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Effect of Some Factors Affecting Selected Carob Strain (*Ceratonia siliqua* L.) Propagation by Grafting

Cross Mark

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ABSTRACT

This study was carried out in the experimental orchard of Horticultural Research Institute, Agriculture Research Center, Giza Governorate, Egypt through 2017 and 2018 seasons. The main aim was to find out the most reliable carob (*Ceratonia siliqua* L.) grafting technique (cleft with apical and axillary buds, saddle with apical and axillary buds, cleft with axillary buds and saddle with axillary buds) and the suitable grafting date (15th February, 15th March or 15th April). Results showed that cleft with axillary buds grafting proved to be the best technique regarding modified success percentage, leaves number, scion and rootstock diameters. Cleft with apical and axillary buds grafting technique recorded the highest modified survival percentage and vegetative growth length values. February was the best grafting date concerning vegetative growth length, leaves and sprouted shoots numbers. Modified survival percentage and grafting union diameter were improved in March. Using saddle with axillary buds grafting in February and March produced the maximum sprouted shoots number, whereas using it in March recorded higher grafting union diameter value. Vegetative growth length and modified survival percentage were improved using cleft with apical and axillary buds grafting technique in February and March, respectively. Both cleft with axillary buds and with apical and axillary buds grafting techniques were the best regarding grafting union examination. It can be recommended that, carob can be vegetatively propagated by using either cleft with axillary buds or with apical and axillary buds grafting techniques. Also, grafting carob can be done in February or March.

Keywords: Carob, Modified success percentage, Modified survival percentage, Grafted seedlings, cleft, Date of grafting.

INTRODUCTION

Long-lived evergreen carob tree (*Ceratonia siliqua* L.) belongs to the Leguminosae family has been described as a plant with a large adaptability in the Mediterranean area (Batlle and Tous, 1997). Genotypes of carob tree show great tolerance to biotic and abiotic stresses such as pests, diseases, drought, salinity, air pollution, high pH (calcareous soil), high summer temperatures (up to 50°C), and low soil fertility (Eshghia *et al*., 2018). Moreover, carob cultivation facilitates the establishment of other plant species, being particularly useful for the rehabilitation of difficult areas, where it can simultaneously play the role of pioneer and productive species due to its ability to preserve and enrich soil fertility (Essahibi *et al*., 2016). In addition to the previously mentioned characteristics, the high economic value of carob's products (used in food, chemicals, cosmetics, processing etc.) described by Omran *et al.* (2018) make this species suitable as a beneficial biological tool to prevent erosion and desertification, as well as a reliable tool for the development of the marginal areas in the Mediterranean basin (Essahibi *et al*., 2016). Carob has been neglected with respect to cultural practices, research and development (Batlle and Tous, 1997) and apart from a few classic works references on this tree remain scarce (Eshghia *et al*., 2018). Carob trees may be male, female and hermaphrodite or play gummous inflorescences, showing high plasticity in inflorescences and flowering characteristics.

Carob tree is traditionally propagated by seeds, which is not recommended because seedlings show high heterozygosis, are slow to become reproductive and about 50% of plants are potentially nonproductive males (Essahibi *et al*., 2016). Moreover, carob seeds are difficult to germinate. The seed coat is extremely hard and not ready to absorb water, so carob seeds need pre-sowing treatments (Zaen El Deen *et al*., 2014). Propagation by cutting is problematic because carob has been described as one of the most difficult to root species (Hartmann *et al*., 1997). This technique has not yet been fully achieved for many woody species including carob (Essahibi *et al*., 2016). Gubbuk *et al.* (2011) concluded that propagation of carob trees by air layering is successful. However, if it is compared to grafting, air-layering is more labor intensive. Only a small number of layers can be produced from a parent plant than when the same plant is used as source of cuttings or scions, however this method is laborious, cumbersome and expensive. Zaen El Deen *et al.* (2014) proved that carob micropropagtion needs further studies to overcome the difficulties in the root induction. Also, El Bouzdoudi *et al.* (2017) reported that carob can be cultured *in vitro* with a survival rate of 40%. Therefore, grafting is the main solution to continue carob vegetative propagation although no commercial rootstock trials have been carried out on cultivars and no rootstock selections are available for carob (Gubbuk *et al*., 2011). Sutter (1994) pointed out that grafting on rootstocks is a valuable technique for improving fruit trees, making it possible to control such important factors as vigorous, resistance to parasites, diseases, adverse environmental factors and adaptation to soil and climatic conditions. There's no available scientific researches dealing with carob grafting until the completion of this study. This investigation was conducted to find the most reliable

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carob grafting technique and the suitable date for propagation of a previously selected strain to optimize the distribution of carob in Egypt.

