INDUCED CHEMICAL MUTAGENESIS IN ROSE UNDER IN VITRO CULTURE

BY

UMA B. B.Sc. (Ag.)

THESIS

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DECLARATION

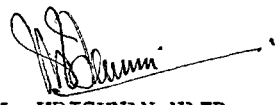
I hereby declare that this thesis entitled "Induced Chemical Mutagenesis in Rose under *in vitro* culture" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

UMA, B.

Vellayani,
3-9-1991.

CERTIFICATE

Certified that this thesis entitled "Induced Chemical Mutagenesis in Rose under *in vitro* culture" is a record of research work done independently by UMA, B. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



Dr. N. KRISHNAN NAIR
Chairman
Advisory Committee
Professor of Agricultural Botany
College of Agriculture

Vellayani,
3 - 9 -1991.

APPROVED BY:

CHAIRMAN

Dr. N. KRISHNAN NAIR

MEMBERS

Dr.(Mrs.) S.T. MERCY

Dr.(Mrs.) D. CHANDRAMONY

Dr K. VASANTHAKUMAR

EXTERNAL EXAMINER

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INTRODUCTION

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The growing realisation of the potentialities of plant cell culture for plant propagation and breeding has itself provided a substantial impetus for research. There are several possible applications of tissue culture technology. The most notable are the use of tissue culture as a tool to make alteration in the genetic make up of plants for plant breeding and the application of tissue culture techniques to the *in vitro* propagation of plants. The major area where tissue culture techniques are presently being successfully applied is in the clonal multiplication of plants. The approach is economically sound and a rapidly increasing number of commercial operations are being devoted exclusively to *in vitro* propagation of ornamentals following the successful multiplication of orchids by shoot meristem culture (Morel, 1968).

In vitro propagation techniques are now often preferred to conventional practices of asexual propagation because of the following potential advantages:

Only a small amount of plant tissue is needed as the initial explant for regeneration of millions of clonal plants in one year.

In vitro propagation provides a potent alternative method for plant species highly resistant to conventional bulk practices.

The in vitro technique provides a method for speedy international exchange of plant materials.

The in vitro stocks can be quickly proliferated at any time of the year while propagation with conventional practices is highly season dependent.

There are three possible routes available for in vitro propagule multiplication (a) enhanced release of axillary buds (b) production of adventitious shoots through organogenesis and (c) somatic embryogenesis. In the present investigation the first method is adopted. The merit of using axillary bud proliferation from meristem, shoot tip or bud culture as a means of regeneration is that the incipient shoot has already been differentiated in vivo. Thus, to establish a complete plant only elongation and root differentiation are required. In vitro organogenesis and embryogenesis, on the other hand must undergo developmental changes which usually involve the formation of callus with subsequent reorganisation into plantlets. The induction of axillary bud proliferation seems to be applicable in many cases where methods of organogenesis

and embryogenesis fail as in the case of carnation (Roest and Bokelmann, 1981) and soybean (Evans, 1981; Kartha et al., 1981b).

If an intermediary callus has been involved, as in the case in most regeneration via organogenesis and embryogenesis, the frequency of genetic changes is increased, especially in the form of polyploidization and aneuploidization resulting from mitotic abnormalities (Bayliss, 1973; Mahlderg et al., 1975). This has been observed in many plant species (Edallo et al., 1981; Novak, 1981; Lester and Berbee, 1977). Plants derived from meristem shoot tip and bud culture are generally phenotypically homogenous, thereby indicating genetic stability. Although the rate of plantlet multiplication by means of organogenesis and embryogenesis is astonishing, their regeneration capacity usually diminishes rapidly after a number of subcultures and eventually the morphogenic potential is completely lost (Kehr and Schaeffer, 1976; Yie and Liaw, 1977). The initial multiplication or axillary bud proliferation on the other hand is rather slow. The rate nevertheless increases during the first few subcultures and eventually reaches a steady plateau during subsequent subculture cycles. The multiplication rate is quite feasible for commercial production of many species. Moreover once a stock of

multiple shoot culture is established, it can continuously serve as the source propagule, instead of having to restart from fresh explant cultures periodically.

The main sources of genetic diversity in crop plants are naturally occurring mutations and hybrids produced from inbreds. Conventional plant breeding procedures however are limited by the need for large amounts of labour, time and space in greenhouse or fields. Genetic manipulation of plants in vitro may help to alleviate some of the problems associated with conventional plant breeding. To have a full spectrum of naturally occurring genetic diversity all the domesticated cultivars and their wild species are screened. If a particular desirable trait is unavailable in the natural populations, it can be created by induction of mutations. Importance of induced mutation, in the field of crop improvement is gaining more attention, recently since many of the sources of valuable naturally occurring germplasm are really disappearing due to monoculture in most of the cultivated crops of the world.

Recent advances made in cell/meristem or tissue culture research appear to offer considerable promise in terms of induced mutations for crop improvement. Cell and tissue culture techniques can contribute for rapid screening of large number of naturally occurring and induced

mutants, fast production of homozygous inbreds and for developing efficient procedures for bringing out suitable modification in the existing cultures. Of all the various factors, the most determinate one is that it is easy to handle a large number of irradiated materials within a small volume. Or in other words induced mutations in relation to tissue culture economise space. It is also an advantage that selection for a particular trait may be done at the tissue level.

The large amount of created variability is being eliminated by the various factors operated on the sequence of mutational events and screening processes. One of the major factor which plays a decisive role in elimination of mutated sector is diplontic selections. Majority of the mutation breeding experiments have been initiated with complex multicellular tissues either with seeds in sexually propagated plants or with cuttings in asexually propagated species. In all these cases plants will develop "chimeras" which will lead to diplontic selection and finally elimination of mutated sectors. In general, smaller the number of initial cells, greater will be the probability for induction of mutations. So cell culture, meristem culture and embryo culture have been adopted to reduce the number of cell involvements during the process of induced mutations

to yield solid mutations and to minimise diplontic selections. Taking into account all these factors, the present investigation was taken up with the following objectives.

1. To standardise a suitable culture medium for in vitro growth and development of axillary buds.
2. To standardise a very successful surface sterilisation technique for axillary buds of rose.
3. To assess the influence of maturity of axillary buds for tissue culture techniques in relation to induced mutagenesis.
4. To standardise the best technique of mutagen treatment while adopting tissue culture technique.
5. To assess the optimum dose requirement for maximum bud take, shoot proliferation and rooting from axillary buds of rose.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Plant cell and tissue culture techniques have developed into very powerful tools for propagation of ornamental species in recent years. The technology had its beginning at the turn of the 20th century with Haberlandt's speculations regarding totipotency of the cells. Haberlandt (1902) suggested that techniques for isolating and culturing plant tissues should be developed and he further postulated that if the environment and nutrition of cultured cells were manipulated, the totipotent cells could recapitulate the developmental sequences of normal plant growth.

Plant tissues were first successfully cultured by White in 1934. By 1939 White, Nobecourt and Gautheret had reported the first successful callus cultures of carrot and tobacco. By 1957, a key paper by Skoog and Miller was published in which they proposed that quantitative interactions between auxins and cytokinins determined the type of growth and/or the morphogenic event that would ensue. Their studies with Nicotiana tabacum indicated that high auxin to cytokinin ratios induced rooting while the reverse induced shoot morphogenesis, but the pattern of response is not universal.

The present work was undertaken for standardising a culture medium for the *in vitro* growth and development of axillary buds of rose and also to standardise a suitable technique of induced chemical mutagenesis under *in vitro* culture. Work in these lines are meagre. So studies which have a bearing on the present investigation are reviewed below.

2.1. Tissue culture technique applied to ornamental species

A major stimulus for application of plant tissue culture techniques for the propagation of ornamental crops may be attributed to the early work by Morel (1960) on the propagation of orchids in culture and to the development of widespread use of a new medium with high concentration of mineral salts by Murashige and Skoog (MS) (1962). Following success with the rapid *in vitro* propagation of orchids, plant cell and tissue culture techniques were applied to other species with varying degrees of success.

2.1.1. Protocols

Shoots of six ornamental species and cultivars of Rosaceae were repeatedly subcultured *in vitro* for nine generations on Linsmaier and Skoog (LS) (1965) medium with the addition of Benzyl Amino Purine (BAP). Shoot proliferation increased over the first few generations and

then gradually declined in all species and at all BAP concentrations tested. A decrease in shoot length and leaf size and an increase in the incidence of callus formation were observed after several subcultures. This apparently irreversible decline could be due to genetic or epigenetic changes resulting from repeated fluxus in cytokinin nutrient status or sucrose, or to the elimination of seasonal environmental fluctuations (Margarete et al., 1985).

Multiplication and subsequent shoot growth of apical or lateral buds of Cercis seliquastrum were optimally induced without changes in a medium containing 10^{-5}m BAP. Rhizogenesis was achieved with 10^{-5}m Naphthalene Acetic acid (NAA) but root elongation was best in half strength medium without growth regulators (Grubisic and Culafic, 1986).

Konchk and Rodeva (1986) found that the bulb scale explants of the lily cultivar 'Yellow Blaze' regenerated in LS medium supplemented with NAA 0.5 mg/l, whereas the presence of 0.5 mg/l Kinetin or 0.1 mg/l BAP in the nutrient medium stimulated callus production.

Pedicel sections from the flowers of Lilium longiflorum cultured on a modified MS medium containing a combination of 5 μ m BAP and 2 μ m NAA produced greatest number of

adventitious buds. Also Indole Acetic Acid (IAA) 10 μ m and Indole Butyric acid (IBA) 10 μ m were most effective in stimulating adventitious roots on shoots developed on these buds (Liv and Burger, 1986).

Michalik and Rogozinska (1986) observed that MS basal medium supplemented with 0.5 mg/l NAA + 0.3 mg/l BAP, 3.0 mg/l IBA + 1.0 mg/l BAP or 1.5 mg/l IAA + 1 mg/l kinetin were good for micropropagation of Kalanchoe blossfeldiana using leaf disc explants.

Nishiuchi (1986), in his studies with the tulip cv Apeldorn, used fully mature bulbs as the source for tissue culture. Buds regenerated from the scale segments when cultured on a medium supplemented with 0.3 mg/l NAA and 0.3 mg/l kinetin. The resulting tissue fragments with many growing buds were further cultured on a modified MS medium adjusted to pH 5.1. The effect of high storage temperature (20-35°C) of the mother bulb on adventitious bud formation and the effect of growth regulators, (Bromocholine Bromide (BCB), Capric acid, 2 thiouracil or GA) on bulb formation during and after a chilling treatment at 5°C were studied. Storage of mother bulbs at 30 or 35°C for 1-2 weeks had a beneficial effect on bulblet forming rate. The number of bulblets increased in the presence of GA (2000 mg/l) or BCB (10^{-4} m) whereas 2 thiouracil had a negative effect.

Pierik and Tetteroo (1987) found that in Begonia venosa, callus could be induced in medium containing BAP and NAA at 0.5 mg/l at a temperature of 21°C and low irradiance. Subculture of organogenic callus was best on a medium containing 0.1 mg/l BAP and 2 per cent glucose. Shoot development from preformed adventitious buds were enhanced by lowering the BAP and glucose concentrations to 0.01 mg/l and 0.5 per cent respectively. Optimal rooting occurred on a growth regulator free medium with low sucrose (0.5 per cent).

Sebastian and Heuser (1987) found that in vitro propagation of Hydrangea quereifolia was possible through differentiation of leaf callus into plantlets and stimulation of axillary bud release from dormant shoots. The plantlets derived from leaf callus appeared to be suitable for commercial propagation, due to their uniform growth pattern and ease of rooting.

2.2. Tissue culture techniques in Rose

There are several routes of in vitro propagation in rose. They include embryo culture, somatic organogenesis, suspension cultures and enhanced release of axillary buds. The works carried out in each of them are reviewed below.

2.2.1. Embryo cultures of Rose

The first tissue cultures of rose established by Lammerts in 1946 were embryo cultures. Asen and Larson (1951) have detailed their procedures for rose embryo culture. Rose has two sets of seed coats and both of them are involved in dormancy. They safely removed the outer seed coat by soaking the seeds for 9-17 hours in Cross and Bevan's solution (1:2 Zinc chloride:Hydrochloric acid w/w). The inner seed coat which is water impermeable must be broken to permit germination. These authors reported that after removing the outer seed coat, the seeds could be soaked in water for about 12 hours which makes the inner seed coat soft enough to permit the penetration of a needle. These two treatments were sufficient to permit good seed germination.

The potential use of embryo culture to rescue a particular hybrid embryo which otherwise abort is one of the goals of a rose breeder. Another very practical use for embryo culture relates to the rapid seed germination which can take place in vitro. Von Abrams and Hand (1956) found that when seeds of a fertile cross were planted in soil, 66.9 per cent of the seeds germinated after 180 days. However, if the seeds were planted on embryo germination medium 98 per cent germination occurred within 14 days.

In embryo culture the seed germination process is shortened and the progeny of a particular cross can be more completely examined.

Graifenberg (1973) has reported that when the achene (fruit) of R. canina is broken and the naked embryo is excised and placed on Knudson C medium, the embryo grows quite well. About 30 per cent of the achenes will yield transplant size plants after about 3-4 months. In contrast, no plantlets were obtained from intact achenes.

2.2.2. In vitro propagation via somatic organogenesis

Callus cultures of rose have been established in many laboratories. The most famous lines were those established from the variety "Paul's Scarlet Rose". Hill (1967) reported the formation of "shoot primordia" in long term callus cultures of hybrid rose. He tried various media to stimulate the growth and proliferation of the shoot like structures, but no full shoots ever developed. Hill did report that on one occasion a trifoliate leaf developed from his cultures.

Jacobs et al. (1968, 1969, 1970 a, b) have investigated the role of growth regulators in callus development from various organs. Mollard and colleagues (Mollard et al., 1976; Mollard and Barnoud 1976 a, b) have utilised tissue

cultures to isolate and study a number of enzymes and plant products of rose.

Kireeva et al. (1977) found that petal, leaf, sepal and embryo of essential oil yielding rose variety "Krymskaya Krasnaya" showed optimum callus formation and tissue growth on MS medium + 2,4-D at 1-4 mg/l and kinetin at 0.05-0.1 mg/l. The callus tissues of petals, leaves and sepals contained essential oil glycoside bound terpenes and aromatic alcohols which were similar in composition to those found in the same organs of intact plants.

Wulster and Sacalis (1980) have studied the influence of auxins and cytokinins on ethylene evolution from rose callus. Both haploid and diploid anther callus have been established by Tabaeezadeh and Khosh Khui (1981).

Khosh Khui and Sink (1982 a) have studied some of the parameters involved in the establishment of callus cultures. They have found that optimal conditions for the production of friable callus include the establishment of callus lines in the dark. They also found that coconut water inhibited callus development and friability. But they failed to get adventitious shoots from their calli.

Recently Tweddle et al. (1984) reported adventitious shoot formation from callus cultures of R. persica x

R. xanthina on MS medium supplemented with 4.4-8.8 μ M BAP and 0.54-1.62 μ M NAA. Callus initiated from internodal segments and subcultured for upto 6 months formed friable light green to yellow calli with cells that were full of starch grains and did not form shoots. However, callus derived from recently initiated shoot cultures transferred to medium containing 13.2 μ M BAP and 0.54-1.62 μ M NAA, formed a hard green callus, that produced adventitious shoots within 4 weeks. Shoots arose from meristemoids beneath the callus surface. Organogenic cells contained few starch grains. After three or more subcultures, the callus lost its organogenic potential and such callus cells contained large numbers of starch grains.

Shoots which had trifoliate leaves and slender stems survived when excised and transferred to proliferation medium. Unifoliate leaved plants with thick fleshy stems leaked phenolic substances into the culture medium and died. There was considerable variation in leaf morphology among the regenerants of R. persica x R. xanthina.

Zieslin et al. (1987) found that the callus growth of the cultivars "Sonia" and "Golden Times" were greatly stimulated when cultured together with callus from Rosa indica var major but to lesser extent by callus from R. canina.

Lloyd et al. (1988) reported results similar to that reported by Tweddle et al. in 1984. They further explained that two other species they tested (R. laevigata and R. wichuriaiana) failed to form adventitious shoots. Cells of these calli, like those from the callus of non-morphogenic R. persica x R. xanthina hybrid contained numerous starch grains.

2.2.3. Suspension cultures of Rose

The use of rose tissues for physiological investigations has been very important since 1957, when Tulicke and Nickell (1959) established a cell line of "Paul's Scarlet rose" from a young stem.

Callus lines of "Pauls Scarlet rose" have been used to study various aspects of cell suspension growth and metabolism (Nash and Davies, 1972), minimal constituents of a tissue culture medium (Nesius et al., 1972), carbon dioxide and pH requirements of non photosynthetic cells (Nesius and Fletcher, 1973), glutamate synthesis (Fletcher, 1974) and the effects of carbohydrate and nitrogen concentration on phenol synthesis (Amorim et al., 1977).

Suspension cultures of R. glauca and R. damascena also have been established and used for studies similar to those of "Paul's scarlet rose". R. glauca suspension

cultures have been used to study the structure of the primary cell wall (Joseleau and Chambat, 1984 a, b) and lignin production (Mollard & Robert, 1984), R. damascena cells have been used to study efflux of K^+ and HCO_3^- ions to the medium (Murphy, 1984).

2.2.4. In vitro propagation via enhanced release of axillary buds

Shoot tips and axillary buds are the most common explants utilised for tissue culture in rose.

Hasegawa (1979) obtained a three fold multiplication of shoots from shoot tips and lateral buds of the rose cv "Improved Blaze" on basic MS medium supplemented with 3.0 mg/l BAP and 0.3 mg/l IAA after 8 weeks of culture. About 50 per cent rooting could be obtained by transferring the shoots to a medium containing 0.3 mg/l IAA and 0 or 0.3 mg/l BAP. Successful transfer of regenerated plants to soil could be done after 2 weeks.

Skirvin and Chu (1979a) achieved shoot proliferation of the glass house rose cv "Forever Yours" using a modified MS high salt medium supplemented with BAP at 2.0 mg/l and NAA at 0.1 mg/l. Rooting was initiated on quarter strength MS medium without hormones.

Davies (1980) developed a method for the rapid micropropagation of seven rose cultivars by the stimulation

of axillary buds. He could obtain a multiplication rate between 3 to 5 shoots in 4 weeks using MS medium supplemented with 0.004 mg/l NAA, 2 mg/l BAP, 0.1 mg/l GA and 4 per cent sucrose.

Hasegawa (1980) found that in vitro derived shoot tips of rose cv "Improved Blaze" proliferated on MS medium (supplemented with 0.5 mg/l thiamine, 0.5 mg/l pyridoxine, 0.5 mg/l Nicotinic acid, 2.0 mg/l glycine, 100 mg/l i-inositol, 3 per cent sucrose, 0.8 per cent Bacto agar, 0.3 mg/l IAA and 1.0, 3.0 or 10.0 mg/l BAP). Root formation and transplantability were enhanced by 0.03 or 0.01 mg/l NAA or 0.1 mg/l IAA added to quarter or half strength of the medium.

Glasshouse roses, outdoor floribundas, miniatures and a range of rootstocks were rapidly and extensively micropropagated by Martin et al. (1981) on a modified MS medium containing auxin, cytokinin and giberillin in quantities varying with the clones and required type of growth.

In in vitro culture, nodes or shoot apices of Rosa indica major (R. chinensis cv Major) produced shoots on MS medium with 0.1 mg/l BAP and roots on MS medium with 0.1 mg/l NAA (Avramis et al., 1982 a). Further experiments were carried out to increase the rooting potential of

in vitro propagated rose shoots of *R. indica* major by Avramis *et al.* (1982 b). They found that best rooting was obtained by pre-treatment with NAA at 0.5 mg/l and/or sucrose at 6 per cent before planting on 1:1 peat:vermiculite.

Hyndnan *et al.* (1982) studied the effect of mineral salt concentration on the rooting of *in vitro* derived shoots of rose cv "Improved Blaze" and found that reduction in Nitrogen salt concentration, rather than the reduction in overall salt was responsible for the improvement in conditions for rhizogenesis.

A comparative study was made between 2 *Rosa hybrida* cvs "Tropicana" and "Bridal Pink" and 2 old world species *R. canina* and *R. damascena* by Khosh Khui and Sink (1982 b). Species variation was observed for growth regulator requirement and rate of multiplication not only between the 2 hybrida cvs, but also between the old world species. Further experiments were carried out by Khosh Khui and Sink (1982 c) using *Rosa hybrida* cv "Bridal Pink" for stimulating root initiation. They found that the best treatments for rooting was 0.1 mg/l NAA + 0.05 mg/l of either IAA or Indole Butyric acid (IBA).

Pitlet and Mancousin (1982) observed that for the establishment phase of lateral buds of rose cultivar

"Joyfulness" IBA at 0.01 mg/l and BAP at 0.5 mg/l were best and for proliferation IBA at 0.1 mg/l and BAP at the same rate were required. By dipping shoots in root promoting substances (NAA at 1 mg/l) for 1 hour before placing them in perlite, 100 per cent rooting was obtained.

Bini et al. (1983) conducted studies on the multiplication of Rosa indica major axillary bud explants and found that BAP and Zeatin gave the greatest increase in multiplication co-efficient while the addition of IBA and GA reduced the multiplication co-efficient. About 60 per cent rooting was obtained using 0.05 mg/l IBA.

According to Priel and Meier-Dinkel (1983) cultivars differ in their ease of in vitro propagation. Dormant axillary buds are more suitable for in vitro cultures than shoot tips and they root in 2 weeks.

