viduals, we are not able to offer an explanation for the lower serum levels of 25-OH-$D_3$ in fishermen.

Vitamin D is transported on a specific plasma-binding globulin and up to 40% is transported by lipoproteins. During hypervitaminosis D these binding sites may become saturated with an increase in the free pool of vitamin D. For evaluating vitamin D status, 25-OH-$D_3$ is considered to be the best index by most workers[16]. Therefore, it is important to measure serum levels of 25-OH-$D_3$ to assess the status of individuals or animals. If serum levels of 25-OH-$D_3$ are increased beyond the normal range, vascular calcification may develop even within normal levels of 1,25$(OH)_2D_3$ (ref. 17). High doses of vitamin $D_3$ when given orally to experimental animals do not always increase the serum levels of 1,25$(OH)_2D_3$. But levels of 25-OH-$D_3$ increase because 1,25$(OH)_2D_3$ levels are closely controlled, their production being determined by parathyroid hormone, calcium and phosphorous levels in blood[17,18]. Hence we did not estimate serum levels of 1,25$(OH)_2D_3$ and instead measured serum levels of 25-OH-$D_3$. Our finding that people working outdoors have higher levels of 25-OH-$D_3$ than those working indoors suggests that tropical sunlight and UV radiation can lead to high serum levels of 25-OH-$D_3$ in humans. The mechanism of acclimatization to prolonged and continued solar exposure in tropical population as well as whether elevation in serum 25-OH-$D_3$ levels is associated with increase in levels of the active metabolite 1,25$(OH)_2D_3$ and increased risk for any pathological conditions in this population need further exploration.

The data reported here are from a small sample and do not contain information on related metabolites like 1,25$(OH)_2D_3$, parathyroid hormone and ionic calcium. However, the results indicate the need for a systematic investigation on vitamin D metabolism in tropical populations.


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# *In vitro* propagation of white marigold (*Tagetes erecta* L.) through shoot tip proliferation

White marigold, belonging to the plant family Asteracae, is one of the important ornamental crops. In spite of its popularity and economic importance, it has not been properly commercialized due to nonavailability of planting materials. Multiplication rate of white marigold is not as fast as that of yellow marigold because of low seed viability and poor germination (30%). White marigold is very delicate and requires extremely favourable climatic conditions for vegetative growth as well as for good blooms. It is difficult to maintain pure line seeds due to its cross-pollinated nature. Tissue culture is the only method to maintain genetically identical clone having snow white flower colour and rapid propagation as well as long-term preservation of germplasm. There are very few reports on the tissue culture propagation of members of Asteracae family[1–5], still fewer on *Tagetes*[6–8] and none on white marigold. The present communication reports the clonal propagation of white marigold through shoot tip culture.

White marigold (cultivar of *Tagetes erecta* L.) was collected from an amateur grower in Lucknow. The explants were collected at two months intervals during different growth periods, i.e. vegetative and flowering. Shoot tips measuring ca. 2 cm in length and the single-node stem

segments measuring ca. 1–1.5 cm in length were taken as the explants for establishment. The explants were washed in running tap water for 30 min, treated with 5% teepol solution, and washed with single distilled water. A quick dip was given in 70% alcohol and then surface-sterilization was done in 0.1% $HgCl_2$ solution for 1–3 min depending on the age and tenderness of the explants. They were thoroughly washed at least thrice at 5 min intervals each with sterilized distilled water. Finally, the explants were inoculated in Murashige and Skoog's (MS)[9] medium for their initial establishment. The MS medium was supplemented with $NH_4NO_3$ (500–1500 mg/l), $(NH_4)_2SO_4$ (100–200 mg/l), L-glutamine (15–25 mg/l) and different concentrations and combinations of growth hormones (in mg/l), 6-benzylaminopurine (BAP, 0.1–10), kinetin (Kn, 0.1–1.0), 3-indoleacetic acid (IAA, 0.1–2.0), $\alpha$-napthaleneacetic acid (NAA, 0.1–2.0), gibberellic acid (GA, 0.5–5.0) and 2,4-dichlorophenoxyacetic acid (2,4-D, 1–10) to find the most suitable concentration and combination of inorganic salts and growth hormones for proliferation of shoots in established explants. Rooting of the isolated excised shoots was done in MS medium having IAA, indole-3-butyric acid (IBA) or NAA (0.05 and 0.1 mg/l each) individually. The pH of all the media used was adjusted to 5.8 before autoclaving at 1.06 kg/cm$^2$. All cultures were incubated at 27 ± 1°C under 3 klux flourescent light.

