

EVALUATING THE SUCCESS OF VEGETATIVE PROPAGATION TECHNIQUES IN LOQUAT cv. MARDAN

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Abstract

Loquat cv. Mardan is a promising variety in loquat growing areas of northern Punjab and Khyber Pakhtoon Khwa. Loquat growers face serious issue of true to type plants availability. An attempt was made to explore different vegetative propagation techniques to find out most successful method for nursery industry. In the study propagation through cutting (during spring) and grafting during different suitable seasons (spring and monsoon) was examined. Cuttings were treated with different growth regulators to promote rooting. PBZ, IBA and their combinations were applied to the cuttings. Highest success percentage (40%) was achieved with PBZ. This limited success in rooting of cuttings was complete failure in getting plant survival at the end of the study. In the second phase of the experiment different grafting techniques i.e. tongue, cleft and side grafting were evaluated during two different seasons i.e., spring and monsoon. Cleft grafting in the months of March/ April proved to be most successful method in terms of success (70%), days taken for shoot growth, no. of leaves, shoots length, no. of branches. While in monsoon season side grafting was significantly better with a success of 85%. Overall, monsoon side grafting proved to be more successful than any other method.

Introduction

Loquat (*Eriobotrya japonica* Lindl.) belongs to the family Rosaceae, subfamily Pomoideae is an evergreen fruit tree. It was originated in China where it is under cultivation for over 2000 years. So far, loquat has been grown in over 30 countries in the world. China is the leading country in loquat cultivation followed by Spain and Japan (Lin *et al.*, 2007). In Pakistan, its production is 13,159 tons (Hussain *et al.*, 2009).

Loquat Fruit matures in early spring season (Cuevas *et al.*, 2008). In Pakistan, its fruit becomes available in the months of March and April, when none of the other fresh fruit is available in the market, hence fetch good returns (Hussain, *et al.*, 2011). Increasing trend in loquat area as well as production indicates its great potential in the country.

Like other fruit plants, loquat if propagated through seed, does not ensure preservation of parental traits. Vegetative methods ensure the true to type production of loquat cultivars. It is fact that the vegetatively propagated plants primarily depend upon proper time, season and a specific grafting method (Khan *et al.*, 2002). Vegetative propagation techniques are the most effective method to produce planting stocks for production of true to type plants (Ismail *et al.*, 2002). Success of grafting method varies from specie to specie. Grafted trees bear fruit in 2-3 years, while a seedling tree will take 5-6 years (Campbell & Malo, 1994). However, grafting for commercial propagation of loquat has been restricted because of poor success. Grafting is done from spring to mid-autumn (Demir, 1987; Campbell & Malo, 1994).

Propagation through cuttings is another option for getting true to type plants. The use of hardwood cuttings is one of the least expensive and easiest methods of vegetative propagation. Endogenous factors such as growth substances, anatomical structure of cutting (Hartmann *et al.*, 2002) and exogenous factors such as, humidity air and light condition, date of cutting are

always required to obtain a satisfactory propagation success (Ercisli *et al.*, 2003). Planting of loquat cuttings in sand and peat moss mixture after the application of IBA under mist propagation unit have been reported to give 50% rooting in green house (El-Shazly *et al.*, 1994).

The traditional propagation methods are time-consuming and limited by space required. In Pakistan, mainly orchards were established through seed due to unavailability of certified and true to type varieties in local nurseries. Poor orchard management techniques and planting system is another important factor in production, multiplication and documentation of promising genotypes in the country (Abbasi *et al.*, 2011).

Keeping in view these problems the research was designed to evaluate different vegetative methods to produce true to type loquat plants. Protocols standardized from this study were conducive for the propagation of elite loquat genotypes at commercial scale which will finally uplift the national horticulture industry & the export potential.

Materials and Methods

The study comprised of two experiments.

