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Research Article

STUDIES ON EFFECT OF PLANT GROWTH REGULATORS ON CALLUS INDUCTION FROM COTYLEDON EXPLANTS OF *CARTHAMUS TINCTORIUS* L.

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ABSTRACT
Carthamus tinctorius in one of the most important oil seed crop belonging to family Asteraceae having valuable antioxidant i.e. alphatocopherol (Vit.E) and red and yellow pigment. The aim of this investigation was to verify effect of different concentrations and combinations of plant growth regulators (auxins and cytokinins) alone and in combinations on callus induction in cotyledon explants of *Carthamus tinctorius* cv. Bhima. All tried concentrations and combinations of plant growth regulators showed initiation of callus. However, best callus induction was observed on MS + 50.0 µM NAA + 2.5 µM BAP in the form of fresh weight (3.55±0.5) and dry weight (0.19±0.06). For maximum callus production the 28th days old callus was subculture on freshly prepared MS + 50.0 µM NAA + 2.5 µM BAP for production of alphatocopherol and pigments.

Keywords: *Carthamus Tinctorius, Germination, Callus Culture, Plant Growth Regulators, Alphatocopherol, Red and Yellow Pigment*

INTRODUCTION
Carthamus tinctorius L. (Safflower, Asteraceae; cv. Bhima) is one of the oil seed crop having higher percentage of linoleic acid. In commercial oil, content of linoleic acid around 78 % of the total fatty acids (Chavan *et al.*, 2010). The major compound of pharmaceutical importance in this species is to be alphatocopherol (Vitamin E) that is used as antioxidant in food and in the treatment of heart disease, cancer and skin related problems (Kanno *et al.*, 1970). Secondly, the water-soluble yellow pigment and water insoluble red pigment from safflower florets have curative effect on coronary heart diseases, myocardial infarction, cerebral thrombosis and some gynecological ailments (Kulkarni *et al.*, 1997).
In recent years, an increasing trend in food and pharmaceutical industries is towards replacing synthetic additives with natural products. Food colors are the most controversial among the food additives used today and restrictions on the use of synthetic dyes have prompted use of and research on natural plant pigments.
Use of natural dyes in food is recommended because of their non-allergic and non-carcinogenic properties (Rudometova *et al.*, 2001) beside these extraction of natural drugs from plants is often tedious and costly since target compounds may be available only seasonally (Kutney, 1998). Because of an increasing use of petals of *Carthamus tinctorius* for pharmaceuticals and food pigments, the yield of petals from naturally grown safflower plants is not enough to meet present needs (Yu and Xu, 1997). Plant callus culture has been considered as an alternative method for solving this problem. Therefore, the objective of present work was to study the response of cotyledon explants on MS medium supplemented with different plant growth regulators for future purpose.

MATERIALS AND METHODS
Germination of Seeds
Seeds of *Carthamus tinctorius* L. (Safflower) cv. Bhima were obtained from NARI (Nimkar Agriculture Research Institute), Phaltan, Maharashtra. Seeds were surface sterilized and inoculated on sucrose (1%) and agar (0.8%) medium. The cultures were incubated at 25±2^{o}C (under dark) for germination. The germinated 7 day old cotyledon explants were used for induction of callus.

Culture Conditions and Callus Initiation

A segment of cotyledon about 1 cm^2 was used as explants. The explants were inoculated on MS medium (Murashige and Skoog, 1962) supplemented with different concentrations and combinations of plant growth regulators. The pH of the medium was adjusted to 5.8 before autoclaving at 1.1 kg cm^2 pressure and 121ºC for 15 minutes. Cultures were incubated at 25 ± 2°C under 16 h photoperiod (50µmol m^{-2} s^{-1}) and about 70 % relative humidity.

Growth of Callus Cultures

After 4th Week of inoculation, the cultures responses were recorded and growth of callus was determined on the basis of fresh and dry weight. The callus was dried in an oven at 60 ºC till constant dry weight was obtained.

Statistical Analysis

All experiments were repeated at least thrice with minimum of 15 replicates per treatment. Significance of treatment effects were determined by using analysis of variance (ANOVA). Variation among treatment means was assessed by DMRT at 5 % level of significance.

RESULTS AND DISCUSSION

For callus induction two week old cotyledonary leaf explants prepared from *in vitro* micropropagated seedling. Initially, the callus was induced from cotyledon explant of *C. tinctorius* on MS medium supplemented with different concentrations of auxins and cytokinins alone and in combinations (2.5 to 50 µM). Based on results about addition of cytokinins (BAP and Kin) the maximum growth of callus was obtained in MS medium supplemented with BAP (10 µM) as compare to Kin (7.5 µM) followed by other combinations of NAA with BAP and Kin (Table 1). Similar result was observed in callus of *Triticum aestivum* (Shah *et al.*, 2003).

