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Using New Micro Grafting Techniques in Grape

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ABSTRACT

Micro-grafted Egyptian grapes were evaluated under *in vitro* conditions. *In vitro* Flame seedless cv. scions were grafted on Freedom rootstock shoots. Two methods were used for micro-grafting; alginate matrix and rhizogenic calli. Fluorescein stain was used to sap transport detection through vessels of both scion and rootstock. Biochemical and molecular analyses were used to evaluate successful micro-grafts. Obtained results showed that, *In vitro* derived shootlets had satisfactory growth. The culture of derived explants formed a successful graft union by alginate matrix (75%) compared to rhizogenic calli (50%) grown shootlets maintained by some roots and leaves formation. There was a significant difference between the two methods in forming a successful graft union and the subsequent growth-related traits. Grafted explants were tested to salinity stress of NaCl: CaCl₂ 1000, 2000 and 3000 ppm. The shoot and root related growth characteristics of the alginate matrix micro grafts were more tolerant to salinity than rhizogenic calli. However, raising salinity level led to growth decrease but alginate plantlets continued forming leaves. RAPD- PCR analysis cleared genetic changes in graft union area reflects a complete scion- rootstock combination, also a hereditary relationship with both was detected and proved micro-grafting success with the two methods. To sum up, micro-grafting is a suitable alternative propagation tool leading to higher growth potential of grafted plants and improving their ability to tolerate different stresses.

Keywords: Grape -Freedom - Micrografting - RAPD - Fluorescein - Antioxidants- Total phenols - Oxidized enzymes- Salinity

INTRODUCTION

Grape (*Vitis* spp.) has a great commercial value in many countries all over the world due to its high income as a favorable fruit. So, it's worthy to find modern methods to maximize its production especially the new breed cultivars.

Since traditional grafting is the main way for grapes propagation, micro-grafting has an extra advantage because it's more flexible for the biomass production.

Thus, micro-propagation techniques are being applied to bring rapid improvement in fruits.

In this regard, there is a priority for conducting much research with both basic cytogenetic and taxonomic problems mentioned by breeders and by demanding cultivars that adapted to various climates and the use (Luo, 2004).

Micro-grafting technique is important mainly for woody species because meristem-tip culture of such plants is often difficult so it has been attempted as adult tissues rejuvenation mean. Also, it's a usual method for vegetative propagation of difficult to root micro shoots, or those which unable to form roots *in vitro* since they can be micro-grafted onto rootstocks to have rooted plants.

In addition, *in vitro* micro-grafting may provide several advantages such as plant production a year-round, the viruses elimination, developing compatibility studies and the correlation between scion and rootstock, extending ecological limits of a cultivar or plant species to tolerate edaphic conditions and making specific genotypic combination that mitigate plant productivity (Pathirana and

McKenzie, 2005 and Dobranszki and Jambor-Benczur, 2006).

Kim *et al.*, (2005) pointed out that the success of micro-grafting primarily depends on the grafting technique.

However, the cleft grafting was the most efficient and successful technique for micro-grafting. Also, working on grapes micro-grafting, Zhu *et al.*, (2007) mentioned that grafts and acclimatized transplants survival differed greatly according the grafting method. Grafting shoot tip/ rooted stem without leaves recorded 90% survival.

Another study set up by Paranychianakis and Angelakis (2008) on Soultanina vines grafted on three rootstocks and irrigated with effluent containing three high salts concentrations. Their finding suggest that grapevines have additional mechanisms cope with salt stress which may control differences in salts uptake and accumulation in shoots.

Also, Peccoux (2011) noticed that climate change and land scarcity to expand producing areas and resources become a need. As for grapes, aside canopy systems and management, plant material choice, root system architecture and its hydraulic properties are important constitutive traits identified between rootstocks. The stress levels x genotypes comparison showed that most of them involved in lipid metabolism and cell wall processes.

Bourrain and Charlot (2014) studied cherries varieties and rootstocks compatibility V-shaped (*In vitro*) shoot tips were scions, while rooted shoots were rootstocks. The highest successful grafts percentages

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obtained using modified MS, glucose and vermiculite in the agar medium.

