

## *In vitro* maintenance of F<sub>1</sub> hybrid

The floriculture industry always looks for novelty in marketing of ornamental crops, but any mutant or hybrid developed requires maintenance, a fast rate of propagation and saving time as well as money for its commercialization. Creativity in ornamentals is required to meet the increasing demand of the market by using various methods of plant breeding<sup>1-3</sup>. Hybrid varieties have been developed and commercially exploited in most of the cross-pollinated crops. Wherever it is commercially feasible, F<sub>1</sub> hybrids may be used directly as a variety. Cytoplasmic/genetic male sterility lines have been developed and used for synthesizing F<sub>1</sub> sterile hybrids for commercial exploitation in *Tagetes*. Few seed companies have their monopoly in producing F<sub>1</sub> hybrids of ornamental and horticultural crops. Breeders will have to maintain the parents and crossing has to be performed every year for raising F<sub>1</sub>. Similarly users will have to purchase fresh seeds every year from breeders as there is no seed setting in F<sub>1</sub> hybrids. The present paper reports how F<sub>1</sub> sterile hybrids of *Tagetes* can be maintained *in vitro*. In normal African marigold (*Tagetes erecta* L., Asteraceae) there are few reports on somatic embryogenesis from cotyledon<sup>4</sup> and on organogenesis from leaves<sup>5-7</sup>, florets<sup>8</sup> and hypocotyls<sup>9</sup>. Recently, *in vitro* clonal propagation of *Paulownia* for F<sub>1</sub> hybrids has been reported<sup>10</sup>.

Seeds of Double African F<sub>1</sub> hybrid of FM569 Inca Yellow marigold were purchased from Sutton & Sons (India) Pvt Ltd, Calcutta. Seeds were divided into three equal parts and sown as follows:

The first group of seeds were sown in the field for studying their performance in natural conditions. The second group of seeds were cultured *in vitro* on MS medium. The third group of seeds were grown *in vivo* on wet filter paper placed in a petri dish, kept in the dark. From the seedlings, shoot tips and single-node stem segments were taken as explants for *in vitro* studies.

Each experiment was conducted with 15 seeds and in three replications.

Explants were washed in running tap water for 30 min and then in a solution of mild liquid detergent for 5 min. Disinfection was done by a quick dip in 70% alcohol and surface sterilization was done with 0.1% HgCl<sub>2</sub> solution for 3-5 min. Three washings were done with sterilized double distilled water. Murashige and Skoog (MS)<sup>11</sup> medium was used for establishment of the explants. The pH of all the medium used was adjusted to 5.8 before autoclaving at 1.06 kg/cm<sup>2</sup>. The cultures were incubated at 27 ± 1°C under 3 klux fluorescent light. For proliferation of shoots half strength MS medium with 30 g/l sugar was used. 6-Benzylaminopurine (BAP, 0.25, 1.0 and 2.0 mg/l) was used along with 0.1 mg/l indole-3-acetic acid (IAA). Nurturing of isolated shoots was done in MS medium supplemented with 10 mg/l adenine sulphate (AdS) only. Roots also developed in MS medium having 0.1 mg/l IAA in 7 days. Rooted shoots were transplanted in the sterilized potted soil (garden soil:leaf manure::3:1) following the procedure reported earlier<sup>12</sup>. Humidity was controlled for the first two weeks by cover-

ing the plants with polythene chambers. The plants were grown under greenhouse conditions.

