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Influence of Grafting Season and Rootstock Age on the Success and Growth of Mango (*Mangifera indica* L.) cv. Apple using Cleft Grafting

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Abstract: **The current study was aimed at determining the effect of grafting season and rootstock age on the success of cleft grafting and growth of mango cv. apple seedlings. A two-factor experiment was conducted using RCBD with three replications under 60% shade net house at Melkassa, Ethiopia. The hot season during March (T.max 30°C; T.min 15.6°C; 50.5% RH) and cool season during November (T.max 28.5°C; T.min 11.4°C; 48.5% RH) were considered; while 12, 16, 20, 24 and 28 weeks old stocks were used. Cleft grafting was performed using scion woods collected from healthy and vigorous mother plants and bud break, grafting success, scion growth parameters viz., scion height, scion diameter, chlorophyll content and dry biomass were evaluated using Analysis of variance followed by mean separation at ($p \leq 0.05$). Cleft grafting performed in the hot season took an average of 16 days for bud break, while significantly longer time (20 days) was required for the cool season. Similarly, grafting success was significantly higher in the hot season (65.88%) as compared to the cool season (59.92%). Hot season grafting improved scion growth characters height and stock diameter albeit only at early growth stage. It has also influenced chlorophyll content with average value of 45.02 and 39.72 nmol/cm^2 for hot and cool season respectively. Older rootstocks (24 and 28 weeks old) improved grafting success with 82.88% and 75.07% graft take respectively, while the lowest success of 42.68% was recorded on grafts made on 12 weeks old rootstocks. Dry matter accumulation was significantly higher for hot season (43.18 g/plant); similarly older rootstocks (28 and 24 weeks) accumulated higher dry biomass (46.19 and 43.46 g/plant) respectively. Growth parameters such as leaf number, scion height and diameter were not affected by the rootstock ages used in the study.**

Keywords: **Mango, grafting season, rootstock age, cleft grafting, graft success.**

1. INTRODUCTION

Mango (*Mangifera indica* L.), proclaimed as the king of fruits, is a multipurpose perennial woody fruit in the 'Anacardiaceae' family and produced widely in the world. It is rich in vitamins and minerals including, vitamin A, folate, ascorbic acid and potassium. It is one of the leading fruits growing in Ethiopia, ranking third in area coverage (14.47%) and occupied second place in terms its volume of production, standing at 13.6% of the total fruit production (CSA, 2014). The vast dry and semi arid lands of Ethiopia, with ample surface water sources, have enormous unexploited potential for mango production. However, the expansion of fruit production is greatly constrained by poor and inadequate supply of improved quality, true-to-type and certified seedlings.

Grafting, one of the oldest propagation technique, is widely practiced for multiplication of true-to-type seedlings; and is crucially important to incorporate genetically superior rootstocks to improve perennial fruit trees. In this regard, cleft grafting has been successfully used as economic, rapid and simple method for true-to-type propagation of mango (Hossain et al., 2016; Pereira et al., 2004; Islam et al., 2004). It is known that grafting utilizes critically determinant rootstock factors that can directly influence developing ability to better adapt to adverse biotic and abiotic factors, reducing juvenility, and increasing crop production (Cassol et al., 2017). Rootstock-scion interaction influences scion growth

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characteristics including vegetative vigor of trees, which ultimately affect future field management and harvesting (Minja et al., 2017; Webster, 1995). Rootstock also modifies plant nutrient uptake and transport to the scion through its influence on the activities of ion transporters (Nawaz et al., 2016).

In the course of grafting, graft healing and acclimatization are vitally important for the survival of grafted plants. Thus, successful union of scion and rootstock depends on the proliferation of callus tissue followed by the union of vascular tissues. Successful grafting take and subsequent growth of scion shoot and development depend on a number of factors including scion variety, rootstock materials, and prevailing environmental conditions (Hartmann et al., 1997). Studies showed that grafting factors, season, and rootstock age influencing grafting success and growth in different crops, including walnut, mango, Brazilian pine, wood apple, and aonla (Gaspar et al., 2017; Singh et al., 2014; Roshan et al., 2013; Mir and Kumar, 2011; Kadam et al., 2005; Islam et al., 2004). Similarly, temperature and relative humidity are widely considered as the most important factors for grafting success and further growth, thus making the grafting process heavily dependent on seasonal variations. Duration for bud break, callus formation, and grafting success can depend on temperature and relative humidity of the grafting nurseries (Paunovic et al., 2011).

