Graft Responses to three different techniques in Mango (*Mangifera indica* L.) under Bhutanese nursery conditions

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ABSTRACT

Veneer grafting (Inarching) is a vegetative method of mass propagation of mango in Bhutan. However, this method of propagation has many setbacks in Bhutanese nursery conditions. The most important disadvantage of this method is high cost of production as grafting is done in field and seedlings are kept in open condition until proper graft union is formed. Providing graft aftercare in open field spread in large areas prove challenging in Bhutanese conditions. This study was aimed at identifying viable alternative methods of propagation to Veneer grafting. The experiment was conducted at Agriculture Research and Development Center, Bajo in 2015 in Horticulture Nursery Block. Three grafting techniques (Veneer, Splice and Wedge) were evaluated for different indicators of shoot, leaf and graft success. The results showed no significant difference ($p<0.05$) among the grafting techniques studied at 95% confidence interval. Initial rate of graft success before 20 DAG was higher for Wedge followed by Veneer or Inarching; however the gap narrowed after 60 DAG. Splice grafting showed higher rate of graft success (60%) and survival percentage (53) after 60 DAG than the veneer grafting (control). Our findings suggest that splice and wedge grafting can be safely followed for mass propagation of mango in Bhutanese nursery conditions. Further work on study of cost of production using different propagating techniques for mango would be useful for coming up with recommendation package.

Keywords: *Graft response, mango, grafting techniques, Bhutanese nursery*

1. Introduction

Mango is one of the important fruits in tropical belt of the world and more importantly in Asia. It is referred to as the King of Fruit in India due to its importance and historical significance. It is also a fruit of choice in Bhutan as it is successfully cultivated in wet tropical (below 600 meters above sea level) and to an extent in warm humid subtropical (600-1200 m.a.s.l.) agro-ecological zone in southern parts of the country. As per the Agriculture Statistics 2014, published by Department of Agriculture (2016), a total number of 57601 trees are in the field while the number of bearing trees is just about one-third (17458). Domestic production (586 ton) meets only about half the country's demand (1190 ton) and more than 582 ton are imported (Bhutan Trade Statistics, 2016). Therefore, enormous opportunity exists to upscale domestic production and to gradually replace import with domestic produce thereby achieving self-sufficiency.

Mango production in Bhutan is not without challenges. Increasing its production would require taking stock of production issues specific to Bhutanese scenarios and careful

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development of mitigation strategy. Mango is recommended to grow below 600 m.a.s.l., and often its production and yield is adversely affected by heavy rainfall especially when it coincides with flowering period. Also, fruit fly is one of the major problems to mango production (Ghalley et al 2014). More importantly, lack of quality seedlings is currently a major hurdle to mango production in the country. Regional Seed Center at Samtenling is the only government seed agency that produces mango seedlings although there are few private nurseries as well. These nurseries currently practice veneer grafting.

Grafting is the most common method of propagating fruit trees in order to obtain trueness-to-type. 'Inarching' or 'Approach grafting' is widely followed as a vegetative propagation technique both within the country and across the border in India (Nayak and Sen, 2000; Lynch and Roy 2015). The technique is proven to be most successful method of grafting (Kumaret al 2015). The graft take depends on several factors (Mulinge 2015) that are inherent to rootstock or scion wood (Pratap et al 2017), stage of scion wood and defoliation (Majeed et al 2014), time or season of grafting (Nayak and Sen 2000; Islam et al 2004; Ullah et al 2017), grafting technique (Kumar et al 2015), potting media (Kumar and Thakur 2016), binding material (Vasara et al 2017) and more importantly the grafting skills of nurserymen.

Inarching is claimed as the most reliable and economical method of mango propagation (Kumar et al 2015; Lynch and Roy 2015). It involves use of scion from the desired tree which is still attached to its own root systems and there is no need for waxing of scion wood as desiccation is less likely to occur resulting in higher rate of graft success (Donovan et al 2016). However, the technique is not without drawbacks. In Bhutanese condition where mother trees are in the field, the graft success depends largely on graft aftercare. In the open condition, managing individual grafted plants spread over an area involves huge costs thus questioning the viability and profitability. Further, the rootstock growing media in the polypot often gets dry at a faster rate and manual irrigation is the only method currently practiced. Thus, seedling mortality is high in Bhutanese nursery condition which in turn increases the unit cost.

Private nurseries import huge quantity of grafted mango seedlings from Indian border to cater to domestic demand. Not much has been done on generation of mango propagation technologies and the little information that is generated has not been extended to private firms resulting in direct import from Indian border. However, some observation trials on mango grafting have been done in Agriculture Research and Development Center, Samtenling. Therefore, this study was aimed at identifying appropriate grafting method for Mango using three different grafting techniques for generation and promotion of alternate propagation methods in the present context of Bhutanese fruit nursery conditions.

