International Journal of Research in Agricultural Sciences
Volume 5, Issue 2, ISSN (Online): 2348 – 3997

Effects of Rootstock Age and Grafting Time on Cleft Grafting in 'Tainung No.2' Papaya

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Date of publication (dd/mm/yyyy): 31/03/2018

Abstract **– This study aimed to evaluate the effect of rootstock age and grafting season on the graft success and initial growth of 'Tainung No. 2' papaya in nursery. The experiment was conducted using an experimental Randomized Complete Block design with factorial scheme 4Ax2B, four blocks and 15 grafted plants per experimental unit. Factor A represented 4 levels of rootstock age (1, 2, 3 and 6 months) and factor B, two grafting times (May, September). The findings showed that rootstock age and grafting season had the significant effect on all the parameters: Time required for take, percentage of graft take, sprout length of scion, number of new leaves. The youngest age of rootstock (1-month) achieved the best results. Grafting in May achieved better results than in September. The best combination was of 1-month-old rootstock and grafting time in May with the minimum time required for take (8.4 days), the highest percentage of graft take (96.7 %), the maximum sprout length of scion (3.9 cm), and maximum number of new leaves (3.1 leaves). Therefore, this combination should be recommended for papaya propagation through cleft grafting.**

Keywords **– Age of Rootstock, Carica Papaya, Cleft Grafts, Propagation, Time of Grafting**

I. Introduction

Papaya (*Carica papaya* L.) is a polygamous species with three sex types: staminate, pistillate and hermaphrodite [1]-[2]. Papaya is one of the few fruit crops still propagated by seed. Papaya seedlings propagated from seed is hindered by problems because of the inherent heterozygosity and dioecious nature of the crop [3]-[4]. In addition, the plants grown from seeds are considerable variation in disease susceptibility, fruit quality, and yield [3]-[5]-[6].

The main advantage of vegetative propagation is the certainty of keeping the characteristics of the mother plant. It had been reported the possibility of developing materials highly productive and resistant to diseases, which can be spread safely keeping intact the characteristics of the papaya mother plants through asexual propagation [7]. The grafted papaya trees bear fruits much lower and earlier and are dwarf in stature with longer economic life cycle [5]-[8]. The case of gynodioecious cultivars, the bisexual types which produce fruits with shape, size, and flavor are preferred to round fruits of female plants as they fetch premium price in the market [3]-[6]. So with vegetative propagation, there is a possibility of maintaining the original characteristics of the parent plant apart from several advantages like higher yield, lower fruiting height, longer cropping span. Papaya grafting propagation has been applied successfully on some cultivars in regions growing papaya. Scion shoots from cultivars 'Co-1' and 'Honey Dew' were successfully cleft-grafted onto uniformly established seedlings [9]. In Malaysia, some growers use grafting to supersede female-fruiting trees of the cultivar 'Eksotika' [10]. A approach of obtaining 100% hermaphrodite stand by cleft grafting papaya seedling using healthy disease free scions was suggested [11]. Allan et al. [12] reported the higher percentage of success (80%) by side grafting obtained after 15 weeks. The variation of results achieved is influenced by different factors. The successful union of scion and stock depend upon proliferation of callus tissue between graft components followed by the union of vascular tissues. This is influenced by some factors: incompatibility, plant species and type of graft, environmental conditions (temperature, moisture, light), growth activity of the rootstock [13]-[14].

In Taiwan, papaya was one of top ten crops growing with 118,822 tons of fruit production and and 2, 469 ha of area harvested in 2013 [15] and papaya cultivars are using mainly as 'Tainung No.2', 'Red Lady', 'Sunrise'. Tainan District Agricultural Research and Extension Station (Taiwan) stated that 'Tainung No.2' papaya is the major cultivar with 90% of growing area. This is the hybrid papaya which is able to grow and develop well in conditional Taiwanese plantation, proving high yield (the fruit weighs about 1.1 kg), good fruit quality (tender and orange-red flesh, good taste, 13% of Brix) and preferred by the local market. Seedlings propagated by sexual method plays a major role in Taiwan papaya production. Plantlets produced from asexual methods (micropropagation, cutting, and grafting) have been conducting in seed center but not yet brought high effect because of the unstable and limited success of propagation. From the literature, there have not been reported the effects of rootstock age and grafting time on grafting success in propagation papaya 'Tainung No.2'. In Taiwan, papaya seedlings can be planted almost all year round. However, the grafted papaya plantlets mainly used from May to June or from September to November in order to reduce the damage of rain or frost in the growth stage of young plants on the field. The main goal of the present study is to determine the best rootstock age and grafting time in the commercial production of 'Tainung No.2' papaya.

