IN VITRO MICROGRAFTING OF RED GLOBE (VITIS VINIFERA L)

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ABSTRACT

This study was carried out to evaluate the micrografting success of Red globe cultivar (Vitis vinifera L.) on three rootstocks Ramsey, Freedom and SO4 and investigate the effect of some factors on the micrografting success. Furthermore, compatibility in the graft union was studied. The results showed that Red globe on un-rooted Freedom achieved the highest percentages of scion survival (100.00 %), scion bud burst (60.00%), graft union formation (60.00%), and rooting (40.00%), also longest shoot (2.00cm), highest root number (8.00) and root length (4.50cm). Murashige and Skoog (MS) medium Supplemented with Indole Butyric Acid (IBA) at 1mgL^{-1} achieved the highest percentages of scion survival (95.20%), scion bud burst (65.43%), graft union formation (82.22%) also, longest shoots (1.84 cm) and maximum average leaf number (2.50). Regarding the effect of MS strength, 25% MS, 50% MS and 75% MS achieved positive effects on all parameters compared with full MS. The anatomical study revealed that Red globe on Freedom combinations were the best due to enough callus formation, less necrotic layers, and a wavy continuity of new cambium were determined. Sterilized 1:1:1 (v:v) peatmoss+perlite+sand mix gave the highest survival rate (100.00%) and showed superior vegetative growth. Freedom micrografts achieved the highest survival rate (100.00%), better growth and longer roots compared with those on SO4 and Ramsey (80, 70%) respectively.

Key words: grape, micrografting, rootstocks, growth regulators, compatibility, cclimatization

التطعيم الدقيق بالأنابيب للصنف ريد جلوب (*VITIS VINIFERA* L.)

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مدرس

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المستخلص

أجريت هذه الدراسة لتقييم نجاح التطعيم الدقيق لصنف ريد جلوب (*Vitis vinifera* L.) على ثلاثة أصول جذرية هي Ramsey، Freedom، SO4 والتحقق في تأثير بعض العوامل على نجاح الطعم الدقيق. علاوة على ذلك ، تم دراسة التوافق الطعم مع الأصل في منطقة الالتحام. أظهرت النتائج أن تركيب الصنف ريد جلوب على الأصول غير المجذرة حققت أعلى نسب بقاء (100.00٪)، وتفتح براعم الطعم (60.00٪)، وتكوين منطقة الالتحام (60.00٪)، والتجذير (40.00٪)، وأطول نمو (2.00 سم)، وعدد الجذور (8.00) وطول جذر (4.50 سم). أدت إضافة منظم النمو إندول بيوتريك أسيد (IBA) عند تركيز 1 ملجم/لتر إلى بيئة MS إلى أعلى نسبة بقاء (95.20٪)، ونمو براعم الطعم (65.43٪)، وتكوين منطقة الالتحام (82.22٪) أيضًا، وأطول نمو (1.84 سم) واكبر متوسط عدد أوراق (2.50). فيما يتعلق بتأثير قوة بيئة MS ، حققت 25% MS و50% MS و 75% MS تأثيرات إيجابية في كل القراءات المدروسة مقارنة مع القوة الكاملة. أظهرت الدراسة التشريحية أن تراكيب الصنف ريد جلوب على Freedom كانت أفضل حيث أعطت أعلى كمية كالس، وأقل طبقات نخرية، وأفضل استمرارية متموجة للكامبيوم الجديد. أعطت بيئة الزراعة المعقمة المكونة من بيتموس+بيرليت+رمل بنسبة 1:1:1 (حجم/حجم) أعلى نسبة بقاء (100.00٪) وأفضل نمو خضري. حققت التراكيب على الأصل Freedom أعلى معدل بقاء (100.00٪) ونمو خضري أفضل وجذور أطول مقارنة بتلك الموجودة في SO4 وRamsey (80، 70٪) على التوالي.

الكلمات المفتاحية: عنب، تطعيم دقيق، أصول جذرية، منظمات نمو، توافق، أقلمة

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INTRODUCTION

Grape is a popular crop and has many cultivars with different purposes in the world around. Red globe is an attractive table grape due to its berry size, color, and favorable for both local market and export. Dormant cuttings are usually used in grapevine propagation besides grafting on phylloxera-resistant rootstocks. However, conventional grafting is slow and does not produce pathogen-free plants perfectly. In vitro micrografting is a new technique applied in aseptic conditions and used on a large scale for plant multiplication as an alternative to the conventional one (32). Micrografting is a rapid process that can produce thousands of healthy plants in a short time as well as improves the yield and grape quality in addition to eliminating the common pathogens (Virus, Phylloxera, Nematodes, root rots..etc) that lead to massive yield and quality losses, and able to overcome some of the anatomical and physiological problems when some species and cultivars merge together (17, 32). Selecting the right rootstocks that adapted to the local climate conditions is very important (19) that affect efficiently the cultivars resistance (18). Micrografting technique has been used on several species including grape (5), Cashew (54), Pistachio (43), and cherry (15). Micrografting success can be affected by several factors including scion origin and age, basal medium formulation, sucrose concentration, micrografting procedure, growth regulators, and rootstock type (5). (24) obtained grafting success of 83 % by using in vitro scion (0.5-1cm cm) of Le Conte pear on *in vitro* decapitated P betulaefolia as rootstock. (47) reported that maximum graft success was observed on Murashige and Skoog liquid medium containing 2% sucrose. (6) stated that the highest graft success (24.55% and 21.89%) was obtained when micrografts were cultured on MS semi-solid media at 3 and 6 % sucrose respectively. (1) used 1 cm of a rootstock and a shoot-tip of a scion and made a good connection of the two pieces then cultured on MS medium solidified with $6gL^{-1}$ agar, 3% sucrose. (49) reported that IBA was insignificantly better than NAA in all parameters, while NAA stimulated forming callus masses on rootstocks, and He recommended Thompson seedless on Freedom micrografts planted on B5 medium contained IBA at 2 mg/l, Flame seedless on Ramsey micrografts planted on B5 medium contained IBA at 3 mg/l. After grafting, compatibility between stock and scion tissues is really important to obtain an optimum grafted plant (22). (31) explained in the study of histological structure of compatible and incompatible plants that necrotic cells layer in incompatible plants was thick, and it was noted the presence of callus as small parts around the grafting area, leading to the separation of scion and stock later. (49) illustrated that Freedom rootstock was more active in cell dividing compared with Harmony rootstock. Moreover, vascular connections were detected although the cambia of both scion and rootstock were in some cases not matched. For the successful transfer to the open field, in vitro plantlets have to pass through a gradual change from high relative humidity and low light intensity of the flask stage into low relative humidity and high light intensity of the open nursery and field. After rooting, the plantlets are transferred into pots filled with rooting substrates, left open and placed inside growth chamber (3), or placed inside propagator units (51). It could also either covered with plastic bags or enclosed inside polyethylene sheet and placed under a glasshouse with natural daylight (30). This study aimed to assess the micrografting success of Red globe grape cultivar on different rootstocks under in vitro conditions and investigate the influence of some factors on the grafting success. Furthermore, determining the compatibility via anatomical study in the graft union. Moreover, acclimatization of the obtained micrografts.

MATERIALS AND METHODS

Plant material and explants preparation

One-year-old transplants were used as mother plants for Red globe grape cultivar and three rootstocks named Freedom, Ramsey, and SO4 for explants. Nodes 1-2 cm long were used as explants. The leaves were removed, and a short part of the petiole left. The explants were washed with water, and a few drops of soap then put under a slow flow of tap water for an hour. Explants were surface sterilized in 1% sodium hypochlorite with 2-3 drops of Tween-20 for 10, 15, and 20 minutes as a surfactant. Then, the nodal cuttings were rinsed 3 times

with sterilized water then divided into separate stem node explants which were cultured (4-5 per jar).

The basal medium and glassware preparation

Full strength and free hormone MS as a basal medium was used (44) in the establishment stage. Sucrose at 3% was used as a source of energy and the medium was adjusted pH 5.8 ±1 before being solidified with purified Agar (Agar-Agar) at 7 g/L, then autoclaved at 100 psi and 121° C for 20 min. Glass jars (400 ml) contained nearly 40 ml medium per jar have been used during micropropagation stage. In addition, glass culture tubes (150×25mm) received 20 ml medium per tube in the micrografting experiments. The glass jars and tubes were washed with detergent then soaked in a 5% solution of sodium hypochlorite (NaOCl) for an hour then rinsed with water before using them.

Scion and rootstock shoots multiplication

In vitro explants from the initiation stage were cultured on a full-strength MS medium supplemented with a plant growth regulator (5 mgL^{-1} 6-furfurylaminopurine Kin, 3% sucrose and 0.7% Agar-Agar). The pH was adjusted to 5.8% before autoclaving (121° C, 20 min).

Micrografting stage

Preparation of scion: 4-week-old In vitro shoots of Red globe from the final subculture with the desired thickness, and internodes have been taken as scions for micrografting. Under aseptic conditions, the selected shoots were cut to 5-10 mm in length and each one had an axillary bud.

