International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(10): 1056-1060
www.biochemjournal.com
Received: 01-07-2024
Accepted: 04-08-2024

SK Dsouza
M.Sc. Scholar, Department of Plant Biotechnology Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

SR Bhalerao
Associate Professor, Department of Plant Biotechnology Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

PV Jadhav
M.Sc. Scholar, Department of Plant Biotechnology Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

SS Fulzalke
B.Tech Scholar, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

DB Gurvpujari
B.Tech Scholar, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Corresponding Author:
SK Dsouza
M.Sc. Scholar, Department of Plant Biotechnology Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

In vitro callus induction in safflower genotypes (*Carthamus tinctorius* L.)

Dsouza SK, Bhalerao SR, Jadhav PV, Fulzalke SS and Gurvpujari DB

DOI: https://doi.org/10.33545/26174693.2024.v8.i10Sm.2640

Abstract
Advancement in plant tissue culture methods, in biotechnology such as callus induction are necessary for further advance studies. This study aimed at establishment of optimized callus induction from safflower genotype PBNS-86 and GMU-6947. An efficient protocol for *in vitro* callus induction was developed using leaves as explant of both genotypes. The most effective combination, T_6 (3.0 mg/l NAA+1.0 mg/l BAP), induced 76% callus formation in PBNS-86 and 70% in GMU-6947. Comparable success was observed with 2,4-D based Treatments, T_3 (3.0 mg/l) and T_4 (4.0 mg/l), which resulted in 73% callus induction for PBNS-86 and 66% for GMU-6947. The genotypes showed a consistent response to Treatments, with PBNS-86 generally exhibiting higher callus induction.

Keywords: Safflower genotypes, callus induction, BAP, 2,4-D, NAA, MS medium

Introduction
Safflower (*Carthamus tinctorius* L.), or kardi, is an important rabi oilseed crop from the Compositae family. Cultivated in India since ancient times, it is referenced as kusumba in historical scriptures (Akashe, 2013) [1]. It is a well-branched, thistle-like winter annual plant, typically featuring numerous long, sharp spines on its leaves (Pascual-Villalobos and Alburquerque, 1995) [2]. The flower colour of cultivated safflower is typically brilliant orange. The leaf morphology is described as alternate, sessile and ovate-lanceolate (Teotia *et al*., 2002) [3]. In India, during 2021-22, safflower production reached 0.45 lakh tonnes from an area of 0.64 lakh hectares, with an average yield of 694 kg/ha. In Maharashtra, safflower was cultivated on 0.23 lakh hectares, producing 0.18 lakh tonnes, with an average productivity of 792 kg/ha (Anonymous, 2022) [4].

Safflower seeds consist of 25-50% oil, 15-20% protein, and 35-45% hull fraction. The typical safflower oil exhibits variation in its fatty acid composition, with approximately 6-8% palmitic acid and 2-3% stearic acid. Safflower oil comes in two types: one high in monounsaturated fatty acid (oleic acid) and the other rich in polyunsaturated fatty acid (linoleic acid). The high oleic variety contains less saturated fat than olive oil, making it ideal for low-cholesterol diets, frying, and preparing frozen foods. The linoleic acid-rich type has the highest linoleic content among vegetable oils, making it one of the best edible oils globally (Knowles, 1958) [5]. However, the plant pigments lately have been looked at again in the food and cosmetic industries because of concerns about safety (Nakano *et al*. 1988) [6]. Traditionally, its flowers were used to color foods, as a spice for flavouring, and in dye making (Gao and Fan, 2000) [7]. The red pigment in the petals has been used as dyes, raw material of cosmetics, a safe coloring agent, and herbal medicine for female-related diseases. The structure of this pigment, which is called carthamin, was identified by Obara *et al*. (1979) [8]. It has a significant medicinal value in traditional Chinese medicine for promoting blood circulation and treating cardiovascular and thrombotic diseases (Bie 2003; Jiang *et al*. 2005) [9, 10].

