IMPROVEMENT OF BALADY MANDARIN (*Citrus reticulate* Blanco) THROUGH SELECTION, EVALUATION AND TISSUE CULTURE TECHNIQUE.

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ABASTRACT

This work was undertaken during the years 2003, 2004, 2005, 2006 and 2007 for improving the productivity of Balady mandarin (*Citrus reticulate* Blanco). Firstly, selection and evaluation of some mandarin seedlings trees were conducted based on yield (fruit quantity and fruit quality); such trees would be considered as mother trees. Secondly, after selection and evaluation, mass production of the selected superior genetic materials (individual trees) was propagated by means of tissue culture technique as it is rapid clonal propagation method to rescue the lose of such limited selected source. The results could be summarized as follows:

I- Selection and evaluation of some superior variants of mandarin included; number of fruits/tree, yield/tree (kg), fruit weight(g), fruit volume (cc), peel thickness (cm), number of seeds/fruit, T.S.S.%, acidity % and vit. C (mg/100ml. juice) content. The results showed that some promising mandarin trees with high yield and good fruit quality which could help in improving mandarin productivity in Fayoum Governorate, Egypt, were detected.

II- Mass production using shoot tip technique. MS basal medium 1962 at full strength supplemented by 1.5 mg/L BA was sufficient for the best shooting proliferation of mandarin. MS basal medium plus 1.5 mg/L NAA significant increased the rooting percentage, number of roots and root length of citrus species

III- Anatomical studies show that MS (Murashinge Skoog Basal Medium) supplemented with 1.5 mg/L NAA enhanced adventitious roots formed from callus and cambium (i.e. high rooting percentage), in comparison to the low concentration 0.5 – 1.0 mg/L which adventitious roots were formed only from cambial zone (low rooting percentage).

Key words: Selection, evaluation of Balady mandarin, In vitro, micro propagation.

INTRODUCTION

Citrus species are the most widely grown fruit crops in Egypt. Although there is a great diversity in the genus citrus, the greatest number of new citrus cultivars which are important today originated from bud mutation and selection from existing cultivars **(Soost & Cameron, 1975).**

In Egypt, attention must be paid to steady expansion in the production of the citrus plants to satisfy the local consumption and for exports. Balady mandarin is one of the most commercially important fruit crops in Fayoum Governorate. The cultivated areas are about 2000 feddans with an average yield of 5 ton/feddan for mandarin.

Most of citrus cultivars in Egypt are budded on sour orange and Balady lime seedling rootstocks. These cultivars with continuous propagation by budding for long time resulted in decreasing, productivity and fruit quality of fruit as well as tumble of growth vigor. These observations are due to both

low activity of RNA and various infections which transmit by scion from infected trees as well as accumulation of mutations.

Propagation of citrus by seeds induced variations when sexual embryo produced trees. Few of these segregations would be of great importance in citrus breeding for improvement of citrus productivity.

The selection from seedlings of fruit trees is considered one of the important methods for improving fruit varieties **(Michurin, 1984).**

Seedling trees of Balady mandarin resulted from sexual propagation in Fayoum Governorate are the main target of the present study as a source of superior genetic materials for higher yield with good fruit characteristics.

After selection and evaluation, i*n vitro* micro propagation is used for mass production of selected superior genetic material (individual tree). Tissue culture would then acts as a rapid clonal propagation technique for such limited source.

The objectives of the present study were:

1- Selection and evaluation of some superior variants among Balady mandarin seedling trees grown in Fayoum Governorate, Egypt.
2- Mass production of selected superior trees by means of tissue culture technique.
3- Anatomical studies to shed a light on the reason behind low survival of some plantlets produced from some tissue culture treatments.

MATERIALS AND METHODS

This research work was carried out during the years 2003, 2004, 2005, 2006 and 2007 on Balady mandarin seedling trees. Trees were about 20 years old, planted 5 meters apart grown in loamy sand soil at a private orchard, Ebshway, Fayoum Governorate, Egypt.

This study was included three parts:

1- Selection and evaluation of some Balady mandarin trees included yield and physical and chemical fruit characteristics.
2- Mass production of superior selected trees using tissue culture technique.
3- Anatomical studies.

Five trees were selected, labeled and punctuated in the orchards and treated individually in sampling and studying.

The following indices were taken into consideration during the present study by:

Part I:

a) Evaluation of yield as total number of fruits/tree and total weight of fruits/tree (kg) at harvest.

b) Fruit quality.