Preparation of rootstocks

8-10-week-old in vitro shoots of Freedom, Ramsey and SO4 rootstocks were used for micrografting. Shoots with a good diameter and long internodes were selected then shortened to (15-20 mm) in length inside the laminar cabinet under aseptic conditions.

Micrografting procedure

Cleft-grafting method was applied. A 0.5 cm slit was made in the rootstock shoot and a wedge from a scion shoot was cut and inserted it in the rootstock making a maximum connection of the two pieces. In all cases, the rootstock and scion should be as one union. Grafted combinations were cultured on MS medium solidified with $7gL^{-1}$ agar and containing $30gL^{-1}$ sucrose. Some experiments were carried out to improve the *in vitro* micrografting success among the studied cultivar and rootstocks. In addition, the factors involved in scions performance and the rooting of micrografts.

Figure 1. Cleft grafting method in vitro

Micrografting parameters

4-6 weeks after grafting, the following parameters were recorded: 1) scion survival percentage (scions stayed green regardless of shoot formation). 2) Scion bud burst percentage (the survived scions formed new shoots). 3) Number of shoots per scion (the number of formed shoots on each scion). 4) Average shoot length**.** 5) Average leaf number per scion**.** 6) Graft union formation percentage (recognized by callus forming in graft union region in coincidence with scion bud burst). **7)** Micrografts rooting percentage (adventitious roots forming on the rootstock stem). 8) Number of roots per micrograft. 9) Average root length per micrograft.

Effect of rooted and un-rooted rootstocks on grafting success: Red globe scions were grafted on both rooted and un-rooted rootstocks and inoculated in a full strength and hormone-free MS medium. The parameters were recorded 4 weeks after grafting.

Effect of IBA concentration on grafting success: A full strength of MS supplemented with IBA at 3 concentrations (0.1, 0.5, and 1 mgL^{-1}) was studied on the rooting percentage and other grafting parameters in order to select the best concentration.

Effect of medium strength

4 different strengths of free hormone MS (25, 50, 75, and full-strength) had been studied to understand their influence on the micrografts performance. Parameters were recorded 4 weeks after grafting.

Anatomical study: Transverse sections were made across the successful graft union of well

rooted and growing micrografts eight weeks after the culture date in order to study the compatibility and incompatibility limits among the studied cultivars and rootstocks. Samples were cut and fixed in formalin-acetic acid–ethanol 70% (FAA) for 48 h, then dehydrated in ascending ethanol. Paraffin wax was infiltrated and samples were embedded (28). Cross-sections were microtomed (12-16 µm) mounted to glass slides and stained with safranin-fast green schedule (50). Sections were examined and photographed with a light microscope (Leica DM 2500).

Incubation conditions

The cultures were incubated at 25±2°C with 16 hours of light using fluorescent lamps (2 lamps per shelf) and 8 hours dark of 2000-2500 lux light intensity at cultures level.

Acclimatization

6-week-old micrografted plantlets had been removed from the culture tubes then washed with running tap water to remove any medium residual then treated with a fungicide solution (Prochloraz 45% EC) $1gL^{-1}$, then potted into a proper sterile medium and kept inside a glass chamber covered with transparent plastic in the incubation room for 4 weeks and watered constantly before being transferred to the glasshouse.

Effect of soil mixture on the acclimatization success: Several soil mixtures of peatmoss:perlite 1:1(v/v), peatmoss: sand 1:1 (v/v) and peatmoss: perlite: sand 1:1:1(v/v) were tested.

Effect of rootstock type on acclimatization success: The objective of this experiment was to investigate the effect of rootstock type on the survival percentage. Micrografted plantlets of Red globe combined with Freedom, Ramsey and SO4 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 and means were compared by Duncan's multiple range test at $p \leq 0.05$ level of confidence**.** (14).

RESULTS AND DISCUSSION

Effect of rooted and un-rooted rootstocks on grafting success : Data in **Table 1** exhibits the effect of rooted and un-rooted rootstocks on some grafting characteristics of Red globe micrografts. It is plain that grafts of Red globe on Freedom rootstock significantly achieved the highest mean scion survival percentage (SS%) compared to those grafted on other rootstocks (100.00%). On the other hand, insignificant differences were recorded between the rooted and un-rooted rootstocks in this parameter. Interaction between the two studied factors was significant as Red globe scions grafted on rooted and un-rooted freedom gave the highest SS% (100.00%), whereas the lowest values were on un-rooted Ramsey and rooted SO4 (60.00%) for each. On the same side, Red globe scions grafted on Freedom gave significantly the highest mean bud burst percentage (BB%), whereas the lowest BB% was in those grafted on SO4(50, 29.5%) respectively. Also, insignificant difference was observed between the rooted and un-rooted rootstocks in BB%. Regarding the interaction, micrografts on un-rooted Freedom significantly gave the highest BB% (60.00%), while the lowest value was in micrografts on un-rooted SO4 (25.00%). It is obviously 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). Concerning the average shoot length (ASL), insignificant differences were observed among rootstocks regardless of roots presence or between rooted and un-rooted rootstocks regardless of rootstocks. Interaction between the studied factors was significant as the highest values were in the grafts of Red globe on un-rooted Freedom and SO4 (2.00cm) for each one, while the lowest values were in rooted Freedom and SO4 (0.50 cm) for each one. The results showed the mean average leaf number (ALN) in the grafts of Red globe on SO4 rootstock (3.35). In terms of roots effects, the grafts of Red globe on un-rooted rootstocks gave the highest mean ALN compared to the rooted one (3.34). Regarding the interactions, there were significant differences between the two factors that appear clearly in grafts of Red globe on un-rooted SO4 (4.40). It was clear that grafts of Red globe on Freedom rootstock showed the highest mean of graft union formation

percentage (GUF%), compared to other rootstocks (50.00%), while the lowest GUF% was on SO4 (25.00%). Also, insignificant difference was recorded between rooted and un-rooted rootstocks regardless of rootstock types. Concerning the interactions, Red globe grafts on un-rooted Freedom showed the highest GUF% (60.00%), while the lowest GUF% was in un-rooted SO4 (25.00%). Concerning micrografts rooting percentage (MR%), Red globe grafts on both Freedom and Ramsey achieved the highest mean of MR% (20.00, 16.50%) respectively with insignificant difference between them . On the other hand, Red globe grafts on un-rooted rootstocks gave the highest MR%, regardless of the rootstocks (24.33%). Regarding the interaction, Red globe grafts on un-rooted Freedom achieved the highest MR%,while the rooted rootstocks did not appear any The results showed that Red globe grafts on Freedom significantly gave the highest mean of average root number (ARN) per micrograft (4.00) followed by the grafts on Ramsey (0.50) then on SO4 (0.00). Regarding the effect of the roots, un-rooted rootstocks were better significantly as gave the highest ARN regardless of the rootstocks (3.00). Concerning the interaction, there were significant differences between the two studied factors which appeared clearly in Red globe grafts on un-rooted freedom (8.00), while the grafts on all rooted rootstocks did not give any new roots (0.00). Red globe grafts on Freedom gave the highest mean of average root length (ARL) followed by the grafts on Ramsey then on SO4 (2.25, 0.50, 0.00 cm) respectively with significant differences among them. On the other side, Red globe grafts on un-rooted rootstocks achieved the highest mean of ARL significantly (1.83 cm) regardless of rootstocks. Interaction between the studied factors revealed that grafts of Red globe on un-rooted Freedom gave the highest ARL (4.50 cm) significantly, while the roots of the rooted rootstocks did not respond or continue their growth**.**

Table 1. Effect of rooted and un-rooted rootstocks on some grafting characteristics of Red globe micrografts

R-stock	Scion survival%			Scion bud burst%			Average shoot number/scion		
	Rooted	Un-rooted	Mean	Rooted	Un-rooted	Mean	Rooted	Un-rooted	Mean
F	100.0a	100.00a	100.00A	40.00bc	60.00a	50.00A	1.00a	1.00a	1.00A
R	80.00b	60.00c	70.00B	50.00ab	33.00cd	41.50B	1.00a	1.00a	1.00A
S	60.00c	80.00b	70.00B	33.00cd	25.00d	29.50C	1.00a	1.00a	1.00A
Mean	80.00A	80.00A		41.00A	39.33A		1.00A	1.00A	
R-stock	Average shoot length (cm)			Average leaf number			Graft union formation%		
F	0.50b	2.00a	1.25A	1.99b	2.61b	2.31B	40.00bc	60.00a	50.00A
Ra	1.00b	1.00b	1.00A	2.00b	3.00b	2.50B	50.00ab	33.00cd	41.00B
S	0.50b	2.00a	1.25A	2.30b	4.40a	3.35A	33.00cd	25.00d	29.00C
Mean	0.67B	1.67A		2.10B	3.34A		41.00A	39.33A	
R-stock	Micrografts rooting%			Average root number			Average root length(cm)		
F	0.00c	40.00a	20.00A	0.00c	8.00a	4.00A	0.00c	4.50a	2.25A
R	0.00c	33.00b	16.50A	0.00c	1.00b	0.50B	0.00c	1.00b	0.50B
S	0.00c	0.00c	0.00B	0.00c	0.00c	0.00C	0.00c	0.00c	0.00C
Mean	0.00B	24.33A		0.00B	3.00A		0.00B	1.83A	

F: Freedom, R: Ramsey, S: SO4. Means followed by the same letter (s) in each column are not significantly different at p≤0.05 level.

