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Research Article
Zeatin and Thidiazuron Induced Embryogenic Calli From *In Vitro* Leaf and Stem of Jojoba (*Simmondsia chinensis*)

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Abstract

Background and Objective: Jojoba is a promising industrial plant, which recommended with pharmaceutical benefits. The present study was conducted to stimulate embryogenic calli formation from jojoba using zeatin and thidiazuron (TDZ), as well as determination of the antioxidant activity of proliferated calli. **Materials and Methods:** For callus induction, leaf and stem explants derived from *in vitro* grown shootlets, were cultured on Murashige and Skoog (MS) medium with different combinations of 0.5 mg L^{-1} benzyl adenine (BA) or kinetin with 2,4-Dichlorophenoxyacetic acid (2,4-D), Naphthalene acetic acid (NAA) and picloram at 0.5 or 1mg L^{-1}. To stimulate embryogenic calli, friable callus were transferred to woody plant medium (WPM) supplemented with different concentrations of zeatin or TDZ. Antioxidant activity of different treatments was determined using hexane or petroleum ether extraction. Data was analyzed as mean±standard deviation (SD). **Results:** The MS medium supplemented with 0.5 mg L^{-1} BA+0.5 or 1 mg L^{-1} picloram was the best treatment to obtain friable calli from both explants types. WPM medium supplemented with 2 mg L^{-1} zeatin gave the highest percentage of embryogenic calli derived from leaf explants. While the highest percentage of embryogenic calli derived from stem explants was registered using 1 or 4 mg L^{-1} TDZ containing medium. Embryogenic calli originated from leaves explants on 1.5 mg L^{-1} zeatin showed promising activity of antioxidant with hexane extraction. However, embryogenic calli originated from stem explants on 1 mg L^{-1} TDZ showed the highest antioxidant activity with petroleum ether extraction. **Conclusion:** TDZ has promising effect on embryogenic callus induction from stem explants. While, zeatin has promising effect on embryogenic callus induction from leaf explants.

Key words: Jojoba, callus induction, embryogenic calli, zeatin, thidiazuron

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jojoba (*Simmondsia chinensis*) plant became one of the important multi-purpose crops cultivated in arid and semi-arid regions. It is adapted to hot and dry climates and it is evaluated as desertification resistant crop. The Optimum growth occurs in the range 27-36°C but wild plants can withstand very high temperature[1]. The cosmetic industry appears to be the principal market for jojoba oil products and the other major industry using jojoba oil is the pharmaceutical sector. Jojoba is a dioecious species, i.e., having separate male and female plants. Only the females, however, give the valuable seeds. As jojoba is a cross pollinated crop, the progeny is highly heterozygous having tendency to generate seedlings of widely varying size, shape and yield, which has raised doubts about the economic feasibility of cultivating jojoba. The success of jojoba growers and indeed of the entire jojoba industry depends upon selection of high yielding genotypes and their multiplication through vegetative means. Direct selection based on identifying potentially promising genotypes and their testing after vegetative propagation has enabled improvement in crop performance[2].

Biotechnology approaches can be utilized for jojoba micro propagation and *in vitro* preservation[3]. Micro propagation offers opportunities for production of elite and true to type plants from the selected stock plant of jojoba in a short time independent of the season[4]. In this respect, considerable differences among clones in response to growth regulators at all stages of *in vitro* propagation have been reported. A nutrient medium may be suitable as a starting point for several genotypes but each clone needs research to optimize it's *in vitro* response[5,6]. There are reports of jojoba on somatic embryogenesis from zygotic embryo[7,8] and leaf explants[9]. On the other hand, proliferation of callus cultures would be other need of jojoba biotechnology. Jojoba oil can be produced from various explants include *in vitro* callus cultures[10]. In this respect, oil from callus, if extracted by suitable methods, were minimize the dependence on seeds for oil production[11]. Otherwise, the development of an *in vitro* regeneration system from unorganized tissues considered very important issue for genetic manipulation of jojoba using modern biotechnology applications. TDZ and zeatin are considered as cytokinins which are a class of plant growth substances that promote cell division. Many physiological and biochemical events in cells are enhanced by TDZ, however, its mode of action is unknown[12]. They are involved in cell growth and differentiation. There are two types of cytokinins, adenine type like zeatin and phenyl-urea type like TDZ[13]. It is well known that seeds which contain the zygotic embryo and also the somatic embryos are the main source of secondary metabolites. Therefore, the present work aimed to recognize an effective protocol for callus induction, maintenance and embryogenic callus induction with examining the anti-oxidant activity of jojoba.

MATERIALS AND METHODS

This study was carried out in Department of Plant Biotechnology, National Research Centre, Giza, Egypt, during the period from January-December, 2016.

