In-Vitro Callus Induction From Two Different Explants Cotyledonary Leaves And Hypocotyl In *carthamus- tinctorius* linn. Var pkv-pink.

S.D. Surbhaiyya[1], M.S. Dudhare[1], R.A. Thakre[1], P.V.Jadhav[1], M.P. Moharil[1], D.R. Dhumale[1] and H.S. Umbarkar[1].

[1]Biotechnology centre, Department of Agricultural Botany, Dr. Panjabrao *Deshmukh Krishi Vidyapeeth-444104, Maharashtra, India.*

(Received : December, 2017 : Revised : January, 2018; Accepted : January, 2018)

Abstract

Callus from Safflower (cv. PKV-Pink.) was raised from two different explants hypocotyls and cotyledonary leaves. The explants were cultured from the one to two weeks old seedlings and transferred to an MS medium supplemented with kinetin and 2, 4-D with different concentration. The highest percentage of callus induction (99.99%) was observed in MS with equal concentration of 2, 4-D and KIN (1 mg/l each) in cotyledonary nodes as compare to hypocotyls and also an attempt are made to standardized the seed surface sterilization protocol by using different sterilizing agents.

Keywords: *Carthamus tinctorius L., Callus, Cotyledonary leaves, Hypocotyls,2, 4-D, KIN*

Introduction

Safflower (*Carthamus tinctorius* L.) one of the most important oil seed crop of the semi-arid regions. Traditionally Safflower has been grown in '*rabi*' or winter dry season in mixture with other '*rabi*' crops such as wheat and sorghum. The genus *Carthamus* is composed of about 25 species that are indigenous to the Mediterranean region and distributed from Spain to North America, West Asia, and India (Knowles, 1969).

Carthamus tinctorius 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) but 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. Tissue culture is an effective way to offers quick and efficient methods to exploit crop or medicinal plants meaningfully to meet the measuring demands of growing population, industries and it also helps to study many basic aspects of cell development and differentiation (Rout et al, 2000).

Advances in plant tissue culture and other innovations in biotechnology have provided opportunities to plant breeders for creating a wide range of useful genetic variability and increased the precision and efficiency of selecting desirable genotypes. In particular, *In vitro* methods are being used progressively more as an adjunct to traditional breeding method for genetic improvement of crops The success of *in vitro* culture depends mainly on the growth conditions of the source material (Zebarjadi et al., 2008 and Ghasempour et al, 2012), medium composition, and culture conditions (Soheilikhah et al., 2013).

In present investigation, callus was raised from two different explants cotyledonary leaves and hypocotyls of plant. The work incorporates to standardize the optimum

Corresponding author's e-mail : riteshthakre23@gmail.com
Published by Indian Society of Genetics, Biotechnology Research and Development,
5, E Biotech Bhawan, Nikhil Estate, Mugalia Road, Shastripuram, Sikandra, Agra 282007
Online management by www.isgbrd.co.in

conditions for induction of callus from above stated explants and also find out the best surface sterilization conditions and agents.

Material And Methods

Plant material

Seeds of safflower (cv. PKV-Pink.) having oil content 33% respectively were obtained from Oilseed Research Unit Dr. Panjabrao Deshmukh Agricultural University, Akola, Maharashtra. The seeds were washed with 1% Tween-20 detergent for 5 min, sterilized in 0.1% mercuric chloride (HgCl2) for 10 min and rinsed in sterile distilled water (3 times). Surface sterilized seeds were then transferred to half strength of MS medium for germination. Cotydedonary and hypocotyl explants were excised from 10 to 12 days old grow seedling and cut into 0.5-1cm^2 (Fig.1a) segments.

Surface sterilization

Seeds of safflower of PKV-Pink were washed with few drops of Tween 20 for 5 min. followed by washing with distilled water for 3-4 times. The seeds were surface-sterilized with 70% ethanol for 1 min and rinse with sterile distilled water for 3-4 times. Seeds were again treated with Bavistin (0.8%) to reduce the fungal contamination. The washed seeds were treated with 0.1% (w/v) $HgCl_2$ for 5 min followed by rinse with sterile distilled water.

Culture medium

Cotydedonary and hypocotyl explants [(0.5-1) cm] were inoculated on Murashige Skoog's (MS) medium containing 3% sucrose and gelled with 0.8% agar supplemented with various concentrations and combinations of 2,4-D and KIN were used. The pH of the medium was adjusted to 5.8 before being gelled with agar and autoclaved for 20 min at 121 °C for 15 lbs pressure.

