

SJIF Impact Factor(2021) : 8.047
ISI I.F.Value : 1.188

ISSN (Online): 2455-3662
DOI: 10.36713/epra2013

Epra International Journal of
MULTIDISCIPLINARY
RESEARCH

Monthly, Peer Reviewed (Refereed) & Indexed International Journal

Volume - 7 Issue - 4 April 2021



I
J
M
R



Chief Editor
Dr. A. Singaraj, M.A., M.Phil., Ph.D.

Managing Editor
Mrs.M.Josephin Immaculate Ruba
Editorial Advisors

1. **Dr.Yi-Lin Yu, Ph. D**
Associate Professor,
Department of Advertising & Public Relations,
Fu Jen Catholic University,
Taipei, Taiwan.
2. **Dr.G. Badri Narayanan, PhD,**
Research Economist,
Center for Global Trade Analysis,
Purdue University,
West Lafayette,
Indiana, USA.
3. **Dr. Gajendra Naidu.J., M.Com, LL.M., M.B.A., PhD. MHRM**
Professor & Head,
Faculty of Finance, Botho University,
Gaborone Campus, Botho Education Park,
Kgale, Gaborone, Botswana.
4. **Dr. Ahmed Sebihi**
Associate Professor
Islamic Culture and Social Sciences (ICSS),
Department of General Education (DGE),
Gulf Medical University (GMU), UAE.
5. **Dr. Pradeep Kumar Choudhury,**
Assistant Professor,
Institute for Studies in Industrial Development,
An ICSSR Research Institute,
New Delhi- 110070.India.
6. **Dr. Sumita Bharat Goyal**
Assistant Professor,
Department of Commerce,
Central University of Rajasthan,
Bandar Sindri, Dist-Ajmer,
Rajasthan, India
7. **Dr. C. Muniyandi, M.Sc., M. Phil., Ph. D,**
Assistant Professor,
Department of Econometrics,
School of Economics,
Madurai Kamaraj University,
Madurai-625021, Tamil Nadu, India.
8. **Dr. B. Ravi Kumar,**
Assistant Professor
Department of GBEH,
Sree Vidyanikethan Engineering College,
A.Rangampet, Tirupati,
Andhra Pradesh, India
9. **Dr. Gyanendra Awasthi, M.Sc., Ph.D., NET**
Associate Professor & HOD
Department of Biochemistry,
Dolphin (PG) Institute of Biomedical & Natural Sciences,
Dehradun, Uttarakhand, India.
10. **Dr. D.K. Awasthi, M.SC., Ph.D.**
Associate Professor
Department of Chemistry, Sri J.N.P.G. College,
Charbagh, Lucknow,

ISSN (Online) : 2455 - 3662
SJIF Impact Factor :8.047
ISI I.F. Value : 1.188
DOI : 10.36713/epra2013



EPRA International Journal of
**Multidisciplinary
Research**

Monthly Peer Reviewed & Indexed
International Online Journal

Volume: 7 Issue: 4 April 2021

Indexed By:



Published By :EPRA Publishing

CC License





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

Varpe S.N¹.

Nutan Arts Commerce and Science College
Rajapur.¹

Sneha Mendhe²

Dr.D.Y.Patil acsw College,
Pimpri.Pune²

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.*

ABBREVIATIONS: IAA: Indol Acetic acid, IBA: Indol butyric acid, NAA: Alpha naphthalene acetic acid, MS: Murashige and Skoog, B5: Gamborg, SH-M: Mitchell 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 used as a leaf vegetable (Anonymous 1950) the oil seed used for industrial and edible purpose. Safflower is considered salt tolerant specially sodium salt.

Modern techniques like embryo rescue and other biotechnological tools 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 polyembryony for varietal improvement and confirmation of apomixis in safflower.

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

In vitro plant regeneration system is a 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; Tejavathi 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

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

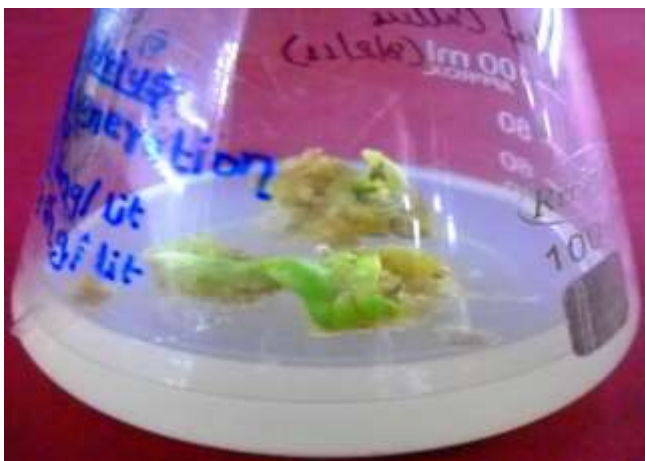
Rooted plantlets were removed from culture vials after agar had been removed by washing with sterile water, the plantlets were planted in a pots containing 1:1 sterilized potting mixture soil and washed sand (with pebble size of 0.5 -1.0 mm).the plants were placed outside in the shade (light max 83.46 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



1.5 Direct multiple shooting from First Leaf



1.5 Direct multiple shooting from First Leaf .



1.6 Hardening-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.

REFERENCES

1. 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.
2. 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.
3. Orlikowska, T.K. and Dyer, W.E. 1993 In vitro regeneration and multiplication of safflower (*Carthamus tinctorius* L.). *Plant Sciences*, 1993: 151-157.
4. 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.
5. 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.
6. Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15: 473-497.
7. 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.



8. 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
9. Orlikowska TK, Dyer WE (1993). *In vitro* regeneration and multiplication of safflower (*Carthamus tinctorius* L.). *Plant Sci.* 93: 151-157.
10. 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.
11. Perbal B (1988). *Expression of cloned DNA sequences in vitro or in prokaryotic and eukaryotic cells. A practical guide to molecular cloning.* 2nd ed. New York: John Wiley & Sons. pp. 731-794.
12. 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.