Although grafting is a very old practice, thus far specific and detail mechanisms of rootstock-scion relations are not fully understood, suggesting for further considerations. Differences in graft success and subsequent growth of grafted mango plants was found to be influenced by rootstock and scion varieties as well as the method of grafting used (Minja et al., 2017; Pereira et al., 2004). Correspondingly, Islam and co-workers (2004) observed high variability in the proportion of survived grafts between mango cvs. 'Amrapali' and 'Gopalbhog' after 120 days of grafting.

In Ethiopia, cleft grafting is the most widely practiced grafting method to obtain true-to-type mango seedlings; however, the effect of grafting season and rootstock age has not been reported in relation to grafting success and growth thereafter. Moreover, environmental factors management during healing and acclimatization is usually done by the empirical knowledge of a grower depending on the season or weather. Hence, the present study aimed at determining the effect of rootstock age and grafting season to improve graft survival rate and initial growth of mango cv. apple under lath-house condition.

2. MATERIAL AND METHOD

The experiment on the effect of rootstock age and grafting season on the success and growth of mango cv. apple was conducted from 2014 to 2015 at the horticulture research department of Melkassa Agricultural Research Centre, Ethiopia. The rootstock seeds were collected from poly-embryonic local accession and were raised on a media composed of soil, sand and a well decomposed farm yard manure of 2:1:1 ratio respectively in a poly bag under 60 % shade net house.

To assess the success of grafting, two factors, grafting season and rootstock age, were considered. For grafting season, hot season during March (T.max 30°C; T.min 15.6°C; 50.5% RH) and cool season during late November (T.max 28.5°C; T.min 11.4°C; 48.5% RH) were considered (Figure 1); while rootstock ages, 12, 16, 20, 24 and 28 weeks were used. Cleft grafting was performed at a height of 25 cm from the collar of the rootstock following standard methods. The scion woods were collected from healthy and vigorous mother plants maintained at Melkassa Agricultural Research Centre, Horticulture Research Department orchard. The grafted seedlings were maintained under 60% shade net house by removing rootstock off-shoots and irrigated at three days interval for the experiment period. The experiment was laid in a randomized complete block design with three replications.

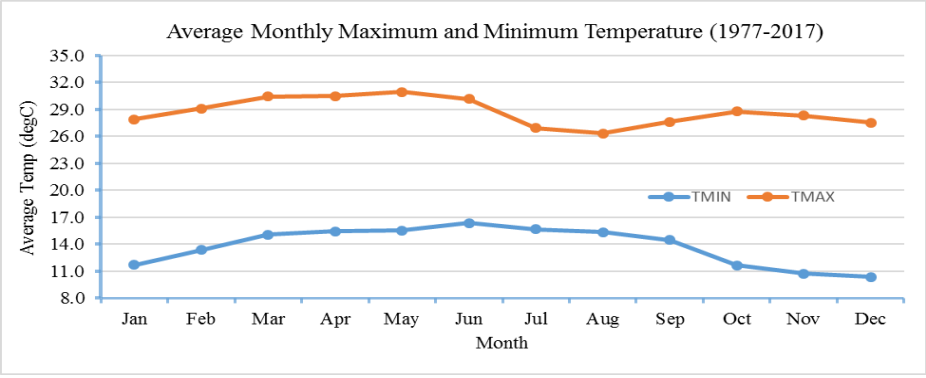

Figure 1: Historical average minimum and maximum temperature from 1977 – 2017 at Melkassa, Ethiopia

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Data on days to bud break since grafting, grafting success at 30 days after grafting, and Scion growth parameters were recorded. Scion growth parameters: scion height (cm), scion diameter (mm), and leaf count were taken monthly between 30 and 120 days after grafting from a randomly selected five grafted seedlings from each experimental unit. Chlorophyll levels were measured using SPAD-502 meter in (nmol/cm^2). The dry biomass was measured by taking the average weight of five dried seedlings in oven at 72^{o}C for 48 hours that were taken at 120 days after grafting. Analysis of variance was carried for the collected parameters using standard least square method while mean separation was carried using Tukey mean separation method at ($p \leq 0.05$).

3. RESULT AND DISCUSSION

Bud breaking and grafting success:

Grafting Season:

In the current study, grafting season affected the grafting success and growth parameters significantly when cleft grafting was performed on mango var. apple ($p \leq 0.05$; Table 1). Table 1 further shows that environmental condition also affected days required for bud break. During hot season, bud break took an average of 16 days, whereas four more days were required in the cool season. Similarly, grafting success was significantly higher in the hot season (65.88%) as compared to cold season (59.92%). Time duration for bud breaking, callus formation and grafting success were reported to depend on temperature and relative humidity of microclimatic atmosphere of the grafting environment (Paunovic et al., 2011).