Experiment I:

Effect of different growth regulators on induction of rooting in loquat cuttings: The experiment was conducted in the Mist Unit and Glass House, Department of Horticulture, PMAS-Arid Agriculture University Rawalpindi. Uniform sized cuttings of loquat (*Eriobotrya japonica* Lindl) about 20-25 cm in length were treated with different concentrations of PGRs for different time intervals as mentioned in Table 1.

Data was taken regarding rooting percentage, No. of roots, length of roots (cm) and time taken for root initiation. Completely randomized design with 2 factor factorial arrangement was used for the experiment with

three replications using five plants per replication. Data was statistically analyzed using ANOVA technique (MSTAT-C software) and means were compared by LSD (Least Significant Difference) test at 5% level of significance (Steel *et al.*, 1997).

Table 1. Different concentrations of PGRs for rooting of loquat cuttings cv. Mardan.

Concentration(ppm)	Dipping time
Distilled water	3 hours
Paclobutrazol	
50	3 hours
60	3 hours
70	8 hours
70	12 hours
Paclobutrazol + IBA	
100 + 2000	30 mints
1000 + 2500	30 mints
5000 + 3000	30 mints
IBA	
5000	5 seconds
6000	5 seconds
600	12 hours

Experiment II:

Comparison of different grafting techniques for the production of true to type loquat plant: This experiment was conducted in the loquat orchard at Kalarkahar District Chakwal during March/April. Loquat plants were used for the investigation of successful grafting technique. Following grafting techniques were used:

Tongue Grafting, Side grafting and Cleft grafting. Each treatment was replicated thrice on ten selected plants. Randomized Complete Block Design (RCBD) was followed in the experiment. Data regarding grafting success percentage, days taken to shoot growth, number of branches plant⁻¹, number of leaves, leaf area and shoot length (cm) were recorded.

Results and Discussion

Experiment I:

Effect of growth regulators on rooting of cuttings and survival percentage of loquat cv. Mardan: Data regarding the effect of growth regulators on rooting percentage of loquat shoot cuttings is presented in Table 2. It is apparent from the data that cutting dipped in paclobutrazol (PBZ 70 ppm) for 12 hours showed significantly higher rooting percentage (40 %) as shown in Fig. 1(a) followed by the cuttings dipped in IBA (600 ppm) for 12 hours, while; all the other treatments were failed to promote the rooting. Though, to some extent the success was achieved in inducing roots but not even a plant survived (Table 2).

It is clear from the results that rooting hormones used in this study enhanced rooting process. The probable reason might be that exogenously applied hormones helped to enhance the activity of root primordia along with rooting cofactors, thus callus formation was increased which is a prerequisite for root formation. Magherini & Sani (1985) got a maximum root formation by the use of IBA. High absorption of PBZ and added dipping time period helped to hold back gibberellins, which generally slow up adventitious roots formation. With such suppression there might be more cell division at the basal portion thus ensuring callus formation and more rooting percentage (Ayaz *et al.*, 2004). PBZ enhance early rooting in tip cutting of Guava (Hafeez *et al.*, 1988 and Mukhtar *et al.*, 1998)

Number of roots per cutting: The number of roots per cuttings was significantly higher (12.56) in IBA (@ 600 ppm) as depicted by Fig. 1(b) followed by PBZ (70 ppm) i.e. 12.56 and 11.80 respectively, while the other treatments did not show any significant results as evident from the data (Table 2).

Our findings are in accordance with the results of Wiesman & Lavee (1995) who found that IBA enhanced root number in comparison with untreated cuttings. It was well documented that a delicate balance between endogenous stimulatory and inhibitory factors control the rooting (Eliason, 1981), while auxin stimulates rooting, cytokinins and gibberellins inhibit it (Hartmann *et al.*, 2002).

Table 2. Effect of different concentration of PGRs (IBA and PBZ) on rooting of loquat cuttings cv. Mardan.