- Physical properties of the fruits. Samples of 25 fruits per tree were collected to study: fruit weight (g), fruit volume (cc), peel thickness (cm) and number of seeds/fruit.
- Chemical constituents of the fruits included T.S.S %, acidity %, and ascorbic acid content (mg/100 ml juice) were determined according to the methods outlined in the **A.O.A.C (1985).**

c) General evaluation of the tested trees: The final evaluation of the tested tree was calculated on the basis of 100 units which were shared between yield (50 units) and fruit quality (50 units) (**Abdallah, 1979 and Mousa, 1981**). The latter units were divided among the various fruit properties on the basis of 10 units for fruit weight, fruit volume and number of

seeds, and 5 units for peel thickness, T.S.S%, citric acid % and ascorbic acid content.

Each tree that had the best results in any character was given the "full mark" specified for this character, while each at the other tested took lower units equal to their qualities. For instance, if any tree produced the highest yield values, it will be given all the 50 units specified for this character. Accordingly, units of any other tested tree for the same character could be calculated as follows:

$$\frac{50 \text{ X yield of the tested tree}}{\text{Yield of the highest tree}}$$

Similarly, units for any concerned character were calculated in the same manner.

Part II

Mass production of the evaluated trees (superior ones):

Preparation and surface sterilization of explants:

Two centimeters long terminal shoots were excised and transferred immediately to soaking in running tap water for 2 hrs. Explants were carried out in aseptic conditions in laminar cabined. Shoots were trimmed to 1 cm. and placed in a flask and disinfected as follows: dipping in 70% ethanol, followed by immersing in Clorox as shown in Table (1) followed by rinses in sterile distilled water. The explants (0.3-0.5 cm. shoot tip) were then cultured in jars (150 ml) containing 35 ml MS basal medium at full strength as mentioned by **Murashige & Skoog (1962)** supplemented with growth substances as follows:

- For shooting proliferation:
  MS basal medium + 0.0 mg/L BA
  MS basal medium + 0.5 mg/L BA
  MS basal medium + 1.0 mg/L BA
  MS basal medium + 1.5 mg/L BA
  MS basal medium + 2.0 mg/L BA
- For adventitious rooting:
  MS basal medium + 0.0 mg/L NAA
  MS basal medium + 0.5 mg/L NAA
  MS basal medium + 1.0 mg/L NAA
  MS basal medium + 1.5 mg/L NAA
  MS basal medium + 2.0 mg/L NAA

All jars plugged with polypropylene closure caps, incubated in growth chamber at 27±1 C^o under 16 hr illuminations of 2500 lux cool white fluorescent lamps.

Average number of shoots/explant was calculated 6 weeks after culturing and average root number as well as root length/explants were recorded. After measurement, the plantlets were treated with antifungal (0.1 g/L Rezolax) to avoid fungal attack. A mixture of sand and peat moss (1:1 v:v) was then used for transplanting in the greenhouse to strengthen the plantlets.

Table (1). Sterilization treatments for mandarin Explants

Treatments	Period (min. & Sec.)
Clorox 30%	10 min.
Clorox 40%	10 min.
Clorox 50%	10 min.
Ethanol 70% + Clorox V. 30%	20 sec. + 10 min.
Ethanol 70% + Clorox V. 40%	30 sec + 10 min.
Ethanol 70% + Clorox V.50%	40 sec + 10 min.

In this part, each treatment was represented by 10 jars and explants were randomly distributed to receive different treatments. The data were statistically analyzed according to **Snedecor & Cochran (1980)**.

Survival of the plantlets:

A hundred of rooted plantlets of mandarin was rinsed carefully to wash off excess culture media (agar) from the roots. Then, they were transplanted in 2-inch plastic pots filled with moistured sterilized mixture of peat-moss and sand (v:v 1:1) and placed under mist condition. After 7 weeks, survived plants were counted and their percentages were calculated.

Part III

Anatomical studies were done to shed a light on the reason behind the low survival percentages of plantlets of Balady mandarin.

Samples of rooted Balady mandarin plantlets were randomly taken from each treatment at weekly intervals to study their anatomical structure. All steps of the anatomical studies were done according to **Johansen (1940)**. The cross sections were examined and microscopically photographed.

RESULTS & DISCUSSION

A selection program on a big number of Balady mandarin seedlings grown in Fayoum governorate was achieved during the years 2003, 2004, 2005, 2006 and 2007 to get a suitable number of promising (superior) seedlings to be utilized as new selected strains for mandarin variety improvement.

1- Yield productivity (fruit number/tree and yield/tree kg):

This part of the study aimed to select superior seedling trees of Balady mandarin.

Data tabulated in Table (2) revealed that some of the studied seedlings exhibited superior yield either as average fruit number per tree or as yield (kg/tree) associated with regular bearing during the three seasons of study. Balady mandarin seedling tree No. 2 produced the highest yield (960 fruits and 130.29 kg/tree).

