INDUCED MUTAGENESIS IN ROSE UNDER *IN VIVO* AND *IN VITRO* CULTURE

BY

D. WILSON

Thesis
submitted in partial fulfilment of the requirement
for the Degree of
DOCTOR OF PHILOSOPHY
Faculty of Agriculture
Kerala Agricultural University

Department of Agricultural Botany
COLLEGE OF AGRICULTURE
Vellayani, Thiruvananthapuram

1993

DECLARATION

I hereby declare that this thesis entitled "**Induced mutagenesis in rose under *in vivo* and *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 to me any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

D. WILSON

Vellayani,

27-03-1993.

APPROVED BY:

CHAIRMAN:

Dr. N. KRISHNAN NAIR

MEMBERS:

1. Dr. (Mrs.) S.T. MERCY

2. Dr. (Mrs.) P. SARASWATHY

3. Dr. (Mrs.) D. CHANDRAMONY

4. Dr. K. RAJMOHAN

EXTERNAL EXAMINER:

CERTIFICATE

Certified that this thesis entitled "**Induced mautagenesis in rose under *in vivo* and *in vitro* culture**" is a record of research work done independently by Sri. D. Wilson under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Dr. N. Krishnan Nair
Chairman, Advisory Committee
Associate Director of Research
Regional Agricultural Research Station
Pilicode - 670 353

Vellayani,
27-03-1993.

ACKNOWLEDGEMENTS

May I express my profound gratitude and indebtedness to Dr. N. Krishnan Nair, Associate Director of Research, Regional Agricultural Research Station, Pilicode and Chairman of the Advisory Committee for suggesting this problem , for his valuable guidance and constant encouragement throughout the course of this investigation and also in the preparation of this thesis.

I wish to express my heartfelt thanks to Dr. S.T. Mercy, Professor, Department of Agricultural Botany for the valuable and excellent suggestions, timely advices and careful scrutiny of this manuscript.

I take this opportunity to thank Dr. P. Saraswathy, Associate Professor (Higher Grade), Department of Statistics for the useful suggestions and help rendered in the analysis and interpretation of the data.

I also express my deep sense of gratitude to Dr. D. Chandramony, Associate Professor, Department of Agricultural Botany for patiently going through the manuscript and making useful corrections.

My thanks are also due to Dr. K. Rajmohan, Associate Professor, Department of Horticulture for the help rendered by him during the course of this investigation at the tissue culture laboratory and also in the preparation of the thesis.

My sincere thanks are also due to Dr. B.R. Reghunath, Associate Professor of Horticulture for the valuable help extended without any reservation in taking photographic exposures, Dr. Sajan Kurien, Assistant Professor of Horticulture for giving a helpful hand in the preparation of thesis and Dr. Sivaprasad, Associate Professor of Plant Pathology for his help in the ex vitro establishment studies.

I am indebted to Dr. S. Ramachandran Nair, Professor and Head, Department of Horticulture for providing the facilities at the tissue culture laboratory and Dr. R. Gopimony, Professor and Head, Department of Agricultural Botany for his valuable help to submit the thesis.

I am also grateful to Mr. C.E. Ajithkumar, Programmer for the tireless help rendered in the analysis of data and to M/s. Athira Computers, Kesavadasapuram for the computerized processing of data and text.

I record my deep sense of gratitude to Prof. K. Gopakumar, Prof. P.V. Prabhakaran, Dr. V. Gopinathan Nair, Dr. N. Saifudeen, Dr. C.S. Jayachandran Nair, Mr. K.N.

Satheesan, Mr. N. Mohan Babu, Dr. Waheed and Smt. Kamalam Joseph for their help extended to me at different stages of investigation.

I also extend my gratitude to the staff and students, Department of Horticulture and Plant Breeding and Genetics for their whole hearted co-operation for the successful completion of the programme.

I remember my wife for her constant help, encouragement and fervent prayers throughout the course of the study.

Above all I humble myself to the Almighty God for giving me strength and grace of knowledge to carryout the project successfully.

D. WILSON

CONTENTS

		Pages
INTRODUCTION	...	1 - 4
REVIEW OF LITERATURE	...	5 - 44
MATERIALS AND METHODS	...	45 - 80
RESULTS	...	81 - 199
DISCUSSION	...	200 - 233
SUMMARY	...	234 - 239
REFERENCES	...	240 - 261
APPENDICES	...	i - xvi
ABSTRACT	...	I - IV

LIST OF TABLES

Table	Title	Pages
1.	Trials on surface sterilization for different explants using mercuric chloride	55
2.	Composition of MS medium used for tissue culture of rose	57
3.	Trials on size of the explants for *in vitro* culture of rose	58
4.	Trials on initial culture establishment with different hormone supplements	61
5.	Trials on *in vitro* rooting of cultured shoots with different auxin supplements	65
6.	Trials on callus induction, callus multiplication, somatic organogenesis and somatic embryogenesis	71
7.	Effect of gamma irradiation of bud woods at different stages on time of bud take	83
8.	Interaction effect among cultivars, bud stages and gamma rays on sprouting (%)	85
9.	Interaction effect among cultivars, bud stages and gamma rays on survival (%)	86
10.	Interaction effect among cultivars, bud stages and gamma rays on plant height (cm)	89
11.	Interaction effect among cultivars, bud stages and gamma rays on number of primary branches per plant	91

Table	Title	Pages
12.	Interaction effect among cultivars, bud stages and gamma rays on prickle density	93
13.	Interaction effect among cultivars, bud stages and gamma rays on number of leaves per plant	94
14.	Interaction effect among cultivars, bud stages and gamma rays on days to flowering	96
15.	Interaction effect among cultivars, bud stages and gamma rays on neck length (cm)	97
16.	Interaction effect among cultivars, bud stages and gamma rays on flower diameter (cm)	99
17.	Interaction effect among cultivars, bud stages and gamma rays on flower weight (g)	100
18.	Interaction effect among cultivars, bud stages and gamma rays on petal weight (g)	102
19.	Interaction effect among cultivars, bud stages and gamma rays on petal number	103
20.	Interaction effect among cultivars, bud stages and gamma rays on number of flowers	105
21.	Floral characters of the mutant obtained from cv. Folklore	110
22.	Effect of surface sterilization (cv. Folklore). Explants: Shoot tip and axillary bud	113
23.	Effect of surface sterilization (cv. Folklore). Explants: Internodal segments and leaf disc	114
24.	Standardization of the size and type of the explants on initiating shoot growth and callus induction.	116

Table	Title	Pages
25.	Effect of bud stages on the *in vitro* establishment of rose (cv. Folklore)	118
26.	Effect of different levels of cytokinin in combination with auxin (2, 4-D or IAA) or GA_3 on initial culture establishment in rose (cv. Folklore)	120
27.	Effect of BAP, kinetin and in combination with GA_3 on shoot proliferation (cv. Folklore)	124
28.	Effect of different levels of IAA, NAA and in combination with 2, 4-D on *in vitro* rooting	130
29.	Effect of activited charcoal in combination with IAA and NAA on *in vitro* rooting	136
30.	Effect of rooting period and potting mixture on survival of plantlets (cv. Folklore)	140
31.	Effect of supply of nutrient solution on growth and survival of explants (cv. Folklore)	144
32.	Effect of mycorrhizae on *ex vitro* establishment of rose (cv. Folklore)	146
33.	Effect of cytokinin (BAP) in combination with auxin (NAA or 2, 4-D) on the induction and growth of callus (cv. Folklore)	151
34.	Effect of different levels of BAP and NAA in combination with 2, 4-D or ascorbic acid on callus Proliferation and organogenesis	155
35.	Effect of gamma rays on percentage of bud break, survival and malformed leaves (cv. Folklore)	159
36.	Effect of gamma rays on days to bud break (cv. Folklore)	163

