IN VITRO PROPAGATION OF CARNATION (*Dianthus caryophyllus* L.)

J. JAGANNATHA

DIVISION OF HORTICULTURE
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J. JAGANNATHA

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CERTIFICATE

This is to certify that the thesis entitled **"*IN VITRO* PROPAGATION OF CARNATION (*Dianthus caryophyllus* L.) "** submitted by **Mr. J. JAGANNATHA**, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) in HORTICULTURE** to the University of Agricultural Sciences, Bangalore, is a record of research work carried out by him under my guidance and supervision, and that no part of the thesis has been submitted for the award of any degree, diploma, associatship, fellowship or other similar titles.

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(P. NARAYANASWAMY)

(B.N. SATHYANARAYANA)

(T.G. PRASAD)

(SURESH. N. SONDUR)

(G.K. MUKUND) 20/6/97.

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Dedicated to my beloved parents

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INTRODUCTION

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.), one of the most fascinating commercial flowers of the world, is indigenous to the Mediterranean region. These prized cut flowers have a great demand in the international market for their attractive form, colour and long vase life. Their ability to stay fresh in cold storage for two to four weeks and to withstand long distance shipment are also important assets of commercial significance (Bhatt, 1989).

In the international flower trade carnation ranked seventh with a value of 81.2 million dollars during the year 1995 (Anon, 1996). Carnation is cultivated on a large scale in France, Holland, Israel, Kenya and Germany mostly in greenhouses. In India, its cultivation is very much limited and is mostly cultivated in open conditions at Himachal Pradesh, Punjab, West Bengal, Jammu and Kashmir and Karnataka. The potential production areas for export of quality carnation cut flowers are places having cool climate like Kashmir, Kalimpong, Bangalore and other places.

The perpetual flowering florists carnations grown for good quality flowers year round can be grouped into two major classes viz., standard and spray types. The standard types produce larger blooms on longer flower stalks. The miniature or spray types on the other hand produce several flowers of smaller size. These are better adapted to warm climate and are less susceptible to diseases, making them easy and more economical for cultivation in tropical conditions, compared to standard types. Also their production cost is less than that of standards. The spray types have recently gained popularity for their use in floral arrangement and also as cut flowers.

Carnation can also be used in bedding, pots, borders, edging and rock gardens. In certain parts of France and Holland, flowers of some cultivars of carnation are used for the extraction of perfume.

Availability of quality planting material is a pre-requisite for the production of good carnation flowers. Traditionally carnations are propagated by soft terminal cuttings. Carnations are also propagated by tissue culture methods. Propagation of carnation by tissue culture as opposed to its propagation by conventional methods by soft terminal cuttings offers manifold advantages. Rapid multiplication of new carnation varieties is possible by micropropagation. Tissue culture is not season dependent and requires only a limited quantity of plant material as a source of initial explant. A steady and guaranteed supply of planting material is ensured which helps in planning the planting time. Allocation of large space for maintaining plantning stock, as in propagation through cuttings is not necessary, ensuring efficient utilization of green house space. Carnation crop is infested by various viral diseases like streak, mosaic, mottle and ringspot. Meristem culture helps in avoiding viral diseases.

Carnation is being recently introduced to India. Many companies are taking up carnation cultivation on a large scale. There is also good scope to grow carnations for export market for which guaranteed supply of good quality and disease free planting material is essential. This could be ensured by following tissue culture propagation. However, information on protocols for *in vitro* propagation of carnation is not readily avaliable. Hence, the present investigation is concerned with the standardization of protocols for rapid multiplication of carnation through tissue culture.

In view of the above points in mind the experimental work on tissue culture was conducted with the following objectives:

I. To standardize the protocol for *in vitro* propagation of carnations,

II. To find the suitability of spray and standard type for *in vitro* propagation,

III. To develop hardening techniques for *in vitro* propagated carnation plantlets,

IV. To standardize the source explant for callus production.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

The concept of cell theory of Schleiden (1838) and Schwann (1839) is the basis of plant cell, tissue and organ culture. It was Haberlandt in 1902 who first attempted cultivation of isolated plant cells *in vitro* on a nutrient medium. This lead to many important discoveries of this century. He introduced the concept of totipotency, according to which all living cells containing a normal complement of chromosomes should be capable of regenerating the entire plant.

Although Haberlandt was not successful in proving his ideas, his basic concepts lead to the successful culture of tomato roots continuously *in vitro* by White (1934). Further, Gautheret (1939), Nobecourt (1939) and White (1939) reported independently that cells in culture can be made to proliferate continuously and also undergo differentiation.

Major breakthrough in plant tissue culture were achieved after the discovery of auxins and cytokinins. Thimann in 1935, made it possible to control the growth of plant cells and tissues. It was not until 1957 that kinetin was discovered and the idea of synergistic effects of auxins and cytokinins in promoting cell division in tobacco triggered the imagination of physiologists (Skoog and Miller, 1957).

Development of nutrient media for tissue culture of tobacco by Murashige and Skoog in 1962 was a great achievement in tissue culture. This medium is now accepted world wide as the classic nutrient medium for tissue culture of most of the plant species .

Commercial application of plant tissue culture techniques in clonal propagation has expanded at a rapid rate. Murashige (1974) was instrumental in giving the technique of *in vitro* culture the status of a viable practical means for rapid and mass propagation of horticultural species. According to him, there are three possible methods available for *in vitro* micropropagation; i) release of axillary buds from the influence of apical dominance, ii) production of adventitious shoots through direct or indirect organogenesis and iii) somatic embryogenesis. In shoot tip and axillary bud culture, genetic stability is maintained to a large extent. Callus mediated organogenesis and somatic embryogenesis are not recommended for clonal propagation since there is a possibility of producing aberrants.

Major stimulus provided for application of plant tissue culture techniques to the propagation of ornamental species may be attributed to the early work by Morel in 1960 on the *in vitro* propagation of orchid. The multiplication of ornamental plants for the horticulture industry has provided the first and at present, by far the largest practical application for the science of plant tissue culture (George and Sherrington, 1984).

The main applications of *in vitro* plant tissue culture in crop production are in micropropagation and production of pathogen free plants. Tissue culture is currently being applied to a large variety of ornamental plants on a commercial basis. Micropropagation techniques have been applied effectively in the clonal propagation of many plant species, including ornamental and crop plants such as ferns, orchids, gerbera, carnation, anthurium, chrysanthemum, strawberry, apple, banana, cardamom and potato.

The use of meristem and shoot tip cultures for the recovery and establishment of pathogen free plants has also become a common practice in the production of virus free stock of vegetatively propagated plants in many commercial nurseries in the developed world (Waithaka *et al.* , 1992).

In carnation, many *in vitro* clonal propagation studies on different methods of propagation, effect of growth regulators and source of explants and hardening techniques have been reported. Research work carried out on the *in vitro* propagation of carnation is reviewed below.

2.1 Direct organogenesis

2.1.1 Regeneration through Meristem tips

Meristem culture is an important way to establish virus free plants. The demonstration of the practical usefulness of this important technique must be credited to Morel and Martin (1952) who for the first time recovered virus free dahlia plants from infected individual by excising and culturing the shoot tips in *in vitro*. Many important horticultural crops (e. g., strawberries, potatoes, orchids) are routinely freed of viral contamination by using this procedure. The true shoot meristem consists only isolated apical dome without any visible primordial leaves attached. The size of the explant measures less than 0.1 mm in height. It is very difficult to obtain such a small explant and also it results in very poor survival and a slow rate of multiplication *in vitro*. The commercial propagators use 5-10 mm stem tip sections. These large explants hasten multiplication but do not necessarily exclude pathogens that may be infecting the stock plants (Murashige 1974). Smith and Murashige (1970) accomplished the first true meristem culture of an isolated angiosperm meristem into a complete plant.

As early as 1963, Stone introduced meristem tip culture for carnation micropropagation. Aseptic multiplication and maintenance of differentiated carnation shoot tissue derived from shoot apices was reported by Hackett and Anderson (1967).

Carnation was successfully propagated by Earle and Langhans (1975) from shoot tips in liquid medium, which when transferred to agar nutrient medium containing 0. 5 mgl^{-1} kinetin and 0. 1 mgl^{-1} NAA formed multiple shoots. Effects of different concentrations of adenine and kinetin on the development of carnation meristem tips was studied by Pennazio (1975). Kinetin at 0. 1 ppm reduced root formation whereas 1 ppm completely inhibited. However kinetin promoted lateral bud development. Adenine at 10 ppm inhibited root formation. Davis *et al.* (1977) showed three stages of clonal multiplication i.e. , i) shoot tip culture as initiation stage ii) shoot multiplication stage and iii) rooting stage. In the initiation stage for multiple shoots they examined, the uses of various inorganic salt mixtures, vitamin mixtures, carbohydrates, growth regulators, agar, pH and additional supplements, for their effect on growth and development. They found that in shoot tips grown on modified MS medium with 10 μM kinetin and 1μM of NAA, apical dominance was counteracted and morphologically similar shoots proliferated rapidly. Shabde and Murashige (1977) observed that in a medium containing both IAA and kinetin apical meristem dome explants produced highest frequency of plants. In an unsupplimented medium they observed that, for the continuous development, the explant should have two pairs of primordial and a pair of expanding leaves. Dabski and Malinowska (1979) studied the influence of kinetin in combination with different NAA concentrations viz. , 0.0, 0.5,1.5, 4.5 and 13.5 μM on *in vitro* growth of meristem tips of carnation cv. Scania 3C. The highest fresh weight of shoots was reported on the medium with 13.5 μM kinetin and 0.5 μM NAA. Hampel (1979), found that culturing shoot apices of carnation cvs. for 20 days on the medium with 6μM BA and transplanting to the medium with 4 μM kinetin was the best for the multiple shoot production.

Dencso (1984), reported that for every 100 meristems, approximately 1300-1500 plants were obtained after 15 weeks. He had observed that carnations were not sensitive to the microelement content of the nutrient medium but the proportion of kinetin to NAA was important, particularly in the propagation stage. Ioannov (1990) studied the production of carnation plants by shoot tip culture in *in vitro* using modified MS medium supplemented with NAA at 0.02 mgl^{-1} and kinetin at 0.5, 1.0, or 20 mgl^{-1}. The highest multiplication rate, averaging 33 plantlets per explant was obtained with the highest (0.2 mgl^{-1}) kinetin concentration.

Meristems cultured on MS medium containing 5.0 mgl^{-1} BA + 1.0 mgl^{-1} IAA produced more axillary buds than other growth regulator combinations. Addition of 0.5 mgl^{-1} GA_3 to the culture medium resulted in shoot elongation (Can and Koc, 1992). Dujakovic *et al.* (1993) studied the development of shoot apex explants of cv. Scania Tina cultured on enriched MS medium further supplemented with 1, 2, 3, 4 or 5 mg IAA + 0.6 or 1.0 mgl^{-1} kinetin. The best growth was produced by 2 mg IAA + 1 mgl^{-1} kinetin and IAA at $\geq$ 3 mgl^{-1} retarded growth.

Frey and Janick (1991) reported that shoot regeneration in carnation was influenced by genotype, explant source, and plant growth regulator balance.

As carnation crop is infested by various viral diseases like streak, mosaic, mottle and ring spot. Meristem culture helps in avoiding viral diseases.

