

Determination of β -carotene in Carrot (*Daucus carota* L.) Plants Regenerated from Stems Callus

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ABSTRACT

This study succeeded in regenerating carrot (*Daucus carota* L.) plants produced from seedling stems callus which transplanted in soil. The results indicated that Murashige and Skoog (MS) salts medium provided with 1.0 mg L^{-1} NAA was superior in callus induction 96.6% after thirty days of culture. Also MS medium containing 1.0 mg L^{-1} of each NAA and BA gave callus up to 83.3%. Numerous shoots that record 417 were differentiated from callus in agar-solidified MS medium supplemented with 1.0 mg L^{-1} NAA and 4.0 mg L^{-1} TDZ. Regenerated shoots were rooted easily in agar-solidified MSO medium of reasonable root systems that sustained adaptation of plantlets successfully in field conditions. Tissue culture plants were better than plants produced from seeds in their growth and morphological characters. They produce normal flowers (Inflorescence) producing fertile seeds and normal storage roots. In addition to their superiority in β -carotene content which recorded 1.988, 1.264 mg / 100 gm fresh weight of callus and roots respectively.

Keywords : carrot plant, shoots regeneration, β -carotene content.

Daucus carota L.

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(*Daucus carota* L.) carrot

1.0	MS	.					
MS	.	30	%96.6			NAA ¹⁻	
.	%83.3	BA	NAA	1-	1.0		
1.0	MS	417					
MSO	.	TDZ ¹⁻	4.0	NAA ¹⁻			

1.988

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100 /

1.264

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INTRODUCTION

Carrot, *Daucus carota* L., is a member of the family Apiaceae, (Ammirato *et al.*, 1986). According to the annual report of FAO, more than 19 million metric tons of carrots were produced worldwide in 2005 (<http://www.fao.org>). Because of its nutritional value for human, it is important to improve the agronomic character of this vegetable crop. Economically, its rank among the top ten of the most important vegetable crops and every part of carrot can be used for different medical purposes (Joy *et al.*, 1998). Carrot, a winter season plant cultivated for its edible storage taproots (Hiltunen and White, 2002), and high content of carotene ($C_{40}H_{56}$). The β -carotene form is the important precursor of provitamine A source (Bohm *et al.*, 2002). Many attempts have been made to establish regeneration systems for *Daucus carota* L. using various parts of carrot seedlings on MS media provided with different concentration of 2,4-D, NAA, IBA and BA (Pant and Manandhar, 2007). A recent study reported the induction of hypocotyl callus on MS media provided with 2,4-D and shoot regeneration from this callus using Kin or NAA with IBA in MS medium (Rabiei *et al.*, 2010), but the number of the produced plants was less than the number obtained in the present study.

This study aimed to find out the efficient conditions to regenerate carrot plants from callus derived from stem of axenic carrot seedlings with the determination of β -carotene content as well.

MATERIALS AND METHODS

Sterile seedlings production:

Seeds of carrot, *Daucus carota* L. were obtained from local market, washed in running water for one hour, soaked in 3.0 % (W/V) sodium hypochloride (NaOCl) with shaking for five minutes, and rinsed thoroughly by sterilized distilled water three times (Al-Mallah and Mohammed, 2012). They were placed on the surface of agar-solidified MS (Murashige and Skoog, 1962) supplemented with 3% sucrose, 0.8% agar and of pH adjusted to 5.8 before autoclaving. Specimens were maintained at $25 \pm 2^\circ\text{C}$ in dark. Seeds germinated after six days of sowing were transferred to the culture room condition with 16 hour light of florescent tube at density $41 \mu\text{mol.m}^{-2}.\text{sec}^{-1}$.

Stems callus initiation:

Two centimeters length of stem explants lacking from nuds were excised from sterile four weeks old carrot seedlings grown on agar-solidified MSO medium. Explants were placed on the surface of 30 ml aliquots in 100 ml glass jars of initiation medium based on MS salt that solidified with 8.0 gm L^{-1} agar and provided with growth regulators. The following stimulation media used were :

MS + 0.5 mg L ⁻¹ NAA + 0.5 mg L ⁻¹ BA	}	(Pant and Manandhar, 2007)
MS + 0.5 mg L ⁻¹ NAA + 1.0 mg L ⁻¹ BA		
MS + 1.0 mg L ⁻¹ NAA + 0.5 mg L ⁻¹ BA		
MS + 0.1 mg L ⁻¹ 2,4-D	}	(Latif <i>et al.</i> , 2007)
MS + 0.5 mg L ⁻¹ 2,4-D		
MS + 1.0 mg L ⁻¹ 2,4-D		
MS + 1.5 mg L ⁻¹ 2,4-D		
MS + 4.0 mg L ⁻¹ 2,4-D	}	(Mousavizadeh <i>et al.</i> , 2010)
MS + 0.5 mg L ⁻¹ IAA		
MS + 1.0 mg L ⁻¹ IAA		
MS + 1.5 mg L ⁻¹ IAA		

