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Effect of Growth Regulators on Callus Induction and Somatic Embryogenesis in Olive.

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ABSTRACT

The present study was carried out to induce callus and somatic embryogenesis Coratina olive cv using different of explants and growth regulators. To induce callus the used explants were cultured on full strength MS medium with 2ip or TDZ combined with either 2, 4-D, IBA or NAA and incubated in dark. The obtained callus was sub-cultured on ½ strangest MS media supplemented with 2ip or TDZ without auxin and incubated either in low or high light intensity conditions. The obtained results showed that callus induction% and callus weight was higher in cotyledons fragment compared with the embryos, the highest callus induction percentage was recorded on media containing TDZ+NAA at 5mg L^{-1}, followed by TDZ+ IBA at 5mg L^{-1}.The highest weight was obtained by using TDZ +IBA at 2.5 mg L^{-1} followed by 2iP +NAA at 2.5 mg L^{-1}. Callus texture and color was not affected by explants type, callus color ranged from white to the brown according to the used growth regulators and the dominant callus type was compact. The highest somatic embryogenesis was obtained with 2ip and low light intensity, the globular embryo developed successfully and germination of mature embryos, achieved on ⅓ MS medium without growth regulators.

Keywords: Olive, *in vitro*, callus, somatic embryogenesis.

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INTRODUCTION

Olive tree plays important role in the economy of the Mediterranean area where 95 of trees are growing [1]. The genetic improvement of olive tree is very limited. The prolonged duration of juvenile phase, low seeds germination, high level of heterozygote and lack of genetic information are the main constrains to olive breeding [2,3]. Recently the genetic improvement of olive tree is based on the selection of individuals adapted to local conditions [4, 5, 6 and 7]. A few cultivars from breeding programs have been reported [8and 9]. For genetic improvement of olive, the *in vitro* somatic embryogenesis techniques provide a good alternate to classic breeding methods [10]. Somatic embryogenesis was achieved from hypocotyls, immature zygotic embryos and seed cotyledons [11, 12]. Growth regulators are an important factor in the induction and development of somatic embryogenesis [2]. Somatic embryogenesis depends on combinations of cytokinin and auxin on culture media [13]. According to Canas and Benbodis [2] BA, NAA, and 2, 4.D affect callus induction of olive embryos, the highest callus induction was achieved on media containing 5 to12.5 µm NAA. The type and concentration of both auxin and cytokinin affected callus type [14].

The aim of the present study is to develop an improved *in vitro* regeneration system for olive *via* somatic embryogenesis using different types of explants and growth regulator combination.

MATERIALS AND METHODS

The present study was performed during two successive years (2015 – 2016) at tissue culture laboratory of Pomology department, Faculty of Agriculture, Cairo University.

Plant materials

Fruits of Coratina olive cv. were harvested when the color begin to change from yellow green to violet. The stones were separated from the fleshy mesocarp and the endocarp was broken according to Sotomayor-Leon and Caballero, [15] the stoneless seeds were used for embryo isolation.

Surface sterilization

The stoneless seeds were surface sterilized with 20% commercial sodium hypochlorite solution for 15 min. transferred to 0.1% $HgCl_2$ solution for 5 min. [16, 17], and washed three times with sterile water.

Embryo isolation

The sterilized seeds were soaked for 48h in sterilized water to promote swelling and faciliting the embryo isolation. Embryo isolation performed in laminar flow hood by cutting into 2 lateral sections, and freeing the embryo from the remaining seed tissue [18, 17]. Embryo axe and cotyledons were separated (cut transversally into apical and basal pieces). The embryo axe and cotyledons were cultured separately into the callus induction media. Three explants were placed individually in sterile jars each containing 30 ml of culture media. The jars were incubated in growth chamber at 25°C with 16 h photoperiod.

Media composition

Callus induction media

Full strength MS medium [19] containing 3% sucrose supplemented with one combination of auxin and cytokinin and solidified by adding 6% agar (Table 1). The media pH was adjusted to 5.8, and autoclaved.