Virus free carnation planting material was produced from meristem cultures by Opera and Pampfil (1982). The studies were conducted with three cvs. using (0.2 to 0.8 mm sized) apical or axillary meristem explants cultured in modified MS medium. Explant regeneration increased from 20 percent (0.2 mm meristems) to 70 percent (0.8 mm meristems). Duration of explant regeneration also decreased from 8-9 weeks with 0.2 mm meristem to 6 weeks in the case of 0.8 mm meristems. Carnation meristem tip culture for preparation of carnation mottle virus free plants was studied by Moshari and Danesh (1983). The best results were obtained when 0.6 to 1 mm long meristem tips were cultured on a medium containing 1 mgl^{-1} of NAA.

2.1.2 Regeneration from Single node

Roset and Bokelmann (1979) cultured stem sections obtained from node, on a medium containing 1 mgl^{-1} BA and 0.1 mgl^{-1} IAA to induce shoots. Vegetative propagation of carnation *in vitro* through multiple shoot development was again attempted by the same authors in 1981. Vegetative shoot cuttings of 6 cultivars of carnation were irradiated with 30 or 60 Gy X-rays. Explants were excised and cultured. Multiple shoot development of *in vitro* cultured nodal stem explants were examined. The *in vitro* propagation method resulted in the production of 2220 plantlets from 209 explants by four months after incubation. Kim and Kang (1986) studied multiplication of carnation by cuttings in *in vitro*. Nodes, leaf buds and shoot tips from plantlets of cv. Scarlet Bell that had been maintained on solid MS medium without growth regulator for a year, were cultured on solid MS medium containing various concentrations (0-10 mgl^{-1}) of NAA and BA for all types of explants, the ability to produce multiple shoots compared favourably with production from axillary buds in liquid culture. Ghosh and Ram (1986) studied multiplication of axillary buds cultured on Gamborg's B_5 medium containing 3 per cent sucrose + NAA 10^{-6} M + BAP 10^{-5} M. Each bud proliferated to yield 13 shoot buds in 2 weeks. Thus begining with a single axillary bud, it was possible to obtain 13^{12} shoot buds in 6 months time.

2.1.3 Regeneration from leaf/stem explant

Lubomski and Jerzy (1989) studied the effects of BA (0:10^{-7} M) and IAA (0:10^{-5} M) on regeneration capabilities of internodal segments. They obtained both adventitious shoots and roots at of 10^{-7} M BA and 10^{-5} M IAA. Nugent *et al.* (1991) reported that in stem explants, morphogenetic capacity was determined mostly by the development stage of the explant. Highest percentage of shoot formation was observed in the youngest stem segment on all the cytokinins tested. Messeguer *et al.* (1993) reported that basal segments were the only part of the leaf that generated shoots when cultured on medium supplemented with 0.01mgl^{-1} NAA and 1mgl^{-1} BA. The per cent regeneration of leaves and the number of shoots/leaf explant improved significantly in dark condition and at 5.5 gl^{-1} agar. Altvorst *et al.* (1994) on MS solid media with 4 μM BA and 1.6 μM NAA. The two leaves from one node of *in vitro* grown plants showed different shoot forming potential, depending on the order in which the leaves were removed from the stem. The leaf removed second formed more shoots and also had a large amount of adhering stem tissues.

2.1.4 Regeneration using Petals

Immature petals and basal flower segments have a high morphogenetic capacity. Adventitious shoots are also induced from the petals (Messeguer *et al.* , 1993). These floral structures are morphogenetic in a determined floral developmental pattern as was observed in petals by Kakehi (1979) and Gimelli *et al.* (1984). When adventitious shoots were regenerated from fragmented flower buds, individual petals and receptacles, the major site of shoot formation was the subepidermal cells at the proximal end of petal. The regeneration medium contained MS basal medium supplemented with 4-8 μM BA (Miller *et al.* , 1991). Frey and Janick (1991) regenerated plants from petals, calyces, nodes internodes and leaves, but only petals, calyces and nodes were regenerative from three cultivars examined (Scania, Improved White Sim and Sandra). Maximum proliferation was achieved with petals on MS medium supplemented with 0.05 μM TDZ and 0.5 μM NAA. Nugent *et al.* (1991) studied the effect of different cytokinin on regeneration from petal and stem explants of cultivar White Sim. They found that thidiazuron was more effective than BAP or kinetin and also stem derived plants grew faster than petal or receptacle derived plants.

Direct shoot differentiation from the base of the petal explant was obtained on MS medium supplimented with 2.0 mgl^{-1} IAA + 1.0 mgl^{-1} BA in which 60 per cent of explant differentiated (Mubarack and Choudhary, 1992).

An efficient procedure for adventitious shoot regeneration from various parts of carnation petals in liquid media was developed by Fisher *et al.* (1993). On the liquid medium shoot regeneration was obtained from nearly all the parts. The adventitious origin of the regenerative shoot was confirmed by scanning under electron microscope. Adventitious shoot regeneration from leaves, basal segment of flower and petals was studied by Messeguer *et al.* (1993). Petals and floral segments exhibited a high morphogenetic capacity in a wide range of growth regulator treatments. Lowering the BA concentration to 0.01 mgl^{-1} and NAA to 0.001 or 0.01 mgl^{-1} resulted in the suppression of the floral development pattern of petal explants and the regeneration of vegetative shoots. Nakano *et al.* (1994) studied adventitious shoot regeneration among the leaf, stem and petal explants on MS medium containing different concentrations of 6-BA and NAA. Regeneration was found to be high in media containing 5-10 μM BA with or without 5 μM NAA. Among the cytokinins tested N-2 chloro-4pyridyl induced organogenesis N'phenyl urea and zeatin on regeneration from petal explants.

2. 2 Indirect organogenesis

Enquind (1972) cultured callus and cell suspensions of carnation using $^1/_2$ MS medium with $3x10^{-6}$ M IAA combined with $3x10^{-6}$ M BAP and 10^{-6} M 2,4-D alone. He observed 100 fold increase in fresh weight with IAA + BAP and also in 2,4-D where cell suspension cultures had a doubling time of about 2 days. Malczewska *et al.* (1979) cultured callus tissues obtained from shoot apices of young plants on $^1/_2$ MS medium. Adventitioous shoots regenerated best on 0.03 μM NAA and 3.0 μM BA. Ruffoni *et al.* (1990) described shoot regeneration from callus and cell cultures of carnation. Friable callus was obtained by culturing segments on solid MS medium with 1 ppm of BA and

NAA. Highest regenarants were obtained in liquid media containing 7 ppm and 2 ppm NAA.

Choudhary and Mubarack (1991) investigated plant regeneration from leaf callus of three varieties viz. , Shoking Pink, William Sim and Arthur Sim. Early callus initiation was observed in 0.5 mgl^{-1} 2,4-D + 0. 5 mgl^{-1} NAA whereas presence of BA in the medium delayed callus initiation. Shoot regeneration occured only in the case of Shocking Pink callus when medium was supplemented with either 0.5 mgl^{-1} kinetin + 0.1 mgl^{-1} NAA or 0.25 mg 2,4-D + 0.5 mgl^{-1} NAA + 0.5 mgl^{-1} BA.

2.2.1 Somatic embryogenensis

Frey *et al.* (1992) induced somatic embryos from internodal callus in liquid basal MS medium supplemented with 3.0 μM 2,4-D followed by transfer to liquid basal medium lacking 2,4-D for embryo development. Nakano and Mii (1993) showed that antibiotics stimulate embryogenesis without plant growth regulators. They tested a range of antibiotics on several cultivars. Among them cefotoxime at 500 mgl^{-1} was most effective, and inducing somatic embryogenesis in 30.7 per cent of cultures after two months of culture. Choudhary and Chin (1995) reported that the occurrance of somatic embryogenesis in cell suspension cultures of White Sim. Callus was generated on MS basal medium suplemented with 2,4-D 5 or 10 μM, lack of 2,4-D during subsequent subcultures of cell suspensions resulted in the formation of somatic embryos.

2.3 Varietal response in carnation

Opera and Pampfil (1982) obtained virus free carnation cultures by meristem cultures of three carnation cvs. viz. , Lena, Scania and White Sim, cvs. Lena and Scania regenerated much better than the cv. White Sim. Radojevic *et al.* (1990) found variation in leaf rosette formation and axillary bud induction in different carnation cultivars, Tangerina, Scania, Park Purple, Arthur Sim, Lena and Telestar gave 42, 50, 73, 76, 90, and 93 per cent regeneration, respectively. They also showed that the choice of cytokinin depended on the cultivar response and kinetin was more favourable for shoot multiplication of Telestar, Lena and Scania while BA was better for Arthur Sim, Dark Purple, Tangerina and White Sim. Fal *et al.* (1991) used Scania, Improved White, Barbaret, Antares, Pink Calipso and Angeline for mass propagation by using meristem cultures and obtained varietal difference in the co-efficient of multiplication which ranged from 3 to 6.

2.4 *In vitro* rooting

For establishment of tissue cultured plants in open conditions, *in vitro* plantlets should have sufficient number of roots for absorption of nutrients and to provide anchorage. In most of the instances the presence of high cytokinin/auxin ratio in stage II inhibits root formation and therefore a separate medium for induction of roots has to be used at stage III. Since auxin is essential for root initiation normally the medium at stage III contains auxins. At the same time very high concentration of auxin inhibits root elongation (Thimmann, 1977) .

leaves. Survival of plantlets was increased by the transfer of plantlets from the uncapped culture vessels to a desiccator for 1-2 weeks before transplanting to soil. Sutter and Hutzell (1984) studied the use of humidity tents and antitranspirants in the acclimatization of tissue cultured chrysanthemum and carnation plants. They found that plants grown in the humidity tents were significantly larger and more vigorous than plants in any other treatment. Treatments with antitranspirants proved ineffective in improving vigour and survival of plants compared with to untreated ones.

Ravindra and Thomas (1995) designed sachet technique for efficient acclimatization of micropropagated plants. In which case 90-100 per cent plantlets established successfully.

2.6 Problems encountered in tissue culture of carnation

2.6.1 Vitrification

Meristem tip culture as a means of freeing carnations from viruses has been carried out since a long time. However, some cultivars when subjected to meristem culture, showed glassiness in the initial phase. In 1977 this became a serious problem in the Netherlands (Hakkart and Versluijs, 1983).

Vitrification is a physiological disorder frequently affecting both herbaceous and woody plants cultured *in vitro*. Vitrified shoots develop abnormally, producing short stem and thick fragile leaves with a translucent or glassy appearance. Debergh *et al.* (1981) made the first scientific examination of vitrification. Their investigations revealed that stage of the mother plant at the time of explantation do not interfere with vitrification.

Hakkart and Versluijs (1983) studied the effect of agar concentration, type of closure and temperature on vitrification. They found that increasing the agar concentration, from the usual 6 gl^{-1} to 12 gl^{-1} decreased glassiness, but at the same time reduced plant growth. The type of caps used for the tubes was also found to affect glassiness, the looser caps showed less glassiness. Exposure of carnation meristem tips to high temperature during the initial periods of culture favoured the development of normal plants.

Kevers *et al.* (1984) hypothesized that initiation of vitrification occurred due to stress induced ethylene production which is controlled by the peroxidase-IAA oxidase system. Excess of ethylene in the atmosphere of stressed plants would retroinhibit its own biosynthesis and as a consequence decrease the activity of PAL and acidic peroxidases, which hinder the lignification process. This also parallels decreases in cellulose synthesis due to conversion of sugar to amino acids. Deficiency of both cellulose and lignin could allow more water uptake due to reduced wall pressure and bring about the hyperhydric malformation.