Moreover, Dumanoğlu *et al.*, (2014) obtained a semi-dwarf pear rootstock that is tolerant to various stresses using micro-grafting. (*In vitro*) shoots used as inter stock and seedlings used as rootstock. Grafted seedlings cultured on ½ strength MS under white fluorescent light for 1.

Aloni *et al.*, (2010) and Grohs *et al.*, (2017) noted that grafting hormonal balance affect grape propagation.

Also, Cookson *et al.*, (2013) characterized the hormonal signaling related to the rootstock/ graft relationship at molecular level, observing that the expression of this signalization is differentiated in time and specific for the rootstock, the grafting and the grafting interface.

Jeanine Risco *et al.*, (2017) investigated the effects of PGRs, holding grafts, scions pre-treatment with BAP and rootstock × scion combination on lucumo micro-propagation and micro-grafting. They indicated that the best graft rate was obtained with a rootstock × scion combination, cooper wire and pre-treated scions.

Sammona *et al.*, (2018) micro-propagated three grape rootstocks till micro-grafted by Superior scions. Results showed that the most efficient sterilizer was NaOCl 1% for 10 or 15 min depending on the rootstocks. Freedom significantly accomplished the highest shoot and leaf No. and tallest shoots on different media. Superior micro-grafts significantly gained the highest survival percentage after acclimatization.

Concerning the chemical composition, Bidabadi *et al.*, (2017) studied the physiological and biochemical changes at the graft union combinations among Iranian rootstocks and Asgari scion in response to BAP and NAA application. Treating the graft cut-surfaces with 500 mg·L⁻¹BAP +200 mg·L⁻¹NAA improved antioxidant activities but reduced lipid peroxidation in scions leaves. Forming a graft union in the presence of 500 mg·L⁻¹BAP + 200 mg·L⁻¹NAA enhanced POX and CAT activities.

However, Singh *et al.*, (2019) pre-treated scion and rootstock with Ki 1.0 mg L⁻¹ and found it more suitable than BAP and further increased micro-grafting success rate. For further micro-grafts growth, MS medium supplied with 0.5 mgL⁻¹ of BAP and 0.1mgL⁻¹ of IAA along with 5% sucrose resulted in maximum response (56.8%).

This study aimed to develop a short term (*In vitro*) micro-grafting method to create a specific grape combination using Flame cv. and Freedom rootstock seedling; which is vigorous and tolerant to different stresses. Also, to study graft union formation of *In vitro* micro-grafted plantlets in complete darkness which may prevent degradation of the internal auxin, by morphological and anatomical (Transmission Electron Microscope) investigations.

MATERIALS AND METHODS

1. Plant material

a. Rootstock and scion selection

The presented study was performed during growth seasons from 2018 to 2019 on (*In vitro*) shoots of Flame grape cv. as a scion, grafted on Freedom as a rootstock by new tissue culture techniques. The explants shoot tips

grown in the green house of Grape Department Orchard, Horticulture Research Institute, A. R. C. The green shoots of both rootstock and scion were selected for culture establishment and micro-grafting.

b. Disinfecting explants

All the micro-grafting explants were washed well with soap and tap water to remove surface dust. Surface sterilization was carried out for 10 min in disinfectant contains 0.2% Tween 80 and shaking on a rotary shaker 50 rpm. (Pathirana and McKenzie, 2005) and subsequently immersing in 70% ethanol for 60 sec., followed by dipping in 15% sodium hypochlorite for 15 min. and rinsing with sterile distilled water (El Shaima, El Botaty, 2012). The sterilized shoots were then cut into single nodes and cultured on MS (Morashige and Skoog 1962) hormones free medium containing (per liter) 25 g sucrose, 0.1 g m yoinstol, 7 g agar and set at a pH 5.7.

