



## EFFECT OF SOME FACTORS ON THE MICROPROPAGATION AND MICROGRAFTING OF SOME GRAPE ROOTSTOCKS *IN VITRO*

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### ABSTRACT

This study aimed to investigate the attitude of three nematode and phylloxera resistant grape rootstocks (Freedom, Ramsey and SO4) through the micropropagation stages till be micrografted by Superior scions. During that the effect of paclobutrazol (PP333) on rootstocks stem diameter, transverse sections in graft union regions and acclimatization of resulted micrografts were studied. The results proved that the most effective sterilization durations using 1% NaOCl were 15 min for Freedom and SO4 explants as they achieved 0.00 and 0.00% of contamination and 100% and 90% of survival percentages respectively, while 10 min duration was sufficient for Ramsey explants as it achieved 0.00 % contamination and 100% survival percentages. Significantly, Ramsey achieved the highest number of shoots (1.25), Freedom gave the tallest shoots (4.13 cm) and SO4 got the highest leaf number (7.33) all on MS medium. In multiplication stage; Freedom rootstock proved to be the most reacted rootstock as it significantly accomplished the highest shoot number (2.00) when planted on BA medium, the tallest shoots (3.54 cm) when planted on TDZ medium and the highest leaf number on Kin medium. Whereas, in order to get thicker shoots for easier grafting; using of PP333 at 5 mg l<sup>-1</sup> significantly was the best for Freedom (3.55 mm) followed by SO4 (3.03 mm) and Ramsey (2.85 mm) rootstocks. In micrografting stage, Superior micrografts significantly achieved the best results on rooted Freedom rootstocks in scion survival (100.00%) and on unrooted rootstocks in scion bud burst (75.00 %), graft union formation (50.00%), rooting (75.00%). On the same trend, Freedom rootstock was more active in cell dividing activity when grafted by Superior cultivar compared to Ramsey and SO4 rootstocks

which were very poor in producing callus cells in graft union. Finally, Freedom rootstock grafted with Superior gained the highest survival (100%) after acclimatization.

### INTRODUCTION

Grapevines (*Vitis sp.*) are grown worldwide for many purposes including fresh fruit, juice, jams, jellies, wine, raisins and other processed products. The cultivation of grapevines dates back several thousand years and remains economically and socially important today. Food and Agriculture Organization (FAO) reported that the production of grape in Egypt reached 1.596169 tons in 2014. The vegetative propagation using hard wood stem cuttings is the common way of grape propagation as many of vineyards are own-rooted plants; so these are highly sensitive to phylloxera and nematodes, leading to heavy yield and quality losses. During this period, the idea has emerged 'to graft fruit-bearing scions onto resistant rootstocks to get infection free vines. Grapevines have been grafted not only under greenhouse but also under *in vitro* conditions (Bouquet & Hevin, 1978 and Walker & Meredith, 1990). However, this also finds application in the rapid propagation of scarce or hard-to-propagate varieties (Lee & Wetzstein, 1990 and Lewandowski, 1991). This tissue culture based grafting system referred to as 'micrografting', was introduced by Tanne et al (1993). The main advantages of this method over other methods have been described as virus elimination as well as improved growth and productivity of resulting grafted plants. In addition, this procedure was able to overcome some physiological and anatomical problems encountered in some species and cultivar combinations. Shoot-tip origin and preparation are main factors in successful micrografting operation. These factors are highly dependent on species and cultivars involved in grafting as well as propagator ability.

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The aim of this study was to investigate the behavior of three important grape rootstocks during the micropropagation stages till they become grafted *in vitro*. Moreover, Study the factors affecting the success of this technique and determining the compatibility degree among the studied rootstocks and important grape cultivar through anatomical study in the graft union. Furthermore, the ability of the resulted micrografts to be acclimatized.

#### MATERIALS AND METHODS

This study was conducted in the Plant Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Ain Shams University during the period of 2014-2016. One year old grape transplants used as mother plants of three nematode and phylloxera resistant grape rootstocks named **Freedom**, **Ramsey** and SO4 and one cultivar named Superior as explants source. Three media have been used in the micropropagation, MS (**Murashige and Skoog, 1962**), WPM (**Lloyd and McCown, 1981**) and C2D (**Chee and Pool, 1982**) and MS medium only in micrografting experiment. Carbon and energy source was sucrose at 3% in all media and propagation stages. The hormonal supplements differed according to experiments. Purified Agar (Agar-Agar) at 7 g l<sup>-1</sup> was used as a solidifying agent. Glass jars (400 ml) contained nearly 40 ml medium per jar of all media have been used during micropropagation experiments. In addition, glass culture tubes (150 x 25 mm) received 20ml medium per tube in the micrografting experiment. The glass jars were washed with detergent then soaked in 5% solution of sodium hypochlorite (NaOCl) for 1 hour then rinsed with water before using them. The cultures were incubated at 25±2°C with 16 hours light using cool white fluorescent lamps (2 lamps per shelf) and 8 hours dark of 2000-2500 lux light intensity at cultures level.

#### Grape micropropagation

##### Establishment stage

##### Effect of surface sterilization durations and rootstock type on contamination and survival percentages of grape explants

Stem node explants 1-2 cm in length were excised from 2-3 weeks old shoots of the mother plants of Ramsey, Freedom and SO4 rootstocks, and trimmed to a bud size and washed in running tap water for 60 minutes. The explants were

soaked in 1 % sodium hypochlorite (NaOCl) solution for 10, 15 and 20 minutes with adding two drops of Tween 20, shaken frequently and rinsed 3 times 5 minutes for each with sterile distilled water. After that they were planted on full strength MS medium. Contamination and survival percentage were recorded after 4 weeks. The experiment was arranged in a factorial experiment (3 duration x 3 rootstocks= 9 treatments)

##### Effect of medium type and rootstocks on establishment criteria

Stem node explants of grape rootstocks have been prepared and inoculated on MS, WPM and C2D media at full strength. After 4 weeks of culture, the proliferation parameters were recorded. The experiment was arranged in a factorial experiment (3 media type x 3 rootstocks= 9 treatments).