Table	Title	Pages
37.	Effect of gamma rays on days to first leaf emergence (cv. Folklore)	165
38.	Effect of gamma rays on induction of multiple shoots (%) (cv. Folklore)	166
39.	Effect of gamma rays on days to induced multiple shoots (cv. Folklore)	168
40.	Effect of gamma rays on number of shoots per culture (cv. Folklore)	169
41.	Effect of gamma rays on inducing morphological variations in culture (%) (cv. Folklore)	172
42.	Interaction effect between bud stages and gamma rays on growth at two weeks intervals (cv. Folklore) i Main shoot	176
43.	Interaction effect between bud stages and gamma rays on growth at two weeks intervals (cv. Folklore) ii side shoot	178
44.	Interaction effect between bud stages and gamma rays on leaf production at two weeks intervals (cv. Folklore)	180
45.	Effect of gamma rays on rooting percentage of culture shoot (cv. Folklore)	183
46.	Effect of gamma rays days to initiate rooting of cultured shoots (cv. Folklore)	184
47.	Effect of gamma rays on number of roots per shoot (cv. Folklore)	186
48.	Effect of gamma rays on length of roots (cv. Folklore)	188

49. Effect of gamma irradiation of multiple shoots on survival, induction of multiple shoots and morphological variations (cv. Folklore) 189

50. Effect of gamma irradiation on days to multiple shoot induction and number of shoots per culture on subculturing (cv. Folklore) 194

51. Effect of gamma irradiation of multiple shoots on rooting efficiency (cv. Folklore) 196

52. Growth analysis of irradiated multiple shoots at two weeks intervals (cv. Folklore) 198

LIST OF PLATES

Plate	Description	Pages
1.	Cultivars (Hybrid teas) selected for induced mutagenesis - cv. Alliance	46
2.	Cultivars (Hybrid teas) selected for induced mutagenesis - cv. Suraga	46
3.	Cultivars (Hybrid teas) selected for induced mutagenesis - cv. Folklore	47
4.	Chlorophyll variations (cv. Alliance)	107
5.	Foliar deformities (cv. Alliance)	107
6.	Foliar deformities (cv. Suraga)	108
7.	Variations in colour (cv. Alliance)	108
8.	Colour mutant (cv. Folklore)	109
9.	Separated petals of reddish yellow mutant & control	109
10.	Variation in size and number of petals (cv. Folklore)	111
11.	Separated petals (control & mutant)	111
12.	Shoot growth (3 weeks after culture in best initial culture establishment medium MS medium + BAP 2.5 mg/l + 2,4-D 0.5 mg/l	126
13.	Multiple shoot (8 weeks after culture) in best shoot proliferation medium. MS medium + kinetin 2.0 mg/l + GA_3 1.0 mg/l	126
14.	Flower bud initiation 6 weeks after culture in MS medium + BAP 2.0 mg/l + GA_3 0.5 mg/l	129
15.	Culture showing absence of rooting at 2 weeks	133
16.	Root initiation in MS medium + IAA 1.0 mg/l + NAA 1.0 mg/l at 2 weeks	133

Plate	Description	Pages
17.	Root growth in MS medium + IAA 1.0 mg/l + NAA 1.0 mg/l at 4 weeks (Mean root length 2.44 cm)	138
18.	Best root growth in MS medium + IAA 1.0 mg/l + NAA 1.0 mg/l + Activated charcoal 500 mg/l at 4 weeks (Mean root length 5.20 cm)	138
19.	Improvised hardening structure consisting of wooden frame and polythene cover	143
20.	Effect of different potting mixtures on hardening and plantlet establishment (Two weeks after planting out)	143
21.	Control (plantlets in mycorrhizae free sand) 4 weeks after planting out	148
22.	Plantlets on sand inoculated with mycorrhizae (VAM) 4 weeks after planting out	148
23.	Plants 8 weeks after culture (inoculated with mycorrhizae	149
24.	Plants 16 weeks after culture (inoculated with mycorrhizae	149
25.	Best callus induction using leaf disc explants in MS medium + BAP 0.5 mg/l + NAA 2.0 mg/l + 2,4-D 0.5 mg/l at 6 weeks after culture	153
26.	Best callus induction using internodal segments in MS medium + BAP 0.5 mg/l + NAA 2.0 mg/l + 2,4-D 0.5 mg/l at 8 weeks after culture	153
27.	Rhizogenesis from leaf callus in MS medium + BAP 0.5 mg/l + NAA 2.5 mg/l + 2,4-D 0.5 mg/l	157
28.	Rhizogenesis from internodal callus in MS medium + BAP 0.5 mg/l + NAA 2.5 mg/l + 2,4-D 0.5 mg/l	157
29.	Effect of Gamma irradiation on initial culture establishment cv. Folklore (3 weeks after culture) - control	162

Plate	Description	Pages
30.	Effect of Gamma irradiation on initial culture establishment cv. Folklore (3 weeks after culture) - 20 Gy control & treated	162
31.	Effect of Gamma irradiation on initial culture establishment cv. Folklore (3 weeks after culture) - 30 Gy control & treated	170
32.	Effect of Gamma irradiation on initial culture establishment cv. Folklore (3 weeks after culture) - 40 Gy control & treated	170
33.	Effect of gamma irradiation on shoot proliferation cv. Folklore - Yellowing of leaf	173
34.	Effect of gamma irradiation on shoot proliferation cv. Folklore - Enlarged leaf blade	173
35.	Effect of gamma irradiation on shoot proliferation cv. Folklore - Deformed leaflets	174
36.	Effect of gamma irradiation on shoot proliferation cv. Folklore - Chlorophyll deficiency of leaf	174
37.	Effect of gamma irradiation on multiple shoot cv. Folklore - Narrow leaves	191
38.	Effect of gamma irradiation on multiple shoot cv. Folklore - deformed striated leaves	191
39.	Deformed leaf with prominant striations	192
40.	Deformed leaves with foliar petiole	192
41.	Deformed leaves with chlorophyll deficiency	193
42.	Plantlet showing altered pigmentation on leaf	193

APPENDICES

i. Effect of gamma irradiation of budwoods of different stages on sprouting

ii. Effect of gamma irradiation of budwoods of different stages on survival

iii. Effect of gamma irradiation of budwoods of different stages on plant height (cm)

iv. Effect of gamma irradiation of budwoods of different stages on number of primary branches per plant

v. Effect of gamma irradiation of budwoods of different stages on prickle density

vi. Effect of gamma irradiation of budwoods of different stages on days to flowering

ix. Effect of gamma irradiation of budwoods of different stages on number of leaves per plant

viii. Effect of gamma irradiation of budwoods of different stages on neck length (cm)

ix. Effect of gamma irradiation of budwoods of different stages on flower diameter (cm)

x. Effect of gamma irradiation of budwoods of different stages on flower weight (g)

xi. Effect of gamma irradiation of budwoods of different stages on petal weight (g)

xii. Effect of gamma irradiation of budwoods of different stages on petal number

xiii. Effect of gamma irradiation of budwoods of different stages on number of flowers

xiv. Effect of gamma rays on growth rate of multiple shoots i Main shoot (cv. Folklore)

xv. Effect of gamma rays on growth rate of multiple shoots (cv. Folklore) ii side shoot

xvi. Effect of gamma rays on leaf production (cv. Folklore)

INTRODUCTION

1. INTRODUCTION

Flowers are among the loveliest objects on this earth, and among flowers rose is eulogised as the 'Queen'. Rose occupies an unique position among flowers, not only of its importance in ornamental gardening, but also of its aesthetic value as cut flower. Fragrance of rose is enchanting and is commercially exploited in perfumery industries.

The genus Rosa is very large. The chromosome number varies from 2n = 14 to 2n = 56; but most species are diploid or tetraploid. The commercially grown cultivars are generally triploid or tetraploid. Rose being highly heterozygous, with a complex genetic constitution, improvement through conventional breeding methods is cumbersome and slow. However, the heterozygosity can be advantageous in mutation breeding as it increases the spectrum of variability.

The general trend in ornamental crops viz., "different is better", certainly applies to rose as well. A slight difference in colour, shade or any other desirable character may open a new avenue for growers and traders.

