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PHYSIOLOGICAL AND ANATOMICAL STUDIES ON BUDDING OF CITRUS

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ABSTRACT

The aim of this experiment is to study the performance of Valencia orange Cv., budded (T-budding) on three types of citrus rootstocks i.e., Volkamer lemon (*C. volkmeriana*), Sour orange (C. *aurantium*) and Balady lime (*C. aurantifolia*). The results showed that, Valencia orange transplants budded on Volkamer lemon rootstocks was statistically the superior. However, the greatest values of success percentage, both scion length and diameter, number of leaves per transplant, largest area of leaves and heaviest weights of fresh and dry shoot system were induced in both seasons. The richest leaves in their mineral contents either macro-elements (N, P, K, Ca and Mg) or micro-nutrients (Fe, Zn and Mn) as well as photosynthetic pigments and indoles were statistically in closed relationship with those Valencia orange Cv. budded on Volkamer lemon rootstocks.

Anatomical examinations of union zone of all investigated treatments proved the success of union process between citrus scions (Valencia orange) from one hand and any of the 3 rootstocks. However, grade of success was relatively varied as the perfect union depending upon the anatomical measurements base was concerned. Hence, anatomical examinations revealed that, budding of Valencia orange scion on Volkamer lemon followed by sour orange rootstocks showed to great extent the perfect union between two graft elements (scion & rootstock). Herein, the widest diameter of the whole section, secondary cortex in either union zone (newly developed tissues between two elements) or tissues of both scion and rootstock themselves, as well as the decrease of vacuoles and necrotic tissues all were clearly observed. The reverse was true with budded transplants on other rootstock (Balady lime rootstock) whereas wider vacuoles and thicker necrotic tissues associated with thinner layers of other union zone tissues were detected.

INTRODUCTION

One of the keys to the profit of horticultural crops is an efficient propagation. Citrus is the most important fruit crops in Egypt as for as acreage, production and exportation potentialities are concerned.

Citrus fruits have higher nutritional value since they contain higher amounts of sugars, vitamins, minerals, organic acids, plant pigments, essential amino acids and pectin as for as using in different industrial purposes to curing people from different diseases (**Hulme, 1971**).

In Egypt, most of the new reclaimed areas are planted with Valencia orange trees and the most of this area is sandy soils, so we must choice the best rootstock for this soil which characterized by poor fertility and low water holding capacity. Citrus orchards occupy 451547 fed. according to the Ministry of Agriculture and Reclamation land statistics in (2009) year.

Citrus volkmeriana or *Citrus aurantium* had significant effect on most parameters of Valencia scion growth, total dry weight of Valencia scions on Volkamer lemon was greater than those on Sour orange **(El-Ezaby, 1994)**. Also, **Hassan *et al*., (2000)** studied growth of young Balady mandarin and Valencia orange trees budded on four citrus rootstocks, namely Sour orange, Volkamer lemon, Cleopatra mandarin and Rangpur lime. They found that Volkamer lemon rootstock promoted number of shoots/tree, shoot length and number of leaves/shoot. Compatible and incompatible combinations of citrus species cannot affect satisfactory union which takes place after a year and it can be detected at an early stage **(Hartmann and Kester, 1978 and Shklarman *et al*., 1994)**.

Navel orange and Balady mandarin scions showed a good compatibility with Volkamer lemon rootstock. Also, they found that, microscopic examination of union zone between sour orange interstock and Volkamer lemon rootstock showed wider parenchymatous vacuoles and necrotic tissues, beside narrower area of different union zone tissues, particularly secondary cortex were presented which gave a logic explanation for the depressive influence of sour orange interstock on most growth parameters **(Bakry *et al*., 2006)**.

The main target in the investigation is to study the effect of three citrus rootstocks i.e., Sour orange, Volkamer lemon and Balady lime on vegetative growth of Valencia orange scion and nutritional status. On the other hand, anatomical studies on budding zone for each rootstock and Valencia was investigated.