Establishment *in vitro* cultures: Female jojoba plants from Egyptian natural oil company were used as plant materials. Nodal segments were washed by running tap water for 30 min and then dipped in ethanol (Sigma) 70% for 30 sec. The explants were then sterilized with Clorox (60%) and mercuric chloride solution (0.2%) for 10 min. After sterilizing, the explants were rinsed in sterilized distilled water (three times) to remove all traces of the disinfectants. All steps of the sterilization producers were conducted under aseptic conditions using laminar airflow cabinet. The explants were aseptically cultured on solidified MS[14] medium supplemented with 0.5 mg L^{-1} Benzyl adenine keeping one explants in a jar. Contaminated cultures were discarded and the proliferated shootlets were used for callus induction.

Callus induction: For callus induction, leaves and stem of *in vitro* jojoba cultures were cut to segments of about 0.5 cm long and placed on MS medium supplemented with different combinations of BA, Kin and 2,4-D, NAA and Picloram (Table 1).

The responses of both explants as callus induction, callus morphology (color and behavior) were registered after 30, 45 and 60 days of culturing.

Table 1: Composition of media used for callus induction

Treatment	Composition
J1	0.5 mg L^{-1} BA+0.5 mg L^{-1} NAA.
J2	0.5 mg L^{-1} BA+1.0 mg L^{-1} NAA.
J3	0.5 mg L^{-1} BA+0.5 mg L^{-1} 2,4-D.
J4	0.5 mg L^{-1} BA+1.0 mg L^{-1} 2,4-D.
J5	0.5 mg L^{-1} Kin+0.5 mg L^{-1} NAA.
J6	0.5 mg L^{-1} Kin+1.0 mg L^{-1} NAA.
J7	0.5 mg L^{-1} kin+0.5 mg L^{-1} 2,4-D.
J8	0.5 mg L^{-1} kin+1.0 mg L^{-1} 2,4-D.
J9	0.5 m L^{-1} BA+0.5 mg L^{-1} Picloram.
J10	0.5 m L^{-1} BA+1.0 mg L^{-1} Picloram.
J11	0.5 mg L^{-1} Kin+0.5 mg L^{-1} Picloram.
J12	0.5 mg L^{-1} Kin+1.0 mg L^{-1} Picloram.

BA: Benzyl adenine, Kin: Kinetin, NAA: Naphthalene acetic acid, 2,4-D :2, 4 Dichlorophenoxyacetic acid

Callus maintenance: The inducted callus, from both leaves and stem, were maintained for two subcultures (each subculture is four weeks) on the same media to specify the treatment that will give friable callus with high rate.

Callus fresh weight (g), behavior and growth value were detected for each subculture[15].

$$\text{Growth value} = \frac{\text{Current weight} - \text{Initial weight}}{\text{Initial weight}}$$

Embryogenic callus induction: In order to embryogenic callus induction, about 0.5 g of friable callus line from leaves and stems were transferred to woody plant (WPM) medium supplemented with different concentrations of TDZ or zeatin (1, 2, 4 mg L^{-1}) for two subcultures (each subculture is four weeks). Callus weight (g), color and behavior were recorded either visually or under binocular.

All the media were supplemented with 30 g L^{-1} sucrose and solidified with 8 g L^{-1} agar. The pH of the media was adjusted to 5.8 with 1N KOH prior to autoclaving (121°C, 20 min). Media were dispensed into glass jars (5×15 cm) with 30 mL in each jar. Embryogenic calli culture were incubated at 24±2°C at darkness for callus induction and maintenance then transferred light at a light intensity of approximately 2000 Lux provided by cool white fluorescent lamps with 16 h photoperiod for embryogenic callus induction.

Determination of antioxidant activity through Radical Scavenging (DPPH) activity

Sample preparation: Two grams of jojoba callus from each treatment were dried in oven at 40°C and for 3 days.

Sample extraction: Seventy five mg of grounded dried samples were extracted with either 1.5 mL hexane or petroleum ether for 24 h. Then the extracts sonication in an ultrasonic water bath (Grant, United Kingdom) for 20 min. Samples were centrifuged (Sigma, 2-16 PK, Germany) for 5 min at 6000 rpm. The supernatants were collected and the pellets were re-extracted twice with 500 µL of the both extraction solvents. The extracts were stored at -20°C until further use[16].

Antioxidant activity through DPPH activity: The DPPH assay according to Gabr *et al.*[16] was used with some the stock reagent solution (1×10^{-3} mol L^{-1}) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at -20°C until use. The working solution (6×10^{-5} mol L^{-1}) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8+or –0.02 at 515 nm, as measured using a spectrophotometer. Extracts in ethanol solutions of different concentrations (0.1 mL of each) were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follow:

$$\text{Radical-scavenging (DPPH)activity}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

A is the absorbance at 515 nm.

