

**Assessment of Preliminary Grafting Compatibility-Incompatibility
between Local Palestinian Table-Grapevine Cultivars and Different
Phylloxera (*Daktulosphaira Vitifoliae*) Resistant Rootstocks**

تقويم التوافق وعدم التوافق في تطعيم اصناف محلية فلسطينية من عنب المائدة على أصول
مقاومة لحشرة فيلوكسرا العنب

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Abstract

This study was conducted to assess levels of grafting-compatibility in the nursery between scions of three Palestinian local table-grapevine cultivars (*Vitis vinifera*) and five grapevine rootstocks resistant to phylloxera. The cultivars inspected were Halawani, Halawani-Baladi, and Red-Romi, whereas, the rootstocks were "Richter-110, B41, Ruggeri-140, Paulsen-1103, and 216/3". For comparison purpose, cuttings of the three cultivars were also grown on their own-roots. The experiment was set up in spring 2006 and, the grafting was done by using indoor tongue grafting technique. Several parameters of graft compatibility-incompatibility including: callus production at the graft union, root development, grafting success in the incubation room as well as in the nursery (the survival percentage), bud burst, and shoot length were evaluated. Results have shown a positive correlation between callus development and grafting success. Indeed, Halawani produced the best results of callus development and graft success, Halawani-Baladi was intermediate, and Red-Romi was worst. After five months in the nursery,

Halawani demonstrated highest compatibility with Richter-110 (100%), Paulsen-1103 (95%), Ruggeri-140 (85%), 216/3 (85%), and B41 (80%) successful grafts. Halawani-Baladi showed medium compatibility (79% successful grafts on Richter-110, 60% on Paulsen-1103, 79% on Ruggeri-140, 80% on 216/3 and 60% on B41). Red-Romi exhibited poor compatibility (25% of successful grafts on Richter-110; 20% on Paulsen-1103; 10% on Ruggeri-140; 5.3% on 216/3 and 45% on B41).

Key words: Grapevines, *Vitis vinifera*, rootstocks, scions, compatibility, grape phylloxera.

ملخص

لقد تم تنفيذ هذه الدراسة بهدف تقويم درجات التوافق و عدم التوافق ما بين ثلاثة اصناف محلية فلسطينية من عنب المائدة (حلواني، حلواني بلدي، رومي احمر) عند تطعيمها على خمسة اصول مقاومة لحشرة فيلوكسرا العنب (ريختر ١١٠، ب ٤١، روجري ١٤٠، بولسن ١١٠٣، ٣/٢١٦). و للمقارنة فقد تم تجذير عقل من الاصناف المحلية كشاهد بدون تطعيم. تم تنفيذ هذه الدراسة خلال ربيع ٢٠٠٦، وتمت عمليات التطعيم بطريقة التطعيم اللساني. لقياس درجات التوافق ما بين الاصناف والاصول فقد تم تسجيل عدة معايير شملت درجة التكلس (callus) في منطقة التطعيم، قوة التجذير، نسبة نجاح الاشتال في الحاضنة وفي المشتل، تطور البراعم وطول الافرع النامية على الاشتال. اظهرت النتائج ان هناك تناسباً طردياً بين درجة التكلس في الطعم و نسبة نجاح الاشتال في الحاضنة (بعد عشرة اسابيع من التطعيم) حيث كانت درجات التكلس اعلى في صنف الحلواني تبعه الحلواني البلدي ثم الرومي الاحمر. كما كانت نسبة نجاح الاشتال في المشتل (بعد عشرين اسبوعاً من التطعيم) ايضاً الاعلى في صنف الحلواني (١٠٠% على ريختر ١١٠، ٩٥% على بولسن ١١٠٣، ٨٥% على كل من روجري ١٤٠ و ٣/٢١٦، ٨٠% على ب ٤١). بينما كانت نسب النجاح في المشتل متوسطة للصنف الحلواني البلدي (٧٩% على ريختر ١١٠، ٦٠% على بولسن ١١٠٣، ٧٩% على روجري ١٤٠ و ٨٠% على ٣/٢١٦، ٦٠% على ب ٤١) ومنخفضة للصنف الرومي الاحمر (٢٥% على ريختر ١١٠، ٢٠% على بولسن ١١٠٣، ١٠% على كل من روجري ١٤٠ و ٥.٣% على ٣/٢١٦، ٤٥% على ب ٤١).