##### Multiplication stage

##### Effect of cytokinin type and rootstocks on some proliferation characteristics during the first subculture

The objective of this experiment was to determine the suitable cytokinin type for the multiplication of Freedom, Ramsey and SO4 rootstocks. Well established cultures of the three rootstocks were subcultured on full strength MS medium supplemented with BA (Benzyl adenine), 2ip (Isopentyladenine), kin(6-furfurylaminopurine) and TDZ (Thidiazuron) at 1 mg l<sup>-1</sup> each individually in four treatments. The multiplication measurements were recorded 4 weeks after subculture date. The experiment was arranged in a factorial experiment contained 4 cytokinin types x 3 rootstocks= 12 treatments each had 4 replicates and 3 explants for each).

##### Effect of Paclobutrazol (PP333) concentrations and rootstocks on some proliferation characteristics and shoot diameter during the first subculture

An experiment was carried out to investigate the impact of PP333 (Paclobutrazol) as a plant growth retardant on the growth and shoot diameter of Ramsey, Freedom and SO4 rootstocks for selecting the best concentration which achieves high shoot diameter to be suitable for micrografting procedure. Well established cultures of them were

subcultured on full strength MS medium supplemented with PP333 at 3 concentrations (1, 5 and 10 mg l<sup>-1</sup>). The proliferation measurements and shoot diameter have been recorded 40 days after the subculture date.

### **Grape micrograftiong**

Four to six weeks old *in vitro* produced shoots of Superior cultivar with desired thickness, and internodes length were used as scions for micrografting. Shoots were cut to 5-10 mm in length each had one bearof axillary bud. Eight to ten week old *in vitro* produced shoots of Freedom, Ramsey and SO4 rootstocks were used for micrografting. Shoots with good internodes diameter and long were selected then shortened to 2-3 cm in length.

### **Micrografting procedure**

Cleft-grafting was applied as a micrografting method. Rootstock shoot was cut off and slit (0.5 cm deep) and scion shoot was cut trying to allow making a wedge and inserted it in the rootstock made to maximum connection of the two pieces. In all cases, the rootstocks and scions were 3 and 1 cm long, respectively. Grafted assemblies were cultured on full strength – hormone free MS medium solidified with 7gl<sup>-1</sup> agar and containing 30gl<sup>-1</sup> sucrose. After four weeks of grafting date, the following parameters were recorded:scion survival percentage (scions stayed green regardless of shoot formation), scion bud burst percentage (the survived scions formed new shoot), number of shoots per scion (the number of formed shoots on each scion), average shoot length, average leaf number per scion, graft union formation percentage (recognized by callus forming in graft union region in coincidence with scion bud burst), micrografts rooting percentage (adventitious roots forming on the rootstock stem), number of roots per micrograft and average root length per micrograft.

### **Effect of rooted and un-rooted rootstock shoots on some grafting characteristics of Superior micrografts.**

This experiment aimed to study the response of the rooted and un-rooted rootstock shoots (Freedom, Ramsey and SO4) when be grafted with Superior scions then cultured in IBA free full strength MS medium supplemented with 7gl<sup>-1</sup> agar and con-

taining 30gl<sup>-1</sup> sucrose and the effect on micrografting success. All grafting parameters were recorded 30 days after grafting.

### **Anatomical study**

Transverse sections were made across the successful graft union of well rooted and growing micrografts eight weeks after culture date in order to study the compatibility and incompatibility limits among Superior cultivar and the rootstocks. Samples were cut and fixed in formalin-acetic acid – ethanol 70% (FAA) for 48 hrs then dehydrated in an ascending way ethanol concentrations. Paraffin wax was infiltrated and samples were embedded (**Johanson, 1940**). Cross- sections were microtomed (12-16 µm) mounted to glass slides and stained with safranin-fast green schedule (**Sander, 1993**). Sections were examined and photographed with a light microscope (**Leica DM 2500**).

### **Acclimatization of micrografted plantlets**

Five to six weeks old grape micrografted plantlets were removed from the culture tubes then the roots washed with running tap water. Culture media were autoclaved for 30 min. plastic pots were washed and chemically sterilized. After planting; the pots were watered with a fungicide solution (Prochloraz 45% EC) using the recommended concentration, then kept inside a glass chamber covered with transparent plastic in the incubation room for 4 weeks and watered constantly before being transferred to the glass house.

### **Effect of rootstock type on the acclimatization success of Superior micrograftedplantlets**

The objective of this experiment was to determine the effect of rootstock type on the survival percentage. Micrografted plantlets of Superior cultivar combined with Freedom and Ramsey rootstocks were planted in a soil mixture of peatmoss: perlite: sand 1:1:1(v/v).

### **Data analysis**

A completely randomized design (CRD) was used and data were analyzed using SASS 9.1 statistical software and means were compared by **Duncan's (1955)** multiple range test at p≤0.05 level of confidence.

