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ABSTRACT

## المجلة العلمية لجامعة الملك فيصل The Scientific Journal of King Faisal University

العلوم الأساسية والتطبيقية Basic and Applied Sciences

# Micrografting Compatibility Between Two Almond Cultivars and Four Wild Species

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التوافق بالتطعيم الدقيق بين صنفين من

رياسة علوم البستنة، كلية الزراعة، جامعة دمشق، دمشق، سوريا قصم علوم اللبوزيات، إدارة بحوث البستنة، الهيئة العامة للبحوث العلمية الزراعية، دمشق، سوريا

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Micropropagation and micrografting techniques are important methods used to obtain high quality plants. This research aimed to investigate the effect of wild almond species (Prunus communis, P. orientalis, P. korschinskii, and P. arabica) on the success of micrografting almond cultivars (Prunus dulcis cvs. Shami Furk and Dafadii)and determine which combination of growth regulators lead to the highest rate of multiplication in micrografted cultivars. The shoot tips were grafted onto the rooted rootstocks by inverted T-budding. The results indicated that Murashige and Skoog (MS) medium, supplemented with 1 mg/l benzyl adenine (BA), 0.1 mg/l indole-3-butyric acid (IBA) and 0.2mg/l gibberellic acid (GA3), achieved the highest shoot multiplication with an average of 5.31 and 3.67 shoots per explant and an average of 6.23cm and 4.98cm shoot length in cultivars Shami Furk and Dafadii, respectively. The highest grafting success rates were 80% and 74.26% obtained from Shami Furk/P. arabica and Dafadii/P. arabica combinations, respectively, while the lowest success rate was 50.63% with the Dafadii/P. orientalis combination. The liquid MS medium supplemented with 0.5 mg/l BA + 0.1 mg/l IBA achieved the highest micrografting success and scion shoot length. This research can be used to improve almond cultivation.

تعد تقنيات التكاثر الخضري الدقيق والتطعيم الدقيق من الطرق الهامة للحصول على نباتات عالية الجودة. يهدف هذا البحث إلى دراسة تأثير أنواع اللوز البرية (الشائع، الشرق، الكورشنسكي، العربي) في نسبة نجاح التطعيم الدقيق لصنفين محليين من اللوز (شامي فرك وضفادعي)، وكذلك لتحديد أفضل التوافقات الهرمونية التي تؤدي إلى أعلى معدل للتكاثر وإلى نجاح التطعيم الدقيق للصنفين المدروسين. تم تطعيم القمم النامية معدل للتكاثر والى نجاح التطعيم الدقيق للصنفين المدروسين. تم تطعيم القمم النامية موراشيج وسكوج (MS) المزود بـ 1ملجم/ل بزيل أدنين 1.0 + (AB) ملجم/ل حمض وراشيج وسكوج (MS) المزود بـ 1ملجم/ل بزيل أدنين 1.0 + (AB) ملجم/ل حمض البوتبريك 2.0 + (ABI) ملجم/ل حمض الجبرلين (GA3) حقق أعلى معدل للتكاثر بلغ المونفين على التوالي، وتم الحصول على أعلى نسبة نجاح للتطعيم الدقيق عند تطعيم الصنفين على التوالي، وتم الحصول على أعلى نسبة نجاح للتطعيم الدقيق عند تطعيم على التوالي، في حين تم الحصول على أعلى نسبة لنجاح التطعيم الدقيق عند تطعيم على التوالي، في حين تم الحصول على أعلى نسبة لنجاح التطعيم الدقيق (S6: على التوالي، في حين تم الحصول على أعلى نسبة نجاح للتطيم الدقيق عند تطعيم على التوالي، في حين تم الحصول على أعلى نسبة لنجاح التطعيم الدقيق (S6: ) عند منهم الم هر رادة على اللوز العربي والتي المعت (S6: ) عند مائمي منهم الدون وضاد على أعلى معدل المنمو على التوالي في حين تم الحصول على أدني نسبة لنجاح التطعيم الدقيق ولدى (S6: ) عند مالم ملم الم الو 1.0 ملجم/ل ABI أعلى نسبة لنجاح التطعيم الدقيق وأعلى متوسط لطول ماجم/ل AB و 1.0 ملجم/ل ABI أعلى نسبة لنجاح التطعيم الدقيق وأعلى متوسط لطول المطاعيم. يمكن الاستفادة من هذا البحث في التوسع بزراعة اللوز.