MATERIALS AND METHODS

This study was carried out in the experimental orchard of Horticultural Research Institute, Agriculture Research Center, Giza Governorate (Latitude 30 03, Longitude 31 13 and Elevation 18.6), Egypt through 2017 and 2018 seasons.

Biological materials and experimental design:

Rootstocks production

The mature carob pods were collected from female trees grown at the experimental orchard of Horticultural Research Institute. Seeds were collected and placed in a cloth bag then were subjected to soaking in running water for 6 days. Germinated seeds were treated with 0.5 % Rizolex-T50%WP fungicide then sown at a depth of 1 cm in polyethylene black bags (1L filled with a mixture of soil and sand [2:1 v/v]) and were placed in a shaded net house with 65% porosity. The germinated seedlings were irrigated regularly and were fertilized biweekly with NPK and micro elements. In January 2017 healthy, sound and vigorous plants (one year old) were selected to serve as rootstocks.

Preparation of scions

Leafy shoots carrying healthy buds were collected from nine years old healthy female carob strain growing at the experimental orchard of the Horticultural Research Institute planted in loamy soil, and it was previously evaluated in 2016 and 2017 seasons and was significantly the highest regarding average weight of seeds/pod, seeds/pod percentage, total sugars, total phenols and fiber percentages (Omran *et al*., 2018) so, it's suitable for many industrial demands which are interested in carob bean gum (CBG which is used in the textile, pharmaceutical, biomedical, cosmetics, nutrition sciences, and food industries), seeds powder and carob germ flour. The scions were prepared from one year old branches (15-20 cm) with 2-4 leaflets and they were kept in a shady place for immediate grafting process.

The grafting process

Two grafting techniques were performed in this study, cleft and saddle grafting using scions containing apical and axillary buds as well as scions containing only axillary buds as shown in Fig. (1). Four grafting techniques were done as follow:

Cleft with apical and axillary buds, saddle with apical and axillary buds, cleft with axillary buds and saddle with axillary buds grafting. Each grafting process was performed at three different dates (15th of February, March and April). Thirty plants were adopted for each grafting technique in every season (10 grafted plants replicated 3 times). Both of the collected scions and the selected rootstocks attained pencil's thickness. Rootstocks were prepared by removing all leaves except 2 leaflets. Grafting was done at 30 cm from soil surface using a sharp knife to make smooth cuts and grafting tapes to keep the grafting union tight and to prevent drying. Scions were moisturized before grafting. The grafted plants were placed under a tight polyethylene tunnels in a shaded net house with 65% porosity for two months. All sprouts arising from rootstock below the grafting position were removed continuously.

Recorded Data

Two months after the grafting process the polyethylene tunnels were removed gradually and the upcoming parameters and measurements were recorded:

- Modified average of success percentage was recorded after two months from grafting (Swamy and Melanta, 1994 and Zenginbal, 2015).
- After one year from grafting, modified average of survival percentage (Abou-Taleb *et al*., 2011), averages of vegetative growth length (cm), number of leaves and number of sprouted shoots were estimated. Also, rootstock, scion and grafting union diameters (cm) were recorded.

Fig. 1. The four-grafting techniques used for the selected carob strain propagation under study.

Unity of grafting union

After one year of grafting, the union zone of survived grafted plants (three seedlings per graft technique) were collected randomly by cutting approximately 2 cm below and above the scion/stock graft union. These stem sections were cut longitudinally into two halves across the union zone using a very thin sow then was softened using a fine sand paper. The collected samples then were observed using a binocular to study the symptoms of compatibility or incompatibility of the grafting union and then were illustrated by photographs (Singer, 1997).

Visual scoring for graft compatibility included a visible union line in wood (scale of 1 to 4: 1 = visible, 2 = faint, 3 = very faint, 4 = absent); browning intensity of deposits at the union interfaces (scale of 1 to 4: 1 = visibly high, 2 = medium, 3 = low, 4 = visibly absent) and amount of callus proliferation (scale of 1 to 4: 1 = high, 2 = medium, 3 = low, 4 = absent) as described by Mng'omba *et al*. (2007).

Statistical analysis

Data recorded in 2017 and 2018 seasons were subjected to analysis of variance (ANOVA) for factorial design (the four grafting techniques and the three dates) in a randomized complete blocks (Snedecor and Cochran, 1980) and the means were distinguished according to the Duncan multiple range test at the level of probability 5% (Duncan, 1955).