Statistical analysis: All analysis were performed in triplicate and data reported as mean±standard deviation (SD). Results were processed by Excel 2010.

RESULTS AND DISCUSSION

Callus induction: In the current study, twelve treatments were used to investigate the effect of different growth regulators on callus induction from jojoba *in vitro* leaves or stem explants during three periods (30, 45 and 60 days). Callus formation from leaves explants was induced after the first period (30 days) with all treatments except J1, J11 and J12 as shown in Table 2. After 45 days, J11 and J12 showed callus induction, while J1 did not induce callus even after 60 days of culturing. Callus color from leaves explants varied from white, creamy, yellow, green or brownish. All treatments gave friable callus except J5, J6, J11 and J12 which gave compact callus. Regarding the stem explants, treatments J4, J7, J8, J9 and J10 induced callus after the first period, 30 days, while the other treatments showed callus induction later. Treatments J1 and J2 did not induce callus even after 60 days of culturing. Callus color from stem explants varies between creamy or brownish. The J3, J7, J9 and J10 gave friable callus while other treatments gave compact callus. It could be concluded that J9 and J10 gave high and early response with both explants. The J3 gave high and early response with leaves explants only while J8 gave high and early response with stem explants Fig. 1, 2. These results are in agreement with those found by Singer[15], who reported that using MS medium supplemented with 0.5 mg L^{-1} BAP+2 mg L^{-1} 2,4-D+100 mg L^{-1} case in hydrolysate gave the highest response of callusing from leaf explants of jojoba. In the same way, Gaber *et al.*[7] stated same results. However, Taha[17] reported that the combination l so between BA and NAA gave the highest response for callogenesis. In this respect, Aftab *et al.*[10] mentioned that auxins and cytokinins ratio plays a great role in regulating of *in vitro* callus induction.

Table 2: Effect of different growth regulators on callus initiation from two explants (leaves and stem) after 30, 45 and 60 days of culturing

	Leaves					Stem				
	Response					Response				
Treatments	30 days	45 days	60 days	Callus color	Callus behavior	30 days	45 days	60 days	Callus color	Callus behavior
J1	-	-	-	-	-	-	-	-	-	-
J2	+	++	++	white	Friable	-	-	-	-	-
J3	+++	+++	+++	Green	Friable	-	-	++	Slight green	Friable
J4	+	++	+++	Brownish	Friable	+	++	+++	green	Compact
J5	+	+	++	Yellow green	Compact	-	+	++	Brownish	Compact
J6	+	+	+	Creamy	Compact	-	-	+	Brownish	Compact
J7	+++	+++	+++	Yellow	Friable	+	++	+++	Creamy	Friable
J8	++	+++	+++	Creamy	Friable	+++	+++	+++	Brownish	Compact
J9	+++	+++	+++	Brownish	Friable	+++	+++	+++	Brownish	Friable
J10	+++	+++	+++	Creamy brownish	Friable	+++	+++	+++	Brownish	Friable
J11	-	+	++	Creamy brownish	Compact	-	+++	+++	Brownish	Compact
J12	-	+	++	Creamy	Compact	-	+	++	Creamy	Compact

- = 0 % response, + = 33.3% response, ++= 66.6 % response, +++= 100.0 % response

Fig. 1: Callus induction from (a) leaves and (b) stem on a medium containing 0.5 mg L^{-1} BA+0.5 mg L^{-1} picloram

Fig. 2: Callus induction from (a) leaves and (b) stem on a medium containing 0.5 mg L^{-1} BA+1.0 mg L^{-1} picloram

Table 3: Effect of different growth regulators on callus weight and growth value for two subcultures of leaf explants

	Leaves					
	First subculture			Second subculture		
Treatments	Callus weight (g)	Callus behavior	Growth value	Callus fresh weight (g)	Callus behavior	Growth value
J3	1.0±0.2	Friable	1.0	1.7±0.6	Friable	2.4
J4	4.3±1.2	Friable	7.6	5.0±0.4	Friable	9.0
J7	5.8±0.6	Compact	10.6	4.0±0.5	Compact	7.0
J8	4.8±0.2	Friable	8.6	3.7±1.0	Friable	6.4
J9	6.4±0.5	Friable	11.8	6.8±0.4	Friable	12.6
J10	5.5±0.8	Friable	10.0	6.4±0.6	Friable	11.8
J11	0.9±0.2	Friable	0.8	1.7±0.3	Friable	2.4
J12	1.9±0.2	Compact	2.8	2.3±0.3	Friable	3.6