The production of secondary metabolites by plant tissue or cell culture has attracted our special interest since these compounds are useful for medicines, food additives and cosmetics. Many papers have been reported about production of secondary metabolites by plant cell culture methods (Fujita *et al*. 1981; Ishimaru *et al*. (1990); Sauerwein *et al*. 1992) [11-13]. Additionally, safflower contains red (water-insoluble) and yellow (water-soluble) pigments, which are utilized in the production of herbal medicines, food colorants,

cosmetics, textiles, and natural dyes (Chavan *et al*., 2010) [14]. Many plant species, even those from diverse taxonomic groups, possess shared biosynthetic pathways for secondary metabolites. Various biotechnological approaches have been proposed and tested to enhance the production of these secondary metabolites in cultured cells, potentially achievable through modifications of standard media and precursor feeding (Whitmer *et al*., 1998) [15].

Plant tissue cultures have shown potential as a complementary approach to traditional agriculture for the industrial production of bioactive plant metabolites (Rao *et al*., 2002) [16]. Callus cells are capable of producing the same secondary metabolites as those found in whole plants, although the concentrations of these compounds often vary (Brown *et al*., 1986) [17]. The variations observed among different parts of the same plant may be due to differing levels of endogenous plant growth regulators in explants taken from various positions (Uranbey *et al*., 2005) [18]. using cotyledon, leaf explants and hypocotyls, Basal medium supplemented with different concentrations of 6 benzyladenine, naphthalene acetic acid and thidiazuron (Belide *et al*. 2011; Shilpa *et al*. 2010) [20].

The present investigation was undertaken to standardise harmone concentration for callus induction in safflower genotypes.

Materials and Methods

Plant material: The experimental material for the present study consists of safflower genotypes PBNS-86 (Purna) and GMU-6947, which were obtained from the All India Coordinated Research Project on Safflower, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (M.S) India.

Treatment details: MS basal medium, supplemented with various combinations of NAA, BAP and 2,4-D was used for the *in vitro* induction of safflower callus.

***Ex vitro* germination:** The seeds of safflower genotypes PBNS-86 and GMU-6947 were sown in pots and watered at regular intervals, ensuring optimal growing conditions.

Media preparation and growth conditions: The callus induction medium consisted of MS basal nutrients and vitamins, supplemented with 30 g/l sucrose and 0.8% agar. The pH was adjusted to 5.8 using 0.1N NaOH or HCl. The prepared explants were aseptically inoculated onto the callus induction medium, which contained MS medium supplemented with various concentrations of growth regulators.

All cultures were incubated in a culture room at 25±2 °C with a relative humidity of 55 ±5%, under a 16 hrs/8 hrs photo and dark period. The light intensity, provided by white fluorescent tubes positioned 50 cm above the bench surface were 3000 lux.

Explant Preparation and Inoculation: Leaves were excised from 14 to 16 day old safflower seedlings. The leaf explants were surface sterilized by sequential immersion in 0.1% Bavistin for 5 minutes, 0.1% Mercuric chloride for 45 seconds, and 70% Ethanol for 30 seconds. Between each treatment, the explants were rinsed with double distilled water for 1 minute to remove chemical residues and then dried on sterile blotting paper. The surface sterilized explants were segmented into 0.5-1 cm using sterile scalpel. In each PTC bottle three leaf segments under Laminar aseptic conditions transferred to the MS medium.

Observations were made at 6,12,18 and 24 days post-inoculation. The efficiency of callus induction was assessed based on the percentage of explants forming callus after 2–3 weeks of incubation.

Results and Discussion

The aim of the experiment was to develop callus induction protocol for obtaining callus from safflower genotypes. Callus induction in safflower genotypes PBNS-86 and GMU-6947 was optimized using leaf to investigate the effect of NAA, BAP and 2,4-D and their combination for callus induction in safflower genotypes.