Other media selected in this investigation were assessed including MS media supplemented separately with 0.5, 1.0, 4.0 mg L⁻¹ NAA. Also, MS media containing 1.0 mg L⁻¹ of each NAA and BA, and MS provided with 1.0 mg L⁻¹ NAA and TDZ at concentration 2.0 mg L⁻¹ and 4.0 mg L⁻¹. Again, MS medium supplied with 0.5 and 1.0 mg L⁻¹ of IBA alone or in combination with 0.5 and 1.0 mg L⁻¹ NAA respectively were examined.

Shoots regeneration

Several samples, one gram each, of green and healthy callus were transferred to the surface of 30 ml of the selected regeneration of agar-solidified medium (MS + 1.0 mg L⁻¹ NAA + 4.0 mg L⁻¹ TDZ) in 100 ml volume glass jars. They were kept in the culture room conditions previously mentioned.

Rooting of regenerated shoots and transfer to soil

The regenerated shoots of 2.5-3.0 cm length carrying three-four leaves were separated individually from the culture. A group of these regenerates was transplanted vertically in agar-solidified MSO and other groups were transplanted in the same manner using half-strength agar-solidified MSO (Rabiei *et al.*, 2010).

Regenerated plantlets of suitable roots were removed from rooting medium, washed carefully with distilled water and transferred to plastic pots 25 gm capacity containing autoclaved mixture of garden soil: peatmoss (1:1, w:w). They were covered with transporence pots and kept in a culture room at the same conditions. After one week, they were transferred into 5 kg pots containing the same mixture and kept in green house for three weeks and finally cultivated in the field.

Growth characters of regenerated carrot plants

The growth and morphological differences between plants produced from callus and those produced from seeds of the same age included, plant height, number of leaves, number and type of inflorescence, petal and sepal number in addition to length, diameter and weight of storage taproots were determined.

β -Carotene content determination

Storage taproots collected from plants produced from seeds and callus were washed, cut into slices of 2mm thickness and 1cm length. Twenty-five gm of each type kept in 4°C for one week (Dutta *et al.*, 2005) and β -Carotene extraction was carried out according to the previous method (Fikselova *et al.*, 2008). Determination of β -carotene in callus tissues

grown on MS medium supplemented with 1.0 mg L⁻¹ NAA and 4.0 mg L⁻¹ TDZ was carried out using the same procedure. β -Carotene content was calculated as in below :

$$\beta\text{-Carotene} = \frac{A \times d \times v}{E_{1\text{cm}}^{1\%} \times w} \quad (\text{Fikselova } et al., 2008)$$

where A: absorbance, d: dilution, $E_{1\text{cm}}^{1\%}$: Coefficient of absorbance (2592 for petroleum-ether), w: weight of sample (g), v: volume (ml)

RESULTS AND DISCUSSION

Callus production

Stems explants, excised from axenic carrot seedlings, produced callus within 3-7 weeks of culture on MS medium supplied with different combinations of growth regulators (Table 1).

Table 1: Assessment of various stimulation media for callus induction from stem explants of carrot, *Daucus carota* L., seedlings

Induction medium (mg L ⁻¹)	No. induction explants	Callus initiation (%)	Period of initiation (weeks)
Control	-	-	-
MS+0.5 NAA	12	40	4
MS+1.0 NAA	29	96.6	4
MS+ 0.1 2,4-D	13	43.3	6
MS+ 1.0 2,4-D	9	30	7
MS+0.5 NAA+ 0.5 BA	14	46.6	5
MS+0.5 NAA+ 1.0 BA	14	46.6	7
MS+1.0 NAA+ 0.5 BA	16	53.3	6
MS+1.0 NAA+ 1.0 BA	25	83.3	3
MS+1.0 NAA+ 2.0TDZ	7	23.3	7
MS+1.0 NAA+4.0 TDZ	15	50	8

thirty explants cultured / treatment

The data in Table (1) proved that all types of tested media stimulate callus formation. The results demonstrated that NAA is essential for the continuity of carrot callus production in certain concentration but the high level of NAA caused cell necrosis. Interestingly, callus obtained from stem explants was of friable type and green in color (Fig. 1-A). Other researchers found the similar effect of NAA in callus formation of Banana (Bidabadi *et al.*, 2010) and with different plant species (Murthy *et al.*, 1998). It seems likely that the presence of BA besides NAA is important to enhance steadily production of callus (Hutchinson *et al.*, 1994). The addition of the low level of 2,4-D and TDZ at 2.0, 4.0 mg L⁻¹ do not greatly encourage the production of callus (Sajid and Aftab, 2009). The addition of growth regulators in the medium interacted with cells hormones present in the explants.