#### KEYWORDS الكلمات الفتاحية Almond, compatibility, growth regulators, microscions, rootstocks, tissue culture أصول وراثية، اللوز، توافق، زراعة أنسجة، طعوم دقيقة، منظمات نمو

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# 1. Introduction

The cultivated almond (*Prunus dulcis* Mill.) belongs to the sub-genus *Amygdalus* from the Rosaceae family and the Prunoideae sub-family and is adapted to dry and semi dry areas (Al-Ghzawi *et al.*, 2009). Almonds have great nutritional value, high fat content, and are added to many nutritional products (Ahrens *et al.*,2005). Martins *et al.* (2004) reported that almond trees are widely spread throughout the Mediterranean region. They are considered one of the most important and ancient nut trees. They also represent the largest nut tree crop produced around the world (Kester and Gradziel, 1996; Sorkheh *et al.*, 2009). In addition to the industrial uses of the almond tree, it is also used as an ornamental tree (Isıkalan *et al.*, 2008).

Syria is rich in genetic diversity among wild species of almond, such as *P. orientalis, P. arabica, P. communis, P. korschinskii* and *P. spartioids* (Ladizinsky, 1999), which are used for stabilizing water sheds and controlling soil corrosion (Mortazavi, 1986).In addition, they are a rich source of important characteristics used by breeding programs to improve cultivars (Gradziel *et al.*, 2001, Rahemi *et al.*,

#### 2010).

Numerous difficulties are encountered in almond cutting during the process of propagation due to the tree's poor rooting ability. The traditional method of almond propagation is through T-budding either in the late spring or in the fall (Hartmann *et al.*, 1997), which is an exhaustive and time-consuming method. Hence, the need for new and rapid methods for propagation is growing, and that is possible only through micropropagation (Jain and Häggman, 2007). The biggest drawback to field grafting is that a whole year of production is lost in order to produce new grafts; therefore, micrografting was used to overcome this problem (Miguelez-Sierra *et al.*, 2016). Micrografting requires much less space and is a much quicker process compared to grafting (Isıkalan *et al.*, 2011).

The micrografting technique is used for several purposes, such as the elimination of viruses, the rejuvenation tissues in plants, the production of plants throughout the year, and the study of compatibility between rootstocks and scions (Richardson *et al.*, 1996). The success of micrografting is affected by several factors, such as scion length, scion source, grafting method, medium used, rootstock age, and the compatibility of reactions between grafting

partners (Hu and Mis, 2015). Liquid media is used to supply more nutrients and growth regulators to the microshoots (Hussain *et al.*, 2014). Micrografting is a safe and alternative way to produce genetically uniform *in vitro* propagated plants. Therefore, the aim of this research was to investigate the effect of the wild rootstock type on the success of micrografting almond cultivars Shami Furk and Dafadii, (which are important, economical woody trees)and to determine the best combination of growth regulators to use in order to achieve the highest rate of multiplication in the micrografted cultivars.

# 2. Materials and Methods

### 2.1. Plant Materials:

This research was carried out in the Laboratory of Biotechnology for Medicinal Plants of the National Commission for Biotechnology/ Damascus, during the period between 2018–2020. The source of plant materials was the *in vitro*-cultured shoots of four wild almond types (*Prunus orientalis* Mill, *P. communis* L,*P. korschinskii*, and *P. arabica* Olivier.) obtained from different origins throughout Syria and nodal segments of two local almond varieties, *Prunus dulcis* cvs. 'Shami Furk' and 'Dafadii' that are grown at the Homs Research Centre of the General Commission for Scientific Agricultural Research. The studied rootstocks and cultivars have a major economical prominence in the breeding process.

