Indian J. Hort. 71(1), March 2014: 82-86

In vitro morphogenesis in marigold using shoot tip as explant

Jayoti Majumder*, Kanwar P. Singh, S.K. Singh, K.V. Prasad and Manjusha Verma*****
Division of Floricultural and Landscaping, Indian Agricultural Research Institute, New Delhi 110 012

ABSTRACT

An effort was made to develop the protocol of *in vitro* mass multiplication in marigold cultivar Pusa Narangi Gainda from the sterilized selfed seeds on Murashige and Skoog medium supplemented with different combinations of BAP, NAA and GA_3. High frequency of germination (84.67%) was obtained 1.0 mg/l BAP and 0.5 mg/l GA_3. The growth of individual shoot tip were significantly enhanced (4.1 cm) when the excised shoot tips were transferred on to MS medium with 2.0 mg/l BAP, 0.1 mg/l NAA and 0.5 mg/l GA_3. Low auxin (0.2 mg/l NAA) in the culture medium in the contrary to high cytokinin (3.0 mg/l BAP) concentration referred the highest shoot multiplication (10.15 shoots/ explant). Half-strength MS medium supplemented with 1.0 mg/l NAA and 1.0 mg/l IBA was observed to be efficient for rooting (87.33%). The rooted plants could be ideally acclimatized in glasshouse conditions for field transfer.

Key words: *In vitro* regeneration, *Tagetes erecta* L., micropropagation.

INTRODUCTION

Amongst commercial loose flowers, marigold (*Tagetes erecta* L.) of the family Asteraceae, native of Mexico, is endowed with large spectrum of commercial potentialities in medicinal and industrial sector (Jothi, 4). Marigold is a sexually propagated crop, though asexual propagation is also successful using herbaceous shoot-tip cuttings. Conventionally, marigold is propagated by seed which is produced after controlled pollination. The potential of tissue culture technique to yield large numbers of true-to-the-type plantlets in a relatively short period of time motivated practice of *in vitro* plant regeneration among all plant species, *e.g.*, petunia (Clapa and Cantor, 2); zinnia (Twumasi *et al*., 11) including marigold (Kumar, 6). *In vitro* propagation was also tried in *Tagetes* sp. (Kothari and Chandra, 5). Earlier, marigold plants have been *in vitro* regenerated from different explant sources, including immature un-pollinated disc florets (Kothari and Chandra, 5), leaf callus and suspension culture (Kothari and Chandra, 5) and proliferated adult plants (Misra and Dutta, 8). It is noteworthy to point out that to our knowledge there are no reports on regeneration through direct organogenesis from selfed seedings in *T. erecta* L.

Development of suitable *in vitro* regeneration protocol though have limited success, it is one of the key pre-requisites for marigold genetic improvement using biotechnological tools. Besides, micropropagation provides the opportunity to improve the availability, quality of planting material and can be an approach for safe, long-term maintenance of valuable germplasm, developed through breeding programmes. In the present study, we report a consistence and reproducible protocol for the regeneration of marigold plantlets from selfed seed derived shoot tip without any loss of morphogenic potential.

MATERIALS AND METHODS

The marigold (*Tagates erecta* L.) var. Pusa Narangi Gainda was selected as it is commercially cultivated for lutein extraction. Today the use of marigold is intensified for its high-value carotenoid pigments, *i.e*. lutein. Bioactive extracts of different *Tagetes* tissues also exhibit nematicidal, fungicidal and insecticidal activity (Lu and Li, 6). Selfed seeds were germinated under field and the shoot tips were cut and cultured in aseptic condition on Murashige and Skoog (MS) medium. Briefly they were surface sterilized with carbendazim (0.1 and 0.2%), 8-hydroxy quinnoline citrate (200 mg/l), and Indofil® (0.1%) on a horizontal shaker (120 rpm) for 45 min. and then treated with (0.1%) mercuric chloride solution for 1-3 min. The sterilized seeds shot-tip were washed with sterile distilled water thrice and single shoot tip was inoculated in each test tube (150 mm × 25 mm) with 7-8 ml of modified Murashige and Skoog (10) (MS) medium, supplemented with 30% sucrose and various combinations of BAP (1.0-2.0 mg/l), NAA (0.1-0.2 mg/l) and GA_3 (0.3-0.5 mg/l). Cultures were incubated in growth chamber (25° ± 1°C) under cool white fluorescent lamps providing light intensity of