كلمات مفتاحية: عنب المائدة، المطاعيم، الاصول، التوافق، فيلوكسرا العنب.

Introduction

Grapevines are considered the second important fruit crop after olive in terms of both areas covered as well as economic returns in Palestinian. Due to the unique geographical and ecological environment for growing high quality table grapes, its growing and production are still restricted to the southern part of West-Bank especially Hebron and Bethlehem areas (MOA, 1998; 1999). According to the recent statistics, the total agricultural area of 170.000 ha, includes 105.000 ha planted with fruit trees, of which 8.900 ha are grown to grapevines cultivation (PCBS, 2004).

Among the major problems this sector faces is the severe damage caused by the grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) which is considered as the most destructive insect pest of cultivated grapes worldwide (Omer, et al, 1999; Read & Sanjun, 2003). This pest attacks the roots of grapevines and gradually destroys the whole rooting system, causing thereby leaf chlorosis, decreased vegetative growth and yield. Consequently, resulting root deformations and secondary fungal infection can also gradually cut off the flow of nutrients and water to the vine which subsequently leads to vine death within three to five years from infestation (Alleweldt and Possingham, 1988; Forneck, et al, 2001; Kellow, et al, 2004).

In Palestine, phylloxera problem was first observed at the beginning of 1980 in Bethlehem district and extended to Hebron area, causing more than 50% yield loss.

Using resistant rootstocks against this pest is of great importance and the only consistently effective and successful strategy in major viticultural countries worldwide (Troncoso, et al, 1999; Omer, et al, 1999). However, choice of rootstock is becoming increasingly difficult as a result of the availability of numerous new rootstocks (Loreti and Massai, 2006). One of the problems when choosing the right scion/rootstock combination is in predicting how the scion and rootstock

genotype will interact (Cus, 2004). Interaction usually results from the mutual translocation of nutrients and growth regulators between the scion and rootstock (Jackson, 2000). Thus, rootstocks must be compatible with local varieties otherwise, it will result in crop losses and economic collapse of many farmers and farms.

Since there is no published data on the performance of different scion/rootstock combinations in Palestine, therefore, an early and accurate prediction of graft incompatibility in the nursery has great importance for our region because incompatibility could be avoided and compatible genotypes could be selected (Petkou, et al, 2004).

The main goal of this work was to investigate levels of compatibility-incompatibility in the nursery between three Palestinian local table-grapevine cultivars (*Vitis vinifera*) and five grape rootstocks resistant to grape phylloxera.

Materials and Methods

1. Planting Material

a. Rootstocks

Cuttings of five phylloxera resistant grapevine rootstocks (Richter-110, B41, Paulsen-1103, Ruggeri-140, and 216/3) were obtained in winter 2006 from the Holly Land Nursery at Sa'er-Hebron that is the only officially licensed grapevine rootstock nursery in Palestine.

To avoid the probability of virus infection, the rootstocks were tested using ELISA method for the following viruses: grapevine leaf roll associated viruses (GLRaV-1-5 and -9), *Fanleaf*, and Corkey-Bark (Al-Moudallal, et al, 1984; Boscia, et al, 1992). The tests were conducted at the laboratory of biotechnology at Bethlehem University.

The cuttings of the five rootstocks were 30-40 cm in length with 3 nodes. The nodes were blinded using sharp sterilized knife. For rooting enhancement, 2cm vertical cuts were done at the bases of each cutting.