#### 2.2. Establishment of In Vitro Rootstocks:

*In vitro*-cultured shoots of *P. communis* and *P. orientalis* in MS medium (Murashige and Skoog, 1962) (Table 1), supplemented with 1 mg/l benzyl adenine (BA) + 0.2 mg/l Gibberellic acid (GA<sub>3</sub>), and micropropagated shoots of *P. arabica* and *P. korschinskii* in MS medium, supplemented with 1mg/l BA + 0.2 mg/l GA<sub>3</sub> + 0.1 mg/l indole-3-butyric acid (IBA) (Figure 1), were used as explants for the establishment of rootstocks.

For root induction, shoots (2–3 cm in length) were excised during the multiplication stage and cultured in ½MS medium supplemented with 1 mg/l IBA. During the rooting stage, the shoots were incubated in constant darkness for one week, then transferred to a growth chamber at  $22\pm1$  °C, with 16h of photoperiod, and a light intensity of 30 µmol m<sup>-2</sup> s<sup>-1</sup> provided by cool daylight fluorescent lamps for three weeks. *In vitro*-rooted shoots were used as rootstocks for grafting (Figure 2).

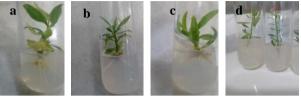
Table 1: Composition of MS Mee	lium (Murashige and Skoog, 1962)
Ingredients	Amounts (mg/ litre)
Macronutrients       NH4NO3       KNO3       CaCL2.H2O       MgS04.7H2O       KH2PO4	1650 1900 0440 0370 0170
Micronutrients	0110
KI KI H3803 MnS04. H20 ZnS04.7H20 Na2Mo04.2H2O CuS04.5H20 CoSo4.6H20 FeS04.7H20 Na2EDTA	0.830 06.20 15.60 08.60 0.250 0.250 0.250 0.025 27.80 37.30
Vitamins	
Thiamine HCl Pyridoxine HCl Nicotinic acid Myo- inositol	0.100 0.500 0.500 100
Others	2 4
Glycine Sucrose Agar pH	2 <b>mg</b> /l 30g/l 7 g/l 5.7

Figure 1. Micropropagation of wild almond species.



a. P. communis, b. P. orientalis, c. P. korschinskii, d. P. Arabica

Figure 2. Rooting of wild almond species in  $\ensuremath{^{/2}\!MS}$  medium supplemented with 1 mg/l IBA.



`a. P. communis, b. P. orientalis, c. P. korschinskii, d. P. Arabica

# 2.3. Establishment of *In Vitro*Shoot Cultures for Scion Source:

New shoots of 15-20cm in length were collected in mid-May from ten-year-old trees of almond cultivars (Shami Furk and Dafadii) and brought to the laboratory. After removing the leaves, shoots were cut into nodal cuttings, which contained 1-2 lateral buds and with lengths that ranged from 0.5 to 1 cm. For the purpose of disinfection, the explants were washed with running water for one hour. Next, they were surface sterilized by dipping in 70% ( $\nu/\nu$ ) ethanol for 30 seconds, then in 0.5% sodium hypochlorite (NaOCl)containing 2 drops of Tween for ten minutes, and in 0.1% mercuric chloride (HgCl<sub>2</sub>) for 30 seconds. Finally, they were rinsed three times (forfive minutes each time) in distilled water. Surface sterilization was performed in sterilized conditions. After sterilization, each explant was cultured in an MS medium. All the cultured tubes were placed and observed for four weeks in a growth chamber at 22±1 °C , with 16 h of photoperiod, and at a light intensity of 30  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> provided by cool daylight fluorescent lamps (Figure 3).



Figure 3. New shoot of Dafadii cultivar at the end of initiation stage.