*Corresponding author's present address: Directorate of Floricultural Research, IARI, Pusa Campus, New Delhi 110 012; E-mail: jayotisarkar1@gmail.com
**Division of Fruits and Horticultural Technology, IARI, New Delhi
***NRC on DNA Fingerprinting, National Bureau of Plant Genetic Resources, New Delhi

47 µmol $m^{-2}s^{-1}$. The photoperiod was adjusted to 16/8 h light and dark cycle. There were 15 test tubes per treatment.

For shoot growth the germinated seedlings were transferred onto fresh medium supplemented with BAP (2.0-4.0 mg/l) and NAA (0.1-0.2 mg/l). After two weeks, micro-shoots (with length ≥ 2.5 cm) were excised from the germinated seedling and transferred to glass-jars (10.5 cm × 5 cm) having 40 ml of half-strength MS medium supplemented along with IBA (0.5 - 1.0 mg/l) and NAA (0.5 - 1.0 mg/l) and solidified with 0.7% agar-agar to enhance plant growth and rooting. All media were supplemented with 30% sucrose and 8.0 g/l agar (Qualigen Chem., Mumbai). The pH of all media was adjusted to 5.8 with 1 N KOH or 1 N HCl prior to autoclaving at 1.05 kg/cm^2, 121°C for 20 min. Cultures were maintained at 25 ± 1°C air temperature in a culture room with a 16-h photoperiod under an illumination of 20 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density provided by cool-white fluorescent light. For hardening rooted plants were transferred after 3-4 weeks to glass jars, with polypropylene caps (Kasablanka, Mumbai) filled with sterile peat, vermiculite and perlite (2:1:1). After 15 days, the caps were loosened and then gradually removed in a week. Thereafter, that the plants were transferred to plastic pots filled with potting mixture. The pots were kept in glasshouse at room temperature (25 to 30°C) under natural illumination at photon flux density of 330 to 350 µmol $m^{-2}s^{-1}$. Three weeks later the plantlets were transferred to shaded place with natural light (572 µmol $m^{-2}s^{-1}$) and then transferred to normal field conditions. The experiments were laid out in completely randomized complete block design (CRBD) with five replications. Treatment means were separated using Fisher's Critical Difference (α = 0.05). The percentage data were subjected to Arc Sin √% transformation before ANOVA.

RESULTS AND DISCUSSION

The shoot tip from the field germinated seed began to grow in terms of culture establishment. Adventitious shoot initiation was evidenced after 12-14 days from the shoot tip in (Fig. 1). There was no difference in days to culture establishment in different pre-treatments. However, there was significant difference in mortality of shoot tip according to the treatment. Cultures were established in high frequency (86.10%) with pre-treatment of carbendazim (0.1%) + Indofil® (0.1%) + 8-HQC (200 mg/l). These fungicides effectively controlled the fungal contamination (Ravindra, 12) and antimicrobial compounds, *viz*., 8-HQC (Prasad, 11) was found to be successful in establishing culture. The surface sterilant $HgCl_2$ produced higher culture establishment (71.3%) and lesser microbial

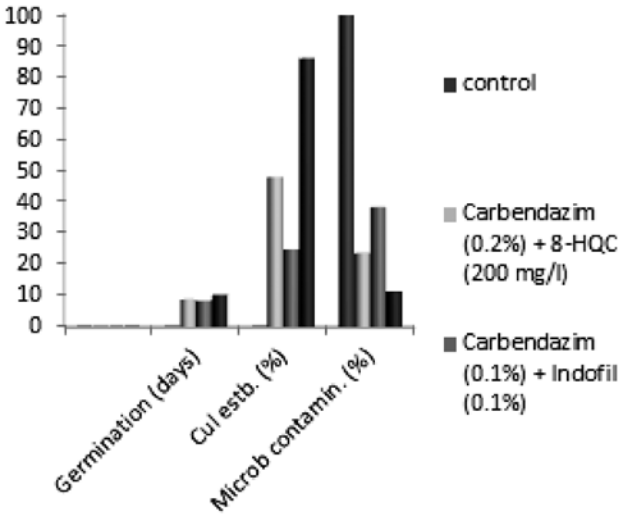

Fig. 1. Effect of different pre-treatments on *in-vitro* seed germination.

contamination (21.3%) in 2 min. duration over 3 and 4 min. (Fig. 2). The maximum culture (84.67%) could survive on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l GA_3 (Table 1). When these shootlets were sub-cultured onto MS medium with respective growth regulator combination for next four weeks, the longer shoots with fully expanded leaves was obtained (Fig. 3).