Furthermore, it could be concluded that the productivity of the selected mandarin trees ranged between 14-20 tons/feddan.

These findings are in harmony with that found by **Higazi and El-Hagah (1984)** on Balady and Soukkari orange selections, **Shaaban (2000)** on seedlings selected from citrus cultivars and **Soliman *et al.* (1991)** on mandarin selections.

These findings would support the opinion that citrus seedlings can give good productivity which may be due to the gene pool resulted from

continuous seed propagation which producing germplasms of indogenous trees suitable to the climate conditions in Fayoum Governorate.

Table (2). Fruit number and yield per tree (kg) of Balady mandarin selections (2003, 2004 and 2005) seasons

Character	Fruit Number/tree				Yield/tree (kg/tree)			
Selections No.	2003	2004	2005	Mean	2003	2004	2005	Mean
No.1	830	900	850	860	107.19	102.17	100.22	103.19
No.2	860	1070	950	960	139.10	128.25	123.52	130.29
No.3	850	1050	950	950	126.53	114.00	111.60	117.37
No.4	800	850	810	820	97.92	95.18	93.49	95.53
No.5	750	870	800	806.66	95.70	92	89.82	95.51
LSD 5%				69.22				11.03

Fruit characteristics:

1- Physical characters:

Data in Table (3) and Plates (1 & 2) indicated that, in general, seedling No.2 gave the highest values of good fruit physical characteristics in comparison to the other ones.

Concerning the average of fruit weight, volume, peel thickness and number of seeds per fruit, the results in this study were in agreement with those reported by **Shahein *et al.* (1986)** on Balady orange, **Soliman *et al.* (1991)** on Balady mandarin and **Shaaban (2000)**on some selected citrus cultivars.

Table (3). Fruit weight, Volume, Peel thickness and Number of seeds per fruit of Balady mandarin selections (2003-2004 and 2005) seasons.

Character	Fruit weight (g)				Fruit volume (cc)				Peel thickness (cm)				Number of seeds/fruit			
Selections No.	2003	2004	2005	Mean	2003	2004	2005	Mean	2003	2004	2005	Mean	2003	2004	2005	Mean
No.1	110.0	119.1	120.2	**116.4**	135.0	139.0	100	**138.0**	0.27	0.28	0.25	**0.26**	13.1	13.5	13.8	**13.46**
No.2	120	130	135	**128.3**	150	140	160	**150**	0.25	0.21	0.21	**0.22**	11.2	10.9	12.9	**11.66**
No.3	110.9	120.5	120.0	**117.2**	140	140.0	150.	**143.3**	0.26	0.26	0.24	**0.25**	12.9	13.1	13.5	**13.16**
No.4	109.2	115.2	117.5	**113.9**	132.0	135.9	135.	**134.3**	0.28	0.28	0.28	**0.28**	14.2	14.9	14.6	**14.54**
No.5	109	110	115	**111.3**	130	130	140	**133.3**	0.30	0.29	0.29	**0.29**	14.9	13.9	14.8	**14.53**
LSD 5%				**4.3**				**N.S.**				**0.02**				**0.98**

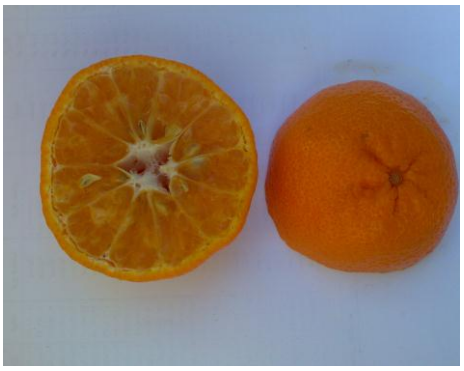

Plate 1: Balady mandarin fruit of seedling number 2.

Plate 2: Balady mandarin fruit of seedling number 5.

2- Chemical fruit characters: (T.S.S %, Acidity and Vitamin C content)

Data in Table (4) indicated that the higher values of chemical fruit quality were obtained by seedling No.2. These results were agreed with those of **Shawky *et al.* (1976), Soliman *et al.*, (1991)** who also selected some promising citrus cultivars on Balady mandarin and **Shaaban (2000)** on some selected citrus cultivars.

Generally, from the aforementioned results concerning evaluation of some selected Balady mandarin seedlings, it was concluded that seedling No. 2 was the most promising one and it would be used as a mother tree for mass production of Balady mandarin through tissue culture technique.

Table (4). Chemical fruit characters of Balady mandarin selections (2003, 2004 and 2005) seasons.

