

Proliferation of Shoot from First Leaf of *Carthamus tinctorius* L. (Safflower)

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ABSTRACT

Callus induction and in vitro plantlet regeneration system for safflower (*Carthamus tinctorius* L.) using the first leaf were optimized by studying the influence on organogenesis of seedling age, media factor, growth regulator and excision orientation. Supplementation medium with auxin and cytokinin ratio >1 enhances the growth rate of callus culture. Growth regulators IAA, NAA, BAP, kinetin in the medium were found effective for callus induction and regeneration in all explants. The BAP 5mg/l, NAA 1mg/l, 5-7 explants and callus derived from cotyledonary. Explants were cut from basal region of cotyledon of 5-7 days old seedlings. As compared to the standard media SH-M and B-5 growth were superior in MS medium. Capitula induction was observed in callus mediated shoot from cotyledon and with sucrose, IAA, NAA and BAP. The well-developed plantlet was transferred to the field.

Key-words: BAP, *Carthamus tinctorius*, Callus, L., Safflower, MS medium, NAA

INTRODUCTION

Carthamus tinctorius L. (Safflower) Asteraceae is an important oil seed crop of semiarid subtropical regions of average temperature 17–20°C, which appear to be best for vegetative growth and optimum temperature of flowering is 24 to 32°C. Due to high content of linoleic acid it occupies unique position among oil seed plants [1]. The young plant is used as a leafy vegetable; seed oil is used for industrial and edible purpose [1,2]. Safflower is considered as salt tolerant specially sodium salt. Flower yield and pigment content of flower have gain economic importance [3,4] to increasing countries and their use in medicine for curing several diseases.

In vitro plant regeneration system is basic necessity for such approaches. Direct somatic embryogenesis from cotyledon explants [4] and *in vitro* shoot regeneration has been reported in safflower [4,5]. Modern techniques like embryo rescue and other biotechnological tool may play

an important role in overcoming such barriers. Development of cytoplasmic-genetic male sterility, system for hybrid breeding, a successful outcome of ongoing efforts to use polyembryony for varietal improvement and confirmation of apomixes in safflower [6].

Genetic transformation of safflower to impart resistance to biotic and abiotic factor in addition to development of seed with altered fatty acid and protein profiles [7]. However cultivar can vary responses and regeneration of whole plant.

MATERIALS AND METHODS

Certified seeds of safflower (*Carthamus tinctorius* L.) were obtained on August 2011 from Department of Botany, National Environment Engineering Research Institute (NEERI) Nagpur, India. Seeds were surface sterilized with 0.1% (w/v) mercuric chloride for (HgCl₂) with 3 minutes constant shaking followed by three washes for 1 minute each in sterilized distilled water. Seeds were then germinated and grown on sucrose 3%, agar 0.8% under photoperiod of fluorescent light. Explants (cotyledon) were isolated- 15–17 mm² from 5 to 7 days old seedlings. The medium was supplemented with BAP and NAA (500 µl BAP and 1250 µl NAA were added

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and volume was made up 250 ml by adding distilled water) then explants were transferred onto callus induction medium.

Induction and Callus- Callus induction was carried out on MS medium supplemented with BAP and NAA in combination. After 21 days of inoculation, completely differentiated dense mass of callus regeneration ability was observed. After three weeks of culture, the responded explants further transferred on fresh medium containing same concentration of BAP and NAA [5]. Each regeneration step was further carried out for period of 21 days subculture onto fresh optimum callus induction [8]. Shoot induction from explants and calli (250 mg–300 mg/culture) was carried out on MS containing BAP 5 mg/L and NAA 3 mg/L. Regenerated shoot was about 1 cm and separated from explants and callus. Rooting of resulting shoots (1–1.5 cm long) from explants and callus was attempted on MS without growth regulator and with sucrose 1–9%, NAA 5 mg/L, BAP 0.25 mg/L (in combination).

Hardening- Rooted plantlets were removed from culture vials after agar had been removed by washing with sterile water, the plantlets were planted in a pot

were placed outside in the shade (light max 83.46 m⁻² s⁻¹ μ m, temperature 25 +/- 4°C) irrigated at 3 days interval with tap water [9].

RESULTS

We select 3–7 days old seedling and first leaf as explant (Fig. 1). Most cotyledonary leaves elongated and formed green yellow coloured compact callus about 18–21 days after culture initiation (Fig. 2). Induction of callus was observed in all media there was no statistically difference among concentration of BAP and NAA (Table 1). After 22–23 days subculture (Fig. 3) and proliferation of shoot from first leaf was visible after 28–32 days in all media tested (Fig. 4) shoot primordia developed into normal shoot after 40–43 days after culture initiation all concentration of BAP and NAA (Fig. 5 and Table 2).

Regeneration response was best on the MS medium supplemented with 1 mg/lit NAA and 5 mg/lit BAP. Callus induction was observed by using in first leaf explants and direct shoot regeneration was observed. Brownish green slow growing friable callus was obtained after 18 days of inoculation & shoot regeneration was obtained after 32 days of inoculation.