## RESULTS AND DISCUSSION

### Grape micropropagation

#### Establishment stage

##### Effect of surface sterilization duration and rootstock type on contamination and survival explants percentages

Data in **Table (1)** showed that there were significant differences among means of durations and rootstocks after using 1% sodium hypochlorite in the contamination and survival percentages. Concerning the interaction among the three studied durations and the rootstocks in contamination percentage, there were significant differences among them and it was noticed that Freedom and SO4 explants gave (0.00) when sterilized with 1% NaOCl for 15 min, while Ramsey explants achieved (0.00) when sterilized for 10 min. On the other hand; the maximum contamination percent-

age was found in 20 min for Freedom explants, 15 and 20 min for Ramsey and in 10 min for SO4. On the other side, the results showed that there were also significant differences among means of the sterilization duration and the rootstock in survival percentage. The interaction among the sterilization durations and rootstocks was significant in survival percentage. The results have shown that the maximum survival percentage was found in Freedom when sterilized for 15 and 20 min while in Ramsey and SO4 when they sterilized for 10 min without a significant difference among them. In this concern; Sodium hypochlorite solution NaOCl exerts its antibacterial effect by inducing the irreversible oxidation of sulfhydryl groups of essential enzymes, and it may also have deleterious effects on DNA and membrane-associated enzyme activity (**McDonnell and Russell 1999**). In agreement, several experiments reported good results with the utilization of NaOCl solutions (**Akbas et al 2004; Jaskani et al 2008; Abido et al 2013 and Samaan, 2013**).

**Table 1.** Effect of surface sterilization duration (min) using NaOCl (1%) on contamination and survival percentages of grape rootstock explants

Duration	Contamination %				Survival %			
	F	R	S	Mean	F	R	S	Mean
10 min	10.00c	0.00d	20.00b	10.00B	77.77c	100.00a	100.00a	92.59A
15 min	0.00d	10.00c	0.00d	3.33C	100.00a	66.66d	90.00b	85.55C
20 min	40.00a	10.00c	10.00c	20.00A	100.00a	77.77c	88.88b	88.88B
Mean	16.66A	6.66C	10.37B		92.59A	81.48B	92.96A	

Means followed by the same letter (s) in each column, each row or interaction are not significantly different at 5% level.

##### Effect of medium type and rootstocks on the criteria of establishment.

Data in **Table (2)** exhibited that there were no significant differences among the means of the studied culture media and the rootstocks in terms of average proliferated shoot number per explants. While Ramsey stem nodes achieved the highest shoot number on MS medium (1.25). The results showed that significant differences were found among the means of the studied media in shoot length as MS medium achieved the highest shoot length (3.33 cm). On the other hand, there were significant differences among the rootstocks as Freedom stem nodes gave the maximum shoot length (3.29 cm), while Ramsey stem nodes gave the shortest shoot. Concerning the interaction,

Freedom stem nodes achieved the longest shoots cultured on MS (4.13 cm), while SO4 stem nodes gave the highest shoot length on MS and C2D media (3.10, 3.00 cm), respectively. The results also showed that there were significant differences in the means of the rootstocks and the media in average leaf number. MS medium achieved the highest leaf number (6.30) with significant difference compared to C2D and WPM. On the other side, SO4 stem nodes gave the highest leaf number with significant difference (5.42) followed by Freedom (5.03). Interaction between the media and the rootstock showed significant differences where Freedom and SO4 stem nodes gave the maximum leaf number on MS (6.78, 7.33), respectively. Our findings went in parallel with those of (**Samaan, 2013**).

**Table 2.** Effect of medium type and rootstocks on the establishment criteria of some grape rootstocks

Medium Type	Rootstocks											
	F	R	S	Mean	F	R	S	Mean	F	R	S	Mean
	Shoot number/explant				Shoot length (cm)				Leaf number/shoot			
MS	1.00b	1.25a	1.00b	<b>1.08A</b>	4.13a	2.75bc	3.10b	<b>3.33A</b>	6.78a	4.78bc	7.33a	<b>6.30A</b>
C2D	1.00b	1.00b	1.00b	<b>1.00A</b>	2.17cd	1.54d	3.00b	<b>2.27B</b>	4.10bc	4.42bc	5.11b	<b>4.54B</b>
WPM	1.00b	1.10ab	1.00b	<b>1.03A</b>	3.56ab	1.92cd	2.43cd	<b>2.63AB</b>	4.22bc	4.61bc	3.83c	<b>4.22B</b>
<b>Mean</b>	<b>1.00A</b>	<b>1.12A</b>	<b>1.00A</b>		<b>3.29A</b>	<b>2.07C</b>	<b>2.84B</b>		<b>5.03AB</b>	<b>4.60B</b>	<b>5.42A</b>	

Means followed by the same letter (s) in each column, row or interaction are not significantly different at 5% level

### Multiplication stage

#### Effect of cytokinin type and rootstocks on some proliferation characteristics during the first subculture

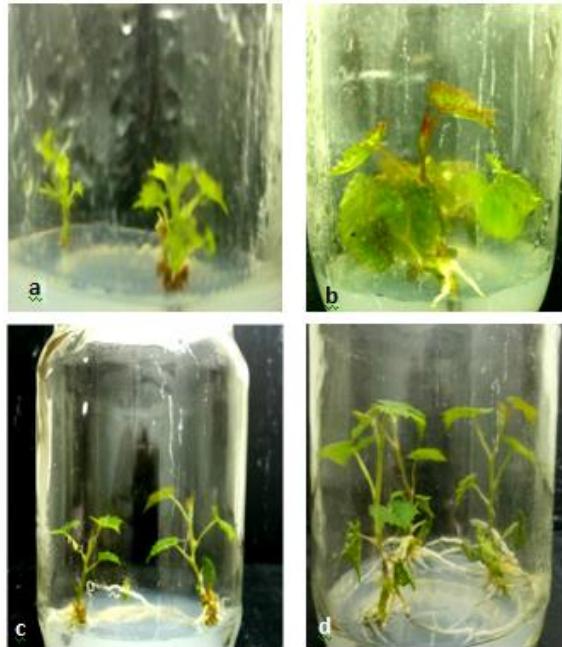
Data in **Table (3)** indicated that there were significant differences among the means of cytokinin types as BA medium achieved the highest average shoot number (1.89) compared to other cytokinins. On the other hand, there were insignificant differences between the means of the all rootstocks in the average shoot number. Regarding the interactions, all rootstocks explants achieved significantly the maximum shoot number when subcultured on BA medium, while no significant differences were observed among the rootstocks and other cytokinin types. Concerning mean average proliferated shoot length (cm), it was indicated that, the maximum shoot length was scored by TDZ followed by Kin without any significant differences between them (2.51, 2.34 cm) respectively while, BA gave the minimum shoot length (1.31 cm). Regarding the rootstocks effect, Freedom achieved significantly the longest shoot (2.41 cm). The interaction showed that, Freedom on TDZ and Kin gave the longest proliferated shoots (3.54, 3.06 cm) respec-

