

**IN - VITRO CALLUS INDUCTION IN LEAF EXPLANTS OF
TAGETES ERECTA, L****NIKAM S.L* . AND KHAN S. J.***Department of Botany, The Institute of Science, Mumbai,-400032, India.***ABSTRACT**

Tagetes erecta is an industrially and commercially important ornamental plant belonging to family Asteraceae. Developing tissue culture technique for *Tagetes erecta* will permit the application of biotechnology to its culture and potential for exploitation of phytochemicals. The present work is based on developing protocols for the callus induction in *Tagetes erecta* from leaf explants. The sterilized explants were inoculated in Murashige and Skoog (MS) media containing various combinations of auxins such as Naphthalene acetic acid (NAA), Indole 3-acetic acid (IAA), Indole 3-butyric acid (IBA), 2,4 Dichlorophenoxyacetic acid (2,4 D) and cytokinins such as Kinetin (KIN) and Benzylaminopurines (BAP). The highest rate of callus induction for *Tagetes* leaf was achieved on media supplemented with 2, 4 D (2 mg/L) and BAP (5mg/L) with compact, fast growing, pale yellow coloured callus. Leaf explants were grown with IAA, IBA, NAA and KIN at different concentrations to evaluate tissue responses such as production of proembryo and roots in the explant.

KEY WORDS: *Tagetes erecta*, callus induction, auxins, cytokinin.**NIKAM S.L.**Department of Botany, The Institute of Science, Mumbai. 15,
Madam Cama road, Mumbai.-40032, India.

*Corresponding author

INTRODUCTION

Tagetes erecta, L is commonly known as marigold belongs to family Asteraceae. It is an ancient plant that has industrial and medicinal importance. The plant is grown as ornamental plant with great public demand and is adapted to several agro-climates. Though originated in North and South America, it is also cultivated in Asian countries like India, Bhutan, Nepal and China.¹ It has long history of usage by the folk system because of its rich medicinal value that have been reported to possess potent antiseptic, stimulant, antifungal, antiinflammatory, antibacterial, antigenotoxic, antiviral and antiulcer properties.² Moreover the plant has number of phytochemicals such as thiophene, flavonoid, carotenoid and triterpenoids. The plant has been shown to contain quercetagenin, a glucosides of quercetagenin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, thienyl and ethyl gallat.³ Most therapeutics use raw material from this species to produce ointments, tincture and fluid extract.⁴ It is also used as poison for non vertebrate and in plant pest control.^{5,6} Due to low seed viability and poor germination it is difficult to fulfill its increasing demand, hence tissue culture was selected as an alternative for large scale commercial propagation of this plant.⁷ Tissue culture is advantageous in producing multiple copies of a plant species within minimum time and space and callus culture is the primary mean for the indirect organogenesis. Most of the genetic engineering technique requires good quality callus production with optimum quantity for transformation and regeneration. Callus or cell suspension culture also could be used for large scale plant cell culture where the bioactive compounds could be extracted. Various *in-vitro* propagation^{9,10,11} studies have been carried out on *Tagetes erecta*, but research is still limited on this valuable medicinal plant. The objective of this work was to evaluate various combinations of different auxins and cytokinins on induction and growth of callus from leaf of *Tagetes erecta* and to enhance the quality and quantity of callus, the basic requisite for all further advance studies.

MATERIALS AND METHODS

Authentic seeds of *Tagetes erecta*, L were purchased from seed market Mumbai were grown in the campus of Institute of Science, Mumbai. From these plant the young leaves were taken and washed thoroughly with running tap water for 30 minutes and treated with liquid detergent Teepol followed by washings with autoclaved distilled water thrice. Further sterilization was done under aseptic conditions in a laminar air flow cabinet. The explants from leaf were surface sterilized with Bavistin (1% w/v) for 10 minutes and with mercuric chloride (0.1% w/v) for 5 minutes followed by washings with sterile distilled water for several times to remove the traces of HgCl₂. The explants were inoculated on MS¹² medium fortified with 30% sucrose and supplemented with various combinations and concentrations of auxins such as IAA (0.5-4.0 mg/L) or IBA(0.5- 4.0 mg/L) or 2, 4-D (0.5-4.0 mg/L) or NAA (0.5-4.0 mg/L) and cytokinin such as kinetin (5.0mg/L), or BAP (5.0mg/L), pH of the media was adjusted at 5.8 by using 0.1N NaOH and 0.1N HCl before gelling the medium with 8.0% agar- agar (Himedia). The cultures were incubated at 25 ± 1°C and photoperiod of 16 h at 3000 lux light intensity of cool white fluorescent light.