Aldrufeu et al. (1984) assessed the in vitro rhizogenesis of Rosa sp in different substrates like cellulose, sand, expanded clay, perlite, vermiculite. Florafort (peat) or TKS-1 (peat) with sucrose and rooting percentages observed were 100, 100, 20, 85, 100, 15 and 80 per cent respectively.

Barve et al. (1984) proposed an effective method for rapid propagation of the cultivars 'Crimson Glory'

and 'Glenfiditch' from axillary vegetative buds with a 10 mm stem portion. Buds proliferated well on MS medium containing kinetin at 0.2 mg/l + BAP at 0.5 mg/l and good rooting was obtained on proliferated shoots by lowering the MS concentration to 1/2 strength and adding IAA + IBA + Indole Propionic acid (IPA) each at 0.5 mg/l.

Cai et al. (1984) found that when stem segments of R. chinensis each with an axillary bud were transplanted on to MS medium plus BAP at 1-3 mg/l, NAA at 0.005-0.1 mg/l and GA at 0-2.0 mg/l, a rosette of shoots was formed after two weeks. Shoots transplanted and cultured on 1/2 strength MS medium + NAA at 0.5-1.0 mg/l for 10-15 days produced roots.

Louwaars (1984) obtained good results with axillary buds of 'Ilona' and 'Red Success' cultivars by culturing them on MS medium supplemented with 4 per cent sucrose, 2 mg BAP/l and 0.004 mg/l IAA.

Successful micropropagation and transfer to the greenhouse was achieved in cultivars Bellona, Bingo, Candia, Cocktail 80 and Sonia using a modified MS medium by Curir et al. (1985). Thiamine at 2 mg/l and Myoinositol at 100 mg/l promoted bud proliferation and IAA at 0.8 mg/l with low sucrose (15 mg/l) promoted rooting.

Leffring (1985) observed that highest percentage of success was obtained using nodal section with a dormant bud that had just coloured, but the position of the bud on the shoot and the season of propagation also influenced the success of tissue culture.

Reist (1985) compared cut flower yields (May to November) of rose bushes (cvs Sonia and Mercedes) established at the same time from cuttings or from in vitro propagated plants and found that flower production was 20 per cent higher in in vitro plants of cultivar Mercedes. The in vitro plants also branched more than plants grown from cuttings.

According to Alekhno and Vysotskii (1986 a) growing the micro cuttings in a horizontal position during shoot proliferation more than doubled the propagation co-efficient compared with growing in a vertical position Later, Alekhno and Vysotskii (1986 b) studied the effect of nutrient medium on micropropagation of rose and found that a combined solid/liquid medium gave improved results due to better accessibility of active substance from the liquid and a large contact area of explants with the medium.

Best results (rate of rooting, number of roots and absence of callus) were achieved by Collet and Le (1987) through the brief induction pretreatment with IAA supplied at the cut ends of the cutting.

Damiano et al. (1987) obtained proliferation rates ranging from 7.2 (for cv Bingo) to 5.8 (for Bellona) by culturing axillary buds on MS medium + 1 mg/l BAP.

Mederos and Rodriguez (1987) studied the factors affecting shoot tip and axillary bud growth and morphogenesis of Rose cv Golden Times.

Valles and Boxus (1987) found that GA at 1 mg/l enhanced axillary branching in their studies with Rosa hybrida cvs.

Alderson et al. (1988) observed that the rooting of the cultured shoots of the cultivars. 'Dainty Dinah', 'Crimson Rosamini' and 'Dujana' was influenced by temperature (15, 20 and 25°C) during the multiplication and rooting phases and by the presence or absence of auxin (IBA) in the rooting medium.

The plant habit, growth and development of 36 dwarf cultivars propagated in vitro and by cuttings were compared by Dubois et al. (1988). They found that plants from in vitro material flowered earlier, had shorter shoots with fewer and shorter internodes and more and longer laterals than plants from cuttings.

Langford and Wainwright (1988) found that shoots maintained on 10, 20 or 40 per cent sucrose showed

decreased levels of carbon dioxide uptake at the higher sucrose concentrations and also vitrification increased with decreasing sucrose concentration.

Li (1988) showed that 86.7 per cent shoots derived from bud explants produced roots in the presence of 2 mg/l 1-aminobenzo triazole (ABT) which was higher than the rooting efficiency of NAA and IBA. Shoots formed on media with small amounts of BAP, rooted and acclimatized better than shoots from media with higher amounts of BAP (Podwyszynska and Hempel, 1988).

Number of shoots per explant was highest when MS medium was supplemented with 0.5 mg/l of BAP and 0.25 mg/l of GA and best rooting was observed in 8 days in liquid MS medium + 0.25 mg/l NAA and 0.1 mg/l 2,4 Dichloro-phenoxyacetic acid (2,4-D) (Rout et al., 1988).

2.3 Induced mutagenesis in Rose

Roses are known to be changeable in the sense that they change themselves either in their colour, form habit etc. Such changes which are hereditary in nature are called mutations.

Spontaneous somatic mutations have played a very importa role in the production of new varieties of rose. The more significant bud-sports in roses include the mutation responsibl for the production of brilliant orange coloured flowers in Dwar

Polyantha roses (Wylie, 1955). It has been estimated that more than 18 per cent varieties of hybrid tea roses were introduced during 1926-1950 and 54 per cent varieties of Dwarf Polyantha originated as sports (Wylie, 1955). During the last fifteen years a number of workers have used ionizing radiations and chemical mutagens for artificial induction of somatic mutations in rose.

Nakajima (1970) observed that best results were obtained by gamma irradiating stem cuttings in late April to late May at about 10 Kiloradiation (Kr) for upto 10 days. He found marked differences in responses between cultivars. "Crimson Glory" and "Golden Masterpiece" rarely mutated whereas many sports were obtained in 'Peace', 'Queen Elizabeth' and 'Kordes Perfecta'. He found that flower colour was most frequently altered by mutation.

Lata and Gupta (1971) recorded the survival of plants and flower, yield of essential oil and production of normal and abnormal flowers in 12 gamma irradiated and non-irradiated scented hybrid tea cvs during the second year after irradiation. Flowers from irradiated plant were generally smaller and contained less oil than those of controls.

Kaicker and Swarup (1972) gamma irradiated the cvs 'Christian Dior', 'Queen Elizabeth' and 'Kiss of Fire' and found that treating dormant buds with 5-10 kr of gamma rays produced best results. Also Ethyl-methane sulphonate (EMS) induced mutants with low petal numbers in "Kiss of Fire".

Based on studies conducted on control and gamma ray induced mutants of rose cultivar "Montezuma", Lata and Gupta (1975) suggested that the variations exhibited by the mutants may be chromosomal and/or genic in nature. Dommergues (1976) suggested that the floral colour changes produced by mutations may be due to a change in the nature of the pigments themselves although most of them are already present in the plant.

Nakajima (1977) observed that of the various doses of gamma rays used for irradiating potted plants of the rose cv 'Peace', 15 kr and 10 kr were the effective treatments. Multicoloured cultivars and those normally grown under protected cultivation mutate more readily than most other cultivars (Haenchen and Gilfert, 1978).

'Madhosh', a mutant variety of rose with blue stripes was obtained by treating the budwood of 'Gulzar' with 0.25 per cent EMS (Kaicker and Swarup, 1978).

Tsvitkov (1978) observed that in crosses with the Kazanlik rose (Rosa damascena) the use of irradiated pollen of such forms as R. canina, R. centifolia and Rose de Mai increased fruit and seed set.

Lata (1980) suggested that the floribunda rose 'Pink Parfait' was the most suitable for the induction of mutations using gamma rays.

Irulappan and Rao (1981) observed that in the vM_1 generation of Edward rose (Rosa bourboniana Desp) individual flower weight and the number and weight of flowers/plant significantly increased in all mutagenic treatments with gamma rays and EMS. In vM_2, increased mean values were observed for number and weight of flowers per plant.

Shepotev and Chernobrivets (1981) observed that in the gamma ray induced mutants of Rosa cinnamomea the leaf blade was significantly thicker than its control.

Buds, gamma irradiated with 3 kr gamma rays and budded on Rosa indica var. odorata produced a white colour mutant, whose flower diameter and petal size were significantly reduced, but the petal number was increased (Datta and Gupta, 1982). Later in 1984 Datta and Gupta produced a new cultivar 'Saroda' by exposing the budwood of the cultivar 'Queen Elizabeth' to 3 kr of gamma irradiation.

Benetka (1985) while studying the isolation of somatic mutations in rose cultivar 'Sonia' found that the optimum gamma irradiation dose was between 4 and 5 kr. With increasing bud generations during the first year after irradiation, the number of non-chimeral mutations decreased and was generally low in shoots of third bud generation.

Datta (1985) found that gamma irradiation of axillary buds resulted in a reduction in the sprouting and survival percentages and it increased with dose. A wide variation was observed in the sensitivity of varieties to gamma irradiation, the cv "Orange Sensation" being the most sensitive and 'Kiss of Fire' the most resistant. His further studies in the rose cv "Contempo" in 1986 showed that though repeated irradiation reduced sprouting, survival and plant height, it increased somatic mutation rate.

Huang and Chen (1986) in their mutation breeding experiments with rose cvs 'Crimson Glory', 'Super Star', 'Condesa de Sastago', 'Peace', 'Pink Peace' and 'South Seas' observed that 3.8-15 per cent of the plants exposed to 3 kr of gamma irradiation bore mutant branches and almost half of them produced chimeric flowers.

Both gamma-irradiation and chemical mutagens (0.1 per cent dimethyl sulphate, 0.05 per cent EMS, 0.5 per cent hydroxylamine) decreased seed germination and no correlation was detected between the use of a particular mutagen and the colour intensity of flowers on the resulting plants. About 10 per cent of plants from treated seeds produced white flowers which were devoid of anthocyanins (Lata, 1987).

2.4. Tissue culture techniques in relation to induced mutagenesis

The application of mutagens to tissues or cell populations cultured in vitro to enhance the rate of spontaneous mutations and the use of direct selection for the screening of spontaneous mutants or variant lines has been used in several laboratories in the last 10 years, with the aim of further recovery of mutated whole plants. Bajaj (1971) studied the direct and indirect effects of gamma irradiation on the seeds, seedlings, callus tissue cultures, excised roots, ovules and embryos and reported that callus tissue cultures are more radio resistant than the intact seedlings.

In 2 cvs of Chrysanthemum, gamma-irradiated at the time of shoot tip culture, the proportion of plants healthy enough to transplant after irradiation was inversely

proportional to the radiation dose. A few of these plants that survived the winter were those irradiated with a dose less than 20 kr. (Mûbuchi and Kuwada, 1975).

An effective chemical mutagenesis procedure for Petunia hybrida cell suspension cultures was reported by Coljin et al. (1979). Of the various chemical mutagens used in cell suspension cultures, grown in MS medium + 1 mg/l 2,4-D, Nitroso guanidine was most effective and cell survival was satisfactory in low dose (5-40 g/ml) of the mutagen.

Unrooted cuttings, callus and suspension cultures of 5 Chrysanthemum clones were treated with gamma rays (1.2-1.8 kr) and 1 clone was treated with 0.5-1.5 per cent EMS (Jung and Horn, 1980). Frequency of variation in flower colour was relatively low in plants from treated cuttings, but it was higher in plants from treated nodes and highest from suspension cultures reaching 38-67 per cent depending on the clone.

As in vitro propagation of two genotypes of Begonia hiemalis was achieved through adventitious shoot formation on cultured leaf disc and subsequent transplantation to soil of explant parts with adventitious shoots. After irradiation of detached leaves with different doses

of X-rays and 2 cycles of adventitious shoot formation on *in vitro* cultured leaf disc explants, plantlets were produced. About 30 per cent of these plants were mutants with respect to the colour, size and form of the leaves and flowers. The great majority of the mutants (98.5 per cent proved to be non chimeric (Roest *et al.*, 1980).

Work was carried out as a part of a *Fuchsia* breeding programme with the aim of producing new forms by somaclonal variation, mutagenic treatments (EMS, hydrazine, sodium azide and methyl nitroso guanidine) and *in vitro* selection using cultivars 'Constance', 'Swingtime' and 'Rose Van den Berg'. Several variants have been isolated, some of which were characterised by an increase in the ploidy level (Bouharmont and Dabin, 1986).

Weigela cv "Bristol Ruby" cultured *in vitro* was gamma-irradiated with doses of 20-60 Gy. Irradiation affected bud survival, rhizogenesis and cutting growth at doses 30 Gy and few buds survived a 60 Gy dose. Mutants produced from irradiated buds appeared to be homogeneous at first but after 2-3 years 40 per cent proved to be periclinal chimeras (Duron and Decourtye, 1986).

Immobilized embryogenic cells of a *Poinsettia* ideotype heterozygous for anthocyanin synthesis (Whwh)

was chosen for X irradiation (10 to 60 Gy) so that induced homozygous 'whwh' mutants could be easily identified. Mutation rates increased with increasing X-ray doses reaching 8.9 per cent at 60 Gy but the survival rates decreased with increasing doses. Application of 2 or 3 doses at 20 Gy separated by intervals of 0.5-25 hours increased survival rates (Kleffel et al., 1986).

A broad spectrum of variability was induced when applying X-ray doses between 25 and 60 Gray (Gy) to basal segments of in vitro derived microshoots of the rose cv 'Ilseta' followed by repeated cutting off of axillary shoots from treated mother explants. The mutations comprised of 73 per cent flower mutants, 14 per cent with altered growth and 13 per cent with modified leaves. The mutant rate increased with increasing irradiation dose (Walther and Saver, 1986a).

Axillary shoots from in vitro derived microshoots of two lines of Gerbera (A 26 and 82/19/6) were irradiated with X-ray doses between 10 and 25. During 16 weeks of post irradiation culture, the following parameters were assessed for their usefulness in estimating radiosensitivity such as explant survival rate, number of developed shoots on the first cut off date (27 days after irradiation) and the cumulative number of axillary shoots on 4 subsequent

dates. Regeneration was dose dependent, the higher X-ray doses resulting in greater inhibition of shoot regeneration. Irradiation induced damage was higher in A 26 than in 82/19/6 (Walther and Saver, 1986b).

Tissue cultures derived from flowering buds of Arctostaphylos were cultivated in the dark on MS medium supplemented with 10 mg/l IBA + 1 mg/l kinetin. Cultures in their 10th to 18th subcultures were gamma-irradiated 5 times at 4 intervals of 4 weeks with doses of 2.5 to 5 or once with doses between 2.5 to 160 Gy from a ^{60}Co source. Compared with untreated controls the growth of the irradiated cultures decreased with increasing radiation dose. The highest dose (160 Gy) killed the calli. None of the radiation treatments induced embryogenesis. The number of very large cells in the calli increased with increasing radiation dose (Duskova et al., 1988).

Shoot tips of the grape cultivars 'Muscet Bailey A', 'Reesling' and 'S 9110' were cultured for 6 weeks in MS medium supplemented with 4.8 MBAP. Treatments with 0.1-2 per cent EMS decreased shoot proliferation and shoot length. In untreated shoot tips, proliferation and length of shoot were greatest in 'S 9110' and lowest in 'Muscet Bailey'. Irradiation with low doses (1 kr) of gamma rays

followed by 72 hr dark treatment increased shoot proliferation rate and shoot length while high doses did not (Kim et al., 1989).

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was undertaken at the Tissue Culture Laboratory attached to the Horticultural Department, College of Agriculture, Vellayani, during 1989-'90. The main objectives were to standardise a culture medium for the *in vitro* growth and development of the axillary buds, and to standardise a suitable technique of induced and chemical mutagenesis under *in vitro* culture for rose.

The details of the various procedures adopted are presented hereunder:

3.1 Materials

3.1.1 Explant

The explant used for the present investigation was axillary buds of rose (*Rosa chinensis* Jacq) at 4 different maturity stages.

1. axillary buds at the time of flower harvest
2. axillary buds 2 days after flower harvest
3. axillary buds 4 days after flower harvest
4. axillary buds 6 days after flower harvest

3.1.2 Medium

The medium tried was MS (Murashige and Skoog, 1962)

medium with different auxins, 2,4-D, GA, NAA, IAA, IBA and cytokinin BAP at concentrations of 0.5, 1.0, 1.5 and 2.0 mg/l.

3.1.3 Mutagen

The mutagen used was Ethyl Methane Sulphonate (EMS) at 0.125, 0.250, 0.375 and 0.5 per cent concentrations.

3.2 Methods

3.2.1 Raising the material

Rose is propagated both by budding and by using cuttings, but the former method is preferred for obtaining better quality and faster growing plants. For the present investigation budded plants of the rose variety "Phor Chlor" were utilised. Large sized pots of about 12" to 17" in diameter and 16" to 18" in height were used for planting the mother plants. They are ideal for the complete growth of the rose plants as they provide enough space for the semi-taproot and adventitious roots to grow. A potting mixture consisting of 2 parts soil, 1 part sand and 1 part cowdung was used for growing the plants. For rose, heavy watering at comparatively long intervals is more useful than frequent light watering and so the plants were irrigated twice daily (morning and evening).

In order to reduce contamination during culturing plants were sprayed with the systemic fungicide Benlate (0.1 per cent) 4 times at 15 days interval before the excision of the explants. The explants were collected one week after the last spraying.

3.2.2 Excision of explant

To obtain nodal segments containing lateral buds, a shallow incision (1-2 mm) was made into the stem and the bud was excised with a small portion of the adjacent stem tissue attached to it as they sprouted better compared to those without the stem portion. The excised buds were immediately transferred to a beaker containing double distilled water to avoid the drying of buds. In the laboratory the buds were given 4-5 washings with distilled water to remove the dust and other extraneous matter adhering on to it.

3.2.3 Standardisation of surface sterilisation

Axillary buds at the time of flower harvest were used for this preliminary investigation. Axillary buds were excised soon after flower harvest with a small portion of the adjacent stem and were washed thoroughly in double distilled water. These buds were then immersed in Mercuric Chloride solution of different concentrations as shown in

Table 1. The Mercuric chloride sterilised buds were inoculated in MS medium under aseptic conditions to study the percentage of buds that remained healthy without contamination.

Table 1. Surface Sterilisation Treatments

Sterilant	Conc (in %)	Time (in min)	Uninfected buds after 10 days (%)
Mercuric chloride	0.06	5	20
		10	46
		15	73
	0.08	5	65
		10	70
		15	90
	0.1	5	25
		10	10
CD value		15	Nil

The survival percentage that resulted due to various concentrations of mercuric chloride indicated that to get maximum number of healthy and growing buds, treating the buds in 0.08 per cent mercuric chloride for 15 minutes was optimum.

The surface sterilised buds were then rinsed 3 times (at 5 minutes interval) in sterile distilled water under aseptic conditions in a Laminar air flow hood. One bud was inoculated per tube containing 15 ml of semi-solid, MS medium and the tubes were incubated at 25°C ± 2°C and 70 per cent humidity under cool white flourescent light of approximately 3000 lux intensity at 16/8 hr light/dark regime.

3.2.4 Standardisation of culture medium

Since a wide varietal variation is observed in the effect of hormones on the in vitro growth of rose as reviewed earlier basic MS medium with several combinations of auxins and cytokinins were tested at 3 different stages of development such as

Stage I Culture establishment

Stage II Enhanced release of axillary buds

Stage III In vitro rooting

The pH of the medium was adjusted to 5.8. Tubes and conical flasks (100 ml and 50 ml) (corning) were used for the study. The medium was autoclaved for 20 min at 121°C and 1.06 kg/cm^2.

3.2.4.1 Culture establishment

Axillary buds (at the time of flower harvest)

excised with a small portion of the adjacent stem were washed thoroughly in distilled water and surface sterilised with 0.08 per cent mercuric chloride for 15 min. After surface sterilization the buds were again given 3 washings (at 5 min interval) with sterile distilled water. These sterilised buds were then inoculated into MS medium with different combinations of the cytokinin BAP and auxin 2,4-D as given below:

BAP (0.5, 1.0, 1.5 + 2.0 mg/l) and 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/l) 4 x 4 diallel set.

All the cultures were incubated under culture conditions explained earlier. Fifteen tubes each were provided for each treatment.

Observations

Days for bud take

After a few days of culturing, the buds swell up slightly and their outer bud sheath separates out. The number of days taken by buds subjected to different treatments to undergo this change was counted and expressed as the number of days for bud take.

3.2.4.2 Enhanced release of axillary buds

After 1 month of inoculation, when the buds grew to a height of 2 cm, they were transferred to MS medium

with BAP alone and with different combinations of BAP and GA to find out the best combination for maximum multiple shoot production. The combinations tried were as follows:

BAP (0.5, 1.0, 1.5 & 2.0 mg/l)

GA (0.5, 1.0, 1.5 & 2.0 mg/l) 4 x 4 diallel set

Observations

Days for multiple shoot production

The number of days taken by each treatment to produce the first multiple shoot was observed and recorded.

Number of shoots/culture

The number of shoots produced in individual culture tubes were counted and recorded.

3.2.4.3 In vitro rooting

The multiple shoots with a height above 4-5 cm were separated from the main shoot and were cultured individually for rooting, in appropriate rooting medium while the smaller shoots were allowed to undergo shoot proliferation again. For rooting, the basic MS salts in half strength or full strength with auxins IBA, NAA and IAA was used. The various media combinations were as given below.

(i) 1/2 strength medium + NAA (0.5, 1.0, 1.5 and 2.0 mg/l)

(ii) " + IBA (0.5, 1.0, 1.5 and 2.0 mg/l)

(iii) " + IAA (0.5, 1.0, 1.5 and 2.0 mg/l)

(iv) full strength medium without hormones

(v) " + NAA (0.5, 1.0, 1.5 and 2.0 mg/l)

(vi) " + IAA "

Observations

Days for rooting

The number of days taken to produce the first root initial was recorded.

