MULTIPLICATION OF SPRAY-CARNATIONS BY AXILLARY BUD CULTURE

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ABSTRACT

Spray-carnations are prized ornamentals many of which normally do not set seeds and are propagated vegetatively. Axillary buds were cultured on Gamborg's B_5 medium containing 3% sucrose (Analar grade)+ NAA (10^{-6} M)+ BAP (10^{-5} M). Each bud proliferated to yield 13 shoot buds in two weeks. Thus, beginning with a single axillary bud, it is possible to obtain 13^{12} shoot buds in 6 months time. Shoots were rooted on both semi-solid and liquid basal medium by reducing sucrose concentration to 1.5%. Use of commercial sugar reduced the rate of bud multiplication from 13 to 11 (per two weeks). The procedure for transplanting *in vitro* plants potted to the field is to be improved. Out of the 105 plants potted 17 survived and produced flowers true to the donor plants.

INTRODUCTION

DOMESTICATION and propagation of attractive ornamental plants are pre-historic practices. Although vegetative propagation ensures maintenance of desirable variants, it is time-consuming and hinders bulking up of elite and disease-free materials. *In vitro* culture of cells and tissues has become an important tool in recent times for the multiplication of heterozygous, sexually incompatible and sterile genotypes on a commercial scale[1–6]

Carnation (*Dianthus caryophyllus*) is a half-hardy perennial with branching stems and linear, glaucous leaves, arranged in an opposite-decussate manner. The inflorescence is a loose cyme; the flowering shoot can be raised in two forms. Either the flower buds developing on short branches in the axils of upper leaves are pinched, leaving a large terminal flower—the standard type—or the terminal bud is excised at an early stage of flower bud growth to allow uniform development of blooms on the lateral branches. The latter is known as the spray type. Spray types are less vigorous and produce fewer flowers in winter than the standard types[7].

Spray-carnations have long stems and large flowers. Many of these normally do not set seed and have low disease resistance. The present goal of carnation breeders is to develop cultivars which flower uniformly throughout the year and are resistant to disease, frost and lodging.

Shoot tip culture of carnations had been suggested not only as a means of eliminating pathogens but also as a method for micropropagation[8–10]. However, plants raised by this technique have been reported to show deviation in normal flower colour, suggesting a disruption of chimeras[11].

Cell suspension cultures from callus obtained from stem pith and young internodes of carnation were filtered and successfully plated on agar in petri dishes[12]. However, single cells failed to divide. Although suspension cultures initiated roots in response to high concentrations of indoleacetic acid (IAA) and α-naphthaleneacetic acid (NAA), all attempts to induce formation of shoots or embryoids gave negative results[12]. An aseptic culture method of multiplying a garden variety of carnations was described by Petrů and Landa[13] in which successful shoot formation was induced using callus tissues isolated from hypocotyl and apical meristem cultures.

We were urged to develop an appropriate tissue culture method (personal communication with Mr M. M. Atavar, Indo-American Hybrid Seed, Bangalore) for propagating and preserving alive carnations as the Indian flower growers experience serious problems in maintaining them. Annual losses are considerable as the cuttings (planted after flowering is complete) have a tendency to wilt in the hot summer months or in the following monsoon season.

MATERIALS AND METHODS

The procedure developed by us for micropropagation, using axillary bud culture is described here. A light pink-flowered variant of spray carnations (referred to as MCP in the text) procured from the

garden of Miranda House, University of Delhi, Delhi served as the source material. The plant material (shoots with top seven nodes) was collected in the second week of April after flowering had stopped. The explant consisted of a node (leaves were removed) with an axillary bud and 1 cm of the internode above and below it. The explants were first washed in running tap water for 10 min and then surface-sterilized on a magnetic stirrer in a solution containing Teepol[a]/Salvon[b]. After a thorough washing in running tap water, the final sterilization was done in a laminar-flow chamber with a 3 min treatment of 2.5% sodium hypochlorite[c] solution. Finally the explants were washed two or three times with sterile distilled water containing 2 ppm of Ambistryn-S[d].

Initially B_5 medium[14] with 3% Analar grade sucrose, gelled with 0.8% agar was used as the basal medium. This was supplemented with 6-benzylamino purine (BAP, 10^{-5} M) and NAA (10^{-6} M). The cultures were kept at 25 ± 2 °C under continuous fluorescent light (1.5 k lux). Both bud break and bud multiplication were promoted in this medium. For initiation of roots, the shoots were placed on sterilized filter paper bridges in liquid B_5 medium containing 0.0–1.5% sucrose. Later Analar grade sucrose was replaced by commercial sugar not only for subculturing but also for rooting. The rate of shoot bud formation on the subcultured medium was estimated. As most of the earlier work on carnations has been done using MS medium[15], the rate of shoot bud formation was estimated on this medium also using the same hormonal supplements.