For cutting disinfection, the cuttings were soaked for 30 minutes in an aqueous solution containing 2 gm/L Bavestin (Carbendazim 50% W.P.), 2 gm/L Merpan (Captan 50% W.P.), 2 ml/L Roger (Dimethoate 40% E.C.), and 1 ml/L Confidor (Imidapride 35% E.C.). After drying, the cuttings were placed into sawdust that was treated also with the same disinfectant solution.

b. Scions

Twelve to fifteen cm long healthy hardwood cuttings (node + internode) of three Palestinian local table-grapevine cultivars namely Halawani, Halawani-Baladi, and Red-Romi were taken from Al-Arroub Agricultural Experimental Station. The cuttings were also dipped in the disinfectant solution for 30 minutes and then placed on benches for drying.

2. Grafting

In February 2006, grafting was done using indoor tongue grafting technique (Todic, et al, 2005), and for comparison, another plot of cuttings were also grown on their own-roots. The grafted area was covered (rolled and tied) with special plastic parafilm and dipped in cooper (5%) for 10 seconds then, the lower parts of the rootstocks were dipped for 5 seconds into 4000ppm Indole-3-Butyric Acid.

For each scion/rootstocks combination, 20 grafts were used and similar number of cuttings for each cultivar was also used for own-rooted vines as control plants.

3. *Incubation Room*

All the grafts as well as own-rooted vines were placed in plastic boxes filled with the treated sawdust and exposed to 22-25°C, 95% RH and 80% darkness (Todic, et al, 2005) in the incubation room. The grafted plants were kept for almost ten weeks inside the room and were then transferred to a nursery for acclimatization.

4. *Nursery*

Successful grafts were transferred into black 2 liter plastic bags with a media of agricultural sand and compost (1:1). The media was treated with a mixture solution of Dimethoate, Confidor, Merpan, and Copper-Sulphate in equilibrium rating. All scions were pruned to two nodes, and then the grafts were arranged in the nursery in a completely randomized design and kept for the whole summer before transplanting successful plants to the open field for further investigation. During this period, all grafts were maintained under nursery conditions (60% shading, 25-30 °C). The plants were watered daily and treated against pests and diseases according to the current agronomic practices.

5. *Measurements*

To determine levels of compatibility-incompatibility of the different scion/rootstock combinations, the following parameters were examined:

- Grade of callus development at the graft union; a scale ranging from 1 to 4 was used: 1 = no callus, 2 = low, 3=intermediate, and 4 = high callus formation on graft union surface.
- Grade of root system development; a scale ranging from 1 to 4 was used: 1 = no roots, 2 = low, 3 = medium, and 4 = high).
- Scion bud-burst: days required to buds burst.

- Percentage (%) of successfully grafted grapevines after ten weeks of incubation using growth of new shoots as an indicator.
- Percentage (%) of survival grafts after five-months in nursery; plants had vegetative growth and the graft union was lignified.
- Average length (cm) of main shoot after five-months in the nursery.

6. *Data Analysis*

Data obtained were statistically analyzed using the one-way analysis of variance ANOVA and means were separated using the Tukey's pairwise comparisons at a significance of $p \leq 0.05$ using the MINITAB package system.

Results and Discussion

In general, Halawani had significantly the highest callus grade of all examined scion/rootstocks combinations followed by Halawani-Baldi and Red-Romi had the lowest grade (Table 1). Consequently, a similar pattern of root development (Table 2) was observed (highest in Halawani, intermediate in Halawani-Baladi, and lowest percentage in Red-Romi). In contrast, control cuttings (own-rooted cuttings) revealed highest rooting in Red-Romi, intermediate in Halawani, and lowest grade in Halawani-Baladi (Table 2).

All grafted plants presented excellent percentage of grafting success almost 100% after ten weeks in the incubation room. This could be attributed to the well-controlled conditions in the incubation room especially the dark conditions and the ideal temperature that are helpful for rooting and callus formation of woody plants (Druart, et al, 1982; Sriskandarajah, et al, 1982; Zhang, et al, 2006). Indeed, dark and proper temperature conditions did most likely improve the activity of peroxidase enzyme which is responsible for the polymerization of *p*-coumaryl alcohols to lignin thus improving grafting compatibility (Wettern, et al,

1998; Pedersen, 2006). Additionally, these environmental conditions may increase free proline content and/or prevent IAA conversion, which will promote rooting (Durand & Nitsch, 1977). Consequently, rooted rootstocks have a positive effect on grafting success (Chanana and Singh, 1974).