Number of roots/culture

The number of roots produced in individual shoots were counted and recorded.

3.2.5 Mutation induction

3.2.5.1 Preparation of mutagen solution

The chemical mutagen EMS, an alkylating agent was used as the mutagenic agent. The various concentrations ranging from 0.125 to 0.5 per cent at 0.125 per cent interval was prepared by dissolving the mutagen in double glass distilled water, immediately before use. A 0.5 per cent stock solution of 0.5 ml EMS in 100 ml distilled water was prepared and from this stock the other concentrations were made as follows:

30 ml of 0.5 per cent stock + 10 ml double distilled water - 0.375 per cent EMS

20 ml of 0.5 per cent stock + 20 ml double distilled water - 0.25 per cent EMS

10 ml of 0.5 per cent stock + 30 ml double distilled water - 0.125 per cent EMS

The pH of the solution was adjusted to neutral using sodium phosphate dibasic salt as buffer.

3.2.5.2 Mutagen treatments

Two methods of mutagen treatments were tried.

3.2.5.2.1 Direct treatment

3.2.5.2.2 Cotton swab method

3.2.5.2.1 Direct treatment

In this method rose buds excised at different stages were directly immersed in EMS solution of different concentrations for 4 hours. After treatment the buds were washed thoroughly in running water for one hour. The buds were then surface sterilised with 0.08 per cent mercuric chloride for 15 min, washed with sterile distilled water and inoculated into MS medium standardised for bud establishment. Buds of 4 different maturity groups were subjected to direct treatment with EMS at 4 different stages of culturing as follows:

(i) Treating the buds immediately before inoculation

(ii) Treating the buds 2 days after culturing

(iii) Treating the buds 4 days after culturing

(iv) Treating the buds 6 days after culturing

3.2.5.2.2 Cotton swab method

In this method the buds were treated before excision, in the plant itself. Different concentrations of the mutagen solutions were prepared as explained earlier. The plants were provided with temporary shade and the buds were wrapped with pieces of cotton dipped in one of the 4 different concentrations of EMS solution. The buds of the same branch were given the same treatment for 4 hours. The cotton was kept wet with the mutagen till the end of the treatment. After 4 hours the cotton wrap was removed and the buds were washed thoroughly with distilled water sprayed with the help of a wash bottle. The next day morning the buds were excised, washed with distilled water, surface sterilised, again washed in sterile distilled water and cultured. The axillary buds were subjected to this method of mutagen treatment at 4 different stages of development as follows:

(i) Treating the axillary buds at the time of flower harvest

As soon as the flowers opened up completely, they were plucked and the leaves below were cut off, retaining

a small portion of the petiole at the base of each axillary bud. The buds were then treated with the mutagen as detailed earlier.

(ii) Treating the axillary buds 2 days after flower harvest

(iii) Treating the axillary buds four days after flower harvest

(iv) Treating the axillary buds six days after flower harvest

3.6.3 Observations

Observations were recorded on the following characters from the treated and cultured explants at different stages of their growth. All the materials used to take observations were sterilised properly before use. The observations taken include

Days for bud take

Number of days for first leaf production

Length of shoots at 15 days interval

Number of leaflets per plant at 15 days interval

Days taken for multiple shoot production

Average number of shoots/culture

Days taken for rooting

Number of roots/shoot

Morphological variation if any

RESULTS

RESULTS

4.1 Standardisation of culture medium

4.1.1 Culture establishment

The number of days required for bud take and the percentage bud take after 10 days under different combinations of BAP and 2,4 D for explants taken at the time of flower harvest are presented in Table 2. Statistical analysis of the data showed significant difference among the treatments for both these factors.

The number of days required for bud take ranged from 4 (BAP 2.0 mg/l + 2,4 D 1.0 mg/l) to 10 (BAP 2 mg/l + 2,4 D 1.5 mg/l). There was no bud take when the level of 2,4 D increased above 1.0 mg/l (1.5 mg/l & 2.0 mg/l) in combination with BAP at 0.5 mg/l, 1.0 mg/l or 1.5 mg/l levels. In all these combinations callusing was observed at the cut ends of the stem portion attached to the bud. In the treatments other than those mentioned above the days required for bud take varied from 6-9 days.

The maximum percentage of bud take (85 per cent) was observed in MS medium supplemented with BAP 2.0 mg/l and 2,4 D 1.0 mg/l. The combinations of BAP 2.0 mg/l + 2,4 D 0.5 mg/l, BAP 1.5 mg/l + 2,4 D 0.5 mg/l and BAP 1.5 mg/l + 2,4 D 1.0 mg/l were on par in the percentage

bud take (82, 80 and 80 per cent respectively). The percentage bud take was very low in the combination BAP 2.0 mg/l + 2,4 D 1.5 mg/l (60 per cent). In the rest of the treatments the percentage bud take ranged from 70 (BAP 0.5 mg/l + 2,4 D 0.5 mg/l) to 78 (BAP 1.0 mg/l + 2,4 D 0.5 mg/l).

4.1.2 Enhanced release of axillary buds

The number of days taken for the initiation of multiple shoot, percentage of cultures with multiple shoots and the number of shoots/culture at different levels of BAP and combinations of BAP and GA are presented in Table 3. Statistical analysis showed significant variation for all the factors.

Days required for initiation of multiple shoots

The combination BAP 2.0 mg/l + GA 1.0 mg/l took only 25 days for multiple shoot production and was superior to all the other combinations. Buds sub-cultured on MS medium containing, BAP at 0.5 - 2.0 mg/l or BAP 0.5 mg/l with GA above 1.0 mg/l (1.5 & 2.0 mg/l) took 34-38 days for multiple shoot production. When BAP was at 1.0, 1.5 or 2.0 mg/l and GA at 0.5 mg/l multiple shoot production occurred within 31-33 days.

Percentage of cultures showing multiple shoots

Addition of BAP (0.5 - 2.0 mg/l) alone to MS medium resulted in poor shoot elongation and multiple shoot formation remained between 59.3 - 88.5 per cent. Incorporation of GA at low concentrations (0.5 - 1.5 mg/l) in the BAP supplemented medium gave a high multiple shoot induction (90.2 - 94.3 per cent). MS medium containing BAP 0.5 - 1.5 mg/l with GA 0.5 and 1.0 mg/l was found to be superior (57.2 - 85.3 per cent) to those cultures supplemented with BAP alone at 0.5 - 1.5 mg/l (59.3 - 82.2 per cent). When BAP level remained at 0.5 - 1.5 mg/l and GA level increased above 1.0 mg/l a negative effect was observed (53.3 - 77.1 per cent). A combination of BAP 2.0 mg/l with GA 0.5 - 1.5 mg/l (90.2 - 94.3 per cent) was found to be superior to only BAP 2.0 mg/l (88.5 per cent). However GA above 1.5 mg/l reduced the percentage cultures with multiple shoots even when BAP was at 2 mg/l (80.2 per cent). The lowest percentage of cultures with multiple shoots (53.3 per cent) was observed when BAP was at 0.5 mg/l and GA 2.0 mg/l followed by BAP 0.5 mg/l + GA 1.5 mg/l (55.1 per cent) and BAP 0.5 mg/l + GA 1.0 mg/l (57.2 per cent)

Number of shoots/culture

The addition of GA to the BAP supplemented MS medium also showed the maximum response for number of shoots/culture.

BAP (1.0 - 2.0 mg/l) with GA below 1.0 mg/l gave an average shoot number of 4 per culture, while BAP (0.5 - 1.0 mg/l) with GA concentration above 1.0 mg/l gave only an average of 1-2 shoots/culture. The combination of BAP 2.0 mg/l + GA 1.0 mg/l enhanced the average number of shoots/culture to 5.25 while BAP 2.0 mg/l alone gave an average of 4.33 shoots/culture. MS medium supplemented with 2.0 mg/l BAP and GA 1.0 mg/l gave the maximum number of cultures with multiple shoots (94.3 per cent) in a minimum period of 25 days and the number of shoots produced per explant was the highest (5.25).

4.1.3 *In vitro* rooting

Rooting was initially tested in 1/4 strength MS medium supplemented with 3.0 mg/l IBA. Here the plantlets took 30-35 days for rooting with a very shy shoot growth and hence the leaves turned yellow and withered. By this time heavy callusing was also observed at the base of the shoots. Further rooting was tried by reducing the IBA concentration (2.0 - 1.0 mg/l). This combination reduced callusing but showed no improvement in shoot growth. Hence the concentration of the base medium was increased to 1/2 strength. The data regarding the days required for root initiation, percentage of shoots with rooting and average number of roots/shoot are presented in Table 4.

The statistical analysis showed significant differences in all the parameters tested.

The hormones tested in 1/2 strength MS medium were NAA, IBA & IAA each at 0.5, 1.0, 1.5 and 2.0 mg/l. The percentage of shoots with rooting was comparatively poor (60.1 - 71.1) in those cultures supplemented with IBA at 0.5 - 2.0 mg/l. Here basal callusing was also observed. The percentage values of IBA at 0.5 and 1.0 mg/l treatments (69.4 - 71.1) were on par but they differed significantly from the other hormonal treatments. The rooting percentages of cultures supplemented with NAA (0.5, 1.5 & 2.0 mg/l) and IAA (0.5, 1.5 & 2.0 mg/l) were found to be on par and the values ranged from 75.5 to 82.3 per cent. In IAA and NAA each at 1.0 mg/l 84.9 and 84.0 per cent of cultures showed rooting with an average of 3.5 and 3.47 roots per culture, but the general health of the shoots was not satisfactory and the yellowing of plantlets was a common feature in all the three rooting hormones tried in 1/2 strength MS medium. A full strength MS medium supplemented with NAA & IAA and without auxins was tried and the data are presented in Table 5. The data collected on the days required for rooting, percentage of shoots with rooting and number of roots/shoot were statistically analysed and showed significant variation among treatments.

In full strength MS medium without hormones, root initiation took a longer period (35 days) and the percentage of shoots showing rooting was very low (46.6 per cent). In NAA (0.5 - 2.0 mg/l) and IAA (0.5 - 1.5 mg/l) supplemented media rooting occurred in 18-20 days. In MS with IAA 2.0 mg/l root initiation was significantly superior (14 days). The lower concentrations of both NAA & IAA (0.5 mg/l and 1.0 mg/l) gave a very low percentage of rooting (59.8 - 65.4) compared to higher concentrations (1.5 mg/l & 2.0 mg/l). In general there was a linear increase in rooting percentage with increase in concentration of both the auxins tried. The treatments also differed significantly regarding the total number of roots produced per shoot. The lowest average number of roots (3.6) was counted in full strength MS medium without the addition of any auxin and it was significantly inferior to all other treatments. The number of roots/shoot in IAA treatments increased with increase in concentration of the hormone and the values ranged from 4.2 to 5.5 in 0.5 and 2.0 mg/l concentrations respectively. The maximum percentage of shoots with rooting (93.1) and the maximum number of roots/shoot (5.5) were both observed in full strength MS medium with 2.0 mg/l IAA.

Table 2. Effect of different levels of BAP and 2,4 D on culture establishment in rose

Levels of BAP & 2,4 D (mg/l)	Days required for bud take	Bud take after 10 days (%)
1. BAP (0.5) + 2,4 D (0.5)	8	70
2. BAP (0.5) + 2,4 D (1.0)	9	75
3. BAP (0.5) + 2,4 D (1.5)	-	-
4. BAP (0.5) + 2,4 D (2.0)	-	-
5. BAP (1.0) + 2,4 D (0.5)	8	78
6. BAP (1.0) + 2,4 D (1.0)	8	75
7. BAP (1.0) + 2,4 D (1.5)	-	-
8. BAP (1.0) + 2,4 D (2.0)	-	-
9. BAP (1.5) + 2,4 D (0.5)	7	80
10. BAP (1.5) + 2,4 D (1.0)	6	80
11. BAP (1.5) + 2,4 D (1.5)	-	-
12. BAP (1.5) + 2,4 D (2.0)	-	-
13. BAP (2.0) + 2,4 D (0.5)	6	82
14. BAP (2.0) + 2,4 D (1.0)	4	85
15. BAP (2.0) + 2,4 D (1.5)	10	60
16. BAP (2.0) + 2,4 D (2.0)	-	-
F value	5.48	68.68
CD	2.289	2.68

* Significant at 5 per cent level

Table 3. Effect of different levels of BAP and GA on shoot proliferation

Levels of BAP and GA (mg/l)	Days required for multiple shoot production	Cultures with multiple shoots (%)	No. of shoots/ culture
1. BAP (0.5)	38	59.3	2.83
2. BAP (1.0)	37	78.1	3.37
3. BAP (1.5)	35	82.2	3.90
4. BAP (2.0)	34	88.5	4.33
5. BAP (0.5) + GA (0.5)	35	64.5	3.25
6. BAP (0.5) + GA (1.0)	35	57.2	2.45
7. BAP (0.5) + GA (1.5)	34	55.1	1.60
8. BAP (0.5) + GA (2.0)	34	53.3	1.33
9. BAP (1.0) + GA (0.5)	33	80.1	4.38
10. BAP (1.0) + GA (1.0)	34	76.3	2.75
11. BAP (1.0) + GA (1.5)	33	73.1	2.20
12. BAP (1.0) + GA (2.0)	32	68.8	1.98
13. BAP (1.5) + GA (0.5)	32	85.3	4.51
14. BAP (1.5) + GA (1.0)	34	79.6	4.35
15. BAP (1.5) + GA (1.5)	31	77.1	2.38
16. BAP (1.5) + GA (2.0)	32	71.1	2.32
17. BAP (2.0) + GA (0.5)	31	91.1	4.65
18. BAP (2.0) + GA (1.0)	25	94.3	5.25
19. BAP (2.0) + GA (1.5)	32	90.2	4.43
20. BAP (2.0) + GA (2.0)	31	80.2	3.33
Significant F value	4.41	166.19	42.71
CD	2.86	2.82	0.517

* Significant at 5 per cent level

Table 4. Rooting of *in vitro* derived shoots in 1/2 strength MS medium supplemented with various levels of NAA, IBA & IAA

Level of auxins in 1/2 strength MS medium (mg/l)	Days required for rooting	Shoots rooting (%)	Number of roots/shoot
1. NAA (0.5)	30	82.2	3.23
2. NAA (1.0)	30	84.0	3.47
3. NAA (1.5)	28	79.1	3.45
4. NAA (2.0)	28	76.5	3.40
5. IBA (0.5)	35	69.4	3.19
6. IBA (1.0)	33	71.1	3.20
7. IBA (1.5)	30	62.3	3.16
8. IBA (2.0)	30	60.1	3.00
9. IAA (0.5)	30	82.3	3.41
10. IAA (1.0)	25	84.9	3.50
11. IAA (1.5)	25	79.3	3.45
12. IAA (2.0)	25	74.5	3.39
Significant F value	3.33	3.12	2.81
CD value	4.985	6.98	0.275

Table 5. Rooting of *in vitro* derived shoots in full strength MS medium supplemented with various levels of NAA & IAA

Level of auxins in full strength MS medium (mg/l)	Days required for rooting	Shoots rooting (%)	Number of roots/shoot
1. Without hormones	35	46.6	3.6
2. NAA (0.5)	20	59.8	4.8
3. NAA (1.0)	20	63.2	5.4
4. NAA (1.5)	18	82.3	5.0
5. NAA (2.0)	18	87.9	5.0
6. IAA (0.5)	18	61.1	4.2
7. IAA (1.0)	18	65.4	4.8
8. IAA (1.5)	18	85.4	5.0
9. IAA (2.0)	14	93.1	5.5
F value	19.66	135.99	9.28
CD	4.003	4.045	0.574

4.2 Mutagen treatments

4.2.1 Direct treatment

The buds treated with EMS immediately before culturing on standardised MS medium showed no bud take. The buds turned brown within a week. Browning of varying intensities was observed in all the 4 different stages of buds. The untreated buds showed bud take within 5 days as well as normal growth and shoot proliferation.

In the buds treated with EMS 2 days after culturing the green colour faded immediately after mutagen treatment. Within one week the buds, turned completely brown. The buds of all the four different stages behaved in a similar manner. The control buds washed with sterile distilled water 2 days after culturing and recultured on fresh MS medium also turned pale yellow after a few days and failed to have normal growth even though no mutagen treatments were given. The few buds which showed bud break performed poorly with a delay in multiple shoot production and a low rate of multiplication.

The buds treated 4 days and 6 days after culturing also exhibited browning as noted above. Of all the four different stages of buds treated, the buds excised at the time of flower harvest were the most affected. Also, the treated and recultured buds were prone to a high rate of

contamination. Though the EMS treatments were repeated by reducing the time of treatment from four to one hour none of them yielded favourable results.

4.2.2 Cotton swab method

4.2.2.1 Culture establishment

The data regarding the days required for bud take, the percentage bud take after 10 days and the number of days taken for the emergence of the first leaf under different treatments are presented in Table 6. Statistical analysis showed significant difference among treatments for percentage of bud take.

Percentage bud take

The four different developmental phases tried differed significantly in the percentage bud take. On an average, buds excised 4 days after flower harvest showed a higher percentage bud take (83.36 per cent) compared to buds excised 2 and 6 days after flower harvest and at the time of flower harvest (79.44, 66.08 and 75.06 per cent respectively). While comparing the effect of the different EMS concentrations (0.5, 0.375, 0.25 and 0.125 per cent) and control it was found that the lower concentrations and control were on par in bud take showing general mean values ranging from 79.65 - 80.03 per cent. Higher

concentrations of EMS (0.375 and 0.5 per cent) differed significantly from each other and from other concentrations and gave 73.53 and 67.08 per cent bud take respectively. The bud take decreased with increase in the concentration of EMS and also with increase in maturity of buds. The interaction between the bud stages and EMS concentrations was not significantly different.

4.2.2.2 Enhanced release of axillary buds

The data concerning the days required for the initiation of multiple shoots, the percentage of cultures with multiple shoots and the average number of shoots/culture in the 4 different bud stages treated with 4 different concentrations of EMS are depicted in Table 7. Statistical analysis of the data gave significant difference for all the parameters considered and also in their interactions.

Days required for initiation of multiple shoots

The higher concentrations of EMS (0.5 and 0.375 per cent) were lethal to the buds excised at the time of flower harvest and these buds failed to produce multiple shoots. In buds excised 2 and 6 days after flower harvest also, the higher concentrations of EMS produced unfavourable results by prolonging the days taken for multiple shoot initiation (53-77 days).

In buds excised 4 days after flower harvest only the highest concentration (0.5 per cent EMS) prolonged multiple shoot initiation (33 days) and 0.375 per cent EMS was on par with that of control (29 days) as it produced multiple shoots in 23 days. The effect of higher concentrations of EMS (0.375 and 0.5 per cent) on the buds excised 2 days after flower harvest was on par with the performance of buds excised 6 days after flower harvest and treated with 0.25 per cent EMS (53, 56 and 56 days respectively). Similarly the days required for multiple shoot initiation by the buds excised at the time of flower harvest and treated with lower concentrations of EMS were on par with the control and lowest concentration (0.125 per cent EMS) in buds excised 6 days after flower harvest (43, 39, 41 and 43 days respectively). In the lower concentrations (0.125 and 0.25 per cent EMS) of the buds excised two and four days after flower harvest and untreated control buds excised at the time of flower harvest, the days for initiation of multiple shoots were on par and ranged from 25-30.

Percentage cultures showing multiple shoots

Comparing the general mean it was found that among the four different bud stages used, the buds excised 4 days after flower harvest gave 86.2 per cent multiple shoots.

This significantly differed from buds excised 2 days after flower harvest (81.48 per cent). These two treatments were followed by buds excised 6 days after and at the time of flower harvest (64.13 per cent and 51.28 per cent respectively). On comparing the EMS concentrations and control it was found that the control gave the highest percentage of cultures with multiple shoots (86.33 per cent). In all the different bud stages, the lower concentrations were on par having general mean values of 81.9 per cent and 81.33 per cent respectively. The general mean of multiple shoot formation in 0.375 per cent and 0.5 per cent EMS concentrations differed significantly (54.19 and 50.12 per cent respectively).

The interactions between the bud stages and EMS treatments were also found to be significant. The untreated buds excised at the time of flower harvest gave the highest percentage of cultures with multiple shoots (94.1) which differed significantly from all the other treatments. The percentage values obtained for treatments of 0.375 and 0.25 per cent EMS in buds excised 4 days after flower harvest were on par with the untreated buds excised 2 days after flower harvest (90.2, 89.8 and 90.1 per cent respectively). The lower concentrations of EMS (0.125 and 0.25 per cent) on buds excised 2 days after flower harvest

and lowest concentration of EMS (0.125 per cent) on buds excised at the time of flower harvest and 4 days later gave similar values (84.3, 82.6, 82.2 and 85.5 per cent respectively). The effect of the highest concentration of EMS on the buds excised 4 days after flower harvest was found to be on par with the performance of untreated buds of the same stage and lowest concentration of EMS (0.125 per cent) in buds excised 2 days after flower harvest (81.2, 84.3 and 84.3 per cent respectively). Similarly the lower concentrations of EMS on buds excised at the time of flower harvest and 0.25 per cent EMS on buds excised 2 days after flower harvest showed similar percentage values (82.2, 80.1 and 82.6 per cent respectively). The effects of 0.5 and 0.375 per cent EMS on buds excised two days after flower harvest and lower concentrations in buds excised six days after flower harvest were on par (76.1, 74.3, 73.3 and 75.1 per cent respectively). But they differed significantly from the effect of 0.25 per cent EMS on buds excised at the time of flower harvest. The percentage multiple shoots produced by higher concentrations of EMS (0.375 and 0.5 per cent EMS) in buds excised 6 days after flower harvest differed significantly from each other (52.3 and 43.2 per cent respectively).