RESULTS AND DISCUSSION

Modified average of success percentage

The available data tabulated in Table 1 represent the effect of grafting techniques and dates on modified average of success percentage during 2017 and 2018 seasons. In the 1st season, using cleft with apical and axillary buds grafting gave the highest value (62.72%) with insignificant difference with cleft with axillary buds grafting (62.67%) whereas saddle with apical and axillary buds grafting recorded the lowest value (34.57%). In the 2nd season, using cleft with axillary buds grafting proved to be significantly the best (66.15%) followed by cleft with apical and axillary buds grafting (58.37%), while saddle with apical and axillary buds grafting (39.29%) was the last with significant differences with the others. Similar results were obtained by Abd El-Zaher (2008), who reported that the translocation between the scion and the rootstock of jackfruit top-cleft grafted seedlings can take place through both the callus bridges and the new vascular elements formed in the grafting union region and consequently the scion continued alive and had a good growth. The opposite anatomical features had shown in the transverse sections of the saddle grafting union region. Moreover, El-Taweel *et al.* (2015) found that cleft grafting gave maximum success percentage of guava grafts in both seasons. On the other hand, the propagation technique played a role and there were numerous possible variables, which may affect the success of operation. Some methods of grafting gave better results than others, or budding may be more successful than grafting, or vice versa (Hartmann *et al*., 2014).

Regarding the effect of grafting dates, as shown in Table 1, the highest significant modified success percentage value was scored in March 15th (67.38%), while grafting in February 15th came next (61.61%) and lastly came grafting in April 15th with significant differences in 2017 season. Modified average of success percentage value 67.82% was recorded in February 15th, 64.76% in March 15th and 23.82% in April 15th with significant differences in 2018 season. The low temperature in February and March in comparable with April date may play an important role in success percentage as high temperature in April may cause dryness of the grafts. The above results are in line with those of Sutter (1994), who reported that the greatest success of grafted olive plants appear to be in February and March with the growth of shoots in spring. Also, Swamy and Melanta (1994) reported that the soft wood grafting of the jackfruit was best carried out in Feb.-March and take 30-60 days after grafting to give the highest success percentage. Moreover, Abou-Taleb *et al.* (2011) found that grafting in March gave increased % of successful grafts of pecan than grafting in February.

Concerning the interaction between the two tested factors (Table, 1), using cleft with apical and axillary buds grafting in March 15th proved to be statistically the best (90.00%) during 2017 season, next came cleft with axillary buds grafting in February 15th (75.18%). In 2018 season, cleft with axillary buds grafting in February 15th scored the highest significant value in this regard (90.00%), while cleft with apical and axillary buds grafting in March 15th recorded the 2nd position (79.84%). Saddle with apical and axillary buds grafting in April scored the least modified success percentage value (0.01%) with statistical differences with the others in both seasons. El-Taweel *et al.* (2015) reported that grafting guava by cleft grafting in Feb. gave relatively higher percentage of successful grafts. Singer (1997) concluded that meristematic cells, in healing zone changed into thin wall parenchyma cells as a result of a wound stimulus for cambial initiation in peach. The successful union of scion and stock depend on the proliferation of callus tissue between graft components followed by the union of vascular tissues. This is influenced by factors such as incompatibility, plant species, type of graft, environmental conditions (e.g., temperature and moisture), and rootstock growth activity (Sadhu, 2005 and Hartmann *et al*., 2014). In this study, maintaining grafted plants in high humidity conditions (tight polyethylene tunnels) to avoid drying out of the scion is very important practice. Shippy (1930) reported that air and moisture levels below the saturation point inhibited callus formation, the rate of desiccation of the cells increased as the humidity dropped of apple grafts.

Table 1. Effect of grafting techniques and dates on modified average of success percentage of grafted carob selected strain in 2017 and 2018 seasons.

Grafting techniques	Modified average success (%)							
	2017				2018			
	15th Feb.	15th March	15th April	Mean	15th Feb.	15th March	15th April	Mean
Cleft*	71.62c	90.00a	26.55i	62.72A	71.58c	79.84b	23.70j	58.37B
Saddle*	46.91g	56.80e	0.01j	34.57C	58.94e	58.94e	0.01k	39.29D
Cleft**	75.18b	65.91d	46.91g	62.67A	90.00a	63.45d	45.00h	66.15A
Saddle**	52.73f	56.79e	33.20h	47.57B	50.77g	56.80f	26.55i	44.71C
Mean	61.61B	67.38A	26.67C		67.82A	64.76B	23.82C	

Means designated with the same letter within columns and rows in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.