tively without significant difference between them, while the shortest shoots were observed by on BA for all studied rootstocks. Regarding mean average leaf number, it is obvious that there were insignificant differences among means of cytokinin types, but it is worth to say that TDZ and Kin gave the highest average leaf number compared to other cytokinins (4.64, 4.61), respectively. Freedom stem pieces achieved significantly the highest leaf number (4.76) in comparison to Ramsey and SO4 rootstocks. Concerning the interactions, explants of Freedom and SO4 on Kin achieved the highest leaf number (5.33, 5.25) respectively, while Ramsey on TDZ scored the maximum leaf number (4.49) without significant differences among other cytokinin types (**Fig 1**). It is remarkable that Kin and TDZ gave the longest shoots and the highest leaf number in the studied rootstocks compared to BA and 2ip. However, Kin was more effective at high concentrations with that above 5  $\text{mg l}^{-1}$  achieved shoots good appearance and color and preferable; similar findings were reported by (**Poudel et al 2005**). Whereas; TDZ was found more effective at low concentrations (0.5-1.0  $\text{mg l}^{-1}$ ) as effectiveness of TDZ at lower level perhaps, was due to induced accumulation of endogenous cytokinins, as reported earlier by **Murthy et al (1995)**.

**Table 3.** Effect of cytokinin types at concentration of 1  $\text{mg l}^{-1}$  and rootstocks on some proliferation characteristics

Cytokinin	Rootstocks											
	F	R	S	Mean	F	R	S	Mean	F	R	S	Mean
	shoot number/explant				shoot length (cm)				leaf number/shoot			
BA	2.00a	1.75a	1.92a	<b>1.89A</b>	1.17e	1.16e	1.14e	<b>1.31B</b>	4.36abc	4.11abc	3.78bc	<b>4.08A</b>
2ip	1.25b	1.00b	1.00b	<b>1.08B</b>	1.85cde	1.42de	1.46de	<b>1.58B</b>	4.33abc	3.83bc	3.75bc	<b>3.97A</b>
Kin	1.00b	1.00b	1.00b	<b>1.00B</b>	3.06ab	1.58cde	2.38bc	<b>2.34A</b>	5.33a	3.25c	5.25a	<b>4.61A</b>
TDZ	1.00b	1.00b	1.00b	<b>1.00B</b>	3.54a	1.79cde	2.2cd	<b>2.51A</b>	5.03ab	4.49abc	4.41abc	<b>4.64A</b>
<b>Mean</b>	<b>1.31A</b>	<b>1.19A</b>	<b>1.23A</b>		<b>2.41A</b>	<b>1.49B</b>	<b>1.79B</b>		<b>4.76A</b>	<b>3.92B</b>	<b>4.3AB</b>	
<b>Cytokinin</b>												

Means followed by the same letter (s) in each column, row or interaction are not significantly different at 5% level.



**Fig. 1.** Effect of cytokinin type on the multiplication of Freedom rootstock.  
a. Freedom on BA. b. Freedom on 2ip. c. Freedom on Kin. d. Freedom on TDZ

#### Effect of Paclobutrazol (PP333) concentrations and rootstocks on some proliferation characteristics and stem diameter during the first subculture

Data in **Table (4)** exhibits the effect of paclobutrazol (PP333) concentrations and rootstocks on some proliferated and stem diameter characteristics of Freedom, Ramsey and SO4 during the first subculture. It is obvious that the multiplication medium supplemented with PP333 at all studied concentrations had insignificant differences on means of average shoot number, in addition to the rootstocks and the interactions among the rootstocks and the PP333 concentrations. The results showed that PP333 addition to the multiplication medium reduced the shoot length and this reduction was increased clearly by increasing the concentration which was at maximum level at 10 mg/l (1.1 cm) comparing with control which achieved the maximum mean average shoot length (2.49 cm) followed by 1mg/l which seemed to be better than 5 and 10 mg/l (1.61 cm). In terms of rootstocks effect, the longest shoot was obtained from Freedom stem pieces with significant differences among Ramsey and SO4 rootstocks (2.02 cm). Regarding the interaction, the best shoot length was in control of all studied rootstocks then in 1 mg/l. It was shown that PP333 apparently affected means of

average leaf number which was revealed as a gradual reduction by increasing PP333 concentrations. Control achieved the highest significant leaf number (5.75) with insignificant differences among other concentrations. On the other side, Freedom formed the highest significant mean of leaf number (4.49), while Ramsey gave the lowest mean leaf number (3.57). Regarding the interactions. The highest insignificant leaf number found by control with Freedom (6.69). For means of average stem diameter, it was clear that PP333at 5 mg/l, significantly achieved the highest mean average stem diameter (3.14 mm). In terms of rootstocks effect, Freedom seemed to be better than other rootstocks as it gave significantly the highest stem diameter (2.17 mm). Regarding the interaction, 5 ppm P333 with Freedom had highest significant value (3.55) (**Fig. 2**).