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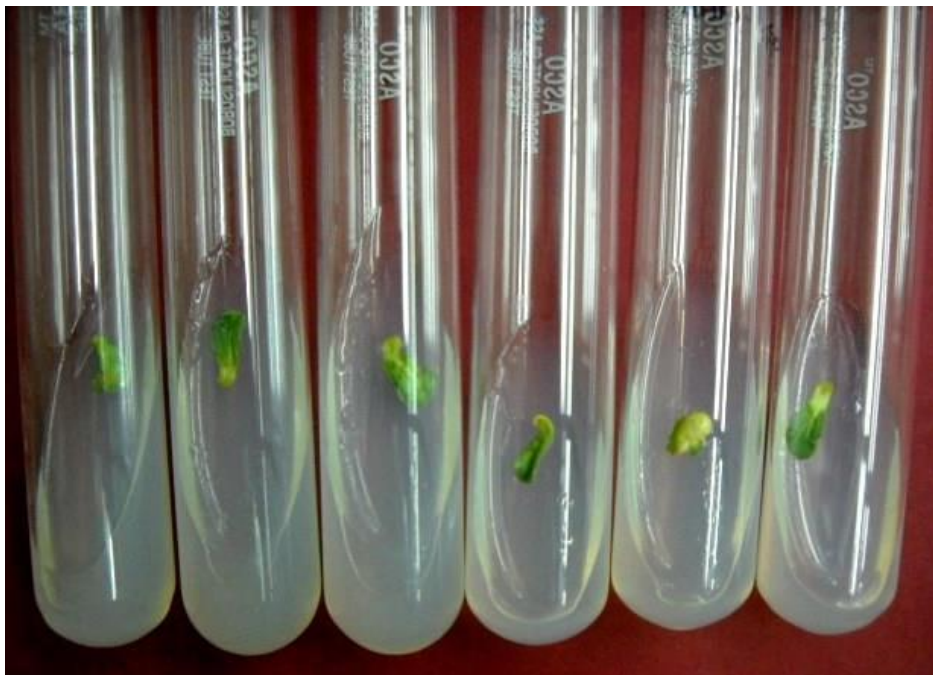


Fig. 1: First Leaf Explant of 5 - 7 days old seedling



Fig. 2: Callus induction from first leaf (18–21 days after culture initiations)



Fig. 3: Sub culturing of calli of leaf explants



Fig. 4: Shoot Proliferation from cotyledonary leaves was visible after 28–31 days



Fig. 5: Direct multiple shooting from First Leaf after 40–43 days after culture initiation



Fig. 6: Hardening acclimatized plant in the pot

Table 1: Observation for Callus induction

Type of explants	Medium	Additional components in medium	Duration			
			7	14	21	28
First leaf	MS	NAA 3 mg/lit BAP 5 mg/lit	S	+	++	+++

Swelling= S, Callus induction= +, Callus Induction and Growth= ++, Callus Induction growth with good response= +++

MS= Murashige and Skoog medium

Table 2: Observation for Shoot regeneration from callus

Type of explants	Medium	Additional components in medium	Duration			
			7	14	21	28
First leaf calli	MS	NAA 1 mg/lit BAP 5 mg/lit	+	S	S	S

S= Shooting, += Primary response, MS= Murashige and Skoog medium

DISCUSSION

According to Neetika *et al.* [2] cultured 10 day old seedling with different concentration of TBZ and IAA induced shoot regeneration from cotyledonary leaves and *In vitro* multiplication in safflower. Most published report [7] described use of TBZ and IAA But we are using only BAP and NAA the best shoot multiplication was achieved in the range of media supplemented with BAP 5 mg/lit and NAA 1 mg/lit. Without using TBZ and IAA we got a good result in BAP and IAA.

The concentration of hormones changed and their growth was studied. For callus induction, different combination of BAP and NAA were taken if we take: BAP 5 mg/L, NAA 5 mg/L, BAP 0.2 mg/L, NAA 5 mg/L, BAP 3 mg/L, NAA 5 mg/L then no response was seen. But in BAP 5 mg/L, NAA 3 mg/L combination best growth was observed. For shoot induction in combination BAP 3 mg/L, NAA 0.5 mg/L no response was seen but in BAP 5 mg/L, NAA 1 mg/L growth was observed. We were used different concentration but explant was very favourable explant with high multiplication ratio 100% at the concentration of BAP 5 mg/lit and NAA 1 mg/lit. All regenerated shoot tip (15–20 mm length) were excised and rooted radially in half strength of MS medium supplemented. Rooting was observed from the cut end of shoots within 40–42 days in most media tested. All developing roots were physically vigorous and healthy (Fig. 6).

CONCLUSIONS

We were concluded that the proliferation of *Carthamus tinctorius* (Safflower) was effectively influenced by sucrose as a carbon source and hormone BAP and NAA. The optimum concentration of carbon source was found to be approximately 8%, which was 8 g/lit by a good response of first leaf cutting. The effective concentration for induction of callus was found to be BAP 5 mg/lit and NAA 3 mg/lit and BAP 5 mg/lit and NAA 1 mg/lit for

shoot induction. *Carthamus tinctorius* (Safflower) was taken as a study of interest as it has many medicinal values so as to make people aware of it. We can produce a genetically modified crop of *Carthamus tinctorius* to make it resistance to biotic and abiotic factor and we can use the stigma of *Carthamus tinctorius* as an alternative to saffron as it is costly.

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CONTRIBUTION OF AUTHORS

The work was designed and performed by Mrs. Sneha Mendhe along with collection of materials and data collection. Ms. Sana Sheikh has analyzed the data and interpreted the work.

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