*** With apical and axillary buds. ** With axillary buds.**

Modified average of survival percentage

The effect of grafting techniques and dates on modified average of survival percentage in 2017 and 2018 seasons is shown in Table 2. Generally, it was apparent that cleft with apical and axillary buds grafting proved to be significantly the best in this concern in both seasons of the study (58.12 and 51.99%, respectively) then cleft with axillary buds grafting (54.47 and 51.20%, respectively). Conversely, saddle with apical and axillary buds grafting (32.66 and 33.25%, respectively). Abd El-Zaher (2008) recommended the using the top-cleft method as a pronounced grafting method in jackfruit seedlings. He also, reported that the most deaths of seedlings during the two years from grafting, were the saddle grafted seedlings. He concluded that this may be due to some incompatibility that illustrated in the incomplete anatomical union between the scions and the rootstocks in spite of the continuity of adhesion between the partners. Moreover, Talukder *et al.* (2015) reported that cleft method showed higher percent of survivability in mandarin.

Regarding grafting dates effect, significant differences were observed between the three dates and this was true in both seasons, March 15th gave the highest values (67.39 and 59.57%, respectively), February 15th came next (55.66 and 54.02%, respectively) then April 15th which gave the least values (17.16 and 15.85, respectively).

Generally, the interaction between the two factors declared that in the 1st season cleft with apical and axillary buds grafting in March 15th recorded the maximum (90.00%) followed by cleft with axillary buds grafting in February 15th (68.60%) with significant difference. In the 2nd season the highest modified average of survival percentage value was significantly given by cleft with apical and axillary buds grafting in March 15th (71.62%). Both cleft with axillary buds and with apical and axillary buds grafting in February 15th were statistically the same (65.91 and 65.94%, respectively) but differed from statistical point of view with the others. In April 15th, saddle with apical and axillary buds grafting technique gave 0.01% in both seasons of study.

Table 2. Effect of grafting techniques and dates on modified average of survival percentage of grafted carob selected strain in 2017 and 2018 seasons.

Grafting techniques	Modified average survival (%)							
	2017				2018			
	15th Feb.	15th March	15th April	Mean	15th Feb.	15th March	15th April	Mean
Cleft*	65.97c	90.00a	18.38i	58.12A	65.94b	71.62a	18.42i	51.99A
Saddle*	41.17f	56.79d	0.01j	32.66D	45.00f	54.74d	0.01j	33.25D
Cleft**	68.60b	65.97c	28.85g	54.47B	65.91b	61.15c	26.55h	51.20B
Saddle**	46.91e	56.79d	21.40h	41.70C	39.23g	50.77e	18.40i	36.13C
Mean	55.66B	67.39A	17.16C		54.02B	59.57A	15.85C	

Means designated with the same letter within columns and rows in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.
*** With apical and axillary buds. ** With axillary buds.**

Average vegetative growth length (cm)

Table 3 shows the effect of grafting techniques and dates on average vegetative growth length. In 2017 and 2018 seasons, cleft with apical and axillary buds grafting proved to be significantly the best (43.33 and 46.73 cm, respectively). Even though, saddle with apical and axillary buds grafting in both seasons scored the minimum rates (26.18 and 28.88 cm, respectively). Both, Talukder *et al.* (2015) and Beshir *et al.* (2019) mentioned similar results.

In this study, vegetative growth length values significantly increased gradually from April 15th (24.03 and 27.07 cm) to February 15th (40.00 and 43.62 cm) in 2017 and 2018 seasons, respectively. This may be due to the early union formation and the long growing season of February grafting date.

The interaction revealed that in both seasons, cleft with apical and axillary buds grafting when was applied in both February 15th and in March 15th were significantly considered the best technique for increasing vegetative growth length (46.89, 50.21, 44.27 and 48.00 cm, respectively) as shown in Table, 3. In both seasons of study, saddle with apical and axillary buds grafting in April showed minimum records, which significantly differed with the others. Results as shown in Table 3 revealed that average vegetative growth length seems to be influenced by apical dominance effect.

Table 3. Effect of grafting techniques and dates on average vegetative growth length (cm) of grafted carob selected strain in 2017 and 2018 seasons.

Grafting techniques	Vegetative growth length (cm)							
	2017				2018			
	15th Feb.	15th March	15th April	Mean	15th Feb.	15th March	15th April	Mean
Cleft*	46.89a	44.27b	38.84c	43.33A	50.21a	48.00b	41.97d	46.73A
Saddle*	39.63c	38.91c	0.01i	26.18D	43.65c	42.98c	0.01h	28.88D
Cleft**	37.54d	36.29e	29.48g	34.44B	41.71d	41.39d	34.32f	39.14B
Saddle**	35.93ef	35.17f	27.78h	32.96C	38.89e	38.48e	31.99g	36.45C
Mean	40.00A	38.66B	24.03C		43.62A	42.71B	27.07C	

Means designated with the same letter within columns and rows in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.
*** With apical and axillary buds. ** With axillary buds.**

Average leaves number

Dealing with average leaves number as affected by grafting techniques and dates in 2017 and 2018 seasons (Table, 4), significant differences were noted between the four grafting techniques in both seasons of study, cleft with axillary buds grafting came first (37.37 and 38.96) followed by saddle with axillary buds grafting (34.29 and 35.78), respectively. Finally came saddle with apical and axillary buds grafting (19.25 in 2017 and 20.45 in 2018). In this concern, the maximum number of leaves was obtained by cleft grafting of mandarin and mango, respectively [Talukder *et al.* (2015) and Beshir *et al.* (2019)].