RESULTS AND DISCUSSION

Callus was initiated from leaf explants of *T. erecta* on MS basal medium with different concentrations of growth hormone IAA, IBA, 2, 4 D, NAA and cytokinins such as KIN and BAP (Table-1). Explants cultured on different hormonal concentrations showed marked difference in their callus induction days to callus size and type (Fig. 1). The observations of leaf were taken after forty five days. Callus was initiated on 7th day from the inoculation of the leaf explants and it was in the range of 7-15 days in most of the hormonal combinations. The callus induction was also found to be hormone dependent. Leaf explants on all the concentration supported better growth. The callus morphology and growth rate varied in different concentrations of hormones. The calli induced from leaf

segment were compact, differentiated in structure, brown or yellow coloured and fast growing in all combinations (Fig. 1). In *Tagetes* leaves it was found that 2, 4 D had a significant effect on callus formation. The maximum callus induction was obtained at concentration 2.0 mg/L 2, 4 D and 5.0 mg/L BAP with compact, pale yellow coloured profuse callus with 100% callus induction frequency (Fig1.2). In the same way concentration of 0.5 mg/L 2, 4 D and 5.0 mg/L BAP showed similar result but formed compact, granular, pale yellow coloured callus which turned brown after three weeks (fig.1.3). At higher concentration i. e. 4.0 mg/L 2, 4 D and 5.0 mg/L BAP the explant produced compact, yellow coloured very slow growing callus with 50 % callus induction frequency. NAA and KIN played very significant role for the induction of proembryo. 2.0 mg/L NAA in combination with 5.0 mg /L KIN produced compact, brown coloured, root differentiated callus with many proembryo (Fig.1.4). Similar results were obtained for 4.0 mg/L NAA and .5.0 mg/L KIN, but at lower concentration i.e. 0.5 mg/L NAA and 5.0 mg/L KIN induced callus was compact, undifferentiated, pale yellow coloured with 100 % average callus induction frequency. It was found that the IAA with KIN influenced the growth and differentiation of proembryo mainly from cut ends of explants and very small quantity of callus produced as compared to other concentrations of growth hormone. Lower level of IAA i.e. 0.5 mg/L in combination with KIN 5.0 mg/L favoured better growth (Fig.

2.5). However callus culture treated with different level of IBA in combination with 5.0 mg/L KIN showed pattern similar to IAA and KIN, with respect to colour and texture (fig. 2.6). The ratio of Cytokinins to Auxins used for callus induction in the present study is higher to those reported in the literature. We intend to use this callus for production of Pyrethrins from cell suspension culture and therefore the amount of Cytokinins was increased. It should be noted that even at this concentration of Cytokinins the callus formation is seen. Tissue culture techniques have been applied to a wide range of ornamental species (about 156 ornamental genera) such as *Begonia*, *Ficus*, *Anthurium*, *Codiaeum*, *Chrysanthemum*, *Rosa*, *Saintpaulia*, *Gerbera* and *Spathiphyllum*¹³. Plant hormones are common among the most important physiological factors affecting the callus growth of plants *in vitro*. Several studies have been reported regarding the effects of plant growth regulators on callus growth of different plants. The major differences in the response of different plant parts lie in the ratio of auxins to cytokinins.¹⁴ Effective callus mass production can be beneficial for high yield of secondary metabolite. *In vitro* generation of callus can encourage *in vitro* mass production of bioactive compounds of health benefits from *T. erecta* plant and consequently will promote the application of plant tissue culture technology in the area of selection, resistance, production of artificial seeds and genetic transformation.