The data in this research also indicated a positive correlation between callus development and grafting success. Indeed, Halawani had 100% of compatibility after five months in nursery with Richter-110, followed by Paulsen-1103 (95%), Ruggeri-140 (85%), 216/3 (85%), and B41 (80%), (Table 3). The high degree of compatible Halawani grafts might be due to its high proliferation rate (Celik, 2000; Todic et al, 2005); great root development (Neves, et al, 1998); cohesion the stock and scion (Moore & Walker, 1981); and vascular connection across the graft union (Moore, 1984).

Once the rootstocks and scions are in contact, the cambial region capable of meristematic activity produces parenchymatic cells and callus tissue that fills the space between the two components (Wang & Kollmann, 1996; Hartmann, et al, 1997). Several researchers consider this step as essential for the development of future vascular connections and have suggested that the primary recognition events occur at the point of cell-to-cell contact (Yeoman, et al, 1978; Yeoman, 1984). The basis of this recognition system would be a protein released from the plasma-lemma forming a complex with catalytic activity resulting in the formation of a successful graft (Errea, 1998; Gokbayarak, et al, 2007).

The results of Celik (2000), suggests that the grade of callus formation at the graft union is the main factor for good compatibility between stock and scion.

With regard to Halawani-Baladi, it gave intermediate level of compatibility, ranging between 60-80% of successful grafts with all rootstocks combinations.

The results showed high significant incompatibility between Red-Romi and all examined rootstock combinations in comparison with the other two cultivars (Table 3). In fact, Red-Romi grafts revealed (Table 3) the significantly lowest percentage of successful grafts. Many grafts died in the nursery by 94.7%, 90%, 80%, 75%, and 55%, for 216/3, Ruggeri-140, Paulsen-1103, Richter-110, and B41, respectively. The high losses could be due to unfavorable environmental conditions in the nursery and/or the graft-incompatibility of cultivar/rootstocks combinations (Celik, 2000).

Environmental conditions to which grafts are subjected (water-saturated atmosphere, low light intensity, heterotrophical nutrition) induce lower plant resistance, resulting in specific morphological and physiological changes (Debergh & Zimmerman, 1991). For example, water loss increases under low relative humidity in nursery (Preece & Sutter, 1991), and plants might eventually die. Nevertheless, throughout the whole experiment the same environmental conditions did not lead to losses in the other cultivars, therefore, graft-incompatibility was likely to cause the losses. In fact, Red-Romi cultivar showed the highest rooting rate of own-rooted cuttings in the incubation room and the lowest survival rate in the nursery when grafted. Our results are also consistent with the results of Manuel, (1948), Bouquet, (1980), and Sarooshi, et al, (1982), who worked with a number of phylloxera-resistant rootstocks, and finding that local cultivars gave a poor scions when grafted and were best on their own roots.

The primary cause of graft incompatibility remains unknown, although some hypothesis have been proposed. It is suggested that biochemical causes, rather than anatomical ones, are responsible for the alterations at the cambial continuity (Mosse, 1962; Errea, 1998). Some of these causes might be insufficient growth of the callus, formation of a necrotic zone at the graft union, defects in phloem differentiation, lignifications or metabolic interactions (Errea, 1998), and overridden graft union by toxins (Moore, 1984). Phenolic compounds also appeared to be implicated in all those events. It has been shown that a continuous

stress situation, caused by a lack of adaptation of the two genotypes forming the graft, may trigger a series of steps resulting in abnormalities to the normal development of the graft union. Changes that affect diffusion and movement of phenols may be implicated in cellular damage and alter the complete cambium system around the graft union. Moreover, in the adaptation mechanism between the two graft partners, a strong accumulation of phenols can lead to a degradation of some of them, which could be oxidized to quinones. Their posterior polymerization could be toxic to a number of metabolic reactions, such as inhibition of the lignin synthesis at the graft union (Errea, 1998). Indeed, one or a combination of many of these factors could explain the graft-incompatibility in Red-Romi and the different examined rootstocks.