The improved bud take time and grafting success in the hot season grafts might be the congenial temperature (T.max 30°C; T.min 15.6°C) as compared the cool season (T.max 28.5°C; T.min 11.4°C), particularly the lower minimum temperature during grafting and subsequent early stages. In agreement to this, cleft grafting made during hot summer season were found with better graft take time, percentage of graft success, sprout length, and formation of new leaves, as compared to cool season, in papaya and mango (Nguyen and Yen**,** 2018; Islam et al., 2004). Similarly, walnut and tomato grafting success was enhanced at higher temperature and relative humidity at grafting and seedling growth by improving wound healing and continuity of cambial and vascular tissues due to rapid sap flow (Vu et al., 2014; Mir and Kumar, 2011). Tomato grafts grown under high light intensity and relative found to have higher photosynthetic capacity, increased leaf thickness and chloroplast cells leading to improved and rapid healing and acclimatization (Jang et al., 2011).

Rootstock age:

The effect of rootstock age is important for the survival and vigor of grafts morphological and anatomical affinity between scion and rootstock, for gene expression patterns, and for reproductive development of the grafts (Gasper et al., 2017; Tzarfati et al., 2013). In the study rootstock age influenced graft success, while the number of days for bud breaking was not affected ($p \leq 0.05$; Table 2). Older rootstocks, 24 and 28 weeks old, improved grafting success, 82.88% and 75.07%, respectively.

Table 1: graft and seedling performance of mango cv. Apple grafted under hot and cool seasons

Grafting season	Bud break in days	Grafting success	Height of scion(cm)				Scion diameter (mm)				Leaf count				Chlorophyll content (nmol/cm^2)	Dry biomass per seedling (gm)
			30 days	60 days	90 days	120 days	30 days	60 days	90 days	120 days	30 days	60 days	90 days	120 days		
Hot	16^a	65.88^a	15.62^a	16.32	17.95	21.05	6.22^a	7.16	7.08	7.64	8.65	12.46	17.18	17.59	45.02^a	43.18^a
Cool	20^b	59.92^b	14.33^b	15.78	18.01	19.38	5.54^b	6.40	6.77	7.12	9.15	11.21	15.57	20.70	39.72^b	29.94^b
Mean	18	62.90	14.98	16.05	17.98	20.21	5.88	6.78	6.93	7.38	8.90	11.84	16.38	19.14	42.37	36.56
P (0.05)	***	*	***	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	*	***

* and *** indicates significant statistical differences at p ≤ 0.05and p ≤ 0.001 respectively; different letters indicate significantly different means

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Table 2: graft and seedling performance of mango cv. Apple grafted on rootstocks with different ages

Rootstock age in weeks	Bud break in days	Grafting success	Height of scion (cm)				Scion diameter (mm)				Leaf count				Chlorophyll content (nmol/cm²)	Dry biomass per seedling (gm)
			30 days	60 days	90 days	120 days	30 days	60 days	90 days	120 days	30 days	60 days	90 days	120 days		
28	18	75.07^{a}	14.87	16.35	17.58	20.55	5.66	6.57	6.95	7.58	7.52	9.78	15.29	15.96	41.50	46.19^{a}
24	18	82.88^{a}	15.35	16.85	19.26	22.45	6.17	7.05	7.27	7.97	9.29	11.89	15.51	18.83	42.35	43.46^{ab}
20	17	61.58^{b}	15.17	16.17	18.37	21.28	5.70	6.48	6.98	7.30	9.31	13.00	15.66	19.44	44.14	37.55^{bc}
16	18	52.31^{bc}	14.68	15.33	17.12	18.47	5.95	7.25	6.77	7.05	9.50	12.69	19.11	22.93	43.89	30.32^{cd}
12	19	42.68^{c}	14.83	15.57	17.57	18.32	5.90	6.55	6.68	6.95	8.75	11.37	16.98	19.10	39.99	25.27^{d}
Mean	18	62.9	14.98	16.05	17.98	20.21	5.88	6.78	6.93	7.37	8.88	11.75	16.51	19.25	42.37	36.56
P (0.05)	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***

* and *** indicates significant statistical differences at $p \leq 0.05$and $p \leq 0.001$ respectively; different letters indicate significantly different means

Grafts made on 20 weeks old rootstocks recorded a subsequent 61.58% graft success, while the lowest success of was recorded on 12 weeks old rootstocks, 42.68%. The higher performance of older rootstocks in graft success was not affected by grafting seasons (Figure 2A & B). Although the interaction of grafting season with rootstock age was poor, as depicted in Figure 2C, the relatively higher grafting success observed during hot season, for the different ages of rootstock, was broken by the 16 weeks old rootstock. Grafting of a 16 weeks old rootstock succeeded better in cool season (54.3%) than hot season (50.4%), unlike the other rootstocks (Figure 2C).

