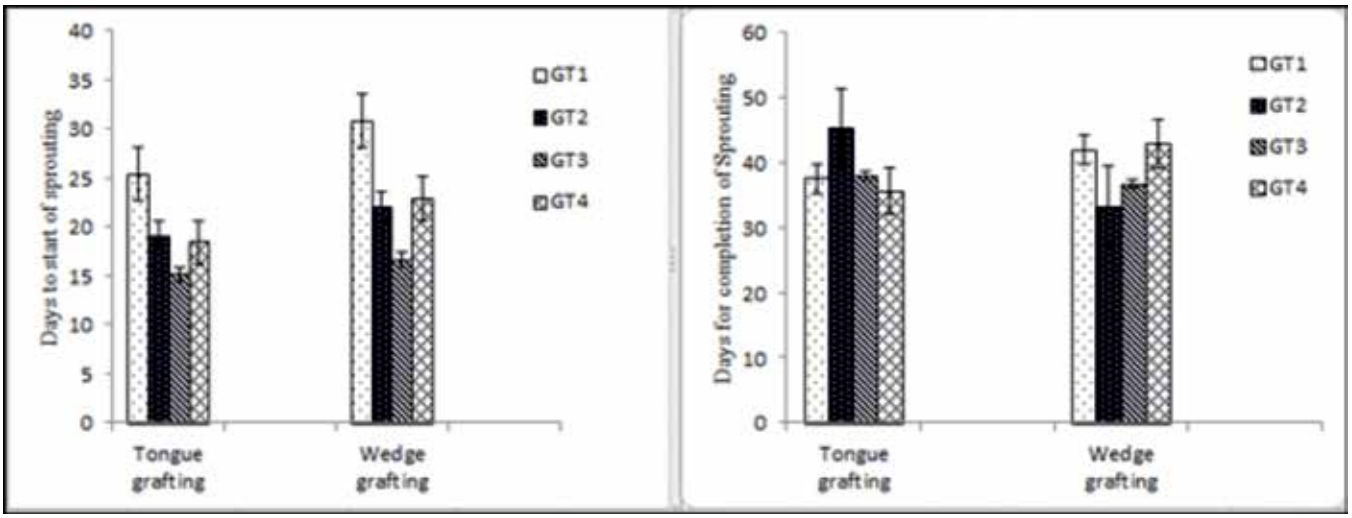

Figure 1. Effect of of method and time of grafting on days to start of sprouting and number of days for completion of sprouting of grape grafts (GT1= 1st week of February, GT2= 3rd week of February, GT3=1st week of August and GT4=1st week of September) Vertical bars indicate standard error of means for five replicates.

