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ORIGINAL ARTICLE

Callus Induction from Anther Explant of Olive (*Olea Europaea* L.) Influenced by Plant Growth Regulators

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ABSTRACT

In order to investigation of effective optimum condition effecting callus induction, close floral bud of olive conservolea cultivar harvested. After disinfection of anther explants cultured on the full MS and modified MS medium with different concentrations of TDZ, NAA, 2,4-D and 2ip. Cultured anthers in the full MS medium a little swallowed and callus not formed. Maximum of callugenesis percentage obtained in MS modified with 0.5 mg/l 2,4-D and 0.1 mg/l 2ip. Using TDZ growth regulator in 2.5 mg/l alone resulted in shoot direct regeneration from anther. Callus production rate from olive anther in dark condition was significantly more than light condition.

Key words: anther, callus induction, in vitro, olive.

Introduction

The Olive (*Olea europaea* L.) belonging to the Oleaceae family. It is native to coastal of the eastern Mediterranean region and northern Iran. In recent years due to high oil nutritional value and tolerance to environmental stresses, its cultivation was increased in most of region of Iran[15].

The tissue culture technique was reported by Guha and Maheshwari on Datura anther culture at first[7,1]. One of the haploid plant production methods is anther culture in vitro used as a complementary suitable method in traditional breeding programs[9,17] that with production of diploid and haploid callus and/or direct regeneration is possible[15]. There are two pathways in anther culture for plant regeneration as including direct and indirect androgenesis. Both of them may be observed in the same culture. Androgenesis type are influenced by factors such as genotype[3,4,6,8,10,17], pollen grain development of stage[2,3,6,8,9,13,18,19], medium composition [3,4,8,17], rate of growth regulators [3,4], illumination regimes[3,5,6] and physiological state of mother plant[6].

Produced haploid lines from microspore and anther culture provide high potential for genetic breeding with development of genetic variation through production of completely homozygous lines in short time [3,4,10,9,14,17]. Haploid plants have high heterosis value that with duplicating chromosome number, very isogenic line obtained[9]. This issue produced by long-term and expensive process of inbreeding, numerous back cross hybridization and controlled self-pollination in traditional breeding programs[9]. Produced haploid and dihaploid plants from anther culture identified with morphological, biochemical and molecular marker[4] and chromosome counting[15].

So for few reports published about olive anther culture and it is essential for conducting such research in this field. The main objective of this research was investigation of growth regulators and

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illumination condition effects on induction and optimum olive conservolea callus and it's evaluation for usable haploid plantlet regeneration in olive breeding program.

Material and methods

In this research Olive conservolea trees (eight ages) available in Kazeroon Olive research station were used. Mother plants were grown in the optimal condition. Branches with flower buds were cut and carried in the plastic bags and transferred to the laboratory. Then buds separated from shoots and used as explants.

Shoots were stripped of leaves and washed with tap water. The explants (flower bud and anther) were disinfected using 2 g/l Benmyle with three drops of Tween 20, shaken on rotary shaker for 20 min and sterilized with NaOCL 10% solutions at 15 min under Air-flow hood and rinsed 3 times sterile distill water.

The half of anthers (1-3 mm length) were separated from floral buds and the rest such as close floral bud were cultured in Petri dishes (9mm width) containing 30 ml MS modified medium (SRASH) (45 g/l sucrose, 8 g/l agar; Germany Merck company, 0.4 mg/l thiamine, 40 mg/l EDTATE, 730 mg/l glutamine). The pH was adjusted to 5.7 by 1N HCl and NaOH before agar addition and autoclaving (121°C and 1.5 atm pressure duration 20 minutes).

The effects of Thidiazuron (TDZ) at 0, 2.5, 5 and 7.5 mg/l and 1- naphthaleneacetic acid (NAA) at 0.02, 0.1 and 0.5 mg/l concentrations separately and in combination each other and 2,4-dichlorophenoxyacetic acid (2,4-D) at 0, 0.5 and 1 mg/l and 6-dimethylamino purine (2ip) at 0, 0.1 and 0.2 mg/l concentrations separately and in combination each other were examined on the callus induction from anther explants of olive conservolea cultivar.
Samples placed in two light (1500 lux provided with cool white fluorescents lamps) and full dark (using aluminum foil) conditions with 16-h photoperiod and 25±2°C. Produced calluses rate after seven week culture were recorded. Then, these produced calluses were subcultured in new medium for plantlet regeneration and storage in growth place.

The effect of different growth regulators concentrations and illumination were investigated on callus induction and growth of olive conservolea cultivar. The rate of callus production was evaluated as percentage and its callus color coded as 1, 2 and 3 representing the white, yellow and brown, respectively. Produced callus quality in the friable face, semi friable and compact coded as 3, 2 and 1, respectively. This research carried out with 10 replications in completely random design that each replication was included 25 explants. Data analysis performed with LSD test in SPSS software, version 13.