Somatic embryogenesis

Somatic embryogenesis media composed of ½ strength MS medium, 3% sucrose and supplemented with 2ip or TDZ at 2 mg L^{-1} plus 0.5g L^{-1} L-glutamine.

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Table (1) Growth regulators combination used in callus induction media

Media	Cytokinine	Auxin	
1	2ip at 0.1 mg L^{-1}	IBA	2.5 mg L^{-1}
2			5.0 mg L^{-1}
3		2,4-D	2.5 mg L^{-1}
4			5.0 mg L^{-1}
5		NAA	2.5 mg L^{-1}
6			5.0 mg L^{-1}
7	TDZ at 0.1mg L^{-1}	IBA	2.5 mg L^{-1}
8			5.0 mg L^{-1}
9		2,4-D	2.5 mg L^{-1}
10			5.0 mg L^{-1}
11		NAA	2.5 mg L^{-1}
12			5.0 mg L^{-1}

Embryo germination

Embryo germination was performed on ⅓ strength MS medium containing 3% sucrose without any growth regulators.

The following parameters were recorded

1. Callus induction percentage.
2. Callus fresh weight (g).
3. Callus color: white-yellowish-brown
4. Callus texture: compact-friable
5. Percentage of somatic embryogenesis (as the development of the globular embryo)

Experimental design and data analysis

The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [20] using MSTAT-C statistical software package [21], and means of the treatments were compared by Least Significant Difference (L.S.D.) according to Duncan [22] at significance level of 0.01.

RESULTS AND DISCUSSION

Data presented in table (2) showed that, callus induction percentage was highly affected by explant type and plant growth regulator. Induction percentage was higher in cotyledon explants compared with the embryos regardless of the used growth regulator. Concerning the effect of growth regulators the highest, induction percentage was recorded on media containing TDZ+NAA at 5mg L^{-1} , followed by TDZ+ IBA at 5mg L^{-1}, while there was no callus induction on media containing 2iP+ NAA at 5mg L^{-1} or TDZ +2, 4, D at 2.5mg L^{-1} with both explants types.

As shown in Table [3] callus fresh weight of Coratina olive cultivar was higher on cotyledons explants compared with the embryos. Concerning the effect of growth regulators the highest callus weight was recorded on TDZ +IBA at 2.5 mg L^{-1} followed by 2iP +NAA at 2.5 mg L^{-1}. The best result was recorded for cotyledons explants cultured on medium containing TDZ +IBA at 2.5 mg L^{-1}.

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Table (2) Effect of explants type and growth regulators on callus induction percentage of Coratina olive cultivar

Growth regulators			Callus induction percentage (%)		
Cytokinine ($0.1mgL^{-1}$)	Auxin (mgL^{-1})		Embryo	Cotyledons	Mean
2ip	IBA	2.5	20.0 l	23 k	**21.50 H**
		5.0	57.5i	60 h	**58.75 F**
	2,4-D	2.5	57 i	91.6 bc	**74.30 E**
		5.0	85 e	73 g	**79.00 D**
	NAA	2.5	22 kl	50 j	**36.00 G**
		5.0	0 n	0 n	**0.00 K**
TDZ	IBA	2.5	88 d	76 f	**82.00 C**
		5.0	76 f	93 ab	**84.50 B**
	2,4-D	2.5	0.0 n	0 n	**0.00 K**
		5.0	0 n	12 m	**6.00 J**
	NAA	2.5	12.5 m	20 l	**16.25 I**
		5.0	95 a	90 cd	**92.5 A**
	Mean		**42.75**	****49.05**	

Means within each column with the same letter are not significantly different at $P < 0.01$

Table (3) Effect of explants type and growth regulators on callus fresh weight of Coratina olive cultivar

