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In-vitro callus induction from two different explants stem and leaf in *Carthamus- tinctorius* Linn.

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ABSTRACT

In present study, callus was raised from two different explants stem and leaf of Carthamustinctorius. The explants were cultured from the 3 week old seedlings and transferred to an MS medium supplemented with different concentration of Auxin, 2, 4-D growth regulator hormones.The highest percentage of callus induction (90%) was observed in all surfaces of leaf explants.The medium supplemented with 2, 4-D was more effective that IAA for callus formation.

Key words: *Carthamus- tinctorius,* leaf, stem, callus, IAA, 2, 4-D.

INTRODUCTION

Carthamustinctorius L. (Asteraceae) is known for its varied economical and medicinal importance throughout Indian subcontinent. With a wide spectrum of pharmacological effects it has been used to treat dysmenorrhea, amenorrhea, postpartum abdominal pain and mass, trauma and joint pains. Rather than medicinal uses it is grown for its seeds and used for coloring and flavoring foods. It is economically important, making red and yellow dyes. Tissue culture tools are considered expensive than conventional mass multiplication method when it comes to commercial and large scale production of needs (Satyapal Singh et al, 2011).

The conservation efforts are of immediate need to save those species in the wild, maintain their wild populations as well as *in vitro* culture by tissue culture methods. Plant tissue culture is an alternative method of propagation and is being used widely for the commercial propagation of large number of species including many medicinal plants (Rout et al. 2000).

Safflower is highly branched, herbaceous, annual plant grow upto a height of 45-130 cm. It's one of the oldest cropscultivated mainly for its seeds(Knowles, 1969). Seeds are used for the production of edible oil commercially and are used in plant industry, (Keso, 1962). In china, it is grown exclusively for its flowers, which are used in treatment for many illnesses. Safflower along-with other herbs has been used to treat respiratory diseases (Kashhara and suzuli, 1919).In the field of biotechnology, the current status and future prospects of safflower cultivation by*in vitro* techniques have also been published (Sujatha M., 2002). In present study, callus was raised from two different explants leaf and stem of plant. The work incorporates to standardize the optimum conditions for induction of callus. The *in-vitro* grown plantlet is with different parts like epicotyls, stem, hypocotyls, cotyledon, leaf and root. It is possible to induce callus from various parts of the plantlet.

MATERIALS AND METHODS

Plant Material

Carthamustinctorius L. belonging to family Asteraceae has great importance both medicinally and industrially. Safflower is commonly known as false saffron a common substitute for the world's most costly spice the true saffron,thus also very important economically.

Source of explants

For initial establishment of callus culture, 3 week old *in vitro* grown seedlings were used as the source of explants along with its different parts. Different parts like leaf and stem from the *in-vitro* grown seedlings were cultured for callus induction.

Culture media

Murashige and skoog agar gelled medium fortified with various concentrations and combinations of growth hormones here after referred to as MS was used as culture medium. Different auxins like 2, 4-D and IAA along-with were used either in isolation or in combination in different concentration were used.

Surface sterilization

All nutrient media, culture vessels and instruments used in handling the tissues as well as the plant material itself must be sterile.

Inoculation and Incubation

Explants are inoculated in the sterilized test tubes containing medium with phytohormones. Inoculated test tubes were labeled with name of the medium, hormone combination, concentrations, with and kept in tissue culture racks.

Culture Conditions

The cultures were incubated at 25$\pm$ 2^0 C temperature under cool, white fluorescent light (2000-300 Lux) and 55 $\pm$ 5% relative humidity. The growth chamber was maintained with 16 hrs. /8 hrs. Photo and dark period.

RESULTS AND DISCUSSION

The induction of undifferentiated cells begin with a small section of plant tissue that is manipulated using plant growth regulators to formation of calli, callus tissue from leaves and stem was obtained at dim light conditions. Callus induction became visible on the surface of the explants and on the wounded edges after 21 days. When segment of stem inoculated on MS media, the segments remained green for about two to three weeks with a little callus at the cut ends; or the segments showing a little callus throughout the tube and sometimes callus did not grow further.

Table 1.Callus induction and organogenesis from *invitro grown* explant stem of *Carthamustinctorius* L. on different concentration of auxins incorporated with MS + 2,4-D (0.5 – 5.0 mg/L)

Explant	Growth regulation 2,4-D (mg/L)	Nature of response	% of response
		Callus	
Stem	0.5	+	75%
Stem	1.5	++	50%
Stem	2.5	+++ Gn	75%
Stem	5.0	++++	80%

The stem segment was cultured on MS medium supplemented with 2, 4-D for studying its effect at various concentrations ranging from 0.5 to 5.o mg/l in almost all the concentrations of 2,4-D callus was induced from both cut ends as well as from entire surface of stem segments. In 2.5 mg/L to 5.0 mg/L of 2,4-D callus responded more(Table 1).

When the margin of the leaf lamina were cultured in the MS medium supplemented with different concentration 0.5 to 5.0 mg/L of 2,4-D there was formation of luxuriantly growing callus, in almost all concentration of 2,4-D (0.5 to 5.0 mg/L) callus on entire surface of leaf. After 3-4 weeks few green nodules were observed on the surface of white transparent nodulated callus. In few cultures transparent globular shaped embryoids emerging from the green

nodules were observed. Forembryogenic callus the most suitable concentration was 0.5 to 5.0 mg/L of 2,4-D (Table2). The amount of the obtained callus from stem segments was significantly less than that from leaf explants. There were significant differences in callus formation depending on the type of auxin. Auxin 2, 4-D in concentration 0.1 mg/L induced differentiation of cells in callus tissues. The highest percentage of callus induction (90%) was observed in all surfaces of leaf explants. The calli were pale-yellow and friable in nature. The medium supplemented with 2,4-D was more effective that IAA for callus formation(Table3).