MATERIALS AND METHODS

The present study was carried out during two successive seasons of (2008 - 2009 and 2009 – 2010) in the green house at experimental farm of the Faculty of Agriculture Moshtohor, Qalyubeia Governorate, Egypt.

Sixty Homogenous healthy and normally growing seedlings of the three investigated citrus rootstocks i.e., Volkamer lemon, Sour orange and Balady lime, each rootstock represented by 20 seedlings were carefully selected. The three investigated citrus rootstock seedlings were transplanted individually i.e., each was planted in black plastic pot (30 cm. in diameter) filled with 20 kg of clay and sand mixture at the ratio 1 : 1 (v : v). These selected transplants were irrigated and recommended nutrient program were applied till budding time (first week of October) during 1st and 2nd experimental seasons, represented by five replicates, each replicate represented by four individually transplants. Accordingly, the different investigated treatments were as follows:

1. Valencia orange Cv. budded on Sour orange rootstock.
2. Valencia orange Cv. budded on Volkamer lemon rootstock.
3. Valencia orange Cv. budded on Balady lime rootstock.

Methodology regarding the response of investigated treatments were being determined as follows:

- Vegetative growth measurements:

The responses of some growth measurements for Valencia orange Cv., pertaining the influence of either 3 citrus rootstocks (Sour orange, Balady lime and Volkamer lemon) were evaluated through determining some parameters at the end of each experiment season i.e., percentage of budding success. scion length (cm), scion diameter (cm), number of leaves, leaf area (cm^2) and fresh and dry weights of shoot system.

- Leaf mineral composition :

Ten healthy and mature leaves per each replicate were taken at the end of each season. Then, leaves were wiped free of dust with a damp cloth and oven dried at 70^oC till a constant weight. After that, 0.2 g of each ground sample was digested**.** The digested solution was used for the determination of N, P, K, Ca, Mg, Fe, Zn and Mn nutrients as described by **(A.O.A.C., 1995).**

- Photosynthetic pigments:

Chlorophyll a, b and carotenoids were Colorimetrically determined in leaves according to the methods described by **Nornal (1982)** and then calculated as mg/g fresh weight.

- Some chemical constituents:

-Total carbohydrates:

Total carbohydrates in dry stem (0.1 gm) were determined photometrically at 490 μm., according to the method described by **Dubois *et al*., (1956).**

- Total indoles:

Total indoles were determined by using the test of P–dimethyl-aminobenzaldehyd (**Larson *et al.,* 1962**) to be estimated calorimetrically. The concentration was calculated from a standard curve of indole acetic acid.

- Anatomical studies:

Anatomical studies of union zone between Valencia orange Cv., (scion) from one hand and three rootstocks (Volkamer lemon, Sour orange and Balady lime) from the other were

previously examined i.e., after 15, 30, 60, 120 and 240 days from carrying out budding process (1st week of October) in order to investigated (microscopically) the structure of newly developed tissues in such area (zone). The samples specimens were taken then killed and fixed in FAA (5ml. formalin, 5ml. glacial acetic acid and 90ml. ethyl alcohol 70%), washed in 50% ethyl alcohol, dehydrated in series of ethyl alcohols 70,90,95 and 100%, infiltrated in xylene, embedded in paraffin wax with a melting point of 60-63°C, sectioned to 20 microns in thickness *(***Sass, 1951),** stained with the double stain method (fast green and safranin), cleared in xylene and mounted in Canada balsam **(Johanson, 1940***)*. Sections were read to detect histological manifestation of noticeable responses resulted from other treatments. The prepared section were microscopically examined, counts and measurements (µ) were taken using a micrometer eye piece. Average of readings from 3 slides/treatment were calculated .

- Statistical analysis:

All data obtained during both seasons of study were subjected to analysis of variance and significant differences among means were determined according to **Snedecor and Corchran (1972).** Significant differences among means were distinguished according to the Duncan`s multiple range test **(Duncan, 1955)**.

RESULTS AND DISCUSSION

1- Some vegetative growth measurements.

