ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In Vitro Propagation Callus Induction and Regeneration of shoot from First Leaf of *Carthamus tinctorius* L.

Sneha Mendhe*.

Department of Botany Dr. D. Y. Patil Arts Commerce and Science College Pimpri Pune -18, Maharashtra, India.

ABSTRACT

Advances in plant tissue culture methods in biotechnology such as callus induction are essential for further advanced studies. A callus induction and in vitro plantlet regeneration system for safflower (Carthamus tinctorius L.) using roots, hypocotyls, first leaf, cotyledons was optimized by studying the effects of plant age, medium composition, growth regulators and pruning orientation on organogenesis. In a medium found effective for callus induction and regeneration in all explants, supplementation medium with auxin and cytokinin ratio >1 increases the growth rate of callus culture growth regulators IAA, NAA, BAP, kinetin. BAP-(6 mg/L), NAA-(2 mg/L) (6-7) explants and cotyledonary derivative callus from 4–5-day old plants resulted in more shoots being formed on explants cut from the basal region of the cotyledons from 4–5-day old plants than on older plants.Capitula induction was observed in callus-mediated shoots on cotyledons, and seedlings with well-developed IAA, NAA, and IBA in shoots on rooting medium containing sucrose were transferred to the field.

Keywords: *Carthamus tinctorious L:* Safflower, organogenesis, callus, explants , First Leaf.

Abbreviation: IAA: Indol Acedic Acid, IBA: Indol butyric acid , NAA: Alpha Naphthalene Acetic Acid , MS: Murashige and Skoog , B5: Gamborg, SH-M: Micchell and Gildow.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Carthamus tinctorius L. (Safflower) belongs to the Asteraceae family. It is an important oilseed crop in the semi-arid subtropical regions. The average temperature for plant growth is considered to be 17-20°C and the optimum temperature for flowering is 24 to 32°C. Safflower occupies a unique position among oilseed crops and is a good alternative source of olive oil due to its high linoleic acid content. [9]

The young plant is used as a leaf vegetable[1] Oilseeds for industrial and edible oil applications. Safflower is considered to be salt tolerant, especially to sodium salt. Modern techniques such as embryo rescue and other biotechnological tools can play an important role in overcoming such constraints. The development of a cytoplasmic genetic male sterility system for hybrid breeding is a successful outcome of ongoing efforts to use polyembryony to improve diversity and confirm apomixis in Safflower.

Flower production and pigment content have become economically important as they are increasingly used in developing countries and in medicine to treat many diseases. Genetic modification of safflower imports resistance to biotic and abiotic factors, as well as the development of seeds with altered fatty acid and protein profiles.

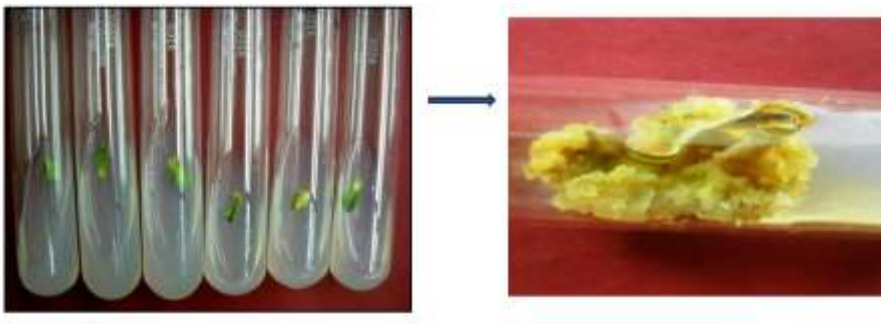
An in vitro plant regeneration system is a basic requirement for such methods. Direct somatic embryogenesis from cotyledon explants [3] and in vitro shoot regeneration in sorghum have been reported[11] . However, the response varies across plant species and regeneration.

MATERIALS AND METHODOLOGY

Certified seeds of safflower (Carthamus tinctorius L.) were obtained from National Environmental Engineering Research Institute (NEERI), Nagpur, India. The seeds were surface sterilized with 0.1% (w/v) mercuric chloride ($HgCl_2$). After continuous shaking for 5 minutes followed by five washes in sterile distilled water for 1 minute each, the seeds were germinated and grown on sucrose (3%) agar 0.8% under fluorescent light. Leaf explants (15-17 mm2) from 4- to 5-day-old plants were isolated from in vitro derived shoots from cotyledon explants, the medium was supplemented with 500µl BAP and 1250µl NAA and made up to 250 ml by adding distilled water. The explants were transferred to callus induction medium.