Culture condition

The growth room conditions maintained for *in vitro* cultures were (25±2)°C and 60%-70% relative humidity, and the light intensity is 3000 lux with a photoperiod of 16 h light and 8 h dark. Each experiment was conducted at least thrice with 20 replicates per treatment.

Sub culturing

Sub culturing was carried out at regular intervals. Visual observations of the cultures were taken for every transfer and the effects of different treatments were quantified on the basis of percentage of cultures showing response.

Results

Surface sterilization

The seed surface sterilization is one of the critical steps for *in-vitro* culture to maintain the aseptic condition. Contamination by the microorganisms should be prevented to facilitate proper response and growth of the inoculated explants. The contamination was prevented by following stringent sterilization and to maintain the asepticness. The frequency of survival of seedlings and callogenic response of explants varied with concentration of Bavistin, HgCl2 and sterilization time. Concentration of 0.1% HgCl2 for 5 min, 70% Ethanol for 30 sec and Bavistin 0.8% for 10-15 min showed highest percent (100%) of survival rate of seedling i.e. no contamination. Among the different treatments used for surface sterilization of safflower PKV Pink the best result without contamination and healthy seed germination were found in some treatment (Table.1) and these treatments were carried out to isolate the explants from germinated seedling.

Culture medium

Generally highest auxin concentration in growth medium induces callus formation. Establishment of callus, which retains high morphogenetic potential, is a preliminary step in tissue culture of any plant species, nature and texture of callus is changed according to use of auxin and cytokinin with different concentration.

The cotyledonary leaves segment was cultured on MS medium supplemented with 2, 4-D for studying its effect at various concentrations ranging from 0.5 to 2.5 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 cotyledonary leaves segments. In 2.0 mg/L to 2.5 mg/L of 2, 4-D callus responded more (Table 2) and

it formed fragile in nature and pale yellow to brownish color. A completely grown, dense, compact mass of callus was obtained after 21 days of culture initiation.
When the hypocotyls segment was cultured in the MS medium supplemented with different concentration 0.5 to 2. 5mg/L of 2, 4-D there was formation of luxuriantly growing callus, in almost all concentration of 2, 4-D (0.5 to 2.5 mg/L) callus on entire surface of hypocotyls. The callus formation percentage increases with increase in the concentration of 2,4-D. Compact callus was observed on 2,4-D supplemented medium and increasing hardness of callus was directly proportionate to the concentration of 2,4-D. For embryogenic callus the most suitable concentration was 1.5 to 2.5 mg/L of 2, 4-D (Table3) and (Fig 1a and Fig 1b).
The callus formation was prominent on MS medium supplemented with 2, 4-D+KIN hormonal combinations for cotyledon explant. Auxin and cytokinin in combination reported to play an important role in callus induction and its proliferation. Among the several concentrations used, 2, 4-D+KIN at 1 mg/l each shown highest callusing response (100%). Callus obtained on equal ratio of 2, 4-D:KIN was fragile in nature and pale yellow to brownish colour. The amount of the obtained callus from cotyledonary leaves segments was significantly less than that hypocotyl segment.The medium supplemented with 2,4-D+KIN was more effective than 2,4-D alone for callus formation (Table 4).

Discussion

High period of exposure with HgCl2 leads to the browning of explants and death. Our results were in tantamount to Monokesh et al. They reported that surface sterilization exceeding 5 min was lethal to explants, whereas the present study showed that above 5 min of surface sterilization was lethal to all of the tested variety of PKV-Pink.

Generally highest auxin concentration in growth medium induces callus formation (Skoog and Miller, 1957). Establishment of callus, which retains high morphogenetic potential, is a preliminary step in tissue culture of any plant species, nature and texture of callus is changed according to use of auxin and cytokinin with different concentration. Auxins and Cytokinins are the most widely used plant growth regulators in plant tissue culture and usually are used in combination (Ghasempour et al, 2012). Result of some studies revealed that auxins played an important role in the callus induction (Baskaran et al., 2006; Ghasempour et al., 2012). Furthermore, they showed that cytokinins facilitated the effect of auxins in callus induction (Ghasempour et al., 2012; Soheilikhah et al., 2013).
The same result of callus formation from hypocotyl explant was reported by (Hamidreza et al. 2014) working on safflower callus culture, reported 2, 4-D, NAA and BAP at 0.5 mg/l concentration along with MS basal medium was best for callus induction (97.79%) after 25-30 days.
Srikanti Kumari et al. 2010 reported the highest mass of callus by using 2, 4-D (0.3ppm-0.5ppm) alone from different parts of explants (hypocotyl, root, leaf) from safflower genotype A-1. Furthermore, (Walia et al. 2007) observed highest incidence of callus on 2, 4-D along with KIN and NAA respectively. Also the same result of callus formation from hypocotyl explant was reported. (Gita rani. 2003) working on safflower callus culture reported the use of 2,4-D at 2 mg/l and KN at 0.5 mg/l concentration along with MS basal medium was best for callus induction (91%) by using hypocotyl explants.