Callus induction

The genotype exhibited the formation of compact calli, with coloration varying from light green and cream to light yellow. Ali and Afrasiab (2014) [21] in his findings reported that the callus produced was greenish, granular, and non-proliferative in appearance. The leaf explant of genotype PBNS-86 and GMU-6947 was treated with different combination of NAA and BAP. The Treatment T_6 (3.0 mg/l NAA+1.0 mg/l BAP) showed maximum response for callus induction, 76% in PBNS-86 and 70% in GMU-6947 (Plate 1 and 2), and Treatment T_3 (3.0 mg/l NAA+0.5 mg/l BAP) ranked second for maximum callus induction 66% in PBNS-86 and GMU-6947. The Treatment T_1 (1.0 mg/l NAA+0.5 mg/l BAP) showed minimum response for callus induction 36% in PBNS-86 and 33% in GMU-6947. For remaining Treatments T_2 (2.0 mg/l NAA+0.5 mg/l BAP), T_4 (1.0 mg/l NAA+1.0 mg/l BAP), and T_5 (2.0 mg/l NAA+1.0 mg/l BAP) callus induction was 40%, 43% and 50% in PBNS-86. Similarly for Treatments T_2 (2.0 mg/l NAA+0.5 mg/l BAP), T_4 (1.0 mg/l NAA+1.0 mg/l BAP), T_5 (2.0 mg/l NAA+1.0 mg/l BAP) in GMU-6947 the callus induction was 43%, 46% and 50% (Table 1 and 2).

Similarly, Ghasempour *et al*. (2014) [22] observed that among all explants, the leaf explant produces significant callus induction (97.79%) on MS + 1.0 mg/l BAP. Ying *et al*. (1992) reported an 80% callus induction rate from leaf-derived calli when cultured on a medium supplemented with 1.0 mg/l NAA and 1.0 mg/l BAP. Rani *et al*. (1996) [23] reported the best combinations for callus growth in the cotyledonary leaves of the M-105 line on MS medium supplemented with 5.0 mg/l NAA +0.25 mg/l BAP and 2.0 mg/l NAA+0.25 mg/l BAP, resulting in callus induction rates of 83.7% and 53.4%, respectively. According to Ali and Afrasiab (2014) [21] the highest callus induction percentages of 100%, 93%, and 96% were observed from leaf explants when treated with the combinations of 10.7µM NAA + 2.2µM BAP and 5.35µM NAA + 2.22µM BAP.

The leaf explant of genotype PBNS-86 and GMU-6947 was treated with different combination of 2,4-D. The Treatment T_3 (3.0 mg/l 2,4-D) and Treatment T_4 (4.0 mg/l 2,4-D) showed maximum response for callus induction, 73% in PBNS-86 and 66% in GMU-6947 (Plate 3 and 4). Treatment T_4 (4.0 mg/l 2,4-D) in PBNS-86 and Treatment T_3 (3.0 mg/l 2,4-D) ranked second for maximum callus induction 63% in PBNS-86 and 53% in GMU-6947. The Treatment T_2 (2.0 mg/l 2,4-D) and Treatment T_1 (1.0 mg/l 2,4-D) showed minimum response for callus induction 43% in PBNS-86 and 36% in GMU-6947. For remaining Treatments T_1 (1.0 mg/l 2,4-D) and T_5 (5.0 mg/l 2,4-D), callus induction was 46% and 53% respectively in PBNS-86. Similarly for Treatments T_2 (2.0 mg/l 2,4-D) and T_5 (5.0 mg/l 2,4-D) in GMU-6947 the callus induction was 40% and 46% (Table 1 and 2).

Similarly, Pandey and kumari (2010) [24] reported the highest callus induction using 2,4-D alone from different explant parts such as roots, leaves, and hypocotyls. Various concentrations of phytohormones (1.0 mg/l, 2.0 mg/l, 3.0 mg/l, 4.0 mg/l, and 5.0 mg/l) were tested, resulting in callus induction ratios of 75%, 50%, 90%, 90%, and 80%, respectively. Ghasempour *et al*. (2014) [22] reported callus induction from leaf segments of *Carthamus tinctorius* L. grown on MS medium supplemented with 1 mg/l and 1.5 mg/l of 2,4-D, this resulted in callus induction rates of 54.53% and 69.09%, respectively, after 25-30 days of culture. Pawar *et al*. (2019) [25] observed the highest callusing rate of 80% on MS media supplemented with 2,4-D at a concentration of 3.5 mg/l. Similarly, a concentration of 3.0 mg/l and 4.0 mg/l resulted in 60% callus induction. Kumari *et al*. (2015) [26] reported callus induction and organogenesis from leaf explants of *Carthamus tinctorius* L. using various concentrations of 2,4-D, including 0.5 mg/l, 1.5 mg/l, 2.5 mg/l, 3.5 mg/l, 4.5 mg/l, and 5.0 mg/l. The corresponding callus induction responses were 47%, 47%, 63%, 50%, 38%, and 31%, respectively.