Table 6. Bud take analysis

INTERACTION

Bud stages	Mutagen concentrations (%)	Days required for bud take	Bud take after 10 days (%)	Days required for first leaf emergence
D_1	Control	4	85.0	9
	0.125	5	82.3	10
	0.25	6	83.0	12
	0.375	7	65.6	13
	0.5	7	60.0	13
D_2	Control	5	83.3	10
	0.125	5	83.9	10
	0.25	6	84.8	11
	0.375	6	75.0	10
	0.5	6	70.2	11
D_3	Control	5	80.2	8
	0.125	5	85.1	9
	0.25	5	86.2	9
	0.375	5	88.3	10
	0.5	6	77.0	10
D_4	Control	3	70.1	7
	0.125	4	67.3	7
	0.25	4	66.1	8
	0.375	5	65.8	11
	0.5	6	61.1	10
	F value	NS	NS	NS

GENERAL MEAN (GM) Table

Percentage bud take

Bud stages	D_1	D_2	D_3	D_4		F value	CD value
GM	75.06	79.44	83.36	66.08		44.93	3.61
EMS concentrations	0.5	0.375	0.25	0.125	C	F value	CD
GM	67.08	73.53	80.03	79.65	79.65	8.05	5.76

Table 7. Shoot proliferation

INTERACTION TABLE

Bud stages	Mutagen concentrations	Days required for multiple shoot initiation	% cultures with multiple shoots	Number of shoots/culture
D_1	Control	25	94.1	5.22
	0.125	43	82.2	3.16
	0.25	39	80.1	3.32
	0.375	-	-	-
	0.5	-	-	-
D_2	Control	28	90.1	4.42
	0.125	27	84.3	3.33
	0.25	30	82.6	3.49
	0.375	53	74.3	2.16
	0.5	56	76.1	2.12
D_3	Control	29	84.3	3.89
	0.125	28	85.5	4.14
	0.25	26	89.8	4.34
	0.375	23	90.2	4.44
	0.5	33	81.2	3.16
D_4	Control	41	76.8	2.81
	0.125	43	73.3	2.53
	0.25	56	75.1	2.62
	0.375	64	52.3	1.49
	0.5	77	43.2	1.17
	F value	45.85	272.62	28.04
	CD	6.603	3.688	0.52

GENERAL MEAN (GM) TABLE

a) Percentage cultures with multiple shoots

Bud stages	D_1	D_2	D_3	D_4		F value	CD value
GM	51.28	81.48	86.2	64.13		620.26	2.127
EMS concentrations	0.5	0.375	0.25	0.125	C	F value	CD value
GM	50.12	54.19	81.9	81.33	86.33	723.15	1.842

b) Number of shoots/culture

Bud stages	D_1	D_2	D_3	D_4		F value	CD value
GM	2.34	3.12	3.99	2.12		56.53	0.37
EMS concentrations	0.5	0.375	0.25	0.125	C	F value	CD value
GM	1.61	2.02	3.44	3.29	4.08	134.12	0.26

Number of shoots/culture

Of the four different bud stages used for EMS treatments, buds excised 4 days after flower harvest gave an average of 3.99 shoots per culture followed by buds excised 2 days, at the time of and 6 days after flower harvest (3.12, 2.34 and 2.12 respectively). Comparing the untreated and EMS treated buds, the untreated buds gave an average shoot number of 4.08, followed by the lower concentrations of EMS (3.44 and 3.29 respectively) which were on par. The higher concentrations of EMS (0.375 and 0.5 per cent) gave an average shoot number of 2.02 and 1.61 respectively which differed significantly.

Interactions between the bud stages and EMS treatments for multiple shoot number showed significant differences. The untreated buds excised at the time of flower harvest gave an average of 5.22 shoots per culture. The number of shoots produced in 0.375, 0.25 and 0.125 per cent EMS treated buds excised 4 days after flower harvest were on par with the untreated buds excised 2 days after flower harvest (4.44, 4.34, 4.14 and 4.42 respectively). The number of shoots produced in the lower concentrations of EMS treated buds excised 4 days after flower harvest was on par with that produced by untreated buds of the same stage (4.14, 4.34 and 3.89 shoots/culture respectively). The shoot numbers observed in the lower concentrations of

EMS (0.125 and 0.25 per cent) in buds excised at the time of and 2 days after flower harvest were on par (3.16, 3.32, 3.33 and 3.49 respectively). They were also on par with the shoot number (3.16) observed in the 0.5 per cent EMS treated buds excised 4 days after flower harvest. The shoot number in control and lower concentrations of the buds excised 6 days after flower harvest were on par (2.81, 2.62 and 2.53 respectively). The higher concentrations of EMS treatments gave a low shoot number (1.17 and 1.49 respectively) and they also differed significantly from the other treatments.

4.2.2.3 Growth Analysis

A. Main shoot

The shoot length measurements taken on the 15th, 30th, 45th and 60th day after bud take under different treatments are presented in Table 8.1. Statistical analysis showed that the bud stages and EMS treatments did not produce any significant difference when considered separately, but their interaction showed significance.

Length of shoot

The shoot length in the buds excised at the time of flower harvest ranged from 1.43 (0.5 per cent EMS) to

3.08 (control). Excepting for treatment with 0.25 concentration all others showed significant difference from the control. In concentrations of EMS 0.125 and 0.25 per cent (2.45 and 2.70 respectively) and 0.375 and 0.5 (1.50 & 1.43 cm respectively) shoot length showed no significant difference between themselves. The higher concentrations of EMS (0.5 and 0.375 per cent) showed significantly lower values compared to all the other treatments.

In buds excised two days after flower harvest there was no significant difference in shoot length between the different concentrations of EMS and also with control. The values ranged from 2.45 cm in 0.5 per cent EMS to 3.0 cm in control. The same trend was noticed in buds excised 6 days after flower harvest where the range was from 2.2 cm in 0.125 per cent EMS to 2.5 cm in 0.25 per cent EMS. In buds excised 4 days after flower harvest. 0.375 per cent EMS showed a maximum value (3.31 cm) which was significantly different from that of the control (2.6 cm), but among the different concentrations of EMS no significant variation in shoot growth was observed.

Comparing the effect of higher concentrations (0.5 and 0.375 per cent EMS) in the 4 different bud stages, it was found that the buds excised at the time of flower harvest showed reduced shoot growth. The different bud

stages did not show significant variation in shoot growth in low concentrations (0.25 and 0.125 per cent) of EMS and controls.

Number of leaves

The number of leaves in the main shoot was also recorded at 15 days interval for 2 months and the tabulated data are presented in Table 8.2. Statistical analysis showed that the bud stages did not have a significant influence on the production of leaves, while the EMS treatments and the interaction of bud stages and EMS treatments showed significant difference.

On comparing the effect of different EMS concentrations and control on the leaf production, it was observed that the general mean in control and lower concentrations (0.25 and 0.125 per cent) were on par (9.19, 8.56 and 8.44 respectively) but control differed significantly from the higher concentrations of EMS (0.5 and 0.375 per cent) which gave an average leaf number of 6.63 and 7.63 respectively.

Considering the interactions it was observed that in buds excised at the time of flower harvest, the higher concentrations of EMS showed a low leaf number of 3.75 and 3.25 respectively and in the lower concentrations and

control the leaf number varied from 9-10. In the buds excised 2 days after flower harvest, the average number of leaves in the highest concentration of EMS (0.5 per cent) was low (5.75) and in the other concentrations (0.375, 0.25 and 0.125 per cent EMS) and control the leaf number was on par (8.75, 8.5, 9.0 and 9.5 respectively). The buds excised 4 days after flower harvest and treated with 0.375 per cent EMS gave a high average leaf number of 11.25 and in the other concentrations of EMS and control the average leaf number was on par and varied from 8.25-9.25. In buds excised 6 days after flower harvest, the average leaf number ranged from 6.75-8.5 in 0.375 per cent EMS and control respectively.

On comparing the higher concentrations of different bud stages it was found that the buds excised 4 and 6 days after flower harvest showed a higher leaf number (8-11). In the first, second and third bud stages treated with lower concentrations of EMS (0.25 and 0.125 per cent) the number of leaves were on par.

B. Side shoots

The height measurements of the multiple shoots recorded on the 15th, 30th, 45th and 60th day from the date of initiation are presented in Table 9.1. Statistical

analysis showed no significance for bud stages, while the EMS treatments and the interaction of the bud stages with EMS concentrations showed significant variation.

Length of shoot

The general mean for length of shoot were on par in the lower concentrations of EMS (0.125 and 0.25 per cent) and control (1.93, 2.09 and 2.20 cm respectively). In the higher concentrations of EMS (0.375 and 0.5 per cent) the general mean differed significantly from each other (1.38 and 1.08 cm respectively) and also from other treatments.

Under the first stage of bud development the shoot length in 0.25 per cent of EMS (2.05 cm) was on par with control (2.43 cm). In the lowest concentration of EMS (0.125) shoot length (1.65 cm) differed significantly from control. The higher concentrations (0.5 and 0.375 per cent EMS) failed to produce any multiple shoots as mentioned earlier. In the second stage of bud development also, the lower concentrations and control were on par and the higher concentrations (0.5 and 0.375 per cent EMS) differed significantly from other concentrations and control.

In the third stage of bud development 0.375 per cent EMS treatment showed a value of 2.68 cm which was significantly different from control value (2.03 cm), but was

on par with lower concentrations of EMS (2.45 and 2.43 cm respectively). Control was on par with shoot length in 0.5, 0.25 and 0.125 per cent EMS concentrations. The shoot length in highest concentration of EMS (1.90 cm) differed significantly from the other EMS treatments. In the fourth stage of bud development, the lower concentrations of EMS and control were on par. But shoot length in control differed significantly from the higher concentrations of EMS.

Comparing the four different bud developmental stages it can be noted that 0.375 EMS treatment showed the highest value (2.68 cm) for shoot length in the third bud stage. The effects of lower concentrations of EMS on the first and second bud stages were on par. The effect of lower concentrations of EMS on second and third bud stages differed significantly from the fourth bud stage. The untreated controls of all the four different bud stages were on par.

Number of leaves

The tabulated data on the number of leaves in the multiple shoot recorded at 15 days interval from the date of multiple shoot initiation is presented in Table 9.2. On statistical analysis, no significance was observed for

the different bud stages but significant difference was observed among the different concentrations and also for the interaction between EMS treatments and bud stages.

Comparison of the general mean for leaf number in different EMS concentrations and control showed that the lower concentrations ie. 0.125 per cent and 0.25 per cent did not differ significantly from control (7.88 and 8.25 respectively). Significant difference was observed in the general mean between 0.375 and 0.5 per cent EMS concentrations (5.75 and 4.31 respectively). Considering the interaction it was found that in buds excised at the time of flower harvest, the higher concentrations of EMS (0.375 and 0.5 per cent) did not produce multiple shoots. So leaf number could not be recorded. In the multiple shoots produced by control and in lower concentrations of EMS the number of leaves ranged from 6-8. In buds excised 2 days after flower harvest, higher concentrations of EMS gave an average of 5.75 to 6.75 leaves in a two month culture period. In lower concentrations of EMS and untreated control buds the number of leaves was 7.0-8.5. In buds excised 4 days after flower harvest the leaf number ranged from 8.0-10.25 in control and lower concentrations of EMS while in 0.5 per cent EMS, the average leaf number was 6.5. In buds excised 6 days after flower harvest, the

lower concentrations and control gave an average leaf number of 7.5-8.5. In higher concentration the multiple shoots showed a lower leaf number (5-6).

In general, the 0.5 per cent EMS treatment showed a low leaf number in all bud stages. The leaf number for the highest concentration was significantly lower to all other treatments in the third bud stage while for the second and fourth bud stages, its leaf number was significantly lower to control and the two lower concentrations of 0.125 and 0.25.

4.2.2.4 Analysis of rooting pattern

The days required for rooting, the percentage of shoots showing rooting and the average number of shoots/culture are presented in Table 10. Statistical analysis showed significant difference for all these characters.

Number of days required for rooting

In shoots separated and cultured on the standardised rooting medium, the number of days required for rooting ranged from 15.67-36.30. In buds excised at the time of flower harvest the average number of days required for rooting was on par in 0.125 and 0.25 per cent EMS (21 and 24.67 respectively). But the shoot from untreated buds

required only an average of 15.67 days for rooting. In buds excised 2 days after flower harvest, the number of days required for rooting was on par in the two lower concentrations and higher concentrations. But the higher concentrations (0.375 and 0.5 per cent EMS) differed significantly from the lower concentrations (0.125 and 0.25 per cent EMS) in the number of days required for rooting (31.33, 31.00, 23.67 and 23.33 respectively). The shoots from untreated buds rooted within 18 days and differed significantly from the number of days required for rooting in the shoots from treated buds. In buds excised 4 days after harvest the shoots from 0.375 per cent EMS and 0.25 per cent EMS treated buds and untreated buds rooting occurred within 21.67 to 22 days. The shoots in 0.5 per cent EMS treated buds differed significantly from all other treatments and required an average of 31.33 days for rooting. In buds excised 6 days after flower harvest, the lower concentrations and control rooted within 22.67-26.00 days. The higher concentrations (0.5 and 0.375 per cent EMS) differed significantly from lower concentrations and control and required an average of 36.3 and 31 days respectively for rooting.

Percentage shoots showing rooting

Among the 4 bud stages used for EMS treatments, the

general mean for percentage shoots rooting was highest in buds excised 4 days after flower harvest (87.41 per cent) followed by buds excised 2 days after flower harvest (78.99 per cent) buds excised 6 days after flower harvest (69.95 per cent) and buds excised at the time of flower harvest (52.13 per cent). Untreated shoots showed a rooting percentage of 85.63 per cent which significantly differed from treated (0.125, 0.25, 0.375 and 0.5 per cent EMS) shoots. EMS treatments of 0.125 per cent and 0.25 per cent showed rooting percentage values of 82.64 per cent and 83.50 per cent respectively which were on par. The higher concentrations (0.375 and 0.5 per cent EMS) showed significant difference in rooting percentages (57.53 and 51.3 per cent respectively).

Comparing the interactions it was found that the shoots from untreated buds excised at the time of flower harvest and shoots from 0.375 and 0.25 per cent EMS treated buds excised 4 days after flower harvest gave rooting percentages which were on par (93.06, 91.17 and 90.83 per cent respectively). In buds excised at the time of flower harvest, the percentage shoots rooting in lower concentrations of EMS and control differed significantly from each other (82.4, 85.2 and 93.06 per cent respectively). The percentage shoots rooting were on par in buds excised

2 days after flower harvest and treated with 0.25 per cent of EMS and control (83.97 and 82.6 per cent respectively). In the higher concentrations of EMS the percentage shoots rooting (74.9 and 72.3 per cent respectively) differed significantly from each other and from the lower concentrations of EMS and control. In buds excised 4 days after flower harvest the rooting percentage in 0.25 and 0.375 per cent EMS were on par (90.83 and 91.17 per cent respectively) but they differed significantly from the other treatments and control. In buds excised 6 days after flower harvest the rooting percentages differed significantly in all the treatments.

Number of roots/shoot

Comparing the general mean for number of roots/shoot in the four bud stages it was found that the buds excised 2 days after and 4 days after flower harvest were on par (4.07 and 4.18 respectively) but differed significantly from buds excised at the time and 6 days after flower harvest (2.75 and 3.09 respectively). The general mean for number of roots/shoot were on par in the lower concentrations of EMS and control (4.10, 4.19 and 4.28 respectively) but differed significantly from the higher concentrations (2.68 and 2.36 respectively).

In buds excised at the time of flower harvest, the number of roots/shoot in control was significantly different from lower concentrations of EMS (5.06, 4.2 and 4.47 respectively). In buds excised 2 days after flower harvest, the number of roots/shoot were on par in control and lower concentrations of EMS (4.57, 4.7 and 4.50 respectively). The higher concentrations were on par among themselves for the number of roots/shoot (3.37 and 3.21 respectively) but differed significantly from the lower concentrations and control. In buds excised 4 days after flower harvest, the number of roots/shoot were on par in control, 0.125, 0.25 and 0.375 per cent EMS (4.04, 4.30, 4.50 and 4.52 respectively) but in 0.5 per cent EMS the number of roots/shoot differed significantly from other treatments. Buds excised 6 days after flower harvest showed the same trend as in buds excised 2 days after flower harvest.

Table 8. Growth Analysis of Main Shoot

8.1. Length of shoot

Bud stages	Mutagen concentrations	Length of shoot (cm)				
		15th day	30th day	45th day	60th day	Mean
D_1	Control	1.4	2.2	3.4	5.3	3.08
	0.125	1.2	1.9	2.8	3.9	2.45
	0.25	1.3	2.1	3.1	4.3	2.70
	0.375	1.1	1.5	1.7	1.7	1.50
	0.5	1.1	1.4	1.6	1.6	1.43
D_2	Control	1.6	2.3	3.2	4.9	3.00
	0.125	1.7	2.2	3.1	4.4	2.85
	0.25	1.5	2.6	3.3	4.6	3.00
	0.375	1.6	2.4	3.0	3.5	2.63
	0.5	1.5	2.3	2.8	3.2	2.45
D_3	Control	1.9	2.1	2.8	3.6	2.60
	0.125	1.8	2.2	3.6	4.7	2.95
	0.25	1.7	2.3	3.2	4.8	3.00
	0.375	2.0	2.6	3.8	5.1	3.31
	0.5	1.9	2.4	3.4	3.6	2.83
D_4	Control	2.0	2.3	2.5	3.1	2.48
	0.125	1.8	2.1	2.3	2.6	2.20
	0.25	1.9	2.4	2.8	3.2	2.58
	0.375	1.6	2.1	2.8	2.9	2.35
	0.5	2.0	2.5	2.6	2.7	2.48
	F value					3.72
	CD value					0.604

Table 8.2. Leaf number

INTERACTION

Bud stages	Mutagen concentrations	Number of leaves				
		15th day	30th day	45th day	60th day	Mean
D_1	Control	4	8	12	28	10.50
	0.125	3	8	10	14	8.75
	0.25	3	7	11	15	9.00
	0.375	3	4	4	4	3.75
	0.5	2	3	4	4	3.25
D_2	Control	6	8	10	14	9.50
	0.125	6	7	11	12	9.00
	0.25	5	7	10	12	8.50
	0.375	6	8	10	11	8.75
	0.5	5	4	6	8	5.75
D_3	Control	5	7	9	12	8.25
	0.125	4	7	10	13	8.50
	0.25	6	8	11	14	9.75
	0.375	6	10	12	17	11.25
	0.5	5	9	10	12	9.00
D_4	Control	5	8	10	11	8.50
	0.125	4	6	9	11	7.50
	0.25	4	5	8	11	7.00
	0.375	5	6	7	9	6.75
	0.5	6	9	9	10	8.50
	F value					6.45
	CD value					2.18

GENERAL MEAN (GM) TABLE

Leaf number

EMS concentrations	0.5	0.375	0.25	0.125	C	F value	CD
GM	6.63	7.63	8.56	8.44	9.19	6.66	1.08

Table 9. Growth Analysis of Multiple Shoots

9.1. Length of shoot

INTERACTION

Bud stages	Mutagen concentrations	Length (cm) from date of 1st multiple shoot production				
		15	30	45	60	Mean
D_1	Control	1.2	1.8	2.6	4.1	2.43
	0.125	0.8	1.3	1.6	2.9	1.65
	0.25	0.9	1.4	2.8	3.9	2.05
	0.375	0	0	0	0	0
	0.5	0	0	0	0	0
D_2	Control	1.1	1.8	2.6	3.8	2.33
	0.125	1.0	1.6	2.1	3.3	2.00
	0.25	1.2	1.9	2.5	3.7	2.33
	0.375	0.6	1.1	2.0	2.4	1.53
	0.5	0.5	0.9	1.8	2.1	1.33
D_3	Control	1.0	1.6	2.1	3.4	2.03
	0.125	1.2	1.8	2.8	3.9	2.43
	0.25	1.5	1.9	2.4	4.0	2.45
	0.375	1.3	1.9	2.9	4.6	2.68
	0.5	1.8	1.5	2.4	2.9	1.90
D_4	Control	1.4	1.9	2.1	2.7	2.03
	0.125	1.2	1.4	1.9	2.0	1.63
	0.25	0.9	1.3	1.8	2.2	1.55
	0.375	0.8	1.1	1.4	1.9	1.30
	0.5	0.4	0.9	1.3	1.7	1.08
	F value					7.78
	CD value					0.533

GENERAL MEAN (GM) Table

EMS concentrations	0.5	0.375	0.25	0.125	C	F value	CD value
GM	1.08	1.38	2.09	1.93	2.2	26.89	0.267

Table 9.2. Leaf number

INTERACTION

Bud stages	Mutation concentration	Number of leaves				
		15th day	30th day	45th day	60th day	Mean
D_1	Control	4	6	8	14	8.00
	0.125	2	5	8	9	6.00
	0.25	3	4	8	10	6.75
	0.375	-	-	-	-	-
	0.5	-	-	-	-	-
D_2	Control	5	8	9	12	8.50
	0.125	3	6	9	10	7.00
	0.25	4	6	10	11	7.75
	0.375	3	5	9	10	6.75
	0.5	2	5	7	9	5.75
D_3	Control	4	7	10	11	8.00
	0.125	4	8	10	11	8.25
	0.25	5	10	11	12	9.50
	0.375	6	10	12	13	10.25
	0.5	3	5	8	10	6.50
D_4	Control	4	9	10	10	8.50
	0.125	4	8	9	10	7.75
	0.25	3	7	10	10	7.50
	0.375	3	6	7	8	6.00
	0.5	2	5	5	8	5.00
	F value					9.30
	CD value					1.646

GENERAL MEAN (GM) Table

EMS concentrations	0.5	0.375	0.25	0.125	C	F value	CD value
GM	4.31	5.75	7.88	7.25	8.25	31.86	0.823

Table 10. Rooting pattern under in vitro conditions

INTERACTION

Bud stages	Mutagen concentration	Days required for rooting	Shoots rooting (%)	Number of roots/shoot
D_1	Control	15.67	93.06	5.06
	0.125	21.00	82.40	4.20
	0.25	24.67	85.20	4.47
	0.375	-	-	-
	0.5	-	-	-
D_2	Control	18.00	82.60	4.57
	0.125	23.67	81.13	4.70
	0.25	23.33	83.97	4.50
	0.375	31.00	74.90	3.37
	0.5	31.33	72.30	3.21
D_3	Control	21.67	84.93	4.04
	0.125	26.33	87.60	4.30
	0.25	21.67	90.83	4.50
	0.375	22.00	91.17	4.52
	0.5	31.33	82.50	3.54
D_4	Control	26.00	81.87	3.45
	0.125	22.67	79.43	3.21
	0.25	26.00	74.00	3.27
	0.375	31.00	64.03	2.82
	0.5	36.30	50.40	2.70
	F value	15.47	958.77	54.37
	CD value	3.85	2.025	0.438

GENERAL MEAN (GM) TABLE

Percentage shoots rooting

Bud stages	D_1	D_2	D_3	D_4		F value	CD value
GM	52.13	78.99	87.41	69.95		789.94	1.75
EMS concentrations	0.5	0.375	0.25	0.125	C	F value	CD value
GM	51.3	57.53	83.50	82.64	85.63	2169.23	1.01

Number of roots/shoot

Bud stages	D_1	D_2	D_3	D_4		F value	CD value
GM	2.75	4.07	4.18	3.09		87.98	0.248
EMS concentrations	0.5	0.375	0.25	0.125	C	F value	CD value
GM	2.36	2.68	4.19	4.10	4.28	148.13	0.219

DISCUSSION

DISCUSSION

The results emanated during the present investigation, to standardise a suitable culture medium, the best developmental phase of the explant material and also to identify the suitable mode of chemical mutagen treatment adopting *in vitro* culture technique for rose are discussed below.

