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IN VITRO GERMINATION AND CALLUS INDUCTION FROM SEEDS OF *CARTHAMUS TINCTORIUS* L.

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ABSTRACT
The seeds of the plant *Carthamus tinctorius* commonly known as Safflower were cultured on a plain MS media for germination. The regenerated different plant parts were cultured in the Murashige and Skoog medium supplemented with different concentrations of phytohormones. The concentrations of different phytohormones taken of 2, 4-D, IBA, IAA and KN either in isolation or combination with each other. MS media was also prepared in combination with coconut milk (20% and 80% v/v). The best callus was induced in different concentrations of 2, 4-D from different parts regenerated from seeds like root, lower hypocotyls, upper hypocotyls, leaf. Callus induction was also found in coconut milk supplemented media and in combination of different Phytohormones like 2, 4-D + IAA, 2, 4-D + IBA, IAA + KN, 2, 4-D + KN. Best result was observed in 2, 4-D in 3.0 ppm – 5.0 ppm.

INTRODUCTION

Carthamus tinctorius L. commonly known as Safflower is a member of family Asteraceae. It is cultivated mainly for its seeds which is used as edible oil and as bird seed (Keso,1962). Traditionally the crop was grown for its flowers used for colouring and flavouring foods making dyes (Gao and Fan, 2000). Safflower is a highly branched, herbaceous, thistle like annual or winter annual, usually with many long sharp spines on the leaves. Plants are 30-150 cm. tall with globular flower heads (capitula) and commonly brilliant yellow, orange or red flowers. Safflower is one of humanity's oldest crop with world seed production around 800000 t per year (Knowles, 1969). Oil has been produced commercially and is used in paint industry. And now a days its edible oil for cooking, margarine and salad oil (Johnson and Bergman, 1999). Whole plant is useful not only for industries but also economically and medicinally (Kellenberger *et al.,* 1951). In China, Safflower is grown almost exclusively for its flowers, which are used in treatment of many illnesses as well as in tonic tea (Kasahara and Suzuki, 1999). Safflower dilates arteries, inhibits thrombus formation. Safflower along with other herbs is used in treatment of many diseases. Two dyes, one water soluble yellow dye, carthamidin and a water insoluble red dye carthamin, which is readily soluble in alkali can be obtained from Safflower florets (Dajue and Hans,1996). There are many wild and weedy relatives of *Carthamus tinctorius* (Norov, 2005). Very limited literature of Safflower tissue culture is available. Though some attempts has been made on tissue culture of this plant (George and Rao,1982; Orlikowska and Dyer,1993; Mandal *et al.,*1995; Sujatha and Suganya,1996; Tejovathi and Das,1997; Nikam and Shitole, 1999; Mandal and Gupta, 2001; Mandal *et al.,* 2003; Neetika *et al.,* 2005; Dilek *et al.,* 2008 etc.). Dilek *et al.,* 2008 investigated the induction of shoot regeneration from cotyledonary leaves and in vitro multiplication in Safflower supplemented with TDZ x IBA. In present investigation, it is planned to study and standardized the optimum conditions for induction of callus tissue from regenerated different parts of seeds grown *in vitro*. Alongwith this, the protocol will also be highly supportive in future as an alternative for the production of new secondary metabolites.

MATERIALS AND METHODS

Culture medium The basal MS medium was prepared by addition of required concentrations from stock solutions of major and minor elements, iron sources, vitamins and different phytohormones in different concentrations and kept under refrigerator and used for such purpose when needed. This was supplemented with 30g/L of sucrose and 8g/L of bacteriological agar. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl prior to adding agar. Phytohormones were added individually or in combination into the medium (Auxin – 2, 4-D, IAA, IBA and Cytokinin – KN). The culture media was poured into culture tubes which are completely sterilized tubes and oven dried. The culture medium was autoclaved at 120ºC and 1.0 x 10 Pa for 15 min. in an autoclave.