For culture establishment from shoot tip, minerals of the culture medium are important components which give a wide scope of choice in combinations of micro- and macro-salt mixtures. Out of different growth hormone combinations tested, 1.0 mg/l of BAP + 0.5 mg/l of GA_3 was found to be most effective. The treatment with GA_3 cause dormancy breaking and shoot morphogenesis (Bespalhok and Hattori, 1). The elongation of the stem is not due to increased formation of nodes and internodes but rapid elongation of internodes, which was because of cell division and cell elongation. The application of GA_3 probably had increased the activity

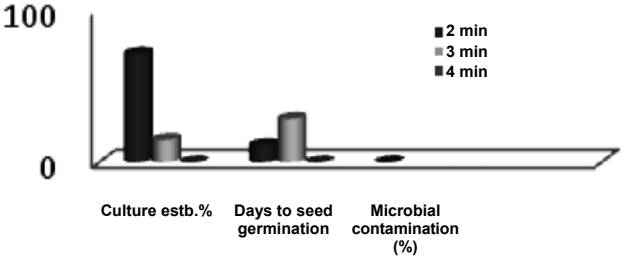

Fig. 2. Effect of different pre-treatment durations on *in vitro* seed germination.

Table 1. Effect of different growth regulators on MS medium for culture establishment and shoot proliferation.

Growth regulators (mg/l)			Culture establishment (%)	Shoots/ explant	Mean shoot length (cm)	Visual observation
BAP	NAA	GA_3				
1.0	-	0.5	67.3 (55.12)	1.03	1.79	Microshoot
2.0	-	0.5	65.33 (53.91)	1.75	1.82	Microshoot
1.0	0.1	0.3	71.46 (57.67)	1.05	2.0	Microshoot, Callus
2.0	0.1	0.5	84.67 (84.67)	2.67	4.1	Microshoot, Callus
1.0	0.2	0.3	67.78 (55.37)	1.33	3.67	Microshoot, Callus
2.0	0.2	0.5	83.67 (66.11)	1.10	3.9	Callus
2.0	0.1	-	-	5.03	3.98	Microshoot, Root
3.0	0.1	-	-	6.33	4.83	Microshoot, Root
4.0	0.1	-	-	5.67	4.09	Microshoot, Root
2.0	0.2	-	-	7.67	5.93	Microshoot, root
3.0	0.2	-	-	10.15	9.97	Microshoot, Root
4.0	0.2	-	-	1.09	7.52	Microshoot, Root, Callus
CD at 5%			2.39	1.25	0.42	-