Each value consists of mean±Standard Deviation

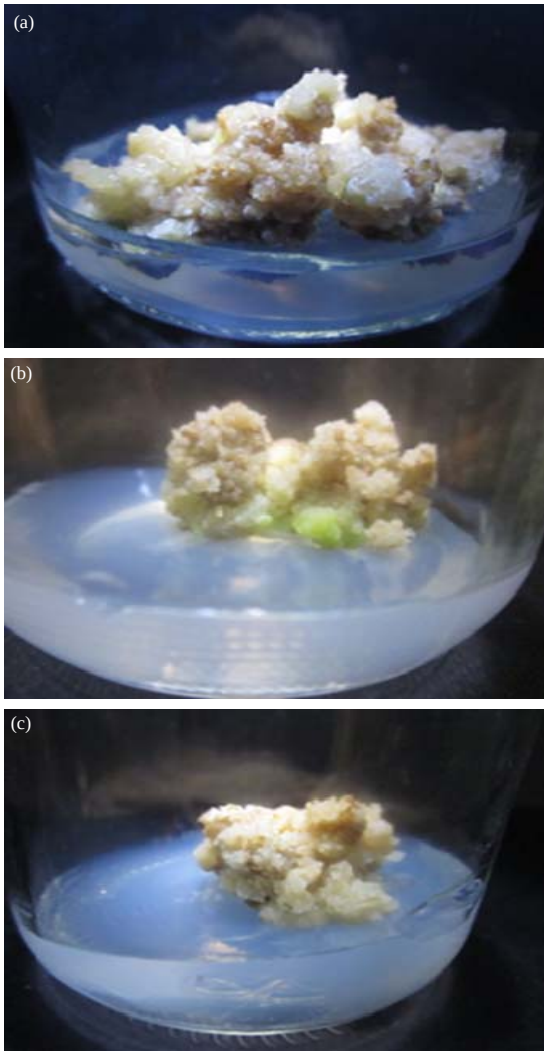

Fig. 3: Callus of jojoba derived from leaves maintained on a medium containing (a) 0.5 mg L^{-1} BA+0.5 mg L^{-1} picloram, (b) 0.5 mg L^{-1} BA+1mg L^{-1} picloram and (c) 0.5 mg L^{-1} BA+1mg L^{-1} 2,4-D

Callus maintenance: In the following experiments, J1, J2, J5 and J6 treatments were excluded since they either did not give response or gave late and low response with compact callus. To specify the maintenance media, induced callus from the previous treatments was sub-cultured two times (each subculture is four weeks) on the same media, which give high friable callus weight and growth value. The J9 and J10 treatments gave the highest callus weight during both subcultures, subsequently the highest growth value and friable callus as shown in the data of Table 3. This was followed by using J4 for the two subcultures Fig. 3. While, J7 and J8 treatments gave high callus weight in the first subculture but decreased in the second subculture and subsequently the growth rate. The lowest callus weight was recorded with treatment J11.

Concerning callus maintenance from stem segments, Table 4 showed that J4 treatment gave the highest callus weight (5.5 and 6.0, respectively) and subsequently the growth value (10.0 and 11.0, respectively) in the first and second subculture. This was followed by J9 and J10 treatments in both subcultures which oppose what was found with the leaves explants (Fig. 4). However, J8 treatment recorded high callus weight in the first subculture but it decreased dramatically in the second subculture. The lowest callus weight and growth value were recorded with treatment J11 in the two subcultures. Then it could be concluded that using a combination of 0.5 mg L^{-1} BAP+0.5 or 1 mg L^{-1} picloram (J9 or J10) could be used as a maintenance media for callus from leaves explants of jojoba which give high weight of friable callus. However, using mg L^{-1} BAP+1 mg L^{-1} 2,4-D (J4) is more preferable with stem segments explants. In this regard, Susan[15] reported that using MS medium supplemented with 0.5 mg L^{-1} BAP+2 mg L^{-1} 2,4-D+100 mg L^{-1} casein hydrolysate could be used as a maintenance medium for callus from leaves, here using a combination of BAP and 2,4-D

Table 4: Effect of different growth regulators on callus weight and growth value for two subcultures of stem segments explants

	Stem					
	First subculture			Second subculture		
Treatments	Callus fresh weight (g)	Callus behavior	Growth value	Callus fresh weight (g)	Callus behavior	Growth value
J3	2.6±0.7	Friable	4.2	1.6±0.2	Friable	2.2
J4	5.5±1.6	Friable	10.0	6.0±1.1	Friable	11.0
J7	2.6±1.1	Friable	4.2	2.9±0.2	Friable	4.8
J8	8.1±2.0	Compact	15.2	4.6±0.5	Compact	8.2
J9	3.9±0.6	Friable	6.4	5.0±1.5	Friable	9.0
J10	5.1±1.9	Friable	9.2	5.6±1.1	Friable	10.2
J11	1.6±0.6	Compact	2.2	1.3±0.4	Friable	1.6
J12	1.5±0.3	Compact	2.0	2.0±0.2	Friable	3.0