Plate 1: Callus induction in PBNS-86 genotype using Treatment T_6 (3.0 mg/l NAA+1.0 mg/l BAP) in leaf explant.

Plate 2: Callus induction in GMU-6947 genotype using Treatment T_6 (3.0 mg/l NAA+1.0 mg/l BAP) in leaf explant.

Plate 3: Callus induction in PBNS-86 genotype using Treatment T_3 (3.0 mg/l 2,4-D) in leaf explant.

Plate 4: Callus induction in GMU-6947 genotype using Treatment T_4 (4.0 mg/l 2,4-D) in leaf explant.

Table 1: Effect of different treatment of growth regulator on callus induction of safflower genotype PBNS-86

NAA+BAP	Treatments	T_1			T_2			T_3			T_4			T_5			T_6			T_7		
	Replication	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
PBNS-86	Leaf Inoculated	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Callus Induction	4	3	4	5	3	4	6	7	7	4	4	5	6	4	5	7	8	8	6	6	5
		36%			40%			66%			43%			50%			76%			56%		
2,4-D	Treatments	T_1			T_2			T_3			T_4			T_5								
PBNS-86	Replication	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3						
	Leaf Inoculated	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10						
	Callus induction	5	5	4	5	4	4	8	7	7	7	6	6	6	4	6						
		46%			43%			73%			63%			53%								

Table 2: Effect of different treatment of growth regulator on callus induction of safflower genotype GMU-6947

NAA+BAP	Treatments	T_1			T_2			T_3			T_4			T_5			T_6			T_7		
	Replication	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
GMU-6947	Leaf inoculated	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Callus induction	4	3	3	5	4	4	7	6	7	3	5	6	4	6	5	7	8	6	4	3	5
		33%			43%			66%			46%			50%			70%			40%		
2,4-D	Treatment	T_1			T_2			T_3			T_4			T_5								
	Replication	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3						
GMU-6947	Leaf inoculated	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10						
	Callus induction	3	4	4	5	4	3	5	5	6	6	7	7	5	5	4						
		36%			40%			53%			66%			46%								

Conclusion

In conclusion, the optimization of callus induction protocols for safflower genotypes PBNS-86 and GMU-6947 demonstrated that specific combinations of NAA, BAP, and 2,4-D significantly enhanced callus formation. The highest induction rates were observed with 3.0 mg/l NAA and 1.0 mg/l BAP (T6) and 3.0 mg/l 2,4-D. These findings align with previous studies, confirming that leaf explants are effective for callus induction in safflower. The successful establishment of these protocols can facilitate further research in plant tissue culture and genetic improvement of safflower.

