



IN VITRO PROPAGATION OF KORONAKI AND CORATINA OLIVE CVS.

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ABSTRACT

Experiments carried out through 2016 revealed that a proper protocol for the in vitro propagation of Koronaki and Coratina olive cvs. as follows : Sterilization of stem node explants was carried out successfully with copper sulphate at 50mg/l for Koronaki cv. And Ampicillin at 150mg/l for Coratina cv. both cultured on Olive medium. Multiplication on MS medium enriched with BAP at 1.5mg/l for both cvs.

Rooting of Koronaki on half strength OM medium enriched with IBA at 0.5mg/l and Coratina on half strength MS medium enriched with NAA at 0.1mg/l.

Acclimatization of Koronaki plantlets derived from half strength OM rooting medium and acclimatized on peat : sand (1:1) and Coratina plantlets derived from MS rooting medium and acclimatized on peat : sand : vermiculite (1:1:1).

INTRODUCTION

Olive (*Olea europaea* L.) is one of the most important fruit tree crop specially in of the Mediterranean sea countries. Olive is usually propagated by the conventional methods which are inefficient and have a limited success for some cultivars (such as Kalamata, Dolcie, Coratina and Koronaki). Propagation through seed is not desirable due to segregation and a long period of juvenility. Consequently, for most olive cultivars, micropropagation is more convenient than traditional propagation (Rugini et al 2001).

Besides the mineral formulation, growth regulators are also one of the most important components of the *in vitro* culture media. Since the pioneering work of OM medium (Rugini, 1984) is considered up-to-now as the most suitable one for olive micropropagation, but they also proved not to be effective for all cultivars (Katerina et al 2002) and (Peixe et al 2007). Acclimatization media is one of the most important factor (İsfendiyaroğlu et al 2007)

Micropropagation is a powerful tool for the quick production of genetically homogenous plants, since it enables rapid propagation and hastens the ability of new cultivars.

In fact, several studies have been conducted with the aim of developing alternative micropropagation methods that can help to overcome the limitations associated with the traditional techniques currently used for the propagation of olive trees (Peixe et al 2007 and Roussos et al 2002).

In vitro propagation of olive is still limited, due to poor growth, poor lateral bud outgrowth, variable rooting ability and acclimatization of the plantlets. Another problem encountered is that of intraspecific variation in tissue culture responses between different cultivars (Cozza et al 1997).

The present study was undertaken in order to develop a protocol for the rapid propagation of Koronaki and Coratina' olive cvs. through *in vitro* technique

MATERIALS AND METHODS

The present study was carried out through the spring of 2016 in order to find a protocol for the *in vitro* propagation of two hard to root olive cvs. Namely "Koronaki and Coratina".

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However the subsequent procedures which practiced here were as follow:

- Explant type

Cuttings of 15-20 cm length from middle parts of one-year shoots were collected from mature Koronaki and Coratina' olive trees of about 15 years old grown in Horticulture Research Institute farm. Giza, Egypt.

Stem node explants were taken from such cuttings then they were washed with tap water for an hour, and soaked in Sodium hypochlorite (NaOCl) 10% for 7 m and Mercury chloride (HgCl₂) 50 mg/l for 7 m. After which they were soaked three times with sterile water (10min for each) then following sterilization treatments were applied.

- Explant Sterelization

Four sterilization treatments were teasted i.e. control (distiled water), ampicillin 150 mg/l, copper sulphate at 50 mg/l, copper sulphate 100 mg/l.

- Multiplication Media

Two nuttonal media i.e. Murashige and Skoog (Murashige and Skoog, 1962) and Olive medium (Ruggini, 1984) were tried where each one was enriched with one of the six following cytokinin treatments, (BAP 0.5,1 and 1.5mg/l) Kin (0.5, 1and 1.5mg/l) besied the control.

- Rooting Media

The same nutrition media applied in the multiplication stage were used here but half strength and enriched with one of the following six auxin treatments (IBA 0.1, 0.3and 0, 5 mg/l), NAA (0.1, 0.3and 0.5 mg/l) besied the control.

- Acclimataziation Media

The successful plantlets derived from the rooting media were transferred instantly to different acclimatization media namely peat moss:sand (1;1) and peat moss:sand :vermiculite(1:1:1) and absorbed for two months at outdoor condition in a mist unit in a glasshouse (Rostami and Shah-savar, 2012).