Character	T.S.S %				Acidity %				Vit. C (mg/100ml. juice)			
Selection Seedling	2003	2004	2005	Mean	2003	2004	2005	Mean	2003	2004	2005	Mean
No.1	10.0	10.0	10.2	**10.06**	1.0	1.1	1.1	**1.06**	29.0	28.1	27.9	**28.33**
No.2	10.9	11.0	11.1	**11.0**	0.91	0.93	0.89	**0.91**	30.1	29.7	29.9	**29.9**
No.3	10.1	10.9	10.0	**10.33**	0.96	0.98	0.99	**0.97**	30.0	29.0	29.0	**29.33**
No.4	10.0	10.0	10.0	**10.0**	1.5	1.3	1.5	**1.43**	26.0	25.9	25.9	**26.3**
No.5	10.0	10.2	10.3	**10.16**	1.1	1.2	1.3	**1.2**	26.7	27.0	27.0	**26.93**
LSD 5%				**0.45**				**0.14**				**0.64**

Mass production of the evaluated Balady mandarin selections:

Rapid clonal propagation *In vitro* technique has been utilized for the selected superior variants of mandarin using shoot tips.

Generally, it could be recommended that using 70% ethanol for 40 sec. followed by 50% Clorox (Sodium hypochlorite) for 10 min. as a best procedure of explants sterilization of mandarin culture (Table, 1), must be followed.

The present results are in harmony with those found by **Guindy (1990)** on some fruit rootstocks, **Shaaban (2000)** on some selected citrus, **Salama *et al.* (2002)** on ''William's" banana and **El-Sayed (2005)** on sweet basil and thyme plants.

Composition of culture medium:

Experiments were carried out to produce healthy and vigorous mandarin seedlings with high percentages of survival.

Shoot tip explants of the selected seedlings were cultured on MS basal medium supplemented with different combinations of BA, then they were maintained in growth chamber at 27±1C° under illumination cycles at light/dark (16/8 hours at 2500 lux) using fluorescent lamps (cool white light). The responses were recorded after 42 days.

Data presented in Table (5) and illustrated by Plates (3 & 4) show that the best medium for micro propagation of mandarin explants (shoot tip) was MS basal medium supplemented with 1.5 mg/L BA.

It is obvious that the best medium which produced the most shoots was MS+1.5 mg/L BA for mandarin explants (Plate, 5). It is evident that increasing the concentration of BA from 0.5 to 1.5 mg/L exerted a pronounced significant increase in average shoot number per explant.

It is clear from Table (5) and Plates (3 & 4) that increasing BA concentration form 0.5 to 1.5 mg/L gave marked increase almost about twice the value of number of shoots obtained with the lower concentration.

Data concerning rooting of mandarin seedlings are shown in Table (6), indicated that applying NAA at 0.5, 1.0, and 1.5 mg/L to MS medium gave a better effect on rooting compared with the control. The most marked effect was obtained with 1.5 mg/L, but the lower concentration had significantly less effect.

Concerning the average number of roots per shoot, the data indicate that NAA at 1.5 mg/L generally increased this parameter than the other tested concentrations.

Regarding the average root length, it is quite clear that NAA at 1.0 mg/L enhanced more root growth than did other concentrations.

Concerning the survival percentage, data indicate that plantlets with better rooting (i.e. average number of roots/plant), had the highest value after hardening and transplanting to the greenhouse. Thus, the shoots rooted in medium containing NAA at 1.5 mg/L gave the highest survival rate.

Table (5). Effect of BA treatments on proliferation shoot tip mandarin explants grown in vitro.

Medium	Number of shoots / explants
MS+0.0 mg/L BA (Control)	0.8±0.75
MS+0.5 mg/L BA	1.0 ± 0.94
MS+1.0 mg/L BA	1.5 ± 1.06
MS+1.5 mg/L BA	2.0±0.05
MS+2.0 mg/L BA	1.41±0.06

- Each value is an average of 4 replicates ± SE
- L.S.D for number of shoot at 0.21 for mandarin

Table (6). Effect of NAA treatments on (rooting %, root number, root length and survival percentage) of mandarin explants shoot tip.

Medium				
	Rooting %	**Root number/ plat let**	**Average root length (cm)**	**Survival %**
MS+0.0 mg/L NAA	25%	1.0±0.01	1.5±0.065	25%
MS+0.5mg/L NAA	56%	2.0±0.05	2.9±0.023	40%
MS+1.0 mg/L NAA	81%	3.02±0.06	3.4±0.031	80%
MS+1.5 mg/L NAA	84%	3.61±0.07	3.0±0.022	85%
MS+2 mg/L NAA	80.0%	3.01±0.06	3.3±0.03	79.9%
L.S.D 5%		0.11	0.02	

- Each value is average of 10 replicates ± SE

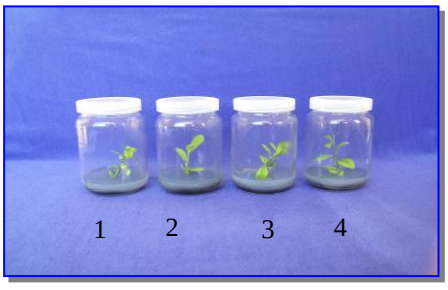

Plate 3. Shows the effect of adding BA to MS basal medium on Balady mandarin shoot tip explants.