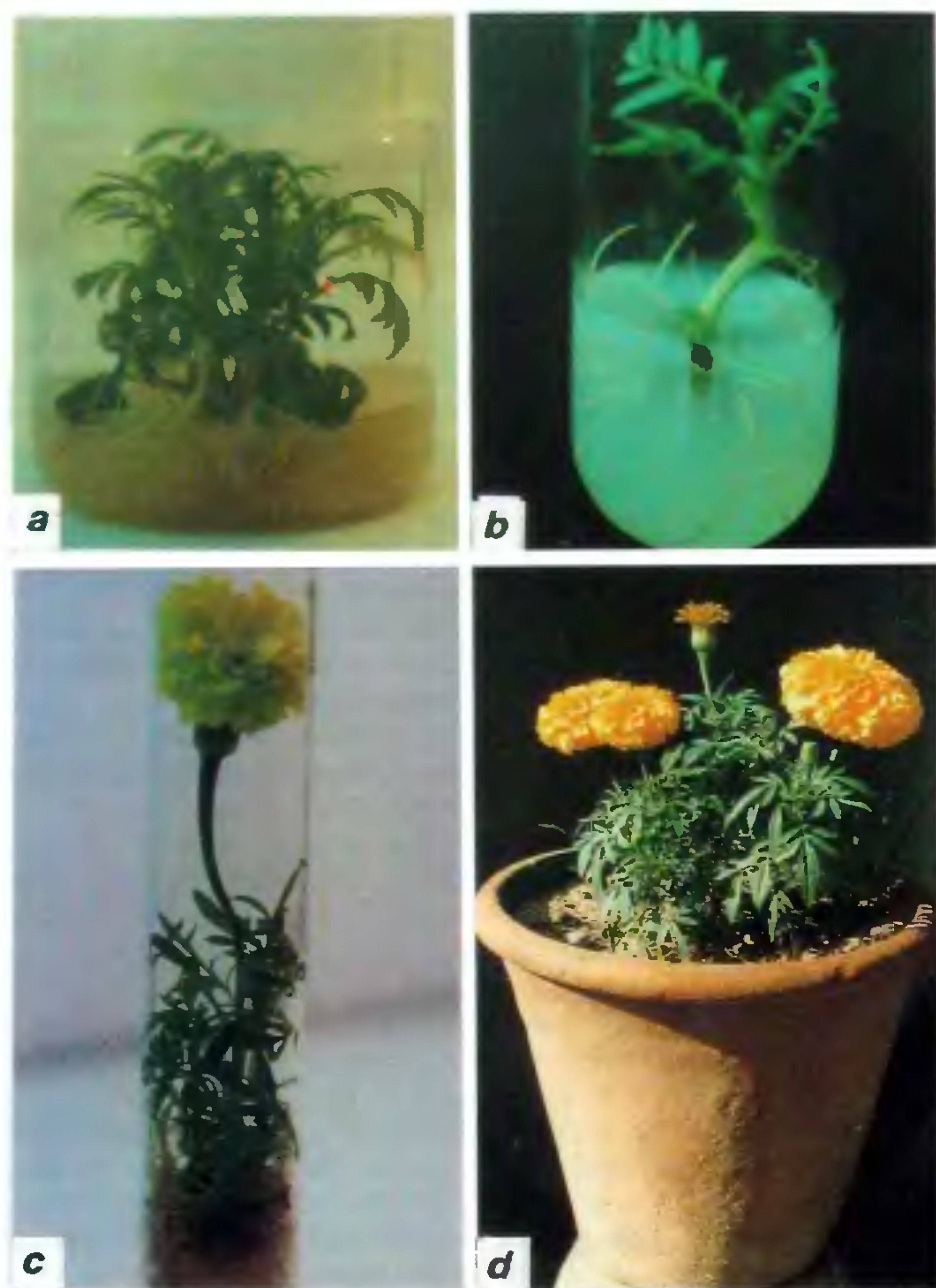
The first group of seeds sown in the field showed 100% germination. Plants were vigorous, early and free flowering. Flowers were extra large and fully double. Plants attained flowering when their height reached up to 12 inches and they were 2-3 months old. The second group of seeds showed very little germination and the germinated seedlings were not of good quality. The third group of seeds showed 100% germination. Shoot tips and single-node stem segments were taken as explants from 3-week-old seedlings.

Aseptically established shoots from the third group of seeds were used for proliferation. In full strength MS medium with the addition of 0.25 mg/l BAP and 0.1 mg/l IAA, callus formation started at the base of the shoots. In this treatment, explants remained vitrified with no proliferation of off-shoots. When the concentration of BAP was increased from 0.25 mg/l to 1.0 mg/l, callus formation was also increased along with the increase in the number of shoots, while the off-shoots remained vitrified. On further increasing the concentration of BAP to 2.0 mg/l, proliferation as well as the callus formation increased while condition of the off-shoots further deteriorated (Table 1). Callusing of tissue during proliferation of shoots is an undesirable feature so far as clonal multiplication is concerned as the callus is reported to be genetically unstable<sup>13</sup>. It was presumed that the concentration of inorganic salts

Table 1. Effects of basal medium and BAP on the proliferation of shoots

| Basal medium        | Conc. of BAP (mg/l) |                           | Number of shoots* | Callus formation | Remarks<br>(Condition of the proliferated shoots) |
|---------------------|---------------------|---------------------------|-------------------|------------------|---|
|                     | Initial culture     | Subculture after one week |                   |                  |   |
| MS                  | 0.25                | -                         | -                 | +                | -   |
|                     | 1.0                 | -                         | 3 ± 0.7           | ++               | Vitrified shoot                                   |
|                     | 2.0                 | -                         | 5 ± 1.2           | +++              | Vitrified shoot                                   |
|                     | 2.0                 | 0.25                      | 6 ± 1.5           | ++               | Vitrified shoot                                   |
| 1/2 MS except sugar | 0.25                | -                         | -                 | +                | -   |
|                     | 1.0                 | -                         | 4 ± 0.7           | +                | No vitrification of shoots                        |
|                     | 2.0                 | -                         | 6 ± 1.0           | ++               | No vitrification of shoots                        |
|                     | 2.0                 | 0.25                      | 7 ± 0.7           | -                | No vitrification of shoots                        |

+Denotes increase in the amount of callus; - denotes decrease in the amount of callus; \*average of 10 replicate cultures ± standard deviation.