2. Material and Methods

The experiment was conducted at Agriculture Research and Development Center Bajo, Wangdiphodrang, which is located at an altitude of 1180 meters above sea level. One year old scion wood was collected from RDC mango germplasm block and grafted after 10 days of scion wood collection by using Wedge, Veneer and Splice Grafting techniques on 1st June, 2015 as higher graft success were reported during June to August (Islam et al 2004). Graft

aftercare and irrigation were provided twice weekly. The wedge and splice grafted seedlings were maintained in 70% green shading net while *Inarching* was done as usual in open field conditions.

Grafting was done following the procedures and guidelines published by Queensland Government and Department of Primary Industries, New South Wales Australia (Donovan et al 2016). The local rootstocks were raised in uniform sized poly pot (2 litre) containing same media mix and only rootstock of uniform size (1.2 cm diameter) at 20 to 30 cm height was used. Scion woods (c.v Deshari) of similar size (about 1 cm) were prepared by defoliating new season shoots (first flush) at least seven days before date of grafting The shoots for *Inarching* were kept intact with mother trees for about a week as defoliation has shown to improve early sprouting (Majeed et al 2014; Ullah et al 2017) while enough defoliated shoots were cut and wrapped with para-film to avoid desiccation for wedge and splice grafting. It was then stored at 12 degree centigrade for 3 days.

2.1. Wedge or cleft grafting

Wedge grafting is also known as cleft grafting. The scion wood was made two clean and sloping cuts (2.5-3 cm long) at its base forming a wedge which was then inserted into the clean-edged cut rootstock (at the height of 20-30 cm above the soil level) to match the cambium layer of scion and rootstock. Then it was tied firmly using grafting tape making air tight to prevent moisture loss and dislodging of scion.

2.2. Splice grafting

Splice grafting is also known as whip grafting. The scion wood was made a single-angled cut through both the rootstock and the scion wood of about equal length. It was then placed together in such a way to match the cambium layer at least at on one side if the size of the scion wood and rootstock were different.

2.3. Veneer grafting

This is also called as ‘Approach grafting’ or ‘Inarching’ and it is a usual method adopted in mango propagation. This technique involves large plant growing in a field as a mother plant and grafted without the scion detached from it. The rootstocks were placed appropriately near mother tree and tied up with the trunk of mother trees where convenient while some grafted seedlings were maintained on the temporary shelf until proper graft union was formed.

2.4. Data collection

The success of the graft in first few months can be determined by the indicators such as bud sprouts, shoot and leaf growth. The scion wood that sprouts from grafted plants put on growth and increases its shoot and leaf number over a period of time. The data was collected only after twenty days of grafting when prominent bud sprouted from the shoots and leaf primordial appeared on the grafted scion wood. A total of 20 plants were grafted for each grafting techniques and the data were collected on shoot growth (cm), number of shoots (SN), number of leaves (LN) and the percent graft take (%), for a duration of two months after twenty days of grafting at 10 days interval. The length and the size were measured using scale and Vernier caliper.

2.5. Data analysis

The indicators of the graft success assessed were the shoot growth referred here as shoot length (SL) and leaf growth as leaf length (LL) in cm. In addition, the number of shoots and the leaves were also evaluated. The graft success was calculated using success percentage for each observation as below:

$$Graft\ success\ (\%) = \frac{\text{Number of grafted plant survived}}{Total\ number\ of\ plants\ grafted}\ x\ 100$$

The variation among the grafting technique was analyzed using One-Way ANOVA using SPSSv16.0, IBM (2007) to assess the level of significance among the three different grafting techniques based on the variables related to graft take, growth of scion wood, leaf number, and growth trend. The variables of leaf and shoot length were compared and the results were presented using descriptive statistics as the corresponding trend in growth. The number of leaf (LN) and shoot number (SN) was also plotted as a performance of the graft for three different grafting techniques.

3. Results and Discussion

Veneer grafting is the only grafting technique that is currently deployed for mass multiplication of mango by nurseries in Bhutan. The grafting technique is preferred mainly for its high graft success and the associated advantages (Islam et al 2004; Kumar et al 2015). However, in Bhutanese nursery conditions, high unit cost per unit is mainly because of the high labor cost. Since *Inarching* involves grafting in the field, high labor is required for watering the plant in the field especially for rootstock raised in small poly pot. Labor requirement for hand watering increases the major chunk of production costs if the graft union is not formed before dry winter season. In addition, preparing stakes and shelf for the rootstock during graft union formation also involves additional cost.