Growth regulators			Callus weight (g)		
Cytokinine ($0.1mgL^{-1}$)	Auxin (mgL^{-1})		Embryo	Cotyledons	Mean
2ip	IBA	2.5	0 i	0 i	**0.0 I**
		5.0	2.6 g	5.8 d	**4.2 E**
	2,4-D	2.5	5.4 e	4.8 f	**5.1 C**
		5.0	5.6 de	0 i	**2.8 G**
	NAA	2.5	0 i	14.2 b	**7.1 B**
		5.0	0 i	0 i	**0.0 I**
TDZ	IBA	2.5	5.4 e	16.7 a	**11.05 A**
		5.0	4.7 f	2.2 h	**3.45 F**
	2,4-D	2.5	0 i	0 i	**0.0 I**
		5.0	0 i	9.4 c	**4.7 D**
	NAA	2.5	2.4 gh	2.1h	**2.25 H**
		5.0	0 i	0 i	**0.0 I**
	Mean		**2.175**	****4.60**	

Means within each column with the same letter are not significantly different at $P < 0.01$

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Table (4) the effect of explants type and growth regulators on callus texture and color of Coratina olive cv.

Growth regulators			Callus texture		Callus color	
Cytokinine (0.1mgL^{-1})	Auxin (mgL^{-1})		Embryo	Cotyledons	Embryo	Cotyledons
2ip	IBA	2.5	------	------	--------	--------
		5.0	compact	compact	Brown	yellowish
	2,4-D	2.5	compact	compact	Brown	Brown
	NAA	5.0	compact	compact	Yellow\Brown	Yellow\Brown
		2.5	------	compact	--------	yellow
		5.0	------	------	--------	--------
TDZ	IBA	2.5	compact	compact	yellow	yellow
		5.0	compact	compact	Yellow\Brown	Yellow\Brown
	2,4-D	2.5	------	------	--------	--------
	NAA	5.0	------	------	--------	--------
		2.5	------	------	--------	--------
		5.0	compact	compact	White\yellow	White\yellow

In study on three olive cultivars Mencuccini and Rugini, [23] reported that the highest callus formation was achieved on MS medium supplemented with TDZ and NAA at 2.5µm. Callus induction reached about 100% for the explants incubated on media supplemented with 2.5µM 2iP and 25µM IBA [24]. The types of callus varied according to the used medium, 2iP or BAP produced small and compact callus [14].

Data presented in table [4] showed that callus texture of Coratina cv. was not affected by explants type or the added growth regulators; the dominant type of callus texture was the compact type.

The callus color of Coratina cv. ranged from white to brown according to the used growth regulators (Table 3and Fig. 1). It is evident that, both kind and concentration growth regulators had a marked effect on callus color; also, that interaction between cytokinine and auxin has evident, effect. Callus color of Coratina cv. was not affected by explants type.

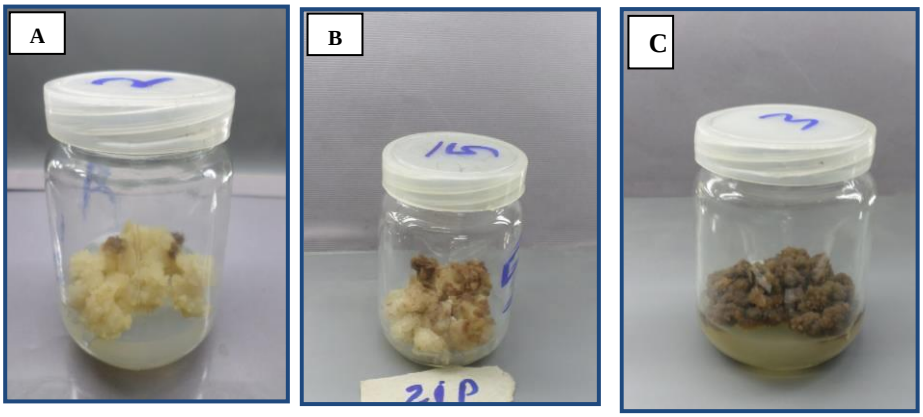

Fig (1).Effect of growth regulators on callus color (A) yellow (2ip+ IBA at 5 mg L^{-1}), (B) yellow\brown (2ip+2,4D at 5 mg L^{-1}) and (C) brown (2ip+2,4D at 2.5 mg L^{-1})

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Table (5) the effect of growth regulators and light intensity on somatic embryogenesis of Coratina olive cv.