RESULTS AND DISCUSSION

Bud break occurred in about two weeks after culture on B_5 + BAP (10^{-5} M) + NAA (10^{-6} M) (figure 1). An analysis of the results indicated that while on an average 13 new shoot buds emerged from a single axillary bud in two weeks' time on B_5, only 9 new buds were produced on MS in the same period. However, when two buds were planted, the number of shoot buds produced was not doubled. An average of 560–580 shoots per flask (of 100 ml capacity) were formed on B_5 in six weeks' time. Thus starting from a single bud it is theoretically possible to obtain 13^{12} shoot buds in 26 weeks (about 6 months). The number of shoot buds forming on a medium (B_5) containing commercial sugar in two weeks' time was 11 (figures 2–4).

In a period of 21 days about 10–12 buds elongated to form shoots, which bore narrow, linear leaves above and broad, ovate leaves towards the surface of the medium (figure 5). With the use of commercial sugar, the leaves became thicker and more intensely green. Subculturing was done at 21-day intervals. So far a continuous bud culture has been maintained without the intervention of a callus through 39 passages.

The initial cluster of shoot buds (from the first culture established) was removed and divided into three or four portions, each with 5–10 shoot buds. Each such piece was transferred to individual tubes containing fresh B_5 + BAP (10^{-5} M) + NAA (10^{-6} M). To assess the rate of shoot bud formation on B_5 containing only auxin, three concentrations of NAA (10^{-5} M, 10^{-6} M, 10^{-7} M) were used. The number of buds formed in two weeks was lower (6 ± 2) than that formed on the original medium containing both cytokinin and auxin. When the shoot buds were placed on B_5 containing only BAP (10^{-5} M, 10^{-6} M, 10^{-7} M) callusing was ensued. The callus formed was fragile and brown. These experiments proved that both BAP and NAA were necessary for bud break and bud proliferation.

To induce rooting, clusters of shoot buds were placed both on semi-solid medium and on sterilized filter paper bridges in liquid medium containing: (i) B_5 (basal); (ii) B_5 + NAA (10^{-5} M) or B_5 + IBA (10^{-5} M) and (iii) B_5 + NAA (10^{-5} M, 10^{-6} M, 10^{-7} M) + IBA (10^{-5} M, 10^{-6} M, 10^{-7} M) with 3% sucrose. No rooting occurred in response to any of these treatments for two weeks. However, when the sucrose level was reduced to 1.5%, prominent tap roots were initiated from the lower end of each shoot bud in all the treatments both on semi-solid and liquid medium (figures 6–8). These put out a few laterals. As roots were formed even on basal medium, the use of hormones was eliminated in the subsequent experiments. Liquid basal medium was advantageous as it was easier to transfer plantlets from the tubes without breaking the roots.