The shoots split into half lengthwise at the node to achieve sufficient multiplication and horizontally cultured with the cut surface contacting the medium and the bud facing upwards (Pathirana and McKenzie, 2005). For micro-grafting, we used the second subculture shoots.

c. *In vitro* Micro-grafting

1. Alginate staking materials

Sodium alginate was prepared by adding 2 g of salts in 100 ml of hot distilled water with continues stirring to complete dissolving. Calcium chloride 20 g was dissolved in 100 ml distilled water and stirred to complete dissolving. All solutions were autoclaved at 121°C for 20 min. The rooted Freedom was de-budded and cut into small pieces 2 – 3 cm, Flame scions were cut into small pieces 2- 3 cm too. The staking matrix was prepared by adding sodium alginate in small sterile wells coated with CaCl₂ and waiting for 30 min till complete polymerization. The matrix was transferred out the wells and the rootstock piece put in a capsule bottom on the oppositeside of the scion piece in the top (Fig. 1-a). The micro-grafted explants were transferred to culture jars containing MS medium plus 0.1 % (v/v) of Flurescein stain. The culture media contained waxy filter paper on their surfaces to isolate the explants from the medium except for the rootstock roots which were immersed in the culture media.

2. Rhizogenic micro-grafting

The small de-budded stem pieces of the rootstock explants were cultured on MS media supplied with 2, 4-D and NAA at 1 mg/l for each. The rhizogenic calli were formed after 4 weeks. The rooted calli were isolated and scion buds were put in the middle of calli and cultured on the medium surface.

d. Plant parameters

1. Morphological characterization

Successful grafts % = [survival count of grafted explants/total grafted explants] x 100

Callusing percentage = [callus formation in grafted area/ total successful grafts] x 100

Rooting percentage = [roots formation in graft area/ total successful graft] x 100

Growth formation organs: scion elongation (mm) and leaves formation.

2. Chemical characterization

a. Total Phenols

Total phenolic compound contents were determined according to Folin-Ciocalteu method (Singleton *et al.*,

1999). Extract samples (0.5 ml of each dilution) were mixed with 2.5 ml of 0.2N Folin-Ciocalteu reagent for 5 min and then 2.0 ml of 7.5% sodium carbonate were added. The reaction absorbance was measured at 760 nm after 2 h of incubation at room temperature. Gallic acid equivalents expressed the results.

b. Total flavonoids

Total flavonoids were estimated using Woisky and Salatino (1998) method by adding 0.5 ml of 2% AlCl₃ methanol solution to 0.5 ml of sample. After an hour at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents; as quercetin were calculated from a calibration curve.

c. Antioxidant activity

Free radical scavenging activity (DPPH –RAS method)

The free radical scavenging activity of methanol extracts was measured by α , α -diphenyl- β -picryl-hydrazyl (DPPH) using Brand-Williams *et al.*, (1995) method. A methanolic DPPH solution was prepared at 0.1 mmol/l and 1 ml of it was added to 3 ml of extracts solution at a concentration of 0.1 to 1.5 g. After 30 min, absorbance was measured at 517 nm. Also, the free radicals concentrations were calculated as the next Equ according to Szabo *et al.*, (2006).

$$C_v = A - 7.499 / 429.03$$

Where A is the absorbance, C_v free radical concentrations (10³/mol dm⁻³).

d. Oxidizing enzymes

1. Assay of Catalase Activity (CAT)

This assay mixture (5ml) contained 300 μ M of phosphate buffer (pH6.8), 100 mM of hydrogen peroxide and 1ml of the enzyme extract. After 5 min incubation at 25° C, the reaction was stopped by adding 10 ml of H₂SO₄ (2%v/v). The residual H₂O₂ was titrated against 0.01 N KMnO₄ until a faint purple colour persistence for at least 15 seconds. A control was run at the same time, in which the enzyme activity was stopped at zero time. Catalyse activity was expressed as μ M destroyed/mg protein/min (Turkan *et al.*, 2005).

2. Assay of polyphenol Oxidas Activity (PPO)

The assay mixture (5ml) was prepared containing 125 μ M of phosphate buffer (pH 6.8), 100 μ M of pyrogallol and 1ml of crude enzyme extract. After incubation at 25° C for 5 min, the reaction was stopped by the adding 1ml H₂SO₄ (10%v/v). The colour intensity was measured at 430 nm and the enzyme activity was expressed as the optical density/ mg protein/ ml change (Beyer and Fridovich, 1987).