After that, the newly-formed microshoots were sub-cultured in MS medium including various types of growth regulators in order to achieve shoot multiplication:

- MS1: MS + 0.5 mg/l BA + 0.2 mg/l GA<sub>3</sub>.
- MS2: MS + 1 mg/l BA + 0.2 mg/l GA<sub>3</sub>.
- MS3: MS + 2 mg/l BA + 0.2 mg/l GA<sub>3</sub>.
- MS4: MS + 1 mg/l BA + 0.2 mg/lGA<sub>3</sub> + 0.1 mg/l IBA.

*In vitro* regenerated shoots were micropropagated and sub-cultured every three weeks. All of the cultivated tubes were placed in a growth chamber at  $22\pm1$  °C, with 16 h of photoperiod, and a light intensity of

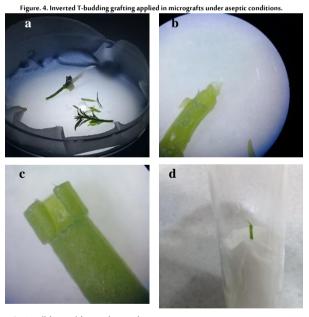
30  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> provided by cool daylight fluorescent lamps. Shoot tips were used as a source of microscions for micrografting experiments.

# 2.4. Micrografting of Scion Cultivars onto Rooted Rootstock:

Inverted T-budding grafting was applied in micrografts under aseptic conditions. Micrografting was achieved by grafting the microscions onto the rooted rootstocks. For the rootstocks, the leaves and their axillary buds were removed from 2-3 cm long stems and all lateral shoots were removed under the stereomicroscope (Figure 4.a). The rootstock shoot was decapitated approx. 1.5-2 cm above the medium surface, and roots were cut to a length of 2-3 cm. After that, the inverted-T slit was done through making a columnar slit of 1 mm length at the decapitation position, followed by a level slit of 1-2 mm width. Shoot-tip explants (meristem plus 2-3 leaf primordia) were excised from the scion donor plants under stereomicroscope (Figure 4.b) and placed directly in contact with the level slit surface of the rootstock (Figure 4.c). Micrografts were cultured in liquid media, with a paper bridge as a fastening device (Figure 4.d), with three different treatments:

- MS0: hormone-free MS.
- MS5: MS + 0.5 mg/l BA + 0.1 mg/l IBA.
- MS6: MS + 0.5 mg/l IBA + 0.1 mg/l BA.

The micrografts were then placed in a growth chamber at  $22\pm1$  °C, with16 h of photoperiod, and a light intensity of 30  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> provided by cool daylight fluorescent lamps for six weeks.Then, successful graft union percentage and shoot growth length were determined.



a. Cutting off the top of the rooted rootstock. b. Preparation of the shoot tip. c. Insertion of the microscion in therootstock.

d. Micrografting shoot in liquid MS media.

#### 2.5. Experimental Design and Statistical Analysis

All experiments were carried out according to completely randomized design. The multiplication experiment contained two almond cultivars, four proliferation treatments, three replicates, and 20 explants per replicate, while the micrografting experiment included two almond cultivars, four wild rootstocks, three micrografting media, three replicates, and 20 micrografts per replicate. The results were analysed using the analysis of variance (ANOVA) method to determine the significant differences between the means of all treatments. Duncan's multiple range test was used at 1% level of significance to assess the significance of difference among means using the Genstat 12 statistical program. Means followed by the same letter are not significantly different.

# 3. Results

#### 3.1. The Effect of Different Plant Growth Regulator Combinations on the Shoot Multiplication of Almond Microscions:

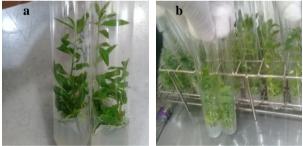
The data in Table 2 show the effect of different concentrations of BA in combination with  $GA_3$  at 0.1 mg/l alone or with  $GA_3$  at 0.1 mg/l and IBA at 0.1 mg/l on the average number of shoots produced per explant as well as the mean length of the shoots.