Cytokinine (2 mg L^{-1})	Somatic embryogenesis %			Somatic embryos developed into plantlet %		
	Low light	High light	**Mean**	Low light	High light	**Mean**
2ip	34 ab	30 bc	**32 A**	13 a	0 d	**6.5A**
TDZ	38 b	26 cd	**32 A**	10 b	0 d	**5.0 B**
BAP	27 bc	19 d	**32 B**	8 c	0 d	**4.0 B**
Mean	**33****	**25**		**10****	**0**	

Means within each column with the same letter are not significantly different at $P < 0.01$

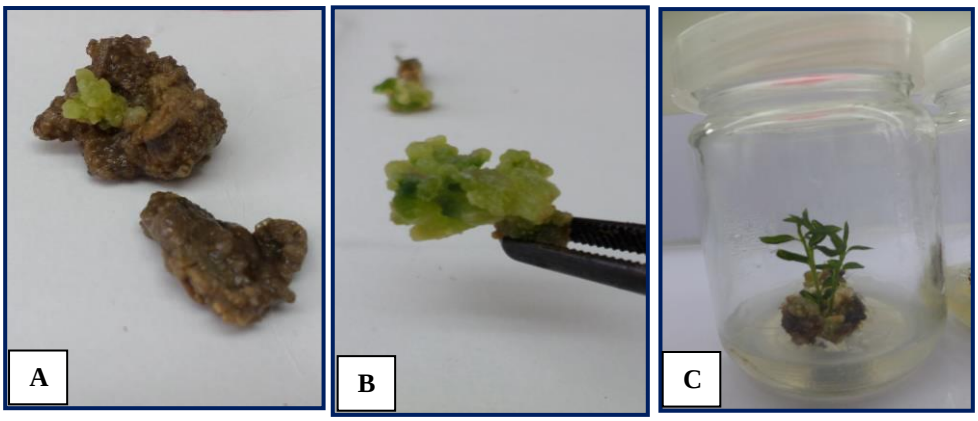

Fig (2) The development of somatic embryo (A. globular somatic embryo form compact callus mass, B. Cotyledonary stage somatic embryo, C. 6-weeks-old developed plantlets.

Data presented in Table (5) and Fig. (2) indicated that the formation of embryogenic callus and the potential of somatic embryo to develop into normal plants was highly affected by the kind of cytokinine. 2ip recorded the highest percentage of developed planted while BAP recorded the lowest. Somatic embryogenesis was developed to plants only at the low light intensity regardless the cytokinine, incubation of the callus under high light condition resulted in the formation of globular embryo, which later turned to dark green and failed to develop to normal embryo.

According to Rugini [12] the elimination of auxin from the somatic embryogenesis media was essential for the normal development of somatic embryos. Shibli et al., [25] in study on somatic embryogenesis of Nabali olive, reported that 2ip resulted in the highest percentage of somatic embryogenesis compared with the other cytokinine. Similar results were reported by Mencuccini and Rugini [23].

Generally dark condition was better for the induction and development of somatic embryogenesis [26].

Mencuccini and Rugini [23] reported that, darkness was essential for successful regeneration of olive somatic embryos. Also, Shibli et al., [25] found that somatic embryogenesis of Nabali olive cv. occurred only under low light intensity. Light condition inhibiting somatic embryogenesis of olive was reported [12, 14]. Benelli et al., [27] attributed the failure of somatic embryo development to histological defects during last growth stages

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