All the shoot tips grew in size and new shoots began to proliferate from their bases within 15 days of incubation. In the nodal segment, the axillary bud grew into a single shoot without any proliferation; proliferation of shoots started after giving a few cuts to the growing axillary shoot. The most suitable basal medium found for proliferation of shoots was MS medium having (in mg/l) 1000 $NH_4NO_3$, 200 $(NH_4)_2SO_4$, 25 L-glutamine–HCl, 15 L-arginine–HCl and 15 adenine sulphate (AdS). When any auxin like IAA or NAA was used in combination with cytokinin for proliferation of shoots, a large amount of callus appeared along with the formation of adventitious shoots. Therefore, to obtain proliferating shoots without any intervening callus, a pulse treatment of few growth hormones like BAP, Kn, GA or 2,4-D was given to the explants (Table 1). It was seen that initial treatment with BAP at the concentration

**Table 1.** Effects of growth hormones on the proliferation of shoots in *Tagetes erecta*

| Growth hormones (conc. mg/l) | | Number of proliferated shoots* | Callus formed | Condition of the proliferated shoots |
|---|---|---|---|---|
| For initial 7 days | For later period | | | |
| 10 BAP | 0.1 BAP | 7 ± 0.548 | ++ | Good |
| 5 BAP | 0.1 BAP | 10.2 ± 0.583 | – | Good |
| 10 Kn | 0.1 Kn | 6 ± 0.316 | ++ | Thin yellow shoots |
| 5 Kn | 0.1 Kn | 8 ± 0.447 | + | Thin yellow shoots |
| 50 GA | 0.5 GA | 5.2 ± 0.374 | – | Browning of shoots |
| 25 GA | 0.5 GA | 3.4 ± 0.245 | – | Browning of shoots |
| 10 2,4-D | 0.1 2,4-D | – | +++ | Necrosis of shoots |

*, Average of 10 replicate cultures; +, positive response; –, negative response.