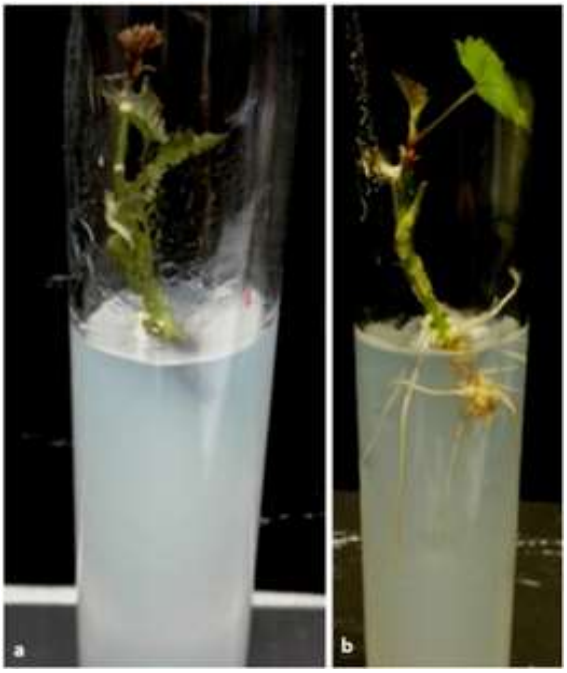

Figure 2. Red globe on Freedom micrgrafts planted on full strength MS medium a. rooted Freedom. b. un-rooted Freedom

Figure 3. Red globe on Ramsey micrgrafts planted on full strength MS medium a.rooted Ramsey. b. un-rooted Ramsey

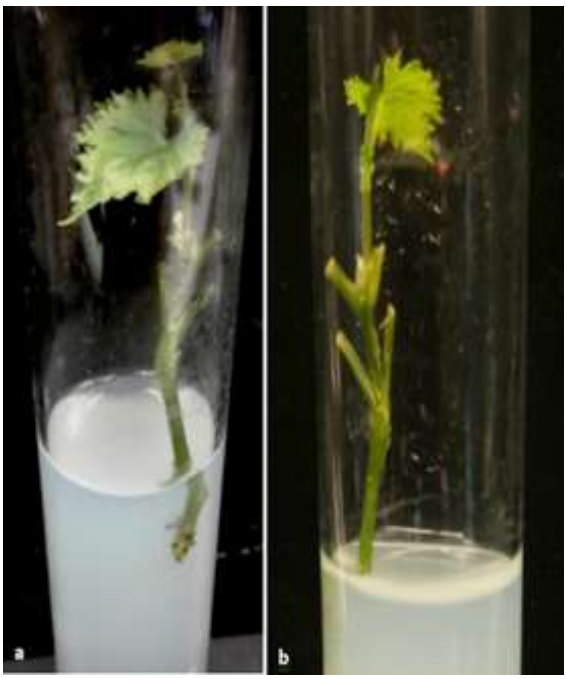

Figure 4. Red globe on SO4 micrgrafts planted on full strength MS medium a.rooted SO4. b. un-rooted SO4

it is clear that Red globe micrografts achieved the best results on Freedom rootstock cultured on hormone-free and full-strength MS medium as their micrografts gave the highest percentages of scion survival (100.00%), scion bud burst (50.00%), graft union formation (50.00%), rooting (20.00%). These findings went in the same line with those obtained by (49) who reported that Freedom proved to be the best rootstock for Superior scions. On the contrary, (16) stated that Ramsey rootstock gave the highest rate of success of Thompson seedless and Superior, if compared to Dog Ridge for both scions. Moreover, the shoot length of Thompson seedless scion on Ramsey rootstock was higher than Thompson seedless scion on Dog Ridge rootstock. In addition, superior cultivar on both Ramey and Dog Ridge showed the same trend. (52) reported that the highest grafting success rate was obtained from Early Cardinal cultivar grafted on 41B followed by Early Cardinal on Ramsey with the rate of 71.4%. Many researchers found that micrografting success varies with the rootstocks because of the compatibility reaction between the grafting partners. (5) found that using Kober 5BB rootstock, the micrografting success rates reached 40.9 % and 68.2% for Hafizali and Emir varieties respectively. On the other hand, the obtained results showed that Red globe micrografts

regardless of the rootstocks were remarkable on un-rooted rootstocks as they gave the best results in all studied parameters compared to the rooted one. It was observed that after the roots had shortened, they turned into brown color and stopped completing their growth which may be attributed to phenolic compounds from the cut surfaces and their oxidation by polyphenol oxidase and peroxidase enzymes caused discoloration of the tissues which resulted in poor micrografting (36). The phenolic compounds present in the cut surfaces inhibit the development of new cells and lead to graft union failure (26). These findings went in parallel with those obtained by (62) who stated that the physiological state of roots extremely influences the survival rate.

Effect of IBA concentration on grafting success: The effect of IBA concentration on some grafting characteristics of Red globe grape micrografts is illustrated in **Table 2**. The micrografts of Red globe on different rootstocks varied in their response to IBA concentrations and that appeared clearly on Freedom rootstock which significantly achieved the highest mean scion survival percentage (SS%) compared to those grafted on other rootstocks (95.20%). On the same trend, significant differences were recorded among IBA concentrations in this parameter, so it was recorded that 1mgL^{-1} IBA scored the highest SS% significantly (95.20%) and the lowest SS% was at 0.1mgL^{-1} IBA (71.47%). Interaction between the two studied factors was significant as Red globe scions grafted on freedom gave the highest SS% at 0.1 and 0.5 mgL^{-1} (100.00%), Ramsey and SO4 at 1mgl^{-1} IBA (100.00%), whereas the lowest values were on Ramsey and SO4 at 0.1 mgL^{-1} (57.20%) for each one. On the same side, Red globe scions grafts on Freedom gave significantly the highest mean bud burst percentage (BB%), whereas the lowest BB% was in those grafted on Ramsey (78.83, 30.67%) respectively. Also, significant differences were observed among IBA concentrations in means BB%, which appeared significantly in 1 mgL^{-1} (65.43%), while 0.5 mgL^{-1} gave the lowest mean BB% (50.00%). Regarding the interactions, micrografts on Freedom significantly gave the highest BB% at 0.1 and 1 mgL^{-1} (87.38, 82.60%) respectively and SO4 at 0.5 mgL^{-1} (83.20%), while the lowest values were in micrografts on Ramsey at 0.5 mgL^{-1} (0.00%) and on SO4 at 0.1mgL^{-1} (0.00%). It was shown that insignificant differences were observed among means of rootstocks or IBA concentrations or the interactions between the two factors in mean average shoot number per scion (ASN). Significant differences in means of average shoot length (ASL) were observed among rootstocks, Freedom significantly scored the maximum ASL compared to other rootstocks (2.46 cm) regardless of IBA concentrations. On the other side, Red globe micrografts cultured on MS supplemented with 1 mgL^{-1} IBA achieved the highest ASL (1.84 cm). Interaction between the studied factors was significant as the highest value was obtained in the grafts of Red globe on Freedom at 1 mgL^{-1} (3.50 cm), while the lowest values were on Ramsey at 0.5 mgL^{-1} and on SO4 (0.00 cm) for each one. The results revealed that significant differences were recorded in the mean of average leaf number (ALN) which appeared significantly in the grafts of Red globe on Freedom rootstock (3.60). In terms of IBA concentration effects, insignificant differences were observed among them although 1 mgL^{-1} gave the highest mean ALN compared to other concentrations (2.50). Regarding the interactions, there were significant differences between the two factors that appear clearly in grafts of Red globe on Freedom at all IBA concentrations but it was better at 0.1 mgL^{-1} (3.80), while the lowest values were on Ramsey at 0.5 mgL^{-1} and on SO4 at 0.1 mgL^{-1} (0.00) for each one. It was obvious that grafts of Red globe on Freedom rootstock showed the highest mean of graft union formation percentage (GUF%), compared to other rootstocks (84.80%), while the lowest GUF% was on Ramsey (55.55%). Also, significant differences were recorded among IBA concentrations regardless of rootstock types, therefore 1 mgL^{-1} gave the highest GUF% (82.22%). Concerning the interactions, the highest GUF% was in Red globe grafts on Freedom at 1 mgL^{-1} (100.00%), on Ramsey at 0.1mgL^{-1}, and on SO4 at 0.5mgL^{-1} (100.00%), while the lowest GUF% was on Ramsey at 0.5 mgL^{-1} and on