3. Assay of Peroxidase Activity (POX)

Five ml of the assay mixture contained 300 μ M of phosphate buffer (pH 6.8), 50 μ M catechol, 50 μ M H₂O₂ and 1 ml of crude extract. After incubation at 25° C for 5 min, the reaction was stopped by 1 ml of 10 % H₂SO₄ (v/v) addition. The colour intensity was read at 430 nm and the optical density/ mg protein/ min change expressed the enzyme activity (Racusen and Foote, 1965).

3. RAPD analysis

Reagents used - Target

DNA (10-100ng), oligonucleotide primers (10-mers Primers OPA 18, OPB 17, OPB 18, OPB 20, OPC 05, OPD 01 and OPW 04), sterile deionised distilled water, Taq polymerase, dNTP mix (dATP, dCTP, dGTP, dTTP), agarose (Sigma, Molecular biological grade), light mineral oil, Molecular marker VI, Gel-loading buffer (ULB- 0.25% bromophenol blue, TBE buffer (X0.5), 0.25% xylene cyanol FF, 30% glycerol in water; stored at 4° C) and Ethidium bromide (10 mg mL⁻¹). This reaction mix was prepared on ice for the PCR analysis. Table II gives the reagents used per PCR tube. The mix was dispensed into the reaction tubes. The Taq Polymerase was last added. A mineral oil drop was added to each tube for evaporation prevention during reaction. The tubes were placed in a thermal cycler and the PCR program for RAPD was then run - reaction initiation at 94°C for 2 min followed by cycles at: 94°C for 1 min, 35°C for 1 min finally 72°C for 1 min and forty such cycles were done.

4. Stain detection by fluorescein

A 0.1- 0.3 drop of fluorescein sodium diacetate (FSD) (C8166, Sigma, St. Louis, MO; stock solution 50 mg ml⁻¹ in acetone) was added to the culture media at a concentration of 0.1 and 50 mg ml⁻¹ in distilled water, respectively. The fluorescence of the dyes was visualized and recorded 5- 30 min after the application using a fluorescence stereomicroscope (SMZ-U, Nikon, Japan) and a CCD camera (DXM1200, Nikon, Japan).

5. Salinity treatments

The successful micro grafted explants were cultured on culture medium containing 1000, 2000 and 3000 ppm of NaCl+CaCl₂ (1:1) for 4 weeks.

6. Statistical analysis

Data was statistically analyzed by analysis of variance (ANOVA), and least significant differences (LSD) at probability of 5% was applied to separate the means using Co-Stat 4.11 software.

RESULTS AND DISCUSSION

1. Successful micro-grafting

a. Morphological changes

The data presented in Table 1 show that using micro-grafting by alginate matrix succeeded to make a union area between rootstock and cultivar by 75 % with callus formation around union area 66.6%. The micro-grafts formed roots on union area reached 100 % for all explants and formed little leaves on this area. After four weeks of micro-grafting process the scion was elongated at the average 0.24 cm from the beginning length. No scion branching recorded.

In this regard, Pathirana and Mckenzie (2005) observed that micrografting grapes two scions on two rootstocks in all possible combinations showed 77.8% success rate.

Table 1. Effect of micro-grafting method on growth characterization

	Successful grafts percentage	Callusing percentage	Rooting percentage	Leaves formation (4weeks)	Scion branching	Scion elongation
Matrix (Alginate)	75.00	66.66	100.0	1.5	0.00	0.24
Rhizogenic	50.00	0.00	0.00	2.4	0.00	0.00
F- Test	*	*	*	*	NS	*