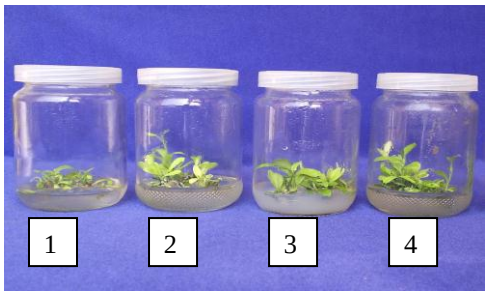

Plate 4. Shows shoots number/explant of Balady mandarin as affected by adding different concentrations of BA to MS basal medium.

Data concerning rooting of mandarin seedlings are shown in Table (6). It could be seen that applying NAA at 0.5, 1.0, and 1.5 mg/L to MS medium gave a better effect on rooting compared with the control. The most marked effect was obtained with 1.5 mg/L and the lower concentration had a significantly less effect.

Concerning the average number of roots per shoot, the data indicate that NAA at 1.5 mg/L generally increased this parameter than the other tested concentrations.

Regarding the average root length of mandarin, it is quite clear that NAA at 1.0mg/L enhanced more root growth than did other concentrations.

Concerning the survival percentage, data in Table (6) also indicate that plantlet with better rooting i.e. average number of roots/plant, had the highest value of survival after hardening and transplanting to the greenhouse. Thus, the shoots rooted in medium containing NAA at 1.5 mg/L gave the highest survival rate.

Generally, the results of the present study tabulated in Table (6) show that the survival values of rooted mandarin plantlets were paralleled to the average number of roots and rooting percentages, especially when NAA was used at the highest concentration (1.5 mg/L).

These findings are in the same line with those found by **Guindy (1990)** on some fruit rootstocks, **El-Sayed (2005)** on sweet basil and thyme and **Shaaban (2000)** on some selected citrus genotypes.

The Anatomical studies:

Cross sections of mandarin explants illustrated in Plate (7), show that the periderm (pr) covers the outer layer of the explant stem and is composed of several phylum layers. This layer is thick. Oil glands (Og) were observed in the cortex of mandarin. Inward to the phylum layers is the cortex with 5-7 layers of mainly parenchymatous and chollenchymatous cells, that vary in cell size and intercellular spaces, Chlorophyll is rarely present in the cortex. Sclereids are observed in the cortex. The outer layers of the cortex sometimes contain resins. Inside the cortex, the vascular system is composed of phloem (ph) (including sieve tubes, companion cells, phloem parenchyma and fibers).

A complete cambium ring (Ca) separates the phloem from the xylem (x). Xylem fibers cover more than 60% of the wood structure. The living cells in the wood are the ray parenchymas which are of uniseriate type (narrow ray) with one cell wide. The area of the rays is small in comparison with the fibers and the vessels. Close to the pith, the xylem is found as a group of narrow vessels. Inside the xylem, the pith is composed of parenchyma cells. This finding is in harmony with that found by **Fahn (1969)** on citrus plants.

Root development:

It was observed that the root primordium initiated from combial zone Plates (8 & 9). This tissue resumed its activity by dividing to give root primordia (rp) and continued in growth giving adventitious roots (ar) which penetrated outward through different tissues of the explant (phloem, cortex and periderm). At that stage, conductive tissues (phloem and xylem) were differentiated and connected with the main vascular tissues of the explants (Plate, 10).

It was observed that some root primordia failed to continue their development due to the presence of sclereids or the formation of resins in the cortex, which formed a mechanical barrier to the developing roots. Such

finding is in line with that found by **Guindy (1990)** on some fruit rootstocks, (Plate, 10).

Effect of adding NAA

Data concerning rooting of mandarin are shown in Table (9). It can be seen that applying NAA at all concentrations; 0.5, 1.0, 1.5 or 2.0 mg/L to the MS medium gave better effects on rooting % when compared with the control. High concentration of NAA promotes formation of some root primordiaum from callus tissues (Plates, 11&12). The most marked effect was obtained with NAA at 1.5 mg/L, i.e., high concentration of NAA (1.5 mg/L) gave the higher percentage of rooting per explant from combial zone and callus, (Plate, 12). These results may explain the reasons behind the high survival percentages of some plantlets produced from MS medium+NAA at 1.5 mg/L when compared with the other concentrations of NAA. These results are in the same line with those found by **Guindy (1990)** on some fruit rootstocks.