Also, significant differences were observed between the three grafting dates in 2017 and 2018 seasons. Grafting in February 15th showed the highest number of leaves (33.51 and 35.22), respectively, while the least records were clear in April 15th (25.35 and 26.21), respectively, this may be due to the long growth period of February grafts. Grafting on 24th of February proved to be successful in No. of leaves/sprouted shoots of pecan more than 2nd of March (Abou-Taleb *et al.*, 2011).

Cleft with axillary buds grafting in March 15th succeeded to score the highest significant value (Table, 4) with insignificant difference with the same grafting technique in February 15th (37.79 and 37.45 in 2017 season as well as 39.45 and 39.31 in 2018 season). Cleft with axillary buds grafting in April 15th recorded the 2nd position (36.87 in 2017 and 38.13 in 2018) with an insignificant difference with cleft with axillary buds grafting in February 15th just in 2017 season, whereas a significant difference was found in 2018 season. While, the lowest values in both seasons were booked by saddle with apical and axillary buds grafting applied in mid of April. Saddle grafting in mid-February was the best for guava seedlings (El-Taweel *et al*., 2015), while for mango, Beshir *et al.* (2019) reported that the highest value for the number of leaves of new growth was recorded by December using cleft technique followed by June grafting time. These contradictions may be resulting from different plant species.

Table 4. Effect of grafting techniques and dates on average leaves number of grafted carob selected strain in 2017 and 2018 seasons.

Grafting techniques	Leaves number							
	2017				2018			
	15th Feb.	15th March	15th April	Mean	15th Feb.	15th March	15th April	Mean
Cleft*	32.00e	31.68e	30.72f	31.47C	32.76de	33.07d	32.05e	32.63C
Saddle*	29.36g	28.39h	0.01i	19.25D	31.10f	30.23g	0.01h	20.45D
Cleft**	37.45ab	37.79a	36.87b	37.37A	39.31a	39.45a	38.13b	38.96A
Saddle**	35.21c	33.87d	33.79d	34.29B	37.72b	34.99c	34.64c	35.78B
Mean	33.51A	32.93B	25.35C		35.22A	34.44B	26.21C	

Means designated with the same letter within columns and rows in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.
*** With apical and axillary buds. ** With axillary buds.**

Average number of sprouted shoots

Table 5 reveals the effect of grafting techniques and dates on average number of sprouted shoots. According to data of both seasons, it's clear that saddle with axillary buds grafting significantly showed the best results (2.52 and 2.31, respectively), followed by cleft with axillary buds grafting (1.93 and 1.32, respectively). The least values were booked by saddle with apical and axillary buds grafting technique (0.78 and 0.74, respectively) with significant differences. The above results proved that the absence of apical dominance encouraged axillary buds sprouting. The above results are in line with El-Taweel *et al.* (2015), who reported that saddle grafting of guava gave the highest number of sprouted shoots.

As shown in Table 5, grafting in February recorded significantly the highest values in 2017 and 2018 seasons (1.85 and 1.60, respectively), while the lowest ones were recorded in April. Similarly, Abou-Taleb *et al.* (2011) noted that grafting of pecan on 24th of February proved to be successful in increasing No. of sprouted shoots.

Dealing with the interactions, in the 1st season insignificant difference was observed between saddle with axillary buds grafting in February and March 15th which gave significantly the higher values in this concern (2.68 and 2.57, respectively). Saddle with axillary buds grafting in April and cleft with axillary buds grafting in February scored the 2nd position with significant differences with the others. In the 2nd season, saddle with axillary buds grafting in February and March surpassed other techniques (2.51 and 2.43, respectively) followed by the same technique in April (2.00) with significant differences. The least number of sprouted shoots was obtained by saddle with apical and axillary buds grafting in April in both studied seasons. Grafting guava using cleft and saddle grafting in March was the best in the 1st season, while in the 2nd it was by using saddle and tongue grafting in mid-January (El-Taweel *et al*., 2015).

Table 5. Effect of grafting techniques and dates on average number of sprouted shoots of grafted carob selected strain in 2017 and 2018 seasons.