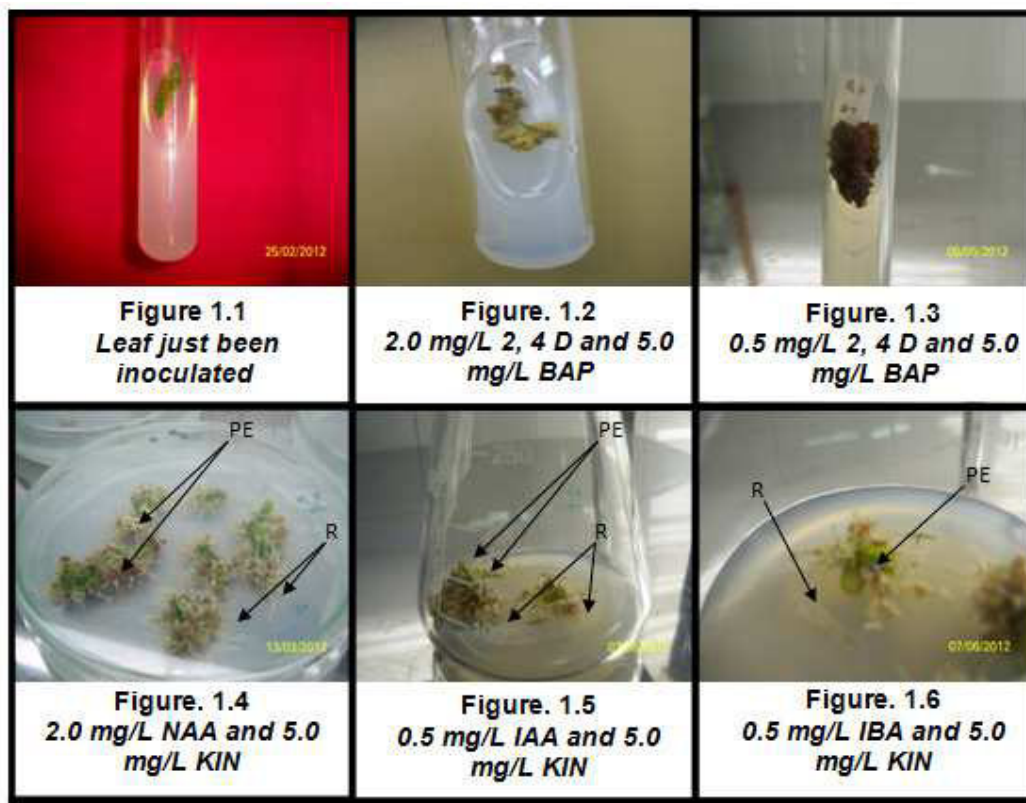
Table 1
Effect of Phytohormones on growth and morphology of calli
Induced from leaf of *Tagetes erecta*, L

Tagetes erecta leaf explant											
Media	Hormonal Combination mg/l		Callus Induction day	Growth of callus / Days					Colour	Texture	Differentiation
				10	20	30	40	50			
MS											
	IAA	KIN									
	0.5	5.0	15		+	+	+	+	brown	compact	proembryo, root
	2.0	5.0	15		+	+	+	+	brown	compact	proembryo, root
	4.0	5.0	15		+	+	+	+	brown	compact	Proembryo, root
	IBA	KIN									
	0.5	5.0	15		++	+	+	+	brown	compact	proembryo, root
	2.0	5.0	15		++	+	+	+	brown	compact	proembryo, root
	4.0	5.0	15		++	+	+	+	brown	compact	proembryo, root
	NAA	KIN									
	0.5	5.0	10		+	+	++	+++	pale yellow	compact	-
	2.0	5.0	10		+	+	++	+++	brown	compact	proembryo, root
	4.0	5.0	10		+	+	++	+++	brown	compact	proembryo, root
	2,4 D	KIN									
	0.5	5.0	15		+	++	+++	++	pale yellow	compact	-
	2.0	5.0	15		+	++	+++	++	pale yellow	compact	-
	4.0	5.0	15		+	++	++	++	pale yellow	compact	-
	2,4 D	BAP									
	0.5	5.0	10		+	+	++	++	pale yellow	compact	-
	2.0	5.0	10		+	+	++	+++	pale yellow	compact	-
	4.0	5.0	10		+	+	+	++	pale yellow	compact	-
	IAA	BAP									
	0.5	5.0	15		+	++	+++	+++	pale yellow	compact	-
	2.0	5.0	15		+	++	+++	+++	pale yellow	compact	-
	4.0	5.0	15		+	++	+++	+++	pale yellow	compact	-
	IBA	BAP									
	0.5	5.0	15		+	++	+++	+++	pale yellow	compact	-
	2.0	5.0	15		+	++	+++	+++	pale yellow	compact	-
	4.0	5.0	15		+	++	+++	+++	pale yellow	compact	-

+ low callus produced, ++ medium callus produced, +++ high callus produced, ++++ profuse callus produced. IAA: Indole 3-acetic acid, IBA: Indole 3-butyric acid, NAA : Naphthalene acetic acid, 2, 4-D: 2, 4 Dichlorophenoxy acetic acid, KIN : Kinetin, BAP : benzylaminopurines,

Figure

Callus induction from leaf explants of *Tagetes erecta* and its growth on MS media supplemented with various hormonal combinations.* R- Roots, PE –Pro Embryo



CONCLUSION

The results obtained in the experiment indicated that the plant growth regulators are important for callus induction in *Tagetes erecta*. The concentration of 2.0 mg/L 2, 4 D and 5.0 mg/L BAP is good for maximum callus induction and the concentration of 2.0mg/L NAA and 5.0 mg/L KIN is the optimum concentration for effective proembryo development. Therefore the results reported in the study would be an important step towards the development of good quality and quantity of callus to provide successful platform for regeneration, cell suspension culture and genetic transformation.

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