Each value consists of Mean±Standard Deviation

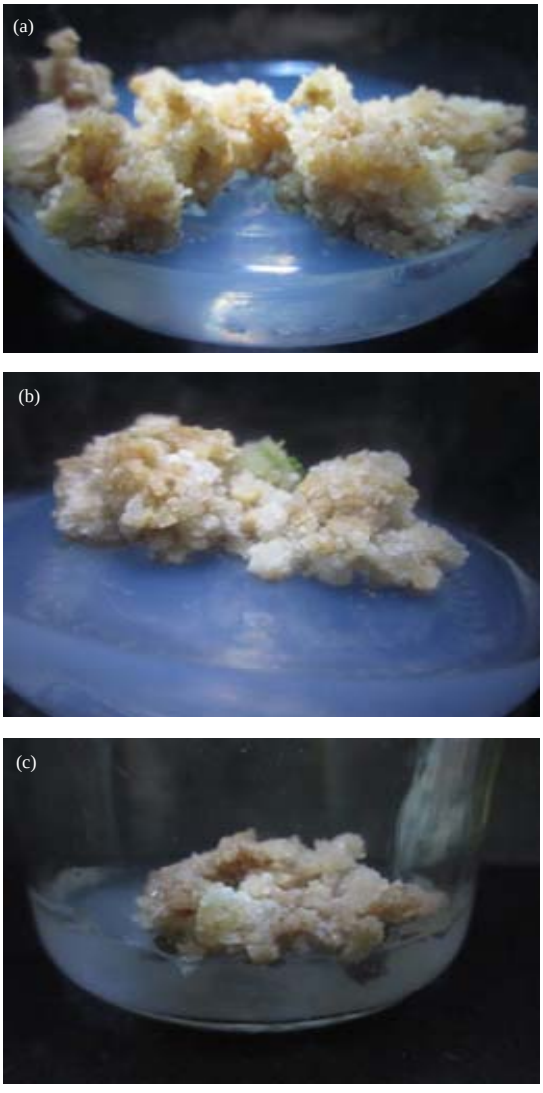

Fig. 4: Callus of jojoba derived from stem explants maintained on a medium containing (a) 0.5 mg L^{-1} BA+1 mg L^{-1} 2,4-D, (b) 0.5 mg L^{-1} BA+0.5 mg L^{-1} picloram and (c) 0.5 mg L^{-1} BA+1 mg L^{-1} picloram

is more preferable with callus from stem segments explant. As for callus from leaves using picloram with BAP gave higher callus weight with high growth rate.

The auxin-like properties of picloram were compared with those of other auxins in tissue cultures of wheat (*Triticum aestivum*), soybeans (*Glycine max*), tobacco (*Nicotiana tabacum*) and jack bean (*Canavalia ensiformis*) explants[18]. There are many problems were associated with the use of 2,4-D in tissue culture but not encountered when picloram were used in the culture medium. In general, picloram was more effective at low concentrations than the other auxins. The data indicated potential uses of picloram in routine callus cultures, in regeneration of plants from calli and in research concerning the physiological development of plant tissues[19]. Kordestani and Karami[19] reported that maximum embryogenesis from leaves of two strawberry cultivars (Selva and Comarosa) were achieved with using 2 mg L^{-1} picloram. While, Benlioglu *et al.*[20] reported that the medium containing 2,4-D gave higher callus weight and callus induction values than that containing picloram with two rice cultivars (Aromatik-1 and Baldo) while using picloram gave higher callus weight and callus induction value was observed with Karadeniz rice.

Embryogenic callus induction: For embryogenic callus induction, 0.5 g of friable callus derived from leaves and stem explants were transferred to woody plant (WPM) medium supplemented with the different concentrations of TDZ or zeatin for two subcultures there was a slight increase in callus weight during the two subcultures with different treatments on callus induction from leaves explants as shown in Table 5. All callus cultured on TDZ were turned green with different grades. Moreover, at the highest concentration (4 mg L^{-1}) of TDZ showed glorying tiny parts (Fig. 5). Using zeatin at different concentrations gives creamy callus with different grades between creamy, or creamy green. Two mg L^{-1} zeatin

Table 5: Effect of TDZ or Zeatin on embryogenic callus induction from callus derived from leaves explants during two subcultures