The various graft success indicators for the variables were assessed up to two months after grafting. Statistically no significant difference (p>0.05) was observed among the grafting techniques deployed with respect to all variables; LN [$F(2,38)=0.29$, $p=0.74$), SN ($F(2,38)=1.13$,$p=0.33$), SG($F(2,38)=0.21$, $p=0.8$), LL ($F(2,38)=0.92$, $p=0.40$), SL ($F(2,38)=0.91$, $p=0.40$]. The highest percentage of survival was found in Splice grafting (53%) and the highest number of shoot was achieved in Wedge grafting (1.834). Similarly, the highest number of leaves at 60 days after grafting (DAG) was recorded for veneer grafting. The overall performance of the grafts is depicted in Figure 1.

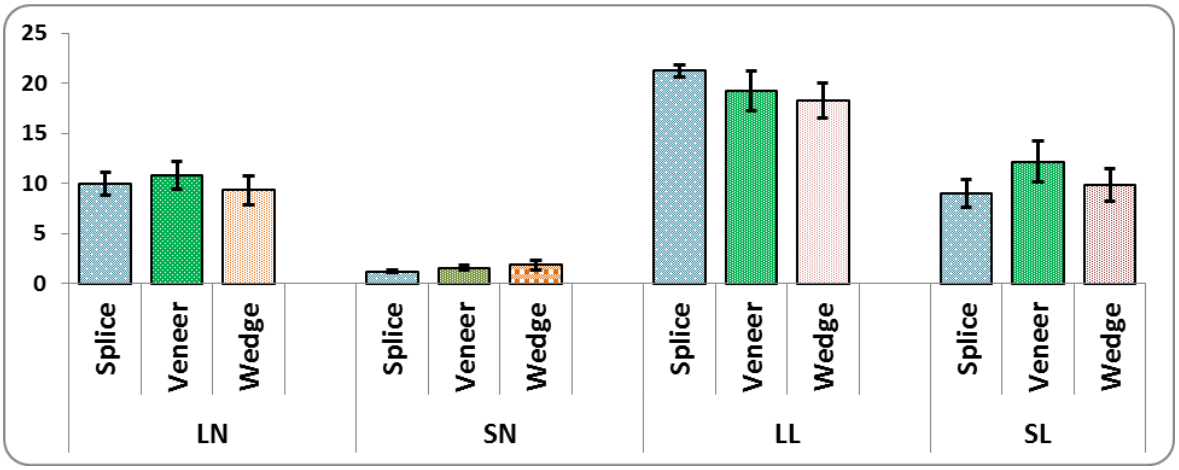


Figure 1.The mean of LN, SN, LL and SL for three grafting techniques 60 day after grafting

Note: (LL and SL refers to leaf length and shoot length, respectively in cm while LN and SN refers to Shoot Number and Leaf number with the corresponding error bar representing the variation in data (not the error in the measurement).

The highest overall shoot length (SL) and number of leaf (LN) at 60 DAG in case of Veneer grafting can be attributed mainly due to its parts still intact with the mother trees for continuous supply of nutrient to scion favoring easy graft union formation unlike the wedge or splice grafting. The lowest shoot length (SL), shoot number (SN), for splice grafting may be due to the time required for callus and graft union formation as scion woods are detached from its root system and there is no nutrient flow from rootstock to scion during the initial graft formation stage.

3.1.Trend of graft success, shoot and leaf growth

The study of trend in grafting success showed that Veneer grafting has lower graft success than splice in this study. It can be due to exposure of the grafted plants directly to outside environment (open condition) as *Inarching* is usually done in Bhutan. This open condition has shown to lower the rate of graft success in mango (Sivudu et al 2014). In real nursery conditions, graft aftercare especially not providing irrigation to the inarched plants suffers from high mortality. Although the differences in graft success were noticed during the initial stages of scion growth among the techniques deployed, their differences narrowed at the later stages (at 60 DAG). Therefore, considering the costs, other two grafting techniques (Splice and Wedge) can be a viable alternative to mango propagation.

We also assessed the growth rate for shoot and leaf at different stages of scion wood development phase. The sprouting began two weeks after the grafting date (1st June 2015). The graft success rate increased over a period of two months. Initially, splice grafting showed the lowest graft take (16%) while the highest (30%) was observed for wedge grafting. Later, splice grafting took a sharp climb in graft success rate for next one month (until 40th day) followed by wedge grafting. The graft success rate in veneer method remained low until 50 day after grafting and finally shot up to 50% at 60 DAG nearly catching up with the splice and wedge techniques. Considering the trend of graft success rate and the final percentage at