Results and discussion

Disinfection treatment used for separated anthers was more effective than floral bud. All of the explants were free fungus and bacteria contaminations. The culture of close floral bud led to 55% and 23% fungus and bacterial contaminations, respectively. Two days after culture, remains of stamen filament developed and penetrated to the medium. The anthers began to swell four days after culture time. Many of the anthers had brown color after ten days following culture time. There are not visible any changes in anthers after six week duration keeping in medium and many of them destroyed.

Callus induction in the all hormonal treatments under full dark condition was significantly more than light condition (data not shown) so that any callus not formed in the light condition. Also unusual anthers were visible in the light condition after three week of culture, resulted in brown and black color that with increasing TDZ concentration to 7.5 mg/l was increased (treatment 15). Two types of callus obtained from olive anther culture that the first at 2-3 weeks and second at 4-6 weeks produced after culture time. Produced calluses were different as quantity, color and quality between treatments so that yellow callus obtained in 20 and 21 medium. The other calluses were white and brown color. Maximum percentage of callus induction obtained under dark condition in 21 treatments (Fig. 1) so that have significant differences with control (1% level) and other treatments (5% level) as presented in Table 1.

Application of NAA growth regulator in a 2.5 mg/l concentration alone resulted in shoot direct regeneration that was effective in high concentration in comparison with other researches. Using 2ip (0.2 mg/l) in combination with 2,4-D (1 mg/l) resulted in embryogenesis. The results indicated that 2,4-D auxin for callus induction is better than NAA that indicate auxin type effect on the callus induction. Callus production accomplished in auxin or cytokinin presence. Induction and optimum growth of Callus accomplished in 5:1 and 10:1 auxin/cytokinin ratio, respectively. Application of 2,4-D and 2ip in 0.5 and 0.1 mg/l concentrations, respectively, could be suggested to callus induction in olive anther culture especially in conservolea cultivar.

Discussion

Response of anther explant to androgenesis process was controlled by genetical and nongenetical factors that influenced by agents such as genotype[3,4,6,8,10,17], development stage of pollen grain[2,3,6,8,9,1318,19], chemical, cold and heat pretreatments[3,17], mother plant condition (from a nutrition, pests, diseases and irrigation point of view), season of explant choice (growth temperature of

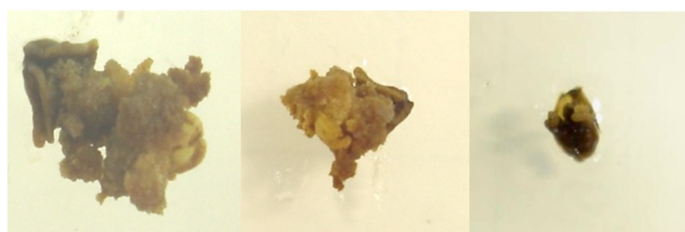

Fig. 1: Callus produced from anther explant of olive conservolea cultivar in MS modified medium.

Table 1: The growth regulators effects in MS modified medium on callus induction from anther explant of olive conservolea cultivar. Color (1: white, 2: yellow, 3: brown); Quality (1: compact, 2: semi friable 3: friable).

Treatment number	Plant growth Regulators (mg/l)		Callus induction (%)	Color	Quality
	TDZ	NAA			
1	0	0	-	-	-
2		0.02	4 d	3	1
3		0.1	4 d	3	1
4		0.5	-	-	-
5	2.5	0	-	-	-
6		0.02	-	-	-
7		0.1	-	-	-
8		0.5	-	-	-
9	5	0	-	-	-
10		0.02	-	-	-
11		0.1	-	-	-
12		0.5	18 bc	1	1
13	7.5	0	20 c	3	1
14	0.02	-	-	-	
15		0.1	19 c	3	1
16		0.5	-	-	-
	2,4-D	2ip			
17	0	0	-	-	-
18		0.1	-	-	-
19		0.2	-	-	-
20	0.5	0	23 b	2	3
21		0.1	37 a	2	2
22		0.2	15 c	1	1
23	1	0	20 b	-	-
24		0.1	23 b	3	2
25		0.2	24 b	3	1

mother plant)[6], medium composition[3,4,8,17], rate of growth regulators[3,4] and dark-light regimes[3,5,6].

Callus induction under dark condition accomplished more than light condition that agreed with Ekiz and Konzak resulted[5]. Callus formation in plant is influence by genotype. Different plant species will indicate different responses to type and growth regulator concentration and auxin\cytokinin ratio so that callus induction was accomplished in eleven mediums from tested 25 mediums. Auxin type was effective on callus induction in conservolea olive cultivar that agreed with Brasileiro *et al*.[3] and in consistent with Haque Aminul *et al*. [8]

The presence of suitable concentration of growth regulators in medium have play vital role in anther culture and many reports have emphasize that auxin and cytokinin are essential constituent of medium. callus formation accomplished in presence of auxin or cytokinin so that view of Brasileiro *et al*. [3] said callus formation accomplished in medium containing auxin and cytokinin it become restitution, although use of GA3 growth regulator in low concentration (0.1 mg/l) in the some species resulted in callus formation. The auxin to cytokinin ratio was effective on callus induction and growth. We can resultant that require suitable concentrations of growth regulators to callus induction for olive anther culture and will not callus formation in medium without growth regulator.

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