Table 1: Effect of Different Concentrations and Combinations of Plant Growth Regulators (Auxins and Cytokinins) on Callus Induction from Cotyledon Explants of *Carthamus Tinctor*ius L.

Cotyledon Explant			
MS Medium +	**FW (g)**	**DW (g)**	**M (%)**
10.0 µM IAA	0.15±0.03^{k}	0.04±0.006^{i}	67.8
10.0 µM NAA	1.44±0.7^{f}	0.09±0.004^{f}	93.4
7.5 µM 2,4-D	1.03±0.4^{g}	0.08±0.004^{fg}	91.4
10.0 µM BAP	1.70±0.5^{d}	0.14±0.05^{c}	93.2
7.5 µM Kin	0.31±0.03^{j}	0.04±0.003^{j}	87.2
5.0 µM IAA + 7.5 µM BAP	0.59±0.04^{i}	0.06±0.003^{h}	88.3
7.5 µM IAA + 7.5 µM Kin	0.64±0.06^{h}	0.08±0.005^{g}	86.6
10.0 µM NAA + 2.5 µM Kin	2.60±0.9^{b}	0.16±0.04^{b}	92.4
50.0 µM NAA + 2.5 µM BAP	3.55±0.5^{a}	0.19±0.06^{a}	93.8
1.2 µM 2,4-D + 7.5 µM BAP	2.25±0.6^{c}	0.13±0.04^{d}	94.1
2.5 µM 2,4-D + 5.0 µM Kin	1.53±0.4^{e}	0.11±0.07^{e}	92.6

The values represent the mean±SE calculated on three independent experiments, each based on minimum of 15 replicates. Values followed by the same letter were not significantly different at 5% level (DMRT). DMRT was applied to fresh weight (FW), dry weight (DW) separately. M (%): moisture percentage.

When NAA and IAA were used alone it shows the rhizogenic callus at 7.5 µM concentration after one week of incubation. At high concentrations, rhizogenesis was completely absent (10 µM) and only callus was induced (Table 1). However, NAA and 2,4-D were shows more growth of callus than IAA in cotyledon explant of *Carthamus tinctorius* L. 2,4-D is important factor in the process of stimulating the cell division. Similar results on effect of NAA and 2,4-D alone for callus was reported by Gopi and

Vatsala (2006), Uddin *et al.,* (2006), Amarasinghe and Yang (2005), Islam *et al.,* (2005), Khar *et al.,* (2005).

On the other hand more callus growth was achieved at combination of NAA+BAP and IAA + Kin as compare to addition of IAA and NAA alone. Among these combinations NAA: BAP has induced more callus growth than NAA: Kin combination. Thus, NAA along with BAP was more effective than NAA with Kin for callus growth. The maximum growth of (mentioned in fresh and dry weight) callus was obtained at 50.0 µM NAA and 2.5 µM BAP was 3.55 ± 0.5g, 0.192 ± 0.06g (Table 1, Figure 1 and figure 2). Thus, the results of present investigation and earlier reports on *Carthamus tinctorius* (Chavan *et al.,* 2010; Nikam and Shitole, 1999) showed that combination of NAA + BAP was superior to other combinations of auxins and cytokinis for proliferation and growth of callus. Plevnes *et al.,* (2006) obtain maximum growth and weight of callus on MS medium containing NAA (1.45g) with BAP (1.11g) in cotyledon explant of *Tomato*. Gray (2003) observed callus induction in *Eucommi aulmoides* on WPM medium supplemented with 6 mg/l NAA with 1 mg/l BA. Yang *et al.,* (2008) observed the combination of NAA and 6-BA could induce callus formation in cotyledon and hypocotyl explant of *Leonurus heterophylus*.

Figure 1: The Callus Induced on Cotyledon Explant in *Carthamus Tinctorius* L.

Figure 2: Callus Maintained in MS + 50.0 µM NAA + 2.5 µM BAP

Conclusion

The effectiveness of callus culture induction depended on the type of growth regulators used and the culture conditions. In a majority of cases, its forming was stimulated by light and the combination of auxins and cytokinins. MS media supplemented with BAP and NAA combination was used in the study

proved to be highly effective in terms of *C. tinctorius* callus induction and enhanced callus growth. The maintained calli were used for further study for extraction of important secondary metabolites.

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