In conclusion, the results showed a clear protocol for developing the selected mandarin seedlings through tissue culture technique. Mandarin explants (shoot tip) seedlings surface sterilized and cultured in MS medium at full strength BA for shooting and NAA for rooting to obtain good plantlets. These findings are cope with of those found by **Guindy (1990)** on some fruit rootstocks, **El-Sayed (2005)** on sweet basil and thyme and **Shaaban (2000)** on some selected citrus cultivars.

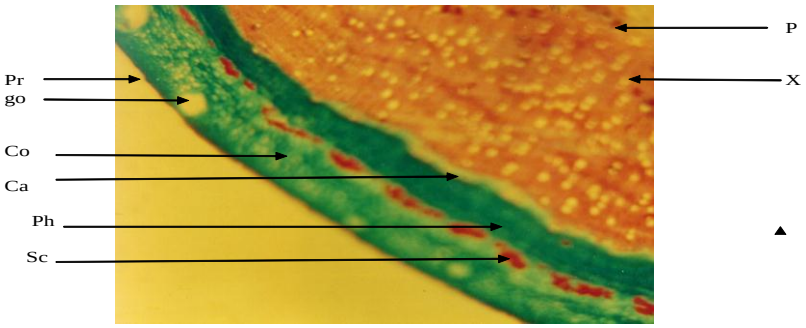

Plate (5). Cross section in Balady mandarin plantlet shows:
a) Periderm (pr); b) Cortex (Co); c) Gland oil (go); d) Sclerieds (Sc); e) Phloem (ph); f) ambium (Ca); g) Xylem (X); h) Pith (p).

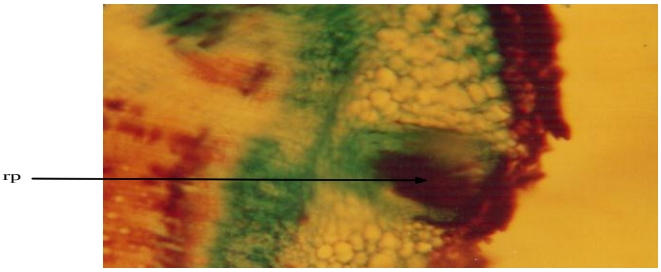

Plate (6). Cross section in Balady mandarin plantlet shows that the root primordia (rp) were initiated from cambial zone as affected by NAA application at low concentrations.

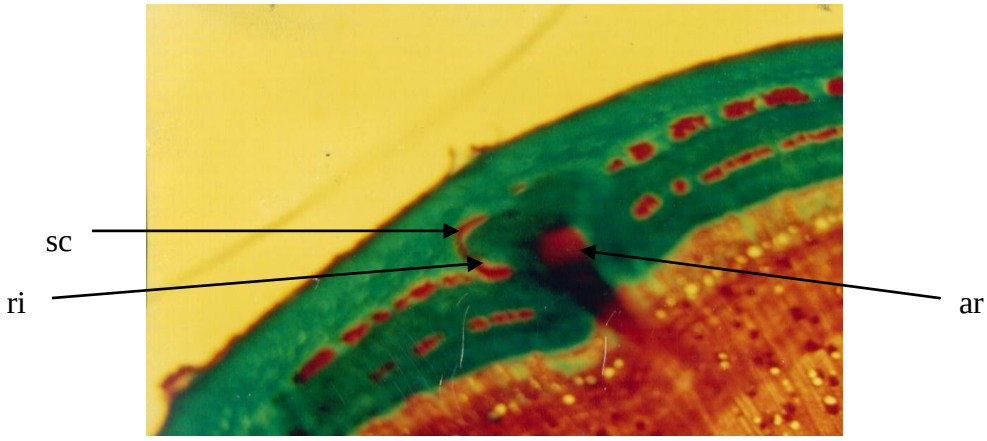

Plate (7). Cross section in Balady mandarin plantlet shows that the root primordia forming adventitious root (ar) and sclarieds (sc) and risens (ri) forming a mechanical barrier for growing.