High concentration of NAA in the culture medium increased and benzylamino purine decreased the proportion of vitrified shoots (Leshem and Sachs, 1985). Leshem *et al.* (1988) showed that use of high concentration of auxin in carnation resulted in bushy and vitrified plants whereas use of high cytokinin resulted in bushy and normal plants. Kim *et al.* (1988) showed that use of higher concentration of agar 15 gl^{-1} in MS medium or the addition of ABA (10^{-6} M) produced normal glacuous plantlets.

Structural changes occured during vitrification of carnation plantlets were studied by Werker and Leshem (1987). They showed three main differences from normal structure viz., i) reduction in the period in which cells remain meristematic in developing parts of the shoot, up to full maturation of the shoot apex itself. ii) hypertrophy of the cells, with a loss of a clear axis of elongation and iii) defective cell walls in which disintegration occurs at certain regions to form cavities.

Choudhary and Prakash (1991) showed that use of 12 per cent of agar was optimal, above which apices developed into small shoots instead of proliferating. They also reported that the proportion of vitrified plants was reduced by lowering the KNO_3 and NH_4NO_3 concentrations but was increased when the $CaCl_2$ concentration was doubled. Carnation plants obtained from vitrified plantlets serve as a source of somaclonal variation (Leshem, 1986).

Vitrification can be controlled by culturing the explant on a medium containing high concentration of agar and by frequent subculturing. Use of loose fitting screw caps for culture tubes is also beneficial. In addition right hormone concentration with a high cytokinin to auxin ratio (Miller *et al.*, 1991) and selection of appropriate media and cultural environment could minimize vitrification. Use of high concentration of penicillin in the medium eliminates or minimizes vitrification (Hu, 1991).

MATERIAL AND METHODS

III MATERIAL AND METHODS

The present study was conducted in the plant tissue culture laboratory (sponsored by Centre for Technology Development, Bangalore) of the Division of Horticulture, University of Agricultural Sciences, G.K.V.K. campus, Bangalore, during the year 1995-96.

3.1 Source of plant material

Two carnation varieties, one each of Spray (Sterlite Dop) and Standard type (IAHS-22) were used. Explants for the spray type were obtained from the polyhouse grown plants of Indian Institute of Horticultural Research, Hessaraghatta, Bangalore. For the standard type, explants were obtained from the plants grown in the polyhouse of the Division of Horticulture, University of Agricultural Sciences, G.K.V.K. campus, Bangalore. Shoot tips with one or two nodes were used as explants in both the cultivars for initial establishment of cultures.

3.1.1 Preparation of explant and surface sterilization

Shoot tips with one or two nodes, collected from healthy plants were immediately put into distilled water before bringing them to the laboratory for further sterilization.

Explants were sterilized by the following steps

1. Washing under running tap water for 1-1$^1/_2$ hour.
2. Dipping in 70 per cent alcohol for 45 seconds.
3. Disinfection with 0.2 per cent mercuric chloride along with two drops of Tween 20 for ten minutes, and
4. Thorough rinsing with sterile distilled water for 3 to 4 times.

Plate 1 : Specimen plants of IAHS-22

3.1.2 Culture initiation and maintenance of explants

The surface sterilized shoot tips were cultured on MS basal medium supplemented with 3 per cent sucrose under aseptic conditions. The cultures were maintained in this medium for about 3 weeks and were selected for further experiments. For the experiments, shoot tips with a single node were used. Explants were placed such that their base was in contact with the media. Cultures were incubated in the culture room at 25 $\pm 2^0$ C under 16 hours light and 8 hours dark conditions.

3.1.3 Stock solutions

Stock solution of macronutrients, micronutrients, vitamins and growth regulators required were prepared separately. They were stored in dark coloured bottles in refrigerator.

Stock solution of growth regulators

Stocks of kinetin and 6-benzylaminopurine (BAP) were prepared by dissolving them first in a few drops of 1 N NaOH and made up to required concentration with double distilled water. Stocks of 3-indolebutyric acid (IBA), 1-napthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were prepared by dissolving them in a few drops of ethanol and made up to required concentration by diluting with double distilled water.

3.1.4 Other media ingredients

Sucrose

The market grade cane sugar was used for all the experiments. Sugar was freshly weighed as required and dissolved in the medium before making up the volume.

Gelling agent

Agar (Bacteriological agar, DH) was used for all the experiments at a concentration of 8 gl^{-1}. To overcome the problem of vitrification 12 gl^{-1} agar was used in the rooting experiment.

3.1.5 Preparation of culture medium

The macronutirents, micronutrients and vitamins drawn from stock solutions were mixed in the required quantity and then volume was made up with distilled water. Sugar was added at the rate of 30 gl^{-1}. The growth hormones were added as necessary. The pH was adjusted to 5.8 by audition of 0.1 N HCl or 1 N NaOH as required. Agar was added at the rate of 8 gl^{-1} to the boiling solution.

For the comparison of media for shoot proliferation and for rooting 15 ml of medium was dispensed into test tubes of size 25x150 mm. For other experiments baby jars were used as culture vessels. For this, 30 ml of media was dispensed into culture vessels. Test tubes and jars were plugged with plastic caps. These vessels were then autoclaved at 121 °C and at a pressure of 1.2 Kg per sq cm for 20 minutes.

3.1.6 Preparation of transfer area for aseptic culture

Aseptic cultural conditions like final surface sterilization, preparation and inoculation of explants and their subculturing were carried out in a laminar air flow cabinet. Initially, before the use of laminar air flow cabinet, the working surface was swabbed with 70 per cent ethyl alcohol. Later the UV lamp was switched on for only 30 minutes followed by the air flow for at least 10 minutes before use. During the course of transfer, between each transfer the dissecting instruments were dipped in 70 per cent ethyl alcohol followed by dipping in glass bead sterilizer for 15-20 seconds and cooled before use. After inoculation, the laminar flow hood was cleaned and sprayed with 70 per cent ethyl alcohol and kept closed.

3.1.7 Incubation room

The cultures were incubated in an air conditioned room at a temperature of 25± 2°C, relative humidity of 60 per cent and under a photoperiodic regime of 16h light and 8h dark cycles.

3.1.8 Hardening of the *in vitro* plantlets

Young plantlets were taken out of the test tubes/baby jars and planted in plastic trays. These plants were hardened either in green house (60-70 per cent relative humidity) or in the mist chamber (relative humidity of 90 per cent and temperature of 34°C).

3.2.1 Selection of media for shoot proliferation

The following media were used

1. Murashige and skoog's medium (1962)
2. Gamborg's B_5 medium (1968)
3. Heller's medium (1953)
4. White's medium (1963)

The composition of all the four basal media is furnished in Appendix-I.

Varieties	:	Two (Sterlite Dop and IAHS-22)
Replications	:	10
Design	:	Factorial CRD

In all the media the growth regulators NAA at 2 µM and kinetin at 10 µM concentrations were used. Shoot tips with single node were cultured by placing them on the media so that their basal end is in contact with the medium.

Observations recorded :

a) Number of shoots

b) Number of leaves

c) Length of shoots

d) Biomass (Fresh weight)

3.2.2 Multiple shoot production with kinetin and 6-Benzyl amino purine(BAP)

The following concentrations of kinetin and BAP were used along with control

Kinetin	:	10, 20 and 40 µM
BAP	:	10, 20 and 40 µM
Control	:	No growth regulator
Varieties	:	Two (Sterlite Dop and IAHS-22)
Replication	:	10
Design	:	Factorial CRD
Explant	:	Shoot tip with a node was used from shoot tip cultures of the best medium.
Medium	:	MS full strength media was used in this experiment as this was found to be the best media for shoot proliferation.

Observations recorded

a) Number of adventitious buds or shoots produced/explant

b) Length of shoots

c) Number of leaves

3.2.3 *In vitro* rooting with auxins

IBA and NAA were tried in different concentrations as given below.

IBA	:	5, 10, 15 and 20 μM
NAA	:	5, 10, 15 and 20 μM
Control	:	No growth regulator.
Varieties	:	Two (Sterlite Dop and IAHS-22)
Replications	:	10
Design	:	Factorial CRD
Explant	:	Shoot tip with one or two nodes were used from the best concentration
Medium	:	MS full strength

Observations recorded

a) Time taken for root initiation

b) Number of primary roots produced per shoot

c) Length of roots

3.2.4 *In vitro* rooting at different media strengths

Media of different strengths as given below with a fixed level of best auxin concentration from the above experiment was tried.

a) Full strength

b) Half strength

c) Quarter strength

Varieties	:	Two (Sterlite Dop and IAHS-22)
Replications	:	10
Design	:	Factorial CRD
Explant	:	Shoot tip with one or two nodes from best concentration
Media	:	MS with 20 μM IBA

Observations recorded

a) Time taken for root initiation

b) Number of primary roots produced per shoot

c) Length of roots

d) Length of shoots

3.2.5 Hardening of *in vitro* rooted plantlets

The *in vitro* rooted plantlets from the best rooting media were transferred to different potting media as mentioned below.

a) Common pot mixture

b) Soilrite TC grade (Perlite: Peat)

c) Vermiculite

The plantlets were then placed either in green house (26°C temperature and relative humidity 65-70 per cent) or in mist chamber (34°C and relative humidity 95 per cent).

Varieties	:	Two (Sterlite Dop and IAHS-22)
Replication	:	10

Observations recorded

a) Percentage survival at fortnight interval

3.2.6 Callus production with different explant source and 2,4-D concentrations

Growth regulators	:	2,4-D at 0, 5, 10 and 20 μM
Media	:	MS medium
Varieties	:	Two (Sterlite Dop and IAHS-22)
Replications	:	10
Explant	:	Leaf, Shoot and Root

Observations recorded :

Amount of callus was recorded by scoring as indicated below.

(-)	:	No callusing
(+)	:	Poor callusing
(++)	:	Medium callusing
(+++)	:	Good callusing

3.2.7 Statistical analysis

Analysis of variance for the effect of different media on shoot proliferation, cytokinins on shoot multiplication, auxins on *in vitro* root production and effect of media strength were performed using Factorial Completely Randomized Design (Factorial CRD). The values presented for the effect of NAA and IBA on root production for the analysis was transformed by $\left(\sqrt{x+1}\right)$. Test for significance was conducted. Critical difference values were used for mean comparison.

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The results of the present studies on *in vitro* propagation of carnation in the two varieties, Sterlite Dop and IAHS-22 carried out at the Plant Tissue Culture Laboratory (Sponsored by Centre for Techonology Development, Bangalore) at the Division of Horticulture, University of Agricultural Sciences, G.K.V.K., Bangalore, are presented here under.

4.1 Shoot proliferation

4.1.1 Effect of different media

Data on the influence of media on the growth parameters of the explant in the two varieties is presented in Table 1.

a) Number of shoots

Significant differences were noticed with respect to number of shoots between the two varieties. The variety Sterlite Dop produced higher number of shoots (3.16) as compared to IAHS-22 (2.44).

Among the different media, highest number of shoots (4.82) was observed in MS medium followed by Gamborg (3.96). MS medium significantly differed from the other three media. On the other hand, least number of shoots was observed in Heller's medium (1.16) which was on par with White's medium (1.27).