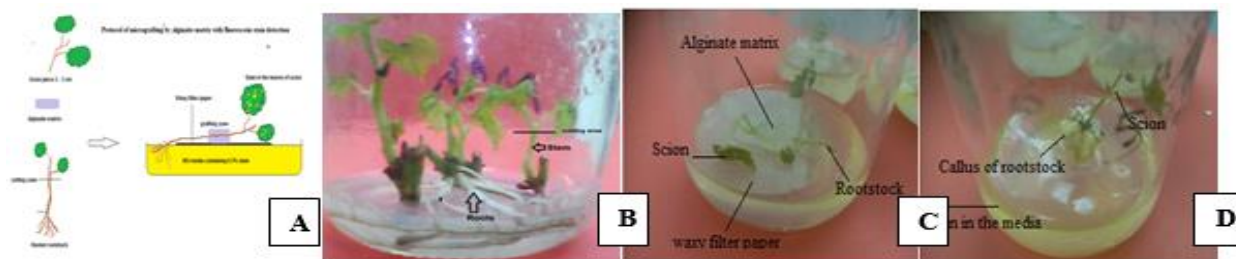


Fig. 1. (a) Micro-grafting steps by using alginate, (b) Root formation in union area (c) After micrograftin by alginate (d) After micro-grafting by rhizogenesis

The callus cells of rootstock containing few roots (Rhizogenic calli) can be used as a rootstock, although these cells haven't transferring vascular, they are a good connector from the media to the scion. So, this method gave a good survival percentage 50 % with new leaves formation 2.4 leaves as an average, but no elongation or branching of scion were observed (Fig. 3-b). In this concern, grafting success was determined by callus formation and rooting. Micro-grafts tended to form calluses at a graft union junction, arising from both, scion and rootstock living cells (Fig. 3-a). Moore and Walker (1983) noticed that adjacent masses of callus tend to graft successfully in vascular differentiation forfeit. However, callus formation was determined by independent grafting success, although callus growth constitutes a key factor in the graft union development. Kim *et al.*, (2005) found that the rootstock Couderc 3309 was successful in rooting, callus formation, and growth with all scion types. Among the rootstocks or scions, they found that grafting compatibilities ranged between 11 % (Tam-nara/Rupestris du Lot) and 100 % (Schuyler/Couderc 3309).

In other words, graft union formation depends on callus initiation process of both rootstock and scion, on callus union, and following differentiation of callus tissue forming the vascular and protective tissue required to form a functional unit from the rootstock and scion parts. Differentiation is not usually considered as a problem, but often there are difficulties at callus initiation and proliferation (Panea *et al.*, 1997). Several authors noticed that, PGRs such as auxins and cytokinins can induce callus initiation and proliferation and new vascular tissue by promoting cell division and/or cell development (Preece and Read, 1993).

b. Dye loading and detection

According to the Fig. (1-c) using alginate gel in micro-grafting connected the scion to the rootstock, fluorescein stain used to detect the cell sap transfer from the rootstock to scion. After five days of micro-grafting, scion leaves were isolated and investigated under UV device to scan fluorescein stain Fig. (2-a). After 10 days the stain still present but light concentration (Fig. 2-b) and decreased after 15 days Fig. (2-c).



Fig. 2. UV scan for fluorescein stain transfer in the grape vessels after 5, 10 and 15 days of micro-grafting process.

Within two weeks of complete darkness incubation, callus tissue between graft elements at graft union in treated samples was dense. The union tissues cells were generally flattened and dispersed. However, parenchymatous cells that produce callus tissue were

formed by large cells with large inter-cellular spaces. Some cells cytoplasm was dense. At this stage, the established cambial relation between graft elements was very clear. New xylem and phloem elements were few in number and they started to differentiate (Figure 3).



Fig. 3. Rhizogenic callus included scion and culture on media containing stain after micrografting (a and b) and after two weeks (c), alginate matrix after two weeks (d)

Methods of grafting and explants types are of main determinants in successful micro-grafting operation. *In vitro* culture derived micronodes showed better response when compared to *In vivo* derived counterparts. These findings meet the results in apple, plum, peach and grape (Tangolar *et al.*, 2003).