Our finding in the experiment regarding type of explants responses is similar to the finding of Dhumale *et. al.* (2015), found the best responses only from young leaves with same texture of callus.

Table1. Standardization of seed surface sterilization protocol by using different sterilizing agents

Serial. numbers	Sterilants	Time	Concentration	Contamination	Contamination status
1	$HgCl_2$	3 min	0.1%	20%	Less bacterial contamination
		5 min	**0.1%**	**0%**	**No contamination**
2	Ethanol	10sec	70%	40%	Less bacterial contamination
		20 sec	70%	20%	Moderate Bacterial contamination
		30 sec	**70%**	**0%**	**No contamination**
3	Bavistin	15 min	0.1%	70%	High Fungal contamination and High Bacterial contamination
		15 min	0.3%	50%	High Fungal contamination and High Bacterial contamination
		15 min	0.5%	20%	Moderate Fungal contamination and High Bacterial contamination
		15 min	**0.8%**	**0%**	**No contamination**

Table 2. Callus induction from *in-vitro grown* explant cotyledonary leaves of *Carthamus tinctorius* L. on different concentration of auxins incorporated with MS + 2,4-D (0.5 – 2.5 mg/L

Explants	MS+2,4-D (mg/L)	No. of explants inoculated	Nature of response	% of response
Cotyledonary leaves	0.5	10	++	56.79
Cotyledonary leaves	1	10	+++	**78.09**
Cotyledonary leaves	1.5	10	+++	74.85
Cotyledonary leaves	2	10	++	66.14
Cotyledonary leaves	2.5	10	++	63.43

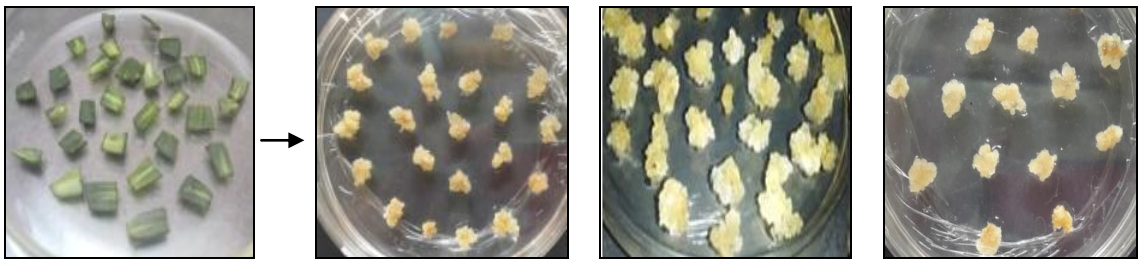
Table 3. Callus induction from *in-vitro grown* explant hypocotyl of *Carthamus tinctorius* L. on different concentration of auxins incorporated with MS + 2,4-D (0.5 – 2.5 mg/L)

Explants	MS+2,4-D (mg/L)	No. of explants inoculated	Nature of response	% of response
Hypocotyl segment	0.5	10	++	65.33
Hypocotyl segment	1	10	+++	80.68
Hypocotyl segment	1.5	10	+++	85.66
Hypocotyl segment	2	10	++++	**92.00**
Hypocotyl segment	2.5	10	++	79.25

Table 4. Callus induction from *in-vitro grown* explants cotyledonary leaves and hypocotyls of *Carthamus tinctorius* L. on different concentration of auxins incorporated with MS + 2,4-D+KIN (0.5 – 2.5 mg/L+0.5-0.1 mg/L)