Grafting techniques	Average number of sprouted shoots							
	2017				2018			
	Feb.	March	April	Mean	Feb.	March	April	Mean
Cleft*	1.27d	1.09d-f	1.00f	1.12C	1.23d	1.00e	1.00e	1.08C
Saddle*	1.25de	1.08ef	0.01f	0.78D	1.22d	1.00e	0.01f	0.74D
Cleft**	2.19b	1.88c	1.71c	1.93B	1.45c	1.33cd	1.19d	1.32B
Saddle**	2.68a	2.57a	2.31b	2.52A	2.51a	2.43a	2.00b	2.31A
Mean	1.85A	1.66B	1.26C		1.60A	1.44B	1.05C	

Means designated with the same letter within columns and rows in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.
*** With apical and axillary buds. ** With axillary buds.**

Average scion diameter (cm)

Table 6 clarifies the effect of grafting techniques and dates on average scion diameter in 2017 and 2018 seasons. Cleft with axillary buds grafting appeared to be the best technique for increasing carob scion diameter (0.777 and 0.833 cm) in both seasons, respectively. The quick and strong union formation, which resulted in higher modified success percentage (Table, 1) and greater uptake of water and nutrients may account for higher scion diameter of cleft with axillary buds grafting. Reversely, saddle with apical and axillary buds grafting showed the minimum values in both seasons. Similar result was reported by Beshir *et al.* (2019) who noted that the highest increment of scion diameter of mango was obtained by cleft grafting technique.

In 2017 season clear significant differences were observed between the three grafting dates, while insignificant difference was noted between grafting in February and March 15th just in 2018 season. Grafting in February 15th tended to have higher values of scion diameter in both seasons (0.780 and 0.800 cm) respectively, while grafting in April 15th showed the least values (0.560 and 0.593, respectively). This may be due to longer growing period of grafting in February.

Concerning the interaction between grafting techniques and dates (Table, 6) in 2017 season, cleft with axillary buds grafting gave the highest significant scion diameter (0.820 cm) in February 15th, also, when was applied in March 15th proved to be the best in 2018 season (0.840 cm). Beshir *et al.* (2019) declared that the highest increment of scion diameter was observed on the grafted plants of March using cleft grafting technique.

Table 6. Effect of grafting techniques and dates on average scion diameter (cm) of grafted carob selected strain in 2017 and 2018 seasons.

Grafting techniques	Average scion diameter (cm)							
	2017				2018			
	15th Feb.	15th March	15th April	Mean	15th Feb.	15th March	15th April	Mean
Cleft*	0.740b	0.730b	0.730b	0.733B	0.810a-c	0.790b-d	0.770cd	0.790B
Saddle*	0.790ab	0.740b	0.01c	0.513C	0.770cd	0.770cd	0.01e	0.517C
Cleft**	0.820a	0.760b	0.750b	0.777A	0.830ab	0.840a	0.830ab	0.833A
Saddle**	0.770ab	0.760b	0.750b	0.760AB	0.790b-d	0.760d	0.760d	0.770B
Mean	0.780A	0.748B	0.560C		0.800A	0.790A	0.593B	

Means designated with the same letter within columns and rows in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.
*** With apical and axillary buds. ** With axillary buds.**

Average rootstock diameter (cm)

Table 7 reveals the effect of grafting techniques and dates on average rootstock diameter (cm) of grafted carob seedlings. Dealing with grafting techniques in both seasons of study, cleft with axillary buds grafting scored the highest significant values (0.813 and 0.839 cm, respectively), while the other grafting techniques showed lesser values. The obtained result was supported by El-Taweel *et al.* (2015) and Beshir *et al.* (2019).

Regarding grafting dates effect, in the 1st season insignificant differences were apparent between grafting in February and March 15th, however in the 2nd season significant differences were apparent between the three grafting dates as grafting in March 15th surpassed others. The least rootstock diameter was given by April 15th date. This result is in harmony with that of Beshir *et al.* (2019).

Referring to the interactions between the two factors, generally, cleft with axillary buds grafting when applied in all grafting dates under study (in February 15th, March 15th, and April 15th) exceeded other combinations in increasing rootstock diameter. Reversely, in both seasons saddle with apical and axillary buds in med-April took the other way around. Beshir *et al.* (2019) concluded that the highest increment of rootstock diameter was observed on cleft grafted mango seedlings in March.

Table 7. Effect of grafting techniques and dates on average rootstock diameter (cm) of grafted carob selected strain in 2017 and 2018 seasons.