**Figure 1.** *F*<sub>1</sub> hybrid of *Tagetes*. *a*, Proliferation of shoots after 40 days of incubation in culture; *b*, Rooting in isolated shoot; *c*, *In vitro* flowering after 2 months of incubation; and *d*, *In vitro*-raised flowering plant after two and half months of transplantation.

became supraoptimal with the increased BAP concentration for proliferating shoots, as callus formation as well as vitrification increased with increase in the concentration of BAP. Therefore, in the next experiment the strength of the MS medium was reduced to half except the sugar concentration which remained 3%. In half strength MS medium when 2 mg/l BAP was used for one week and then subcultured in a lower concentration of 0.25 mg/l BAP, a good proliferation of shoots with no intervening callus and no vitrification of shoots was found. Paques and Boxus<sup>14</sup> have shown in some species that media rich in mineral nutrients like MS promote vitrification, while half strength MS salts improved plant development in carnation and cucumber<sup>15,16</sup> Ca 7–8 shoots developed

from one nodal segment in one month after establishment. In the proliferation medium the number of leaves formed were less; so 10 mg/l AdS was added to the MS medium resulting in good number and growth of leaves (Figure 1 *a*). Roots also developed in the same MS medium having 0.1 mg/l IAA within 7 days (Figure 1 *b*). Flowering was achieved when the shoots were allowed to grow in the same nurturing medium (Figure 1 *c*). After attaining a height of 8–10 cm with 4–5 leaves, rooted plantlets were transplanted in the sterilized potted soil and grown under glass-house conditions. They grew well and flowered within two to two and a half months after transplantation. Yellow coloured flowers appeared exactly like their parent plants (Figure 1 *d*).

In the present investigation it has been possible to regenerate and to maintain *F*<sub>1</sub> sterile hybrids of marigold through tissue culture. This would decrease the cost of production by reducing unnecessary field maintenance of parental varieties and laborious crossing every year. This is perhaps the first approach for *in vitro* maintenance of *F*<sub>1</sub> hybrids of floricultural crops. The present study will open a new vistas for safe preservation of commercially important sterile *F*<sub>1</sub> hybrids.

1. Hutchinson, J. F., Kaul, V., Maheswaran, G., Moran, J. R., Graham, M. W. and Richards, D., *Aust. J. Bot.*, 1992, 40, 765–787.
2. Jain, S. M. and De Klerk, G. J., *Plant Tissue Cult. Biotech.*, 1998, 4, 63–75.
3. Khoshoo, T. N., *Indian Hortic.*, 1990, 34, 25–29.
4. Beshpalhok, F. J. C. and Hattori, K., *Plant Cell Rep.*, 1998, 17, 870–875.
5. Ketel, H. D., *Physiol. Plant.*, 1986, 93, 298–304.
6. Kothari, S. L. and Chandra, N., *Hort-Science*, 1984, 19, 703–705.
7. Kothari, S. L. and Chandra, N., *J. Plant Physiol.*, 1986, 122, 235–241.
8. Kothari, S. L. and Chandra, N., *J. Plant Physiol.*, 1984b, 117, 105–108.
9. Belarmino, M. M., Abe, T. and Sasahara, T., *Jpn. J. Breed.*, 1992, 42, 835–841.
10. Sharma, S. K. and Dhiman, R. C., *Phytomorphology*, 1998, 48, 167–172.
11. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, 15, 473–497.
12. Misra, P. and Chaturvedi, H. C., *Plant Cell Tissue Org. Cult.*, 1984, 3, 163–168.
13. D'Amato, F., in *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture* (eds Reinert, J. and Bajaj, Y. P. S.), Springer-Verlag, Berlin, 1977, pp. 343–357.
14. Paques, M. and Boxus, P., *Acta Hortic.*, 1987, 212, 193–210.
15. Ziv, M. Schwartz, A. and Fleminger, D., *Plant Sci.*, 1987, 52, 127–134.
16. Ziv, M. and Gadasi, G., *Plant Sci.*, 1986, 47, 115–122.

**ACKNOWLEDGEMENT.** We thank the Director, National Botanical Research Institute, Lucknow for the facilities provided.

PRATIBHA MISRA  
S. K. DATTA\*

*Floriculture Section,  
National Botanical Research Institute,  
Rana Pratap Marg,  
Lucknow 226 001, India*

\*For correspondence. (e-mail: Manager@nbri.sirnetd.ernet.in)

Received 20 October 1999; accepted 23 December 1999.