Natural mutation has played a very important role in the evolutionary history of rose cultivars. The occurrence of spontaneous mutation or 'budsports' is at the mercy of nature and is quite often retrogressive. Hence organized and controlled efforts to induce variation by artificial means have been resorted to increase the frequency of such events. The rapid growth in theoretical understanding and technical advancement in the induction and recovery of mutations have triggered a dynamic phase in the use of induced mutations as a supplement to, or rather a substitute for, the conventional method of breeding. Genetic improvement of ornamental plants, particularly vegetatively propagated plants through induced mutation is one of the potential areas of research, as it is possible to improve one or a few characters of an otherwise excellent cultivar (Broertjes, *et al.* 1976).

One of the major difficulties in the mutation breeding of higher plants is the formation of chimeras. Plants with drastic induced variation have been naturally eliminated due to vigorous diplontic selection (Swaminathan *et al.*, (1970), and Goud *et al.*, 1970). These problems can be solved to a great extent by adopting *in vivo* and *in vitro* propagation methods which enable the successful production of solid mutants, that can be recognized sooner and propagated more easily.

Plant cell culture has provided a rapid and exciting option for obtaining increased genetic variability. *In vitro* techniques are becoming important in mutation breeding to obtain desirable mutants and to restrict chimera formation (Broertjes, *et al.* 1976, Roest, 1977).

Callus culture is also being used for irradiation. Compared to a cell in a well organized apex, a mutated cell in a mass of cells will have more chance of survival and regeneration. The majority of the mutants produced by this method will be solid, especially if the shoots are regenerated from a repeated subculture of irradiated callus, since the number of cells from which adventitious shoots originate *in vitro* seems to be restricted. *In vitro* propagation by callus culture has been reported in many ornamentals like Chrysanthemum morifolium (Ben.Jaacov and Langhans 1968), Gladiolus sp. (Simonsen and Hilderbrandt,1971) Petunia sp. (Binding, 1971).

Irradiation of explant like axillary bud, leaf disc, petiole, pedicel and peduncle is another possible means for the production of mutants, especially when adventitious shoots are regenerated *in vitro* from them.

The present investigation was undertaken with the following objectives.

1. To assess the optimum dose of gamma rays for inducing variations under *in vivo* and *in vitro* culture techniques.

2. To find out the effect of gamma rays on various morphological and biometrical attributes.

3. To standardise *in vitro* culture conditions and techniques for *in vitro* mutagenesis in rose.

4. To isloate mutants induced by gamma rays both under *in vivo* and *in vitro* culture.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

In numerous ornamentals the appearance of sports is a source of variability often used in developing new cultivars. Such sports in vegetatively propagated plant species may be caused by uncovering of and rearrangements in existing periclinal chimeras, by replacement of the epidermal layer with deeper tissue or by spontaneously arising mutations in single cells. Over a period of 25 years, about 20 per cent of all rose cultivars were developed from sports (Krussmann, 1974). Amongst 5819 cultivars marketed from 1937 to 1976, 865 have developed from bud mutation, out of which 289 were climbers (Haenchen and Gelfert, 1978). Multicoloured cultivars and those normally grown under protected cultivation mutate more rapidly than others. Only five cultivars have been known to be produced by induced mutation namely 'Desi', 'Flemingo Queen', 'Milena', 'Permoser', and 'September Wedding'.

Heslot (1964) reported that spontaneous mutations occur more frequently, and have caused a valuable increase in genetic variation especially in Hybrid Tea group. The more significant bud sport in rose include the mutation in the Centifolia group (Hurst and Breeze, 1922), the induction of climbing character in Hybrid Tea roses (Morey, 1954) and the mutation responsible for the production of brilliant orange

coloured flowers in dwarf polyantha roses (Wylie, 1955a,b). Wylie, 1955a reported that the climbing Hybrid Teas had occasionally arisen, following a cross between two varieties, but more commonly from bud mutations. Examples of stable Hybrid Tea climbing sports are 'Climbing Crimson Glory' and 'Climbing Mrs Sam Mc Greedy'. She also reported that Hybrid Tea varieties of bush habit, in addition to climbing form, have frequently arisen by bud mutation. She estimated that more than 54 per cent varieties of dwarf polyanthas originated as sports.

Saakow (1960) studied the history of bud mutation, in a number of cultivars on the time of mutation in relation to the age of the variety, the degree of mutation in relation to the genotype as well as the tendency of the flower colour to mutate, in relation to the flower colour of the initial cultivar. Climbing types have been found to result from mutation rather often. He reported that 'Ophelia' produced 22 varieties through bud sporting for seventeen years from 1916, and 'Mme Butterfly', the best of 'Ophelia' sports had given rise to seven new varieties in the same way. Saakow (1960) and McFarland (1958) showed further examples of sport groups of rose varieties such as 'Radiance' and 'Mme Caroline Test Out' group. He also established that rose groups such as Hybrid Tea, Polyantha and Peretiana have a tendency to induce most bud mutations spontaneously.

The world famous rose variety 'Peace' also produced several sport varieties including 'Chicago Peace' and 'Peace Port' (Nakajima ,1965). Malik and Singh (1980) reported 'Nav Sadabahar' and 'Pink Montezuma' to be natural bud sports originated from cultivars 'Sadabahar' and 'Montezuma'.

2.1. INDUCED MUTATION IN ROSE

Mutagenic action of X-rays was discovered by Muller in 1927 on Drosophila and in 1928 by Stadler in barley (H. vulgare) and maize (Z. mays). In 1944 Auerbach and Robson showed that nitrogen mustards produced mutation in Drosophila. Subsequently, a number of chemicals with mutagenic action were described.

Important ionising radiations were effective on biological materials are X-rays, gamma rays, fast neutron, alpha rays and beta rays. In rose, different plant parts namely budwood, whole plants, pollen grains, seeds and cuttings can be used for mutagen treatment.

2.1.1. Mutation induction by X - rays

Gelin (1955) reported induction of mutation in rose by X-rays. Experiments conducted by Streitberg (1964, 1966 a,b and 1967) with 42 rose cultivars revealed a linear relationship of bud mortality to X-ray dose but varieties

differed in their sensitivity to irradiation. Several mutants with different flower colour, form and size of the flower were produced. Out of a total of 14.9 per cent of mutation produced, 5.8 per cent showed new variations, the remaining 9.1 per cent representing repetitions. The most suitable range of X-rays for rose shoots was 1.5 to 3.5 kR.

After irradiation of five cultivars namely 'Peace', 'Queen Elizabeth', 'Better Times', 'Baccaria' and 'Tropicana' with X-ray doses of 7-8 kR, Chan (1966) reported several mutations that affected flower colour and growth habit. The mutant 'Desi' characterised by dark red stripes on yellow petals was produced by X-ray irradiation of variety 'Gloria Dei' at 3 kR.

Harney (1976) obtained 'Flamingo Queen' as a mutant of 'Queen Elizabeth' when treated with 7-8 kR X-ray and Strietberg (1977), obtained the mutant 'Permoser' from 'Kordes Perfecta' by treating the buds at 1.5 kR X-rays.

2.1.2. Mutation induction by gamma rays

2.1.2.1. Cuttings

Latha and Gupta (1971) irradiated the stem cuttings with doses 2,4,6, and 8kR. They recorded the survival of plants, flower and essential oil yield and production of

normal and abnormal flowers in 12 gamma irradiated and non-irradiated scented hybrid tea cultivars during the second year after irradiation. They reported that flowers from irradiated plants were generally smaller and contained less oil than those of control.

Gupta *et al.* (1982) irradiated the cuttings of Rosa damascena with gamma rays at 1 and 2 kR and reported reduction in sprouting, sprout number and plant height.

Guo *et al.* (1983) irradiated the cuttings of rose cultivars 'Gruss an Berlin', 'Super Star' and 'John Strong' with 2,4,6, and 8 kR gamma rays. Flower colour mutations were observed in 'Gruss an Berlin' and 'John Strong' 6 months after flowering.

Benteka (1985) irradiated single node cuttings of variety 'Sonia' with 2, 3, 4, 5 and 6 kR and rooted them under mist. He reported that the optimum dose of irradiation was found to be between 4 and 5 kR. He observed the reduction in the number of non-chimeral mutation in shoots of third bud generation. The third bud generation was found to be the most suitable material to obtain non-chimeral shoots.