SO4 at 0.1 mgL^{-1} (0.00%). Concerning micrografts rooting percentage (MR%), Red globe grafts on Freedom significantly achieved the highest mean of MR% (84.12%). On the other hand, 0.1 mgL^{-1} gave the highest mean MR% regardless of the rootstocks (47.60%). Regarding the interaction, Red globe grafts on Freedom at 0.1 mgL^{-1} achieved the highest MR% (100.00%), while the lowest MR% was on Ramsey at 0.5 and 1 mgL^{-1} (0.00%) and on SO4 at 0.1 mgL^{-1} (0.00%). The results showed that Red globe grafts on Freedom significantly gave the highest mean of average root number (ARN) per micrograft (11.20). Regarding the IBA concentrations effect, insignificant differences were observed in the mean ARN regardless of the rootstocks. Concerning the interaction, there were significant differences between the two studied factors which appeared clearly in Red globe grafts on freedom at 0.1 and 1 mgL^{-1} (12.60, 13.00) respectively, while the grafts on Ramsey at 0.5 and 1 mgL^{-1} or on SO4 at 0.1 mgL^{-1} did not give any roots (0.00). Red globe grafts on Freedom and SO4 gave the highest mean of average root length (ARL) significantly (3.74, 4.00 cm) respectively with insignificant differences among them. On the other side, insignificant differences were recorded among IBA concentrations in the mean of ARL regardless of rootstocks. Interaction between the studied factors revealed that grafts of red globe on SO4 gave the highest ARL at 0.5 mgL^{-1} (7.00 cm) significantly, whereas Freedom and Ramsey gave the highest ARL at 0.1 mgL^{-1} (4.69, 3.71 cm) respectively**.**

Table 2. Effect of IBA concentration on some grafting characteristics of Red globe micrografts on three rootstocks

IBA mgL^{-1}	Scion survival%				Scion bud burst%				Average shoot number/scion			
	F	R	S	Mean	F	R	S	Mean	F	R	S	Mean
0.1	100.00a	57.20c	57.20c	71.47C	87.38a	50.00c	0.00e	45.79C	1.00a	1.00a	0.00b	0.67A
0.5	100.00a	85.70b	85.70b	90.47B	66.50b	0.00e	83.20a	50.00B	1.00a	0.00b	1.00a	0.67A
1.0	85.60b	100.00a	100.00a	95.20A	82.60a	42.00d	71.70b	65.43A	1.00a	1.00a	1.00a	1.00A
Mean	95.20A	80.97B	80.97B		78.83A	30.67C	51.63B		1.00A	0.67A	0.67A	
IBA Mgl^{-1}	Average shoot length (cm)				Average leaf number				Graft union formation%			
0.1	2.00b	0.90cd	0.00d	0.96B	3.80a	2.20ab	0.00c	2.00A	87.40b	100.00a	0.00e	62.47B
0.5	1.88b	0.00d	1.10bc	0.99B	3.50a	0.00c	2.60ab	2.03A	67.00d	0.00e	100.00a	55.67C
1.0	3.50a	0.84cd	1.20bc	1.84A	3.50a	1.40ab	2.60ab	2.5A	100.00a	66.66d	80.00c	82.22A
Mean	2.46A	0.58B	0.77B		3.6A	1.2B	1.73B		84.8A	55.55C	60.00B	
IBA Mgl^{-1}	Micrografts rooting%				Average root number				Average root length(cm)			
0.1	100.00a	42.8d	0.00f	47.60A	12.60a	1.40c	0.00d	4.67A	4.69b	3.71bc	0.00d	2.80A
0.5	66.66c	0.00f	14.30e	26.98C	8.00b	0.00d	1.00c	3.00A	2.87c	0.00d	7.00a	3.29A
1.0	85.7b	0.00f	14.30e	33.33B	13.00a	0.00d	1.00c	4.67A	3.67bc	0.00d	5.00b	2.89A
Mean	84.12A	14.27B	9.53C		11.20A	0.47B	0.67B		3.74A	1.23B	4.00A	

F: Freedom, R: Ramsey, S: SO4. Means followed by the same letter (s) in each column are not significantly different at p≤0.05 level.

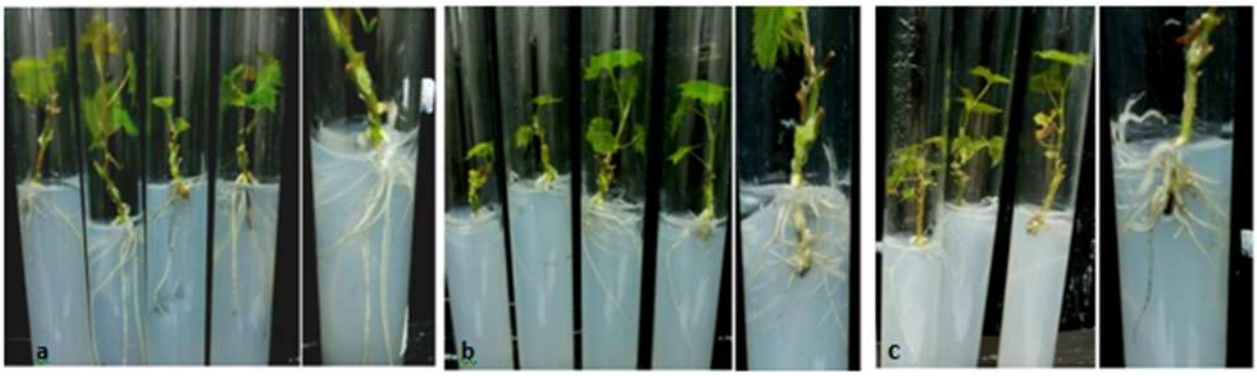

Figure 5. Red globe on Freedom micrografts cultured on full strength MS medium supplemented with concentrations of IBA . a. 0.1 mgL^{-1} . b. 0.5 mgL^{-1}

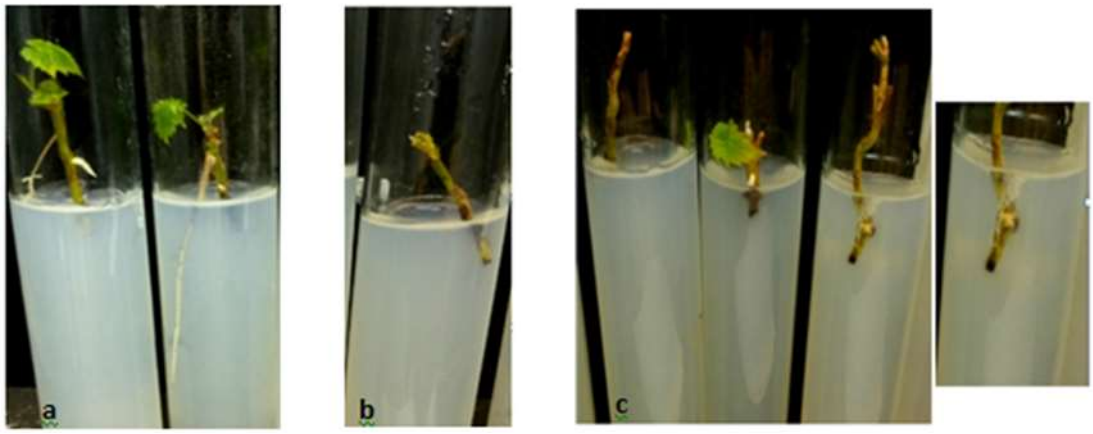

Figure 6. Red globe on Ramsey micrografts cultured on full strength MS medium supplemented with different concentrations of IBA. a. 0.1 mgL^{-1} . b. 0.5 mgL^{-1} c. 1 mgL^{-1}

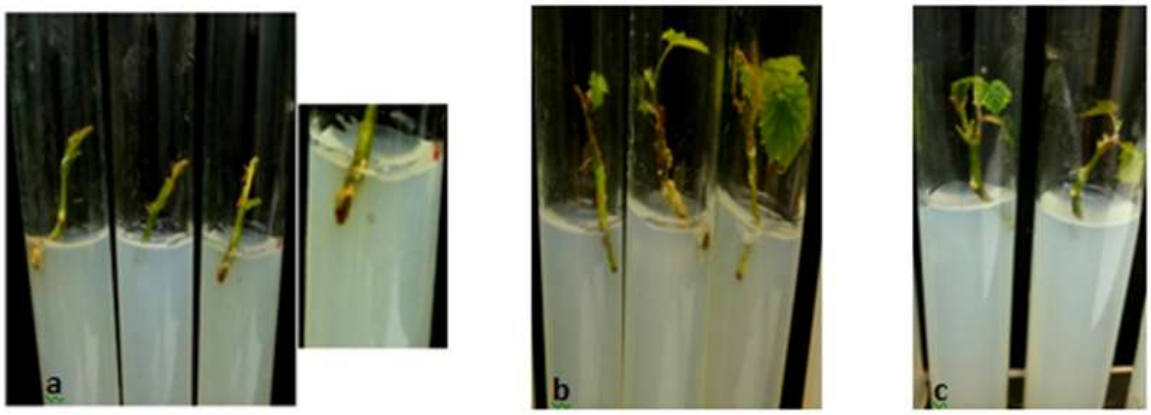

Figure 7. Red globe on SO4 micrografts cultured on full strength MS medium supplemented with different concentrations of IBA. a. 0.1 mgL^{-1}. b. 0.5 mgL^{-1} c. 1 mgL^{-1}