This preserve all the requirements needed for cells division and its developments to differentiation cellular aggregates (Bidabadi *et al.*, 2010).

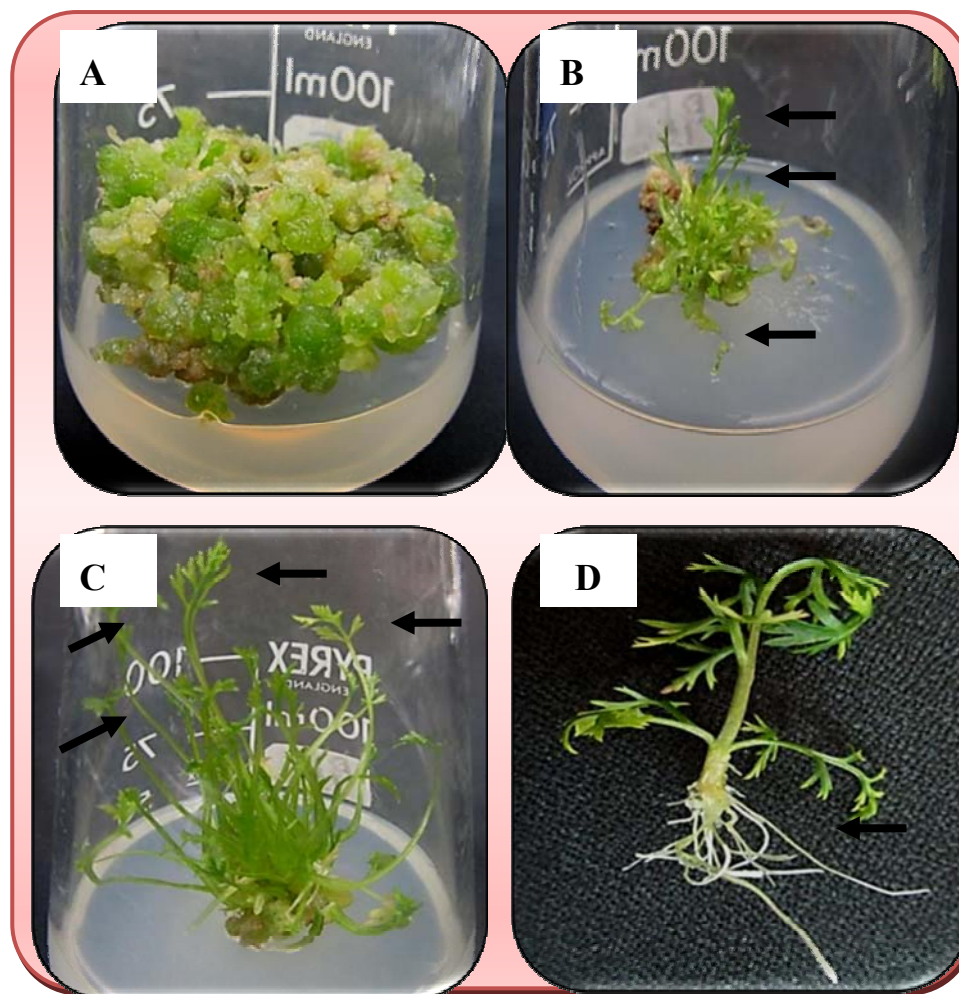


Fig. 1: Regeneration of carrot, *Daucus carota* L., plants from stem callus.

- (A) Culture of stem callus (6 weeks old) grown in agar- solidified MS+1.0 mg L⁻¹ NAA medium.
- (B) Young shoots regeneration (arrows) in MS+1.0 mg L⁻¹ NAA + 4.0 mg L⁻¹ TDZ after 4 weeks of culture.
- (C) Development of regenerated shoots in (B) after 7 weeks of culture (observe number and length of shoots).
- (D) Sample of rooted shoot in (C) 2 weeks after transferring to MSO medium (note the number and length of roots).

All other media mentioned in stems callus culture formation paragraph (in materials and methods) don't enhance callus initiation and explants died.

Shoots regeneration:

The obtained results expressed that stem callus placed on agar-solidified MS medium supplemented with 1.0 mg L^{-1} NAA in the presence of 4.0 mg L^{-1} TDZ led to the great capacity of shoots regeneration (Table 2).

Table 2: Effect of addition of 1.0 mg L^{-1} NAA and 4.0 mg L^{-1} TDZ in agar-solidified MS medium in regeneration of carrot, *Daucus carota* L., shoots

Regeneration medium	No. of				Period of shooting (weeks)	Shoot formation (%)
	callus fragment	regenerated fragment	shoots formation	shoots/ callus fragment		
MSO (control)	50	0	0	0	0	0
MS + NAA + TDZ	100	73	417	6.1	4	73

The shoots started in regeneration after 4 weeks of callus transfer on regeneration medium. Numerous Shoots were raised subsequent in the same medium (Fig. 1-B, C). In this studies the addition of TDZ with NAA in MS medium led to great shoots formation.