Table 2.Callus induction and organogenesis from leaf explant of *Carthamustinctorius* L. at different concentration of auxins in MS + 2,4-D (0.5 – 5.0 mg/L)

Explant	Growth regulation 2,4-D (mg/L)	Nature of response	% of response
		Callus	
Leaf	0.5	+++	47%
Leaf	1.5	+++	47%
Leaf	2.5	++	63%
Leaf	3.5	+	50%
Leaf	4.5	++	38%
Leaf	5.0	+++	31%

Table 3.Effect of different combinations of phytohormones on Callus induction

Growth Regulator Mg/L	Concentration	Callusing ability of explants		Nature of callus
2,4 - D + KN	1.0 + 0.1	++++	++++	Extra ordinarily high rate of callus induction
	1.5 + 0.2	++++	++++	
	2.0 + 0.3	++++	+++	
	2.5 + 0.4	++++	++	
	3.0 + 0.5	++++		
2,4-D + IAA	1.0 + 0.1	+++	++	Callus along with shoot observed
	1.5 + 0.2	+++	++	
	2.0 + 0.3	+++	++	
	2.5 + 0.4	+++	++	
	3.0 + 0.5	+++	+++	
2,4-D + IBA	1.0 + 0.1	++++	+++	High rate of white modulated callus
	1.5 + 0.2	++++	++++	
	2.0 + 0.3	++++	++++	
	2.5 + 0.4	++++	+++	
	3.0 + 0.5	++++	++++	
2,4 - D + KN	1.0 + 0.1	+++	++	High rate of callus and medium amount
	1.5 + 0.2	+++	++	
	2.0 + 0.3	+++	+++	
	2.5 + 0.4	+++	++	
	3.0 + 0.5	+++	+++	
MS + Coconut water	80% + 20%	++++		High rate of callus

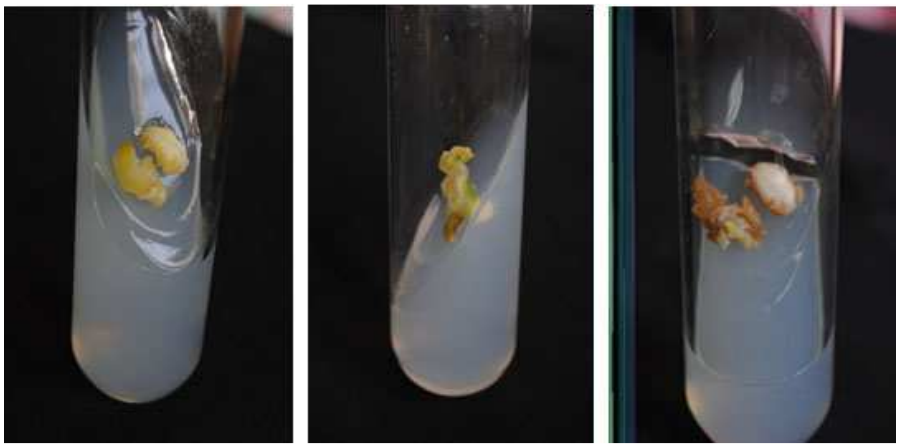

Fig. 1 Photographs showing Callus from Stem as explants at 2.5 mg/L – 5.0 mg/L of 2,4-D

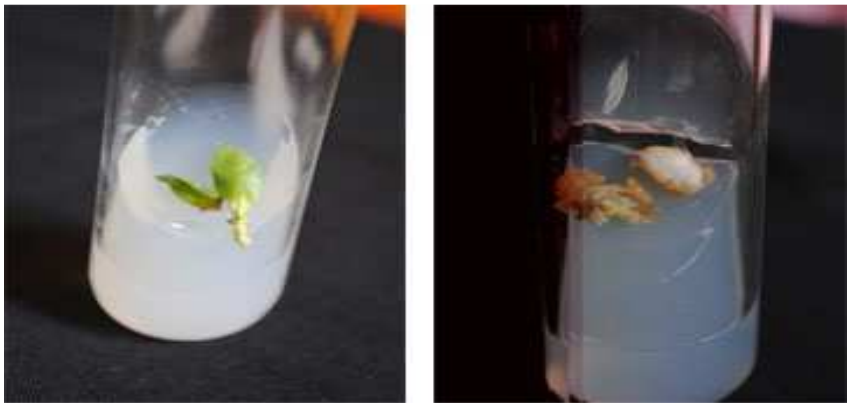

Fig. 2 Photographs showing Callus from Leaf as explants at 2.5 mg/L – 5.0 mg/L of 2,4-D

After this, when compared to the other members of family Asteraceae regarding *in vitro* regeneration and callus induction such explants (leaf) were ineffective. This is reported in *Achilliamillefolium*. The efficacy of exogenous 2,4-D has also been reported in other medicinal plants by various authors. The results are in accordance with the finding made by using this synthetic plant growth regulator in the culture medium for callus induction *withaniasomnifera*, cardio spermumhalicacabum Linn and Abrusprecatorious respectively. (Rani et al., 2003; Thomas and Masseena2006).Synergism of 2,4-D and NAA found by the authors are in accordance with results obtained by Nikolaeve et al. (2009) in *Camellia Chinensis.*

Our finding in the experiment regarding type of explants responses is similar to the finding of Hassan *et.al.* (2009), found the best responses only from young leaves with same texture of callus.

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