Plant growth regulators are one of the factors that may have an influence on graft success (55). Some workers used plant growth regulators to improve graft success through improving the callus formation, and vascular tissue differentiation. Auxins are the first of the major plant hormones to be discovered. (37). In the present study IBA addition to MS medium had been found effective for improving the percentages of scion survival, scion budburst, graft union formation, and rooting of the Red globe micrografts regardless of the rootstocks. These findings show the correlation between the successful graft unions and the cell activity and growth that improve callus formation (37). Auxin plays a vital role in the development of compatible unions and enhances the differentiation of vascular tissues (55). Auxin enhances many developmental effects by regulating gene expression (60). (58) Found that NAA was effective in improving the micrograft success in walnut**.** (49) Reported that IBA was insignificantly better than NAA in all parameters, while NAA stimulated forming callus masses on rootstocks. The obtained results indicated that the effects of auxin, IBA, on the measured parameters were dependent on applied concentrations where treatment of 1.0 mgL^{-1} had significantly enhancing effects. The highest percentages of scion survival, scion budburst, graft union formation and shoot length were observed in 1.0 mgL^{-1} treatment, whereas 0.1mgL^{-1} had a positive effect on rooting%, the average number of roots and root length. This might be related to the fact that higher concentrations of IBA are inhibitory both to root induction and elongation. (2) Indicated that the high concentrations of auxin enhances ethylene production which inhibits the rooting. Auxin has a great role in many developmental activities, and can impact cell division, cell growth, or cell differentiation (60). (25) Indicated that IBA at 0.5 ppm decreased the rate of establishment, shoot, and root values in the grafts with Rupestris du Lot, but had a favorable effect on the grafts with 140 Ruggeri at doses up to 4 ppm. (49) Recommended Thompson seedless on Freedom micrografts

planted on B5 medium contained IBA at 2 mgL^{-1}, Flame seedless on Ramsey micrografts planted on B5 medium contained IBA at 3 mgL^{-1}. On the other hand, the obtained results illustrated that Red globe on Freedom micrografts gave significantly the best results compared to other rootstocks in all studied parameters. These findings went in parallel with the results obtained by (49).

Effect of medium strength

Data in **Table 3** exhibit the effect of medium strength on some grafting characteristics of Red globe grape micrografts. It is distinct that Freedom significantly achieved the highest mean scion survival percentage (SS%) compared to those grafted on other rootstocks (93.75%) regardless of medium strength. On the other side, significant differences were observed among medium-strength levels in this parameter, so it was recorded that full MS scored the highest SS% significantly (86.67%) and the lowest SS% was at 50% MS (58.20%). Interaction between the two studied factors was significant as Red globe scions grafted on freedom gave the highest SS% at 25% MS and full MS (100.00%), also on Ramsey at 25% MS (100.00%) and on SO4 at full MS(80%). On the other hand, Red globe scions grafts on Freedom gave significantly the highest mean scion bud burst percentage (SBB%), whereas the lowest SBB% was in those grafted on Ramsey (73.88, 33.33%) respectively. Also, significant differences were recorded among medium strength levels in mean SBB%, which appeared significantly in 75% MS (71.97%), while Full strength gave the lowest mean SBB% (30.00%). Regarding the interaction, micrografts on SO4 significantly gave the highest SBB% at 75% strength MS (100.00%), while at 50% MS and 75% MS on Freedom without significant difference between them (85, 7, 83.30 %) respectively and at 25% MS on Ramsey (50.00 %). The results indicated that insignificant differences were observed among means of rootstocks or medium strength or the interactions between the two factors in the mean average shoot number per scion (ASN). It is clear that significant differences were observed among rootstocks in mean of average shoot length (ASL). Superior on SO4 and on Freedom micrografts significantly scored the maximum ASL compared to Ramsey (2.15, 2.03 cm) regardless of medium strength. On the other side, Red globe micrografts cultured on 25% MS achieved the highest ASL (2.67 cm) regardless of rootstocks. Interaction between the studied factors was significant as the highest value was obtained in the grafts of Red globe on SO4 at 25% MS (3.50 cm) and the lowest values were on Freedom and on SO4 at full MS (0.50 cm) for each one. It was revealed that insignificant differences were recorded among the rootstock in mean of average leaf number (ALN). In terms of medium strength effect, significant differences were observed among them as micrografts of Red globe at 25% MS, 50% MS, and 75% MS gave the highest mean ALN without significant difference among them. Regarding the interactions, there were obvious differences between the two factors that appear clearly in grafts of Red globe on Freedom at 75% MS (4.2), on SO4 and on Ramsey at 25% MS (4.80, 3.80) respectively, while the lowest values were on SO4 at full MS (2.00). Obviously found that grafts of Red globe on Freedom and Ramsey rootstocks showed the highest mean of graft union formation percentage (GUF%) (88.75, 93.75%) respectively, while the lowest GUF% was on SO4 (66.63%). Also significant differences were recorded among medium-strength levels regardless of the rootstock types which were clearly shown at 50% MS as gave the highest GUF% (100.00%). Concerning the interaction, the highest GUF% was on Freedom at 50% MS and full MS (100.00%), on Ramsey at all strength MS (100.00%) except 75% MS (75.00%) and on SO4 at 25% and 50% MS (100.00%) while the lowest GUF% was at full MS (0.00%). Concerning micrografts rooting percentage (MR%), the grafts on Freedom significantly achieved the highest mean of MR% (70.83%). On the other hand, 50% MS gave the highest mean MR% regardless of the rootstocks (91.73%). Regarding the interaction, the grafts on Freedom at 25% and 50% MS, on SO4 at 50% MS and on Ramsey at 25% MS achieved the highest MR% (100.00, 100.00, 100.00, 87.38%) respectively, while the lowest MR% was on all rootstocks at full MS (0.00%). Red globe grafts on Freedom significantly gave the highest mean of average

root number (ARN) per micrograft (10.50). Regarding the medium strength effect, significant differences were observed in mean ARN regardless of the rootstocks which shown that 25%, 50 and 75% MS gave the highest ARN without significant differences among them (6.50, 8.00, 7.13) respectively. Concerning the interaction, there were significant differences between the two studied factors which appeared clearly on freedom at 50% and 75% MS(15.20, 14.80) respectively, the grafts on Ramsey at 25% and 50% MS (4.20, 5.20) respectively, while SO4 at 25%, 50%, and 75% MS have high ARN without any significant differences among them, whereas the lowest value was observed on all rootstocks at full MS which did not give any new roots (0.00). The results indicated that insignificant differences were recorded among the rootstocks in the mean of average root length (ARL). On the other side, significant differences were recorded among medium-strength levels in the mean of ARL regardless of rootstocks and it appeared significantly at 25% MS (2.26 cm). Interaction between the studied factors revealed that the highest values of ARL were on Freedom at 75% MS, (2.76 cm), on Ramsey, and SO4 at 25% MS.

Table 3. Effect of medium strength on some grafting characteristics of Red globe micrografts

MS	Scion survival%				Scion bud burst%				Average shoot number/scion			
Strength	F	R	S	Mean	F	R	S	Mean	F	R	S	Mean
25 %	87.50b	100.00a	50.00d	79.17B	66.50c	50.00d	40.00e	57.17B	1.00a	1.00a	1.00a	1.00A
50%	87.50b	50.00d	37.10e	58.20D	85.70b	25.00f	33.30e	48.00C	1.00a	1.00a	1.00a	1.00A
75%	100.00a	75.20c	37.10e	70.77C	83.30b	33.30e	100.00a	71.97A	1.00a	1.00a	1.00a	1.00A
100%	100.00a	80.00c	80.00c	86.67A	60.00c	25.00f	25.00f	36.67D	1.00a	1.00a	1.00a	1.00A
Mean	93.75A	76.30B	51.10C		73.88A	33.33C	49.58B		1.00A	1.00A	1.00A	
	Average shoot length (cm)				Average leaf number				Graft union formation%			
25 %	2.50ab	2.00b	3.50a	2.67A	3.20bcd	3.80ab	4.80a	3.93A	75.00b	100.00a	100.00a	91.67B
50%	2.50ab	1.90bcd	2.50bc	2.3AB	3.20bcd	3.60bc	4.00ab	3.60A	100.00a	100.00a	100.00a	100.00A
75%	2.60ab	1.80bcd	2.10bc	2.17AB	4.20ab	3.40bc	3.40bc	3.67A	80.00b	75.00b	66.50d	73.83C
100%	0.50d	1.00cd	0.50d	0.67C	2.40cd	3.00bcd	2.00d	2.47B	100.00a	100.00a	0.00a	66.67D
Mean	2.03A	1.68B	2.15A		3.25A	3.45A	3.55A		88.75A	93.75A	66.63C	
	Micrografts rooting%				Average root number				Average root length(cm)			
25 %	100.00a	87.38b	70.20e	85.86B	12.00b	4.20c	4.20c	6.80A	1.49b	2.59a	2.68a	2.26A
50%	100.00a	75.20d	100.00a	91.73A	15.20a	5.20c	3.60cd	8.00A	1.72b	1.48b	1.66b	1.62B
75%	83.30c	25.20f	87.38b	65.30C	14.80a	2.40d	4.20c	7.13A	2.76a	1.75b	1.23b	1.89AB
100%	0.00g	0.00g	0.00g	0.00D	0.00e	0.00e	0.00e	0.00B	0.00c	0.00c	0.00c	0.00C
Mean	70.83A	46.95C	64.37B		10.50A	2.95B	3.00B		1.50A	1.46A	1.40A	

F: Freedom, R: Ramsey, S: SO4. Means followed by the same letter (s) in each column are not significantly different at p≤0.05 level.