Data on interaction effects clearly showed that the variety Sterlite Dop produced significantly highest number of shoots in MS medium(5.80). Least number of shoots was observed in Heller's medium (1.15). In IAHS-22 significantly highest number of shoots was produced in MS medium (3.85) and least number of shoots in White's medium was observed (1.17).

b) Shoot length

The cultivars differed significantly with respect to shoot length. It was significantly higher in case of Sterlite Dop (1.58 cm) as compared to IAHS-22 (1.25 cm).

Among the different media, maximum shoot length was obtained in Gamborg medium (1.54 cm). Least shoot length was recorded in Heller's medium (1.27 cm).

Interaction effect revealed that Sterlite Dop produced highest shoot length in Gamborg medium(1.89 cm) followed by MS medium (1.61 cm). Least shoot length was recorded in MS medium (1.30 cm). Whereas in IAHS-22 highest shoot length was observed in Heller's medium (1.30 cm) and least was observed in Heller's medium (1.16 cm).

c) Number of Leaves

Both the varieties differed significantly with respect to number of leaves. The variety Sterlite Dop produced higher number of leaves (14.11) as compared to IAHS-22 (12.86).

Among the different media, maximum number of leaves was recorded in MS medium (21.57) followed by Gamborg medium (18.17). MS medium differed

Table 1 : Growth parameters of the explants as influenced by different media in the two carnation varieties

	No. of shoots/explant	Shoot length (cm)	No. of leaves	Biomass (mg)
Varieties				
V_1: Sterlite Dop	3.16	1.58	14.11	260
V_2: IAHS-22	2.44	1.25	12.86	146
S. Em±	0.04	0.02	0.19	3
C.D at 5%	0.14	0.07	0.57	9
Medium				
M_1: MS	4.82	1.40	21.57	415
M_2 : Gamborg's	3.96	1.54	18.17	253
M_3: Heller's	1.16	1.27	6.97	75
M_4: White's	1.27	1.29	7.25	70
S.Em±	0.06	0.03	0.27	4
C.D at 5%	0.19	0.11	0.81	12
Interaction				
V_1 x M_1	5.80	1.61	23.12	551
V_1 x M_2	4.47	1.89	18.60	330
V_1 x M_3	1.15	1.40	7.25	89
V_1 x M_4	1.25	1.42	7.50	73
V_2 x M_1	3.85	1.30	20.02	280
V_2 x M_2	3.45	1.20	17.75	176
V_2 x M_3	1.17	1.15	6.70	61
V_2 x M_4	1.30	1.16	7.00	69
S.Em±	0.09	0.05	0.40	6
C.D at 5%	0.28	0.15	1.17	17
C.V %	6.87	7.81	5.44	5.78

Observations recorded after 4 weeks
Number of replications :10
Biomass=Fresh weight

Photo is not there book.

Sterlite Dop

IAHS-22

Plate 2 : Effect of different media on growth of *in vitro* cultures of carnations (MS=Murashige & Skoog's GB_5=Gamborg's HE=Heller's & WH=White's media)

significantly with all the other media. Least number of leaves was observed in Heller's and White's media (6.97 and 7.25, respectively), which were on par.

Interaction data revealed that in Sterlite Dop significantly highest number of leaves was recorded in MS medium (23.12) and least number of leaves was recorded in Heller's medium (7.25). For IAHS-22 highest number of leaves was observed in MS medium (20.02) and least was recorded in Heller's medium (6.70).

d) Biomass (Fresh weight)

Significant difference was noticed with respect to biomass production between the two varieties. It was significantly higher in the case of Sterlite Dop (260 mg) as compared to IAHS-22 (146 mg).

Among the different media, highest biomass production was in MS medium (415 mg) followed by Gamborg (253 mg). MS medium differed significantly with all the other three media. Least biomass production was observed in white's medium (70 mg) which was on par with Heller's medium (75 mg).

Data on interaction effect revealed that in Sterlite Dop highest biomass (551 mg) was recorded in MS and least in White's medium (73 mg). In IAHS-22 highest biomass (280 mg) was recorded in MS medium and least in Heller's medium (61 mg) which was on par with White's medium (69 mg).

4.1.2 Shoot proliferation with kinetin and BAP

Kinetin and 6-benzylaminopurine were used at different concentrations for induction of multiple shoots from the shoot tips. The results of the influence of kinetin and BAP on induction of multiple shoots in the two varieties are presented in Tables 2 and 3 respectively.

4.1.2.1 Shoot proliferation with kinetin

a) Number of shoots

Multiple shoot production differed significantly between the two varieties. Sterlite Dop produced 4.99 shoots per explant while IAHS-22 produced 2.89 shoots per explant.

Among the different kinetin concentrations significantly highest number of shoots (6.70) was recorded at 10 μM, which differed significantly from all the other treatments. No multiple shoot was observed in control.

Data on interaction effect revealed that the variety Sterlite Dop produced maximum number of shoots with 10 μM kinetin (10.20), whereas IAHS-22 produced maximum number of shoots at 20 μM of kinetin (4.50). In both the cultivars, in the absence of kinetin multiple shoot production did not occur.

b) Shoot length

Significant differences were noticed with respect to shoot length between two the varieties. Maximum shoot length of 2.32 cm was observed in Sterlite Dop and it was 2.11 cm in the case of IAHS-22.

Among the different treatments, the control recorded maximum shoot length (3.66

Table 2 : Growth parameters of the explants as influenced by kinetin in the two carnation varieties

	No. of shoots/explant	Shoot length (cm)	No.of leaves
Varieties			
V_1 : Sterlite Dop	4.99	2.32	21.37
V_2 : IAHS-22	2.89	2.11	17.21
S.Em ±	0.07	0.01	0.21
C.D at 5%	0.21	0.04	0.62
Kinetin Concentrations (µM)			
C_1 :Control	1.00	3.66	10.75
C_2 : 10	6.70	2.05	28.12
C_3 : 20	4.68	1.78	23.50
C_4 : 40	3.38	1.36	14.81
S.Em ±	0.10	0.02	0.30
C.D at 5%	0.30	0.06	0.87
Interactions			
V_1 x C_1	1.00	3.80	13.00
V_1 x C_2	10.20	2.10	35.55
V_1 x C_3	4.87	1.84	22.00
V_1 x C_4	3.90	1.53	15.00
V_2 x C_1	1.00	3.52	8.56
V_2 x C_2	3.21	2.00	20.75
V_2 x C_3	4.50	1.72	25.00
V_2 x C_4	2.87	1.20	14.62
S.Em±	0.14	0.03	0.42
C.D. at 5%	0.42	0.09	1.24
C.V.%	7.46	2.94	4.41

Observations were recorded after 5 weeks
Number of replications: 10

Sterlite Dop

IAHS-22

Plate 3 : Effect of kinetin on growth of *in vitro* cultures of carnations (Control, 1=10 μM, 2=20 μM & 3=40 μM)

cm) followed by kinetin at 10 μM (2.05 cm). On the other hand, least shoot length was observed in kinetin at 40 μM (1.36 cm). All the treatments differed significantly from one another with respect to shoot length.

Interaction data revealed that shoot length was highest in the case of control for both Sterlite Dop and IAHS-22, (3.80 and 3.52 cm, respectively). Both the varieties recorded least shoot length at 40 μM kinetin (1.53 and 1.20 cm in Sterlite Dop and IAHS-22, respectively).

c) Number of leaves

The two varieties differed significantly with respect to number of leaves. It was highest in Sterlite Dop (21.3) and least in IAHS-22 (17.21).

Kinetin at 10 μM gave significantly highest number of leaves (28.12). The least number of leaves was observed in the case of control (10.75).

As regards the interaction effect, Sterlite Dop with kinetin at 10 μM produced the maximum number of leaves (35.55) and control produced the least (13.00). IAHS-22 recorded a maximum of 20.75 leaves at 20 μM kinetin and least in control (8.56).

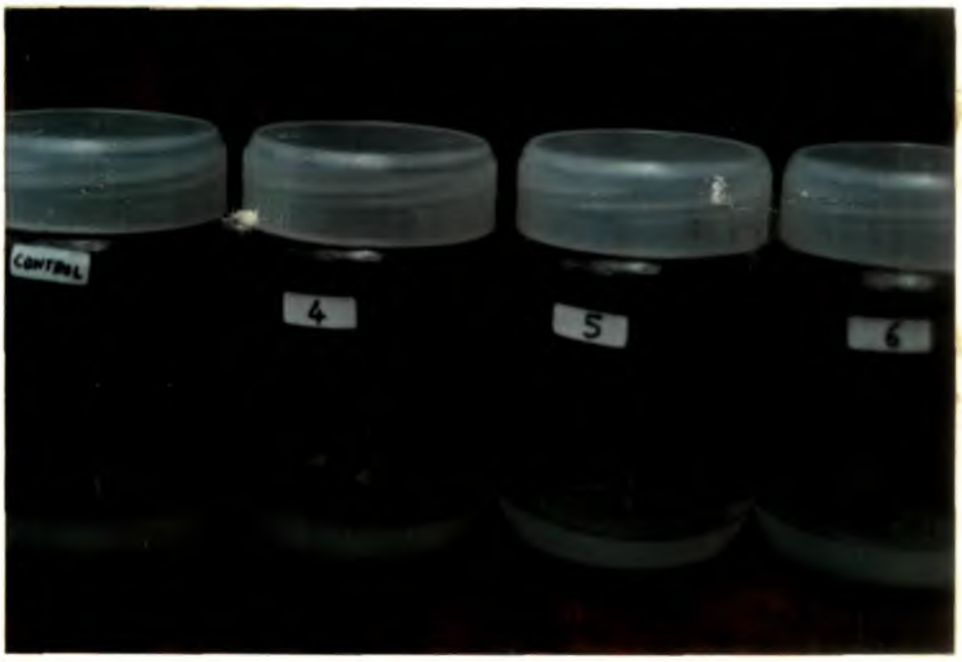
4.1.2.2 Shoot proliferation with BAP

The response of shoot tips of the two varieties of carnation to different concentrations of 6-benzylaminopurine is presented in Table-3

Table 3 : Growth parameters of the explant as influenced by BAP in the two carnation varieties

	No. of shoots/explant	Shoot length (cm)	No. of leaves
Varieties			
V_1: Sterlite Dop	3.98	2.25	17.28
V_2: IAHS-22	3.53	1.93	15.56
S.Em±	0.05	0.03	0.13
C.D.at 5%	0.14	0.09	0.40
BAP Concentrations (μM)			
C_1 : Control	1.00	3.62	10.93
C_2 : 10	7.26	1.74	26.75
C_3 : 20	3.90	1.57	15.75
C_4 : 40	2.87	1.45	12.25
S.Em±	0.07	0.04	0.19
C.D.at 5%	0.20	0.13	0.57
Interactions			
V_1 x C_1	1.00	3.75	13.12
V_2 x C_2	6.52	1.80	25.00
V_1 x C_3	4.80	1.72	16.00
V_1 x C_4	3.62	1.75	15.00
V_2 x C_1	1.00	3.50	8.75
V_2 x C_2	8.00	1.68	28.50
V_2 x C_3	3.00	1.42	15.50
V_2 x C_4	2.12	1.15	9.50
S.Em±	0.10	0.06	0.27
C.D.at 5%	0.29	0.19	0.81
C.V.%	5.31	6.25	3.39

Observations were recorded after 5 weeks
Number of replications :10



Sterlite Dop

IAHS-22

Plate 4 : Effect of BAP on growth of *in vitro* cultures of carnations (Control, 4=10 μM, 5=20 μM & 6=40 μM)

a) Number of shoots

Significant difference was observed with respect to number of shoots between the two the varieties. The variety Sterlite Dop recorded highest number of shoots (3.98) as compared to IAHS-22 (3.53)

Highest number of shoots was recorded with 10 μM BAP (7.26). This treatment differed significantly with all the other treatments. Least number of shoots was observed in 40 μM BAP (2.87). There was no multiple shoot production in control.