5.1 Standardisation of culture medium

The standardisation of different levels of hormones was done at 3 phases of explant development viz. culture establishment, axillary bud proliferation and root development by using MS as the basal medium. Cytokinins and auxins are the two types of hormones required for shoot growth. Although shoots grown *in vitro* (Koda and Okazawa, 1980) are capable of synthesising a small quantity of cytokinin, roots are the principal site of cytokinin biosynthesis. It is unlikely that the meristem shoot tip and bud explants have sufficient endogenous cytokinin to support growth and development and so cytokinins must be added to the culture medium. In the case of auxins, if relatively large shoot tip explants from actively growing plants are used exogenous auxins are not needed in the establishment phase (Kusey *et al.*, 1980; Lane, 1979a). But if resting buds and meristems of 0.4 mm size or less

are used enough endogenous auxins for shoot growth may not be produced and hence in these cases auxins need to be added (Dale, 1977b; Ziv, 1979; Evans, 1981).

5.1.1 Culture establishment

In the culture establishment phase different combinations of the cytokinin BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and auxin 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/l) were tried. The results clearly indicated that the combination of BAP, 2.0 mg/l + 2,4-D 1.0 mg/l was the best compared to other combinations as it gave a maximum bud take of 85 per cent in a minimum of 4 days. The auxin 2,4-D was used successfully in combination with cytokinin in other plants also such as Dactylis glomerata, and Festuca sp., (Dale, 1977b) and Lolium multiflorum (Dale, 1977a). The most potent auxin is 2,4-D which stimulates callus formation but at the same time strongly antagonises organised development. This might be the reason for the heavy callusing observed when 2,4-D level increased above 1.0 mg/l in the present study. Hill (1967b), Street (1979) and Engvild (1978) suggested that high levels of auxins and in particular 2,4-D tend to suppress morphogenesis. Induction of callus by higher levels of auxin was also reported by Kireeva et al. (1977) in rose variety Krymskaya krasnaya. It was also found that an increase

in 2,4-D level accompanied by a simultaneous increase in BAP reduced callusing. So when the combination of BAP 2.0 mg/l + 2,4-D 1.5 mg/l was used, bud take occurred but with a delay of about 6 days. The addition of auxins other than 2,4-D (IBA, NAA etc.) to BAP to obtain better culture establishment has also been reported in Arachis hypogea (Kartha et al., 1981b), Coffea arabica (Kartha et al., 1981a), Dianthus caryophyllus (Roest and Bokelmann, 1981), Malus sp. (James and Thurbon, 1981), Rosa hybrida (Hasegawa, 1980), Rosa sp. (Skirvin and Chu, 1979a), rose cultivar 'Joyfulness' (Pitlet and Mancousin, 1982), Santalum sp. (Barlass et al., 1980) and Solanum etuberosum (Towill, 1981).

5.1.2 Axillary bud proliferation

In axillary bud proliferation cytokinin is utilised to overcome the apical dominance of shoots and to enhance the branching in lateral buds from leaf axils (Hasegawa, 1980). The effective concentration of exogenous cytokinin required to reverse apical dominance varies with the culture systems. In general BAP appears to be the most effective cytokinin for stimulating axillary shoot proliferation (Hasegawa, 1979; Davies, 1980; Skirvin Chu, 1979b).

In the present study, the addition of BAP 2.0 mg/l alone to the medium gave a fairly high percentage of

cultures with multiple shoots and the average number of shoots per culture was around 4. Good shoot proliferation was reported in a number of cases when BAP alone was used as in Anthurium andreanum (Kunisake, 1980), strawberry (Kartha et al., 1980), Gypsophila paniculata (Kusey et al., 1980), Phaseolus vulgaris (Kartha et al., 1981b), Prunus cistena (Lane, 1979a), Santalum sp. (Barlass et al., 1980), Spinea bumalda (Lane, 1979b), Stevia rebaudiana (Yang, 1981), Rosa indica (Avramis, 1982a) and hybrid tea (Alekhno, 1980a).

The addition of low concentrations of GA to the BAP supplemented medium gave positive results. GA at the level of 0.5 mg/l along with BAP 0.5, 1.0, 1.5 or 2.0 mg/l gave better results compared to BAP alone added to the medium. But when the level of GA increased and BAP remained at the concentrations 0.5, 1.0 or 1.5 mg/l, the percentage of cultures with multiple shoots and the average number of shoots/culture were lower than that obtained when BAP alone was added to the medium. BAP at 2.0 mg/l along with GA at the concentrations from 0.5 to 1.5 mg/l produced favourable results. A further increase in GA reduced the percentage of cultures with multiple shoots and the average shoot number/culture. This indicates the existence of some sort of interaction between BAP and GA. A similar

effect due to increase in GA concentrations has also been reported by Rout et al. (1989) in the Rosa hybrida cv. Landora. Cai et al. (1984) also observed effective shoot proliferation when a combination of BAP and GA was used in Rosa chinensis. Wochok and Sluis (1980) observed that a topical treatment of shoot explants of Atriplex with GA was effective not only in stimulating shoot elongation but also in enhancing shoot multiplication beyond that of the most effective auxin-cytokinin combination. Valles and Boxus (1987) found that GA at 1.0 mg/l enhanced axillary branching in Rosa hybrida cvs. A combination of BAP and GA was also successful in other crops such as Beta vulgaris, Fragaria virginiana x F. chiloensis, Phlox subulata, and raspberry (Atanassov, 1980; James, 1979; Schnabdrauch and Sink, 1979).

Efficient shoot proliferation was reported by several workers in rose when a combination of BAP and an auxin in low concentrations was used (Hasegawa, 1979; Skirvin and Chu, 1979; Davies, 1980; Avramis et al., 1982a; Khosh Khui and Sink, 1982b; Pitlet and Mancousin, 1982; Bini et al., 1983; Barve et al., 1984; Louwaars, 1984 and Damiano et al., 1987).

In vitro rooting

The purpose of this stage is to induce de novo

regeneration of adventitious roots from shoots obtained in the previous stage and this root initiation depends on a low cytokinin - high auxin ratio.

When the salt concentration in the medium is lowered to 1/2, 1/3 or 1/4 of the standard strength, rooting becomes abundant (Lane, 1979b; Skirvin and Chu, 1979). In the present study rooting was first tried in 1/4 strength MS medium supplemented with 3.0 mg/l IBA as reported by Vijaya et al. (1985) in rose. Here rooting occurred after 1 month but the leaves gradually turned yellow and dropped off. Although, lower salt concentration in a medium may be beneficial to root induction, it sometimes results in poor top growth. Wang (1978) observed that a 1/4 strength of MS medium stimulated 100 per cent rooting in Cryptomeria japonica, but resulted in poor shoot growth. Callusing also was observed at the base of the shoot when 3.0 mg/l IBA was used. When the auxin concentration is too high, callus will form at the shoot base which inhibits normal root development (Lane, 1979a). Thimann (1977) reported that in general high auxin concentration will inhibit the root elongation. A reduction in the auxin concentration decreased the callus formation, but the yellowing of leaves continued. This may be due to the reduction in salt concentration.

When rooting was tried in 1/2 strength MS medium, the leaves retained their green colour for a little longer than the previous trial, but still had an unhealthy appearance. The auxins tried were NAA, IBA & IAA each at 0.5, 1.0, 1.5 and 2.0 mg/l levels. Of these, IBA supplemented cultures exhibited a comparatively poor performance. But successful rooting has been reported in Coffea arabica and Malus sp. using low concentration of IBA in 1/2 strength MS medium (Kartha et al., 1981a; Snir and Erez, 1980). Several species such as Cynara scolymus (Ancora et al., 1981), Grevilba rosmarinfolia (Ben Jaacov and Dax, 1981), Prunus cistena (Lane, 1979a), Pyrus communis (Lane, 1979c), Spirea bumalda (Lane, 1979b), rose cvs 'Forever Yours' (Skirvin and Chu, 1979), 'Improved Blaze' (Hasegawa, 1980) and Rosa indica major (Avramis et al., 1982b) produced an efficient root system in NAA supplemented MS medium. Khosh Khui and Sink (1982c) and Barve et al., 1984) obtained good rooting using IAA in rose cvs. "Bridal Pink" and "Crimson Glory".

In the present study in vitro derived shoots of rose were also cultured on full strength MS medium supplemented with the auxins NAA and IAA (0.5, 1.0, 1.5 and 2.0 mg/l) to improve the top growth. Here it was found that root initiation occurred much earlier than the previous

treatments. IAA (2.0 mg/l) initiated roots in just 14 days. When 1/2 strength medium was used, higher doses of auxins decreased the percentage rooting and the average number of roots/shoot. But, in full strength medium higher doses gave better results than lower doses. This indicates that when the salt concentration is low a lower level of auxin can initiate rooting, but when salt concentration increases, the same auxin is required at a slightly higher dose to initiate rooting. The shoots appeared healthy which may be due to the increase in salt concentration. Good rooting was obtained in full strength MS medium supplemented with IAA in Atriplex canaescens (Wochok and Sluis, 1980), Citrullus lanatus (Barnes, 1979), Dianthus caryophyllus (Roest and Bokelmann, 1981) and Tectona grandis (Gupta et al., 1980). Brassica campestris, Carica papaya, Gladiolus sp., Hosta decorata, Phlox subulata, Willow and Stevia rebaudiana produced good root system in MS medium supplemented with NAA (Kuo and Tsay, 1977; Litz and Conover, 1978; Ziv, 1979; Papachatzi et al., 1981; Sehnabelrauch and Sink, 1979; Bhojwani, 1980; Yang, 1981).

Successful rooting in full strength MS medium without hormones has been reported in Chrysanthemum morifolium (Earle and Langhans, 1974) and Eucalyptus citriodora (Gupta et al., 1981).

In the present study in MS medium without hormones, though rooting occurred it was at a delayed date. The rooting percentage was poor and average number of roots/shoot was also low compared to those in which hormones were added. This indicates the importance of hormones in initiating roots from in vitro derived shoots.

5.2 Mutagen treatments

5.2.1 Direct treatment

The axillary buds responded very poorly to this method of mutagen treatment. The buds of all the 4 maturity groups showed browning but at varying degrees. The browning was severe in the buds excised at the time of flower harvest followed by buds excised 2 days, 4 days and 6 days after flower harvest. The intense browning observed in the buds excised at the time of flower harvest may probably be due to the small size of the buds. Though browning occurred in buds excised 2,4 & 6 days after the harvest, its intensity was low. Since the control buds showed bud take and normal growth, the browning can be attributed to the direct effect of the mutagen. Similar damage due to higher dose of mutagen (EMS) has also been reported in Weigela cv Bristol Ruby (Duron & Decourtye, 1986) and Arctostaphylos (Duskova et al., 1988). In the buds treated

2 days, 4 days and 6 days after culturing also, browning occurred after EMS treatment. The buds cultured on MS medium remained green and intact upto the EMS treatment and when the treated buds were recultured on fresh MS medium, browning occurred within one week. Browning occurred at all stages of buds with varying intensities. The control buds washed in sterile water and recultured also failed to produce normal growth. This indicates that even the slightest disturbance of the bud during the first few days of culturing can be fatal. The treated and recultured buds were also prone to high rate of infection. During treatments, the buds belonging to a particular treatment were washed together and so there was a high chance of contamination. Moreover usually contamination occurs in culture medium during the first 10 days.

5.2.2 Cotton swab method

5.2.2.1 Culture establishment

The buds seemed to respond well to this method of mutagen treatment. The bud take analysis revealed no pronounced difference in the days required for bud take and the days required for the initiation of the first leaf between the various treatments tried. In buds excised 4 days after harvest, the treated as well as the control

buds showed a higher bud take percentage than in buds excised at the time and 2 days after flower harvest.

The bud take percentage was lowest in buds excised 6 days after flower harvest probably because of a higher rate of contamination observed. The greater incidence of contamination in buds excised 6 days after may be due to lack of proper sterilisation as the size of the bud or explant was slightly large. Report of infection due to a larger size of explant was made by Mellon and Stace-Smith (1977). They found that buds more than 0.7 mm long were prone to greater infection in potato.

The bud take percentages of 0.125 and 0.25 per cent EMS and control were on par while those of 0.375 and 0.5 per cent EMS was much lower indicating the sensitivity of the buds to higher concentrations of the mutagen. Similar decrease in survival percentages with increase in mutagen concentration was reported by Kleffel et al. (1987) in Poinsettia.

5.2.2.2 Enhanced release of axillary buds

The multiple shoot production was remarkably influenced by the bud stages as well as the EMS treatments. A delay in the initiation of multiple shoots was observed with an increase in the size or maturity of the bud and

concentration of EMS. The buds excised 6 days after flower harvest and treated with higher doses of EMS required the maximum time for initiation of multiple shoots. In buds excised 4 days after flower harvest an increase in EMS concentration upto 0.375 per cent did not produce much delay in the initiation of multiple shoots and they were on par with the control. In buds excised 2 days after flower harvest the delay in multiple shoot initiation was pronounced above 0.25 per cent EMS. In buds excised at the time of flower harvest even low concentrations of 0.25 per cent EMS delayed multiple shoot initiation while it was completely inhibited at higher doses. In mustard, George and Rao (1979) reported that EMS had an inhibitory effect on shoot regeneration while gamma rays above 2 kr suppressed shoot regeneration but stimulated callus growth. High EMS doses decreased shoot proliferation in grapes (Kim et al., 1989).

The buds excised 6 days after flower harvest exhibited a poor performance compared to the buds excised after 4 days, 2 days and at the time of flower harvest. The higher doses of EMS still worsened their performance while lower doses were on par with control. EMS treatment at a concentration of 0.375 per cent had a stimulatory effect on the buds excised 4 days after flower harvest.

Here the buds treated with 0.375 and 0.25 per cent EMS gave a higher percentage of cultures with multiple shoots and the average number of shoots/culture was also greater than that of the control. Here the buds treated with concentrations upto 0.375 per cent far excelled the control in multiple shoot production. In the buds excised 2 days after flower harvest doses upto 0.25 per cent did not hinder the multiple shoot production and similar effect was observed in buds excised at the time of flower harvest. But in buds excised at the time of flower harvest EMS doses above 0.25 per cent completely inhibited shoot regeneration and the effect of higher doses was not so intense in buds excised 2 days after flower harvest. Walther and Saver (1986b) had reported a similar effect due to higher doses of X ray in Gerbera and Kim et al. (1989) in grape.

5.2.2.3 Growth Analysis

In the analysis of growth in the main shoots derived from the treated and control buds significant interaction was observed between the bud stages and treatments. The buds excised at the time of flower harvest and treated with 0.5 and 0.375 per cent EMS showed poor growth. The concentrations were on par with the control. But in the case of buds excised 4 days after harvest, 0.375, 0.25 and 0.125 per cent EMS treatments far excelled the control.

The buds excised 6 days after harvest exhibited a comparatively poor growth at all EMS levels.

The growth of multiple shoots also was greatly influenced by the different concentrations of EMS. The growth of multiple shoots was poor in the two higher concentrations of EMS. The buds excised 4 days after flower harvest were not greatly retarded by higher concentrations of EMS. Here the buds treated with 0.375 and 0.25 per cent EMS, excelled the control. Kim et al. (1989) has reported that higher doses of EMS decreased shoot length in grape.

5.2.2.4 In vitro rooting

The number of days required for rooting increased with the increase in EMS doses and increase in the maturity of the bud. In the lower concentrations of EMS and control the days required for initiation of roots were on par. The percentage shoots rooting also decreased with increasing doses of EMS. But in buds excised 4 days after flower harvest EMS doses upto 0.375 produced favourable results. In this stage a slightly higher dose was found to have a stimulatory effects. The number of roots/shoot also decreased with increasing doses of EMS.

In short of the various stages of buds considered, buds excised at the time of flower harvest excelled all

other stages, but when treated with EMS their growth rate, shoot proliferation and rooting were highly suppressed. For mutagen treatment, the buds excised 4 days after flower harvest were found to be the best. Here EMS concentrations upto 0.375 per cent were favourable and they were found to have a stimulatory effect on the buds as they excelled their control. In buds excised 2 days after flower harvest the lower concentrations of EMS were on par with control. The buds excised 6 days after flower harvest exhibited a poor performance in normal and treated populations.

SUMMARY

SUMMARY

The present investigation to standardise a suitable *in vitro* culture technique for induced mutagenesis in rose (*Rosa chinensis*) was conducted during 1989-'90 at the Tissue Culture Laboratory attached to the Department of Horticulture, College of Agriculture, Vellayani. The main objectives were to standardise the *in vitro* technique in rose using axillary bud as the explant, to standardise surface sterilisation techniques, to standardise the correct stage of the bud for mutagen treatment, to standardise the technique of treatment and also to assess the dose requirement of the chemical mutagen, ethyl methane sulphonate (EMS). The dose effect was assessed based on various growth indices including days taken for bud take, percentage of cultures producing multiple shoots, length of shoot at 15 days interval, number of leaves per plantlet at fifteen days interval and rooting percentage. Based on the results eminated from the investigation the following conclusions are made.

1. Of the various concentrations of mercuric chloride solutions tested and various time intervals adopted for treatment, the treatment 0.08 per cent mercuric chloride for 15 min was found to be the best, based on survival percentage.

2. In the culture establishment phase a combination of BAP 2 mg/l + 2,4-D 1 mg/l in MS medium was found to be the best for maximum bud take.

3. For shoot proliferation or enhanced release of axillary buds, BAP 2 mg/l + GA 2 mg/l was the apt dose for obtaining maximum multiple shoot production.

4. In 1/4 and 1/2 strength MS medium, root initiation was delayed and cultures showed poor top growth.

5. In full strength MS medium, IAA at 2 mg/l gave maximum rooting percentage and the shoots had a healthy appearance.

6. An increase in the maturity of buds delayed multiple shoot production.

7. Buds excised at the time of flower harvest produced maximum multiple shoots.

8. Direct treatment of the axillary buds with EMS was not found to be feasible in rose at any stage of maturity viz. at the time of flower harvest, 2, 4 and 6 days after flower harvest. The buds turned brown and no further development was observed.

9. A decrease in survival percentage was noted with an increase in mutagen concentration during the early stages of development.

10. Higher concentrations of EMS curbed multiple shoot production in buds excised at the time of flower harvest, and prolonged multiple shoot production in mature buds.

11. The lower concentrations of EMS (0.125 and 0.25 per cent) gave an insignificant result compared to untreated control.

12. No significant variation was observed in shoot length with increase in maturity of buds.

13. Rooting percentages decreased with increasing concentrations of EMS.

14. The percentage shoots rooting and the number of roots/shoot decreased with increase in the maturity of buds.

15. Buds excised at the time of flower harvest was found to be the best stage of explant for *in vitro* culture in rose, but mutagen concentrations above 0.25 per cent proved to be fatal.

16. Treatment of buds excised four days after flower harvest was found to be more suitable for mutagenic treatment compared to buds excised 2 and 6 days after flower harvest.

17. Buds excised 6 days after flower harvest was not found to be suitable for *in vitro* culture due to their poor performance.

18. The best concentrations for the maximum mutational events in rose while exploiting *in vitro* culture techniques seems to be between 0.125 and 0.375 per cent of EMS.

Based on the results emanated during the present investigation it is recommended that for detailed analysis of induced mutagenesis in rose adopting *in vitro* culture technique, MS medium with buds excised 4 days after flower harvest and a concentration of EMS solution between 0.125 and 0.375 per cent will result in maximum mutated events to give an economic response to induced variability by minimising diplontic selection.