- Percentage of success

With regard to the effect of investigated citrus rootstocks seedlings under study i.e., (Volkamer lemon, Sour orange and Balady lime) on the success percentage, data tabulated in **Table (1)** revealed clearly that, the greatest value of success percentage was resulted by the Valencia orange Cv. budded on Volkamer lemon rootstock seedlings followed statistically in a descending order by Valencia orange Cv. budded on both Sour orange and Balady lime rootstocks, respectively.

The obtained data regarding the effect of different investigated citrus rootstock seedlings on the success percentage are in general agreement with those reported by **Martinez *et al.* (1994), Gonzalez and Figueroa (1996), and Dubey *et al.* (2004).**

- Scion length and diameter (cm):

Concerning the response of scion length and diameter (cm) of Valencia orange Cv. to the different studied citrus rootstock seedlings used in this work, obtained data represented in the abovementioned **Table (1)** showed clearly that, the highest values of scion length and diameter were in closed relationship with Valencia orange Cv. budded on Volkamer lemon rootstock seedlings.

The obtained results concerning the effect of some citrus rootstocks on scion length and diameter go in line with those mentioned by **Monteverde *et al.* (1990), Salem *et al.* (1994), Gonzalez and Figueroa (1996) and Dubey *et al.* (2004).**

- Number of leaves and Leaf area (cm^2):

Data obtained during both seasons as shown in **Table (1)** revealed obviously that, the highest number of leaves and leaf area (cm^2) were in closed relationship with Valencia orange transplants budded on Volkamer lemon rootstocks, while the opposite was noticed with those which budded on Sour orange rootstocks.

The present results are in a general agreement with those mentioned by some investigators, **Martinez *et al.* (1994), Dawood (1996) and Dubey *et al.* (2004).**

- Fresh and dry weight of shoot system:

As for the response of fresh and dry weights of shoot system of Valencia orange transplants to citrus rootstocks, data obtained during both 2008 and 2009 experimental seasons are presented in **Table (1)**. It could be generally detected that, the heaviest fresh and dry weights of shoot systems were significantly coupled with Valencia orange transplants budded on Volkamer lemon rootstock during two seasons.

The obtained results concerning the response of both fresh and dry weights for shoot to the various investigated citrus rootstocks were supported by the findings of several investigators **El-Ezaby (1994), Martinez *et al.* (1994), Abou-Rawash *et al.* (1995) and Dawood (1996).**

2- Leaf mineral contents (nutritional status):

The obtained results during both 2008 and 2009 seasons were tabulated in **Tables (2)** and revealed that, significant differences between the three studied citrus rootstocks in this investigation. The richest leaves in their mineral contents either macro-elements (N, P, K, Ca and Mg) or micro-nutrients (Fe, Zn and Mn) were statistically in closed relationship with those Valencia orange Cv. budded on Volkamer lemon rootstocks during both 2008 and 2009 seasons.

The obtained results concerning the effect of some citrus rootstocks on leaf mineral content go in line with those mentioned by **Salem *et al.* (1990), Saad-Allah *et al.* (1993)**, **Kaplankiran and Tuzcu (1994), Abou-Rawash *et al.* (1995), Azab (1995) and El-Shazly (1996).**

3- Photosynthetic pigments:

Data represented in **Table (3)** revealed clearly that, Valencia orange budded on Volkamer lemon rootstocks exhibited the richest leaves in their chlorophyll A and B as well as carotenoids contents in two seasons of study. Meanwhile, the reverse was found with Valencia orange Cv. budded on Balady lime rootstocks which induced the poorest ones in their chlorophyll A, B and carotenoids contents in the first and second seasons of study.

The obtained results concerning the effect of some citrus rootstocks on photosynthetic pigments content go in line with those mentioned by **Bakry *et al*., (2006)**.

4- Some chemical constituents.

Obtained results from **Table (3)** indicated obviously that, the greatest and the highest values of total carbohydrates and total indoles contents were significantly in relationship with Valencia orange budded on Volkamer lemon rootstocks followed statistically in a descending order by those budded on Sour orange and Balady lime rootstocks. The differences between three investigated citrus rootstock were significant as compared each other during both experimental seasons.