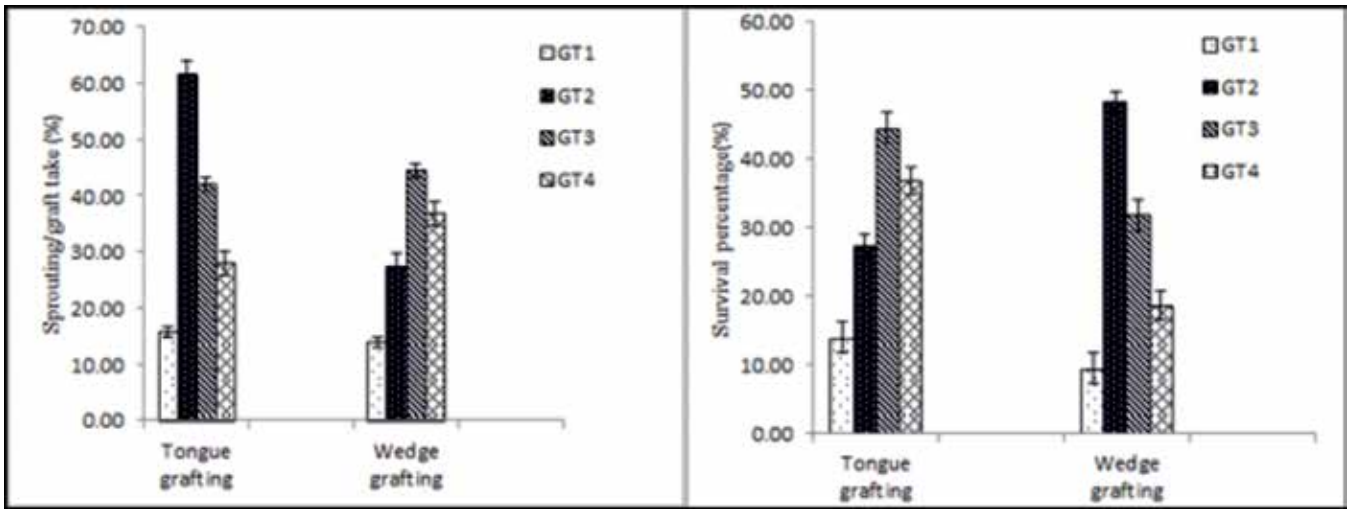

Figure 2. Effect of method and time of grafting on sprouting percentage and survival percentage of grape grafts (GT1= 1st week of February, GT2= 3rd week of February, GT3=1st week of August and GT4=1st week of September). Vertical bars indicate standard error of means for five replicates.

scion compatibility between the each partner. It is evident from the data that the stock: scion ratio was near 1 in cuttings grafted in First week of February month which is at par with August planted cuttings (Table 1). The lowest scion stock ratio was recorded in cuttings grafted in third week of February using tongue grafting technique. Stock: scion ratio plays an important role in maintaining the uniformity in trunk thickness. The uniform size of stock and scion also indicates the easy flow of food material in the plant system thus predicting the longevity of a vine. The ratio of Flame Seedless grafted onto Dog Ridge rootstock observed here corroborates with the one reported by Somkuwar *et al* (2006).

The time of grafting had no effect on scion shoot length of grape grafts (Table 4). The highest scion shoot length was recorded in wedge grafted plants made in the month of August. On the other hand, lowest scion shoot length was observed in tongue grafted plants planted in first week of February. A

positive correlation between shoot length, internode length, and leaf numbers indicated that vigorous rootstock greatly influences the shoot growth of scion (Hartman *et al,* 2002). A strong correlation between shoot growths, stock to scion ratio, and callus development indicates that growth of grafted grapevine could be different in respect to grades of callus development (Celik, 2000) and rootstock genotype (Soar *et al*, 2006).

The numbers of leaves per shoot were recorded at 60 after grafting (Table 1). Significantly higher numbers of leaves per shoot (17.8) was found with February third week grafts compared to February first week, August and September made grafts. The increment may be due to accumulation of greater photosynthates leading to better growth parameters. The findings are similar with that of Kudmulwar *et al* (2008) who reported that in guava the grafting performed on 15th February recorded the highest number of leaves, maximum diameter of scion, highest success percentage and maximum length of scion. Verma *et al* (2012) reported that marked variation in leaf area in Pusa Urvashi cultivar of grapes following its grafting on to different rootstocks. The total chlorophyll content of leaves was estimated after 60 days of grafting. Significant differences were found for the total chlorophyll contents in with different timing of grafting (Table 1). The average total chlorophyll content at 60 days after grafting was recorded to be in 1.39, 1.31, 1.34 and 1.25 mg/ g on fresh weight basis in February first week, February third week, August first week and September first week, respectively. The mean total chlorophyll contents was highest (1.42 mg/g) in tongue grafted plants from first week of February while the minimum was registered in wedge grafted plants from August first week grafting. The variations observed in the chlorophyll contents of vine leaves might be attributed to the varying interception of light during their development at different stages after grafting. It is an established fact that cytokinins prevent chlorophyll degradation. The root tips are known to be the predominant sites of cytokinin biosynthesis, thus, variations observed in chlorophyll contents of could be attributed to the differences in the levels of cytokinins produced by the roots of rootstock which were at different stages of healing. Bica *et al* (2000) suggested that this parameter to be influenced by the stionic combination and graft healing process.

CONCLUSION

The present study clearly indicated that both grafting time and technique had significantly influenced days required to bud break, rootstock diameter, scion diameter, scion length, number of leaves per graft and graft success of grapes. The tongue grafting performed on grape cuttings during the third week of February exhibited better performance in terms of sprouting percentage and other vegetative growth parameters.

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