II. Materials and Methods

The study was performed in National Pingtung University of Science and Technology nursery, located at Pingtung city, southern Taiwan (lat. 22° 70'N, long. 120° 55'E). The Meteorological data during the experimental period in the nursery were presented in Table 1.

Table 1. Meteorological data during grafting time (May, September) in nursery condition at Pingtung, Taiwan.

Meteorological data	Grafting time	
	May	Sept.
Daylength (hours/day)	13.4	12.3
Monthly average temperature (°C)	28.6	27.5
Monthly mean max. temperature (°C)	34.4	33.5
Monthly mean min. temperature (°C)	25.2	24.8
Relative humidity (RH) (%)	88.4	89.1

A. Preparation of the Scions

Hermaphrodite papaya plants 'Tainung No.2' planted in the net house were collected as source material to produce scions this study. The selected plants for supplying scions were developing normally and free from diseases. Before collecting the scions, the mother plants were topped and sprayed on term with solution of 6-Benzylaminopurine (500 mg/l) and Gibberellic Acid (100 mg/l) three times at weekly intervals to induce side shoot production [16]. After about 1 month, shoots 7 - 10 mm in diameter were selected as scions in grafting.

The selected scions without pest and diseases were used for softwood grafting. Shoots were collected directly from selected healthy papaya trees in the morning hours from 7 to 9 a.m. on the day of grafting. The bases of the shoots were kept in water to avoid desiccation.

B. Preparation of the Rootstocks

Seeds of variety 'Tainung No.2' sowed in sowing trays. After 20 days, uniform seedlings having two mature leaves were transferred to soil pots (10cm x 15cm) with growth medium consisted of peat: soil (1: 2) and were placed in nursery as rootstock. Rootstocks were taken care in a nursery until reaching to grafting age being suitable for each experimental treatment. The morphological characteristics of rootstocks at different ages showed in Table 2.

Table 2. The morphological characteristics of rootstocks.

Age of rootstocks	Number of leaves (Leaves)	Height of stem (cm)	Diameter of stem (mm)
1 month	13.9	56.2	7.6
2 months	22.2	97.7	9.9
3 months	30.5	101.5	10.3
6 months	36.4	105.4	11.2.

C. Conduction of the Experiment

The grafting methods used were cleft methods [8]-[13]. The base of this scion was trimmed to a sharp wedge using a sharp and clean knife. The leaves were trimmed to reduce transpiration loss. The cuts of 2-3 cm were trimmed to a smooth surface. These seedling rootstocks were topped-off at about 10 cm from soil level and a slit of about 2-3 cm was made at the middle of the stem. The prepared scions were then inserted into the rootstock in a manner to ensure that both cambiums were aligned and then whole graft wrapped firmly with sealing parafilm to ensure good contact. Parafilm was a suitable wrapping material because of its stretchable properties and would break up by itself later as the plant grows. Prior to 1 week after grafting, the soil in pots was adequately watered and the plant carefully handled to avoid wetting the grafted parts. High humidity was necessary to reduce transpiration water loss.