Treatments	Time taken for rooting (days)	Rooting %age	No. of roots per cutting	Root length (cm)	Survival %age
Control	0.00 c	0.00 c	0.00 c	0.00 c	0.00
PBZ (50 ppm) for 3 hrs	0.00 c	0.00 c	0.00 c	0.00 c	0.00
PBZ (60 ppm) for 3 hrs	0.00 c	0.00 c	0.00 c	0.00 c	0.00
PBZ (70 ppm) for 8 hrs	0.00 c	0.00 c	0.00 c	0.00 c	0.00
PBZ (70 ppm) for 12 hrs	66.07 b	40.27 a	11.80 b	3.55 a	0.00
PBZ + IBA (100 + 2000 ppm) for 30 min	0.00 c	0.00 c	0.00 c	0.00 c	0.00
PBZ + IBA (1000 + 2500 ppm) for 30 min	0.00 c	0.00 c	0.00 c	0.00 c	0.00
PBZ + IBA (5000 + 3000 ppm) for 30 min	0.00 c	0.00 c	0.00 c	0.00 c	0.00
IBA (5000 ppm) for 5sec	0.00 c	0.00 c	0.00 c	0.00 c	0.00
IBA (6000 ppm) for 5sec	0.00 c	0.00 c	0.00	0.00 c	0.00 c
IBA (600 ppm) for 12 hours	82.33 a	30.13 b	0.00	12.56 a	2.75 b
LSD _{0.05}	1.2567	0.7577	0.00	0.2052	0.4224

Means in a column sharing same letter are not statistically different at $p < 0.05$



Fig. 1. (a) Rooting percentage in PBZ (70 ppm) for 12 hrs (b) Root length in PBZ (70 ppm) for 12 hrs and (c) Number of roots/cutting in IBA (600 ppm) for 12 hours.

In present study, PBZ (70 ppm) induced fewer number of roots (11.80) as compare to IBA, these results are showing disparity with Mukhtar *et al.*, (1998) where he found maximum number of roots per plants (136) with 100 ppm PBZ in guava while Hafeez *et al.*, (1988) got 30.19 roots with 3 ppm PBZ treatment of softwood cuttings of guava. Singh (1998) got 48.3 primary roots and 138.54 secondary roots by stooling with the aid of 2500 ppm paclobutrazol. Increase in concentration and dipping time of growth regulators induced high number of roots as compared with low concentration. It was also recorded by Mukhtar *et al.*, (1998) that the number of roots with high concentration of root promoting growth regulator and longer dipping period may have allowed the cutting to absorb it well.

Root length (cm): Data presented in Table 2 advocates that root length was un-affected by most of the treatments applied. However, significant difference was observed in PBZ (70 ppm) and (IBA 600 ppm) with root length of 3.55 cm (Fig. 1C) and 2.75 cm respectively, while other treatments did not show any significant response. None of the cuttings treated with low concentrations of IBA and PBZ showed any rooting at all, while the bases of the cuttings treated with high concentration and longer dipping period alone showed a high number of rooting with good length. Moreover, from the rooting data (Table 2) it was clear that the rooting increased with increase in dipping period. Thus more number of roots might be due to absorbing more IBA due to longer time of dipping.

The results of the study are in consonance with those of Darwesh *et al.*, (2013) who reported that growth regulators help in stimulating the roots in cuttings when the endogenous and climatic factors are favorable. With regard to treatments with and without PBZ, no significant differences were observed, i.e., they did not contribute towards the root regeneration process. Thus plant growth retardant has effects on the partition of photo assimilates, on the water status of the cuttings, and has been described as a promoter of adventitious roots in many species (Steffens & Wang, 1986). Paclobutrazol, in addition to stimulating root growth, as also mentioned by Davis *et al.*, (1985) and Steffens & Wang (1986) increased rooting potential.



Fig. 2. Result of different grafting techniques.