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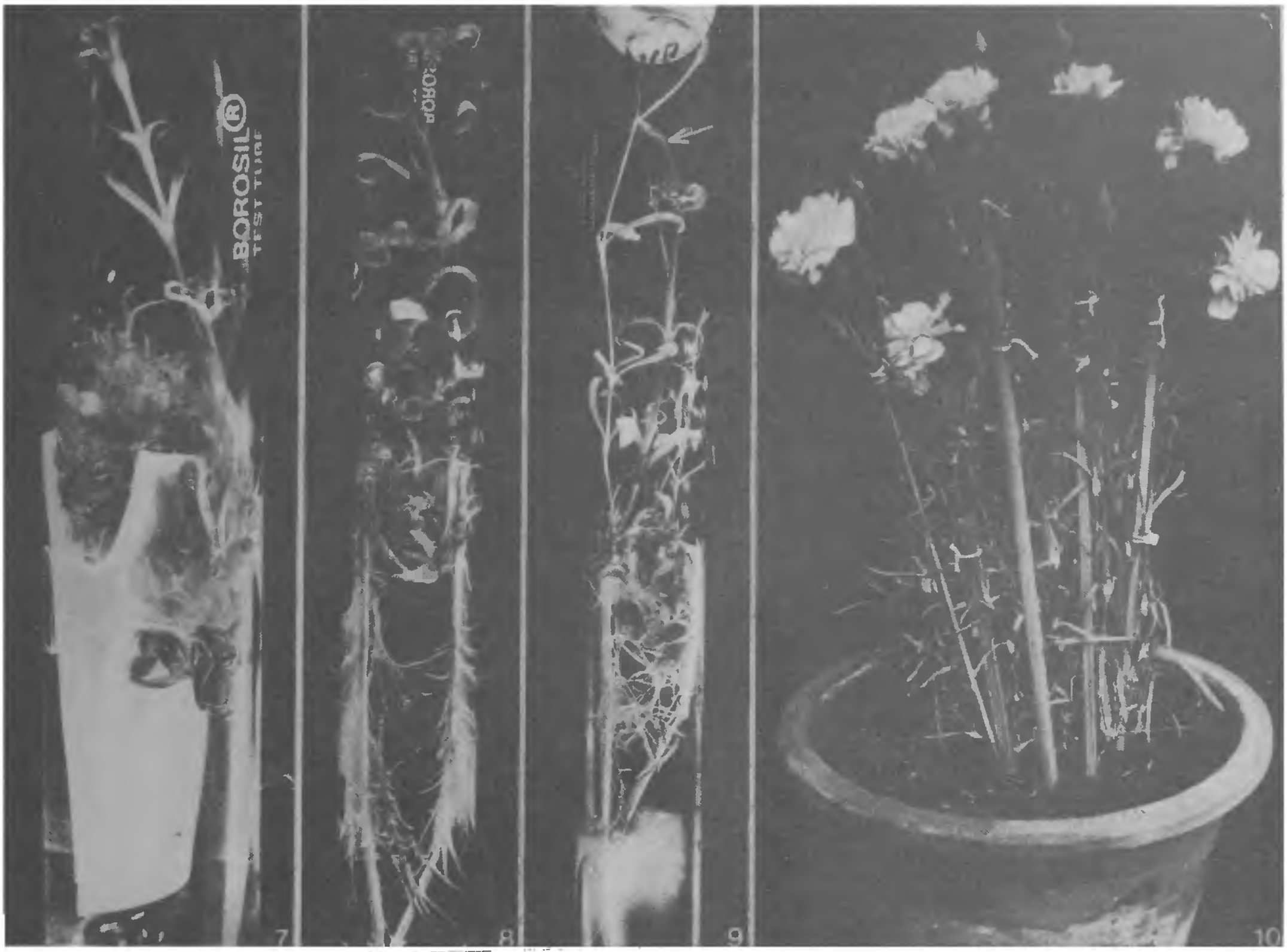
[a] B–300, detergent manufactured by SHELL Development Corporation, USA marketed in India by National Organic Chemical Industries Limited, Bombay, India.

[b] Liquid detergent originally manufactured by ICI, UK Marketed in India by IEL Limited, Calcutta, contains chlorohexidine gluconate solution, strong cetrimide and isopropyl alcohol.

[c] Marketed by Polypharm Private Limited, Bombay.

[d] Streptomycin sulphate marketed by Sarabhai Chemicals, Baroda.

Figures 1–6. *Dianthus caryophyllus.* **1–5.** Culture of axillary buds on B_5 + BAP (10^{-5} M) + NAA (10^{-6} M). **1.** Formation of new shoot buds (arrows) from the original bud. **2–4.** Proliferation of shoot buds over a period of six weeks. **5.** Elongation of shoots in 28 days old culture. Note one shoot bearing linear leaves towards the top (arrow) and broad leaves towards the base. **6.** Formation of roots in B_5 semi-solid medium containing 1.5% sucrose.



Figures 7–10. *Dianthus caryophyllus*. **7–8.** Formation of roots in B_5 liquid medium. **9.** Formation of floral bud in B_5 liquid medium (arrow). **10.** *In vitro* grown plants transferred to pot have bloomed (photographed slightly late in the season. The size of the blooms was larger 4 weeks earlier).

Formation of roots stimulated elongation of shoots. In each culture tube 2 or 3 shoots took lead and attained a height of 9–12 cm in ten days. These elongated shoots developed terminal flowers but no axillary shoots (figure 9). Elongation of at least three internodes was necessary for floral bud initiation. However, the time of flower initiation *in vitro*, coincided with the time of flowering of the donor plants maintained under natural conditions. The percentage of cultures that showed flowering on B_5, B_5 + IBA, B_5 + NAA and B_5 + IBA + NAA was 25, 25, 17 and 8 respectively.