REFERENCE

REFERENCES

Alderson, P.G., Mckinless, J. and Rice, R.D. (1988). Rooting of cultured rose shoots. Acta Horticulturae. 1 (226) pp 175-182.

Aldrufeu, A., Pages, M., Messeguer, J. and Mele, E. (1984). In vitro rhizogenesis of Rosa species in different substrates. Acta Horticulturae (150) pp 315-323.

Alekhno, G.D. and Vysotskii, V.A. (1986a). Clonal micropropagation of roses. Fiziologiya i Biokhimiya kul' turnykh Rastenii. 18 (5) pp 489-493.

Alekhno, G.D. and Vysotskii, V.A. (1986b). Effect of nutrient medium on the micropropagation of roses. In Sortoizuchenie i Razmnozhenie Dekorativnykh kul'tur 57-61 from Referativnyi Zhurnal 6: pp 855-858.

Amorim, H.V., Dougall, D.K. and Sharp, W.R. (1977). The effect of carbohydrate and nitrogen concentration on phenol synthesis in Paul's Scarlet rose cells grown in tissue culture. Physiol. Plant. 39 pp 91-95.

Ancora, G., Belli-Donini, M.L. and Cuozzo, L. (1981). Globe artichoke plants obtained from shoot apices through rapid in vitro micropropagation. Sci. Hort. 14: pp 207-213.

Arya, K. (1987). In vitro techniques in relation to induced mutations in groundnut. M.Sc. Thesis, KAU.

Asen, S. and Larson, R.E. (1951). Artificial culturing of rose embryos. Penn. State Coll. Progr. Rep. (40) 4 pp.

*Atanassov, A.J. (1980). Method for continuous bud formation in tissue cultures of sugarbeet (Beta vulgaris L.). Z. Pflanzenzuchtg 84: pp 23-29.

Avramis, T., Hugard, J. and Jonard, R. (1982a). In vitro propagation of the rose rootstock Rosa indica major. Science de. la. Vie. 294 (1) pp 63-68.

Avramis, T., Hugard, J. and Jonard, R. (1982b). Increased rooting potential of in vitro propagated rose shoots by soaking them in mineral solutions with or without sucrose and NAA before direct planting in horticultural substrate. Sciences de la Vie. 294 (3) pp 679-682.

Bajaj, Y.P.S. and Saettler, A.W. (1971). Gamma irradiation studies on seeds of Phaseolus vulgaris. Radiat. Bot. 10: pp 35-37.

Barlass, M., Grant, W.J.R. and Skene, K.G.M. (1980). Shoot regeneration in vitro from native Australian fruit-bearing trees - Quandong and plum bush. Aust. J. Bot. 28: pp 405-409.

Barnes, L.R. (1979). In vitro propagation of watermelon. Sci. Hort. 11: pp 223-227.

Barve, D.M., Iyer, R.S., Kendurkar, S. and Mascarenhas, A.F. (1984). An effective method for rapid propagation of some budded rose varieties. Indian J. Hort. 41 (1/2) pp 1-7.

Bayliss, M.W. (1973). Origin of chromosome number variation in cultured plant cells. Nature 246: pp 529-530.

Benetka, V. (1985). Some experiences of methodology with the isolation of somatic mutations in the rose cultivar Sonia. Acta Pruhoniciana 50: pp 9-25.

Ben-Jaacov, J. and Dax, E. (1981). In vitro propagation of Grevillea rosmarinifolia. Hort. Sci. 16: pp 309-310.

Bhojwani, S.S. (1980). Micropropagation method for a hybrid willow (Salix matsudana x alba NZ-1002). New Zealand J. Bot. 18: pp 209-214.

Bini, G., Leva, A.R.C. and Nicese, F.P. (1983). Studies on micropropagation of roses. Ortoflorofrutticoltura Italiana. 67 (1) pp 1-13.

Bouharmont, J. and Dabin, P. (1986). Application of in vitro culture for the breeding of fuchsia by mutation. In Nuclear Techniques and in vitro culture for plant improvement IAEA pp 339-347.

Cai, J., Cai, M.Y. and Qian, D.L. (1984). Induction of multiple shoots and rapid propagation of clones of China rose (Rosa chinensis). Plant Physiology Communications (5) pp 37-38.

Coljin, C.M., Kool, A.J. and Nijkamp, H.J.J. (1979). An effective chemical mutagenesis procedure for Petunia hybrida cell suspension cultures. Theor. Appl. Genet. 55 (3/4) pp 101-106.

Collet, G.P. and Le, C.L. (1987). Role of auxin during in vitro rhizogenesis of rose and apple trees. Acta Horticulturae 1 (212) pp 273-280.

Curir, P., Damiano, C. and Cosmi, T. (1985). In vitro propagation of some rose cultivars. Annali. dell' Instituto Sperimentale. per. la. Floricultura. 16 (1) pp 69-73.

Dale, P.J. (1977a). The elimination of ryegrass mosaic virus from Lolium multiflorum by meristem tip culture. Ann. Appl. Biol. 85, pp 93-96.

Dale, P.J. (1977b). Meristem tip culture in Lolium, Festuca, Phleum and Dactylis. Plant Sci. Lett. 9: pp 333-338.

Damiano, C., Ruffoni, B., Constantino, C. and Bregliano, R. (1987). In vitro propagation of 7 rose cultivars. Annali dell Instituto Sperimentale per la Floricultura. 18 (1) pp 43-55.

Datta, S.K. (1985). Radiosensitivity of garden roses. J. Nucl. Agri. Biol. 14 (4): pp 133-135.

Datta, S.K. (1986). Effects of recurrent gamma irradiation on rose cv "Contempo". J. Nucl. Agri. Biol. 15 (2) pp 125-127.

Datta, S.K. and Gupta, M.N. (1982). Gamma ray induced white flower mutant in rose cv 'Junior Miss'. J. Nucl. Agri. Biol. 11 (1) pp 32-33.

Datta, S.K. and Gupta, M.N. (1984). Saroda and Sukumari - new rose cultivars evolved by gamma radiation. Science and Culture 50 (6) pp 200-201.

Davies, D.R. (1980). Rapid propagation of roses in vitro. Sci. Hort. 13 (4) pp 385-389.

Dommergues, P. (1976). Induced mutagenesis in roses. Horticulture Francaise 74: pp 13-15.

Dubois, L.A.M., Vries, D.P. and De Roggemans, J. (1988). Comparison of plant habit of pot roses propagated in vitro and by cuttings. Acta Horticulturae. 11 (226) pp 611-613.

Duron, M. and Decourtye, L. (1986). Biological effects of gamma radiation applied to Weigela cv Bristol Ruby plants cultured in vitro. In Nuclear techniques and in vitro culture for plant improvement IAEA pp 103-111.

Duskova, J., Sovova, M., Dusek, J. and Jamodar, L. (1988). The effect of ionising irradiation on the tissue culture of Artostaphylos uva-ursi. Pharmazie 43 (7) pp 518-519.

Earle, E.D. and Langhans, R.W. (1974). Propagation of Chrysanthemum in vitro 1. Multiple plantlets from shoot tips and the establishment of tissue culture. J. Am. Soc. Hort. Sci. 99: pp 128-132.

Edallo, S., Zucchinali, C., Petenzin, M. and Salamini, F. (1981). Chromosomal variation and frequency of spontaneous mutation associated with in vitro culture and plant regeneration in maize. Maydica. 26: 39-56.

Engvild, K.C. (1978). Substituted indoleacetic acids tested in tissue cultures. Physiol. Plant 44: p 345.

Evans, D.A. (1981). Soybean tissue culture. Soybean Genetics Newsletter, 8: 21-29.

Fletcher, J.S. (1974). Metabolism of glutamate in suspension cultures of Paul's Scarlet rose cells. Plant Physiol. 54: pp 503-505.

*Gautheret, R.J. (1939). Sur la possibilite de realizer la culture Indefinie des tissus de tubercules de carrote, C.R. Acad. Sci., 208: pp 118.

George, L. and Rao, P.S. (1979). In vitro regeneration of mustard plants (Brassica juncea var. RA.5) on cotyledon explants from non-irradiated, irradiated and mutagen-treated seed. Ann. Bot. 46 pp 107-112.

*Graifenberg, A. (1973). Coltura 'in vitro' di embrioni e di parti di seme in Rosa canina. Riv. Ortofluoro Fruitticoltura Ital. 57: 374-380.

Grubisic, D. and Culafic, L. (1986). In vitro vegetative propagation of Cercis siliquastrum. Bulletin Scientifique 31: 0316.

Gupta, P.K., Mascarenhas, A.F. and Jagannathan, V. (1981). Tissue culture of forest trees - Clonal propagation of mature trees of Eucalyptus citriodora Hook, by tissue culture. Plant Sci. Lett. 20: pp 195-201.

Gupta, P.K., Nadgir, A.L., Mascarenhas, A.F. and Jagannathan, V. (1980). Tissue culture of forest trees: clonal multiplication of Tectona grandis L. (teak) by tissue culture. Plant Sci. Lett. 17: pp 259-268.

*Haberlandt, G. (1902). Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. Mat - Nat. KI. Kais. Akad. Wiss. Weien. 111: p 69.

Haenchen, E. and Gelfert, E. (1978). The part played by mutation in the development of rose cultivars especially during the last 40 years. Archiv fur Gartenbau 26 (8) pp 231-241.

Hasegawa, P.M. (1979). In vitro propagation of rose. Hort. Sci. 14 (5) pp 610-612.

Hasegawa, P.M. (1980). Factors affecting shoot and root initiation from cultured shoot tips. J. Am. Soc. Hort. Sci. 105 (2) pp 216-220.

Hill, G.P. (1967a). Morphogenesis of shoot primordia in cultured stem tissue of garden rose. Nature 216: pp 596-597.

Hill, G.P. (1967b). Morphogenesis in stem-callus cultures of Convolvulus arsensis L. Ann. Bot. (London) 31: p 437.

Huang, S. and Chen, Y. (1986). Mutation Breeding in Rose. Mutation Breeding Newsletter 27: p 14.

Huang, S.C. and Millikan, D.F. (1980). In vitro micrografting of apple shoot tips. Hort. Sci. 15: pp 741-743.

Hu, C.Y. and Wang, P.J. (1983). Meristem, shoot tip and bud culture. In: Handbook of plant cell culture. Vol. 1. Techniques for Propagation and Breeding (D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada, eds). Macmillan Publishing Co., New York. pp 177-228.

Hyndman, S.E., Hasegawa, P.M. and Bressan, R. (1982). Stimulation of root initiation from cultured rose shoots through the use of reduced concentrations of mineral salts. Hort. Sci. 17 (1) pp 82-83.

Irulappan, I. and Rao, V.N.M. (1981). Induced mutations in Edward rose (Rosa bourboniana Desp) Mean performance in vM_1 and vM_2 generations. S. Indian Hort. 29 (4) pp 206-210.

Jacobs, G., Allan, P. and Bornman, C.H. (1969). Tissue culture studies on rose: Use of shoot tip explants I. Auxin: cytokinin effects. Agroplantae 1: pp 179-187.

Jacobs, G., Allan, P. and Bornman, C.H. (1970a). Tissue culture studies on rose: Use of shoot tip explants II. Cytokinin gibberellin effects. Agroplantae 2: pp 25-28.

Jacobs, G., Allan, P. and Bornhan, C.M. (1970b). Tissue culture studies on rose. Use of shoot tip explants III. Auxin: gibberellin effects Agroplantae 2: pp 45-50.

Jacobs, G., Bornman, C.H. and Allan, P. (1968). Tissue culture studies on rose: Use of pith explants. S. Afr. J. Agric. Sci. 11: pp 673-678.

James, D.J. (1979). The role of auxins and phloroglucinol in adventitious root formation in Rubus and Fragaria grown in vitro. J. Hort. Sci. 54: pp 273-277.

James, D.J. and Thurbon, I.J. (1981). Shoot and root initiation in vitro in apple rootstock M.9 and the promotive effects of phloroglucinol. J. Hort. Sci. 56: pp 15-20.

Joseleau, J.P. and Chambat, G. (1984a). Structure of the primary cell walls of suspension-cultured Rosa glauca cells. I. Polysaccharides associated with cellulose. Plant Physiol. 74: pp 687-693.

Joseleau, J.P. and Chambat, G. (1984b). Structure of the primary cell walls of suspension cultured Rosa glauca cells. II. Multiple forms of xyloglucans. Plant Physiol. 74: pp 694-700.

Jung-Heiliger, H. and Horn, W. (1980). Variation following mutagenic treatment of cuttings and in vitro culture of chrysanthemum. Zeitschrift fur Pflanzenzuchtung 85 (3) pp 185-199.

Kaicker, U.S. and Swarup, V. (1972). Induced mutation in roses. Ind. J. Genetics Pl. Breed. 32 (2) pp 257-265.

Kaicker, U.S. and Swarup, V. (1978). Induced mutation in the rose cultivar Gulzar and the effect of chemical and physical mutagens on plant growth. Acta Agronomica Academiae Scientiarum. Hungaricae 27 (1/2) pp 43-48.

Kartha, K.K., Leung, N.L. and Pahl, K. (1980). Cryopreservation of strawberry meristems and mass propagation of plantlets. J. Am. Soc. Hort. Sci. 105: pp 481-484.

Kartha, K.K., Mroginski, L.A., Pahl, K. and Leung, N.L. (1981a). Germplasm preservation of coffee (Coffea arabica L.) by in vitro culture of shoot apical meristems. Plant Sci. Lett. 22: pp 301-307.

Kartha, K.K., Pahl, K., Leung, N.L. and Mroginski, L.A. (1981b). Plant regeneration from meristems of grain legumes: Soybean, Cowpea, Peanut, Chickpea and Bean. Can. J. Bot. 59: 1671-1679.

Kehr, A.E. and Schaeffer, G.W. (1976). Tissue culture and differentiation of garlic. Hort. Sci. 11: 422-423.

Khosh-Khui, M. and Sink, K.C. (1982a). Callus induction and culture of Rosa. Sci. Hort. 17: pp 361-370.

Khosh-Khui, M. and Sink, K.C. (1982b). Micropropagation of new and old world rose sp. J. Hort. Sci. 57 (3) pp 315-319.

Khosh-Khui, M. and Sink, K.C. (1982c). Rooting enhancement of Rosa hybrida for tissue culture propagation. Sci. Hort. 17 (14) pp 371-376.

Kim, S.K., Hwang, J.K., Ko, K.C. and Lee, Y.I. (1989). In vitro growth and mutagenesis of grape shoot tips as affected by chemical and physical mutagen. Abstract of communicated papers Korean Society for Horticultural Science. 7 (1) pp 140-141.

Kireeva, S.A., Bugorskii, P.S. and Reznikova, S. (1977). Tissue culture of essential oil rose and terpenoid accumulation. Fiziologia Rastenii 24 (4) pp 824-831.

Kleffel, B., Walther, F. and Preil, W. (1987). X-ray induced mutability in embryogenic suspension cultures of Euphorbia pulcherrina. Nuclear Techniques and in vitro culture for plant improvement, IAEA pp 113-120.

Koda, Y. and Okazawa, Y. (1980). Cytokinin production by Asparagus: shoot apex cultured in vitro. Physiol. Plant 49: pp 193-197.

Konar, R.N. and Oberoi, Y.P. (1965). In vitro development of embryoids on cotyledons of Biota orientalis, Phytomorphology. 15: pp 137.

Konchk, I. and Rodeva, V. (1986). Effect of some growth regulators on in vitro production of regenerated Lilium plants. Genetika i Selektsiya 19 (5) pp 394-395.

Kunisaki, J.T. (1980). In vitro propagation of Anthurium andreanum Lind. Hort. Sci. 15: pp 508-509.

Kuo, C.G. and Tsay, J.S. (1977). Propagation of Chinese cabbage by axillary bud culture. Hort. Sci. 12: pp 459-460.

Kusey, W.E., Jr., Jammer, P.A. and Weiler, T.C. (1980). In vitro propagation of Gypsophila paniculata L. "Bristol Fairy". Hort. Sci. 15: pp 600-601.

Lammerts, W.E. (1946). The use of embryo culture in rose breeding. Plants. Garden 2: p 11.

Lane, W.D. (1979a). The influence of growth regulators on root and shoot initiation from flax meristem tips and hypocotyls in vitro. Physiol. Plant 45: pp 260-264.

Lane, W.D. (1979b). In vitro propagation of Spirea bumalda and Prunus cistena from shoot apices. Can. J. Plant Sci. 59: pp 1025-1029.

Lloyd, D., Roberts, A.V. and Short, K.C. (1988). The induction in vitro of adventitious shoots in Rosa. Euphytica 37 (1) pp 31-36.

Louwaars, N.P. (1984). Propagation of roses in vitro is not without problems. Vakblad voor de Bloemisterij 39 (47) p 49.

Mabuchi, T. and Kuwada, H. (1975). Radiation effects on shoot tip culture of chrysanthemum II. Plant growth in the field Kagawa Dargaku Nagabubu Gakuzyutu Hokuku. 26 (2) pp 78-82.

Mahlderg, P.G., Turner, F.R., Walkinshaw, C., Venketeswaran, S and Mehrotra, B. (1975). Observations on incomplete cytokinesis in callus cells. Bot. Gaz. 136: pp 189-193.

Margarete, Norton and Colin, R. Norton (1985). Change in shoot proliferation with repeated in vitro subculture of shoots of woody species of Rosaceae, Plant Cell Tissue Organ Culture. 5: pp 181-197.

Martin, C., Carre, M. and Vernoy, R. (1981). Vegetative propagation in vitro of cultivated woody plants; the case of roses. Sciences de. la. Vie. 293 (2) pp 175-177.

Mederos, S. and Rodriguez Enriquez, M.J. (1987). In vitro propagation of "Golden Times" roses. Factors affecting shoot tips and axillary bud growth and morphogenesis. Acta Horticulturae 11 (212) pp 619-624.

Mellor, F.C. and Stace Smith, R. (1977). Virus-free potatoes by tissue culture. In Applied and Fungamental Aspects of Plant Cell, Tissue and Organ Culture (J. Reinert and Y.P.S. Bajaj, eds.) Springer-Verlag, Berlin. pp 616-637.

Michalik, P. and Rogozinska, J. (1986). In vitro regeneration from excised leaf discs of Kalanchoe blossfeldiana, Genetika i Selektsiya 19 (5) pp 403-404.

Lane, W.D. (1979c). Regeneration of pear plants from shoot meristem-tips. Plant Sci. Lett. 16: pp 337-342.

Langford, P.J. and Wainright, H. (1988). Influence of sucrose concentration on the photosynthetic ability of in vitro grown rose shoots. Acta Horticulturae (227) pp 305-310.

Lata, P. (1980). Effect of ionising radiation on roses: induction of somatic mutations. Environ. and Exp. Bot. 20 (4) pp 325-333.

Lata, P. (1987). Effects of mutagens on rose pigments. Genetica Iberica. 39 (1/2) pp 171-186).

Lata, P. and Gupta, M.N. (1971). Mutation breeding of garden roses. Effect of gamma rays on essential oil content in some scented roses. Perfumerie and Kosmetik 52: pp 267-70.

Lata, P. and Gupta, M.N. (1975). Effects of ionizing radiation on roses: meiotic studies on control and gamma ray induced mutants of rose cultivar Montezuma. Cytologia 40: pp 213-219.

Leffring, L. (1985). In vitro propagation of roses. Vakblad. voor. de. Bloemisterij. 40 (27) p 35.

Lester, D.T. and Berbee, J.G. (1977). Within-clone variation among black poplar trees derived from callus culture. For. Sci. 23: pp 122-131.

Li, Z.P. (1988). Studies on factors affecting in vitro propagation and transplantation of roses. Acta Horticulturae Sinica 15 (2) pp 131-134.

Litz, R.E. and Conover, R.A. (1978). In vitro propagation of papaya. Hort. Sci. 13: pp 241-242.

Liv, L. and Burger, D.W. (1986). In vitro propagation of Easter lily from pedicels. Hort. Sci. 21 (6) pp 1437-1438.

*Mollard, A. and Barnoud, F. (1976a). Une xyloglucane hemicellulosique B 1-6 dans les cellules de Rosa New Dawn cultivees in vitro. Physiol. Veg. 14: pp 241-249.

*Mollard, A. and Barnoud, F. (1976b). Une glucane hemicellulosique B 1-3 dans les parois des cellules de Rosier cultivees in vitro. Physiol. Veg. 14: pp 233-240.

*Mollard, A. and Robert, D. (1984). Etude de la lignine parietale et extracellulaire des suspensions cellulaires de Rosa glauca. Physiol. Veg. 22: pp 3-17.

Morel, G.M. (1960). Producing virus free Cymbidium. Am. Orch. Soc. Bull., 29: 495.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant. 15: 473.

Murphy, T.M. (1984). Effect of sulhydryl reagents on K^+ efflux from rose cells. Plant Physiol. 75: pp 138-141.

Nakajima, K. (1970). Gamma irradiation induced mutants of the rose cv. Peace. Technical News. 4: 2 pp.

Nakajima, K. (1977). Studies on effective methods of inducing mutations in vegetatively propagated plants by gamma-irradiation. Acta Radiobotanica et Genetica. 49 (5) pp 80-95.

Nash, D.T. and Davies, M. (1972). Some aspects of growth and metabolism of Paul's Scarlet rose cell suspensions. J. Exp. Bot. 23: pp 75-91.

Nemeth, G. (1981). Adventitious root induction by substituted 2-chloro 3 phenyl-propionitriles in apple rootstocks cultured in vitro. Sci. Hort. 14: pp 253-259.

Nesius, K.K. and Fletcher, J.S. (1973). Carbondioxide and pH hydrogen ion concentration requirements of non-photosynthetic culture cells. Physiol. Plant 28: pp 259-263.