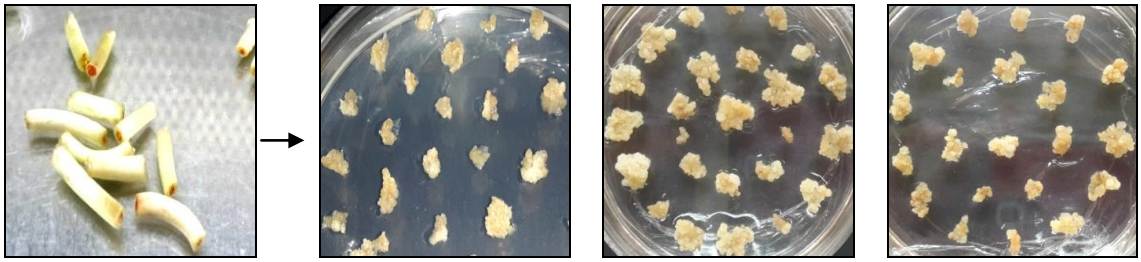
Explants	MS+2,4-D+KIN (mg/L)	No. of explants inoculated	Nature of response	% of response
Cotyledonary leaves	0.5+1.0	10	++	70.22
Cotyledonary leaves	1.0+1.0	10	++++	**99.99**
Cotyledonary leaves	1.5+1.0	10	++++	90.66
Cotyledonary leaves	2.0+1.0	10	+++	86.00

Cotyledonary leaves	2.5+1.0	10	++	75.26
Hypocotyl segment	0.5+1.0	10	++	66.49
Hypocotyl segment	1.0+1.0	10	++	75.33
Hypocotyl segment	1.5+1.0	10	++	82.35
Hypocotyl segment	2.0+1.0	10	++++	88.00
Hypocotyl segment	2.5+1.0	10	++	81.14



Cotyledonary leaves explant MS+2,4-D+KN(0.5/1mg/L) MS+2,4-D+KN(1/1mg/L) MS+2,4-D+KN(1.5/1mg/L)

Fig. 1a Photographs showing callus from cotyledonary leaves as explants at 1 mg/L of KN with 1.5 mg/L – 2.5 mg/L of 2, 4-D after 28-30 days of inoculation.



Hypocotyl explant MS+2, 4-D (1.5 mg/L) MS+2, 4-D (2mg/L) MS+2, 4-D (2.5 mg/L)

Fig. 1b Photographs showing callus from hypocotyls as explants at 1.5 mg/L – 2.5 mg/L of 2, 4-D after 28-30 days of inoculation.

REFERENCES

1 Satyapal Singh et al, 2011. *Adv. Appl. Sci. Res.,* 2011, 2 (3):47-52.

2 Rout, G. R., 2004. *Biol. Lett*. 41 (1): 26.

3 Knowles P.F, 1969. *Journal American Oil Chem. Soc.* 46:130-132.

4 Ghasempour H.S, Dabiri and Cheraghi M, 2012. *Iranian Journal of Plant Physiology*, 3(1): 539-546.

5 Soheilikhah Z.N, Karimi H.R, Ghasmpour and Zebarjadi A.R, 2013. *Australian Journal of Crop Science*. 7(12): 1866-1874.

6 Sujatha M. and Dinesh Kumar,V.2007 :Biologia Plantarum, 51(4): 782-786 (2007).

7 Skoog F and Miller CO, 1957. In: Biological Action of Growth Substances. Symp. Soc. Exp. Biol. 11:118-131

8 Zebarjadi,A.L, Borjian L. Ghasempour H. R. and Kahrizi D. 2008. *Journal of Biotechnology*. P.116-Y-048.

9 Baskaran P.B, Raja Rajeswari and Jayabalan N. 2006. *Turk. J. Bot*. 30: 1-9.

10 Hamidreza G, Zhaleh S, Ali R, Saba G, and Naser K. 2014. Iranian Journal of Plant Physiology.4(2):999-1004

11 Kumari S and Pandey R.K. 2010. The bioscan. 5(2): 247-250.

12 Walia N, kaur A, and Babbar S.B. 2007. Biomedical and life science, biologia platarum. 51(4):749-753.

13 Gita Rani, Virk G.S. and Avinash N. 2003. *In-vitro* Cellular and Developmental Biology-Plant 39(5):468-474

14 Dipti D.R, Dudhare M.S, Mohite N.R, Shingote P.R, Jadhav P.V and Moharil M.P. 2015. Journal of Cell and Tissue Research Vol. 15(1):4849-4854