To make the rooted plants autotrophic before transplantation, they were transferred at weekly intervals to liquid medium containing decreasing percentage of sucrose (1.0, 0.75, 0.50, 0.25 and 0.00). Finally they were put in paper cups (of capacity 150 ml) containing a mixture of vermiculite and garden soil in the ratio of 1:1. Individual cups were covered with perforated polythene bags and kept in a culture room at 25±2°C under continuous fluorescent light (1.5 k lux). The plantlets were irrigated daily with 30 ml of liquid B_5 medium (1/5 original strength) minus sucrose. The bags were removed for 90 min each day for one week. The plantlets remained green and erect for one week after which they gradually wilted. Later some plantlets were transferred to plastic pots containing Jiffy plus (a potting mixture containing nutrients used by American horticulturists for easy rooting of plants) but these also failed to survive. One hundred and five rooted plantlets in a sucrose-free medium were given to Dr Vishnu Swarup (Indo-American Hybrid Seed, New Delhi) for transplantation. The procedure followed by him is as follows: The plantlets were removed from the cultured tubes and washed with distilled water. These were transferred to thumb pots containing sterilized peat moss and

were irrigated for 7 to 10 days with 1/5 strength B_5 mineral solution. After being transferred to 10 cm wide earthen pots they were supplied with tap water. When the plants had attained a height of 15 cm they were moved to larger pots [15 cm wide and containing very fine organic matter (leaf mould or farmyard manure)]. The surviving plants were finally transferred to pots of 22.5 cm across and stored in plastic houses whose temperature was 8–10°C lower than the ambient temperature. Of these, 17 plants survived under natural conditions, attained full size and bore flowers which could be compared with those of the donor plants (figure 10).

Commercial application of *in vitro* propagation is important because using a single bud as the starting material, a large number of plants can be obtained. This feature helps in maintaining a rare, scarce, elite or seedless ornamental plant. It also leads to a reduction in maintenance cost of donor or stock plants under green house conditions. Tissue cultured plants do not usually exhibit strong apical dominance and are reported to be more attractive than the conventionally propagated plants[16].

A method for multiplying spray-carnations (MCP) using axillary buds has been described in this paper. The merit of the present work lies in the fact that axillary buds can be obtained at the end of the flowering season without sacrificing the terminal bud. As the material was collected after flowering, there was no apical dominance and the laterals were pre-conditioned to grow. More importantly, there was no callusing, which ensured minimization of variability in the regenerated plants. Previous workers have used MS medium for micropropagation of carnations. We have demonstrated that the use of B_5 medium enhances the rate of bud multiplication by 30% at each step, which over a long period would be a substantial gain. Furthermore, the present work has shown that commercial sugar can be used for *in vitro* propagation which substantially brings down the cost.

Increasing the ratio of cytokinin to auxin in the medium enhances the proliferation of shoot buds as reported in *Gerbera*[17], *Gloxinia*[18] and *Chrysanthemum*[19, 20] and carnations (present work). If this ratio is reversed rooting is favoured[21]. In the present work rooting occurred even on a hormone-free medium, suggesting that the endogenous levels of both auxin and cytokinin are adequate for initiation as well as sustenance of the root system.

Having established a technique for mass multiplication of spray-carnations, efforts are being made to ensure high survival rates of true to type plants. Methods are also being worked out to store planting material of high quality carnations in limited space at low cost, using low temperature and other treatments. Our success in the micropropagation of Sim's carnations will be the subject matter of another paper.

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The Third World Academy of Sciences has decided to institute a Prize of US dollars 10,000 to be awarded to the best research essay which brings to light the scientific achievements of a Third World scientist prior to the 20th century, whose work has not been hitherto clearly recognised.

The research essay should summarise the major contributions of the Third World scientist.

It should indicate to what extent the contributions of the scientist were recognised within his/her community and should establish in what way the achievements are linked to the sources of modern scientific thought. It should present evidence which should be acceptable to the contemporary scientific community regarding the originality and relevance of the Third World scientist's achievements. Arrangements will be made for the essay to be published in the form of a book by the Third World Academy of Sciences.

Essays for the Prize can be submitted by all scholars in the world.

The Prize will be awarded in 1988, and the closing date for announcement of intention by those planning to present an essay is 30 October 1987.

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A National Seminar on Physiology and Biochemistry of Oil Seed Plants, will be held at the Department of Botany, Sri Venkateswara University, Tirupati 517 502, Andhra Pradesh, India, during November 27–29, 1986.

The programme will include invited lectures as well as sessions of free communications.

Scientists interested in participating in the Seminar are requested to contact the Director, Prof. G. Rajeswara Rao, Head of the Department of Botany, S. V. University, Tirupati 517 502, India.