Paclobutrazol (PP333) is one of the plant growth retardants which had been used in the present trial. The results showed that PP333 induced several morphological changes in the regenerated material, including a drastic reduction in length and leaf number and increase in the thickness of stem. The reduction in shoot length and leaf number increased obviously in parallel with the increase in PP333 concentration from  $1\text{mg l}^{-1}$  to  $10\text{ mg l}^{-1}$ . On the other side, there was remarkable increase in stem diameter with the increase in PP333 concen-

tration to  $5 \text{ mg l}^{-1}$  which achieved the highest of average stem diameter (3.14 mm) compared to control (1.11mm), and then reduced at  $10 \text{ mg l}^{-1}$  in all studied rootstocks (Fig. 2). These findings are in agreement with those obtained by Nowello et al (1992) who observed that 1 ppm paclobutrazol applied in the same way to cultured shoots of *Vitis vinifera* caused plantlets to have leaves with a re-

duced area and bearing smaller stomata than usual. Stem length was also reduced. When roots were formed, they were thicker and more numerous than those on the control shoots. Kofidis et al (2008) found that treated plants with PP333 possessed thicker leaves, wider stems with more collenchyma tissue, and more vessels in the vascular bundles.

**Table 4.** Effect of PP333 concentrations and rootstock type on some proliferation characteristics of rootstocks during the first subculture

PP333 $\text{mg l}^{-1}$	Rootstocks							
	Average proliferated shoot number/explants				Average proliferated shoot length (cm)			
	F	R	S	Mean	F	R	S	Mean
0	1.00a	1.00a	1.00a	<b>1.00A</b>	3.63a	1.75bc	2.10b	<b>2.49A</b>
1	1.00a	1.00a	1.00a	<b>1.00A</b>	1.75bc	1.13ef	1.96bc	<b>1.61B</b>
5	1.00a	1.00a	1.00a	<b>1.00A</b>	1.56cde	1.08f	1.21def	<b>1.28C</b>
10	1.00a	1.00a	1.00a	<b>1.00A</b>	1.13ef	1.03f	1.13ef	<b>1.10C</b>
<b>Mean</b>	<b>1.00A</b>	<b>1.00A</b>	<b>1.00A</b>		<b>2.02A</b>	<b>1.25B</b>	<b>1.59B</b>	
Average stem diameter (mm)				Mean	Average leaf number/ proliferated shoot			
0	e151.	0e11.	e081.	<b>D111.</b>	6.69a	4.78bc	5.25b	<b>5.57A</b>
1	1.93d	1.98d	1.95d	<b>1.95C</b>	4.10cd	3.50defg	4.17cd	<b>3.92B</b>
5	3.55a	2.85b	3.03b	<b>3.14A</b>	4.00de	3.00g	3.84def	<b>3.61B</b>
10	2.45c	2.45c	2.55c	<b>2.54B</b>	3.17fg	3.00g	3.25efg	<b>3.14B</b>
<b>Mean</b>	<b>A272.</b>	<b>2.10B</b>	<b>B152.</b>		<b>4.49A</b>	<b>3.57C</b>	<b>4.13B</b>	

Means followed by the same letter (s) in each column, row or interaction are not significantly different at 5% level.



**Fig. 2.** Effect of PP333 concentrations on the shoot diameter of Freedom rootstock

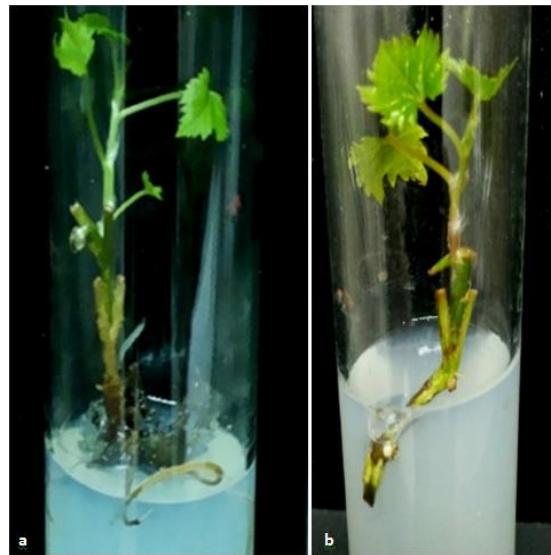
### Grape micrografting

#### **Effect of rooted and un-rooted rootstockshoots on some grafting characteristics of Superior micrografts**

Data in **Table (5)** illustrated the effect of rooted and un-rooted rootstocks on some grafting characteristics of Superior micrografts. The results showed that insignificant differences were recorded among the grafts of Superior on all rootstocks in mean scion survival percentage (SS%), on the other hand, grafting on un-rooted rootstocks resulted in the highest value in comparison to rooted rootstocks (92.67%). Interaction between the two studied factors was significant as Superior scions grafted on rooted Freedom and un-rooted Ramsey and SO4 achieved significantly the highest survival percentage (100.00%). On the same line, micrografts on Freedom were significantly the best in mean bud burst percentage (BB%), whereas the lowest (BB%) was in micrografts of SO4(57.50, 22.50%), respectively. Also un-rooted roots tocks significantly gave the higher (BB%) in comparison to rooted ones (45 & 30%), respectively. Regarding the interaction, micrografts of un-rooted Freedom significantly gave the highest BB% (75.00%), while the lowest values were in micrografts of rooted Ramsey and SO4 (25.00%) for each and in un-rooted SO4 (20.00%). It is plainly shown that insignificant differences were observed among means of rootstocks or rooted and un-rooted rootstocks or the interactions between the two factors in mean average shoot number per scion (ASN). For average shoot length (ASL), Superior grafts on Ramsey and Freedom gave the highest mean (ASL) without significant difference between them (1.75, 1.50 cm), respectively. On the other side Superior scions grafted on un-rooted rootstocks achieved the higher ASL significantly (2.00 cm). The interaction between the studied factors were significant as the highest values were in the grafts of superior on un-rooted Freedom and Ramsey (2.50 cm) for each one, while the lowest values were in rooted Freedom and SO4 (0.50 cm) for each one.