Grafting techniques	Average rootstock diameter (cm)							
	2017				2018			
	15th Feb.	15th March	15th April	Mean	15th Feb.	15th March	15th April	Mean
Cleft*	0.770b	0.780ab	0.770b	0.773B	0.790de	0.820a-d	0.800cd	0.803B
Saddle*	0.770b	0.780ab	0.010c	0.520C	0.790de	0.800cd	0.010f	0.533C
Cleft**	0.800ab	0.820a	0.820a	0.813A	0.840ab	0.827a-c	0.850a	0.839A
Saddle**	0.770b	0.790ab	0.790ab	0.783AB	0.760e	0.820a-d	0.810b-d	0.797B
Mean	0.778A	0.793A	0.598B		0.795B	0.817A	0.618C	

Means designated with the same letter within columns and rows in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.
*** With apical and axillary buds. ** With axillary buds.**

Average grafting union diameter (cm)

Table 8 shows the effect of grafting techniques and dates on average grafting union diameter in 2017 and 2018 seasons. The perusal of grafting data indicates that, in both seasons it's clear that saddle with axillary buds grafting statistically recorded the highest values (1.130, and 1.147 cm, respectively). Cleft with axillary buds grafting recorded 1.010 and 0.987 cm, respectively with insignificant differences with cleft with apical and axillary buds grafting (0.987 and 1.020 cm, respectively). Our results are in agreement with those of El-Taweel *et al.* (2015), who proved that graft union zone diameter of guava seedlings using saddle grafting significantly surpassed others in the 1nd season.

Table 8. Effect of grafting techniques and dates on average grafting union diameter (cm) of grafted carob selected strain in 2017 and 2018 seasons.

Grafting techniques	Average grafting union diameter							
	2017				2018			
	15thFeb.	15thMarch	15thApril	Mean	15thFeb.	15thMarch	15thApril	Mean
Cleft*	0.960d	1.010cd	0.990cd	0.987B	1.050cd	1.030c-e	0.980de	1.020B
Saddle*	1.140ab	1.170a	0.010e	0.773C	1.170ab	1.150ab	0.010f	0.777C
Cleft**	0.980d	1.040bc	1.010cd	1.010B	0.960e	1.020c-e	0.980de	0.987B
Saddle**	1.150a	1.160a	1.080b	1.130A	1.170ab	1.180a	1.090bc	1.147A
Mean	1.058B	1.095A	0.773C		1.088A	1.095A	0.765B	

Means designated with the same letter within columns and rows in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.
*** With apical and axillary buds. ** With axillary buds.**

Grafting in February and March 15th were more pronounced and recorded higher values with significant difference between them just in the 1st season (1.058 and 1.095 cm in 2017 season as well as 1.088 and 1.095 cm in 2018 season). Grafting in April 15th differed statistically with the two other dates (Table, 8).

Dealing with the interaction, the analysis of variance indicated clear significant respond to the interaction between grafting methods and dates. Saddle with apical and axillary buds or saddle with axillary buds illustrated the maximum values in both February and March 15th compared to other treatments in both seasons. The least value 0.010 cm was recorded by saddle with apical and axillary buds grafting in April 15th in both seasons as a result of failing to succeed and survive (Table, 1 and 2). From the above results, it can be concluded that scoring the higher positions of grafting union diameter by using the both saddle grafting techniques is an indicator of poor grafting union unity.

Unity of the grafting union

Regarding the visual scoring (Fig. 2 and Table 9), cleft with axillary buds grafting's photo shows absent visual line between partners, both medium browning intensity, callusing and healing, while cleft with apical and axillary buds grafting's photo gave a faint visual line between partners, low browning intensity and medium callus proliferation. Errea *et al*. (2001) reported that, the first important stage in graft union of apricot is to make tight contact between rootstock and scion, matching cambium tissues, then the occurrence of callus just after the grafting. Rapid cambial activity played a role in transformation as well as production of secondary tissues, which joined the vascular tissues of both stock and scion. The success of grafted plants depends on the used technique and the ability of healing callus to proliferate rapidly (Hartmann *et al*., 2002). On the other hand, both saddle with apical and axillary buds and saddle with axillary buds grafting showed a very clear visible line between partners, visibly high browning intensity and low callus proliferation amounts at the union zone as well as cell necrosis, which resulted in poor union that may affect the modified success and survival percentages as the two grafting techniques scored lesser percentages. (Table, 1 and 2). Similarly, Abd El-Zaher (2008) reported that the transverse sections of the saddle grafts illustrated some incompatibility between the scion and the rootstock of jackfruit showed in the line of necrotic layer on the margins of the graft union and existence of the collapsed cells at the union region.