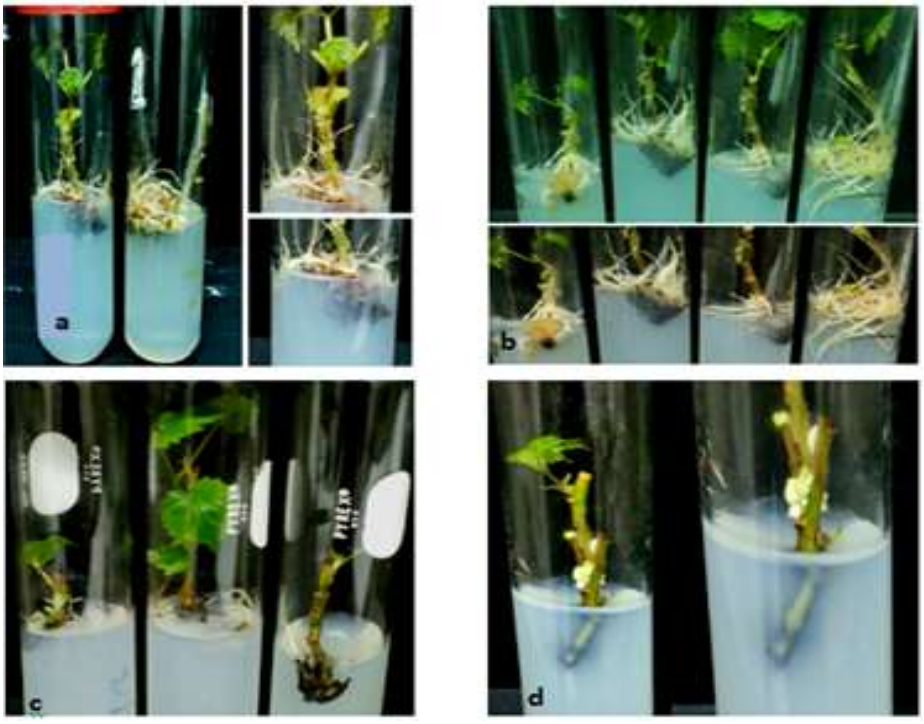

Figure 8. Red globe on Freedom micrografts cultured in different strength of MS a.25%, b.50%, c. 75%, d.100%

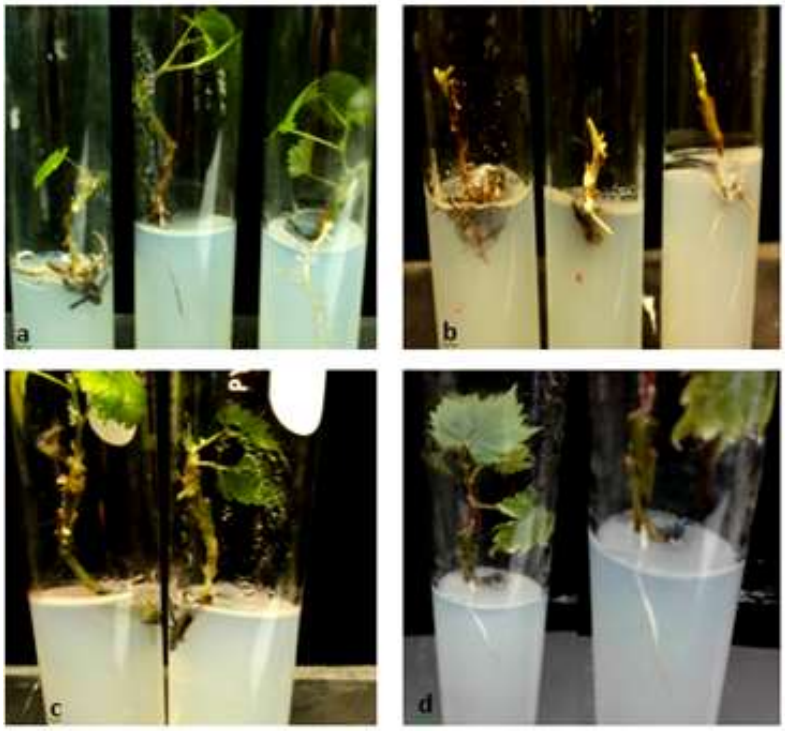

Figure 9. Red globe on Ramsey micrografts cultured in different strength of MS a.25%, b.50%, c. 75%, d.100%

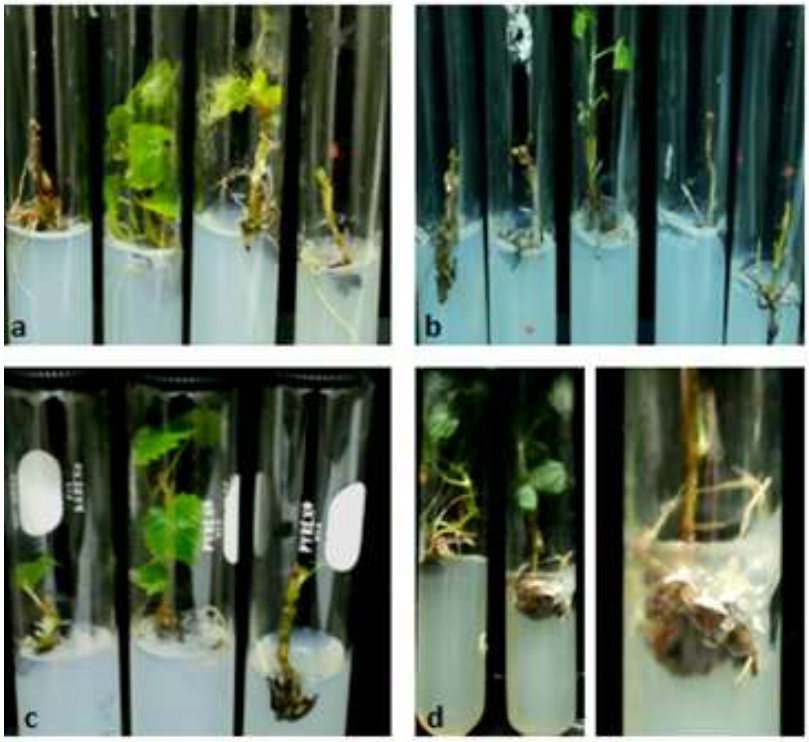

Figure 10. Red globe on SO4 micrografts cultured in different strength of MS a.25%, b.50%, c. 75%, d.100

The graft success as a means of plant propagation is greatly influenced by the nature of the culture medium used. The purpose of the culture medium, is to provide optimum conditions for the growth of the grafts. The most widely used culture medium is MS medium, because most plant cell cultures react to it favorably. It is classified as a high salt medium in comparison to many other formulations, with high levels of nitrogen, potassium, and some of the micronutrients, particularly boron and manganese (11). The findings of this experiment showed that the strength of the medium had a significant effect on all parameters measured. The highest values for all parameters measured were

obtained from the micrografts cultured on 25%, 50% and 75% MS medium showing a significant difference compared to full MS which obtained the lowest values except scion survival percentage. It was clear from the obtained results that the graft growth was better using lower strength of MS. The highest percentage of scion budburst was obtained on 75% MS, while the highest percentages of graft union formation and rooting were obtained on 50% MS, whereas the longest shoot and root were obtained on 25% MS. On the contrast, full MS produced the lowest values in all studied parameters and prevented the rooting in all micrografts on the studied rootstocks. These findings are in agreement with those obtained by (7) who stated That the highest root length and root number in micro cuttings of tea were obtained with application redundancy MS medium (25% macro element). The lowest root length and root number in micro cuttings of tea were obtained with application full MS medium. (53) stated that the reduction in the strength of MS medium resulted in the increase of in vitro shoot and root formation from blue berry. (44) reported that root number and root length of Stevia rebaudiana plant cultures were significantly influenced by the strength of MS medium (25%MS, 50% MS and full strength MS). Several researchers in the tissue culture of grapes used MS medium (52, 1, 49). (35) reported that rooting 'Marechal Foch'grapevine in half-strength MS salts was superior to rooting in full-strength MS salts. The effect of the medium strength could be possibly associated with particular components of the culture medium. For example, even minor changes in the concentration of trace elements can affect plant organogenesis in vitro. (10) observed a radical reduction in the number and length of roots induced from white poplar (Populus alba) cultures when zinc concentration in the culture medium was increased.