Incubation Conditions

In each of three stages Establishment, Multipliation and Rooting explants were subcultured on fresh medium supplemented with the same concentrations of cytokinins, auxins and incubated in growth chamber at $22 \pm 1^{\circ}\text{C}$, with a photoperiod of 16 h and a light intensity of 3000 lux (Katerina et al 2002) and adjusted pH media with HCl and NaOH to 5.8 pH before autoclaving.

The experiment was factorial comprised of 3 factors i.e. cultivars, media, and treatments .Where each treatment represented by 4 replicates each one had 4 jars each contains 3explants.

Rooted plantlets were then transferred to a growth-cabinet with the same temperature, photoperiod and light intensity, but with progressive decreasing air humidity (HR) from 100 to 65%. Acclimated plants were then transferred to a greenhouse.

All data parameters studied were analyzed as Randomized Complete Blocks Design in factorial arrangement with three replications. All data were subjected to statistical analysis as described by (Snedecor and Cochran 1989). Mean separation were carried out using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Data present in Table (1) reveals the effect of different sterilization materials on survival % of Koronaki and Coratina explants cultured on MS and OM media in spring of 2016.

Results indicated that copper sulphate at 50 mg/l gained the significantly greteest survival % regardless of of two factors (cultivars and media). On the other hand, Koronaki cv. Showed significantly higher survival % than Coratina cv. irrespectiv to media and treat factors.

However, OM medium surpassed significantly than MS one in survival %, regardless of cultivars and treatments.

Regarding of the interation between the three studied factors (treatments , cvs. and media) it is apparent that Koronaki expanta sterilized with copper sulphate at 50 mg/l and cultured on OM medium achieved the highest survival % .(60%) Such results are in approval with the (Hassan et al 2016)

In Table (2) one can notice that different studied cytokinins significantly induced shoot formation on studied olive cvs. than control without significant difference among them.

Table 1. Effect of Different Sterilization Materials on Survival (%) of Koronaki and Coratina explants cultured on MS and OM media in spring 2016

	Koronaki		Coratina		Treatments Mean
	M.S	OM	M.S	OM	
Control	40.00 d	20.00 f	00.00 h	10.00 g	17.50 C
Ampicillin 150 mg/L	40.00 d	43.33 c	20.00 f	60.00 a	40.83 B
Copper Sulphate at 50 mg/L	40.00 d	60.00 a	50.00 b	50.00 b	50.00 A
Copper Sulphate at 100 mg/L	60.00 a	30.00 e	30.00 e	50.00 b	42.50 B
Cv. Mean	41.67 A⁻		33.75 B⁻		
Media Mean	35.00 B⁺		40.42 A⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each table

Table 2. Effect of Cytokinens, cultivars and media type on number of Shoot/ explant in the 1st subculture of multiplication stage in spring 2016

	Koronaki		Coratina		Treatments Mean
	M.S	OM	M.S	OM	
Control	0.00 c	0.00 c	0.00 c	0.00 c	0.00 B
BAP 0.5 mg/L	1.66 ab	1.33 ab	1.66 ab	2.00 ab	1.66 A
BAP 1.0 mg/L	1.33 ab	1.33 ab	1.66 ab	1.66 ab	1.50 A
BAP 1.5 mg/L	2.00 ab	1.33 ab	2.33 a	2.00 ab	1.91 A
kin 0.5 mg/L	1.00 bc	1.00 bc	1.66 ab	1.66 ab	1.33 A
kin 1.0 mg/L	2.00 ab	1.33 ab	2.00 ab	1.33 ab	1.66 A
kin 1.5 mg/L	1.66 ab	2.33 a	1.33 ab	1.33 ab	1.66 A
Cv. Mean	1.452 A⁻		1.333 A⁻		
Media Mean	1.310 A⁺		1.476 A⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each table

In addition, the two studied cvs. as well as the two practiced multiplication media failed to differ significantly.

However, Koronaki cv. Cultured on OM medium enriched with Kin at 1.5 mg/l as well Coratina explants cultured on MS medium enriched with BAP 1.5 mg /l showed the greatest number of shoots /explants (2.33). Such results are in accordance with the finding of (Roussos and Pontikis, 2002) and (Katerina et al 2002).

As shown in **Table (3)** BAP at 1 and 1.5 mg/l gave the tallest shoots without significant difference than BAP 0.5 g/l regardless of other studied factors.

In addition koronaki cv was better than coratina one irrespective to other studied factors.

Meanwhile, no significant difference was detected between both studied multiplication media.