Interaction effect due to variety and BAP concentration was significantly different with respect to number of shoots. Both Sterlite Dop and IAHS-22 produced maximum number of shootswith 10 μM of BAP (6.52and 8.00, respectively). Multiple shoots were not produced in control.

b) Shoot length

Significant difference was observed with respect to shoot length between the two varieties. Higher shoot length was recorded in Sterlite Dop (2.25 cm) as compared to IAHS-22 (1.93 cm).

Among the different BAP concentrations, maximum shoot length was recorded in control (3.62 cm). Least shoot length was recorded in 40 μM BAP (1.45 cm).

Both Sterlite Dop and IAHS-22 recorded highest shoot length of 3.75 and 3.50 cm, respectively in control. Sterlite Dop recorded least shoot length at 20 μM BAP (1.72 cm) and which was on par with 40 μM BAP (1.75 cm) whereas, IAHS-22 recorded least shoot length (1.15 cm) at 40 μM BAP.

c) Number of leaves

Differences were significant for number of leaves between the two varieties. Sterlite Dop recorded maximum number of leaves (17.28) followed by IAHS-22 (15.56).

Maximum number of leaves was recorded at 10 μM BAP (26.75) followed by 20 μM BAP (15.75) and it was least in control (10.93). All the treatments differed significantly with one another.

Data on interaction effects revealed that Sterlite Dop produced maximum number of leaves at 10 μM BAP (25.00) and least in control (13.12). In IAHS-22 maximum number of leaves was recorded at 10 μM BAP (28.50) and least in case of control (8.75).

4.2 *In vitro* rooting

4.2.1 *In vitro* rooting with auxins.

In this experiment effect of NAA and IBA at different concentrations on the induction of adventitious roots in the microshoots was studied in the two varieties. The results of the experiments are presented below.

The data regarding the effects of NAA and IBA on number of days taken for rooting, number of roots per explant and length of the roots after 5 weeks of incubation are presented in Tables 4 and 5.

4.2.1.1 *In vitro* rooting with NAA

The data in Table 4 shows that significant difference was seen with respect to days taken for rooting between the two varieties. Early rooting occured in Sterlite Dop (9.82 days) and it was delayed in IAHS-22 (17.12 days).

Among the different NAA concentrations NAA at 10, 15 and 20 μM took equal number of days (10.00) for root initiation followed by NAA at 5 μM (10.43 days). Shoots in control took maximum number of days for rooting (26.62 days).

Interaction effect indicated that earliest rooting was observed in Sterlite Dop at 20 μM of NAA (7.00 days) whereas in control it was (18.25 days). In IAHS-22 early rooting was observed at 10 μM NAA (11.62 days) whereas in control even on 35th day there was no rooting. In both the varieties, the higher concentrations (15 and 20 μM) encouraged high callus production followed by rooting.

b) Number of roots

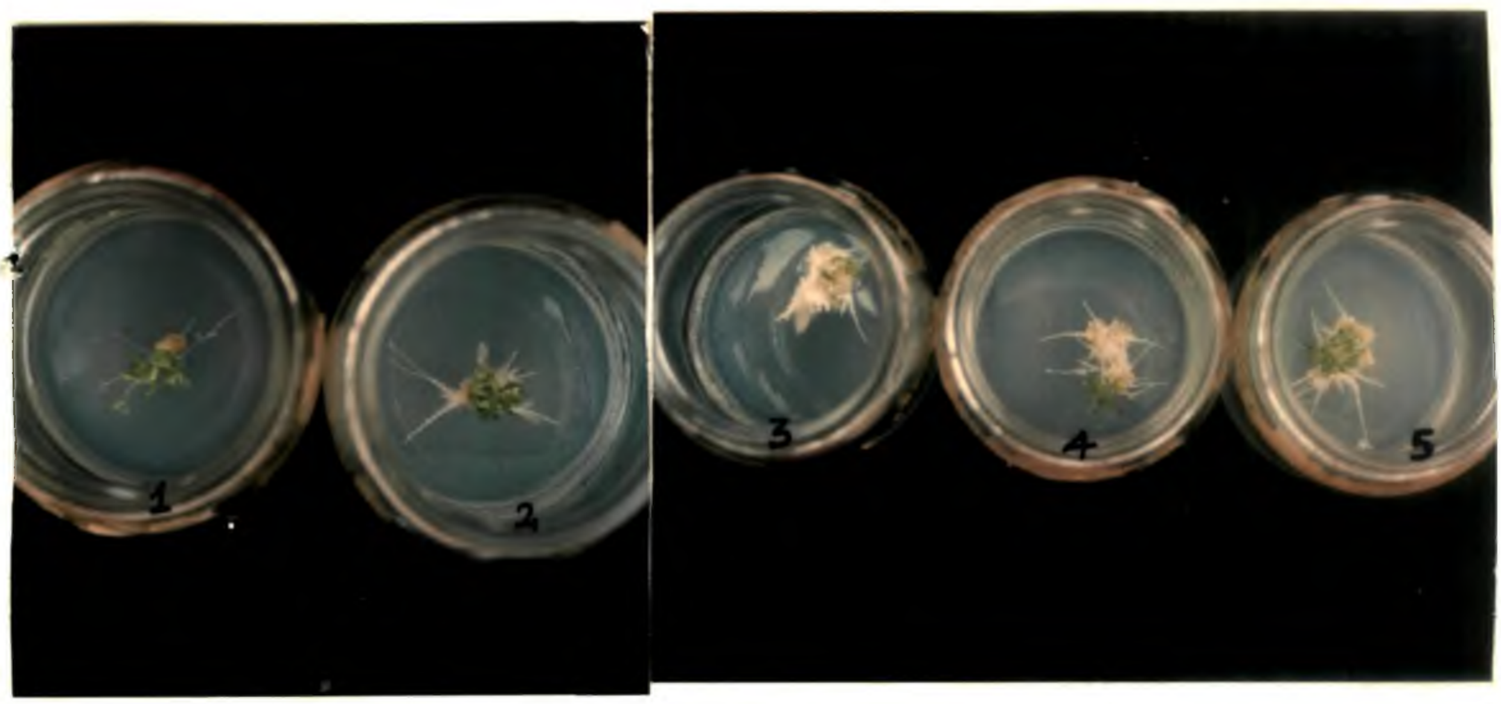
Significant differences were noticed with respect to number of roots between the two varieties. Sterlite Dop produced higher number of roots (5.12) as compared to IAHS-22 which produced 3.75 roots.

Among the different NAA concentrations highest number of roots was recorded at 15 μM (6.37), followed by 20 μM (6.18). It was least in control (1.75). All the treatments differed significantly from one another. As the concentration of NAA increased, callus formation also increased.

Table 4 : Effect of NAA on *in vitro* rooting in the two carnation varieties

	No. of days taken for rooting	No. of roots	Root length (cm)
Varieties			
V_1 : Sterlite Dop	9.82 (3.23)	5.12 (2.45)	0.96 (1.39)
V_2: IAHS-22	17.12 (4.15)	3.75 (2.07)	0.50 (1.21)
S.Em±	0.02	0.03	0.07
C.D.at 5%	0.06	0.11	0.02
NAA Concentrations (μM)			
C_1 : Control	26.62 (5.19)	1.75 (1.55)	0.59 (1.23)
C_2 : 5	10.43 (3.36)	3.21 (2.01)	0.78 (1.32)
C_3 : 10	10.00 (3.29)	4.75 (3.37)	1.19 (1.47)
C_4 : 15	10.00 (3.29)	6.37 (2.71)	0.57 (1.25)
C_5 : 20	10.00 (3.29)	6.18 (2.67)	0.52 (1.23)
S.Em±	0.03	0.06	0.01
C.D.at 5%	0.09	0.17	0.03
Interactions			
V_1 x C_1	18.25 (4.35)	3.50 (2.11)	1.18 (1.47)
V_1 x C_2	8.12 (3.02)	3.75 (2.17)	1.17 (1.47)
V_1 x C_3	8.25 (3.04)	6.00 (2.64)	1.50 (1.58)
V_1 x C_4	7.57 (2.91)	6.00 (2.64)	0.50 (1.22)
V_1 x C_5	7.00 (2.82)	6.37 (2.71)	0.47 (1.21)
V_2 x C_1	35.00 (6.00)	0.00 (1.00)	0.00 (1.00)
V_2 x C_2	12.75 (3.70)	2.50 (1.86)	0.40 (1.18)
V_2 x C_3	11.62 (3.55)	3.50 (2.10)	0.86 (1.37)
V_2 x C_4	12.50 (3.67)	6.75 (2.78)	0.65 (1.28)
V_2 x C_5	13.75 (3.83)	6.00 (2.64)	0.57 (1.25)
S.Em±	0.04	0.08	0.01
C.D.at 5%	0.13	0.24	0.04
C.V %	2.52	7.42	2.49

The figures in the parentheses indicate the transformed values
Observations were recorded after 5 weeks
Number of replications :10



Sterlite Dop

IAHS-22

Plate 5 : Effect of NAA on growth of *in vitro* cultures of carnations
(1=Control, 2=5 µM, 3=10 µM, 4=15 µM & 5=20 µM)

Data on the interaction effect revealed that in Sterlite Dop 6.37 roots were produced with NAA at 20 μM which was the highest. Least was observed in control (3.50). IAHS-22 produced maximum number of roots at 15 μM NAA (6.75) and no root was produced in the absence of NAA.

c) Root length

Significant difference was observed for root length between the two varieties. Maximum root length of was observed in Sterlite Dop (0.96 cm) and in IAHS-22 it was 0.50 cm.

Among the different NAA concentrations, all the treatments differed significantly with respect to root length. Maximum root length was recorded at 10 μM of NAA (1.19 cm). Least root length was recorded at 20 μM NAA (0.52 cm). Extensive callusing was observed under higher concentrations of NAA (15 and 20 μM).

Data on interaction effect revealed that Sterlite Dop produced maximum root length in control (1.18 cm) and least root length in 20 μM NAA (0.47 cm). IAHS-22, recorded the maximum root length at 10 μM of NAA (0.86 cm). Rooting did not occur in control.

4.2.1.2 *In vitro* rooting with IBA

Data on the effect of IBA on *in vitro* rooting is presented in Table 5.