	First subcultures			Second subculture		
Treatments	Callus fresh weight (g)	Callus color	Callus behavior	Callus fresh weight (g)	Callus color	Callus behavior
1 mg L^{-1} TDZ	3.4±0.3	Dark creamy and slight green	Friable	3.5±05	Creamy and slight green	Friable
2 mg L^{-1} TDZ	4.5±0.4	Dark creamy and green	Friable	4.8±0.4	Dark creamy and green	Friable
4 mg L^{-1} TDZ	4.1±0.2	Dark green creamy	Friable	4.3±0.7	Dark green creamy with tiny glorying parts	Friable
1 mg L^{-1} Zeatin	2.5±0.5	Dark creamy	Friable	2.7±0.2	Dark creamy	Friable
2 mg L^{-1} Zeatin	4.0±0.3	Creamy green	Friable	4.2±0.3	Creamy green with glorying parts	Friable
4 mg L^{-1} Zeatin	2.4±0.3	Creamy brownish	Friable	2.6±0.2	Creamy brownish with white small pins	Friable

Each value consists of Mean±Standard Deviation

Table 6: Effect of TDZ and zeatin on embryogenic callus induction from callus derived from stem explants for two subcultures

	First subculture			Second subculture		
Treatments	Callus fresh weight (g)	Callus color	Callus behavior	Callus fresh weight (g)	Callus color	Callus behavior
1 mg L^{-1} TDZ	2.9±0.7	Creamy green	Friable	3.3±0.5	Creamy green with white tiny pins	Friable
2 mg L^{-1} TDZ	2.0±0.2	Dark creamy	Friable	2.4±0.3	Creamy green with glorying parts	Friable
4 mg L^{-1} TDZ	2.9±0.6	Creamy green	Friable	3.2±0.4	Green with white tiny parts and creamy red	Friable
1 mg L^{-1} Zeatin	1.5±0.2	Creamy brown	Friable	2.2±0.2	Creamy green	Friable
2 mg L^{-1} Zeatin	1.7±0.3	Brownish green	friable	2.4±0.4	Dark creamy brown	Compact
4 mg L^{-1} Zeatin	1.0±0.2	brownish	Friable	2.0±0.4	Creamy	Friable

Each value consists of Mean±Standard Deviation

Fig. 5: Callus derived from leaves grown on (a) 1 mg L^{-1}, (b) 2 mg L^{-1} and (c) 4 mg L^{-1} TDZ under binocular

showed tiny glorying parts. While, 4 mg L^{-1} zeatin gave creamy brownish with small pins (Fig. 6). It could be concluded that, zeatin containing treatments was more effective with callus from leaves explants.

Embryogenic callus induction from stem explants was presented in Table 6. There was a slight increase in callus weight during the two subcultures with different TDZ or zeatin treatments. TDZ turned callus green with different grades. Using 1 mg L^{-1} TDZ gave white tiny pins a creamy green friable callus. While glorying parts appeared with 2 mg L^{-1} TDZ. Small white tiny pins appeared on the callus cultures as increasing TDZ to 4 mg L^{-1} many (Fig. 7). Otherwise, all zeatin treatments gave callus with different creamy grades without any obvious other noticeable description (Fig. 8). TDZ and

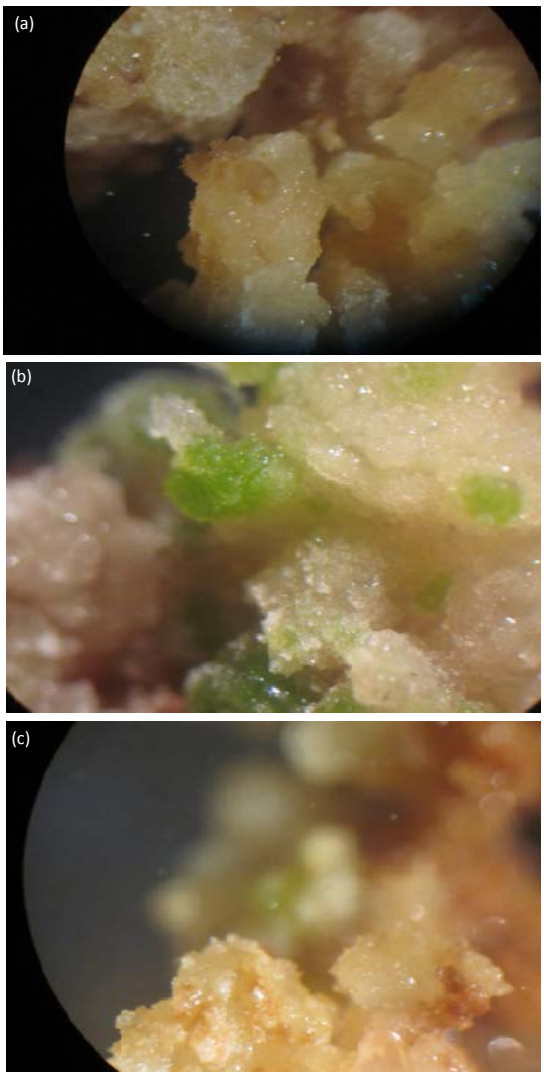

Fig. 6: Callus derived from leaves grown on (a) 1 mg L^{-1}, (b) 1.5 mg L^{-1} and (c) 4 mg L^{-1} zeatin under binocular