Buds of grafted Halawani opened 33 days after grafting with all examined rootstocks. However, Halwani-Baladi had a high variability ranging from 33 days with Richter-110, B41, and 216/3; 38 days with Ruggeri-140; and 47 days with Paulsen-1103 (Table 4). Consequently, Red-Romi took 45 days with Ruggeri-140 and 47 days with all other rootstocks. In comparison to the grafted scion cultivars, own-rooted cuttings took longer time (62, 66, and 66 days) for Halawani, Halawani-Baladi, and Red-Romi, respectively (Table 4). Differences in bud burst between grafted (shorter time) and own-rooted plants (longer time), is possibly because of the increased concentration of plant growth regulators (PGRs) especially cytokinins and gibberellins, which resulted from blinding rootstock nodes. These PGRs probably moved down thereby affecting the vegetative growth via initiating root primordium formation and thus resulting in earlier bud burst (Hackett, et al, 1997).

A strong correlation was also found between callus, root development, and shoot lengths. In fact, for all examined rootstocks, measurements of shoot (stem) lengths presented in table 5, showed the longest average shoot length with Halawani followed by Halawani-Baladi and Red-Romi respectively. These results indicate that, growth of grafted grapevines could be different in respect to grade of callus development (Celik, 2000), grade of root development (Boscia, et al,

1992; Lima-da-Silva, et al, 2000), and to rootstock genotype (Soar et al. 2006; Yetisir, et al, 2007). Since the same consequence of callus as well as root development was exhibited (table 1, 2) with all examined rootstocks, therefore, it is clearly linked. On the other hand, rootstock can have a substantial influence on the vegetative growth, gas-exchange and on the water status of the scion (Paranychianakis, et al. 2004; Soar, et al. 2006); however, there is no convincing mechanistic explanation for this phenomenon. Genetically, determined rootstock characteristics were found to have an effect on vegetative growth via root distribution (Williams and Smith, 1991; Smart, et al. 2006), vine hormonal status (Nikolaou, et al. 2003; Soar, et al. 2006), water and nutrient-uptake efficiencies (Bavaresco, et al. 2003; Alvarenga et al. 2004; Wolpert et al. 2005), and associated differences in root hydraulic conductivity (Bavaresco and Lovisolo, 2000; Atkinson, et al. 2003). These factors could have direct and indirect effects on leaf gas-exchange and plant water status and thereby, vegetative growth. Additional effect of other processes could also play a role in explaining the superior shoot lengths in Halawani with all examined rootstocks.

Conclusions

Halawani presented good compatibility with all examined grapevine-rootstocks used in this study. Therefore, propagation of this cultivar on the evaluated rootstocks can be recommended. In contrast, and due to their poor performance and low success, combinations of Red-Romi with the examined rootstocks are not recommended.

Further field investigations are needed in order to continue observation on compatibility and effects on yield and quality.

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Table (1): Grade* of callus development on graft union surfaces in different scion/rootstock combinations after ten weeks of grafting. (Mean** \pm S.E).

Rootstocks	Scion		
	Halawani	Halawani-Baladi	Red-Romi
Richter-110	3.85 ^a \pm 0.11	2.94 ^b \pm 0.12	2.05 ^c \pm 0.15
Paulsen-1103	3.74 ^a \pm 0.13	2.10 ^b \pm 0.1	1.20 ^b \pm .09
Ruggeri	3.8 ^a \pm 0.12	1.89 ^b \pm 0.13	2.10 ^b \pm 0.16
B41	3.8 ^a \pm 0.09	2.05 ^b \pm 0.11	2.05 ^b \pm 0.18
216/3	3.95 ^a \pm 0.05	1.9 ^b \pm 0.07	1.10 ^c \pm 0.07

* Grade of callus development on graft union surfaces was evaluated on a scale 1 to 4: 1=no callus, 2=low, 3=intermediate, and 4=high.