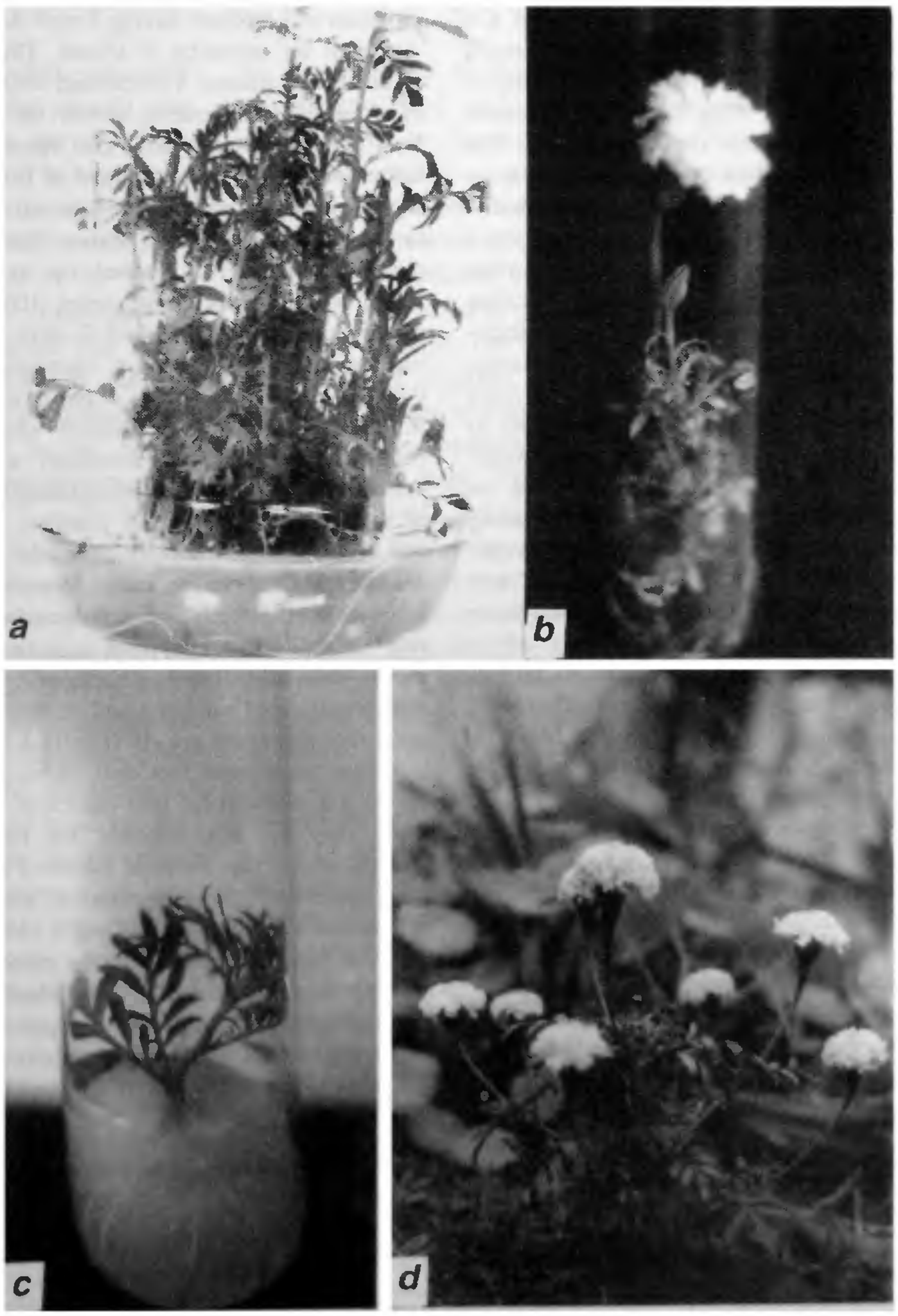


**Figure 1.** Cultures of *Tagetes erecta* showing *a*, proliferating shoots; *b*, *in vitro* flowering; *c*, rooting of isolated shoot; *d*, *in vitro*-raised plant of *Tagetes erecta* showing snow white blooms after 45 days of transplantation.

**Table 2.** Effect of season on the growth pattern of the explants

| Month | Formation of the vegetative shoots in the culture | Formation of the floral axis in the culture |
|---|---|---|
| September–October | +++ | – |
| November–December | +++ | + |
| January–February | ++ | + |
| March–April | + | ++ |
| May | + | +++ |

+, positive response; –, negative response.

of 5 mg/l for 7 days followed by transfer to fifty times lower concentration, i.e. 0.1 mg/l for the later period proved most beneficial for induction of shoot buds among all the growth hormones used. Ca. 10 shoots proliferated from one nodal segment within 30 days of incubation (Figure 1 *a*). With Kn although shoots proliferated, new shoots were very thin and yellowish in colour and were associated with callus which was undesirable for clonal multiplication. In the presence of GA all shoot tips turned brown, while in the presence of 2,4-D excessive callus formation was found without any differentiation of shoots. In the present material, if explants were collected during early vegetative phase, i.e. from September to December, a good proliferation of vegetative shoots was found, whereas in explants collected during the flowering stage, i.e. from December onwards, vegetative growth was inhibited with less number of proliferated shoots and more number of floral axis (Table 2), which developed full blooms within 60 days of establishment. But this *in vitro* flowering was not controlled by growth hormone supplement used exogenously (Figure 1 *b*). Here *in vitro* flowering depends mainly on the season of collection of the explants, which is crucial for its favourable response *in vitro*[10]. Growth of the vegetative shoots in the culture is required as these shoots are easy to transplant and in due course of time they flowered in the field.

The proliferated shoots were excised and the isolated shoots, very thin in appearance, were nurtured, while the remaining base of the proliferated shoots was divided into 3–4 parts and reused for proliferation of shoots in the same basal medium having 0.25 mg/l BAP. The same modified MS medium having 5 mg/l AdS was used for nurturing of shoots. Thus, these cultures became a continuous source of obtaining proliferating shoots for at least 5–6 subcultures, after that regenerative potentiality declined and a fresh source of shoots was prepared according to the method described above. When nurtured shoots had reached up to a height of 3–4 cm, they were rooted. 100% rooting was achieved within 7 days of incubation in MS medium containing 0.05 mg/l NAA. Although rooting was achieved in any of the auxins used, callusing preceded root formation was undesirable for successful transplantation of the *in vitro*-regenerated plantlets to soil. Hence efforts should be made to minimize the intervening callus formation and to obtain a fresh and well-branched root system[11]. Just after root induction, shoots were subcultured in hormone-free medium to avoid callusing and to obtain good root and shoot growth (Figure 1 *c*). It is an established fact that although auxins are essential for root induction in stem, they are not required for root growth, which may even be inhibited in their presence[12]. Rooted plantlets were acclimatized in the liquid Knop's solution and then transplanted to the potted soil by exposing them to a gradually decreasing humidity regime over a period of 15 days under glasshouse conditions. The plants flowered within 30–45 days of transplantation to the field with snow white blooms (Figure 1 *d*). Being a highly cross-pollinated genus, pure line seeds could be produced only by selfing and bagging of the flowers. It is, therefore, necessary to maintain the germplasm of this cultivar in tissue culture so that a similar clone could be provided at the time of plantation of the crop. Flowers produced by this method were also of the same colour as those of the mother plant[13].


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