It is clearly shown that mean of average leaf number (ALN) was in the highest values in the grafts of Superior on both Ramsey and Freedom (4.00, 3.90), respectively. In terms of roots effects, the grafts of Superior on un-rooted rootstocks gave the higher mean (ALN) compared to the rooted one (4.60). Regarding the interactions, there were significant differences between the two factors that

appear clearly in grafts of Superior on un-rooted Freedom and Ramsey (5.00) for each one, while (ALN) was at the lowest value in rooted SO4 (2.00). It was clear that Grafts of Superior on Freedom rootstock showed the highest mean of graft union formation percentage (GUF%), (45.00%). Also the positive effect of un-rooted rootstocks appeared obviously when achieved the higher mean of GUF% (36.67%) regardless of rootstock types. Concerning the interactions, Superior grafts on rooted and un-rooted Freedom showed the highest GUF% followed by Un-rooted Ramsey (50.00, 40.00, 40.00%), respectively, while the lowest GUF% was in rooted SO4 (15.00%). Concerning micrografts rooting percentage (MR%), Superior grafts on Freedom achieved significant mean of MR% (37.50%). On the other hand, Superior grafts on un-rooted rootstocks gave the highest MR% regardless of the rootstocks (38.33%). Regarding the interaction, Superior grafts on un-rooted Freedom achieved the highest MR%, while the rooted rootstocks did not reflect any response by continuing the roots growth or giving new roots (75.00, 0.00%), respectively. Rooting of SO4 showed that it was hard to root by not giving any roots (0.00%) when planted on free hormones full strength MS medium. On the same side, Superior grafts on Freedom gave the highest mean of average root number (ARN) per micrograft (4.50) followed by the grafts on Ramsey (3.00) then on SO4 (0.00) with significant differences among them. Regarding the roots effect, un-rooted rootstocks were better significantly as they gave the highest ARN regardless of the rootstocks (5.00). Concerning the interaction, there were significant differences between the two studied factors which appeared clearly in Superior grafts on un-rooted Freedom (9.00), followed by un-rooted Ramsey (6.00), while the grafts on all rooted rootstocks did not give any new roots (0.00). On the same trend, Superior grafts on Freedom gave the highest mean of average root length (ARL) followed by the grafts on Ramsey then on SO4 (2.15, 1.25, 0.00 cm), respectively with significant differences among them. On the other side, Superior grafts on un-rooted rootstocks achieved the highest mean of ARL significantly (2.27 cm) regardless of rootstocks. Interaction between the studied factors revealed that grafts of Superior on un-rooted Freedom gave the highest ARL (4.30 cm) significantly, while the grafts on all rooted rootstocks did not respond or continue the roots growth (**Fig.3**). As a conclusion, Superior micrografts achieved the best results on Freedom rootstock planted on hormone-



**Fig. 3. Superior on SO4 micrografts planted on full strength MS medium.**  
(a-Superior on rooted SO4. b. Superior on un-rooted SO4)

free and full strength MS media as their micrografts gave the highest percentages of scion survival (100.00, 78.00%), scion bud burst (40.00, 75.00%), graft union formation (40.00, 50.00%), rooting (0.00, 75.00%), on rooted and unrooted rootstocks, respectively. These findings go along with those obtained by **Samaan (2013)** who reported that Freedom proved to be the best rootstock for Superior scions which achieved the best results on B5 medium lack of IBA supplements. In contrary, **EI-Hammady et al (2012)** stated that Ramsey rootstock gave the higher rate of success of Thompson seedless and Superior, if compared to Dog Ridge for both scions. On the other hand, the obtained results showed Superior micrografts regardless the rootstocks were remarkable on unrooted rootstocks as they gave the best results in all studied parameters compared to the rooted one.

It was observed that after the roots had been shortened, they turned into brown color and stopped completing their growth which may attributed to phenolic compounds from the cut surfaces and their oxidation by polyphenoloxidase and peroxidase enzymes caused discoloration of the tissues which resulted in poor micrografting (**Martinez, 2010**). Browning of the cut surfaces inhibits the growth and development of new cells and lead to poor graft union (**Hossein et al 2008**). This finding went in parallel with that obtained by (**Chancel et al 1979**) who reported that MS alone or with agar forms asphyxia which prevents formation of lateral roots.

**Table 5. Effect of rooted and un-rooted rootstocks on some grafting characteristics of Superior micrografts**

Rootstocks	Scion survival%			Scion bud burst%		
	Rooted	Un-rooted	Mean	Rooted	Un-rooted	Mean
F	100.00a	78.00b	<b>89.00A</b>	40.00b	75.00a	<b>57.50A</b>
R	80.00b	100.00a	<b>90.00A</b>	25.00c	40.00b	<b>32.50B</b>
S	78.00b	100.00a	<b>89.00A</b>	25.00c	20.00c	<b>22.50C</b>
Mean	<b>86.00B</b>	<b>92.67A</b>		<b>30.00B</b>	<b>45.00A</b>	
Rootstocks	Average shoot length (cm)			Average leaf number/scion		
	0.50b	2.50a	<b>1.50A</b>	3.00bc	5.00a	<b>3.90A</b>
F	1.00b	2.50a	<b>1.75A</b>	3.00bc	5.00a	<b>4.00A</b>
R	0.50b	1.00b	<b>0.75B</b>	2.00c	4.00ab	<b>3.00B</b>
Mean	<b>0.67B</b>	<b>2.00A</b>		<b>2.67B</b>	<b>4.60A</b>	
Rootstocks	Average shoot number/scion			Average root number/shoot		
	1.00a	1.00a	<b>1.00A</b>	0.00c	9.00a	<b>4.50A</b>
F	1.00a	1.00a	<b>1.00A</b>	0.00c	6.00b	<b>3.00B</b>
R	1.00a	1.00a	<b>1.00A</b>	0.00c	0.00c	<b>0.00C</b>
Mean	<b>1.00A</b>	<b>1.00A</b>		<b>0.00B</b>	<b>5.00A</b>	
Rootstocks	Micrografts rooting%			Graft union formation%		
	0.00c	75.00a	<b>37.50A</b>	40.00ab	50.00a	<b>45.00A</b>
F	0.00c	40.00b	<b>20.00B</b>	25.00cd	40.00ab	<b>32.50B</b>
R	0.00c	0.00c	<b>0.00C</b>	15.00d	20.00cd	<b>17.50C</b>
Mean	<b>0.00B</b>	<b>38.33A</b>		<b>26.67B</b>	<b>36.67A</b>	
Rootstocks	Average root length(cm)					
	0.00c	4.30a	<b>2.15A</b>			
F	0.00c	2.50b	<b>1.25B</b>			
R	0.00c	0.00c	<b>0.00C</b>			
Mean	<b>0.00B</b>	<b>2.27A</b>				