** Means within rows with different letters are significantly different at p value \leq 0.05.

Table (2): Grade* of root development of grafted and own-rooted plants after ten weeks of grafting. (Mean** \pm S.E)

Rootstocks	Scion		
	Halawani	Halawani-Baladi	Red-Romi
Richter-110	3.85 ^a \pm 0.11	2.94 ^b \pm 0.12	2.05 ^c \pm 0.15
Paulsen-1103	3.74 ^a \pm 0.13	2.10 ^b \pm 0.1	1.20 ^c \pm .09
Ruggeri	3.8 ^a \pm 0.12	1.89 ^b \pm 0.13	2.10 ^c \pm 0.16
B41	3.8 ^a \pm 0.09	2.05 ^b \pm 0.11	2.05 ^b \pm 0.18
216/3	3.95 ^a \pm 0.05	1.9 ^b \pm 0.07	1.10 ^c \pm 0.07
Control (own-roots)	2.80 ^b \pm 0.09	1.9 ^c \pm 0.07	3.80 ^a \pm 0.07

* Grade of root development on graft union surfaces was evaluated on a scale 1 to 4: 1 =no roots, 2=low, 3=intermediate, and 4=high.

** Means within rows with different letters are significantly different at p value \leq 0.05.

Table (3): Percentage of successful grafted and own-rooted plants after five months in nursery. (Mean* ± S.E).

Rootstocks	Scion		
	Halawani	Halawani-Baladi	Red-Romi
Richter-110	100 ^a ±0.0	79 ^a ±9.6	25 ^b ±9.9
Paulsen-1103	95 ^a ±5.3	60 ^b ±11.2	20 ^c ±9.2
Ruggeri	85 ^a ±8.2	79 ^a ±4.6	10 ^b ±6.7
B41	80 ^a ±9.2	60 ^a ±11.2	45 ^a ±11.4
216/3	85 ^a ±8.2	80 ^a ±9.2	5.3 ^b ±5.3
Control (own-roots)	50 ^{ab} ±11.5	30 ^b ±10.5	80 ^a ±9.2

* Means within rows with different letters are significantly different at *p* value ≤ 0.05.

Table (4): Number of days required for bud-burst of grafted and own-rooted plants. (Mean* \pm S.E).

Rootstocks	Scions (Local Cultivars)		
	Halawani	Halawani-Baladi	Red-Romi
Richter-110	33.60 ^b \pm 0.17	33.42 ^b \pm 0.20	47.20 ^a \pm 0.28
Paulsen-1103	33.52 ^b \pm 0.19	47.60 ^a \pm 0.18	47.65 ^a \pm 0.19
Ruggeri	34.75 ^c \pm 0.30	38.47 ^b \pm 0.19	45.50 ^a \pm 0.22
B41	33.60 ^b \pm 0.25	33.65 ^b \pm 0.20	47.60 ^a \pm 0.29
216/3	33.50 ^b \pm 0.26	33.55 ^b \pm 0.26	48.00 ^a \pm 0.34 \pm
Control (own-roots)	62.55 ^b \pm 0.18	66.55 ^a \pm 0.22	66.20 ^a \pm 0.12

* Means within rows with different letters are significantly different at p value \leq 0.05.

Table (5): Average shoot length (cm) of grafted plants after five months in nursery. (Mean* ± S.E).

Rootstocks	Scion		
	Halawani	Halawani-Baladi	Red-Romi
Richter-110	119.55 ^a ±16.8 (20)	95.27 ^a ±12.3 (15)	47.6 ^a ±3.88 (5)
Paulsen-1103	102.78 ^a ±8.95 (18)	76.83 ^{ab} ±5.52 (12)	50.75 ^b ±13.4 (4)
Ruggeri	110.65 ^a ±7.31 (17)	75.87 ^b ±4.24 (14)	48.50 ^c ±2.5 (2)
B41	125.06 ^a ±22.30 (16)	81.33 ^{ab} ±9.52 (12)	17.55 ^b ±2.01 (9)
216/3	123.12 ^a ±.553 (17)	49.88 ^b ±4.11 (15)	51.00 ^b ±0.0 (1)

* Means within rows with different letters are significantly different at *p* value ≤ 0.05.