Taken together, the ontogenic age of mango rootstock affected the bud take and graft success, regardless of the grafting season. Nalage and Padihar (2017) reported that the rootstocks in mango produce most of the callus of the graft union, signifying the role of a rootstock age. This suggests that older rootstocks having a well-developed root system for absorption of water and nutrients might play a significant role in graft success. Likewise, grafting success was found heavily affected in wood apple rootstocks aged 45 and 60 days than aged 75 and 90 days, due to delayed callus formation and bud breaking caused by the succulent nature of younger stock (Kadam et al., 2005). Therefore, the success of cleft grafting in mango can be significantly improved by taking 24 and 28 weeks old rootstocks as compared to more juvenile rootstocks.

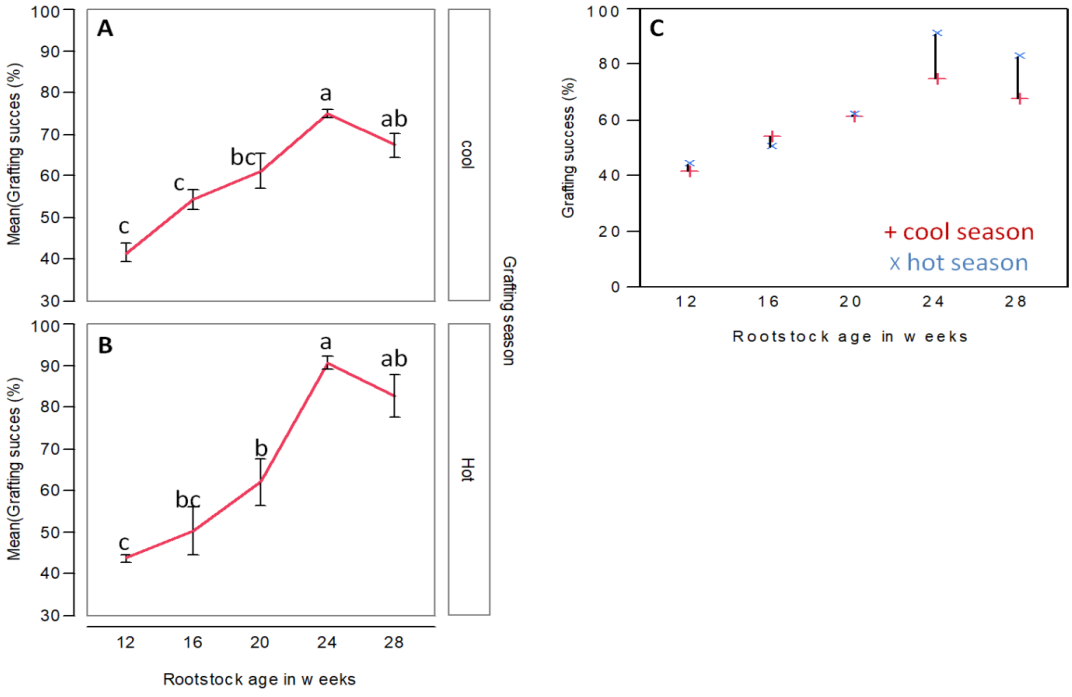

Figure 2: percentage of grafting success of mango cv. Apple at different rootstock ages: A) under cool grafting season, B) under hot grafting season and C) patterns of grafting success under hot and cool grafting seasons.

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Growth Characters of the scion:

Scion growth characters:

Grafting season influenced the scion growth character, height and stock diameter of the scion albeit only at early growth stage, 30 days after grafting; whereas the number of leaves per plant was not affected at all seedling growth stages studied ($p \leq 0.05$; Table 1). Mango scions grafted in hot season attained 15.6 cm scion height and 6.22 mm diameter after 30 days of grafting as compared to a respective value of 14.33 cm and 6.22 mm for cool season grafting. This can be attributed to lower minimum temperature of immediately after cool season grafting, which can slow or cease growth and development of the plants. Historical data on average monthly temperature also indicates that December recoded the lowest minimum average temperature, below 11.0 °C, over the last four decades (Figure 1). Contrastingly, the increased minimum temperature during subsequent growth stages after December might explain the comparable growth characteristics exhibited by the treatments.