Meanwhile, *In vitro* derived shoot-tips recorded higher successful graft union compared to *In vivo* grown ones in grapes (Aazami and Hassanpouraghdam, 2010). Providing a solution for some anatomical or physiological difficulties between related species and cultivars is one of micro-grafting main aims. This has been answered by several authors in many fruits. Another common problem is the low percentage of successful graft union. For the majority of plants and in most cases this is due to the shoot-tip organ small size, excision complications, handling, grafting and subsequent maintenance of grafted assembly and leading to the drying shoot-tips and low graft integration. Despite that, *In vivo* derived shoot-tips had

larger size than *In vitro* derived ones, so handling them was easier (Baydar and Celik, 1999).

c. Chemical changes

According to the data in Table 2 the total antioxidants determined in both rootstock, scion and union area gave insignificant effect between them, the total antioxidants gave the highest level on scion 90.15 mg/100 g FW followed by the union area 85.71 and rootstock 80.21 mg/ 100 g FW. The total soluble phenols were accumulated in the union area after grafting recorded 0.036 mg/ 100 g FW compared to both scion and rootstock 0.017 and 0.015 mg/ 100 g FW, respectively. Phenols accumulation in union area increased oxidized enzymes activity PPO and POD 0.031 and 0.023 mg/ml protein, respectively compared with rootstock and scion (0.0021; 0.001 and 0.0011; 0.002 mg/ml protein, respectively). Catalase activity and total flavonoids in all explants have no significant difference among them.

Table 2. Effect of micro-grafting methods on chemical composition of rootstock, scion and union area

	Total antioxidants (mg/100 g FW)	Total solublephenols (mg/100 g FW)	Total flavonoids (mg/ 100 g FW)	CAT (mg/ml protein)	PPO (mg/ml protein)	POD (mg/ml protein)
Rootstock	80.21	0.015	0.143	7.33	0.002	0.001
Scion	90.15	0.017	0.097	6.33	0.001	0.002
Union area	85.71	0.036	0.110	6.83	0.031	0.023
LSD 5%	9.016	0.021	0.019	0.93	0.001	0.001

CAT: catalase activity , PPO : polyphenyl oxidase activity , POD : peroxidase activity

However, their higher concentrations of phenolic compounds and hormonal contents results in higher polyphenol oxidases and peroxidases activities and hence higher browning and drying of fresh tissue just before and beyond integration of grafting elements (Jonard *et al.*, 1983).

d. Genetic changes

DNA amplified bands were scored with all of selected RAPD primers (Table 3) and each “Flame” primer yielded amplification products ranging in size from 300bp to 1000bp, from 400 to 1300 in “Freedom”, and from 200 to 1000 in “Union area”. Number of bands of each primer stable about 10 primers UBC-809, UBC 809, UBC 811, UBC 812 and UBC 817. These 5 primers used in RAPD analysis yielded a total of 25 scorable bands averaged of 62.5 bands per primer in union area, 24 scorable bands

detected in “Freedom” averaged of 60 bands and 17 scorable bands detected in “Flame”, with an average of 42.5 bands per each primer. Totally, 23 monomorphic and 8 polymorphic (34.7 %) bands in union area were produced with all of the tested primers. The highest number of three polymorphic bands was detected by primer UBC-807, whereas other primers resulted in monomorphic bands in union area. Cluster analysis was held on the basis of Jaccard’s similarity coefficients between union area and each of rootstock and scion by means of UPGMA. The similarity values ranged from 0.907 to 1.000 with a mean of 0.945 in union area, they ranged from 0.948 to 1.000 with a mean of 0.976 in Freedom, and from 0.963 to 1.000 with a mean of 0.982 in Flame.