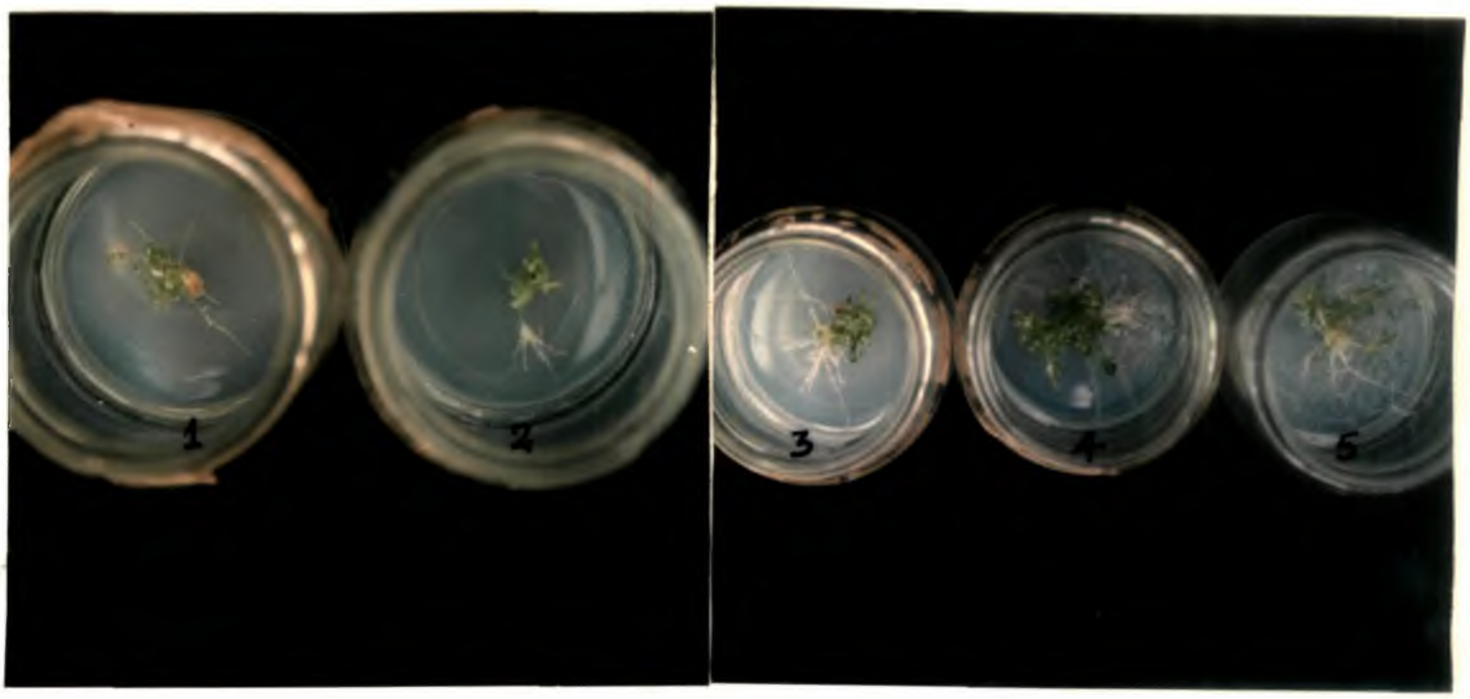
a) Days taken for rooting

Significant difference was observed with respect to days taken for rooting

Table 5 : Effect of IBA on *in vitro* rooting in the two varieties of carnation

	No. of days taken for rooting	No. of roots	Root length (cm)
Varieties			
V_1 : Sterlite Dop	12.75 (3.67)	5.98 (2.61)	2.32 (1.80)
V_2 : IAHS-22	23.22 (4.88)	2.96 (1.91)	1.08 (1.43)
S.Em±	0.01	0.01	0.01
C.D.at 5%	0.04	0.04	0.04
IBA Concentrations (µM)			
C_1 : Control	26.50 (5.17)	1.55 (1.50)	0.62 (1.24)
C_2 : 5	18.81 (4.43)	3.43 (2.07)	1.55 (1.61)
C_3 : 10	15.62 (4.03)	4.62 (2.37)	1.90 (1.61)
C_4 : 15	15.56 (4.02)	5.75 (2.59)	2.00 (1.68)
C_5 : 20	13.43 (3.75)	7.00 (2.82)	2.45 (1.71)
S.Em±	0.02	0.02	0.02
C.D.at 5%	0.07	0.07	0.06
Interactions			
V_1 x C_1	18.00 (4.35)	3.12 (2.02)	1.25 (1.49)
V_1 x C_2	15.50 (4.06)	4.92 (2.43)	2.20 (1.78)
V_1 x C_3	10.50 (3.39)	6.25 (2.69)	2.57 (1.88)
V_1 x C_4	10.62 (3.40)	7.25 (2.87)	2.70 (1.91)
V_1 x C_5	9.12 (3.18)	8.37 (3.06)	2.90 (1.95)
V_2 x C_1	35.00 (6.00)	0.00 (1.00)	0.00 (1.00)
V_2 x C_2	22.12 (4.80)	1.95 (1.71)	0.90 (1.44)
V_2 x C_3	20.75 (4.66)	3.99 (1.99)	1.22 (1.48)
V_2 x C_4	20.50 (4.68)	4.25 (2.29)	1.30 (1.51)
V_2 x C_5	17.75 (4.33)	5.62 (2.57)	2.00 (1.72)
S.Em±	0.03	0.03	0.03
C.D.at 5%	0.10	0.10	0.09
C.V %	1.64	3.19	3.95

The figures in the parantheses indicate the transformed values
Observations were recorded after 5 weeks
Number of replications :10



Sterlite Dop

IAHS-22

Plate 6 : Effect of IBA on rooting of *in vitro* cultures of carnations
(1=Control, 2=5 μM, 3=10 μM, 4=15 μM & 5=20 μM)

between the two varieties. In Sterlite Dop early rooting was observed (12.75 days), whereas IAHS-22 took longer time for rooting (23.22 days).

Among the different IBA concentrations earliest rooting was observed at the highest IBA concentration of 20 μM (13.43 days) followed by 15 and 10 μM (15.56 and 15.62 days, respectively). Microshoots took longer time for rooting (26.50 days) in control.

Data on interaction effect revealed that in Sterlite Dop early rooting occurred at 20 μM IBA (9.12 days). In control it took maximum number of days (21.62). In IAHS-22 also early rootingwas observed at 20 μM IBA (17.75 days) and in control even on 35th day no rooting was observed.

b) Root number

Significant difference was noticed with respect to root number between the two varieties. Of the two varieties, Sterlite Dop produced higher number of roots (5.98) as compared to IAHS-22 (2.96).

Among the different IBA concentrations significantly highest number of roots was recorded at 20 μM of IBA (7.00). This was followed by 15 μM IBA which produced 5.75 roots. Least number of roots was recorded in control (1.55).

Interaction effect revealed that Sterlite Dop produced highest number of roots 20 μM IBA (8.31). Least number of roots was recorded in control (3.12). IAHS-22 also recorded highest number of roots at 20 μM of IBA (5.82). But no root formation was observed in the control.

c) Root length

Significant difference was noticed with respect to root length between the two varieties. Sterlite Dop recorded maximum root length (2.32 cm) whereas in IAHS-22 it was 1.08 cm.

Among the different IBA concentrations, all the treatments differed significantly with respect to root length. Maximum root length was recorded at 20 μM (2.45 cm) followed by15 μM (2.00 cm). Least root length of 0.62 cm was recorded in control.

Data on interaction effect revealed that Sterlite Dop recorded maximum root length at 15 μM IBA (2.90 cm) and least root length was recorded in control (1.25 cm). IAHS-22 recorded maximum root length at 20 μM IBA (2.00 cm) and no rooting was observed in control.

4.2.2 Media strength and *in vitro* rooting

The results on the effect of different strengths of MS media on rooting of micro shoots of the two varieties are presented in the Table-6. IBA at 20 μM was used in the media for both the varieties.

a) Days taken for rooting

Significant difference was noticed with respect to days taken for rooting between the two varieties. Early rooting was observed in Sterlite Dop (9.41 days), whereas IAHS-22 took longer duration to root (18.45 days).

Table 6: Effect of media strength on *in vitro* rooting in the two carnation varieties

	No. of days taken for root initiation	No. of roots	Root length (cm)	Shoot length (cm)
Varieties				
V_1 : Sterlite Dop	9.41	6.93	2.01	3.00
V_2 : IAHS-22	18.45	4.48	1.78	2.76
S. Em±	0.15	0.10	0.03	0.03
C.D at 5%	0.46	0.29	0.10	0.09
Media Strength				
M_1: Full strength	13.93	6.18	2.27	2.51
M_2: Half Strength	14.00	5.86	2.16	4.40
M_3: QuarterStrength	13.87	5.07	1.27	1.73
S. Em±	0.19	0.12	0.04	0.04
C.D at 5%	NS	0.36	0.12	0.11
Interaction				
V_1 x M_1	9.50	7.25	2.45	2.57
V_1 x M_2	9.37	7.02	2.37	4.38
V_1 x M_3	9.37	6.52	1.35	2.05
V_2 x M_1	18.37	5.12	2.10	2.45
V_2 x M_2	18.62	4.70	1.96	4.42
V_2 x M_3	18.37	3.62	1.20	1.42
S. Em±	0.27	0.17	0.06	0.05
C.D.at 5 %	NS	0.51	0.18	0.16
C.V.%	3.43	6.63	6.68	3.95

Observations were recorded after 5 weeks
Number of replications :10
NS: Non significant

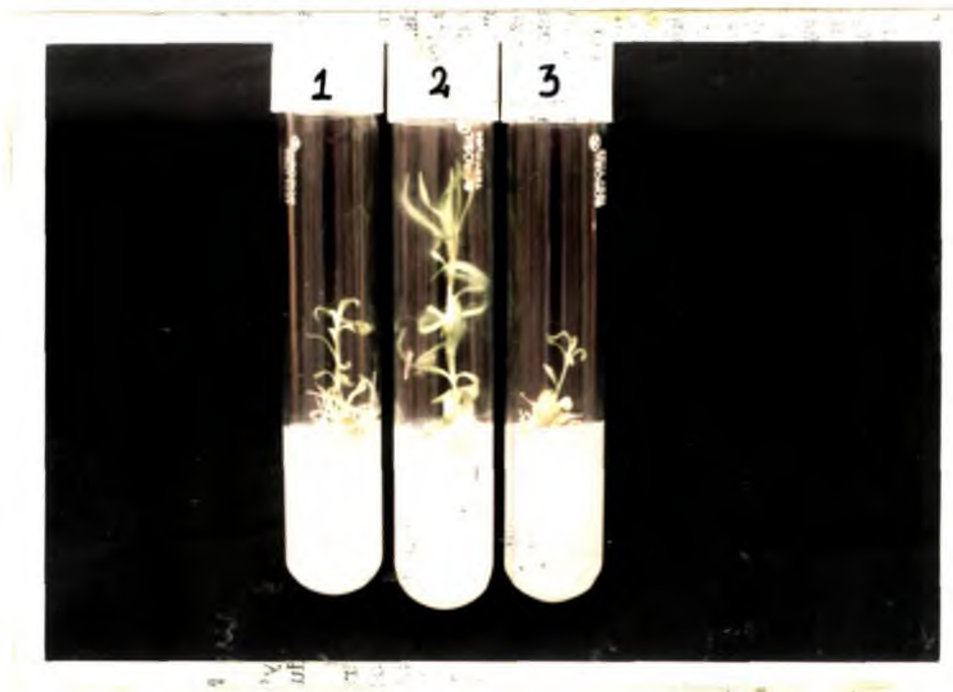


Plate 7 : Effect of media strength on rooting of *in vitro* cultures of Sterlite Dop carnation
(1=Full strength, 2=Half strength & 3=Quarter strength)

Results were not significantly different for media strength, and the interaction between variety and media strength.

b) Root number

Significant difference was noticed with respect to root number between the two varieties. Sterlite Dop produced higher number of roots (6.93), whereas IAHS-22 produced the least (4.48).