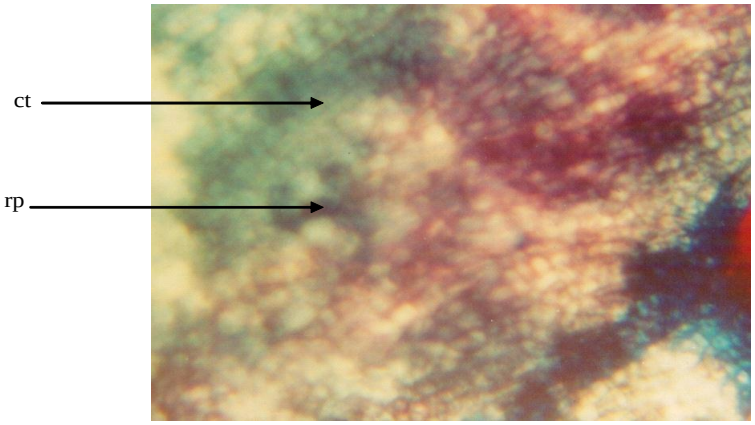

Plate (8). Cross section in stem of Balady mandarin plantlet shows that the root primordia was initiated from callus tissue (ct) as affected by NAA application at 1.0 mg/L.

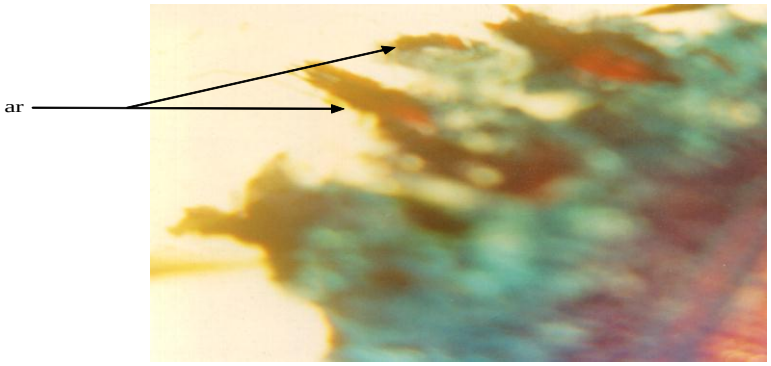

Plate (9). Cross section in stem of Balady mandarin plantlet shows that the root primordium forming adventitious roots (ar) at 1.0 mg/L NAA.

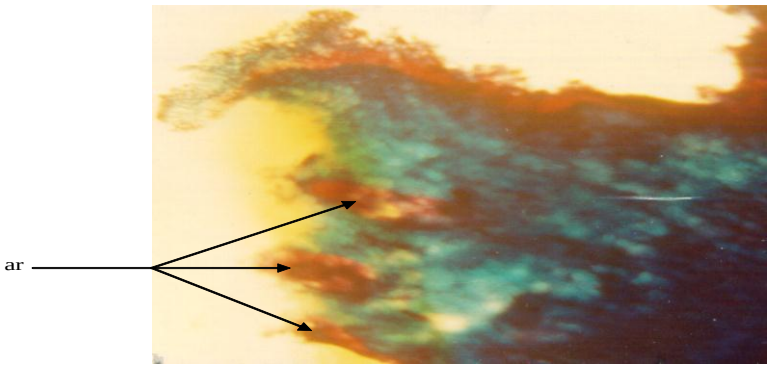

Plate (10). Cross section in stem of Balady mandarin plantlet shows that the root primordia forming the highest number of adventitious roots as affected by the high concentration of NAA (1.5 mg/L).

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تحسين محصول اليوسفي البلدي باستخدام الأنتخاب والتقييم وتقنية زراعة الانسجة

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من المعروف جيدا ان الاكثار الخضرى المستمر للموالح يؤدى الى نقص فى المحصول كما ونوعا بسبب انتقال الأمراض من الأشجار التي تؤخذ منها عيون الطعم والانحدار السلالى الناتج عن النقص المتزايد فى فاعلية RNA.

ومن هنا برزت فكرة هذا البحث بهدف الأنتخاب من الأشجار البذرية لليوسفى البلدى المنتشر زراعتها فى بعض حدائق محافظة الفيوم بغرض إنتاج سلالات عالية المحصول كما ونوعا وذلك بأتباع الخطوات التالية:

١- انتخاب وتقييم بعض الأشجار عاليه الإنتاج (كما و نوعا) من اليوسفى البلدى النامية في بعض الحدائق الخاصة بمحافظة الفيوم واستخدامها كأمهات.

٢- الانتاج الكمى المعملى لهذه الامهات عالية الانتاج كما ونوعا بأستخدام تقنية زراعة الأنسجة و العمل على نشر زراعتهابالمحافظة وذلك لتحسين أنتاج محصول اليوسفى.

٣- اجراء دراسة تشريحية لتفسير بعض النتائج المتحصل عليها ومنها سبب إرتفاع أو أنخفاض نجاح إنتاج بعض الشتلات و تأثير نسب مكونات البيئات المختلفة عليها.