Data obtained were supported by the findings of **Saad-Allah *et al.* (1985), and Faiz *et al.* (1993).**

5- Anatomical study of citrus union zone.

Data in **Tables (4,5 and 6)** and **Figs.(1, 2 and 3)** indicated that, anatomical measurements (in microns) of cross section in union zone i.e., diameter of whole section, rootstock thickness, scion thickness, cortex thickness in rootstock, scion cortex thickness, cambium thickness in rootstock and xylem tissue thickness in rootstock were increased with increase the transplant age. On the other hand, vacuoles and necrotic tissues decreased with increase the transplant age.

Generally, it could be safely concluded from comparison between three rootstocks, data show that, the best results were obtained from budded Valencia orange scion on Volkamer lemon rootstock. Hence, all anatomical measurements especially the directly correlated ones with enhancing the process of compatibility to take place successfully were obviously higher with the Volkamer lemon x Valencia orange rather than two other ones (Valencia orange x Sour orange or Balady lime).

These results are in line with those obtained by **Fortanzza and Rugini (1983)** and **Bakry *et al*., (2006)**.

Table (1): Some vegetative growth measurements of Valencia orange transplants in response to citrus rootstocks used during both 2008 and 2009 seasons.

Type of citrus rootstock	Success %	Scion length	Scion diameter	No. of leaves/plant	Leaf area (cm.)	F.W. of shoot (gm.)	D. W. of shoot (gm.)
The first season (2008)							
Volkamer lemon	96.89A	27.80A	0.260A	26.80A	24.04A	20.33A	7.468A
Sour orange	92.77B	12.40C	0.100C	10.20C	14.90C	14.28C	4.758B
Balady lime	79.46C	21.00B	0.180B	14.80B	18.84B	17.10B	5.211B
The second season (2009)							
Volkamer lemon	97.47A	24.60A	0.280A	25.00A	24.17A	19.67A	10.28A
Sour orange	89.64B	9.60C	0.120B	9.40C	15.24C	13.70C	5.018B
Balady lime	81.34C	19.20B	0.160B	13.00B	20.04B	17.37B	5.158B

Means followed by the same letter/s within each column are not significantly different from each other at 0.05 level.

Table (2): Leaf elements content of Valencia orange transplants in response to citrus rootstocks used during both 2008 and 2009 seasons.

Type of citrus rootstock	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)
The first season (2008)								
Volkamer lemon	2.56A	0.264A	1.78A	3.67A	1.60A	46.94A	71.83A	46.94A
Sour orange	2.49AB	0.228B	1.51B	3.50A	1.44B	34.81B	58.46B	34.81B
Balady lime	2.38B	0.215B	1.22C	3.13B	1.21C	31.90C	49.16C	31.90C
The second season (2009)								
Volkamer lemon	2.71A	0.287A	1.83A	3.74A	1.48A	711.8A	45.86A	69.59A
Sour orange	2.53B	0.216B	1.64B	3.61A	1.26B	513.2B	39.73B	64.34B
Balady lime	2.29C	0.223B	1.20C	3.17B	1.19B	262.5C	35.81C	57.81C

Means followed by the same letter/s within each column are not significantly different from each other at 0.05 level.

Table (3): Leaf photosynthetic pigments and some chemical constituents content of Valencia orange transplants in response to citrus rootstocks used during both 2008 and 2009 seasons