Rootstock age and grafted season are 2 experiment factors in this research. The first factor represented 4 levels of rootstock age (1, 2, 3 and 6 months) counted from the date of transplantation. And the second factor was two grafting times (May, September). In this the rootstocks were collected from uniformly, vigorously growing seedling of different ages (1 month, 2 months, 3 months and 6 months) were used as rootstocks for cleft grafting. The study was performed in May and September under nursery condition in Pingtung city, Taiwan. Experiments were analyzed as randomized complete block design with four replications, each replication conducted 15 samples per treatment. The data was recorded following parameters: Time required for take was calculated from the time of operation to when the formation of grafting union with showing of the expansion of the first leaves. Percentage of graft take, sprout length of scion per graft and number of new leaves per graft were estimated at the end of 4 weeks. And percentage of graft take was computed by using the following formula:

$$\text{Percentage of graft take (\%)} = \frac{\text{Number of successful grafts}}{\text{Total no. of graft age done}} \times 100$$

Statiscal Analysis

Analysis of variance was performed to test for significant effects of rootstock age and grafting time on time required for success, percentage of graft take, sprout length of scion, and number of new leaves. Means were compared by Duncan's multiple range test (SAS 9.0 software) at $P<0.01$.

III. Results and Discussion

Table 3. ANOVA for grafting time and rootstock age for papaya cleft grafting.

Variation factor	DF	Mean square			
		Time required for take	Percentage of graft take	Sprouting length of scion	Number of new leaves
Grafting times (S)	1	2.97**	612.41**	0.23**	0.12**
Rootstock age (A)	3	61.74**	4097.64**	10.95**	3.55**
S×A	3	0.25*	82.91**	0.04**	0.01*
Error	21	0.06	16.20	0.003	0.003
CV, %		2.23	5.41	2.07	2.21

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 4. Effect of rootstock age on cleft grafting and growth of papaya plants.

Rootstock age	Time required for take (days)		4 weeks after grafting					
			Percentage of graft take (%)		Sprout length of scion (cm)		Number of new leaves (leaves)	
1 month	8.4	a	94.2	a	3.8	a	3.1	a
2 months	9.5	b	90.8	a	3.3	b	2.7	b
3 months	12.3	c	66.7	b	2.4	c	2.3	c
6 month	14.6	d	45.8	c	1.1	d	1.5	d

In plant propagation, the age of rootstock, time of grafting are factors affecting grafting success [13]-[17]. In papaya propagation, this opinion is agreed by the ANOVA results shown in Table 3. The grafting time and rootstock age affected the success of cleft grafting (parameters: Time required for take, percentage of graft success) and growth of grafted plants (parameters: Sprouting length of scion, number of new leaves) were significant ($P<0.01$). The summary of the ANOVA showed interaction between grafting time and rootstock age factors for the parameters.

A. Effect of Rootstock Age on Cleft Grafting and Growth of Papaya Plants

The parameters as time required for take, percentage of graft take, sprout length of scion, number of new leaves varied significantly with respects to rootstock age ($P<0.01$). The results showed in Table 4. The best results of time required for take, percentage of graft take, sprout length of scion and number of new leaves were obtained grafting 1-month-old rootstock with 8.4 days, 94.2 %, 3.8 cm and 3.1 new leaves respectively. For effects of rootstock age, the results revealed that younger rootstocks were obtained more success in observed parameters. Percentage of graft take gave the highest rate when grafted onto 1-month-old rootstock in comparison of older rootstock (2, 3, 6-month-old). This opinion was in agreement with Islam et al. [18] while working in mango, and by Dadzie et al. [19] while working in kola. Finding showed that younger rootstocks need minimum time required for success during working in different ages (1, 2, 3, and 6 of months). This was consentaneous with claimed research of cashew grafting of Mahunu et al. [20], jackfruit grafting of Islam et al. [21], mango grafting of Alam et al. [22] and Upadhya et al. [23].

Table 5. Effect of grafting time on cleft grafting and growth of papaya plants

Grafting time	Time required for take (days)		4 weeks after grafting					
			Percentage of graft take (%)		Sprout length of scion (cm)		Number of new leaves (leaves)	
May	10.9	a	78.8	a	2.7	a	2.5	a
September	11.5	b	70.0	b	2.6	b	2.3	b

Means within a column followed by different letters are significantly different according to Duncan's multiple range test at $P<0.01$.