Time taken for root initiation: The data regarding the number of days taken for rooting (Table 2) shows significant difference between the treatments at $p < 0.05$ for the time taken for rooting. The results showed that PBZ (70 ppm) produced roots in short period of time (66 days) followed by IBA (600 ppm) where rooting started in about 82 days while other treatments did not show any significant effect on root induction.

In the previous studies higher concentration of IBA (4000 ppm) resulted in, maximum number of roots per cutting in olive (Mukhtar *et al.*, 1998) but in our study it was observed that at lower concentration (600 ppm) for 12 hours dipping period gave better results. Our findings are supported by the findings of Pan and Zhao (1994), who suggested that PBZ may counter the inhibitory effect of gibberellins on root primordia formation. PBZ was found to affect the metabolism of IBA (Wiesman *et al.*, 1994) and increased sink capacity of the base of the cuttings (Davis *et al.*, 1985). Free auxins, provided by conjugates of auxin is required during all the stages of the rooting process (Wiesman *et al.*, 1989). Rooting is generally inhibited by gibberellins as observed by Brain *et al.*, (1960) and Kefford (1973). While treatments with anti-gibberellins compounds promoted root formation (Chin *et al.*, 1969). Success of rooting with paclobutrazol is a good breakthrough in vegetative propagation (Wally *et al.*, 1981).

Experiment II: Comparison of different grafting techniques for the production of true to type loquat plants

Grafting success and survival percentage: Three promising grafting techniques were used to evaluate the grafting success in loquat (Fig. 2). From the data presented in the Fig. 3 it is evident that highest plant survival percentage (86.67%) was observed in side grafting during monsoon while survival percentage during spring (March/April) was high in cleft grafting (71.24%) followed by side grafting (61%).

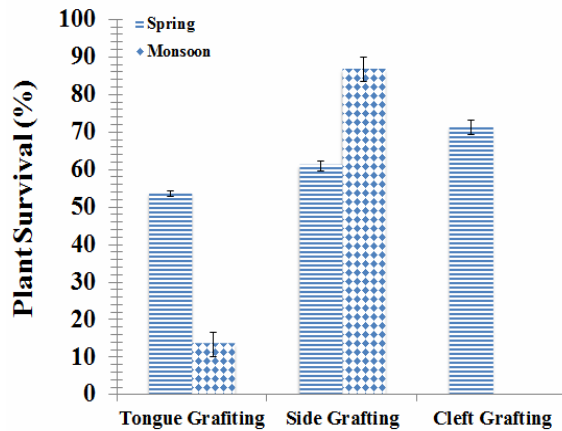


Fig. 3. Effect of different grafting techniques on plant survival percentage during spring and monsoon seasons. Bars represent standard error.

Mian (1971) reported highest success during April which is contrary to the results of present study. Although grafting can be done any time during the dormant season. However, the chances for successful healing of the graft union are better when it is done in early spring and during monsoon season.

Days taken to sprouting: Data presented in Fig. 4 showed a significant difference among various grafting methods in both seasons (spring and monsoon) regarding the days taken to sprouting. Results showed the minimum days (36) taken to sprouting were recorded in the plants in which side grafting was used during monsoon season followed by cleft (49 days) and side grafting (50 days) in the spring season at the same root stock.

The present results fully support the findings of Tekintas & Dolgun (1996), who noted that cambial continuity and vascular transformation were clearly observed in 45 and 60 days samples of nectarin/almond combination. Tekintas (1991) also reported in citrus that cambial continuity reoccurred in 45 days after grafting.

Success of grafting depends on several factors and conditions such as temperature, humidity and plant species which influence callus formation. Some species form callus quickly and these are likely to unite if properly grafted while those that form callus slowly are less likely to unite. It is possible that callus tissue becomes weak when the rootstock is weak or dwarf. Vascular differentiation begin after establishment of cambial continuity and the strong connection occur in a short time in the compatible grafts. A good established vascular connection provides a good water and nutrient flow from rootstock to bud (Ben-Ya'acov *et al.*, 1992).