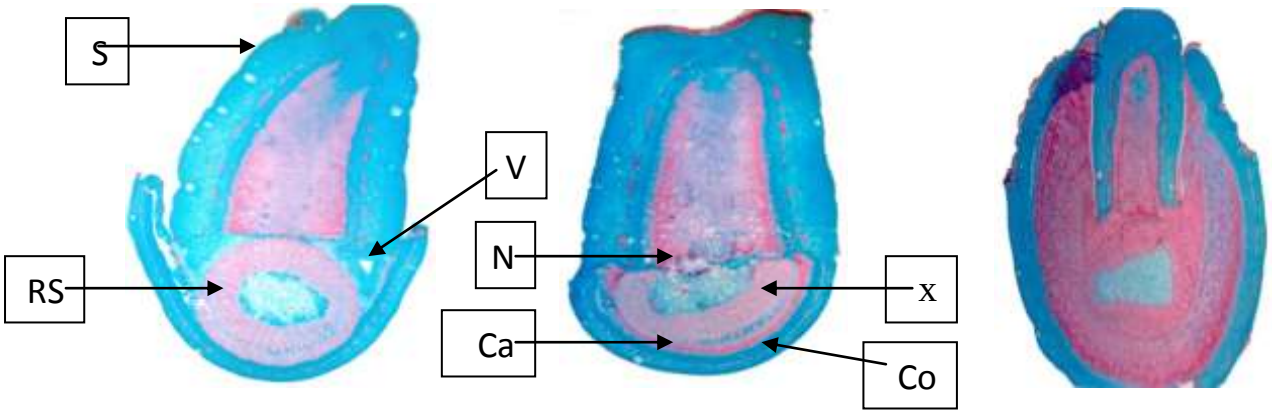
Type of citrus rootstock	Chlorophyll (a)	Chlorophyll (b)	Carotenoids	Total carbohydrates	Total indoles
The first season (2008)					
Volkamer lemon	7.03A	6.91A	3.99A	23.98A	11.43A
Sour orange	5.66B	5.27B	3.64A	19.36B	6.39B

Balady lime	4.85C	4.27C	2.93B	18.63C	6.15C
The second season (2009)					
Volkamer lemon	6.86A	6.74A	4.21A	24.61A	11.67A
Sour orange	5.14B	4.93B	3.61B	21.33B	6.76B
Balady lime	4.24C	4.18C	2.40C	17.46C	5.97C

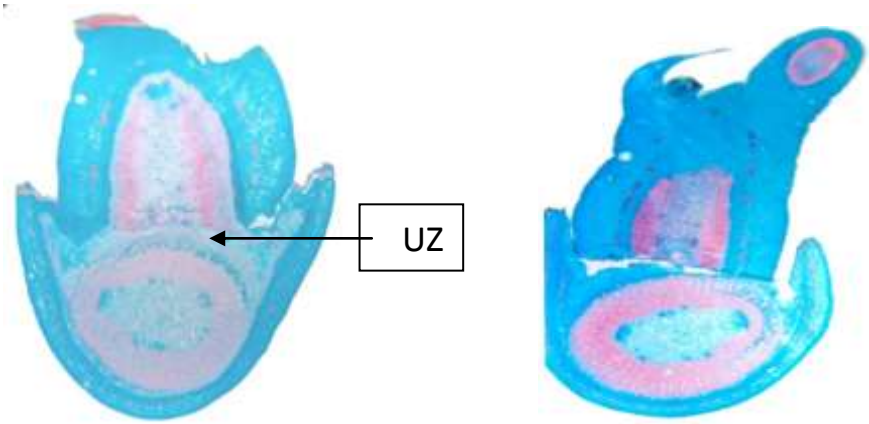
Means followed by the same letter/s within each column are not significantly different from each other at 0.05 level.

Table (4): Some anatomical measurements of cross section in union zone for Valencia orange scion on Volkamer lemon rootstock.

Transplant age / Measurements (microns)	15 days	30 days	60 days	120 days	240 days
Diameter of whole section	2984.08	3173.54	3417.45	5139.00	7524.00
Rootstock thickness	1613.77	1767.46	1818.69	2502.00	4815.00
Scion thickness	1216.62	1559.77	1598.76	2637.00	2709.00
Cortex thickness in rootstock	70.38	116.28	120.60	129.60	191.80
Scion cortex thickness	141.66	141.66	148.74	155.83	156.20
Cambium thickness in rootstock	27.00	40.50	48.60	51.30	84.60
Xylem tissue thickness in rootstock	307.66	317.43	390.68	562.50	1287.90
Secondary cortex thickness	125.82	217.08	253.96	255.60	270.45
Union zone thickness	122.40	140.76	153.00	161.40	239.70
No. of vacuoles	1	1	-	-	-
Vacuoles thickness	29.70	8.10	-	-	-
Thickness of necrotic layer	30.60	14.40	-	-	-