Culture material The seeds of *Carthamus tinctorius* L. (A-1 variety) had been collected from Birsa Agriculture University, during the month June – July, 2008. The explants such as leaf, stem, hypocotyls, root were obtained from in vitro grown plants in aseptic condition. Explants were washed in the liquid detergent (Teepol 5 – 8 drops 100mL) in a vial under gently agitating conditions. Subsequently, these were again washed with distilled water to remove trace of detergents. After washing

explants were surface sterilized by soaking 0.1% Mercuric Chloride for 5 min., followed by 4 - 5 times ringing with sterilized distilled water. Vigorous and healthy seeds were used for culture in tubes of 20 x 150 mm. (Borosil) containing MS media with different phytohormones.

Culture condition The culture condition was completely sterilized as good results are favoured by sterile condition. Aseptic culture condition was made by autoclaving and sterilizing glasswares and using UV light and 70% ethyl alcohol.

Explant culture technique The different parts regenerated *in vitro* were root, leaf, hypocotyls, stem which were placed aseptically into sterile petridishes for cutting into small pieces for culture. Then these pieces were cultured in MS medium with different concentrations of phytohormones either in isolation or in combination. These were IAA, IBA, 2, 4-D and KN.

Callus maintenance medium Cultures were incubated at 25 + -2ºC under cool fluorescent light (1500 – 2000 Lux) with a 16 hrs/8 hrs light/dark cycle. Each treatment consisted with minimum of 24 cultures and were repeated at least thrice.

RESULTS AND DISCUSSION

In *in-vitro* condition, seeds were germinated to form plantlets in MS – basal medium without any growth regulators. Explants were taken from 10-16 days old grown seedlings and cultured on the MS media with different plant growth regulators for the callus tissue (Bohidar and Thirunavoukkrasu , 2008). White, compact, embryogenic calli were produced from roots, leaf and hypocotyls of the explants within 15 days of inoculation in 2, 4-D supplemented media. Highest callus was seen in 2, 4-D alone from different parts of explants with different concentrations of phytohormones initiating with 0.5 ppm to 5 ppm. The present findings are in accordance with Bahrany (2002) and Hayati (2004). In 0.5 ppm to 2.5 ppm 2, 4-D only callus was observed on the surface of the cultured explants. In 2.5 mg/L to 5.0 mg/L 2, 4-D callus responded more .The present findings are in accordance with Mahato *et al.,* 2009.Results are shown in Table 1. Good callus was also induced in combination of 2, 4-D and kinetin (Balarama and Padmaja, 2003). Slight decrease or increase in plant growth regulators concentration gave varied results during present work. In lower concentration of IBA (0.5 mg/L) growth of white

Table 1: Influence of 2, 4-D on callus induction

Growth regulator	Nature of explant and callusing ability	No. of explants responded / cultured explants	Nature of callus
2, 4-D (mg/L)	(Root, leaf and hypocotyl)		
0.5	+ + +, + + +, + + +	15/20	On the surface of explant callus responded
1.0	+ + +, + + +, + + +	15/20	
1.5	+ + +, + + +, + + +	10/20	
2.0	+ + +, + + +, + + +	12/20	
2.5	+ + +, + + +, + + +	15/20	
3.0	+ + + +, + + + +, + + + +	18/20	Good callus responded
3.5	+ + + +, + + + +, + + + +	18/20	
4.0	+ + + +, + + + +, + + + +	18/20	
4.5	+ + + +, + + + +, + + + +	17/20	
5.0	+ + + +, + + + +, + + + +	16/20	

Table 2: Effect of different combinations of phytohormones on callus induction

Growth regulator (mg/L)	Concentration	Callusing ability of explants (Root, leaf, hypocotyl)	Nature of callus
2, 4-D + KN	1.0 + 0.1	+ + + +, + + + +, + + + +	Extraordinarily 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 alongwith 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 nodulated callus.
	1.5 + 0.2	+ + + +, + + + +, + + +	
	2.0 + 0.3	+ + + +, + + + +, + + + +	
	2.5 + 0.4	+ + + +, + + +, + + +	
	3.0 + 0.5	+ + + +, + + + +, + + + +	
IAA + 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