Anatomical study

Red globe grafted on freedom rootstock

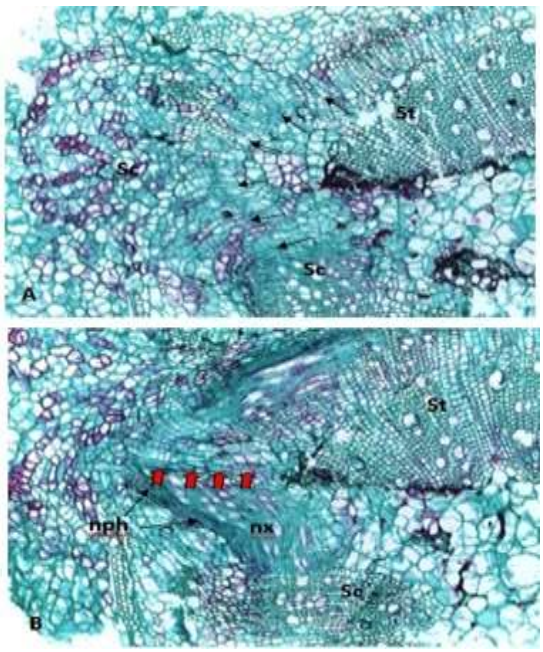

Figure 10. Cross section in the graft union of red globe/freedom
Sc: scion, St: stock, nph: new phloem, nx: new xylem

Figure 10 shows two stages in connection cambium development. ″A″ an early stage, the cambium is well developed (arrows). In ″B The development of vascular tissues (xylem and phloem) from the activity of the connection cambium. Although the graft is apparently successful but there is a weak zone lacking the vascular tissues indicated with arrow heads in ″B″. Figure 11 illustrates that there was an advanced stage similar to figure ″B″, the two partners of cambium produced considerable amounts of vascular tissues, these tissues were not connected (the zone facing the two arrows).

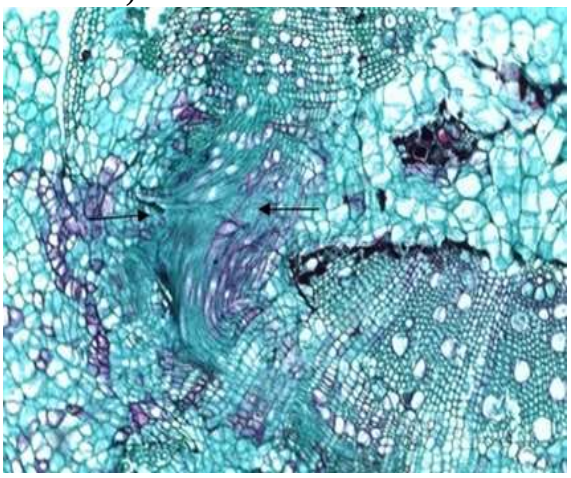

Figure 11. Cross section in the graft union of Red globe/Freedom shows an advanced stage

Red globe grafted on Ramsey rootstock
Figure 12 reveals the initial cells of the callus. It initiated from the ray parenchyma of the xylem, from the cambial derivative cells and from the phloem parenchyma.

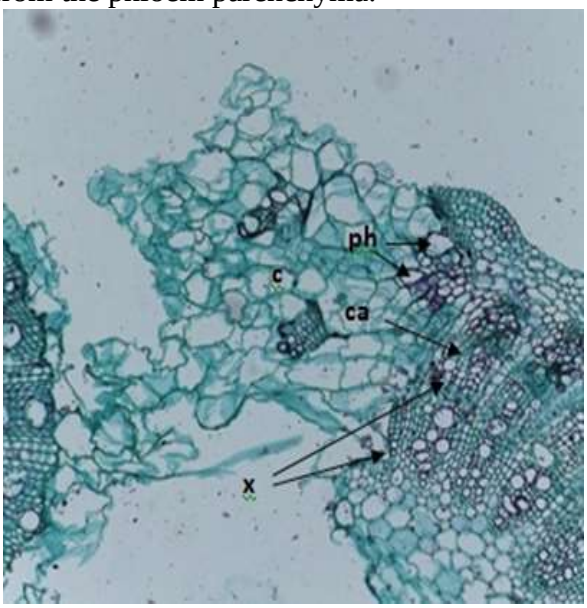

Figure 12. Cross section in the graft union of Red globe/Ramsey micrograft
C: callus, ca: cambium, ph: phloem, x: xylem

Red globe grafted on SO4 rootstock
Figure 13 indicated that the graft was unsuccessful as all the callus cells have their origin from the scion which is clearly observed in ″B″. Firstly, this failure due to the wide space between stock and scion. Secondly, to the un-matched vascular tissues of stock and scion.

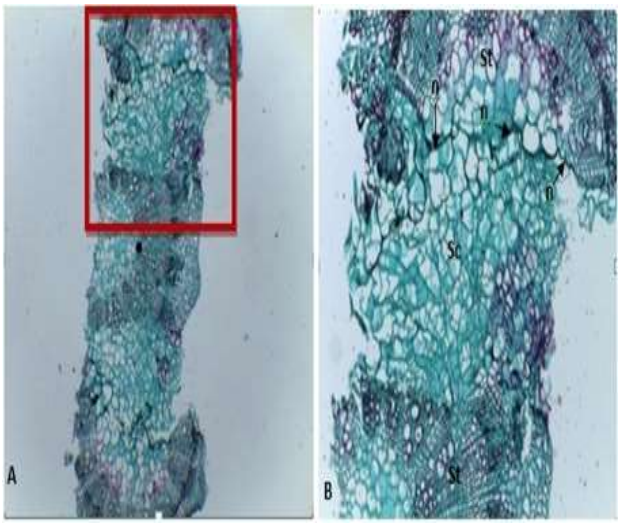

Figure 13. Cross section in the graft union of Red globe/SO4
n: necrotic layer, St: stock, Sc: scion. B: enlarged view of the part A

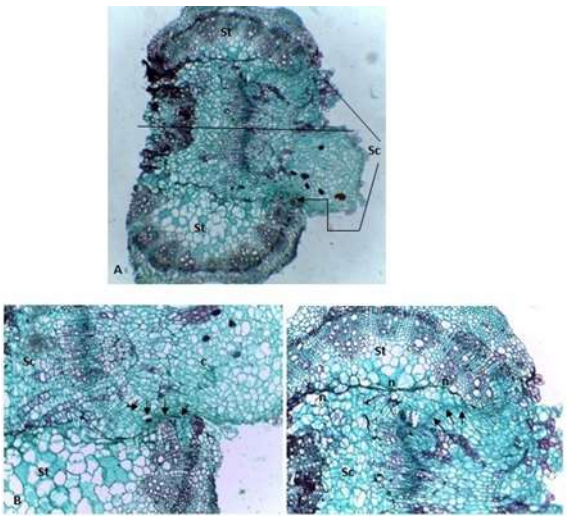

Figure 14. Cross section in the graft union of Red globe/SO4 micrograft
St: stock, Sc: scion, n: necrotic layer, c: callus, B: an enlarged view of the lower part of A. C: an enlarged view of the upper part of A

In **Figure 14,** B shows an early stage of connection cambium differentiation (arrows). A and C , somewhat later stage of cambium development connecting the two partners of the graft (arrows in C). Compatibility is extremely important for The success of grafting on the of the graft union to make fast development of vascular match between the stock and the scion (42), which will allow continuing the growth of both the root and the canopy (23, 34). The callus is a tissue produced to cover wound surfaces of the plant and formed by parancimatic cells differentiating from the phloem zone near the cambium. In further periods there are cellular differentiation in callus, some cells transform to produce cambium and cambium produces xylem and phloem. Thus vascular tissue between stock and scion is formed and water and food transfer is provided (37, 59, 8). Researchers reported that after grafting, callus continues development thus the gaps between stock and scion filled and necrotic layers break and disappear (12, 61) as small pale-colored necrotic masses while other parts of it remained as very dark-colored tissue. According to (9) in situations where necrotic layers have not broken, cambial differentiation took a long time. In this study, Freedom rootstock activity was clearly observed in cell dividing when grafted compared to Ramsey and SO4 which were producing limited 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 (49). In this situation, the anatomical observations indicated that successful grafting was observed when Red globe grafted on Freedom rootstock as both stock and scion were sharing together the formation of callus cells to fill the gaps between them in parallel with breaking the necrotic layers and a new cambium produced perceptible amounts of vascular tissues (xylem and phloem) these observations in agreement with that stated by **(**42, 49). Regarding Red globe on Ramsey rootstock combinations, graft union formation was more difficult because of less callus formation and dense necrotic layers caused more wavy cambial contact and relatively weak vascular tissues (4, 23). On the same side, the combinations of Red globe on SO4 showed that the vascular tissues of the stock and scion were unmatched although the stock and scion contributed to the formation of callus and intermingled, no connecting cambium was established and dense necrotic layers were observed. (9, 13, 45). It is observed that Red globe is quite compatible with Freedom rootstock supported by the anatomical observations that declared enough callus formation, less necrotic layers, and a wavy cambial continuity of new cambium. Whereas on Ramsey and SO4 combinations where has relatively less callus formation, dense necrotic layers caused quite wavy cambial continuity result to from the weak and late formation of vascular tissues. These findings also revealed that different stock scion combinations gave different levels of callus formation and rooting compatible with the previous studies.