(A) **(B)** **(C)**

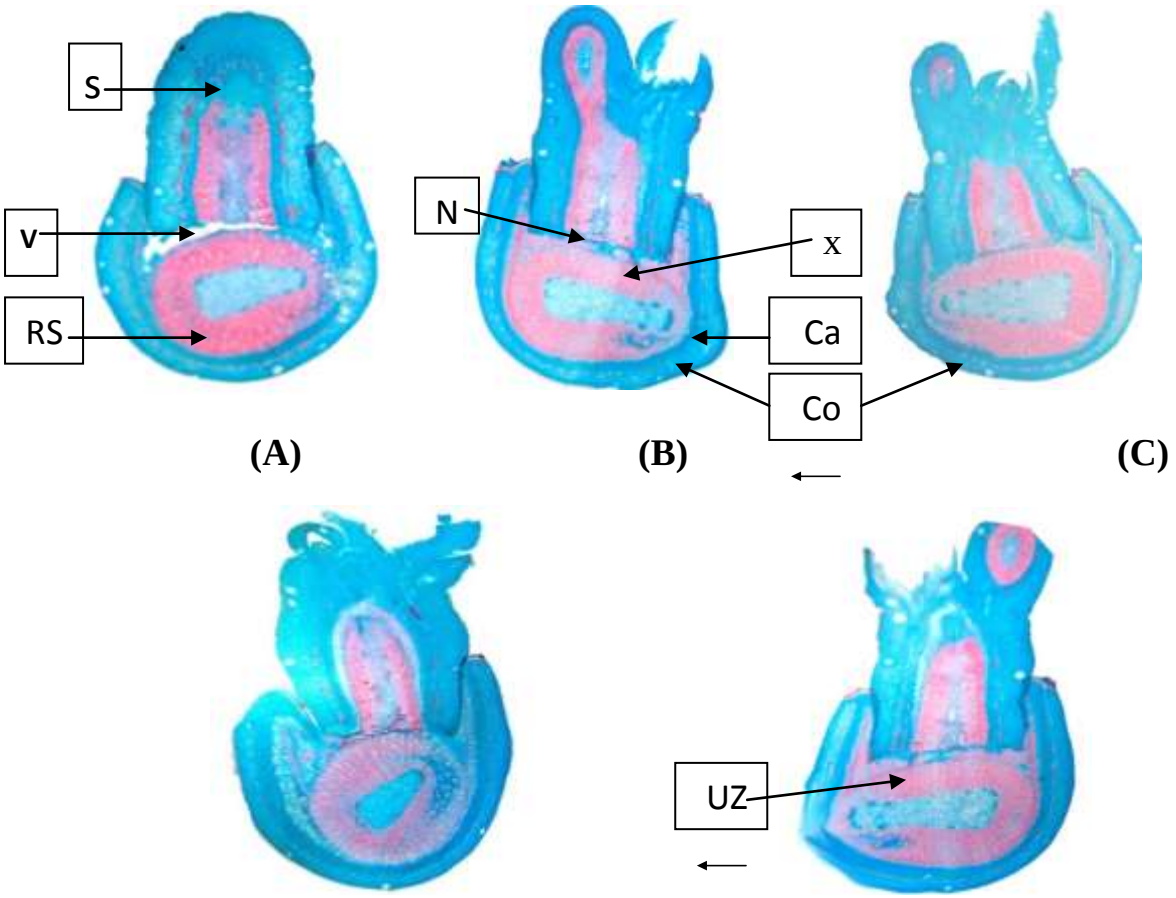


(D) **(E)**

Fig.(1): Cross section in union zone for Valencia orange scion on Volkamer lemon rootstock (X= 50).
A- After 15 days age. B- After 30 days age. C- After 60 days age.
D- After 120 days age. E- After 240 days age.
S = Scion, R.S. = Rootstock, Co = Cortex, Ca= Cambium X. = Xylem, U.Z. = Union zone, V. = vacuoles, N. = Necrotic layer.