Regarding the interaction between the three studied factors, one can observe that Koronaki explants cultured on Ms medium enriched with BAP at 0.5 mg/l exhibited the tallest shoots (5.86 cm).

As the effect of cytokinins on the number of leaves /explant, **Table (4)** exhibited that BAP at 1.5mg/L gained the significantly greatest number regardless of other studied factors.

On the other hand, both cvs. And media variables failed to affect such parameter irrespective to cytokinin factor.

However, Koronaki explants cultured on MS medium supplemented by BAP 1.5mg/L gave the greatest number of leaves /explants (23.43).

The obtained results are in agreement with the finding of (Ansar et al 2009 b); Hassan et al 2016).On the contrary, the present results differ than the finding of (Manisha et al 2014).

Table 3. Effect of Cytokinens, cultivars and media type on mean of Shoot length (cm) in the 1st subculture of multiplication stage in spring 2016

	Koronaki		Coratina		Treatments Mean
	M.S	OM	M.S	OM	
Control	2.45 i	2.43 i	2.16 i	2.69 i	2.43 C
BAP 0.5 mg/L	5.86 a	3.92 e-h	4.81 b-d	3.49 h	4.52 AB
BAP 1.0 mg/L	5.46 ab	4.70 b-e	5.33 a-c	4.45 d-g	4.98 A
BAP 1.5 mg/L	4.92 b-d	4.13 d-h	5.45 ab	3.44 h	4.48 AB
kin 0.5 mg/L	3.70 gh	3.48 h	4.71 b-e	3.95 e-h	3.96 B
kin 1.0 mg/L	4.48 d-g	3.55 h	4.60 c-f	3.74 gh	4.09 B
kin 1.5 mg/L	3.51 h	3.84 f-h	4.58 c-f	4.21 d-h	4.03 B
Cv. Mean	4.433 A⁻		3.718 B⁻		
Media Mean	4.033 A⁺		4.118 A⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each Table

Table 4. Effect of Cytokinens, cultivars and media type on number of leaves/ explant in the 1st subculture of multiplication stage in 2016

	Koronaki		Coratina		Treatments Mean
	M.S	OM	M.S	OM	
Control	6.00 o	4.66 o	5.66 o	5.33 o	5.41 D
BAP 0.5 mg/L	20.03 bc	14.23 i-m	15.53 g-k	13.60 j-l	15.85 BC
BAP 1.0 mg/L	16.24 f-i	20.50 b	16.75 e-h	14.87 h-l	17.09 B
BAP 1.5 mg/L	23.43 a	17.57 d-g	18.88 b-e	18.43 b-e	19.58 A
kin 0.5 mg/L	11.60 n	13.00 l-n	18.09 c-f	19.45 b-d	15.53 BC
kin 1.0 mg/L	13.40 k-n	14.03 i-m	13.84 j-n	14.72 h-m	14.00 C
kin 1.5 mg/L	13.73 j-n	15.87 g-j	12.49 mn	15.83 g-j	14.48 C
Cv. Mean	14.69 A⁻		14.44 A⁻		
Media Mean	14.59 A⁺		14.53 A⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each Table

The beneficial effect of cytokinins in improving and hastening the number of shoots and their length as well as number of leaves per explant may be due to their chemical structure which contain nitrogenous bases especially adenine which considered as a component of nucleic acids which in turn play an important role of breaking apical dominance of the terminal bud and inducing branching.

Regarding the effect of auxins on rooting %, number of roots / explant and root length, **Tables (5, 6 and 7)** indicated that both IBA and NAA at any concentration significantly increased the three studied parameters than the control regardless of the other two factors.

On the contrary, both cvs. and rooting media failed to affect the three considered parameters irrespective to auxin treatments. As far the effect of

the interaction between the auxin treatment, cvs. and rooting media, it is apparent that Koronaki cv. cultured on half-strength OM medium enriched with 0.5 mg IBA/L as well as Coratina cv. cultured on half-strength MS medium supplemented with 0.1 mg/L NAA achieved the significantly highest rooting % (80.0).

Meanwhile, Koronaki explants grown on OM medium enriched by 0.1 mg/L IBA gained the greatest number of roots / explants. (7.33). On the other hand, Koronaki explant cultured on MS medium plus 0.3 mg/L NAA showed the tallest root length (18.67 cm). Such results are in accordance with those found by (Binet et al 2002), (Mencuccini 2002) and (Ali et al 2009). Whereas, they differ than the results of (Katerina et al 2002), (Ansar et al 2009a) and (Manisha et al 2014).