Among the different strengths of MS media, full strength medium produced highest number of roots (6.18) and quarter strength medium recorded the least (5.07).

Data on interaction effect revealed that Sterlite Dop recorded maximum number of roots in full strength medium (7.25) and least in quarter strength medium (6.52). IAHS-22 produced maximum number of roots in full strength medium (5.12) and least in quarter strength medium (3.62).

c) Root length

The two varieties differed significantly for root length. Sterlite Dop recorded higher root length (2.01 cm) as compared to IAHS-22 (1.78 cm).

Among the different media strengths maximum root length was recorded in full strength medium (2.27 cm), followed by half strength medium which was on par with full strength medium (2.16 cm). Least root length was observed in quarter strength medium (1.27 cm).

Data on interaction effect revealed that Sterlite Dop recorded maximum root length in full strength MS medium (2.45 cm) and least in quarter strength medium (1.35 cm). IAHS-22 also recorded maximum root length in full strength medium (2.10 cm) and least in quarter strength medium (1.20 cm).

d) Shoot length

The two varieties differed significantly for shoot length. Sterlite Dop recorded the maximum shoot length (3.00 cm) and IAHS-22 recorded the least shoot length (2.76 cm).

Among the different media strengths maximum shoot length was recorded in half strength MS medium (4.40) followed by full strength MS medium (2.54 cm). Least shoot length was recorded in quarter strength (1.73 cm).

Data on interaction effect revealed that Sterlite Dop recorded maximum shoot length in half strength MS medium (4.38 cm) and least in quarter strength medium(2.05 cm). The variety IAHS-22 also showed maximum shoot length in half strength medium (4.42 cm) and least in quarter strength medium (1.42 cm).

4.3 Hardening and establishment of *in vitro* grown plantlets

Slight vitrification of microshoots occured when they were rooted in the medium containing 0.8 per cent agar. Hence microshoots were rooted *in vitro* in the medium containing 1.2 per cent agar. These plantlets were thoroughly washed to remove the media adhering to the root system . Three different potting media viz., common pot mix, soilrite and vermiculite were used to harden *in vitro* rooted carnation plantlets, in mist chamber and greenhouse. Survival percentage of the plantlets was taken at 15 and 30 days after planting.

Table 7 : Survival of plantlets in different potting media under mist chamber

Media	Survival percentage			
	Strerlite Dop		IAHS-22	
	15 days	30 days	15 days	30 days
Common pot mix	40	0	25	0
Soilrite	100	100	80	75
Vermiculite	40	20	20	0

Number of replications : 10

Sterlite Dop

IAHS-22

Plate 8 : Weaned *in vitro* cultures of carnations

4.3.1 Establishment in greenhouse

In the greenhouse all the plantlets of both the varieties, which were transfered to different potting media failed to survive even for 15 days.

4.3.2 Establishment in mist chamber

In the mist chamber the plantlets of both the varieties survived and established. Sterlite Dop showed 100 per cent survival on 15th day in soilrite followed by 40 per cent survival in both common pot mix and vermiculite media. Whereas in the case of IAHS-22 only 80 per cent of the plantlets survived in soilrite mix, followed by 25 per cent survival in vermiculite and 20 per cent survival in common pot mix.

On 30th day Sterlite Dop showed 100 per cent survival in soilrite medium and 20 per cent survival in vermiculite medium but in common pot mix all the plants died. On the contrary IAHS-22 showed 75 per cent survival in soilrite medium but none of the plantlets survived in both common pot mix and vermiculite media.

4.4 Callus production with different explant source and 2,4-D concentrations

The effect of 2,4-D on callus induction of leaf, stem and root explants of the two carnation varieties, viz., Sterlite Dop and IAHS-22 were investigated and the data are presented in Table-8

The auxin 2,4-D at various concentrations induced callus in leaf stem and root explants of both the varieties.

Table 8 : Callus production in the two carnation varieties as influenced by explant source and 2,4-D concentrations

2,4-D Concentrations (μM)	Leaf	Stem	Root
Sterlilte Dop			
Control	+	+	-
5	+++	++	+
10	+++	++	++
20	++	+	+
IAHS-22			
Control	+	+	-
5	+++	+++	+
10	+++	++	++
20	++	++	+

(-) : No callusing

(+) : Little callusing

(++) : Medium callusing

(+++) : Good callusing

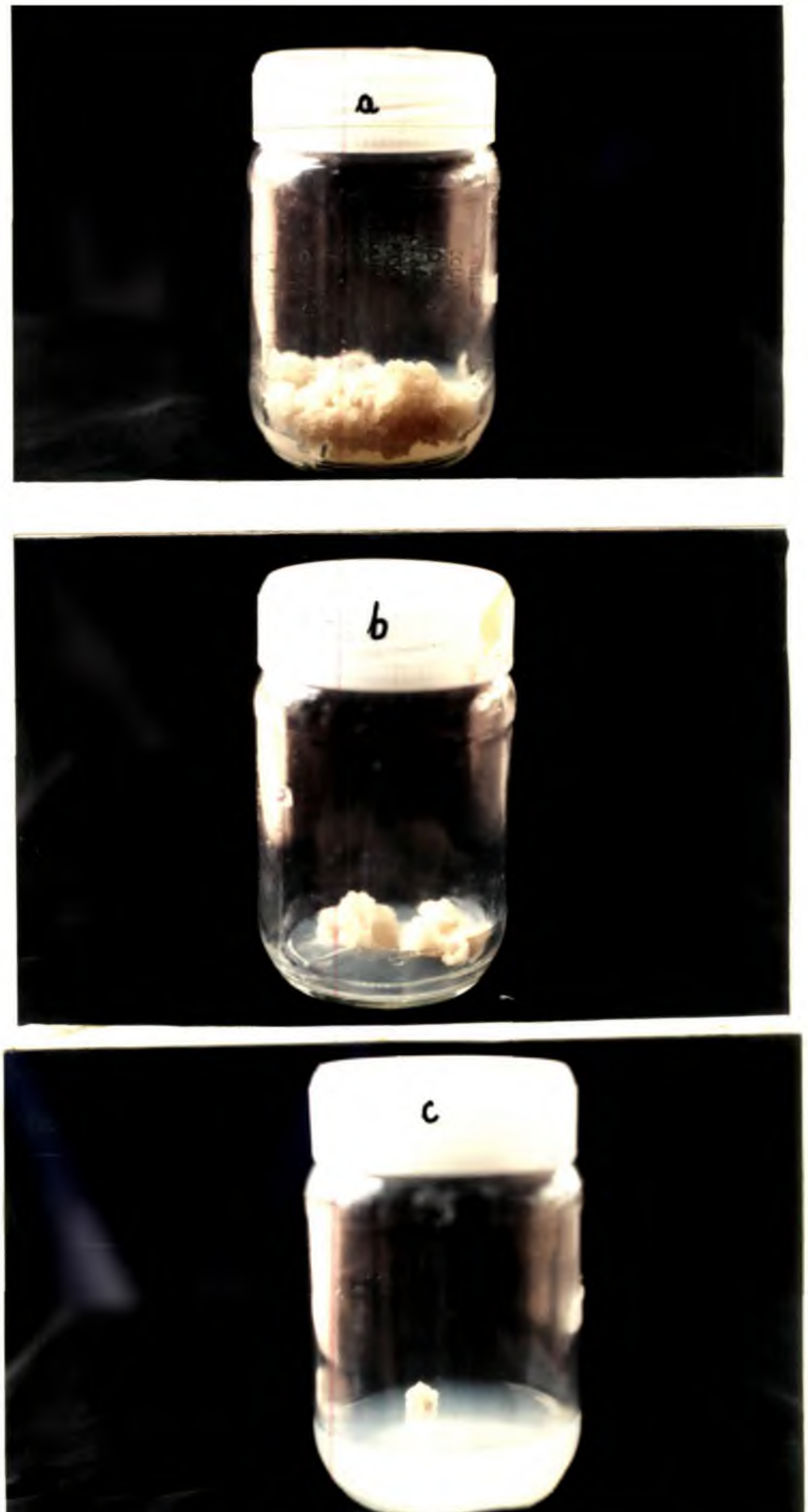


Plate 9 : Callus obtained from different explants of carnations (a=Leaf, b=Stem & c=Root)

a) Leaf

In both Sterlite Dop and IAHS-22 when leaf was used as an explant good callusing (+++) was observed at 5 and 10 μM of 2,4-D and it was least (+) in control for both the varieties.

b) Stem

Sterlite Dop produced good callus from stem explant, at 5 μM of 2,4-D and little callusing was observed both in control and 20 μM 2,4-D. Whereas IAHS-22 produced good amount of callus(+++) at 5 μM 2,4-D and least callusing (+) was observed in control.

c) Root

Both Sterlite Dop and IAHS-22 produced moderate amount of callus (++) at 10 μM, 2,4-D and no callusing (-) was observed in the absence of 2,4-D.

DISCUSSION

V DISCUSSION

The present studies on *in vitro* propagation of carnation (*Dianthus caryophyllus*) was carried out to standardize protocols for multiple shoot production, *in vitro* rooting, hardening of *in vitro* plantlets and callus production in the two carnation varieties Sterlite Dop and IAHS-22. The results of the experiments are discussed here under.

5.1 Shoot proliferation in different media

Good shoot proliferation was observed in MS and Gamborg media. Similar result was obtained by Choudhary (1992), who observed good shoot production in carnation, in solid and liquid media of both MS and Gamborg. The explant produced highest number of shoots (4.82) and leaves (21.57), and maximum biomass (415 mg) in MS medium. Increased number of shoot and leaves, and biomass obtained may be due to the higher concentration of salts and nutrients in MS medium as compared with Gamborg. MS medium has been successfully used in tissue culture of carnation (Meristem and shoot tip culture: Shabde and Murashige, 1977, Ioannov, 1990; Node culture : Roset and Bokelmann, 1981; Organogenesis from petals: Miller *et al.*, 1991 and : Somatic embryogenesis : Frey *et al.*, 1992).

Varieties Sterlite Dop and IAHS-22 responded well for shoot proliferation. Sterlite Dop produced higher number of shoots (3.16) than IAHS-22 (2.44). Sterlite Dop is a spray type where as IAHS-22 is a standard type. Hence the difference in *in vitro* shoot production between these two varieties appear to be due to the inherent varietal characters.

Gamborg medium was significantly superior to Heller's and White's media in inducing shoot proliferation. But the shoots in this medium turned yellowish green after four weeks. This may probably be due to lower concentrations of nutrients as compared with MS media. Shoot proliferation was very poor with Heller's and White's media. This could also be accounted to comparatively low concentration of nutrients.

5.2 Shoot proliferation with cytokinins

The effect of cytokinins is most noticeable in tissue cultures as they appear to be necessary in plant cell division. Cytokinins added to shoot culture media overcome apical dominance and release lateral buds from dormance. Despite the occurrence of endogenous cytokinins in whole plants, many tissues and small organs isolated from them and cultured in *in vitro* are unable to synthesize sufficient quantities of these substances to sustain growth . Hence a low level of cytokinins is frequently required to be added to the culture medium. Addition of cytokinin to the media induces the growth of several tiny shoots from each explant within a span of 4 to 6 weeks. The effect of cytokinins on tissue and organ cultures can vary according to the particular compound used, the type of culture and the variety from which it was derived . Keeping this in view, efficacy of two different cytokinins viz., kinetin and BAP at different concentrations on multiple shoot production was studied in the second experiment of the present investigations (Tables 2 and 3)

Varieties Sterlite Dop and IAHS-22 differed in their response to different source of cytokinins. Sterlite Dop produced higher number of shoots (4.99) with kinetin while IAHS-22 produced higher number of shoots (3.53) with BAP as a cytokinin source.