According to the data, the MS medium culture supplemented with 1 mg/l BA plus 0.2 GA<sub>3</sub> plus 0.1 IBA, resulting in a mean number of 4.49shootswith an average shoot length of 5.60 cm, is the most suitable treatment. As for the interactions between studied cultivars and growth regulator combinations, the results clarified that the highest significant average number of shoots/explant (5.31 and 3.67) and the longest shoot (6.23 and 4.98 cm) for both Shami Furk and Dafadii cultivars, respectively, were obtained in a medium supplemented with BA at 1 mg/l plus GA<sub>3</sub> at 0.2 mg/l plus IBA at 0.1 mg/l (Figure 5).

Table 2. Micropropagation of Shami Furk and Dafadiimicroscions in MS medium supplemented

with different plant growth regulators combinations									
Media	Avg No of shoo	ts/ explants	mean	Avg length of	mean				
Meula	Shami Furk	Dafadii	mean	Shami Furk	Dafadii				
MS1	1.34 f	1.12 g	1.23 d	2.53 d	2.40 g	2.46 c			
MS2	3.22 c	3.15 d	3.18 b	2.62 c	2.43 f	2.52 b			
MS3	2.34 e	1.07 h	1.7 c	2.46 e	2.02 h	2.24 d			
MS4	5.31 a	3.67 b	4.49 a	6.23 a	4.98b	5.60 a			
I.S.D	Cultivars			0.01					
(0.01)	Media	0.17							
(0.01)	Interaction	0.24							

Figure 5. Shoot culture at the end of multiplication stage in MS medium supplemented with BA at 1 mg/l plus GA3 at 0.2 mg/l plus IBA at 0.1 mg/l:a. Dafadii cv., b. Shami Furk cv.



# 3.2. The Effect of Different Types of Rootstocks and Scions on the Success of Micrografting:

In this study, the success rates of grafting were investigated for different cultivar/rootstock combinations. It was found that the P. arabica was a more compatible rootstock for the tested almond cultivars compared to the rest of the rootstocks tested in micrografting. Two different scion types (Shami Furk and Dafadii) showed clear differences in micrografting success, as Shami Furkperformed a higher success rate (70.28%) than Dafadii (62.65%) (Table 4). The interaction between the rootstock and scion was significant (Table 3). The highest grafting success rate was obtained from Shami Furk cultivar grafted on P. Arabica (80%), which was followed by the combination of Dafadii/P. arabica with a rate of 74.26% (Figure 6). The lowest success rate was with the Dafadii/P. orientalis combination (50.63%). In addition, the micrografts growth differed significantly relying on the rootstock type. The shoot length ranged between1.14 cm for Shami Furk/P.korschinskii and 0.50 cm for Dafadii/P. orientalis (Table 4).

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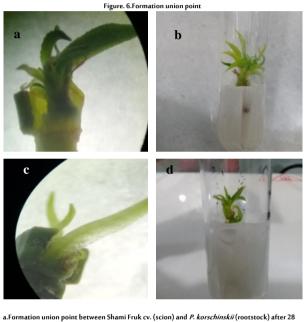
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Table 3. Analysis of variance (ANOVA) for the effects of the rootstock type and cultivar on the success of in vitro micrografting

Source of variation	Degrees of Freedom (D.F)	Sum of Squares (S.S)	Mean Square (M.S)	F-Value	P-Value
Rootstocks	3	1646.9535	548.9845	4388.80	<.001
Cultivars	1	349.3014	349.3014	2792.46	<.001
Interaction (Rootstocks. Cultivars)	3	12.3933	4.1311	33.03	<.001
Residual	16	2.0014	0.1251		1
Total	23	2010.6496			
Table 4. The effects	of the rootstock	type and cultivar	on the success of	<i>in vitro</i> micros	rafting and