Callus between rootstock and scion was produced in cleft and side grafting, however in tongue the callus showed an irregular distribution. Initial development of callus provides a bridge between scion and root stock, which is the first step for the success union of the grafting (Tekintas, 1988). In grafting techniques, the callus tissue had higher density at the rootstock than at the scion,

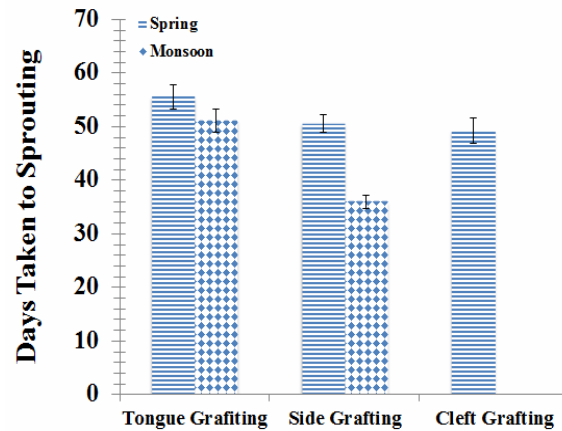


Fig. 4. Effect of different grafting techniques on days taken to sprouting during spring and monsoon seasons. Bars represent standard error.

which agrees with previous studies (Tekintas, 1988; Polat & Kaska, 1992). This was also observed by Ding *et al.*, (2002) Necrotic layers were observed at high density on cut surfaces especially cortex areas. Loquat fruit contain high concentrations of polyphenols and high activity of polyphenol oxides, which can explain the amount of the necrotic layers at cutting area.

Number of branches/plant: Significant differences were observed in grafting methods (Fig. 5) during different seasons on the production of lateral shoots. The number of shoots per plant was significantly higher (2.4) at in side grafting during monsoon followed by cleft method (1.5) during spring. It is clear from the data that side and cleft grafting methods gave higher number of lateral shoots per plant during the different seasons. These findings are supported by the observations of Abou-Rayya *et al.*, (2009) who reported that successful sprouted grafting not only increase the number of leaves but also produced more shoots. Results of this experiment indicated that accumulation of carbohydrates and seasonal variation contributes in grafting success and helpful in production of lateral shoots.

Number of leaves: Data taken during study (Fig. 6) showed that different grafting techniques significantly affected number of leaves per plant.

Maximum number of leaves (5.26) appeared in plants propagated by side grafting method during monsoon followed by cleft (2.5) during spring.

Our results are supported by the findings of Abou-Rayya *et al.*, (2009); he reported that number of leaves per plant of *Neplus ultra* almond scion, in cleft grafting gave the highest average number of leaves per plant compared other methods of grafting. He also observed similar trends during the second season as cleft grafting gave higher number of leaves/plant. Higher number of leaves obtained in cleft and side grafting could be due to the time lag which involved preparation of the scion wood.

Leaf area (cm²): The results showed that plants developed from side grafting were having maximum leaf area (11.27 cm²) followed by tongue grafting (5.49 cm²) during monsoon season (Fig. 7). Tongue and cleft grafting provide union in line with xylem and phloem vessel that provides good translocation of water and nutrients.

Shoot length (cm): Different grafting techniques and seasons significantly affected shoot length in plants. Maximum shoot length (6.98 cm) was achieved in side grafting followed by tongue grafting which produced 4.15

cm shoot length during monsoon (Fig. 8). Ram & Sirohi (1989) reported similar findings and suggested the use of relatively longer scions.

It can be concluded that Paclobutrazol can be used successfully to initiate rooting in loquat cuttings but the ultimate survival of the plant remained the major issue which can be focused in future research programs. In case of grafting techniques, the tongue grafting developed strong contact between rootstock and scion and promoted scion growth during spring season. While in monsoon season side grafting showed significantly better results.