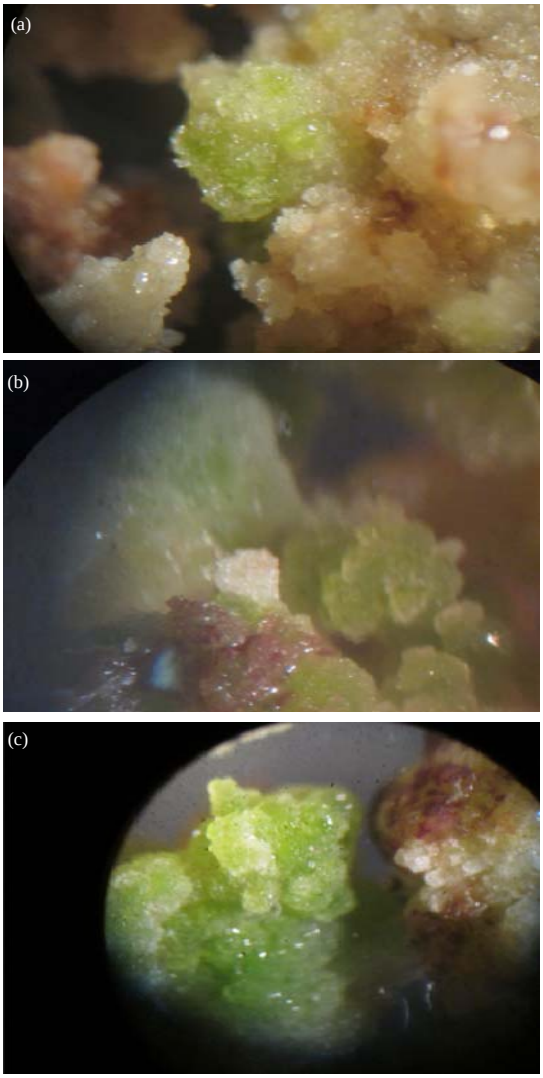

Fig. 7: Callus derived from stem grown (a) 1 mg L^{-1}, (b) 2 mg L^{-1} Z and (c) 4 mg L^{-1} TDZ under binocular

zeatin are considered as cytokinins which are a class of plant growth substances that promote cell division. Many physiological and biochemical events in cells are enhanced by TDZ, however, its mode of action is unknown[12]. They are involved in cell growth and differentiation. There are two types of cytokinins, adenine type like zeatin and phenyl-urea type like TDZ[13]. In this respect, Gairi and Rashid[21] reported that using a medium containing 10 μM from TDZ resulted in regeneration of somatic embryos and shoots in 30% of the non-responsive caryopses of rice cultures within 10-15 day. Mithila *et al.*[22] reported that low concentration of TDZ induced shoot organogenesis of African violet explants, whereas at higher doses (5 to 10 μM) somatic embryos were formed. TDZ was produced firstly for defoliation of cotton (*Gossypium hirsutum*)[23]. It was classified as a cytokinin. It gave many responses similar to that induced by natural cytokinins.

Antioxidant activity through DPPH: To examine the antioxidant content of callus among the different treatments, two extraction solvents (hexane or petroleum ether) were used. DPPH percentages among different concentrations of TDZ or zeatin the highest DPPH percentage of callus derived from leaf explants is registered with callus treated with 2 mg L^{-1} zeatin which extracted with hexane followed by the callus cultured on 4 mg L^{-1} TDZ and extracted by petroleum ether as shown in Table 7. While, the lowest DPPH percentage is registered with callus cultured on 4 mg L^{-1} zeatin and extracted with hexane. For callus

Table 7: Effect of TDZ and zeatin on DPPH percentage of callus derived from leaf or stem explants