Acclimatization of micrografted plantlets

Effect of soil mixture : The survival rates of micrografted Red globe on Freedom transplants after acclimatization planted on different artificial soil mixtures were presented in **Figure 15.** It is clear that the plantlets affected by the characteristics of the mixture, which the sterilized 1:1:1 (v:v) peatmoss+perlite+sand mix were found to be effective of *in vitro* plantlets hardening which significantly gave the highest survival (100%) and showed superior vegetative growth compared to other soil mixtures, whereas the lowest rate was in 1:1 (v:v) peatmoss+perlite (50%). It seemed that organic matter represented with peatomoss strongly affected the plantlets survival, whereas the presence of sand with peatmoss as 1:1 (v:v) may modify the concentration in the mixture leading to improvement in survival rate (80%) Immediately after transplantation, visible wilting was observed. However, the water status of plants could still stable after some days or weeks.

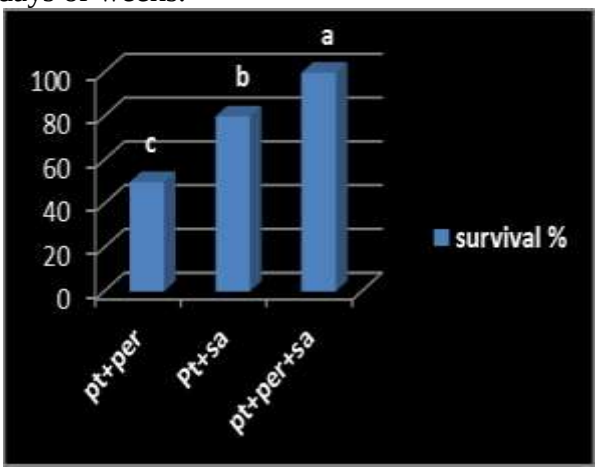

Figure15. The survival rates of micrografted Red globe on Freedom transplants after acclimatization planted on different artificial soil mixtures. Pt: peatmoss, Sa: sand, Per: perlite

The physical and chemical properties of the soil media have obviously different impact on the plantlet survival. Therefore, different workers have suggested different media such as soil-vermiculite mixture (19)**,** soil (33), and sand-peatmoss (21). Among various potting mixtures tried, the mixture containing sand+peatmoss+perlite (1:1:1) was found to be the most suitable. Physical, chemical and biological properties of the potting mixture display a vital role in the establishment of in vitro produced plantlets (27). The better performance of peatmoss might be attributed to its ability to improve the biological properties of the mixture. Sand may be responsible for providing sufficient aeration and high water holding capacity besides satisfied water drainage and good ventilation providing by perlite. Hence mixing sand, peatmoss, and perlite in equal volumes might have helped in giving a better grip for the

roots, ample aeration, and sufficient organic matter. Similar results have also been obtained by (48, 41, 21).

Figure16. Acclimatization of Red globe grafted on Freedom rootstock in 3 kinds of artificial soil mixture inside the glass chamber

Figure17. Red globe/Freedom planted on peatmoss+perlite mixture (1:1) 4 weeks after the acclimatization

Figure18. Red globe/Freedom planted opeatmoss + sand mixture (1:1) 4 weeks after the acclimatization

Figure 19. Red globe/Freedom planted on peatmoss+perlite+sand mixture (1:1:1) one month after the acclimatization

Figure 20. Red globe on Freedom micrografts planted in plastic bags in the glasshouse

Effect of rootstock type on the acclimatization success

The results presented in **Figure 21** show that Freedom rootstock is considered more effective for Red globe micrografts than SO4, as it achieved the highest survival rate (100%) compared to those on SO4 (80%). Moreover, a difference in vegetative growth was recorded which was vigorous and higher on Freedom than on SO4. Furthermore, Freedom was obviously had the highest number of roots and longer than in SO4 rootstock.

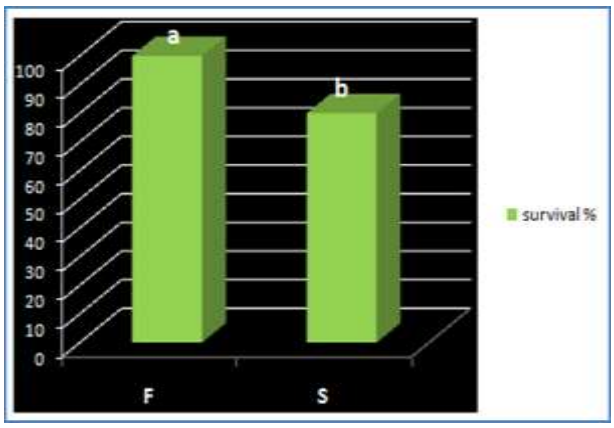

Figure 21.The survival rates of micrografted Red globe on Freedom (F) and SO4(S) transplants after acclimatization

Figure 22. Acclimatization of Red globe on Freedom and SO4 rootstocks inside the glass chamber at the incubation room

Figure 23. Plantlets of Red globe/Freedom 4 weeks after the acclimatization

Figure 24. Plantlets of Red globe/SO4 4 weeks after the acclimatization

Acclimatization success is greatly associated with the plantlets survival and considered the best indicator for the selection of suitable genotype and soil medium. On the other hand, the low growth rate during the acclimatization stage could be handled by proper fertilization and irrigation schedule after transferring the survived plants to nursery beds or bigger pots (21). The remarkable growth and roots of the survival plantlets could be explained by the successful acclimatization and these results is in agreement with those obtained by (37) who reported that rooting of grafted plants may happen when the roots first initiated in vitro and after that grown outdoor on root growing media and those recorded by (46, 29) who stated that the more successful transplantation of plantlets, the high growth can be achieved**.** Furthermore, different stages were noted during the acclimatization firstly, an adaptation stage with slow shoot growth and root formation, secondly a fast growth of roots and shoot (56). These present results went in parallel with (49) who reported that Freedom micrografts achieved a satisfied response to transplant in sterile soil mixture through Superior on Freedom micrografts gave the highest survival rate (70%) followed by Flame seedless and Thompson seedless (60%).

CONCLUSION

The findings of this study showed that Red globe on un-rooted Freedom rootstock

significantly achieved the highest percentages of scion survival (100.00%), scion bud burst (60.00%), graft union formation (60.00%), rooting (40.00%), shoot length (2.00 cm), root number (8.00) and root length (4.50 cm). Regarding the response to IBA concentrations, Red globe on Freedom was better than other studied rootstocks which achieved the highest values in all studied parameters. It is obvious that the micrografts on $1mgL^{-1}$ IBA achieved the highest percentages of scion survival (95.20%), scion bud burst (65.43%), graft union formation (82.22%) also, longest shoots (1.84 cm), and maximum average leaf number (2.50), while on $0.1mgL^{-1}$ IBA gave the highest rooting percentage (47.60%). Red globe on Freedom micrografts on MS medium supplemented with 0.1 mgL^{-1} IBA recorded the highest percentages of scion survival (100.00%), scion bud burst (87.38%), rooting (100.00%), and the highest average root number (12.60). on the other side, the results of the medium strength experiment showed that 25% MS, 50% MS, and 75% MS achieved positive effects on all parameters compared with full MS. The results of the anatomical study have shown that the callusing level in graft union was higher in Freedom compared to Ramsey, and SO4. It was clearly noticed that Red globe on Freedom combinations was Successfully survived as enough callus formation, less necrotic layers, and a wavy cambial continuity of new cambium were identified. Acclimatization results revealed that 1:1:1 (v:v) peatmoss+perlite+sand mix significantly gave the highest survival (100.00%) and showed superior vegetative growth. Red globe on Freedom micrografts achieved the highest survival rate (100.00%), better vegetative growth, and longer roots compared with those on SO4 and Ramsey (80.00, 70.00%) respectively.

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