60 DAG, splice grafting technique proved superior against the widely adopted and recommended veneer technique.

The vigor of the scion growth also determines the success of graft at the later stages. In some crop species, early sprouting can be due to the stored nutrient of the scion wood without actually forming the graft union. We studied the ability of the scion to sprout before graft formation by Deshari Mango using stored nutrient in the scion wood. The growth rate of shoot and leaf length showed that veneer grafting attained highest shoot growth (6 cm) within 20 days of grafting followed by Wedge and Splice which again is due to continuous supply of nutrient from rootstock and scion mother tree. Although the growth rate increased for all the three techniques under evaluation, veneer grafting proved best (about 12 cm) at the end of 60 days followed by Wedge (10 cm) and Splice (8 cm) techniques. The study on leaf length for different techniques showed some variations at the beginning (20th day) however, over the period of two months, no significant difference in leaf growth was observed. Also, the grafted plants for all three techniques that did not form graft union showed no sprouting due to store scion nutrient as in other tree crops. However, the scion remained green for about a month after grafting as if it was still under graft union formation but eventually wrinkled and died. The trend in graft success, growth of shoot and leaf for different grafting techniques are shown in Figure 2.

Treatment	Graft success (%) Final	Date of observation	Graft take (%)	Shoot length (cm)	Leave length (cm)
Wedge	40	20/06/2015	30	2.5	7.0
		30/06/2015	30	3.7	13.3
		10/07/2015	40	6.0	14.9
		20/07/2015	40	6.2	16.0
		30/07/2015	40	9.9	18.3
Veneer	40	20/06/2015	20	5.0	10.0
		30/06/2015	30	5.3	11.8
		10/07/2015	30	5.8	13.7
		20/07/2015	30	8.0	17.3
		30/07/2015	40	11.2	17.9
Splice	60	20/06/2015	10	2.4	8.4
		30/06/2015	40	2.4	11.3
		10/07/2015	50	5.0	14.8
		20/07/2015	50	5.1	17.1
		30/07/2015	60	11.1	18.9

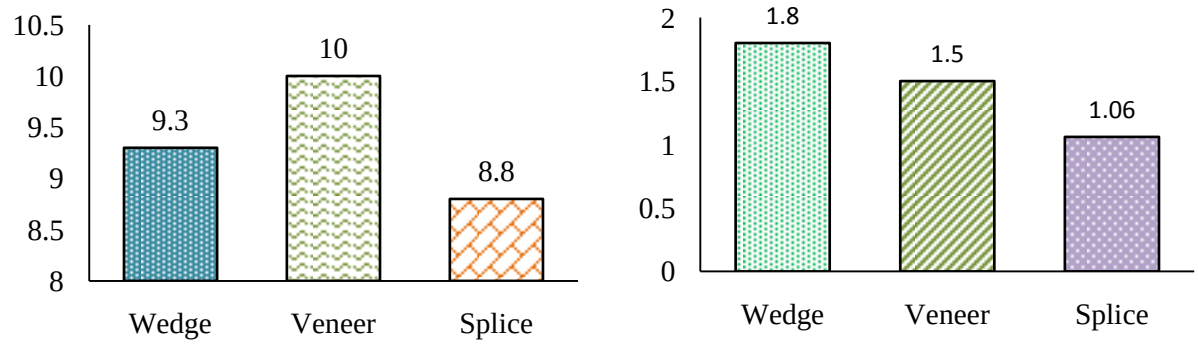
Figure 2.The trend in Graft success (A), shoot length (B) and Leaf length (C) for three grafting techniques over a period of two months after grafting

3.2. Shoot Number and Leaf Number

The number of leaves per graft was high for veneer grafting (10) followed by Wedge (9.3) and Splice (8.8). However, number of shoots per graft was highest for Wedge (1.8) followed by Veneer (1.5) and finally the Splice (1.06). As reported by Omer et al (2016), there was no

significant effect of grafting techniques on number of leaf but our result differed slightly in terms of number of shoots as variation was observed among the three techniques.

The difference in number of shoot and leaf as affected by three grafting techniques under evaluation is shown in Figure 3.



A. Number of leaf per graft B. Number of shoot per graft

Figure 3. Number of leaves and the shoots per graft for different grafting techniques

4. Conclusion

Our assessment for different grafting techniques showed statistically insignificant differences among the grafting techniques (Veneer, Splice and Wedge) indicating that other grafting techniques (Splice and Wedge) can be viable alternative to currently adopted veneer grafting. The percentage of graft success and the trend of graft success also showed no significant difference. Similarly, no variations were found for number of leaf and shoot per graft for different techniques. All the grafts that were successful showed sprouting from scion wood within two weeks after grafting. Considering the parameters (graft success, variables of leaf and shoots) in this study, Splice or Wedge grafting can serve as alternative methods for propagating mango so that the unit cost for mango seedlings can be reduced. However, further economic studies on the cost of production of mango seedlings may provide further insights about the feasibility of the wedge and splice method.

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