		Dried callus (in oven)	
		Extraction solvent	
Callus origin	Treatments	Hexane	Petroleum ether
Leaf	1 mg L^{-1} TDZ	20.2±0.05	13.0±0.1
	2 mg L^{-1} TDZ	19.3±0.2	16.7±0.1
	4 mg L^{-1} TDZ	22.6±0.1	25.4±0.1
	1 mg L^{-1} Zeatin	10.9±0.05	12.3±0.2
	2 mg L^{-1} Zeatin	27.1±0.2	17.9±0.2
	4 mg L^{-1} Zeatin	10.3±0.05	17.6±0.4
Stem	1 mg L^{-1} TDZ	20.1±0.15	24.9±0.1
	2 mg L^{-1} TDZ	10.1±0.15	20.3±0.1
	4 mg L^{-1} TDZ	20.7±0.3	15.6±0.2
	1 mg L^{-1} Zeatin	8.5±0.2	15.3±0.15
	1.5 mg L^{-1} Zeatin	15.7±0.15	8.1±0.1
	4 mg L^{-1} Zeatin	5.7±0.3	15.5±0.4

Each value consists of Mean±Standard Deviation

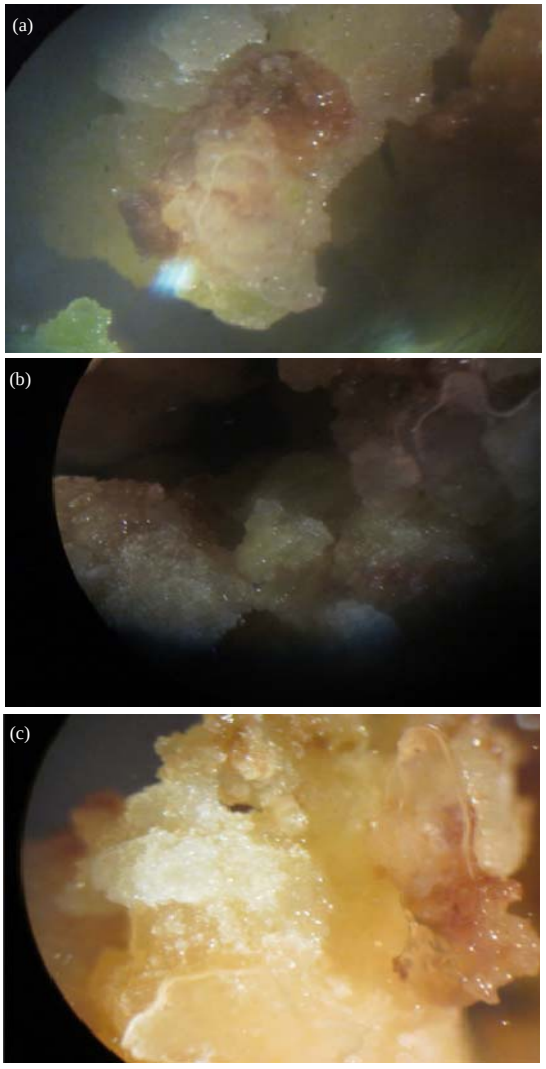

Fig. 8: Callus derived from stem grown on (a) 1 mg L^{-1}, (b) 1.5 mg L^{-1} and (c) 4 mg L^{-1} zeatin under binocular

originated from stem explants, it was found that the highest DPPH percentage was registered with callus cultured on 1 mg L^{-1} TDZ and extracted with petroleum ether followed by the callus cultured on 4 mg L^{-1} TDZ and extracted by hexane. While, the lowest DPPH percentage is registered with callus cultured on 4 mg L^{-1} zeatin and extracted with hexane.

Finally, it could be noticed that DPPH percentage in callus originated from leaves is higher than that from callus originated from stem with using hexane as extraction solvent with all treatments. However , the embryogenic calli from leaves originated on 2 mg L^{-1} zeatin showed promising activity with hexane extraction. However, embryogenic calli originated from stem explants on 1 mg L^{-1} TDZ showed the highest antioxidant activity with petroleum ether extraction. In this respect, Ali and Abbasi[24] reported that the highest DPPH radical scavenging activity of *Artemisia absinthium* L. was observed with callus cultured on 1.0 mg L^{-1} TDZ on day 42. In vitro technology has been used widely for the antioxidant compounds synthesis from medicinal plants[25-27].

CONCLUSION

It could be concluded that, zeatin containing treatments was more effective with callus from leaves explants in inducing embryogenic callus. While TDZ containing treatments was more effective in inducing embryogenic callus from stem explants. Also, it could be noticed that DPPH percentage in callus originated from leaves is higher than that from callus originated from stem with using hexane as extraction solvent with all treatments. The embryogenic calli from leaves originated on 2 mg L^{-1} zeatin showed promising activity with hexane extraction.

SIGNIFICANCE STATEMENT

This study described the possibility to induct a semantic embryos from *in vitro* leaf or stem. This is the first report on successful induction of somatic embryo initiated from *in vitro* leaf and stem.

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