the choot length

			e snoot le	0				
		Success	of microg		Shoot length (cm)			
	Rootstocks	Shami Furk	Dafadii	Mean (Rootstocks)	Shami Furk	Dafadii	Mean (Rootstocks)	
	P. communis	70.57 d	61.42 f	66 c	0.85 c	0.72 d	0.78 b	
	P. orientalis	57.37 g	50.63 h	54 d	0.54 f	0.50 g	0.52 d	
	P. korschinskii	73.18 c	64.29 e	68.73 b	1.14 a	1.04 b	1.09 a	
	P. arabica	80 a	.7426b	77.13 a	0.70 d	0.57 e	0.63 c	
	Mean (Cultivars)	70.28 a	62.65b		1.80 a	1.70 b		
	Cultivars	0.42			0.011			
L.S.D	Rootstocks			0.016				
0.01	Interaction (Cultivars*Rootstocks)	0.84			0.023			



days of *in vitro* grafting. b. Successful Shami Furk cv. micrografted on *P. korschinskii* rootstock after 45 days of *in vitro* 

grafting in MS liquid media + 0.5 mg/l BAP + 0.1 IBA.

c. Dafadii cv. micrografted on *P. arabica* rootstock after 21 days of *in vitro* grafting. d. Successful Dafadii cv. micrografted on *P. korschinskii*rootstock after 45 days of *in vitro* grafting.

# 3.3. The Effects of BA and IBA on the Success of Micrografting:

The data illustrated in Tables 5 and 6show a significant effect of the media on the success of micrografting and the shoot mean length (cm) of two tested scions types grafted onto rootstocks in this experiment. The maximum micrografting success rate (73.85%), and the longest length of micrografts (0.90 cm) were obtained when all of the grafted rootstocks were cultured in an MS medium supplemented with 0.5 mg/l BA + 0.1 mg/l IBA, while the lowest rate was recorded inthe controlled medium (58.46%). As for the interaction between the two scions and rootstocks in this experiment, both of the tested cultivars (Shami Furk and Dafadii) showed the highest micrografting success rates (86.83 and 79.50%, respectively) when grafted on P. arabica and planted in an MS medium containing BA at 0.5 mg/l plus 0.1 mg/l at IBA. Meanwhile, the tested cultivars achieved the longest shoot lengths (1.50 and 1.33 cm, respectively) when grafted onto P. korschinskii and planted in an MS medium containing BA at 0.5 mg/l plus IBA at 0.1 mg/l, with significant differences from the rest of the treatments.

Table 5. Effects of culture media on the success of micrografts (%) after six weeks of micrografting Success of micrografts (%)

		Sham	i Furk		Dafadii				Mean (Media)
Media	P. communis	P. orientalis	P. korschinskii	P. arabica	P. communis	P. orientalis	P. korschinskii	P. arabica	
MS0	55.50 s	48.50 v	65.30 n	74.50 h	51.74 t	46.85 w	56.83 q	68.44 k	58.46 c
MS5	78.77 c	67.301	78.71 d	86.83 a	68.82 j	60.55 p	70.33 i	79.50 b	73.85 a
MS6	77.44 e	56.33 r	75.50 f	78.70 d	64.71 o	50.50 s	65.71 m	74.86 g	67.97b
	Rootstocks		0.008						
L.S.D	Cultivars		0.006						
0.01	Media		0.007						
	Interaction				0.02	21			

Table 6. Effects of culture media on shoot mean length (cm) after six weeks of micrografting

	Shoot mean length (cm)									
	Shami Furk				Dafadii				Mean (Media)	
Media	P. communis	P. orientalis	P. korschinskii	P. arabica	P. communis	P. orientalis	P. korschinskii	P. arabica		
MS0	0.75 g	0.48 lm	0.89 e	0.64 hi	0.60 ij	0.45 m	0.85 e	0.501	0.64 c	
MS5	1.03 c	0.60 ij	1.50 a	0.80 f	0.80 f	0.55 k	1.33 b	0.65 h	0.90 a	
MS6	0.78 fg	0.55 k	0.55 k 1.03 c 0.65 h 0.76 tg 0.501 0.95 d 0.56 jk 0							
	Rootstocks				0.01					
L.S.D	Cultivars		0.012							
0.01	Media		0.015							
	Interaction				0.04	13				

## 4. Discussion

Micropropagation is a convenient and rapid procedure to obtain a large number of genetically identical plants (Antonopoulou *et al.*, 2005). Prior research indicates that almond is difficult to micropropagate effectively from mature explants (Akbas *et al.*, 2009). However, each plant species propagated *in vitro* needs different requirements and concentrations of plant growth regulators. Most of them are based on BAP and auxins IBA, IAA and NAA (Channuntapipat, 2002). Brison *et al.* (1995) found that the simultaneous presence of cytokinin, giberellin and auxin in the media was more effective for *Prunus* rootstocks *in vitro*.