*Arc Sin √% transformed data

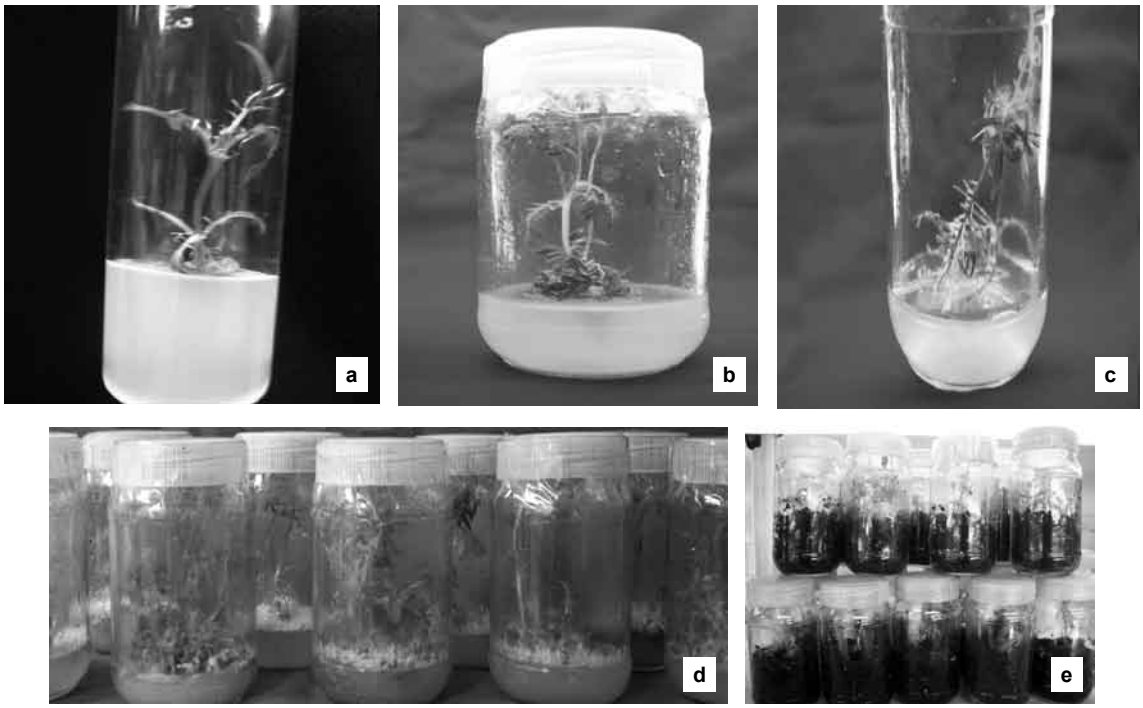

Fig. 3. Stages of micropropagation in marigold var. Pusa Narangi Gainda (a) Culture establishment from shoot tip, (b) Proliferation of micro-shoots, (c) rooting of micro-shoot, (d) Mass multiplication, and (e) hardening stage.

of endogenous auxin and auxin-derivatives (Prasad, 11). Benzyladenine being synthetic in nature it is more stable at even lower concentration (Kothari and Chandra, 5). Misra and Dutta (8) also stated that BAP in combination with GA_3 was better for culture establishment in *Tagetes minuta*. After *in vitro* culture establishment, majority of tissue survived on food material from the explant tissue itself. The application of GA_3 probably had increased the activity of endogenous auxin (Prasad, 11).

There was a considerable increase in shoot number on lower NAA (0.1 or 0.2 mg/l) and higher BAP (2.0, 3.0 or 4.0 mg/l) levels. Higher levels of NAA along with higher BAP at all concentrations were less effective in shoot regeneration. MS medium supplemented with 3.0 mg/l BAP and 0.2 mg/l NAA resulted in maximum number of micro-shoots (10.15 days) in short time (Table 1) as compared to other treatments (Fig. 3). It also gave highest average shoot length measuring 9.97 cm (Table 1). The shoot growth was accompanied with callus formation and rooting but with slight delay in proliferation in higher BA level (4.0 mg/l). Clumps of shootlets from leaf segments were sub-cultured on to MS medium devoid of growth regulators for next 4 weeks and the full grown plantlets were obtained (Fig. 3). Herbaceous species usually show good shoot proliferation with high cytokinin and low auxin is low in medium through their optimal concentration varies considerably with the genotype. Cytokinins when applied with auxins do show an improved shoot prolificacy but cytokinin at high concentration or with a low amount of auxin may give rise to glassiness and other deformities (Ziv *et al*., 14). Earlier, Mujib *et al*. (9) found that in carnation, the production of adventitious shoots is triggered with auxin and BAP. In *Tagetes* too, BAP and NAA was shown to be effective for shoot bud formation (Kothari and Chandra, 5). The rooted plantlets could survival at most 68.10% after their transfer to glass jars with polypropylene caps filled with peat vermiculite and Soilrite® (2:1:1) (Fig. 3) into glasshouse conditions in 40.03 days.