Means followed by the same letter (s) in each column, row or interaction are not significantly different at 5% level

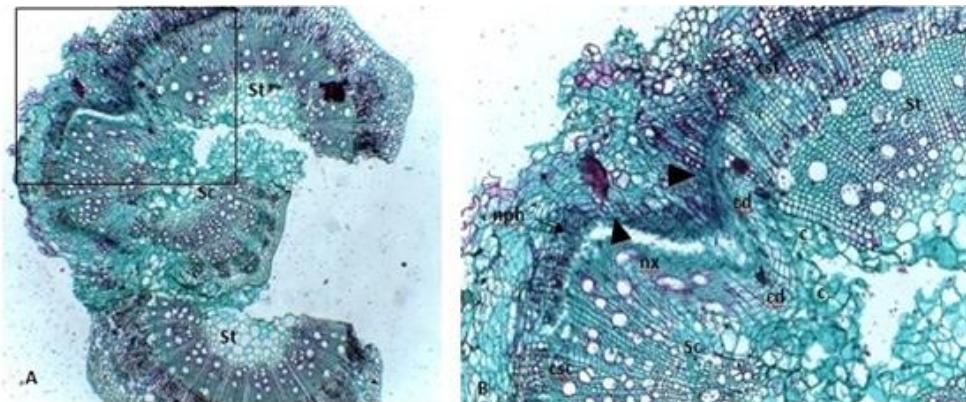
**Rehman and Gill (2014)** reported that medium composition and its phase has significant effect on graft success, necrosis, vigor and verification of Patharnakh micrografts. Maximum graft success was observed on MS liquid medium containing 20 g/l sucrose. **Bhatt et al (2013)** while investigating effect of different media formulations on graft success reported highest micrografting success rate using MS semisolid media followed by MS liquid media and MS liquid plus vermiculite media. Higher graft success (24.55% and 21.89%) was obtained when micrografts were cultured on MS semi-solid media at 3 and 6 % sucrose, respectively. **Samaan (2013)** mentioned that B5 medium was better than MS medium in the presence of IBA at 1 mg l<sup>-1</sup> regarding the grafting parameters and rooting percentage.

### Anatomical study

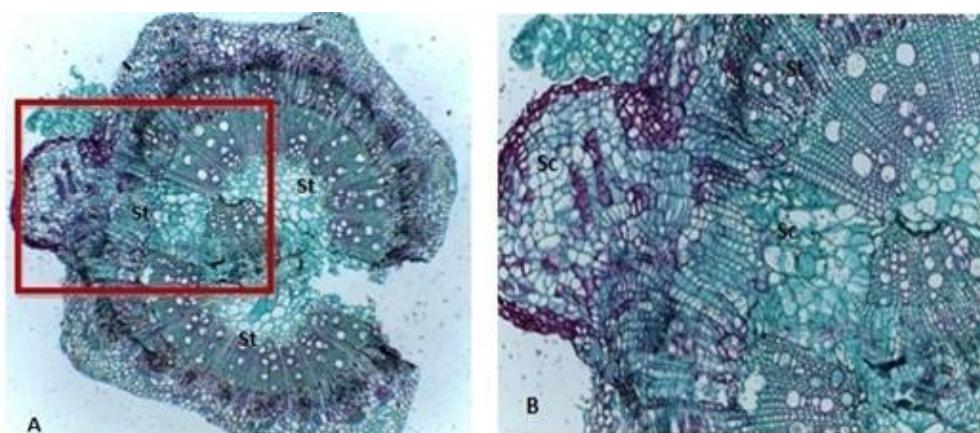
#### Superior cultivar grafted on Freedom rootstock

**Figure (4)** indicated that a successful graft was observed since a new vascular cambium initiated from the callus (between two arrow heads in "B") in line with the cambium in the stock and the scion. The new vascular cambium forms xylem (nx) and phloem (nph) in continuity with the same tissues in the stock and scion. As shown in figure "A" the union between stock and scion took place at one side only. The other side failed in union since there was a poor matching of cambia of stock and scion.

**Figure (5)** shows that successful graft was occurred from one side only, while another side of the scion did not match the vascular tissues of the stock.



**Fig. 4.** Cross section in the graft union of Superior/Freedom micrograft  
B: an enlarged view of A, St: stock, Sc: scion, cd: cambium derivatives, csc: vascular cambium of scion, cst: vascular cambium of stock, nph: new phloem, nx: new xylem, c: callus.