Similar observations of differential response of varieties to different source of cytokinin was also observed by Radojevic *et al.* (1990). They showed that kinetin was more favourable for shoot multiplication of Telestar, Lena and Scania cultivars of carnation while BA was better for cultivars Arthur Sim, Dark purple, Tangerina and Whit Sim.

Both kinetin and BAP induced the production of maximum number of shoots (6.70 and 7.26, respectively) at 10 μM concentration. When BAP and Kinetin were compared at a concentration of 10 μM, BAP was found to be the best source of cytokinin for shoot proliferation.

Sterlite Dop produced maximum number of shoots (10.20) with kinetin at 10 μM, where as IAHS-22 produced maximum number of shoots (8.00) with BAP at 10 μM. Concentration of 10 μM kinetin (Davis *et al.*, 1977) and 5 to 15 μM of BA (Miller *et al.*, 1991) have been shown to be the best for shoot proliferation in carnation.

In the media without cytokinin no shoot proliferation was observed, but the growing shoots were significantly taller compared with shoots produced in media containing cytokinins. This increased shoot length in media without cytokinins is likely to be due to apical dominance in the shoot.

The variety Sterlite Dop also produced roots in the cytokinin free media. It appears that the variety Sterlite Dop is auxin habituated.

5.3 *In vitro* rooting with auxins

NAA induced early rooting in both Sterlite Dop and IAHS-22 as compared with IBA. Early rooting observed with NAA may probably be due to the fact that NAA is a stronger auxin and hence may have caused early root initiation. Though NAA produced early rooting there was an increased amount of callus production with increase in NAA concentration. But in IBA even at the highest concentration of 20 μM, callusing was not observed. NAA at 15 μM produced highest number of roots (6.37) but mean root length was only 0.57 cm. Root length was appreciable at 10 μM (1.19 cm). The increased callus production observed at 15 μM might have suppressed the development of roots. It is also now known that auxins generally inhibit the development of roots. Hence it is also likely that NAA was detrimental to development of roots. IBA, being considered a comparatively weaker auxin, may have delayed initiation of roots and it may not have influenced the development of roots much.

Early rooting was observed in Sterlite Dop with both NAA and IBA medium as compared with IAHS-22. Number of roots were also more and longer in Sterlite Dop. This difference between these two varieties may be due to the varietal specificity.

Sterlite Dop has been observed to root even in the absence of any growth regulator. In IAHS-22 even on 35th day none of the shoots were able to produce any roots in control. Sometimes tissues, organs or strains of cells arise in cultures which are able to grow without the addition of any auxin to the medium. They are said to be auxin autonomous or auxin habituated (George and Sherrington 1984).

5.3.1 *In vitro* rooting at different media strength

Rooting was observed in all the three media strengths. The time taken for rooting did not differ significantly among the three media strengths. There was no significant differences in number of roots and root length, between full and half strength MS media which were superior to quarter strength medium. However length of the shoot was significantly higher in half strength MS media (4.40 cm). The external appearance of the shoot was good. The plants rooted in half strength MS medium grew quickly and had reached transplantable height within 35 days after culture. The growth of the shoot with full and quarter strength MS were comparatively poor and had not reached transplantable height by 35th day. This suggests that for plant growth and development, requirement of nutrients varies at different stages. At shoot development stage, plant growth was better in high salt concentration and at rooting stage low salt media is preferred by plantlets.

5.4 Hardening and establishment of *in vitro* grown plantlets

The microshoots rooted in 0.8 per cent agar showed vitrification. These plantlets were unable to survive in both green house and mist chamber. Hence the microshoots were rooted in 1.2 per cent agar. These plantlets showed no vitrification. These *in vitro* produced plantlets were then transferred to greenhouse or mist chamber for further hardening.

None of the plants kept in greenhouse survived while a good percentage of plantlets transferred to mist chamber survived. This may probably be due to lower humidity level in the green house (65-70 per cent). It is known that plantlets under *in vitro* conditions are exposed to 90-100 per cent humidity and hence transfer of this plantlets to 65-70 per cent humidity as seen in the greenhouse may not have been congenial for their survival. Low survivability of carnation plantlets under greenhouse was also observed by Sutter and Langahans. (1979).

In the soilrite medium 100 per cent survival was observed in Sterlite Dop and 75 per cent in IAHS-22 after 30 days. Higher survival in soilrite might be due to the optimum conditions of aeration, WHC and nutrients present in this media, as soilrite media contained peat and perlite. In vermiculite only 20 per cent survival was observed in Sterlite Dop and none in IAHS-22. Poor survivability in vermiculite compared with soilrite could be accounted to high WHC and consequent waterlogged conditions in mist chamber. In common pot mix initially, for 15 days plants were normal and green but after that all the plants started dying. In this compact media delicate *in vitro* roots of carnation probably could not establish.

5.5 Callus production with different explant source and 2,4-D concentrations

Callus production was obtained from leaf, stem and root explants of carnation with the use of 2,4-D. Use of 2,4-D for callus induction is well documented. In carnation also, 2,4-D has been successfully used for induction of callus (Choudhary and Chin, 1995 ; Frey *et al.*, 1992 ; Enquind, 1972).

In the present experiment leaf as an explant performed better. Good callusing (+++) of the leaf explant was obtained for both the varieties at 5 and 10 μM of 2,4-D. The stem explant in IAHS-22 also showed good callusing at 5 μM 2,4-D. In root explant medium callusing was observed at 10 μM 2,4-D in both the varieties. Use of leaf as an expalnt for callus production is well documented in literature (George and Sherrington 1984).

SUMMARY

VI SUMMARY

The present study was conducted to standardize appropriate *in vitro* propagation strategies for spray and standard type carnations. The experiments were conducted at the Plant Tissue Culture Laboratory of the Division of Horticulture, University of Agricultural Sciences, G.K.V.K. campus, Bangalore.

Murashige and Skoog's (1962) medium was found to be the best for shoot proliferation for both the varieties. In this medium more number of shoots, leaves and higher biomass production was observed. Variety Sterlite Dop performed better as compared with IAHS-22.

Proliferation of shoots was better in Sterlite Dop compared with IAHS-22. Sterlite Dop and IAHS-22 appear to be specific in their requirement of cytokinin type. In Sterlite Dop shoots proliferated better with kinetin while IAHS-22 preferred BAP (4.99 and 3.53, respectively). As regards to concentration of cytokinin, BAP and kinetin at a concentration of 10 µM, induced better shoot proliferation. In the variety Sterlite Dop kinetin at the concentration of 10 µM gave highest number of shoots (10.22) while in IAHS-22 BAP at 10 µM gave highest number of shoots (8.00).

Rooting of shoots was observed both in NAA and IBA media. However, IBA was superior to NAA. NAA, though hastened rooting, the roots were small and short in length. NAA also induced callus production at 15 and 20 µM. IBA, though delayed root initiation gave increased number of roots and longer roots. Unlike NAA, IBA did not induce callusing. IBA at a concentration of 20 µM produced highest number of roots and maximum root length in both the varieties.

Half strength and full strength MS media were superior to quarter strength media in influencing rooting of microshoots. Though there was no significant difference between half strength and full strength media, with number of roots and root length, the rooted plantlets produced with half MS had a good growth at 35 days and hence were highly suited for hardening.

Rooted *in vitro* plantlets failed to survive in the greenhouse while a good per cent of plantlets survived in the mist chamber. (100 per cent survival in Sterlite Dop and 75 per cent in IAHS-22, on 30th day).

Callus production was observed in leaf, stem and root explants with 2,4-D as auxin. Leaf explant was found to be the best for both Sterlite Dop and IAHS-22, which showed good callusing with 2,4-D at a concentration of 5 and 10 μM.

In general, Sterlite Dop, a spray type, responded well to all *in vitro* propgation treatments as this variety showed better shoot proliferation, rooting and establishment.

The results recorded from the investigations as presented in this thesis show that carnations could be propagated successfully through tissue culture as an alternative means for conventional propagation. The protocol developed from the investigation could be used for large scale multiplication of Sterlite Dop, a spray type and IAHS-22, a standard type varieties of carnation. It is also evident from the studies that the two varieties differed in their response to different *in vitro* propagation treatments. Hence suitable modification in the present protocol should be made to suit each variety.

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* Original not seen.

APPENDICES

Appendix I : Composition of MS, Gamborg's, White's and Heller's media (mgl^{-1})

Basal media	MS	Gamborg's	Heller's	White's
Macro nutrients				
KNO_3	1900	3000	-	80
NH_4NO_3	1650	-	-	-
$Ca(NO_3)_2 . 4H_2O$	-	-	-	300
$Ca Cl_2 . 2H_2O$	440	150	75	-
$MgSO_4.7H_2O$	370	500	250	720
KH_2PO_4	170	-	-	-
$(NH_4)_2SO_4$	-	134	-	-
KCl	-	-	750	65
$NaH_2PO_4.H_2O$	-	150	125	16.5
Na_2SO_4	-	-	-	200
$NaNO_3$	-	-	600	-
Micro nutrients				
$MnSO_4 . 4H_2O$	22.3	10	0.1	7
$ZnSO_4.7H_2O$	8.3	2	1	3
H_3BO_3	6.2	3	1	1.5
KI	0.83	-	0.01	0.75
$CuSO_4 . 5H_2O$	0.25	0.025	0.03	-
$Na_2MoO_4 . 2H_2O$	0.25	0.25	-	-
$CoCl_2 . 2H_2O$	0.25	0.025	-	-
$FeSO_4.7H_2O$	27.8	-	-	-
Na_2EDTA	37.3	-	-	-
Fe(as sequestrene).	-	28	-	-
$Fe_2(SO_4)_3$	-	-	-	2.5
$NiCl_2 . 6H_2O$	-	0.03	-	-
$AlCl_3$	-	0.03	-	-
$FeCl_3 . 6H_2O$	-	1	-	-
Vitamins				
Myo inosital	100	100	-	-
Thiamine HCl	0.1	10	1	0.1
Nicotinic acid	0.5	1	-	0.5
Pyridoxial HCl	0.5	-	-	-
Glycine	2	-	-	3
Pyridoxine Hcl	-	-	-	0.1
Calcium panthothenate	-	-	-	1
Cystenic HCl	-	-	-	1

Appendix : II Abbreviations of the terms used in the text:

BA	: 6-benzyladenine
BAP	: 6-benzylamino purine
^{o}C	: Degree Celsius
cm	: Centimeter
2,4-D	: 2,4-Dichlorophenoxyacetic acid
gm	: Grams
IAA	: Indole 3-acetic acid
IBA	: Indole 3-butyric acid
mg	: Milligram (s)
mm	: Millimeter
ml	: Millilitre
mgl^{-1}	: Milligram per litre
μM	: Micromolar
MS	: Murashige and Skoog's medium
NAA	: Napthalene acetic acid
%	: Per cent
pH	: Hydrogen ion concentration
RH	: Relative humidity
WHC	: Water holding capacity