اجريت هذه الدراسة خلال المواسم الاتية ٢٠٠٣ ، ٢٠٠٤ ، ٢٠٠٥ ثم ٢٠٠٦ ، ٢٠٠٧ على أشجار يوسفى بلدى بذرية نامية بمزرعة خاصة بمحافظة الفيوم حيث اجريت دراسات مبدئية أولا لتحديد عدد من الاشجار العالية الانتاج كما نوعا ثم تم اختيار افضل ٥ اشجار من اليوسفى البلدى البذرية وكانت جميع هذه الاشجار المنتخبة تخضع لمعاملات متماثلة من ري وتسميد.. الخ وذلك بأتباع الخطوات الأتية :

ا- انتخاب الأشجار الجيدة الصفات في المحصول كماً ونوعاً حيث تم التقييم كالاتى :

- المحصول (عدد الثمار ووزن الثمار) لكل شجرة.
- الصفات الطبيعية للثمار (وزن الثمرة حجم الثمرة سمك القشره وعدد البذور).
- الخصائص الكيماوية للثمار(المواد الصلبة الذائبة والحموضة وفيتامين C).

ب- الانتاج الكمى باستخدام زراعة الانسجة للاشجار المتميزه:

١- الغرض منها: استخدام تقنية زراعة الأنسجة للإنتاج الغزير والحصول على اعداد كبيرة من الأشجار المنتخبة والمتميزة (كما ونوعا) وذلك باستخدام زراعة القمم الميرستمية لنموات هذه الأشجار على بيئه غذائية معقمة (موراشيح - سكوج) حيث ان هذه الاشجار محدودة (شجره واحدة) حيث تم استخدام ٠.٣- ٠.٥ سم من القمة النامية وتم تطهيرها ثم زراعتها فى بيئه مورا شيج - سكوج الاساسية مضافاً إليها البنزايل أدينين بتركيزات مختلفة للحصول على الافرع الخضرية ثم اضافة نفتالين حمض الخليك بتركيزات مختلفة للحصول على الجذور.

وبعد الحصول على الشتلات المناسبة فى النمو الخضرى والجذري ثم أقلمتها و تم زراعتها في اصص صغيرة بها تربة مكونة من (الرمل + البيت موسى) بنسبة ١:١ حجما.

٢- أجريت تجربة تحدد نسبة بقاء الشتلات حيه وصالحه للزراعة

جـ- أجريت دراسة تشريحية:

الغرض منها تحديد الاسباب التى ادت الى انخفاض أو أرتفاع نسبة بقاء الشتلات حية فى بعض المعاملات.

وكانت اهم النتائج على النحو الاتى:

١. اظهرت النتائج تفوق بعض أشجار اليوسفى البلدى المنتخبة من حيث المحصول تفوقاً معنويا اذا ما قورنت بالمتوسط العام لأنتاج الأشجار الأخرى بمحافظة الفيوم.
٢. اظهرت النتائج تفوقا كبيرا فى صفات الجودة لثمار الأشجارالتي تم انتخابها من حيث الصفات الطبيعية (وزن الثمرة – حجم الثمرة – سمك القشرة – عدد البذور) ومن حيث الصفات الكيماوية (نسبة المواد الصلبة الذائبة – الحموضة – نسبة فيتامين ج).
٣. اظهرت النتائج زيادة عدد الشتلات الناتجة من زراعة الأنسجة للأشجارالمنتخبة عند زراعة القمم النامية للأفرع في بيئة موراشيج - سكوج الأساسية كاملة القوة مضافا اليها كل من بنزايل أدينين بمعدل ١.٥ مجم/لتر للحصول على اكبر عدد من النموات الخضرية ونفثالين حمض لخليك بمعدل ١.٥ملجم/لتر للحصول على اكبر كم من الجذور.
٤. اظهرت النتائج زيادة النسبة المئوية للشتلات الحية عند إضافة ١.٥ ملجم بنزيل أدينين/لتر الى بيئة موراشينج و سكوج وعلى العكس انخفضت هذه النسبة فى باقى المعاملات.
٥. فسرت الدراسة التشريحية ان زيادة تركيز نفتالين حمض الخليك في بيئه التجذير (١.٥ مجم/اللتر) شجع تكوين مبادئ الجذور من نسيج الكالوس بالاضافة الى تكوينها من الكامبيوم مما أدى الى زيادة كمية الجذور وهذا قد يفسر زيادة نسبة بقاء الشتلات حية والناتجة من هذه الاضافة.

ومن هذه الدراسه يتضح انه يمكن تحسين صفات أشجار اليوسفى البلدى المنتشرة فى محافظة الفيوم عن طريق انتخاب أمهات ذات جودة عالية فى المحصول (كما ونوعا) واستخدام تقنية زراعة الانسجة للحصول على الإكثار الكمي السريع من هذه الأشجار المنتخبة.