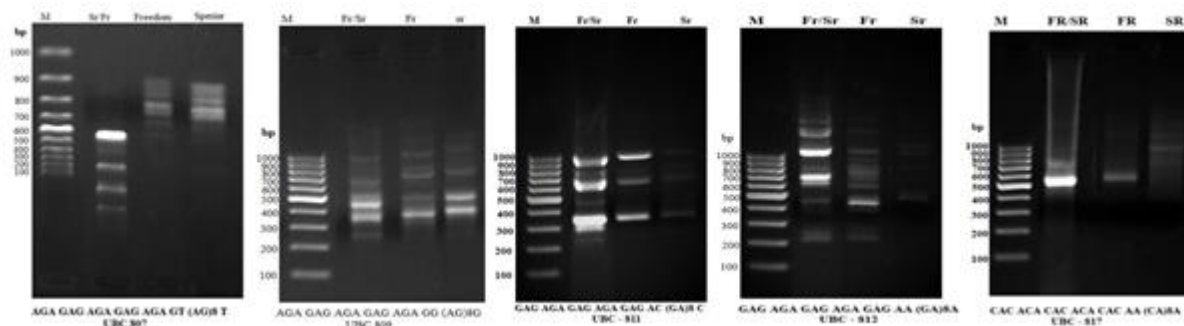


Fig. 4. DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for union area, Freedom and Flame with random oligonucleotide primers.

Data of Table 3 show that variation occurred in both rootstock and scion during micrografting process was presented because two wounded tissues staked by chemical

or physical stress that performs some mutagenic effect on union area especially when callus formed.

Table 3. DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for union area, Freedom and Flame with random oligonucleotide primers.

No	M.W (bp)	UBC-807			M.W (bp)	UBC-809		
		AGA	GAG	AGA GT(AG8)T		AGA	GAG	AGA GT(AG8)G
		Sr/Fr	Fr	Sr		Sr/Fr	Fr	Sr
1	1000	0	1	1	1000	1	1	1
2	900	0	1	1	900	1	1	1
3	800	0	1	1	800	1	1	1
4	700	0	1	1	700	1	1	1
5	600	1	0	0	600	1	1	1
6	500	1	0	0	500	1	1	1
7	400	1	0	0	400	0	0	0
8	300	1	0	0	300	0	0	0
9	200	1	0	0	200	0	0	0
10	100	0	0	0	100	0	0	0
Total		5	4	4		6	6	6

No	M.W (bp)	UBC-811			M.W (bp)	UBC-812		
		AGA	GAG	AGA GT(AG8)C`		AGA	GAG	AGA GT(AG8)A
		Sr/Fr	Fr	Sr		Sr/Fr	Fr	Sr
1	1000	1	1	1	1000	1	1	1
2	900	1	0	0	900	1	1	1
3	800	1	1	1	800	1	1	1
4	700	0	0	0	700	1	1	1
5	600	0	0	0	600	1	1	0
6	500	1	0	0	500	1	1	0
7	400	1	1	1	400	1	1	0
8	300	0	0	0	300	1	1	0
9	200	0	0	0	200	1	0	0
10	100	0	0	0	100	0	0	0
Total		5	3	3		9	8	4

Table 4. DNA polymorphism and monomorphism using randomly amplified polymorphic DNA (RAPD) for union area, Freedom and Flame.

Primer Name	Polymorphic bands	Monomorphic bands	Total Amplified bands	Polymorphism %
UBC – 807	4	0	10	40
UBC – 809	-	6	10	0
UBC – 811	1	9	10	11
UBC – 812	1	9	10	11
Total	6	24	40	62

2. Salinity tolerance

The data tabulated in Table 5 show the effect of micro-grafting process on morphological characteristics of scion union with rootstock. Using alginate in micrografting decreased survival percentage with salinity increasing (75, 50 and 33.3 % for 1000, 2000 and 3000 ppm,

respectively.) these explants formed callus on union area 66.6, 25 and 0.0 %, respectively. Moreover, average of leaves formation were 1.0 leaf / scion and some roots formed on union area. On the other hand, rhizogenic methods gave survival percentage but no leaves formation and growth formation.