Huang and Chen (1986) irradiated the green shoots of several cultivars including 'Crimson Glory', 'Super Star', 'Condesa de Sastago', 'Peace', 'Pink Peace' and 'South Seas'.

Four new cultivars namely 'Ji Guanj', 'Xia Guang Wan Dao', 'Zhen Jie and 'Nan Hai Lang Hua' were established from stable mutant clones.

2.1.2.2. Budwood

Gupta and Shukla (1970) irradiated budwood of 'Montezuma' and 'Super Star' with 4, 5 and 6 kR of gamma rays. Two years after irradiation they observed mutation in flower colour and shape in 'Montezuma' Dark coloured, light pink coloured and compact petalled mutation, were induced from 'Montezuma' after gamma irradiation. The highest number of mutation was produced after exposure to 5 kR gamma rays. The cultivar 'Super Star' was found to be more radio sensitive than 'Montezuma'.

Gupta and Shukla (1971 a, b) irradiated bud wood of twelve scented cultivars of garden rose with 4 kR gamma rays. Somatic mutations were induced in flower colour in 'Bettina', 'Lady Florence Stronge', 'Mc Gredy's Sunset' and 'President Poincare'. They also suggested that the irradiated plants should be kept under observation for several years to detect mutation.

Kaicker and Swarup (1972) induced colour mutations in three cultivars namely Christian Dior', 'Queen Elizabeth' and 'Kiss of Fire' by treating the dormant buds with gamma

rays at 5 - 10 kR. They also found that 10 kR gamma rays was toxic to all survived under Delhi conditions.

Usenbaev and Iman Kulova (1974) irradiated buds of four cultivars with 0.5 to 3.0 kR of gamma rays and grafted them on Rosa canina. They obtained 20 sports with changes in flower colour and petal number after five years.

Based on the studies conducted on control and gamma ray induced mutants of Montezuma, Lata and Gupta (1975) suggested that the variation exhibited by the mutants may be chromosomal and / or genic in nature.

Dommergues (1976) explained that the changes in flower colour might be due to a change in the nature of pigments themselves although most of them were already present in the plant.

Lata (1980) irradiated bud wood of seven rose cultivars viz. 'Caledonia', 'Oklahoma', 'Papa Meilland', 'Pink Parfait', 'Prelude', 'Quebec' and Virgo with 3, 4, and 5 kR of acute gamma rays. She isolated three mutations one in growth habit, and two in flower colour. The results indicated that the floribunda rose 'Pink Parfait' was the most suitable one for the induction of mutation.

Datta and Gupta (1982a) irradiated bud wood of rose cv. 'Junior Miss' with 3, 4, and 5 kR of gamma rays, and

reported a flower colour mutation from 3 kR treated population in the second year after drastic pruning. The original colour of 'Junior Miss' was pink, whereas the mutant colour was almost white.

Datta and Gupta (1983) obtained 3 mutants 'Empire Yellow', 'Tangerine Orange' and 'Deep Pink' from cv. 'Contempo' by irradiating the buds 3 - 5 kR gamma rays. The 'Contempo' flowers are orange with an yellow eye at the base of petals. The 'Empire Yellow' mutant was almost similar to the yellow eye colour of 'Contempo'. They explained that somatic flower changes in 'Contempo' were due to qualitative and quantitative changes in biosynthetic pathway of pigments induced by irradiation.

Datta and Gupta (1984) reported two mutant varieties viz., 'Saroda' and 'Sukumari' induced by exposing budwood of cv. 'Queen Elizabeth' and 'Junior Miss' to 3 kR. The mutant tissue detected in vM_1 was isolated and multiplied by repeated budding. The mutant 'Saroda' produced very light pink flowers, and 'Sukumari' almost white flowers.

Datta (1985) irradiated budwood of nine cultivars at 3, 4 and 5 kR gamma rays. He found differential sensitivity among the rose cultivars. The cultivar 'Orange sensation' was found almost sensitive and 'Kiss of Fire' the most resistant to irradiation. Somatic mutations in flower

colour and types were recorded in chimeric forms. He isolated six mutation in pure form from four cultivars. Datta (1986b) studied the effect of recurrent gamma irradiaton on rose cv. 'Contempo'. Recurrent gamma irradiaton showed cumulative effect on sprouting, survival and plant height. Percentage of somatic mutation and spectrum of mutations were higher after recurrent irradiaton, in comparison to single irradiation.

Kaicker and Dhyani (1986) and Kaicker (1988) found that the most effective dose was 2.5 kR with cultivar 'Folklore' and there was a decrease in mutated sector in 5 kR treatment. They obtained a mutant with reduced number of petals from 'Doris Tysterman' after 4 kR treatment. They detected five stable mutants in cultivar 'Folklore'.

2.1.2.3. Whole Plants

Dommergues *et al.* (1967) exposed a number of varieties belonging to the diploid, triploid and tetraploid groups to gamma rays. They compared the reactions of diploid cultivars 'Gloria Mundi' and 'Border King' with those of triploid 'Orange Triumph' and tetraploid 'Peace' and several others to gamma rays. A large number of mutants had been induced, isolated and multiplied by grafting. One year old plants were treated with gamma rays 8-9 kR and two years

after treatment, mutations in number and shape of the petal, flower colour and growth habit were observed. The survival, rearrangement of pre-existing periclinal chimeras and mutations in the cultivars were different, both between and within the ploidy groups.

Heslot (1964, 68) treated plants of diploid, triploid and tetraploid cultivars with 4 and 8 kR gamma rays. High frequencies of mutation were obtained in cultivars 'Orange Triumph' and 'Gloria Mundi' due to pre-existing periclinal chimerism, whereas several mutatnts also appeared in other cultivars.

Nakajima (1970) irradiated plants at 10 kR gamma rays upto 10 days. The cultivars showed difference in response to radiation. The cultivar 'Crimson Glory', 'Golden Master Piece' rarely mutated, whereas many sports were produced by 'Peace', 'Queen Elizabeth' and 'Kordes Perfecta'. Flower colour was characteristic and most frequently mutated.

Desai (1973) subjected 36 cultivars of rose to chronic and semichronic gamma irradiation and found that doses of 8 to 10 kR in 10 to 20 days to be the most effective to bring about mutations for flower colour and number of petals.

Nakajima (1977) exposed potted plants of cv. 'Peace' to gamma rays at 15 kR and shoots cut back 6-8 times

for 2 years to produce 9 mutants showing changes in flower, leaf and thorn characters. Six mutants were induced by treatment with 10 kR. He also reported that high radiation dosages in early April were most lethal to 'Kordes Perfecta', less to 'Peace' and least to 'Super Star'. Plants of Rosa wichuriana and R. rugosa were all killed by 10 days exposure to 20 kR gamma rays in spring, where as over 50 per cent of normal R. multiflora and thornless clones survived exposure to 30 kR.

Desai and Abraham (1978) irradiated one year old 250 potted plants belonging to 30 cultivars of rose with chronic gamma rays. A dose of about 8 kR chronic gamma rays was found optimum for induction of mutations. They isolated 3 mutants with complete flower colour change from two cultivars namely 'Saroya' and 'Confidence'.

2.1.2.4. Pollen Grains

Klimenko *et al.* (1974) reported that gamma irradiation of pollen at 5 to 20 kR raised the germination percentage but higher doses lowered it. The critical irradiation dose (LD_{70}) was between 40 and 600 kR and LD_{100} between 75 and 650 kR. The pollen of cultivars 'Kordes', 'Sondermeldung' and 'Dortmand' showed outstanding resistance to radiation.

2.1.2.5. Seeds

Klimenko *et al.* (1975) reported that gamma irradiation at 1 to 50 kR, generally depressed or inhibited seed germination, and seedling emergence in five rose cultivars. In some cases, however, low doses stimulated seed germination, plant growth, vigour and induced morphological changes.

2.1.2.6. Effect of gamma rays on morphological characters

In general, gamma irradiation of budwood resulted in delayed sprouting of buds, lower percentage of bud sprout, and reduction in plant height [Gupta and Shukla, (1970); Kaicker and Swarup (1972, 78); Lata (1980); Gupta and Datta (1982); Gupta *et al.*, (1982); Datta, (1986, 1987 and 1988)].