Growth parameters such as leaf number, scion height, and diameter were not significantly affected by different rootstock ages used. However, in other study, rate of shoot extension and timing of termination of active extension growth of grafts of deciduous fruit crops were influenced by rootstock ontogeny (Webster, 1995). The comparable number of leaves and chlorophyll content recorded for the grafts made on all rootstock ages in the current study might have contributed by dictating the source capacity to supply various assimilates.

Chlorophyll content and dry matter accumulation:

Chlorophyll content and dry matter accumulation were influenced by grafting season ($p \leq 0.05$; Table 1). The average chlorophyll content of the hot and cool season grafting was 45.02 and 39.72 nmol/cm^2, respectively; and dry matter accumulation was 43.18 and 29.94 g per seedling, in respective order. Likewise, rootstock age affected the accumulation of dry matter but nit chlorophyll content ($p \leq 0.05$; Table 2). Plants grafted on older rootstocks, 28 and 24 weeks, accumulated a comparable average dry weight of 46.19 and 43.46 g per plant, respectively. However, a significantly lower dry weight of 30.32 and 25.27 g per plant was recorded for grafts made on 16 and 12 weeks old rootstocks, respectively.

High temperature and relative humidity during the hot grafting season (T.max 30°C; T.min 15.6°C; 50.5 % RH) might have contributed to increased photosynthetic rate and growth of the grafts unlike to the cool season. Moreover, the above micro-climatic factors promote rootstock-scion union for rapid sap flow in stock and scion to advance higher growth and development (Mir and Kumar, 2011). Similarly, higher photosynthetic capacity, increased leaf thickness and chloroplast cells formation were reported during high light intensity and relative humidity (Jang et al., 2011).

Interaction effects:

Grafting season and rootstock age interactions were not found significant for scion height and dry matter (Table 3). During hot season grafting, rootstock age significantly affected scion height at 30 and 60 days after grafting, but no such effect was observed in cool season ($p \leq 0.05$; Table 3). Grafting made on 24 weeks old rootstock recorded the highest average scion height of 16.54 and 18.23 cm at 30 and 60 days after grafting respectively followed by a significantly comparable scion height for 28 and 20 weeks old rootstocks. In a similar pattern, the dry biomass weight was positively affected by older rootstocks under both seasons ($p \leq 0.05$; Table 3).

Table 3: height and dry biomass of mango cv. Apple under different combination of grafting season and rootstock age

Grafting season	Rootstock age in weeks	Height of scion(cm)				Dry biomass per seedling (gm)
		30 days	60 days	90 days	120 days	
Hot	28	16.03^{a}	17.11^{a}	18.07	22.49	54.33^{a}
	24	16.54^{a}	18.23^{ab}	20.21	25.17	55.16^{a}
	20	16.28^{ab}	16.58^{abc}	18.50	22.80	45.50^{ab}
	16	14.41^{bc}	14.72^{bc}	16.33	17.80	34.22^{bc}
	12	14.87^{c}	14.97^{c}	16.62	17.02	26.70^{c}
	Mean	15.62	16.32	17.95	21.05	43.18
	P (0.05)	*	*	ns	ns	*

Cool	28	14.77	16.16	18.54	19.60	38.06 [a]
	24	14.96	15.94	17.87	19.11	31.76 [ab]
	20	14.07	15.79	18.23	19.79	29.61 [bc]
	16	14.13	15.46	18.30	19.73	26.42 [bc]
	12	13.70	15.57	17.10	18.65	23.85 [c]
	Mean	14.33	15.78	18.01	19.38	29.94
	P (0.05)	ns	ns	ns	ns	*
Season x Rootstock ages		ns	ns	ns	ns	ns

* indicates significant statistical differences at $p \leq 0.05$; different letters indicate significantly different means

4. CONCLUSION AND RECOMMENDATION

In the current study, cleft grafting success and subsequent growth and developments were significantly improved by hot season grafting as compared to cool season. Similarly, rootstock age exerted influence particularly on the grafting success with subsequent increment observed in older rootstocks. As a result, nurseries are encouraged to graft their stock in hotter months of the year around March using older stocks (24 - 28 weeks old) to have better success of cleft grafting of mango cv. Apple.

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