Nesius, K.K., Uchytil, L.E. and Fletcher, F.E. (1972). Minimal organic medium for suspension cultures of Paul's Scarlet rose. Planta 106: pp 173-176.

Nishiuchi, Y. (1986). Multiplication of tulip bulb by tissue culture in vitro. Acta Horticulturae 1 (177) pp 279-283.

*Nobecourt, P. (1939). Sur la perennite de l'augmentation de volume des cultures de tissus vegetaux, C.R. Soc. Biol. (130) p 1270.

Novak, F.J. (1981). Chromosomal characteristics of long-term callus cultures of Allium sativum L. Cytologia 46: pp 371-379.

Papachatzi, M., Hammer, P.A. and Hasegawa, P.M. (1981). In vitro propagation of Hosta decorata "Thomas Hogg" using cultured shoot tips. J. Am. Soc. Hort. Sci. 106: pp 232-236.

Pierik, R.L.M. and Tetteroo, F.A.A. (1987). Vegetative propagation of Begonia venosa Skan in vitro from inflorescence explants. Plant Cell Tissue and Organ Culture 10 (2) pp 135-142.

Pitlet, H. and Mancousin, C. (1982). Rose, in vitro micropropagation. Revere Horticole Suisse 55 (3) pp 67-69.

Podwyszynska, M. and Hempel, M. (1988). The factors influencing acclimatization of Rosa hybrida plants multiplied in vitro to greenhouse conditions. Acta Horticulturae 11 (226) pp 639-642.

Priel, W. and Meier-Dinkel, A. (1983). Propagation of roses by tissue culture - an expanding new method? Deutsche. Baumschule 35 (5) p 202.

Reist, A. (1985). In vitro culture in rose nurseries; is this an alternative to propagation by cuttings or grafting of varieties? Revue Suisse de. Viticulture. d', Arboriculture. et. d'. Horticulture. 17 (6) pp 361-364.

Roest, S. and Bokelmann, G.S. (1981). Vegetative propagation of carnation in vitro through multiple shoot development. Sci. Hort. 14: pp 357-366.

Roest, S., Van Berkel, M.A.E., Bokelmann, G.S. and Broertjes, C. (1980). The use of an in vitro adventitious bud technique for mutation breeding of Begonia x Hiemalis. Euphytica 30: pp 381-388.

Rout, G.R., Debata, B.K. and Das, P. (1988). In vitro mass scale propagation of Rosa hybrida cv. Landora. Curr. Sci. 58 (15) pp 876-879.

Sax, K. (1955). The effect of ionising radiations on plant growth. Am. J. Bot. 42: pp 360-364.

Schnabdrauch, L.S. and Sink, K.C. (1979). In vitro propagation of Phlox subulata and Phlox paniculata. Hort. Sci. 14: pp 607-608.

Sebastian, T.K. and Heuser, C.W. (1987). In vitro propagation of Hydrangia quereifolia. Sci. Hort. 31 (314) pp 303-309.

Shepotev, F.L. and Chernobrivets, V.T. (1981). Aspects of the anatomical structure of the leaves in mutants of Rosa cinnamomea. Referativnyi Zhurnal 9: pp 65-82.

Shimada, T., Sasakuma, T. and Tsunewaki, K. (1969). In vitro culture of wheat tissues. I. Callus formation, organ redifferentiation and single cell culture. Can. J. Genet. Cytol. 11: p 294.

Skirvin, R.M. and Chu, M.C. (1979a). In vitro propagation of 'Forever Yours' rose. Hort. Sci. 14 (5) pp 608-610.

Skirvin, R.M. and Chu, M.C. (1979b). Tissue culture propagation of rose. Illinois State Florists' Association Bulletin (284) pp 22-23.

Skoog, F. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol. 11: p 118.

Snir, I. and Erez, A. (1980). In vitro propagation of Malling Merton apple rootstocks. Hort. Sci. 15: pp 597-598.

Street, H.E. (1979). Embryogenesis and chemically induced organogenesis. In: Plant Cell and Tissue Culture, Principles and Applications (Sharp, W.R., Larsen, P.O., Paddock, E.F. and Raghavan, V., Eds.). Ohio State University Press, Columbus. p 123.

Tabaeezadeh and Khosh-Khui, M. (1981). Anther culture of Rosa. Sci. Hort. 15: pp 61-66.

Thimann, K.V. (1977). Hormone action in the Whole Life of Plant. Univ. of Massachusetts Press, Amherst.

Towill, L.E. (1981). Solanum etuberosum: A model for studying the cryobiology of shoot-tips in the tuber-bearing Solanum species. Plant Sci. Lett. 20: pp 315-324.

Tsvitkov, R. (1978). Radiosensitivity of pollen of the essential oil rose. Selektsiya 11 (4) pp 265-270.

Tulecke, W. and Nickell, L.G. (1959). Production of large amounts of plant tissue by submerged culture. Science 130: pp 863-864.

Tweddle, D., Roberts, A.V. and Short, K.C. (1984). In: Plant Tissue and Cell Culture Application to Crop Improvement (F.J. Novak, L. Havel and J. Dolezel, eds.), Czechoslovak Academy of Science, Prague. pp 529-530.

Valles, M. and Boxus, P. (1987). Micropropagation of several Rosa hybrid L. cultivars. Acta Horticulturae 11 (212) pp 611-617.

Vijaya, N., Satyanarayana, G. and Reddy, C.S. (1985). Propagation of rose through tissue culture. Indian Rose Annual 5: pp 136-146.

Von Abrams, G.J. and Hand, M.E. (1956). Seed dormancy in Rosa as a function of climate. Am. J. Bot. 43: pp 7-12.

Walther, F. and Saver, A. (1986a). In vitro mutagenesis in roses. Acta Horticulturae (189) pp 37-46.

Walther, F. and Saver, A. (1986b). Analysis of radiosensitivity, a basic requirement for in vitro somatic mutagenesis II. Gerbera jamesonii. In Nuclear Techniques and in vitro culture for plant improvement IAEA. pp 155-159.

Wang, P.J. (1978). Clonal multiplication of Cryptomeria japonica D. Don in vitro. In: Studies and Essays in Commemoration of the Golden Jubilee of Academia Sinica, Taipei, Taiwan. pp 559-566

White, P.R. (1934). Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol., 9: p 585.

White, P.R. (1939). Potentially unlimited growth of excised plant callus in an artificial nutrient. Am. J. Bot. 26: p 59.

Wochok, Z.S. and Sluis, C.J. (1980). Gibberellic acid promotes Atriplex shoot multiplication and elongation. Plant Sci. Lett. 17: pp 363-369.

Wulster, G. and Sacalis, J. (1980). Effects of auxins and cytokinins on ethylene evolution and growth of rose callus tissue in sealed vessels. Hort. Sci. 15: pp 736-737.

Wylie, A.P. (1955). The History of Garden Roses. J. Roy. Hort. Soc. 80: 8-24 pp 77-87.

Yang, Y.W., Hsing, Y.I. and Chang, W.C. (1981). Clonal propagation of Stevia rebaudiana Bertoni through axillary shoot proliferation in vitro. Bot. Bull. Academia Sinica 22: pp 57-62.

Yie, S.T. and Liaw, S.I. (1977). Plant regeneration from shoot tips and callus of papaya. In vitro 13: pp 564-568.

Zieslin, Gavish, N.H. and Ziv, M. (1987). Growth interactions between calli and explants of rose plants *in vitro*. *Plant Sci.* 49: pp 57-62.

Ziv, M. (1979). Transplanting *Gladiolus* plants propagated *in vitro*. *Sci. Hort.* 11: pp 257-260.

* Original not seen

PLATE 1. Growth in bud, after 15 days culture in culture establishment medium (MS medium + BAP 2 mg/l + 2,4 D 1 mg/l)

PLATE 2. Growth in bud, after 15 days culture in shoot proliferation medium (MS medium + BAP 2 mg/l + GA 1 mg/l)

PLATE 3. Growth in bud, 1 month after first subculture in shoot proliferation medium

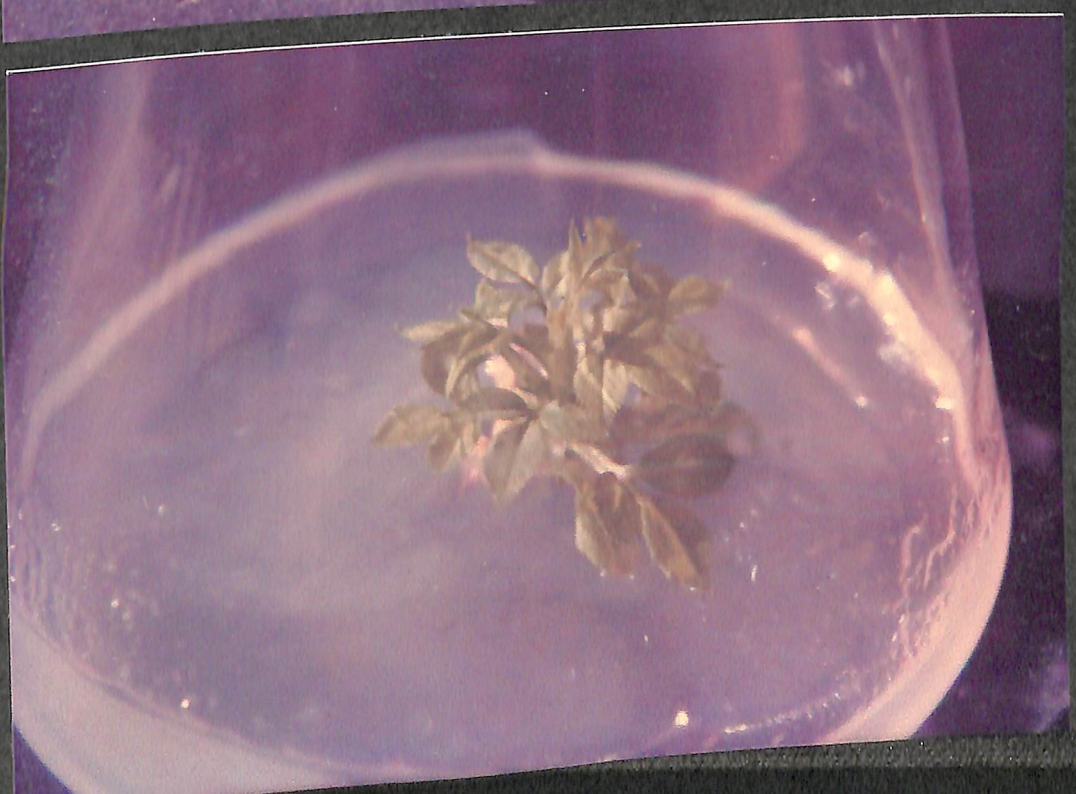


PLATE 4. Multiple shoot production in 4 month old culture

PLATE 5. Root distribution in shoot cultured in full strength MS medium + IAA 2 mg/l

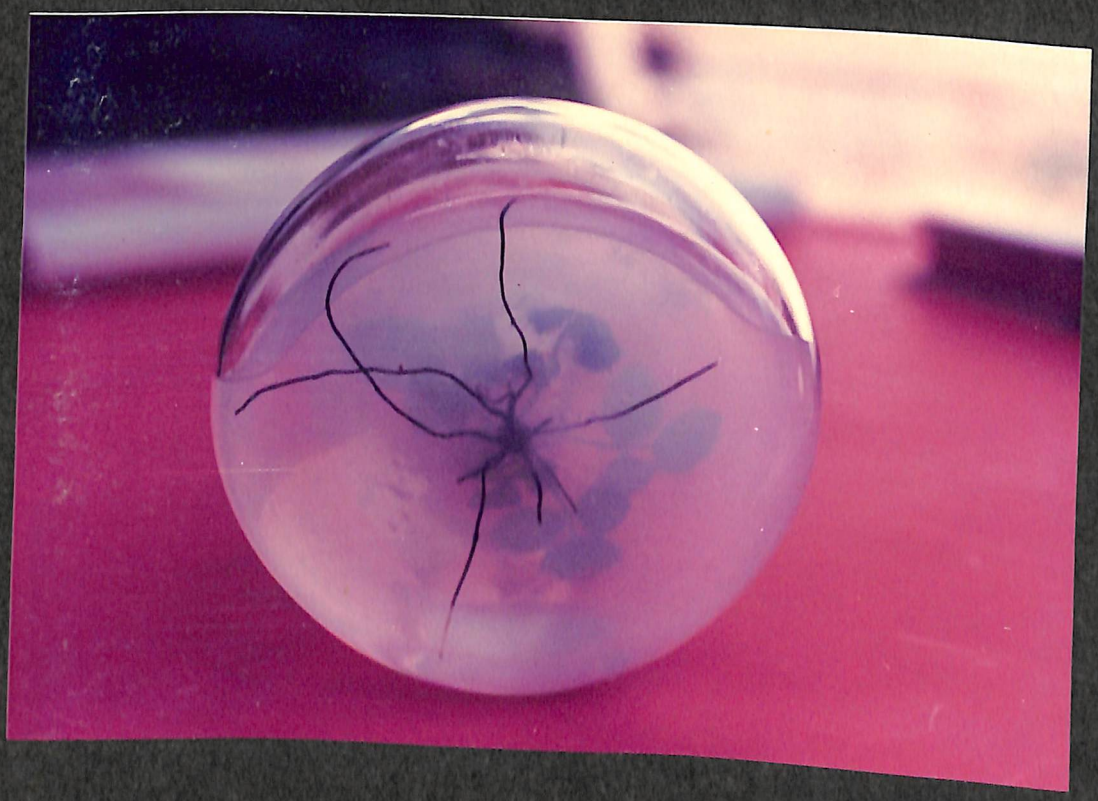
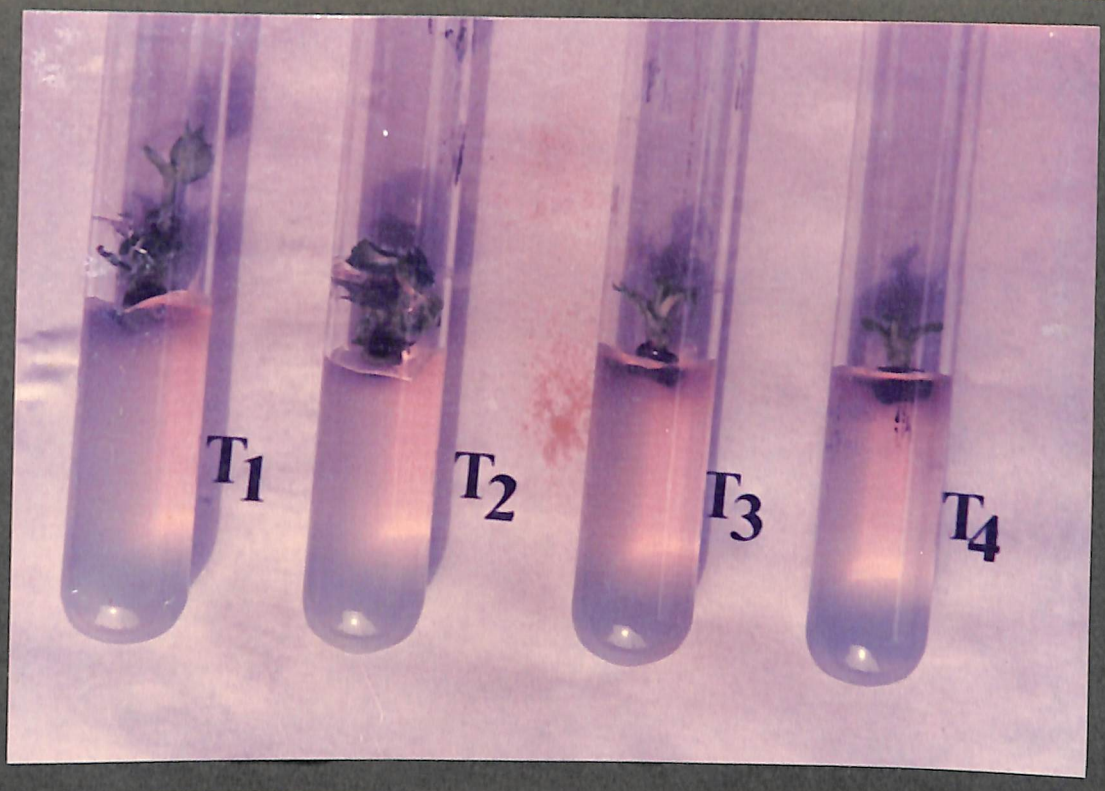


PLATE 6. Treated buds excised at the time of flower harvest, 30 days after culture

PLATE 7. Treated buds excised 2 days after flower harvest, 30 days after culture

PLATE 8. Treated buds excised 4 days after flower harvest, 30 days after culture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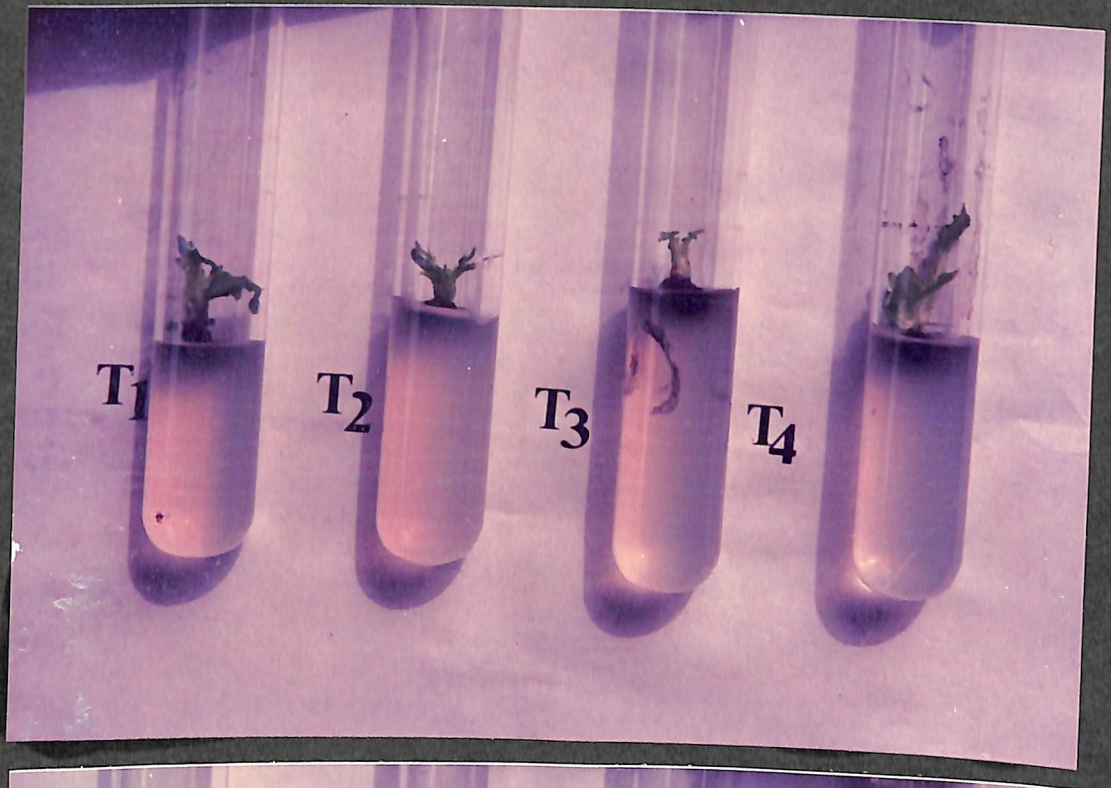
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T3
T4

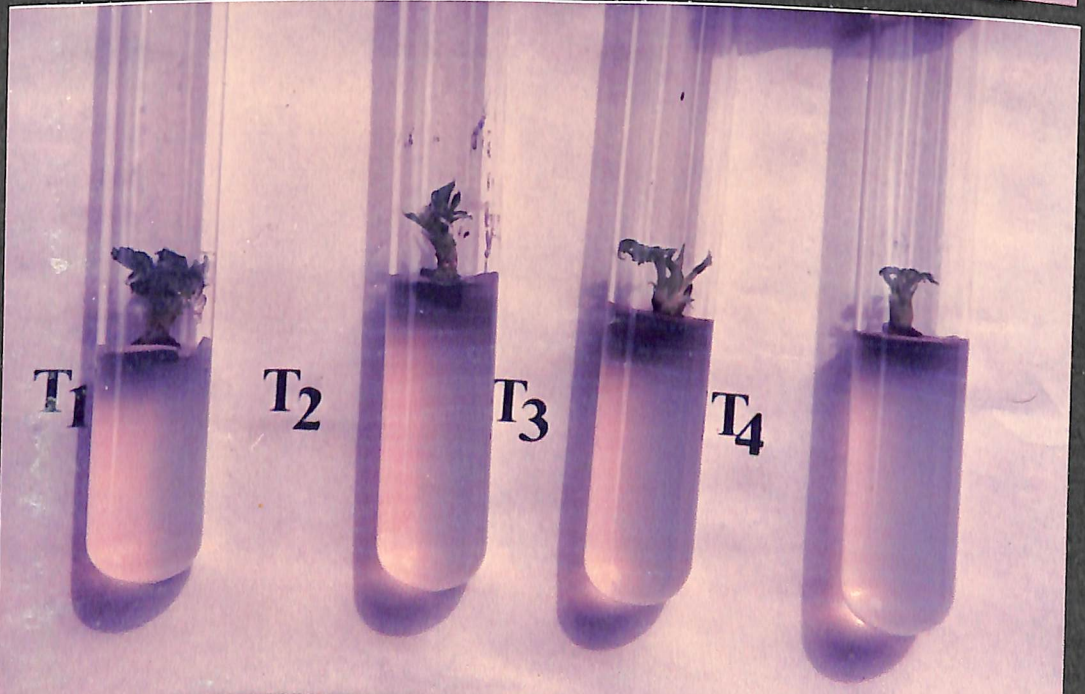

T1
T2
T3
T4

PLATE 9. Treated buds excised 6 days after flower harvest, 30 days after culture

PLATE 10. Control buds 30 days after culture

T_1 - 0.5% EMS treated buds

T_2 - 0.375% " " "