Kaicker and Swarup (1972, 1978) observed that, irradiation with gamma rays delayed sprouting of buds from six to nine months in 'Christian Dior' and by one year in 'Kiss of Fire'.

Lata (1980) found that the LD_{50} for white and mauve flowered cultivar was lower than that for yellow, red or pink flowered ones, the latter being more prone to mutations. Many phenotypically detectable variations in leaf, flower and growth habit were recorded in irradiated population.

Datta (1985) reported differential sensitivity to rose cultivars with respect to sprouting, survival and plant height as a result of irradiation.

Kaicker and Swarup (1972), Deasai and Abraham (1978, Gupta and Datta (1982) observed several leaf abnormalities like simple, narrow, thickened, chlorophyll mosaic leaves, leaves with forked and joined leaflets, and unequal development of leaf lamina.

Nishida *et al.* (1967) found delay in time of flowering after irradiation in 'Crimson Glory' under chronic gamma ray exposure. The number of petals decreased at higher exposure rates. Delay in time of flowering was also reported by Kaicker and Swarup (1972) with higher doses of irradiation. Stimulation in flowering was also reported by Gupta and shukla (1971 a) in cultivars 'SuzonLothe' 'Priscilla and 'Hadley' after irradiation. Kaicker and Swarup (1972) observed forked flower bud initiation as one of the primary effects of radiation in some shoots.

Gupta and Shukla (1970) Datta and Gupta (1982 a, b) and Guo *et al.* (1983) reported reduction in the total number of flowers produced and petal weight after irradiation.

Irulappan and Rao (1981) assessed the effect of gamma rays on edward roses. In vM_1 generation, flower weight

and number and weight of flowers per plant were significantly increased in all mutagenic treatments. In the vM_2 generation, increased values were observed for number and weight of flowers per plant. Desai and Abraham (1978) found that lower doses of irradiation produced bigger flowers.

2.1.2.7. Effect of radiation on flower pigments

The floral pigments of induced mutants and that of original cultivars of rose were studied by Heslot (1968). He found that the nature of pigment did not alter, but the pigments showed either an increase or decrease of one or several of the pigments found in control.

Kaicker and Pandey (1973) evolved a method for the extraction of pigments florochrome A and B from ornamental plants. They studied the florochrome contents of two rose cultivars 'Christian Dior' and 'Gulzar' and reported minute quantitative changes in pigment content in mutant.

Dommergues (1976) explained that the changes in flower colour might be due to a change in the nature of pigment themselves although most of them were already present in the plant.

Gupta and Datta (1982), Datta and Gupta (1982 a b) and Datta (1986 c) studied the pigments of petals in some of the mutants by thin layer chromatography and

spectrophotometric method. They confirmed that mutation might have taken place in four major directions. In the first case, the mutagen might result in either increase or decrease or both in concentration of one or more existing pigments. In the second case the difference might be due to blockage of one or more pigments' synthesis, and this might be associated with increase or decrease in concentration of one or more existing pigments. In the third case, the mutation might give rise to a new pigment which may be associated with increase or decrease in the concentration of one or more existing pigment. In the fourth case mutation might result in synthesis of a new pigment as well as in blocking of development of one or more existing pigments. The situation may be associated with either increase or decrease or both in concentration of one or more existing pigments.

2.1.2.8. Cytological effect of irradiation

Tackholm (1920) reported that cytologically Rosa forms a regular polyploid series from 2x to 8x. Besides various ploidy levels, the occurrence of aneuploidy, structural hybridity and irregular meiosis are common in garden roses (Shahare and Shastry, 1963).

Meiotic studies on control and gamma ray induced mutants of rose cultivar 'Montezuma' were carried out by Lata

and Gupta (1975). They observed that, 'Montezuma' and two of its gamma ray induced mutants presented a marked difference in their cytological features. Their Pollen Mother Cell (PMC) meiosis revealed great variation with regard to chromosome association at metaphase I, which comprised of univalents, bivalents trivalents and quadrivalents. Ring shaped quadrivalents were more common than chains. The frequency of quadrivalents was enhanced in mutant plants. The percentage of PMCs with precocious separation, laggards and bridges at anaphase was found to be higher in mutants as compared with the control plants. The pollen fertility decreased in pink flowered but increased in reddish orange flowered mutant compared to the control.

2.2. *IN VITRO* CULTURE OF ROSE

Traditionally most of the ornamental quality Rosa species have been propagated by budding. Budding is slow and cumbersome. The development of tissue culture technology has been accelerated by its commercialization. Tissue culture propagation offers many advantages over the conventional methods. It offers a method to increase valuable genotypes rapidly and expedite the release of improved varieties.

There are three major routes of *in vitro* propagation viz., enhanced release of axillary buds, organogenesis and somatic embryogenesis, (Murashige, 1974). All the different methods have been attempted in rose, of which the first method reported to be most successful.

2.2.1. Embryo Culture

Embryo culture in rose was first established by Lammerts in 1946. Asen and Larson (1951) have detailed their procedure for embryo culture. The potential use of embryo culture to rescue particular crosses which otherwise might abort was the goal of the rose breeder. Another very practical use of embryo culture relates to the rapid seed germination *in vitro*.

Von Abrams and Hand (1956) reported 98 per cent germination of seeds of a fertile cross in embryo germination medium in 14 days, on comparison to 66.9 per cent germination of seeds in 180 days when sown in soil.

Graifenberg (1973) reported that, when the achene (seed) of R. canina was broken and the naked embryo was excised and grown on Knudson medium (KC), the embryos germinated quite well. About 30 per cent of the fruits yielded transplant size plants in about 3-4 months. In contrast no plants were obtained from intact achenes.

2.2.2. Anther Culture

Tabaeezadeh and Khosh-Khui, (1981) studied the response of anthers of two Rosa spp to various levels of auxins and cytokinins at different bud stages and light conditions. They found that Murashige and Skoog (MS) medium with 2.0 mg/l IAA (Indole Acetic Acid) and 0.4 mg/l kinetin was generally best for anther culture of R. damascena, Mill, while medium with 7.5 mg/l (IAA) and 0.8 mg/l kinetin was optimum for R. hybrida. Culture of anthers of R. damascena when a few petals are visible on the flower bud and of R. hybrida when the flower bud is completely close has been recommended. Both species produced more callus in the dark than in light, at both stages of bud development.

2.2.3. Protoplast Culture

Protoplasts were first isolated from Paul's Scarlet' rose cultivars in 1973 by Pearce and Cocking. Strauss and Potrykus (1980) isolated protoplasts from cell suspension cultures and achieved sustained deviation of protoplasts by plating them on agar solidified M x G medium. The first change in plated protoplasts was deviation from spherical shape due to cell wall synthesis on the third day at 27^{o}c. From these first cells, micro and macrocolonies were developed. The macrocolonies were plated to form calli.

Frequent subculturing in the early stages of division was essential.

2.2.4. Suspension Cultures

The use of rose tissue for physiological investigations has been very important since 1957, when Tulecke and Nickell (1959) established a cell line of Paul's Scarlet rose from the young stem. Cell lines of this cultivar have been used to study various aspects of cell suspension, growth and metabolism (Nash and Davies, 1972), minimal constituents of tissue culture medium (Nesius *et al.*, 1972), carbondioxide and pH requirement of non-photosynthetic cells (Nesius and Fletcher, 1973), the effects of carbohydrate and nitrogen concentration on phenol synthesis (Amorim *et al.*, 1977) and glutamate synthesis (Feletcher, 1974).

Suspension cultures of R. glauca and R. damascena have also been established and used for studies similar to those for Paul's Scarlet rose. Suspension cultures of R. glauca were used to study the structure of primary cell wall (Joseleau and chambat, 1984a,b) and lignin production (Mollard and Robert,1984).