Induction and callus

Callus induction was performed on MS, SH-M, B5 [6] medium supplemented with BAP and NAA[5] alone or in combination. After 21 days of inoculation, completely differentiated dense masses of callus showing more regeneration potential were taken as a standard measure to calculate the percentage of cause[12]. Each regeneration stage was subcultured on fresh optimal callus induction for a period of 21 days[7]. After three weeks of culture, the explants were further transferred onto fresh medium containing the same concentrations of BAP and NAA.



1.1 Explant -First Leaf **1.2 Induction of Callus from First Leaf**

Shoot induction from explants and callus (250 mg-300 mg/culture) was performed on MS, SH-M medium containing BAP-6 mg/liter and NAA-2 mg/liter. The regenerated shoots were about 1 cm and were separated from the explants and callus[8].Multiple shootings are developed[10]

ISSN: 0975-8585

1.3 Subculturing of Callus from first Leaf

1.4 Proliferation of Shoot from Callus from First Leaf

1.5 Direct Multiple Shooting

Rooting of long shoots (1-1.5 cm) from explants and callus was attempted on MS SH-M and B5 without growth regulator and in a mixture of sucrose (1-9%) NAA- 5 mg/liter both BAP- 0.25 mg/liter

Hardening

After washing with sterile distilled water and removing the agar, the rooted seedlings were removed from the culture vial. The seedlings were planted in pots containing 1:1 sterile soil and washed sand (with 0.5 -1.0 mm pebble size). The seedlings were kept outside in the shade (maximum light 83.46 m-2 s-1µm, temperature 25 +/- 4 °C) and watered with tap water at 3-day intervals.

1.6Hardening- Adapted plant in pot culture

RESULTS AND DISCUSSION

The regeneration response was best on MS medium, in which 2 mg/liter NAA and 6 mg/liter BAP were used and callus induction was given in first leaf explants and direct shoot regeneration was observed in the first leaf. Brownish green slow growing delicate callus was obtained after 17 days of inoculation and shoot regeneration was obtained after 33 days of inoculation.

ACKNOWLEDGEMENT

The authors extend their gratitude to the Principal of Dr. D. Y. Patil Arts Commerce and Science College, Pimpri, Pune for their continuous support in carrying out this work. We also express our heartfelt thanks to our parents for their boundless encouragement and motivation.

REFERENCES

[1] Anonymous (1950) Carthamus tinctorius L. In: Wealth of India, Indian raw materials, vol CSIR, Indian, Bhopal, pp 83–88"
[2] Mandal, A.K. and Gupta, D.S. 2001 Direct shoot organogenesis formation and plant regeneration in safflower. In Vitro Cell. Dev. Biol. 37: 50–54.
[3] Mandal, A.K. and Gupta, D.S. 2003 Somatic embryogenesis of safflower: influence of auxin and ontogeny of somatic embryos. Plant Cell, Tissue and Organ Culture 72: 27–31.
[4] Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 473-497.
[5] Neetika W, Amandeep K, Babbar SB (2005). In vitro regeneration of a high oil-yielding variety of safflower (Carthamus tinctorius var HUS-305). J. Plant Biochem. Biotechnol.14(1): 65-68
[6] Nikam TD, Shitole MG (1999). In vitro culture of Safflower L. cv. Bhima:initiation, growth optimization and organogenesis. Plant Cell Tissue Organ Cult. 5: 15-22.5
[7] Orlikowska, T.K. and Dyer, W.E. 1993 In vitro regeneration and multiplication of safflower(Carthamus tinctorius L). Plant Sciences, 1993: 151-157.
[8] Özgen M, Özcan S, Sevimay CS, Sancak C, Yıldız M (1998). High frequency adventitious shoot regeneration in sainfoin. Plant Cell, Tissue Organ Cult. 52: 205-208.
[9] Perbal B (1988). Expression of cloned DNA sequences in vitro or in prokar*yotic and* eukaryotic cells. A practical guide to molecular cloning. 2nd ed. New York: John Wiley & Sons. pp. 731-794
[10] Singh R, Srivastava K, Jaiswal HK, Amla DV, Singh BD (2002). High frequency multiple shoot regeneration from decapitated embryo axes of chickpea and establishment of plantlets in the open environment. Biol. Plant. 45: 503-508.
[11] Tejowathi, G. and Anwar, S.Y. 1987 Plantlet regeneration from cotyledonary cultures of safflower (Carthamus tinctorius L.). In: Reddy, G.M., ed. Plant cell and tissue culture of economically important plants. Osmania University, Hyderabad, India 1987; 347-353.
[12] Ying, M., Dyer, W.E. and Bergman, J. 1992 Agrobacterium tumefaciens mediated transformation of safflower (Carthamus tinctorius L) cv 'Centennial'. Plant Cell Reports,11: 581-585.