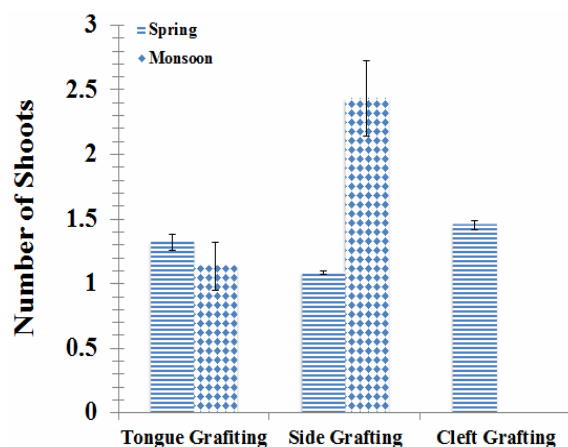


Fig. 5. Effect of different grafting techniques on number of shoots during spring and monsoon seasons. Bars represent standard error.

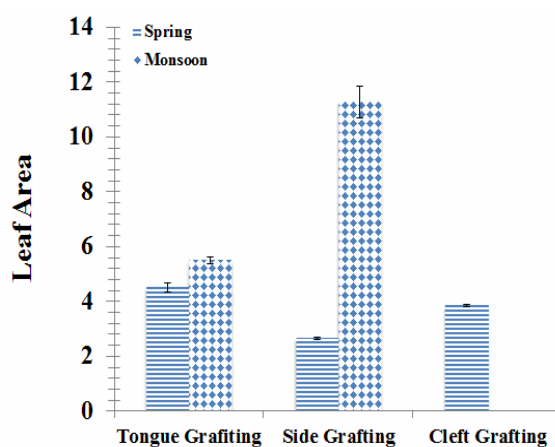


Fig. 7. Effect of different grafting techniques on leaf area during spring and monsoon seasons. Bars represent standard error.

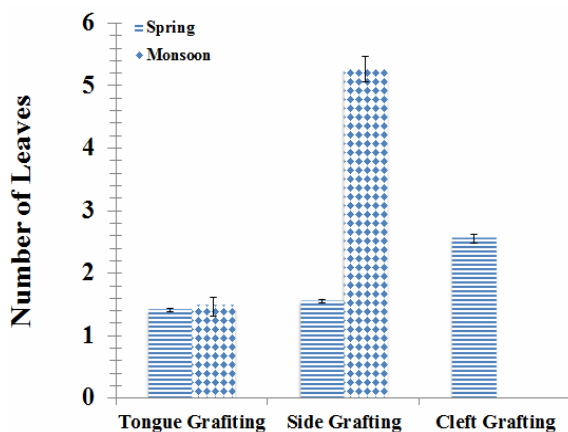


Fig. 6. Effect of different grafting techniques on number of leaves during spring and monsoon seasons. Bars represent standard error.

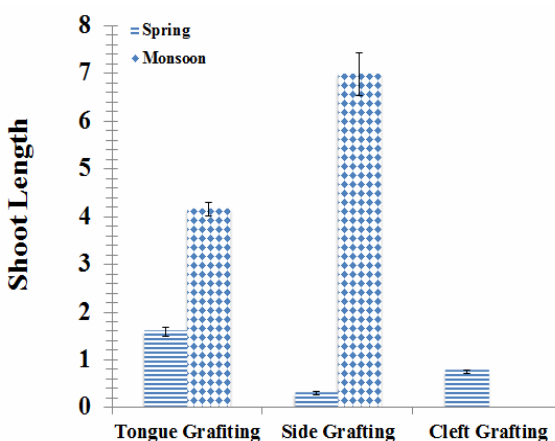


Fig. 8. Effect of different grafting techniques on shoot length during spring and monsoon seasons. Bars represent standard error.

Acknowledgment

Higher Education Commission of Pakistan is acknowledged for providing financial support for this research study.

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