2.2.5. Meristem Culture

Elliot (1970) reported successful culturing of rose

meristem tips on a defined medium containing inorganic salts, sucrose, thiamine, inositol and a cytokinin. Excised shoot apices (0.6 or 1.0 mm) of R. multiflora were grown in axenic culture on defined media including auxins, gibberellic acid and cytokinins. In the presence of zeatin (10^{-8}–10^{-7}M) and 6-benzylaminopurine (BAP) (10^{-7} – 10^{-6} M), apices grew into plantlets, but two other cytokinins [Kinetin and 6- (3- methyl but -2- enyl) - aminopurine] were ineffective. Auxins and gibberellic acid (GA_3) were either inhibitory or had no effect on differentiation.

2.2.6. Callus culture and somatic organogenesis

Callus culture of rose have been established in many laboratories. The most famous lines were those established in 'Pauls Scarlet'rose cultivar.

Hill (1967) reported the formation of 'shoot primordia' in long term stem callus cultures of hybrid tea rose. He failed to produce normal shoots by frequent subculturing on various media, however, he got a bud with trifoliate leaves in one culture.

Jacobs *et al.* (1968) reported that, a modified Knob and Berthelot medium supplemented with Indole Butyric Acid (IBA) proved satisfactory for the growth of pith callus. Addition of IBA 1.0 mg/l resulted in bud formation although

no roots were produced. Jacobs *et al.* (1969, 1970) studied the effect of auxins, cytokinin and gibberellin on the growth of callus and stated that callus growth was dependent on the presence of Naphthalene Acetic Acid (NAA), and growth was further stimulated by the addition of kinetin at low concentrations (0.05 to 0.5 mg/l). The amount of callus produced was influenced by both the ratio between NAA and kinetin and their concentration.

Kireeva *et al.* (1977) found that petal leaf, sepal and embryo of essential oil yielding rose variety "Krymskaya Krasnaya" showed optimum callus formation on MS medium supplemented with 2, 4-Dichloro phenoxyacetic acid 1 - 4 mg/l, and kinetin 0.05 - 0.1 mg/l. They also observed the presence of essential oil, glycoside bound terpenes and aromatic alcohols in the callus produced from different explants except embryo, which are similar in composition to those found in the same organs of intact plant.

Wulster and Sacalis (1980) studied the effect of auxin and cytokinin on ethylene evolution and growth of rose callus tissue, and reported that ethylene evolution from callus tissue of rose Rose hybrida grown in air tight vessels was not enhanced by the presence of auxin and cytokinin in the culture medium. Callus growth was not adversely affected by increased ethylene levels.

Khosh-Khui and Sink (1982 b) used leaf and stem explants for the production of callus. They observed faster callus initiation in the dark than in light, but callus deteriorated when continuously subcultured in the dark.

Li (1983) studied the effect of 'Phloridzin' on *in vitro* culture of R. hybrida and reported that callus formation and bud differentiation can be improved by the treatment of 'phloridzin' 3.0 mg/l.

Adventitious shoot formation from callus cultures of R. persica and R. xanthina on MS medium supplemented with 4.4 - 8.8 uM BAP and 0.54 - 1.62 uM NAA was reported by Tweddle *et al.* (1984) and Lloyd *et al.* 1988. Shoot formation was obtained from fresh callus produced from internodal segments. Shoots with trifoliate leaves and slender stems survived, whereas unifoliate leaved plants with thick fleshy stems failed to survive. There was considerable variation in leaf morphology among the regenerants of R. persica x R. xanthina. They also observed that organogenic cells contained few starch grains and callus which lost organogenic potential contained large numbers of starch grains.

Gavish *et al.* (1986) and Zieslin *et al.* (1987) reported that growth of callus of rose cultivars 'Sonia' and

'Golden Times' was extensively promoted when cultured together with callus of Rosa. indica major but to a lesser extent by callus from Rosa canina inermis. It was explained that a diffusible promotive factor which affected the growth of callus of cv. Sonia exists in the tissue of rose rootstock cv. R. indica major and to a lesser extent in R. canina inermis.

Lloyd *et al.* (1988) further explained that other species which they tested (R. laevigata and R. wichuriana) failed to form adventitious shoots. Cells of these calli, like those of nonmorphogenic R. persica x R. xanthing, contained numerous starch grains.

Rout *et al.* (1992) reported successful *in vitro* regeneration of shoots from callus cultures of Rosa hybrida L.cv. Landora. Internodal segments from pot-grown plants and leaf disc from *in vitro* proliferated shoots were induced to form callus on modified MS salts reduced to half strength, 2 mg/l BAP, 0.01 mg/l NAA, 10 mg/l GA_3 AND 600 mg/l L - proline or L - glutamine. Adventitious shoot buds differentiated within 5-6 weeks, by subculturing the calli on modified induction medium consisting of half strength MS supplemented with 0.2 mg /l BAP, 0.01 mg/l NAA, 5, 10, or 20 mg/l GA_3 and 600 mg/l of either L - proline or L - glutamine. Regenerated shoots were successfully rooted within 10 days on liquid medium containing half strength MS

basal salts and 0.1 mg/l NAA. The rooted plants after hardening could be transplanted to pots in the green house with 75 to 80 per cent survival.

2.2.7. Somatic Embryogenesis

Rout *et al.* (1989c) developed the protocol for the induction of somatic embryogenesis in callus cultures of Rosa hybrida cv. Landora. Friable callus was obtained from immature leaf and stem internode segment on MS medium supplemented with 0.5 mg/l BAP, 1.0 mg/l NAA, 0.5 - 2.0 mg/l 2.4-D and 30 g/l sucrose. Somatic embryos were initiated on half strength MS supplemented with 0.5 mg/l BAP 0.01 mg/l NAA, 0.1 mg/l GA_3 and various concentrations of l-proline (200 - 800 mg/l). But the embryos showed abnormalities in shape structure and number of cotyledons.

Rout *et al.* (1991) observed that some of the somatic embryos were morphologically normal showing distinct cotyledons and radicles. The embryos were loosely attached to the mother callus with short suspensor like structures at the basal end. Somatic embryos also arose from the basal region of other embryos in clusters, indicating a clear case of secondary somatic embryogenesis. On subculturing the somatic embryos on a regeneration medium, 12 per cent of the embryos elongated and formed leaves, but failed to develop into a plantlet.

2.2.8. Shoot tip culture

In shoot tip culture, the explants may be the whole or part of an apical or lateral growing point of a stem or it may be a stem section of several nodes. Shoots tips and axillary buds are generally used for tissue culture in rose.

Skirivin and Chu (1979 a,b) achieved shoot proliferation of 'Forever Yours' greenhouse rose (Rosa hybrida L.) using modified MS salt medium complemented with BAP 2.0 mg/l and NAA 0.1 mg/l. Rooting of the shoots was reported on one quarter strength MS medium without hormones and the plantlets were transferred to soil and grew well in the greenhouse.

Hasegawa (1979) reported three-fold multiplication of shoots from shoot tip, and lateral buds of rose cultivar 'Improved Blaze' on MS medium supplemented with 3.0 mg/l BAP and 0.3 mg/l IAA after 3 weeks of culture. Roots could be initiated from 50 per cent of shoots after transfer to a medium containing 0.3 mg/l IAA alone or with 0.3 mg/l BAP. Regenerated plants were successfully transferred to soil after 2 weeks.

Hasegawa (1980) obtained six fold multiplication of shoots from shoot tip cultures of 'Improved Blaze' on a medium containing MS salt, thiamine HCl (0.5 mg/l), Pyridoxine HCl (0.5 mg/l), Nicotinic acid (0.05 mg/l),

glycine (2.0 mg/l), bacto - agar (8 g/l), IAA (0.3 mg/l) and BAP 1.0, 3.0, or 10.0 mg/l. No further increase in multiplication rate was obtained by extending the length of culture period. *In vitro* proliferated shoots cultured for 10 - 14 days on the MS medium without growth regulators initiated roots and could be transplanted to the soil. However, root formation and transplantability were enhanced by NAA (0.03 or 0.1 mg/l) or IAA (1.0 mg/l), and by lowering the MS salt concentration to one quarter or half strength.

A method for rapid micropropagation of seven rose cultivars by the stimulation of axillary bud was developed by Davies (1980). He obtained a multiplication rate of 3 - 5 in a 4 weeks period, over a series of subcultures. In addition to rooting *in vitro*, he achieved rooting of cultured shoots under standard greenhouse condition on transferring to 1:1 mixture of sterilized peat and perlite moistened with water or liquid MS medium without hormones.