Table (5): Some anatomical measurements of cross section in union zone for Valencia orange scion on Sour orange rootstock.

Transplant age / Measurements (microns)	15 days	30 days	60 days	120 days	240 days
Diameter of whole section	2678.85	2780.92	3227.53	3427.79	4220.10
Rootstock thickness	1665.00	1793.07	1972.38	1998.00	2492.10
Scion thickness	1013.85	987.85	1255.15	1429.79	1216.62
Cortex thickness in rootstock	129.60	137.63	138.00	153.00	175.95
Scion cortex thickness	208.64	222.55	236.45	292.09	306.00
Cambium thickness in rootstock	25.38	27.00	45.90	50.40	102.60
Xylem tissue thickness in rootstock	280.80	256.38	316. 52	410.21	948.60
Secondary cortex thickness	222.15	253.80	301.95	320.43	321.30
Union zone thickness	112.50	162.18	168.60	189.72	198.90
No. of vacuoles	1	1	-	-	-
Vacuoles thickness	38.70	6.66	-	-	-
Thickness of necrotic layer	-	30.60	-	-	-

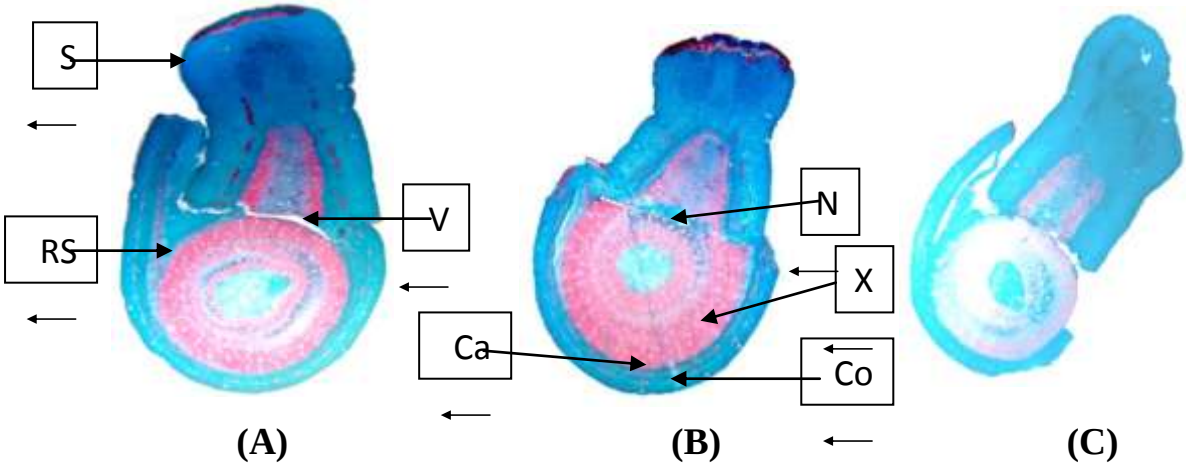


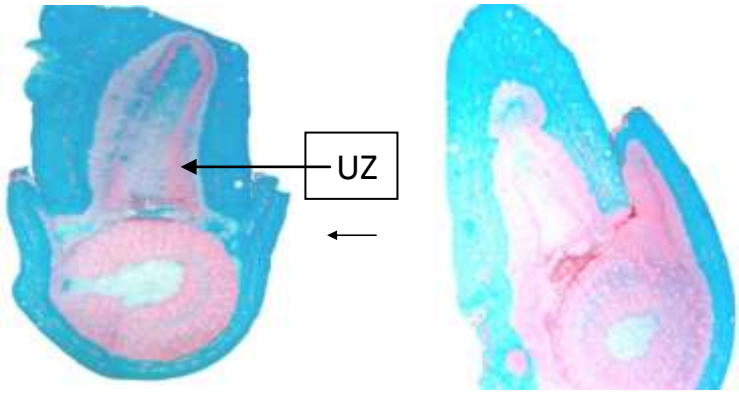
(D) (E)

Fig.(2): Cross section in union zone for Valencia orange scion on sour orange rootstock (X= 50).
A- After 15 days age. B- After 30 days age. C- After 60 days age.
D- After 120 days age. E- After 240 days age.
S = Scion, RS. = Rootstock, Co = Cortex, Ca= Cambium X. = Xylem, Uz. = Union zone, V. = vacuoles, N. = Necrotic layer.