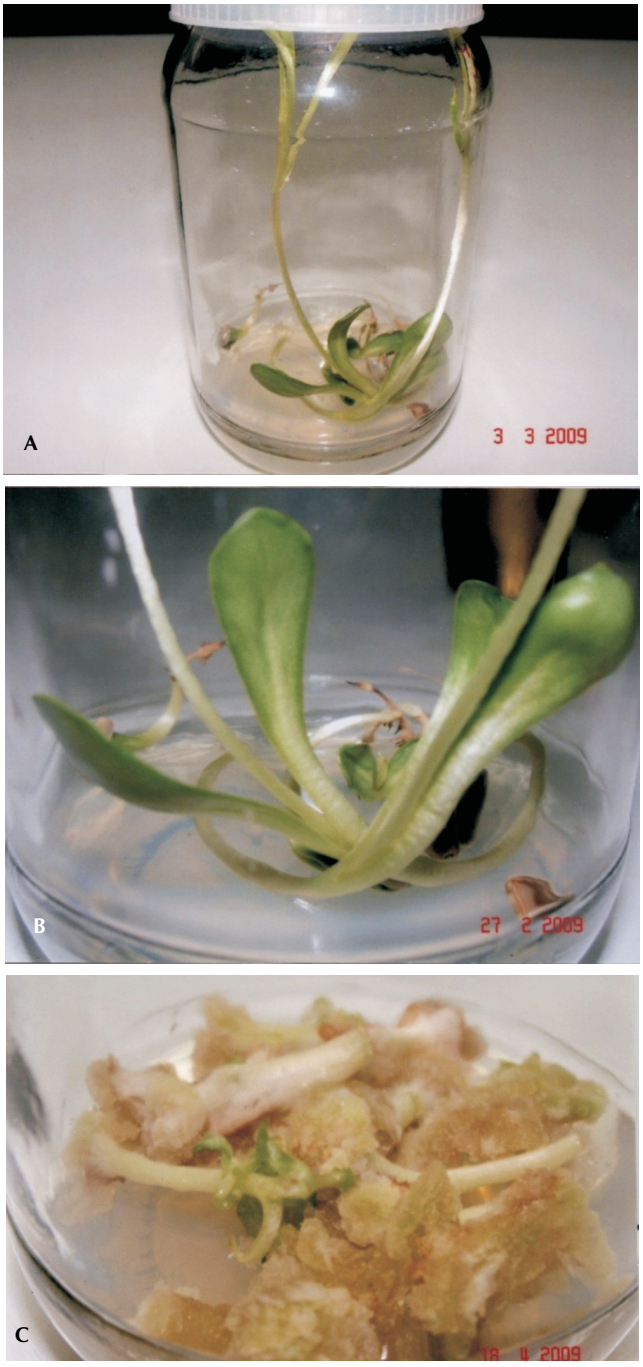

Figure1a, b and c: Plantlet regeneration in plain MS media from seeds of Safflower and callus induction after 10 -15 days

nodulated callus was observed whereas in a combination of IBA (2.5 mg/L) and BAP (2.5 mg/L) caused brown callus. This finding is in accordance with *Patel et al.,* 2007). Some amount of callus was also induced in combination of 2, 4-D + IAA in 1.00 mg/L to 3.00 mg/L of 2, 4-D and 0.1-0.5 mg/L of IAA. But in 2, 4-D + IBA callusing was more in same concentration. Slightly high rate of callus induction was seen in different concentrations of the combination of IAA + KN. Callus alongwith shoot was also observed in 2, 4-D + IAA, shown in Table 2. The expressions of totipotentiality also vary with the physiological state of the explants derived from the same plant like meristematic region responded well to yield calli having higher regenerability. Media containing coconut water (20% v/v) showed good results as Coconut water contains a number of cell division factors and free amino acids. Coconut water when added 20% v/v on MS media showed development of callus on root and hypocotyls explants. Shoot growth and multiple shoot production induced by medium containing coconut water has been reported in a number of plants of family Fabaceae (Murashige and Skoog, 1962; Chandra and Rai, 1989). One of the example of this family is *Clitoria ternatea* L. reported by Rout (2004). Finally, it was observed that there was different responses of callus growth according to the concentration of phytohormones taken either in isolation or in combination. The callus was also left for one year for observation and it was found that there was development of brown coloured soft callus. It was retained for further experiment.

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