Martin *et al.* (1980) reported large scale micro propagation of glasshouse roses, outdoor floribundas, miniature roses and a range of rootstocks, on a modified MS medium containing auxin, cytokinin and gibberellin in quantities varying with the clones. The micropropagated plants were compared for 3 years with the grafted plants and it was reported that micropropagated plants produced 10 per

cent more flowers during the first year and 20 per cent more in the second and third years.

Avramis *et al.* (1982 a,b) reported *in vitro* culture of rose root stock Rosa indica major. Nodes and shoot apices produced shoots on MS medium with 0.1 mg/l NAA. They also reported that increased rooting efficiency was obtained by pre treatment with NAA 0.5 mg/l and /or sucrose at 6 per cent before planting in an 1:1 peat vermiculate mixure.

Bressan *et al.* (1982) studied the factors affecting *in vitro* propagation of rose cultivars 'Golden Glow' and 'Improved Blaze', and observed that, the node position from which axillary buds were isolated markedly affected their growth and development. Buds nearest to and farthest from the apex either failed to develop, or took the longest time to develop. They also reported that plants which initiated roots at 16,21 or 26°C had the highest level of transplant survival and root initiation was adversely affected by the length of time the cultures were maintained in the multiplication medium prior to transfer to rooting medium.

Hyndman (1982) found that lowering the total mineral salt level in nutrient medium provided a more favourable nitrogen salt concentration for rhizogenesis than that provided by MS salt formulation. The number and length

of roots in *in vitro* derived shoots of cv. 'Improved Blaze' increased as the concentration of total nitrogen in the MS salt formulation was reduced from 60 mM to 7.5 mM.

Pittet and Mancousin (1982) conducted *in vitro* propagation studies in rose cultivar 'Joyfulness' and found that IBA at 0.01mg/l and BAP at 0.5 mg/l were the best for the establishment phase of lateral buds while for shoot proliferation IBA at 0.1 mg/l and BAP at 0.5 mg/l were found to be better. They had also obtained 100 per cent rooting by dipping the shoots in NAA 1.0 mg/l for 1 hour before planting them in perlite.

Micropropagation comparisons were made between two Rosa hybrida cv. 'Tropicana' and 'Bridal Pink' and two old world spp. R. canina and R. damascena Mill by Khosh-Khui and Sink (1982 a). They observed variation in growth regulator requirement and rate of multiplication not only between two Rosa hybrida cvs. but also between the old world spp. Khosh-Khui and Sink (1982 c) studied the effect of combinations of auxin sources and concentrations, temperature shift, light intensity and light reduction on shoot tip cultures of Rosa hybrida L. 'Bridal Pink' in relation to root formation. They reported that IBA alone did not stimulate rooting while IAA and NAA singly and in combinations with them were more effective in stimulating rooting. An additive effect on

rooting existed between NAA and IAA in many of the concentrations tested. Reducing light intensities to 1.0 k lux or lower and incubating the cultures for 1 week at 5°C, helped to enhance rooting.

Aldrufeu *et al.* (1983) reported the rooting efficiency of *in vitro* plantlets of Rosa cv. 'Rufa' on different substrates added to the medium. With the culture medium containing sacchrose, the percentage of rooted plants was 100 per cent in cellulose, sand and vermiculate and 80 per cent in perlite and peat (TKS.1). However, there was variation in the number and size of the roots grown in each substrate.

Preil and Meier Dinkel (1983) suggested that dormant axillary buds are more suitable for *in vitro* culture than shoot tips and they root in 2 weeks. Bini *et al.* (1983), in their studies on multiplicatin of Rosa indica major using axillary bud explants observed that BAP and zeatin gave the greatest increase in multiplication coefficient, but zeatin imparted a chlorotic and glassy appearance to the explant.

Barve *et al.* (1984) reported an effective method for rapid propagation of rose cvs. 'Crimson Glory' and 'Glenfiditch' from axillary vegetative buds with 10 mm stem portions. Buds proliferated well on MS medium containing

kinetin at 0.2 mg/l + BAP 0.5 mg/l. Good rooting was obtained on proliferated shoots by lowering the MS concentration to half strength, and adding IAA, IBA and indole propionic acid (IPA), each at 0.5 mg/l.

Lauwaars (1984) reported successful *in vitro* propagation of 'Ilona' and 'Red Success' rose cultivars with axillary buds on MS medium supplemented with sucrose 4 per cent, BAP 2 mg/l and IAA 0.004 mg/l. Cai *et al.* (1984) reported rapid propagation of clones of china rose (Rosa chinensis) using axillary bud explants. The cultivars showed differences in the proliferation rate and growth of shoots.

Lefering (1985) reported that highest percentage of success was obtained in rose cultivars 'Montrea', 'Disco', 'Madelon' and 'Ilona' using nodal sections with dormant bud that had just coloured, but the position of the bud on the shoot and the season of propagation also influenced success. The shoots were directly rooted on rock wool or compost under plastic cover in the glass house following a 2 hours soak in IBA solution.

Sauer *et al.* (1985) tested the suitability for *in vitro* propagation of six tetraploid rose cultivars viz., 'Gabriella', 'Lorena', '8151-1', 'Ilseta', 'Mercedes', 'Duftwolke' and three diploid lines viz., 79/81-18,

80/240-1, 83/299-1 on MS medium containing 0.1 ppm NAA and 2 ppm BAP, and found that the number of plantlets regenerated were 278 and 1472 respectively. The productivity increased during subsequent subcultures. Micro propagated shoots of all cultivars and lines rooted in 9-14 days in one third strength MS medium containing 2 ppm IAA.

Curir *et al.* (1985) reported successful *in vitro* propagation of rose cultivars 'Bellona', 'Bingo', 'Candia', 'Cocktail-80' and 'Sonia'. Bud proliferation was achieved using modified MS medium with thiamine 2 mg/l, myoinositol 100 mg/l and rooting was promoted by IAA 0.8 mg/l with low sucrose. Three days of culture in presence of activated charcoal followed by transfer to the fresh medium was found highly effective in enhancing growth of primary explant.

Alekhno and Vysotskii (1986,87) reported that by growing micro-cuttings in a horizontal position during shoot proliferation, the propagation coefficient can be doubled, compared with growing in vertical position. They also observed more than doubled propagation coefficient compared to solid medium, when a combined solid/liquid medium comprising a 7-8 mm solid layer with MS salts, 0.5 mg/l thiamine, 0.5 mg/l inositol, 0.2 to 1.0 mg/l BAP, 30 g/l sugar and 15-20 g agar/l, covered with a liquid medium of same composition without agar. They explained that the improved results were due to better accessibility of active

substance from the liquid and a large contact area of the explants with the medium.

Mederos and Rodriguez (1987) studied the factors affecting shoot tip and axillary bud growth and morphogenesis in *in vitro* propagation of rose cv. 'Golden Times'. They observed that the type and age of the explant, its position on the mother plant, and the physiological stages of the same had an influence on the multiplication rate of roses *in vitro*. They found that shoots growing from herbaceous stem at vegetative stage developed better than those from the corky and older stems. Buds located at the middle of the stem grew best, and presence of small petiole fragments inhibited the development of their adjacent axillary buds. They also observed that development of shoot was best when the buds were taken at the flowering stage.

Alderson *et al.* (1988) observed that the rooting of cultured shoots of rose cvs 'Dainty Dinah', 'Crimson Rosamini' and 'Dicjana' was influenced by temperature during multiplication and rooting phases and by auxin (IBA) in the rooting medium. They found that cellulose moistened with liquid medium can be used successfully as the rooting medium. They also reported that inclusion of fungicide in the liquid medium was essential for the control of fungi which caused

breakdown of the cellulose rods and were detrimental to the growth of young plants during establishment in compost.

The factors influencing acclimatization of Rosa hybrida plants, multiplied *in vitro* were studied by Podwyszynska and Hempel (1988). They found that shoots of all rose cultivars formed on the media with smaller concentration of BAP (0.25 and 0.5 mg/l) rooted and acclimatized better than shoots from the media with higher concentration of BAP (1.0 and 2.0 mg/l). They also observed that absence of IAA in the rooting medium significantly improved plantlet acclimatization.