References

1. Akashe VB, Gud MA, Shinde SK, Kadam JR. Biodiversity of insect pests of safflower and their natural enemies in Maharashtra. Bioinfolet-A Quarterly Journal of Life Sciences. 2013;10(4b):1389-1392.
2. Pascual-Villalobos MJ, Alburquerque N. Genetic variation of a safflower germplasm collection grown as a winter crop in southern Spain. Euphytica. 1995;92:327-332.
3. Teotia DS, Kumar A, Kumar V, Singh S. Agroecological characteristics and ethanobotanical significance of safflower (*Carthamus tinctorius* L.): An overview. Science. 2002;2(3):228-231.
4. Anonymous. Agricultural Statistical Information of Maharashtra, Part-II; c2022. p. 228.
5. Knowles PF. Safflower. Advances in Agronomy. 1958;10:289-323.
6. Nakano K, Sekino Y, Yomo N, Wakayama S, Miyano S, Kusaka K, *et al*. High-performance liquid chromatography of carthamin, safflor yellow A and a precursor of carthamin: Application to the investigation of an unknown red pigment produced in cultured cells of safflower. Journal of Chromatography A. 1988;438:61-72.
7. Gao WY, Fan L, Paek KY. Yellow and red pigment production by cell cultures of *Carthamus tinctorius* in a bioreactor. Plant Cell, Tissue and Organ Culture. 2000;60:95-100.
8. Obara H, Onodera JI. Structure of carthamin. Chemistry Letters. 1979;8(2):201-204.
9. Bie XD. Clinical observation on treatment of traumatic intracranial hematoma by Flos Carthami combined with Radix Acanthopanacis Senticosi injection. Chinese Journal of Integrative Traditional and Western Medicine. 2003;23:296-297.
10. Jiang TF, Lv ZH, Wang YH. Separation and determination of chalcones from *Carthamus tinctorius* L. and its medicinal preparation by capillary zone electrophoresis. Journal of Separation Science. 2005;28(11):1244-1247.
11. Fujita Y, Hara Y, Suga C, Morimoto T. Plant Cell Rep. 1981;1:61-3.
12. Ishimaru K, Satake M, Shimomura K. Plant Tissue Cult Lett. 1990;7:159-163.
13. Sauerwein M, Yoshimatsu K, Shimomura K. Further approaches in the production of secondary metabolites by plant tissue cultures. Plant Tissue Culture Letters. 1992;9(1):1-9.
14. Chavan SP, Lokhande VH, Nitnaware KM, Nikam TD. Influence of growth regulators and elicitors on cell growth and α-tocopherol and pigment productions in cell cultures. 2010;89:1701-1707.
15. Whitmer S, Verpoorte R, Canel C. Influence of auxins on alkaloid accumulation by a transgenic cell line of *Catharanthus roseus*. Plant Cell, Tissue and Organ Culture. 1998;53:135-141.
16. Rao SR, Ravishankar GA. Plant cell cultures: chemical factories of secondary metabolites. Biotechnology Advances. 2002;20(2):101-153.
17. Brown JT, Charlwood BV. The control of callus formation and differentiation in scented pelargoniums. Journal of Plant Physiology. 1986;123(5):409-417.
18. Uranbey S, Sevimay CS, Özcan S. Development of high frequency multiple shoot formation in Persian clover (*Trifolium resupinatum* L.). Plant Cell, Tissue and Organ Culture. 2005;80:229-232.
19. Belide S, Hac L, Singh SP, Green AG, Wood CC. *Agrobacterium*-mediated transformation of safflower

and the efficient recovery of transgenic plants via grafting. Plant Methods. 2011;7:1-13.
20. Shilpa K, Dinesh Kumar V, Sujatha M. *Agrobacterium*-mediated genetic transformation of safflower (*Carthamus tinctorius* L.). Plant Cell, Tissue and Organ Culture (PCTOC). 2010;103:387-401.
21. Ali N, Afrasiab H. Effect of TIBA and other plant growth regulators on callogenic response from different explants of safflower (*Carthamus tinctorius* L.). International Journal of Agriculture and Biology. 2014;16(6):1112-1126.
22. Ghasempour HR, Soheilikhah Z, Zebarjadi AR, Ghasempour S, Karimi N. *In vitro* micro propagation, callus induction and shoot regeneration in safflower L. cv. Lesaf. Iranian Journal of Plant Physiology. 2014;4(2):999-1004.
23. Rani KJ, Rao TN, Raghunatham G, Rao PV. Studies on callus growth and differentiation in safflower. Indian Journal of Genetics and Plant Breeding. 1996;56(4):458-461.
24. Pandey R, Kumari S. *In vitro* germination and callus induction from seeds of *Carthamus tinctorius* L. The Bioscan. 2010;5(2):247-250.
25. Pawar NS, Rathod BU, Majhi PK, Gotamwad VE. *In vitro* callus induction of safflower (*Carthimus tinctorius* L.). International Journal of Molecular Biology and Biochemistry. 2019;1:11-12.
26. Kumari S, Pandey RK, Uttam K. *In vitro* callus induction from two different explants stem and leaf in *Carthamus tinctorius* Linn. European Journal of Experimental Biology. 2015;5(2):1-4.