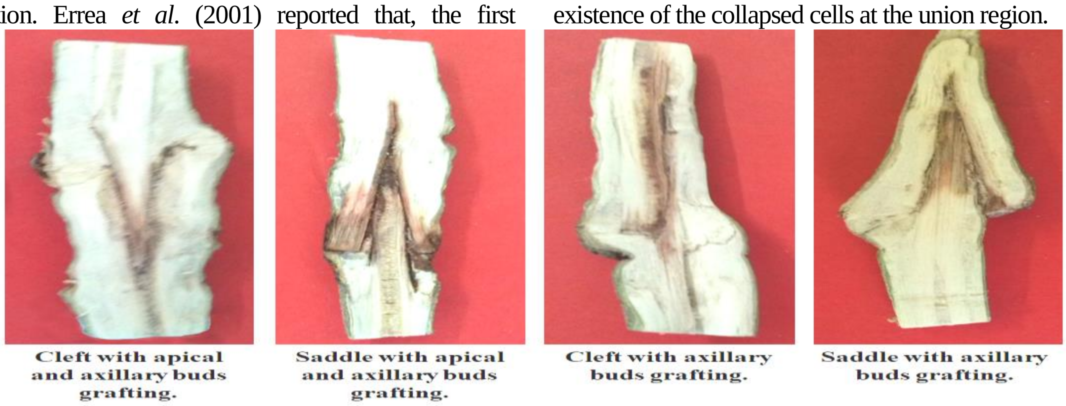

Fig. 2. Grafting union zone of the different grafting techniques of carob selected strain.

Various phenolic compounds of prunus species are known to affect cell division, development and differentiation at the graft union (Gainza *et al*., 2015). Keeping the scions in a shady place for immediate grafting and moisturizing scions before grafting in this study were very beneficial practices regarding union continuity and healing process as was reported by Hartmann *et al*. (2014) that maintaining water film at the union during grafting is necessary for callus formation. This water film could possibly dilute some phenols as they accumulate below the union, especially water soluble phenols. This could aid in phenols breaking up by prolific callus tissues and consequently, grafted partners are able to establish cambial continuity.

Table 9. The effect of grafting techniques on union formation with respect to absence or presence of visible line in the wood, browning intensity and callus proliferation of grafted carob selected strain.

Characteristics	Grafting techniques			
	Cleft with apical and axillary buds	Saddle with apical and axillary buds	Cleft with axillary buds	Saddle with axillary buds
Visual union in the wood	Faint	Visible	Absent	Visible
Browning intensity	Low	Visibly high	Medium	Visibly high
Callus proliferation amount	Medium	Low	Medium	Low

CONCLUSION

According to our results, it could be concluded that

1. Carob can be vegetatively propagated by using either cleft with axillary buds or with apical and axillary buds grafting techniques.
2. Grafting carob can be done in February or March.

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تأثير بعض العوامل المؤثرة على إكثار سلالة خروب منتخبة بالتطعيم

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أجريت هذه الدراسة خلال موسمى 2017 و 2018 فى الحديقة البحثية بمعهد بحوث البساتين، مركز البحوث الزراعية- الجيزة- مصر، وكان الهدف الرئيسي من هذه الدراسة هو الوصول لأفضل طريقة لتطعيم الخروب (تطعيم بالشق مع براعم طرفية وإبطية- تطعيم سرجي مع براعم طرفية وإبطية - تطعيم بالشق مع براعم إبطية - تطعيم سرجي مع براعم إبطية) وكذلك أفضل ميعاد للتطعيم (منتصف كل من فبراير أومارس أوإبريل). وقد أظهرت النتائج أن طريقة التطعيم بالشق مع براعم إبطية كانت الأفضل فيما يتعلق بالنسبة المئوية المعدلة لنجاح التطعيم وعدد الأوراق وسمك كل من الطعم والأصل. سجلت طريقة التطعيم بالشق مع براعم طرفية وإبطية أعلى قيمة لكل من النسبة المئوية المعدلة للبقاء وطول النمو الخضري. كان ميعاد التطعيم في فبراير الأفضل فيما يتعلق بطول النمو الخضري وعدد الأوراق والأفرع الجانبية النامية. تحسن كل من نسبة البقاء المعدلة وسمك منطقة التطعيم في شهر مارس. أعطى استخدام طريقة التطعيم السرجي مع براعم إبطية في فبراير ومارس أعلى عدد من الأفرع الجانبية النامية في حين سجل استخدامها في مارس قيمة مرتفعة لسمك منطقة التطعيم. تحسن كل من طول النمو الخضري ونسبة البقاء المعدلة باستخدام طريقة الشق مع براعم طرفية وإبطية في كلٍ من شهري فبراير ومارس على التوالي. أظهر فحص منطقة التطعيم أن طريقتي التطعيم بالشق مع براعم إبطية ومع براعم طرفية وإبطية كانتا الأفضل. طبقاً للدراسة يمكن التوصية بإكثار الخروب خضرياً باستخدام طريقتي التطعيم بالشق مع براعم إبطية أو التطعيم بالشق مع براعم طرفية وإبطية. كما يوصى بتطعيم الخروب خلال شهري فبراير أو مارس.