Using younger rootstock gave the better results in all parameters of time required for take, percentage of graft take, sprout length of scion, and number of new leaves. This may be because of relationship with regenerating ability of a plant part, which is found in younger rootstocks. This is because of higher activity of meristematic cells resulting in faster callus formation and quick healing of grafting union. In general, the lower graft union success could be attributed to the lack of intimate contact of cambial region of both stock and scion and to interference of exudation of latex [24]. It was reported that rapid formation of callus (parenchymatous) tissues allows translocation of vital biochemical compounds stock and scion might be the reason for minimum days to graft union [25]. Other reason for growth of scion after graft was due to younger stocks store more carbohydrates and other food substances and this leads to more vegetative growth in terms [26]. Additionally, translocation of vital biochemical compounds stock and scion might be the reason for increasing scion height and number of leaves per graft in younger old rootstocks. Older tree accumulates high latex and phenolic exudates that may reduce the rate of growth of grafted plants. On the other hand, presence of more concentrated latex and hardiness of aged rootstock which hindered to the graft union process might be the reason for maximum days to complete graft union [27].

B. Effect of Grafting Time on Cleft Grafting and Growth of Papaya Plants

Several researches have reported that environmental condition directly affects graft union success. In this study, the results of influences of environmental condition in two grafting time (May, September) on grafting success were showed in Table 5. The parameters of time required for take, percentage of graft take, sprout length of scion, and number of new leaves showed that grafting in May achieved better results than in September ($P<0.01$).

Temperature and humidity are two important environmental factors that influence greatly to the healing of graft union by callus formation [13]-[14]. In this research, the results in May were better than in September for parameters of time required for take, percentage of grafting take, sprout length of scion, number of new leaves. This could be explained: the firstly, daily mean temperature in May (28.6^{o}C) was higher than in September (27.5^{o}C). And Sadhu [14] reported that temperature that between 5^{o}C and 32^{o}C rate of graft union increases with the rising

temperature. Thus, it is true that temperature in May is a cause to get better results in comparison with September. Secondly, The maintain high humidity is very important to prevent the scion from drying [14]. Difference of humidity between May (88.4%) and September (89.1%) may be not enough big to make difference of scion desiccation after grafting. Meanwhile, light effects on the callus formation, photosynthesis of plants [28]. It may be cause for faster graft union success and stronger growth of grafted plantlets in May with more day length.

C. Combined Effect of Rootstock Age and Grafting Time on Cleft Grafting and Growth of Papaya Plants

The results showed that combination of rootstock age and different grafting time made different results in papaya graft. Using 1-month-old rootstock in May or in September achieved the best results for all parameters (Table 6). For time required for take, the minimum period (8.4 days) was recorded from the combination of 1-month-old rootstock and May which were statistically similar to the grafts of 1-month-old rootstock and September, 2-month-old rootstock and May. The highest percentage of graft take (96.7%) was achieved when grafted onto 1-month-old rootstock in May which was statistically similar to the grafts of 1-month-old rootstock in September, 2-month-old rootstock in May. The maximum sprout length of scion (3.9 cm) was recorded in combination of 1-month-old rootstock and May which was statistically similar to the grafts of 1-month-old rootstock in September. The maximum number of new leaves was found with 1-month-old rootstock grafted in May (3.1 leaves) or September (3.1 leaves).

The experimental results elucidated that combination of rootstock age and different environment at grafting time made different results in papaya graft. The percentage of graft take of 91.7% or higher are higher than the previous results claimed by Chong et al. [8], who reported that percentage of success was 80% in cleft grafting on papaya 'Eksotika' and by Ramkhelawan and Baksh [29], who obtained the percentage of success (75%) by side grafting and (85%) by chip budding. Additionally, the results of growth of papaya plantlets after grafting onto 1-month-old rootstock in May or September are satisfactory for producing commercial plantlets. The outstanding results achieved in the research because responsibility of 1-month-old rootstock with environmental condition might be the best favorable for the rate of callus formation and healing of grafting union.

IV. Conclusion

Our findings of study indicated that the success in propagation of 'Tainung No.2' papaya through cleft grafting was affected by age of rootstock and grafting time. Among tested rootstock ages, 1-month-old rootstock showed the best graft success. Grafting in May was better than in September. The results also revealed that grafting papaya onto 1-month-old rootstock in both May and September achieved the best results for all parameters, including time required for take, percentage of graft take, sprouting length of scion and number of new leaves. This study suggests that 1-month-old rootstock could be applied for cleft grafting in both May and September to produce large number grafted plantlets.

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