T_3 - 0.25% " " "

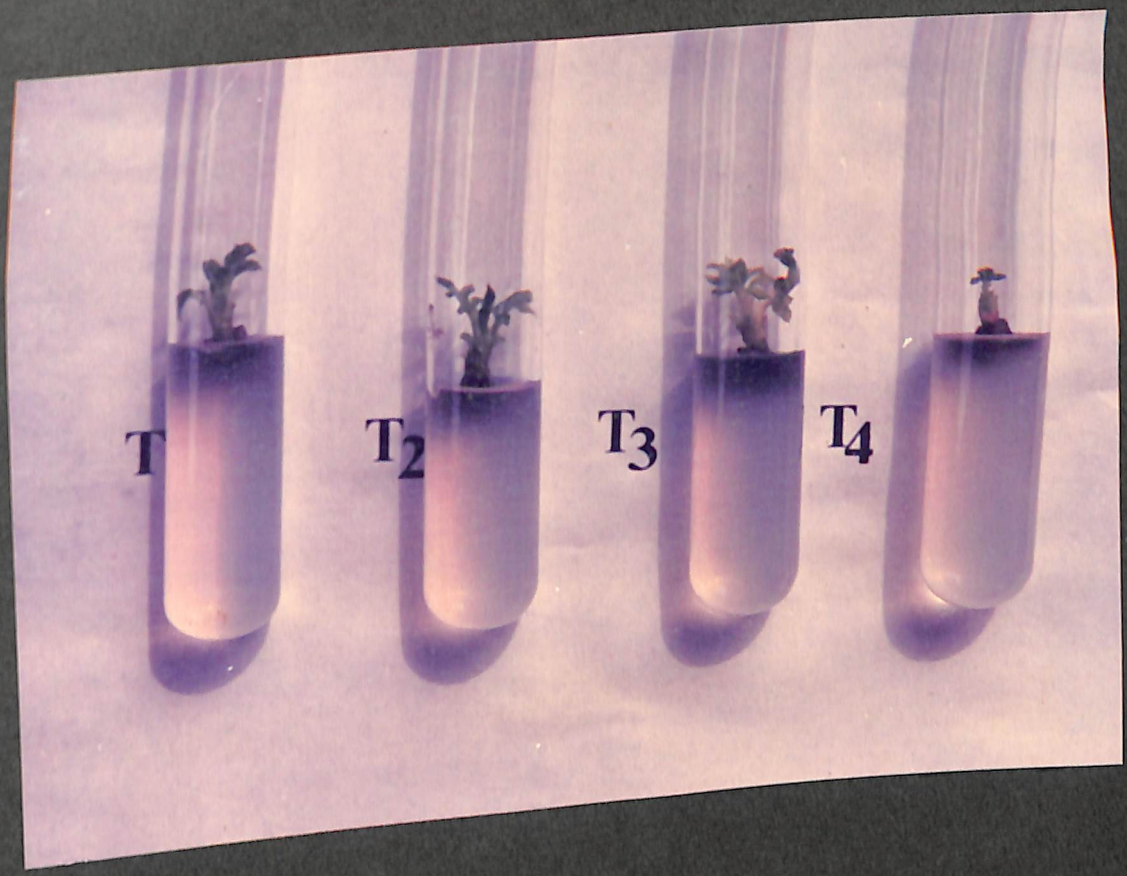
T_4 - 0.125% " " "

D_1 - Buds excised at the time of flower harvest

D_2 - Buds excised 2 days after flower harvest

D_3 - Buds excised 4 days after flower harvest

D_4 - Buds excised 6 days after flower harvest


T
T2
T3
T4

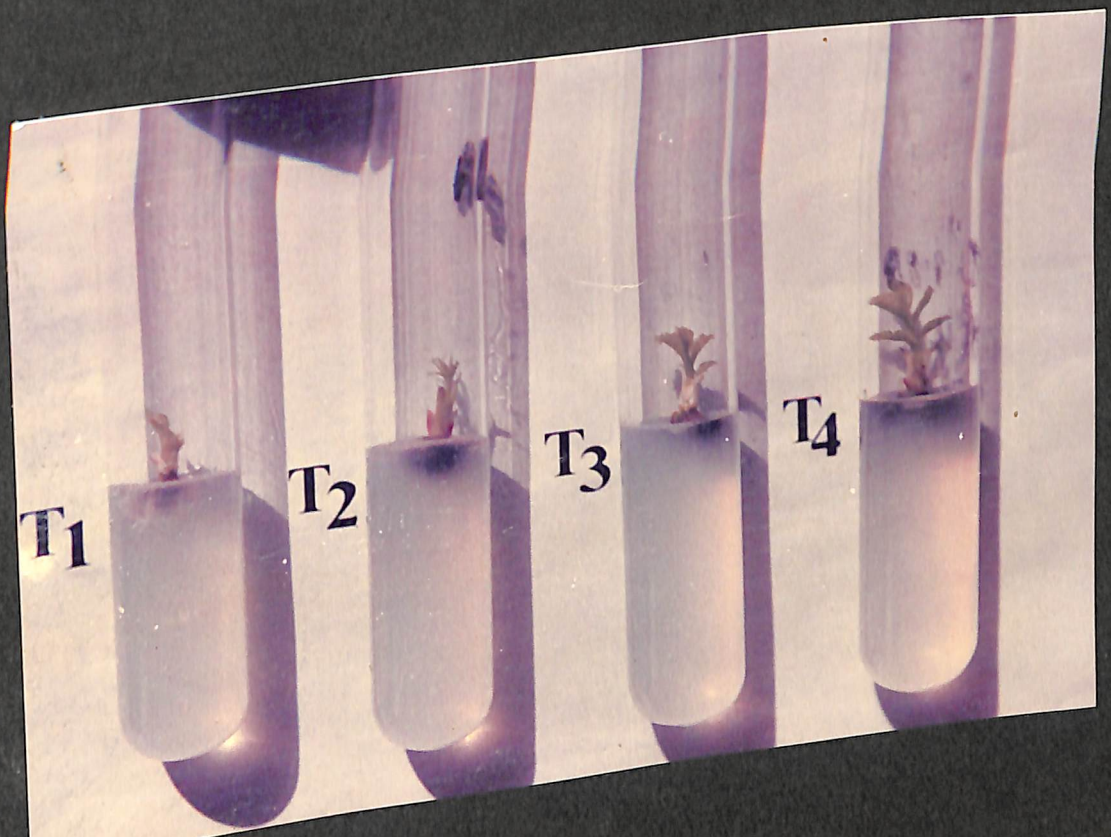

T1
T2
T3
T4

PLATE 11. Growth comparison in treated and control buds excised at the time of flower harvest, 1 month after 1st subculture. In higher concentrations multiple shoot production did not occur and the shoots exhibited poor growth

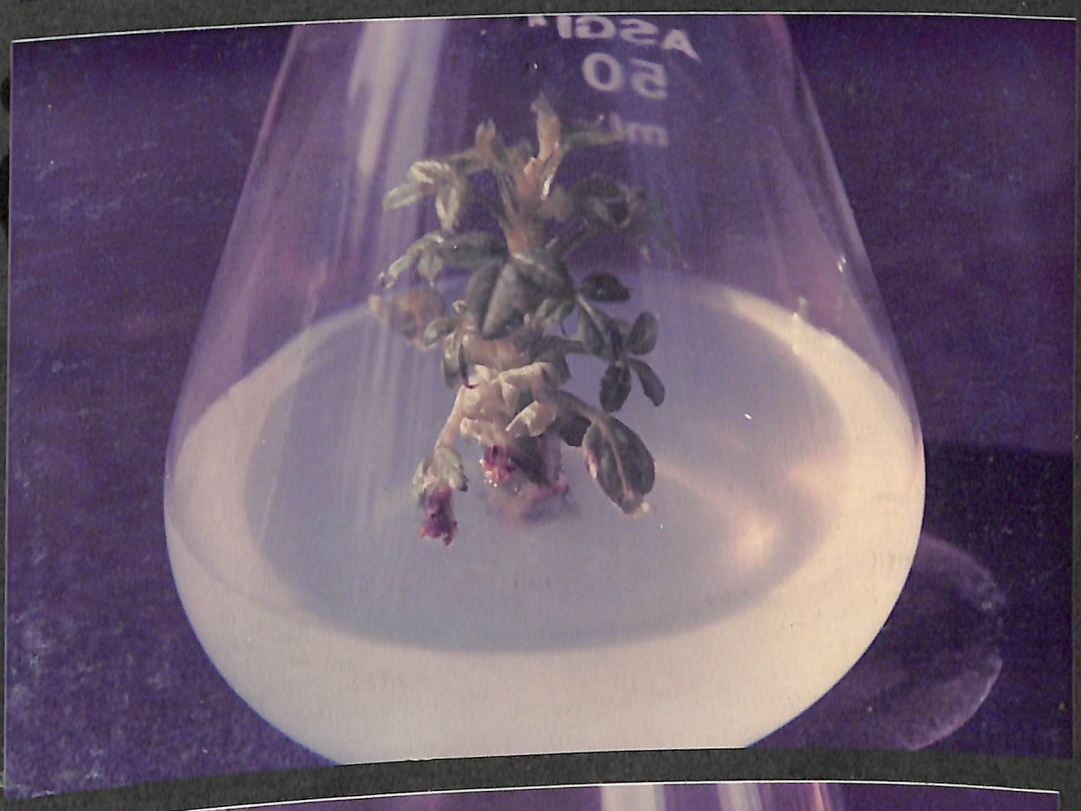

ASTM
50
ml

PLATE 12. Growth comparison in treated and control buds excised 2 days after flower harvest, 1 month after 1st subculture

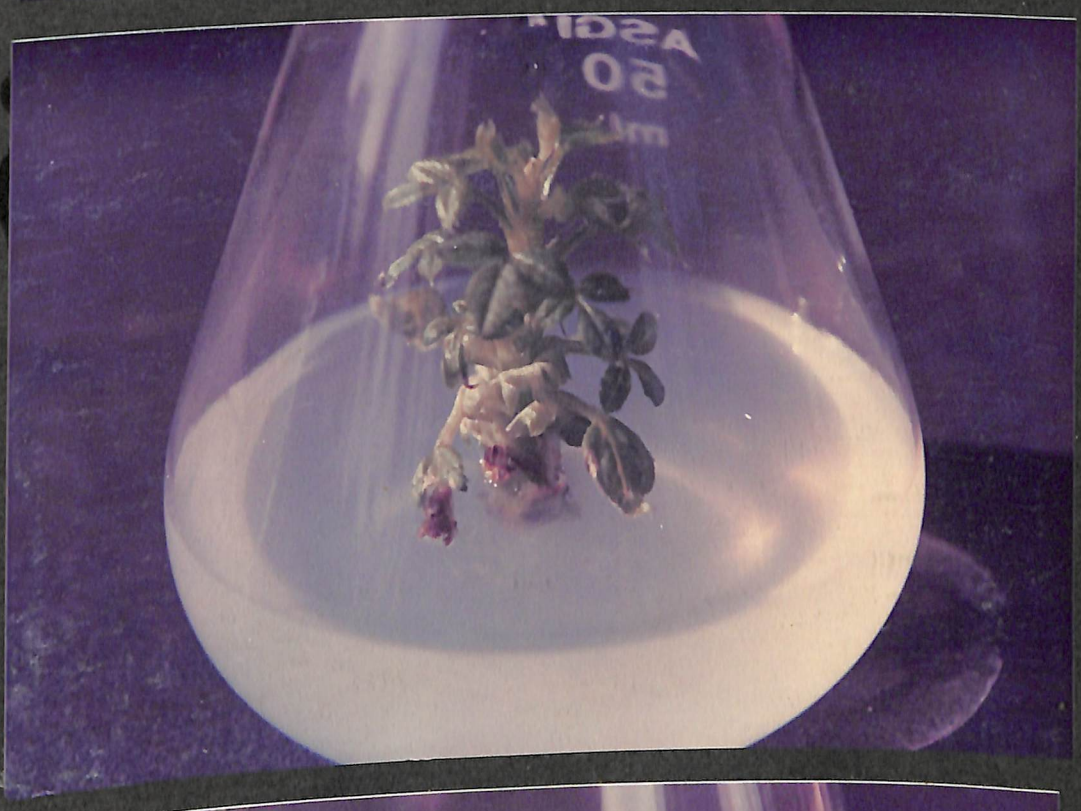

ASTM
50
ml

PLATE 13. Growth comparison in treated and control buds excised 6 days after flower harvest, 1 month after 1st subculture

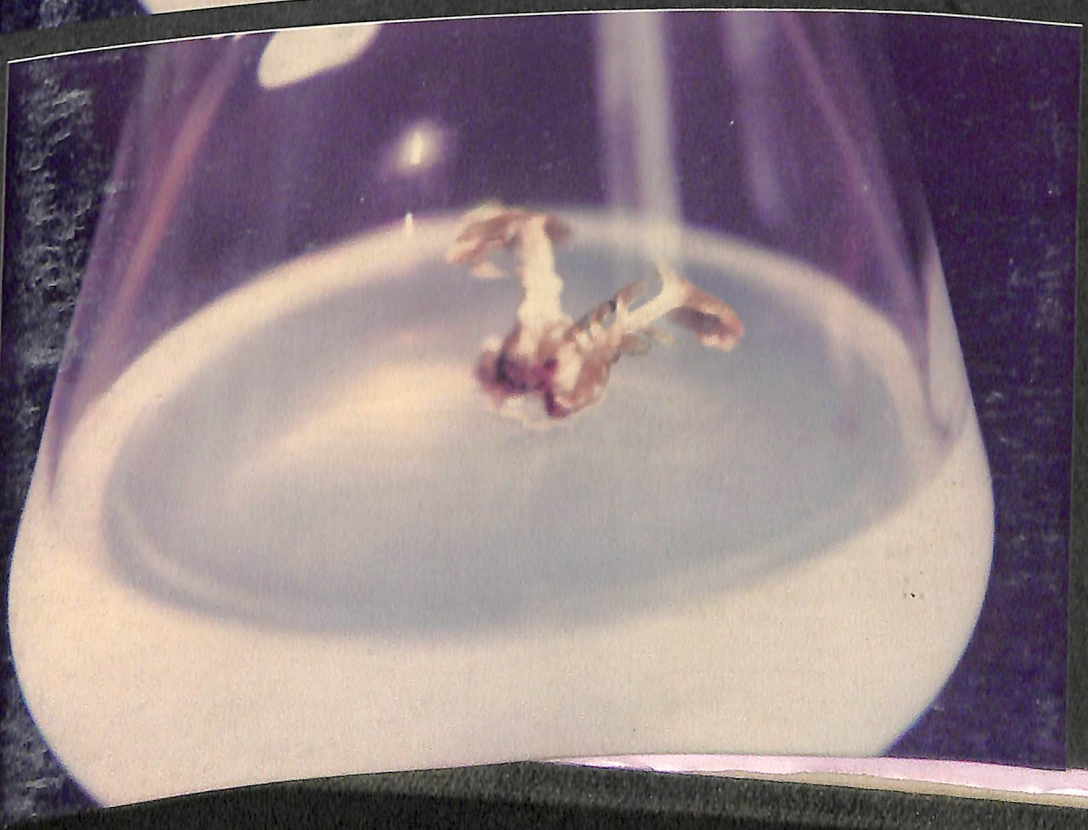
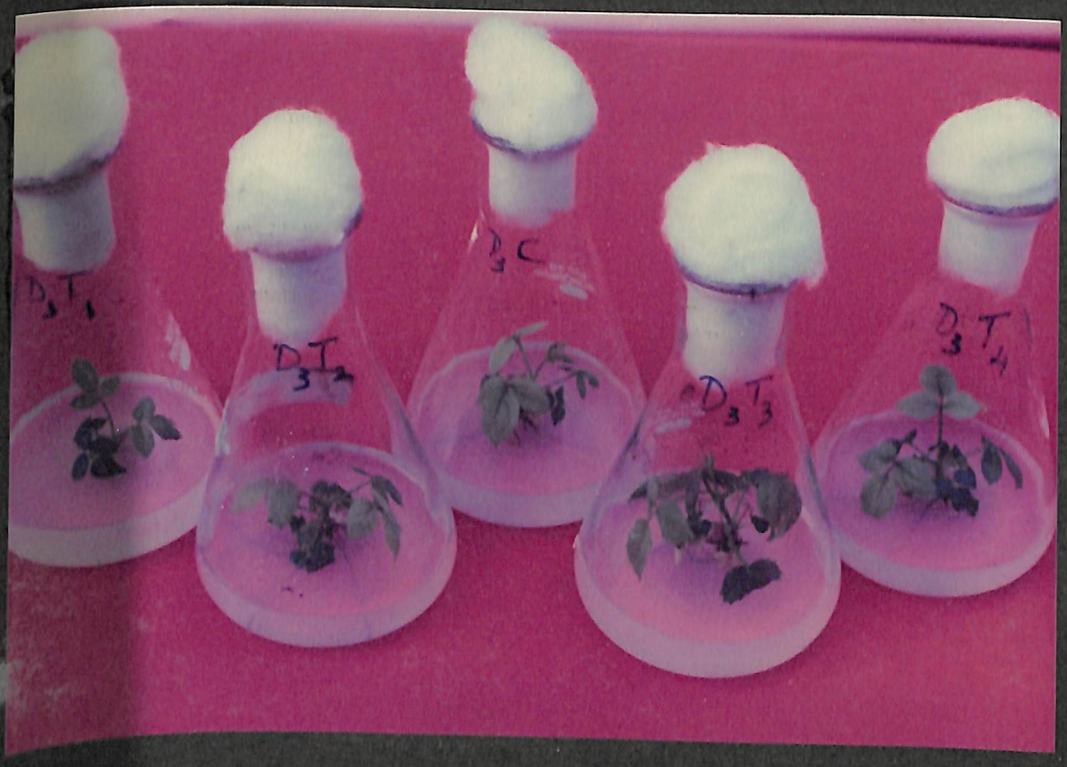


PLATE 14. Treatment effects in buds excised 4 days after flower harvest 1 month after 1st subculture

PLATE 15. Shoots from buds excised 4 days after flower harvest, after rooting


D_3T_1
D_3T_2
D_3C
D_3T_3
D_3T_4

INDUCED CHEMICAL MUTAGENESIS IN ROSE UNDER IN VITRO CULTURE

BY

UMA B. B.Sc. (Ag.)

ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT
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ABSTRACT

The present investigation entitled "Induced chemical mutagenesis in rose (Rosa chinensis) under in vitro culture" was carried out in the Tissue Culture Laboratory attached to the Horticultural Department, College of Agriculture, Vellayani during 1989-90. The main objectives of the experiment were to standardise a suitable culture medium for the growth and development of axillary buds and to standardise a successful method of chemical mutagenesis in rose under in vitro culture using the most potent chemical mutagen, ethyl methane sulphonate.

The standardisation of hormone levels in the culture medium (MS) was done at three stages of explant development viz. culture establishment, axillary bud proliferation and in vitro rooting. Surface sterilisation of axillary buds were standardised by using mercuric chloride selecting out three concentrations 0.06, 0.08 and 0.1 per cent and 3 periods of treatment 5, 10 and 15 minutes. The axillary buds used were of 4 maturity stages ie. axillary buds at the time of flower harvest and 2, 4 and 6 days after flower harvest. The various concentrations of ethyl methane sulphonate tested include 0.125, 0.25, 0.375 and 0.5 per cent. Two methods of mutagen treatments were tried ie. direct treatment and cotton swab method. In the direct treatment

the axillary buds were subjected to EMS treatment at different periods, treating the buds at the time of culturing, 2 days after culturing, 4 days after culturing and 6 days after culturing. In the cotton swab method buds were treated with EMS in the plant itself, at various stages ie. at the time of flower harvest and 2, 4 and 6 days after flower harvest.

Surface sterilisation of axillary buds was found to be most successful with mercuric chloride at 0.08 per cent for 15 minutes. Of the various levels of hormonal combinations tested BAP 2 mg/l + 2,4-D 1 mg/l was found to be the best for culture establishment and BAP 2 mg/l + GA 1 mg/l for shoot proliferation. Maximum rooting was obtained in full strength MS medium supplemented with IAA 2 mg/l. Of the two methods of mutagen treatments tried direct treatment of axillary buds with EMS was not found to be effective as the buds turned brown and no further development occurred.

In the cotton swab method, lower concentrations of EMS (0.125 and 0.25 per cent) gave a better performance based on days taken for bud take, multiple shoot production and rooting percentage. A decrease in survival percentage was noted with increase in mutagen concentration. Higher concentration of EMS (0.375 and 0.5 per cent) curbed multiple shoot production in buds excised at the time of flower

harvest and delayed multiple shoot production in other stages. The percentage cultures showing, rooting and the number of roots/shoot also decreased with increase in concentration of EMS. Increase in maturity of buds also delayed multiple shoot production and decreased rooting percentages. Of the 4 stages of buds used for *in vitro* culture, buds excised at the time of flower harvest was found to be the best. But mutagen treatment retarded their performance. For mutagen treatment buds excised 4 days after flower harvest was found to be the best, followed by buds excised 2 days after flower harvest. Buds excised 6 days after flower harvest showed a poor performance in the normal and treated populations. The experiment clearly demonstrated that induced mutagenesis in rose can be successfully done adopting *in vitro* culture techniques.