Table 5. Effect of different auxin concentrations on rooting percentage of “Koronaki “ and “Coratina” cvs shoots after 5 weeks on rooting medium 2016

	Koronaki		Coratina		Treatments Mean
	M.S	OM	M.S	OM	
(control)	33.33 i	33.33 i	23.33 j	30.00 ig	30.00 C
0.1 mg/L IBA	76.67 ab	66.67 c-e	76.67 ab	56.67 f-h	69.17 AB
0.3 mg/L IBA	80.00 a	76.67 ab	63.33 d-f	53.33 gh	68.33 AB
0.5 mg/L IBA	66.67 c-e	80.00 a	76.67 ab	66.67 c-e	72.50 A
0.1 mg/L NAA	66.67 c-e	66.67 c-e	80.00 a	73.33 a-c	71.67 AB
0.3 mg/L NAA	70.00 b-d	66.67 c-e	53.33 gh	70.00 b-d	65.00 AB
0.5 mg/L NAA	60.00 e-g	50.00 h	53.33 gh	60.00 e-g	55.83 B
Cv. Mean	62.86 A⁻		60.71 A⁻		
Media Mean	63.81 A⁺		59.76 A⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each Table

Table 6. Effect of different auxin concentrations on number of root/explant of “Koronaki “ and “Coratina” cvs shoots after 5 weeks on rooting medium 2016

	Koronaki		Coratina		Treatments Mean
	M.S	OM	M.S	OM	
Control	3.00 bc	5.33 a-c	6.33 ab	5.67 a-c	5.083 A
0.1 mg/L IBA	5.67 a-c	7.33 a	2.00 C	5.33 a-c	5.083 A
0.3 mg/L IBA	5.00 a-c	6.33 ab	6.67 ab	4.67 a-c	5.667 A
0.5 mg/L IBA	5.66 a-c	4.33 a-c	5.00 a-c	3.67 a-c	4.667 A
0.1 mg/L NAA	4.33 a-c	4.00 a-c	3.33 a-c	4.67 a-c	4.083 A
0.3 mg/L NAA	3.67 a-c	4.67 a-c	4.33 a-c	5.33 a-c	4.500 A
0.5 mg/L NAA	4.67 a-c	6.00 a-c	3.67 a-c	5.33 a-c	4.917 A
Cv. Mean	4.524 A⁻		5.190 A⁻		
Media Mean	5.000 A⁺		4.714 A⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each Table

Table 7. Effect of different auxin concentrations on mean of root length (cm) of “Koronaki“ and “Coratina” cvs shoots after 5 weeks on rooting medium 2016

	Koronaki		Coratina		Treatments Mean
	M.S	OM	M.S	OM	
(control)	2.17 j	3.33 h-j	2.50 ij	3.33 h-j	2.83 B
0.1 mg/L IBA	5.00 e-j	11.50 bc	9.33 b-h	9.00 b-h	8.71 A
0.3 mg/L IBA	7.17 c-j	11.33 b-d	8.33 b-i	8.67 b-h	8.88 A
0.5 mg/L IBA	4.33 f-j	7.17 c-j	10.67 b-e	9.50 b-g	7.92 AB
0.1 mg/L NAA	10.80 b-e	6.17 c-j	6.17 c-j	10.00 b-g	8.28 AB
0.3 mg/L NAA	18.67 a	10.17 b-f	4.00 g-j	8.33 b-i	10.29 A
0.5 mg/L NAA	14.17 ab	4.83 e-j	6.50 c-j	5.33 d-j	7.71 AB
Cv. Mean	7.843 A⁻		7.762 A⁻		
Media Mean	8.343 A⁺		7.262 A⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each Table

The mode of action of auxin as mentioned by previous investigations as increasing the elasticity and plasticity of cell wall by activating the cellulose and pectinase and other hydrolytic enzymes, as well as inducing cell division and formation of adventitious roots on stem and leaves tissues.

As for the achieved experiment **Table (8)** illustrates that Koronaki plantlets derived from OM rooting medium and transplanted into peat moss: sand (1:1) showed significantly higher survival % (66.67) opposite to Coratina plantlets derived from the same rooting medium and transplanted into any acclimatization medium (20%).