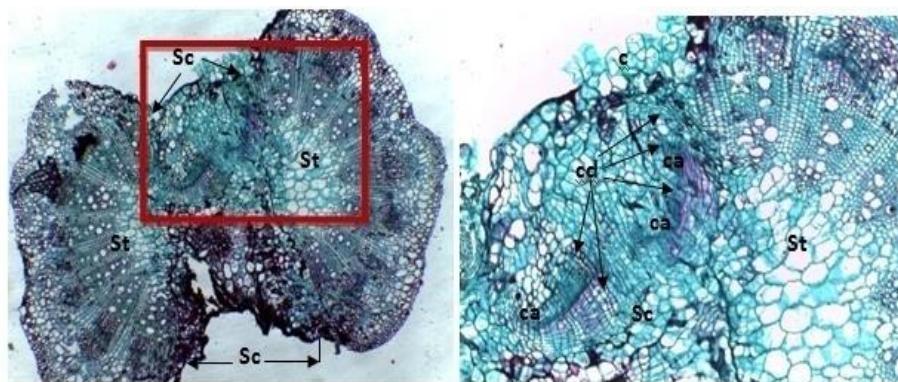
**Fig. 5.** Cross section in the graft union of Superior/Freedom micrograft  
B: an enlarged view of A, St: stock, Sc: scion

#### Superior cultivar grafted on Ramsey rootstock

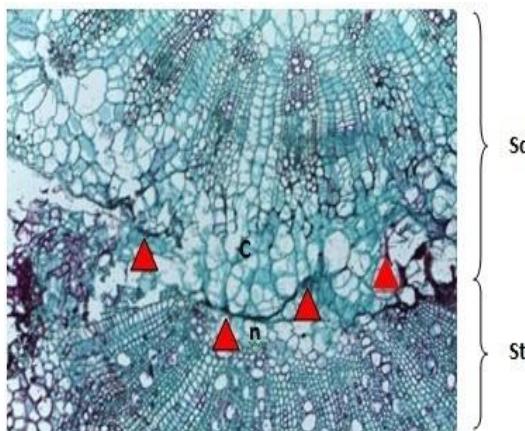
**Figure (6)** shows that few callus cells were observed. The cambia of scion gives many derivatives. Although these derivatives reached the margins of the scion, no connection between this cambium and that of the stock occurred (**Fig. B**). These

observations agree with that reported by (Balta et al 1996, Hartmann et al 1997).

**Figure (7)** shows that few callus cells were produced from the scion. The stock did not contribute in the callus development. Also necrotic layer were found between the stock and the scion (indicated by arrowheads).



**Fig. 6.** Cross section in the graft union of Superior/Ramsey micrograft  
B: an enlarged view of A, St: stock, Sc: scion, cd: cambium derivatives, ca: cambium, c: callus



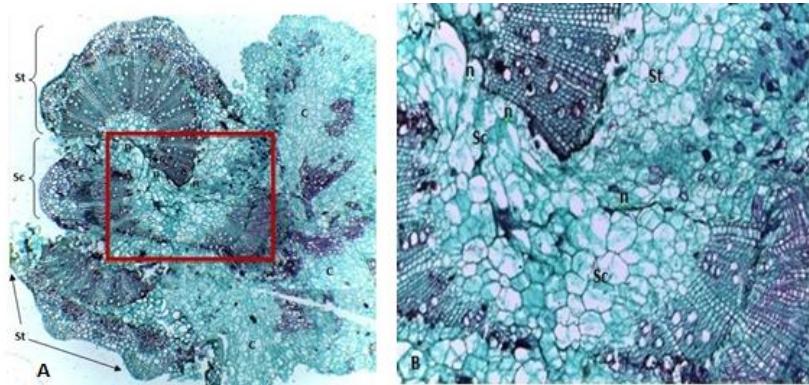
**Fig. 7.** Cross section in the graft union of Superior/Ramsey micrograft  
St: stock, Sc: scion, c: callus, n: necrotic layer

#### Superior cultivar grafted on SO4 rootstock

**Figure (8)** illustrates that the scion and the stock contributed in the formation of callus as was

observed from figure "A" the contribution of the scion was found to predominate that of the stock. It was noticed that interrupted necrotic layer and dividing activity were found which indicate that the divisions may be the initiated cells of the connecting vascular cambium between stock and scion. The callus cells produced from the stock were completely intermingled with those produced from the scion but no connecting cambium was established.

In this study, Freedom rootstock was more active in cell dividing activity when grafted by Superior cultivar compared to Ramsey and SO4 rootstocks which were very poor in producing callus cells in grafting union. However the anatomical observations in this study went harmonically with the present results that the highest bud burst and graft union formation percentage of the studied cultivars scions were obtained when grafted on Freedom rootstock. These observations were similar to those obtained by (Cantos et al 1995 and Samaan, 2013).

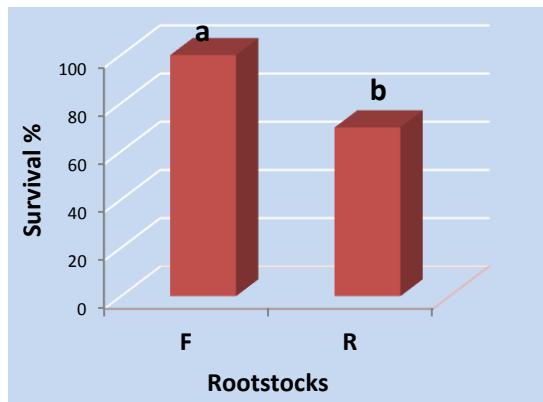


**Fig. 8.** Cross section in the graft union of Superior/SO4 micrograft  
B: An enlarged view of the delimited rectangle in A. St: stock, Sc: scion, n: necrotic layer

#### Acclimatization of micrografted plantlets

##### Effect of Freedom and Ramsey rootstocks on the acclimatization success of Superior micrografted plantlets

**Figure (9)** showed that the highest survival rate was remarkably observed by Superior on Freedom micrografts (100%) with a better foliage and roots length **Fig. (10)** than those on Ramsey rootstock which achieved the lower survival rate (70%).



**Fig. 9.** The survival rates of micrografted Superior on Freedom and Ramsey transplants after acclimatization

These present results went in parallel with **Samaan (2013)** who reported that Freedom micrografts achieved satisfied response to transplant in sterile soil mixture though Superior on Freedom micrografts gave the highest survival rate (70%) followed by Flame seedless and Thompson seedless (60%).



**Fig. 10.** Plantlets of Superior/Freedom one month after the acclimatization

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