Many investigators stated that TDZ acts as a potent regulator of in vitro plant morphogenesis in cotton bolls (Murthy *et al.*, 1998). This may due to the TDZ which consider a cytokinin stimulate shooting but its high concentration might act as a modifier of the endogenous cytokinin metabolism and auxin (Taha *et al.*, 2009).

Shoots Rooting:

The results in Table (3) indicate that the regenerated shoots were rooted easily in both full (Fig. 1. D) and half strength agar-solidified MS lacking hormones.

Table 3: Rooting of regenerated carrot, *Daucus carota* L., shoots in agar-solidified MSO medium

Medium	No. of			Period of rooting (weeks)	Roots formation (%)
	cultured shoots	rooted shoots	roots / shoot		
MSO (control)	100	56	4.3	3	56
1/2 MSO	20	4	2.2	2	20

The successful rooting of the regenerated carrot shoots probably due to suitability of MSO salts concentration that were enough to shoot rooting. It is clear from the results of this study, that the number of rooted shoots will decrease when salts concentration reduced

to the half. Callus differentiation may explained by the formation of meristemoid clump, with synthesis of rhizocallin compound, that developed to embryos or buds forming the shoots that initiated from pericycle (Wilson, 1988). Researchers mentioned that rooting of shoots in MSO probably due to the endogenous auxin that encourage growing zones in shoots which to roots formation (Jennifer *et al.*, 2004 ; Davie, 2004).

Adaptation and transfer of regenerated plants to soil.

Data showed a successful adaptation of carrot plantlets regenerated from callus when primarily transferred, at the fourth week, in small plastic pots (Fig. 2-A) containing peatmoss: garden sand (w:w), then transported to large pots (Fig. 2-B) in green house conditions. These plants formed numerous roots (Fig. 2-C) and finally cultivated in garden soil (Fig. 2-D, E).

These tissue cultured plants showed the ability to grow actively and tolerate environmental conditions and produced flowers (Fig. 2-F,G) that produced fertile seeds (Fig. 2-H, I).

Phenotypic characters

Table (4) indicates some differences between carrot plants regenerated from callus and those produced from seeds.

Table 4: Phenotypic characters of carrot plants regenerated from stem callus and those produced from seeds

Characters	Regenerated plants	Seeds plants
Plant height (cm)	123.1	80
No. Flowers		
Umbel / plant	19	4
Umbellet / umbel	50	25
Flowers / umbellet	70	40
Petal / colour	5 / white	5 / white
Sepal / colour	Not found	Not found
Taproots		
Length (cm)	10.8	4
Diameter (cm)	1.87	0.37
Weight (gm)	9.05	0.5

Each value represents the rate of five replicates



Fig. 2: Successful transplantation of regenerated carrot, *Daucus carota* L., plants in field conditions

- (A) A carrot plant (4 weeks) transferred in peatmoss in plastic pot.
- (B) Plant in (A) was grown in 5 kg soil pots.
- (C) Numerous roots production of plant in (B).
- (D) Plant in (B) of seven weeks old transferred to the garden soil.
- (E) Greatly grown (19 weeks) carrot plant in (C) in garden soil. Note number and length of shoots and beginning of inflorescence formation (arrows).
- (F) Typical development of inflorescence in (E), 22 weeks (observe umbellets).
- (G) Shizocarps production (arrows) in plant in (F).
- (H) Liberation of seeds from mature shizocarps in (G).
- (I) Close shot of shizocarps in (H).

Carotene Content:

The results indicate that β -carotene content was increased with the time of extraction (Table 5) in both callus tissue and storage roots of the regenerated plant compared with control sample.

Table 5: β -carotene contents (mg/100 gm) in various extraction time of stem callus and taproots of regenerated carrot, *Daucus carota* L., plant.

Samples	Extraction time (hour)			
	1	2	3	4
Storage (Tap) root produced from seed plants (control)	0.220	0.352	0.800	0.756
Storage (Tap) root of regenerated plants	0.276	0.400	1.264	0.984
Stems callus	1.308	1.832	1.988	1.926

Each value represents the rate of three replicates

β -Carotene, the bioavailable compound, is widely known as provitamine A, having the role as antioxidant and other benefits (Bohm *et al.*, 2002). The difference in its amount in callus and taproot depended on the processing of the sample. The actual increase depends on the increase of the bioavailability effects of callus, whereas the reduced amount may due to the oxidation in cells (Livny *et al.*, 2003).

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