In this research, MS medium containing 1.0 mg/l BA, 0.1 mg/l IBA, and 0.2 GA3 was chosen as the optimum medium for multiplication and development of Shami Furk and Dafadii shoots. When BA and IBA were added together to the medium, the shoots number multiplied and their growth increased. Gürel and Gülşen (1998) obtained the best rate of shoot multiplication for almond by using the combination of 0.1 mg/l IBA and 1.0 mg/l BA. However, on the contrary, Isikalan et al. (2008) obtained the highest rate of shoot multiplication for almond cultivar 'Nonpareil' in an MS medium supplemented with 1.0 mg/l BA. It was indicated that the cytokinin, such as BA, encourages cell division by activating DNA synthesis, inducing growth of lateral buds, and promoting shoot formation (Dobranszki and Silva, 2010). The selection of BA as a cytokinin was due to its effect in vitro with several woody plants (Bennett and Davies, 1986). In this regard, it is important to observe that the multiplication medium should be supplied with more cytokinin in relation to auxin (Murashige, 1974). The effect of cytokinins on tissue or organ cultures differs based on the culture type, the cultivar used, and explant age (George *et al.*, 2007). The auxins control cytokinin levels through repressing its synthesis ratio and its gathering size (Nordstrom et al., 2004). Kodad et al. (2020) showed that the regulators' best concentration and type depends on the genotype to get a successful multiplication rate of various almond explants. The results obtained from Isikalan et al. (2011) proved that the medium supplied with only BAP were more beneficial than themedium containing IBA and BAP for shoot development of almond cultivars (Nonpareil).

In this research, micrografting both Shami Furk and Dafadii microscions on *P. arabica* rootstock showed higher success than the other studied rootstocks. This may be due to either the different genotypes and the variation of genes responsible for the numbers of formed cells or their size in plants in terms of growth and development (Guo *et al.*, 2009). Meanwhile the micrografting of both Shami Furk and Dafadii microscions on *P. korschinskii* rootstock showed longer shoot length of micrografts than the other studied rootstocks and indicates the strength of this rootstock, which was reflected in the length of the microscions. This variation in the success

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of micrografting and the shoot length of micrografts may be due to the different genotypes of the wild species used (Al-Ghzawi *et al.,* 2009).

Forming the graft union depends mainly on putting the microscion correctly onto the rootstock in order to ensure perfect contact (Wu *et al.*, 2007). Izadi *et al.* (2014) showed that the compatibility between rootstock and scion has a significant role for the complete union of the two. Channuntapipat *et al.* (2003) achieved a micrografting success rate of 50% and 65%, respectively, when they micrografted 1.5 cm long almond scions 'Nonpareil 15- 1' and 'Ne-Plus Ultra' onto rootstock stems and cultured in a rooting medium. These results also agreed with Wu *et al.* (2007), who obtained the highest micrografting success rate by using untreated microscions. Moreover, applying an equiponderant rate of auxins and cytokinins *in vitro* will have a critical impact on the proportion of micrografting success.

### 5. Conclusions

This study indicated the possibility of investing in wild almond species *P. Arabica* and *P. korschinskii*as rootstocks for grafting almond varieties Shami Furk and Dafadii. This micrografting technique can be used to produce almond cultivars Shami Furk and Dafadii widely and spread their cultivation. The protocol described in this research is easy and inexpensive as it does not demand any preprocessing of microscions before grafting, and it is important for the effective rejuvenation of adult plants upon implementation of genetic diversity to the almond.

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