Table 5. Effect of salinity on growth morphology of micrografted grape (cv. Flame x rootstock Freedom)

NaCl + CaCl ₂ (ppm)	Matrix				Rhizogenic			
	Survival	callus	Leaf formation	roots	Survival	callus	Leaf formation	Roots
Cont.	100	100.0	2.0	100	50.0	100	0.00	100
1000	75.0	66.66	1.0	100	50.0	100	0.00	100
2000	50.0	25.0	1.0	100	0.0	0.0	0.00	0.0
3000	33.3	00.00	1.0	75	0.0	0.0	0.00	0.0
LSD 5 %	11.29	8.02	0.29	10.18	7.54	6.09	0.00	9.23

In vitro culture conditions provide a great advantage due to independence upon growing season. Furthermore, the possibility of performing micro-grafting in any desired time. Another principal benefit over *In vivo* grown plants is attaining a high number of shoot-tip explants presenting possible frequent individual shoots subcultures under controlled conditions. Other than other asexual propagation ways, micro-grafting produce disease-free, mainly virus-free plants, plus possible benefits of scion rootstock combinations Youssef *et al.*, (2009). In

grape, micro-grafting considered a high-tech method to produce disease-free materials for cultivation such as in breeding programs for virus infections detection Youssef *et al.*, (2009). This propagation method yields homogenous clonal disease-free plants populations, which are capable of high establishment and performance potential in field conditions.

This technique is particularly useful as a rapid method for viral contamination detection in grapevines since it has been applied successfully to detect GRLAVIII

and corky bark viruses within 8- 12 weeks (Pathirana and McKenzie, 2005). Among the limited experimental evidences available to date, it seems that the incompatibility seen in other grafting methods less to micro-grafting. Whereas, selecting the proper indicator rootstock is important to maximize the chances of detecting the viruses because not all the rootstocks used in woody grafting produce similar results. Validation of this method could support grapevine industry by increasing the rate and ease diseases detection that have a serious economic impact on both domestic and imported grapevine material.

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إستخدام تقنيات حديثة للتطعيم الدقيق في العنب

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أجريت هذه الدراسة لتقييم التطعيم الدقيق لصنف العنب الفليم سيدلس تحت ظروف معمل زراعة الأنسجة النباتية و حفظ الأصول الوراثية خلال الفترة من 2018- 2019، و ذلك باستخدام المنفصلات النباتية من صنف فليم سيدلس كطعم على أصل فريدم بطريقتين هما الأجنة والكالس الجذري، كما استخدمت صبغة الفلوروسين للتأكد من سريان العصارة ما بين أوعية الأصل و الطعم وتم تقييم مدى نجاح التطعيم بإجراء التحليلات الجزيئية والبيوكيميائية وأظهرت النتائج أن: الفروع الناتجة بزراعة الأنسجة متميزة بقوتها. وأظهر التطعيم الدقيق نجاح تكوين منطقة الالتحام بين الطعم والأصل بواسطة الأجنة بنسبة 75% مقارنة بالكالس الجذري 50% مع نمو الفروع جيدا و تكوين الأوراق و الجذور وسجل فارق معنوي بين الطريقتين في نسبة نجاح التطعيم و بالتالي صفات النمو. وقد تم إختيار درجة تحمل النباتات الناتجة من التطعيم للملوحة بواسطة محلول أملاح كلوريد صوديوم: كلوريد كالسيوم بتركيزات 1000، 2000، 3000 جزء في المليون، و بالرغم من أن النمو الجذري و الخضري للنباتات المطعومة بواسطة الأجنة أكثر تحملاً للملوحة إلا أنه تناقص نتيجة التعرض للملوحة العالية ومع ذلك استمر في تكوين الأوراق بعكس النباتات المطعومة بالكالس الجذري فشلت في ذلك. أوضح الفحص الوراثي RAPD-PCR وجود تغيرات وراثية بمنطقة التلاحم الطعم بالأصل وهو ما يعني إندماجها تماما واختلاط التركيب الوراثية لكليهما كما أظهرت منطقة التطعيم أيضا قرابة وراثية مع كلا من الطعم والأصل مما يثبت نجاح عملية التطعيم الدقيق بهاتين الطريقتين. بناء على هذه الدراسة، يعتبر التطعيم الدقيق طريقة بديلة ومناسبة للإكثار لارتفاع معدل النمو، و استخدامه في تحسين قدرة الأصناف على تحمل الظروف البيئية غير الملائمة.

الكلمات الدالة: العنب- فريدم- التطعيم الدقيق- RAPD- الفلوروسين- مضادات الأكسدة- الفينولات- إنزيمات الأكسدة- الملوحة.