Table (6): Some anatomical measurements of cross section in union zone for Valencia orange scion on Balady lime rootstock.

Transplant age / Measurements (microns)	15 days	30 days	60 days	120 days	240 days
Diameter of whole section	2602.76	2835.20	2975.33	3188.01	3401.52
Rootstock thickness	1536.92	1639.38	1716.23	1831.50	2023.62
Scion thickness	1065.84	1143.83	1195.82	1377.90	1481.78
Cortex thickness in rootstock	97.92	100.92	104.04	137.70	137.70
Scion cortex thickness	167.84	178.33	188.82	188.82	209.80
Cambium thickness in rootstock	24.30	45.90	51.30	52.65	54.00
Xylem tissue thickness in rootstock	316.52	415.09	456.61	463.93	512.77
Secondary cortex thickness	168.48	174.84	181.32	228.42	231.78
Union zone thickness	91.80	183.60	195.84	198.90	260.10
No. of vacuoles	1	1	-	-	-
Vacuoles thickness	20.70	18.45	-	-	-
Thickness of necrotic layer	38.25	30.60	-	-	-





(D) (E)

Fig.(3): Cross section in union zone for Valencia orange scion on Balady lime rootstock (X= 50).
A- After 15 days age. B- After 30 days age. C- After 60 days age.
D- After 120 days age. E- After 240 days age.
S = Scion, RS. = Rootstock, Co = Cortex, Ca= Cambium X. = Xylem, Uz. = Union zone, V. = vacuoles, N. = Necrotic layer.

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الملخص العربي

دراسات فسيولوجية وتشريحية علي التطعيم فى الموالح

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الهدف الرئيسي من هذه التجربة هو دراسة تطعيم البرتقال الصيفي (فالنشيا) على ثلاثة أصول (ليمون الفولكاماريانا – النارنج – الليمون البلدى). أوضحت النتائج المتحصل عليها أنه لوحظ أن جميع قياسات النمو الخضرى المدروسة (نسبة نجاح التطعيم، طول وقطر الطعم، وعدد الأوراق/نبات، مساحة الورقة، الوزن الجاف والطازج للمجموع الخضرى (الطعم) قد استجابت معنوياً. وأظهرت شتلات البرتقال الفالنشيا المطعومة على أصل الفولكاماريانا أعلى القيم لتلك القياسات الخضرية سالفة الذكر مقارنة بالأصليين الآخرين (النارنج – الليمون البلدى)، خلال موسمي الدراسة. وكانت أوراق البرتقال الفالنشيا المطعومة على أصل الفولكاماريانا هى الأغنى فى مستواها من العناصر المدروسة (النيتروجين، الفوسفور، البوتاسيوم، الكالسيوم، الماغنسيوم، الحديد، الزنك، المنجنيز) ومن كلوروفيل أ، ب والكاروتينات والأندولات الكلية خلال موسمي الدراسة. كما أوضحت القياسات التشريحية والقراءات الميكروسكوبية التى أجريت على منطقة التطعيم فى البرتقال الفالنشيا المطعوم على ثلاثة أصول أن هناك علاقة موجبة بين نجاح التطعيم وقوة الالتحام بين الأصل والطعم والذي يتمثل فى قلة الفجوات بين كل من الأصل والطعم فى منطقة الالتحام وكذلك قلة عدد الخلايا الميتة وكان ذلك واضحاً عند تطعيم البرتقال الفالنشيا على أصل الليمون الفولكاماريانا. والعكس كان صحيحاً عند تطعيم البرتقال الفالنشيا على الليمون البلدي المالح.