Root formation occurred on almost every combination of NAA and IBA tried (Table 2). Maximum number of roots per plantlet (20.67) was produced with 0.5 mg/l NAA and 0.5 mg/l IBA at the basal end of shoots, which were vigorous (87.33%) and sufficient for acclimatization (Fig. 3). Induction of roots was earlier (13.93 days) with higher rooting (87.33) and micro-shoot exhibited higher average root length (3.68 cm) in treatment combination of NAA (0.5 mg/l) and IBA (0.5 mg/l) (Table 2). It was observed that auxin supplementation markedly improved the rooting as well as root quality. Supplementation of auxin (s) is essential in different plant species to have an early rooting (Hemper, 3). NAA concentration affects the number, length and root morphology of *in vitro* regenerated herbaceous plants (Ziv *et al*., 14).

REFERENCES

1. Bespalhok, F.J.C. and Hattori, K. 1998. Friable embryogenic callus and somatic embryo formation from cotyledon explants of African marigold (*Tagetes erecta* L.). *Plant Cell Rep*. **17**: 870-75.

2. Clapa, D. and Cantor, M. 2006. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*, **63**: 23-35.

3. Hemper, M., Petos-Witkowska, B. and Tymoszuk, J. 1985. The influence of cytokinins on

Table 2. Effect of different growth regulators supplemented in half-strength MS medium on rooting of micro-shoots.

Treatment (mg/l)		Days for root initiation	Rooting (%)	Av. root length (cm)	Av. No. of roots/ shoot
IBA	NAA				
0.0	0.0	20.33	54.09 (47.81)	1.33	1.33
1.0	0.0	18.67	72.33 (58.24)	3.33	15.67
0.0	1.0	14.4	80.33 (63.35)	2.64	19.67
0.5	0.5	13.15	87.33 (69.12)	3.68	20.67
1.0	1.0	18.7	62.07 (51.94)	3.10	8.09
CD at 5%		0.95	5.38	0.46	2.45

*Arc Sin √% transformed data

multiplication and subsequent rooting of gerbera *in vitro*. *Acta Hort*. **167**: 301-5.

4. Jothi, D. 2008. Extraction of natural dyes from African marigold flower (*Tagetes erecta* L.) for textile coloration. *Autex Res. J.* **8**: 49-53.

5. Kothari, S.L. and Chandra, N. 1986. Plant regeneration in callus and suspension cultures of *Tagetes erecta* L. (African marigold). *J. Pl. Physiol*. **122**: 235-41.

6. Kumar, A., Raghava, S.P.S., Singh, S.K. and Misra, R.L. 2007. Micropropagation of male sterile line in marigold (*T. erecta* L.). *J. Hort. Sci. Biotech*. **85**: 75-83.

7. Lu, S. and Li, L. 2008. Carotenoid metabolism: Biosynthesis, regulation, and beyond. *J. Integrat. Pl. Biol.* **50**: 778-85.

8. Misra, P. and Datta, S.K. 1999. *In vitro* propagation of white marigold (*Tagetes erecta* L.) through shoot tip culture. *Curr. Sci.* **77**: 1141-44.

9. Mujib, A., Pal, A.K. and Jana, B.K. 1993. Effects of growth regulators on plantlet regeneration of carnation from shoot tip and node cuttings *in vitro*. *Maharashtra J. Hort*. **7**: 96-98.

10. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. **15**: 473-97.

11. Prasad, K.V. 1995. Studies on *in vitro* regeneration of roses. Ph.D. thesis submitted to P.G. School, Indian Agricultural Research Institute, New Delhi.

12. Ravindra, K. 2005. Symbiotic effect of arbuscular mycorrhizal fungi on survival, growth and flowering of *in vitro* raised plantlets of chrysanthemum (*Dendrathema grandiflora* Tzelev.). M.Sc. thesis submitted to P.G. School, Indian Agricultural Research Institute, New Delhi.

13. Twumasi, P., Schel Jan, H.N., Ieperen, W. van, Woltering, E., Kooten, Olaf, V. and Emons Anne, M.C. 2009. Establishing *in vitro Zinnia elegans* cell suspension culture with high treachery element differentiation. *Cell Biol. Int*. **33**: 524-33.

14. Ziv, M., Meir, G. and Halevy, A.H. 1983. Factors influencing the production of hardened glaucous carnation plants *in vitro*. *Plant Cell Tissue Organ Cult*. **2**: 55-65.

Received: June, 2013; Revised: December, 2013;
Accepted: January, 2014