Table 8. Effect of acclimatization medium on survival (%) of "Koronaki" and "Coratina" plantlets developed on MS and OM rooting media after (2 months) acclimatization

	Koronaki		Coratina		Acclimatization Mean
	M.S	OM	M.S	OM	
Peat moss: sand (1:1)	60.00 b	66.67 a	33.33 e	20.00 f	44.44 A
Peat moss: sand : vermiculite (1:1:1)	31.10 e	40.00 d	53.33 c	20.00 f	36.67 B
Cv. Mean	49.44 A ⁻		31.67 B ⁻		
Rooting Media Mean	45.00 A ⁺		36.11 B ⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each Table

Regarding the effect of acclimatization medium on shoot length of the developed plantlets, **Table (9)** showed that no significant differences were detected between the three studied factors.

But in regarding the interaction between these variables, one can notice that Coratina plantlets derived from MS rooting medium and transplanted into any considered acclimatization medium gained the tallest shoots (13.67 and 15.00 cm).

Table 9. Effect of acclimatization medium on shoot length (cm) of "Koronaki" and "Coratina" plantlets developed on MS and OM rooting media after (2 months) acclimatization

	Koronaki		Coratina		Acclimatization Mean
	M.S	OM	M.S	OM	
Peat moss: sand (1:1)	13.00 ab	12.33 ab	13.67 a	12.67 ab	12.92 A
Peat moss: sand :vermiculite (1:1:1)	8.33 c	10.00 bc	15.00 a	12.00 ab	11.33 A
Cv. Mean	10.91 A ⁻		13.33 A ⁻		
Rooting Media Mean	12.50 A ⁺		11.75 A ⁺		

Means having the same letter (s) in each row, column or interaction are insignificantly different at 5% level in each Table

CONCLUSION AND RECOMMENDATION

The main target of the present research work is developing protocols for in vitro propagating of two hard to root olive cvs. Namely, Koronaki and Coratina, where such cvs. are too famous as oil cvs. where fruit oil % reaches about 24% as fresh weight basis but unfortunately they are difficult to be propagated by stem cutting by the use of auxin treatment.

However, the recommended explant type for olive cvs. (stem node) was adopted, and different sterilization multiplication, rooting and acclimatization methods were tried here finally, such work ended to the proper protocol for the In vitro propagation for each cv. individually as follows.

Koronaki alive cv. sterilization of explants with copper sulphate 50mg/l. Multiplication on OM medium enriched with Kin 1.5mg/l. Rooting on OM

medium enriched by IBA at 0.5mg. Acclimation on peat moss: sand (1:1).

Coratina alive cv. sterilization of explants with ampicillin 150mg/l. Multiplication on MS medium enriched with BAP at 1.5mg/l. Rooting on MS medium enriched by 0.1mg/l NAA. Acclimation on peat moss : sand : vermiculite (1:1:1)

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الإكثار المعملّي لصنفي الزيتون كروناكي وكوراتينا

[132]

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موراشيخ وسكوج بأضافة بنزيل أمينو بيورين بتركيز 1.5 ملجم/لتر لكلا الصنفين. بالنسبة للتجدير لصنف كروناكي كان على بيئة الزيتون نصف قوة مزودة باندول حامض البيوتريك 0.5 ملجم /لتر والكوراتينا كان على بيئة موراشيخ وسكوج نصف قوة مدعمة بنفثالين حامض الخليك بتركيز 0.1 ملجم /لتر ومرحلة أقلمة النبيتات لصنف الكروناكي الناتجة من بيئة الزيتون للتجدير نصف قوة أقلمت في بيت : رمل (1:1) ونبيتات الكوراتينا الناتجة من بيئة تجدير موراشيخ وسكوج نصف قوة أقلمت في بيت : رمل : فرميكيولايت (1:1:1).

الكلمات الدالة: الزيتون، كرواتينا، كروناكي، الأكتار المعملّي، التعقيم، التضاعف، التجدير، الأقلمة

الموجز

أجريت هذه التجربة في 2016 للأكتار الدقيق والوصول الى برتوكول مناسب لأكتار صنفى الزيتون كروناكي وكوراتينا كالأتى :تم تعقيم العقد الساقية بكبريتات النحاس 50 ملجم /لتر لصنف كروناكي وأمبيسلين 150 ملجم / لتر لصنف الكوراتينا وكلاهما زرعاً على بيئة الزيتون. أما التضاعف كان على بيئة

1816



مجلة اتحاد الجامعات العربية
للعلوم الزراعية
جامعة عين شمس ، القاهرة
مجلد(26)، عدد (2C)، عدد خاص ، 1809 - 1816، 2018

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