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In Vitro Organogenesis – Regeneration of Shoot From *Carthamus tinctorious*

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ABSTRACT

Callus induction and in vitro plantlet regeneration system for safflower (Carthamus tinctorious L) using root, hypocotyls ,first leaf ,cotyledon 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 enhance growth rate of callus culture growth regulators IAA,NAA,BAP, kinetin in the medium where found effective for callus induction and regeneration in all explants .

The BAP-(5mg/l) ,NAA-(3mg/l) (5-7) explants and cotyledonary derived callus more shoots were produced on explants cut from more basal region of cotyledon from 5-7 day old seedling than from older seedlings are more distal cut side MS medium was superior to SH - M and B5.

Capitula induction was observed in both callus mediated shoots on cotyledon and shoots on rooting medium with sucrose IAA,NAA and IBA well developed plantlet were transferred to the field.

KEYWORDS: ***Carthamus tinctorious L: Safflower, organogenesis, callus, explants.***

ABBRIVATIONS: IAA: Indol Acedic acid, IBA: Indol butyric acid ,NAA: Alpha naphthalene acetic acid ,MS: Murashige and Skoog , B5: Gamborg, SH-M: Micchell and Gildow.

INTRODUCTION

Carthamus tinctorious L (Safflower) Asteraceae, is an important oil seed crop of semiarid subtropical regions average temperature of 17 - 20°C appear to be best for vegetative growth and optimum temperature for flowering is 24 to 32 °C. Safflower occupies unique position among oilseed crops and due to high content of linoleic.

The young plant is use as a leaf vegetable (Anonymous 1950) the oil seed use for industrial and edible purpose. Safflower is considered salt tolerant specially sodium salt.

Modern techniques like embryo rescue and other biotechnological tool may play an important role in overcoming such barriers. Development and cytoplasmic genetic male sterility system for hybrid breeding a successful outcome of ongoing efforts to use polyembrony for varietal improvement and confirmation of apomixes in safflower.

Flower yield and pigment content of flower have gain economic importance due to increasing countries and their use in medicine for curing several diseases. Genetic transformation of safflower to import resistance to biotic and a biotic factor in addition to development of seed with altered fatty acid and protein profiles.

In vitro plant regeneration system is basic necessity for such approaches direct somatic embryogenesis from cotyledon explants (mandal et.al. 1995) and in vitro shoot regeneration has been reported in safflower(George and Rao, 1982; (Tejovathi and Anwar 1996)
However response varies cultivar and regeneration of whole plant.

MATERIALS AND METHODS

Certified seeds of safflower (*Carthamus tinctorious* L) were obtained from National Environment Engineering Research Institute (NEERI) Nagpur ,India. Seeds were surface sterilized with 0.1 % (w/v) mercuric chloride for (HgCl2). 3 min with constant shaking followed by three washes for 1 min each in sterilized distilled

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water seeds were then germinated and grown on a sucrose (3%) agar 0.8 % under ih photo period of fluorescent light. Explants were isolated cotyledon – (15-17 mm2) from 3 to 5 days old seedlings leaf explants (15-17 mm2) were isolated from the shoot obtained in vitro from the cotyledon explants on medium supplemented with 500µl of BAP and 1250µl of NAA were added and volume was made up 250 ml by adding distilled water. Explants were transferred onto callus induction medium.

Induction and callus

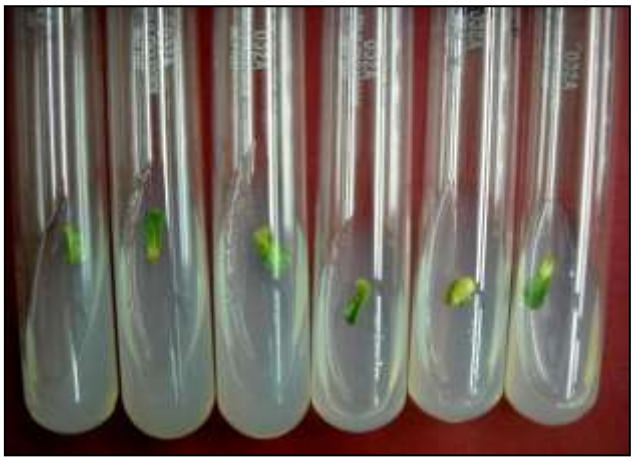
Callus induction was carried out on MS, SH-M, B5 medium supplemented with BAP and NAA either alone or in combination. After 21 days of inoculation completely differentiated dense mass of callus showing further regeneration ability were taken as a standard measure to calculate percentage of causing each regeneration step was further carried out for period of 21 days subculture onto fresh optimum callus induction .After three weeks of culture responded explants where further transferred on fresh medium containing same concentration of BAP and NAA.

Shoot induction from explants and calli (250 mg- 300mg/culture) was carried out on MS, SH-M medium containing BAP-5mg/L and NAA 1 mg/L. regenerated shoot were about 1cm and were separated from explants and callus.

Rooting of resulting shoots (1-1.5 cm) long from explants and calli was attempted on MS SH-M, and B5 without growth regulator and with sucrose (1-9%) NAA- 5mg/L both combination of BAP- 0.25 mg/L.

Hardening

Rooted plantlets were removed from culture vials after agar had been removed by washing with sterile water, the plantlets were planted in a pots containing 1:1 sterilized potting mixture soil and washed sand (with pebble size of 0.5 -1.0 mm).the plants were placed outside in the shade (light max 83.46 m-2 s-1µm, temperature 25 +/- 4 °C) irrigated at 3 days interval with tap water.



1.1 First Leaf Explant

1.2 Callus induction from first leaf

1.3 Sub culturing of calli of leaf explant.

1.4 Shoot Proliferation

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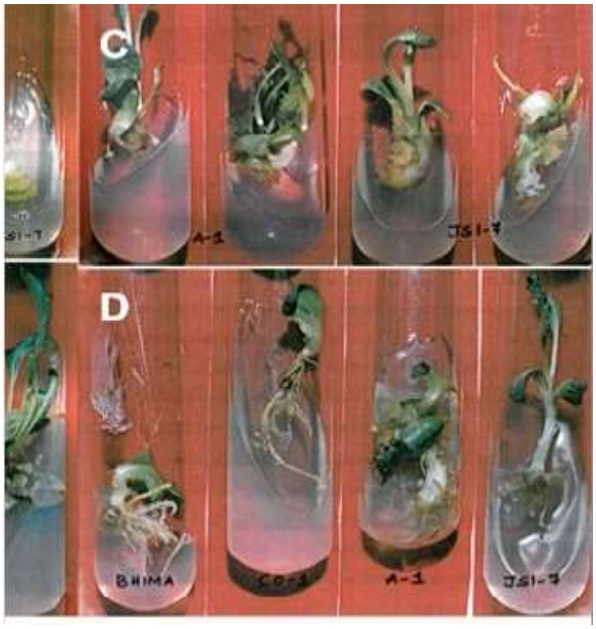
1.5 Direct multiple shooting from First Leaf



1.5 Direct multiple shooting from First Leaf .

1.6 Hardaning-Acclimatized Plant in the pot

RESULT AND DISCUSSION

Regeneration response was best on the MS medium supplemented with 3mg/lit NAA & 5mg/lit BAP gave callus induction in first leaf explants and direct shoot regeneration was observed in first leaf. 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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