Dubois *et al.* (1988) compared the plant habit, growth and development of 36 dwarf rose cvs., propagated *in vitro* and by cuttings, and found that plants from *in vitro* material flowered earlier, had shorter shoots, fewer and shorter internodes and more number of longer laterals than plants from cuttings.

Li (1988) reported that the rooting efficiency of 1-aminobenzo triazole (ABT) was higher than NAA and IBA. He found that 86.7 per cent shoots derived from bud explants produced roots in the presence of ABT 2 mg/l.

Rout *et al.* (1989a) reported *in vitro* mass scale multiplication of Rosa hybrida cv. Landora using dormant

axillary bud explants. The number of shoots produced per explant was the highest on the medium containing 0.5 mg/l BAP, and 0.25 mg/l GA_3 and best rooting was observed in about 8 days in medium containing 0.25 mg/l of NAA and 0.1 mg/l 2, 4-D. Rout *et al.* (1989b) observed shoot multiplication from axillary buds of Rosa hybrida L. cv. 'Queen Elizabeth' using MS medium supplemented with BAP 0.1 mg/l. Micropropagated shoots were easily rooted in half strength MS liquid medium supplemented with 0.25 mg/l of NAA. Rooted microshoots were successfully acclimatized, and more than 70 per cent survived upon transfer to potted soil.

Rout *et al.* (1990) compared the shoot multiplication response of six Rosa hybrida L. cultivars viz., ~Landora', 'Queen Elizabeth', 'Happiness' 'Virgo', 'Sea Pearl', 'Super Star' and thornless root stock Rosa multiflora. They found that all the cultivars and thornless rootstock exhibited axillary bud proliferation on MS medium supplemented with BAP, GA_3, NAA, IAA at varying concentrations and combinations. The cultivars and species showed considerable variation with respect to growth regular requirement and rate of multiplication.

Campos and Salome (1990) reported mass propagation of dwarf rose cultivar 'Rosamini' using shoot tip and axillary bud explants. Multiplication rates of six to seven fold were reached every 4 weeks. Multiplication rate

decreased with prolonged maintenance of shoots without subculture. They also observed that flowerbud break occured in early stage after transfer of rooted plant to pots and occasionally occured in culture vessels.

Bhat (1992) reported shoot proliferation of Rosa hybrida L. in a medium supplemented with NAA 0.1 mg/l and GA_3 0.5mg/l. The maximum rooting efficiency was obtained in a medium supplemented with IBA 0.5 mg/l. He also found out that for successful *ex vitro* establishment the *in vitro* grown plantlets required a constant incubation at 70-80 per cent relative humidity for seven days and then it can be reduced to 50 per cent for the subsequent three days.

2.3. INDUCED MUTATION ADOPTING *IN VITRO* CULTURE

Induced mutation in biotechnology has great potential in contributing to crop improvement by introducing new variation and thereby widening the genetic base. Plant cells can be exposed to physical and chemical mutagens for inducing variability followed by selecting desirable mutants.

Bajaj (1971) based on a study of direct and indirect effect of gamma irradiation on the seeds, seedlings, callus tissue cultures, excised roots, ovules and embryos has

observed that, callus tissue cultures are more radio-resistant than intact seedlings.

In two cvs. of Chrysanthemum, Mabuchi and Kuwada (1975) reported that, gamma irradiaton of shoot tip cultures resulted in the production of plants healthy enough to transplant. The higher the radiation dose the lesser was the survival rate. A few of the plants that survived the winter were those irradiated with a dose less than 20 kR.

An effective chemical mutagenesis procedure for Petunia hybrida cell suspension cultures was reported by Coljin *et al.* (1979). Among the various chemical mutagens tested Nitroso guanidine was the most effective one.

Johnson (1980) using gamma irradiation achieved *in vitro* induced separation of chimeral genotypes in carnation (Dianthus caryophyllus) from meristem cultures and macerated shoot tip cultures.

Roest *et al.* (1980) irradiated the detached leaves of two genotypes of Begonia hiemalis with different doses of X-rays and when the leaf disc explants were cultured *in vitro* about 30 per cent of plantlets produced after two cycles were mutants with respect to colour, size and form of leaves and flowers. Majority of the mutants (98.5 per cent) were found to be non-chimeric.

Unrooted cuttings, callus and suspension cultures of five Chrysanthemum clones were irradiated with gamma rays at 1.2 - 1.8 kR (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.

Sunnio *et al.* (1984, 1986) proposed a procedure for *in vitro* mutation breeding of potato. Two hundred and thirtyfive plants obtained from buds of cultivar 'Desiree' cultured on modified MS medium were irradiated with 3kR of gamma rays and single node pieces were subcultured twice. After about 40 days, vM_1 plants were cut into single node pieces and transferred to fresh medium. Among the 1094 plants established, 158 mutations were detected; 36 of leaf size and shape, 39 of leaf colour (dark green, pale green), 24 of flower colour, (white or dark purple) 1 of flower shape (exerted style), 7 of anthocyanin deficient stems, 5 of dwarf type and 46 of tuber skin colour (yellow, dark purple or spotted). Of 102 mutants, 78 were apparently homogeneous while 24 appeared to be chimeric.

Duron and Decourtye, (1986) reported that when Weigela cv 'Bristol Ruby' cultured *in vitro* was gamma irradiated with doses 20-60 gray (1 Gray = 100 rads) bud

survival, rhizogenesis and cutting growth were found to be affected at doses 30 Gy and few buds survived at 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.

Axillary shoots from *in vitro* derived microshoots of two lines of Gerbera (A26 and 82/19/16) were irradiated with X-ray doses between 10 and 25 Gy (Walther and Sauer, 1986c). During 16 weeks of post-irradiation culture, the radiosensitivity was estimated based on the 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 four subsequent dates. They observed that higher X-ray doses resulted in greater inhibition of shoot generation and radiation induced damage was higher in A26 than in 82/19/16.

Kleffel *et al.* (1986) obtained homozygous 'whwh' mutants in Poinsettia, by X-irradiation (10-60 Gy) of immobilized embryonic cells heterozygous for anthocyanin synthesis (Whwh). Mutation rates increased with increasing X-ray doses reaching 8.9 per cent at 60 Gy, but the survival rates decreased with increasing doses.

Tissue cultures derived from flowering buds of Arctostaphylos were cultivated in the dark on MS medium

supplemented with 10 mg/l IBA and 1.0 mg/l kinetin. The cultures were gamma irradiated in their 10th to 18th subculture five times at an interval of four weeks with doses of 2.5 to 5 Gy or with doses between 2.5 to 160 Gy. Compared with the controls, the growth of the irradiated cultures decreased with increasing radiation doses. The highest dose (160 Gy) was lethal to the calli. None of the radiation treatments induced embryogenesis. The number of very large cells in the calli increased with increasing radiation doses (Duskova *et al.* 1988)

2.3.1. Induced Mutation in Rose Adopting *In vitro* Culture

Walther and sauer (1986 a) observed that tetraploid rose cultivars responded in a different manner to X-ray irradiation of *in vitro* derived microshoots. Based on their studies with six tetraploid rose cultivars, it was suggested that radiosensitivity of any cultivar can be estimated by determining the survival rate of explants, the productivity of axillary shoots and inhibition of shoot development on the first day of cut off.

A broad spectrum of variability was induced by applying X-ray doses between 25 and 60 Gy to basal segments of *in vitro* derived microshoots of rose cultivar 'Ilseta' followed by repeated cutting of axillary shoots from treated

mother plants. The mutation comprised of 73 per cent flower mutants with variation in size, colour, and number of petals, 14 per cent with altered growth and 13 per cent with modified leaves. They also observed that a period of about 9 months was required to select the mutants ready for grafting into rootstock.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation on induced mutagenesis in rose under *in vivo* and *in vitro* culture was carried out at the Department of Agricultural Botany and the Tissue Culture Laboratory attached to the Department of Horticulture, College of Agriculture, Vellayani, during the period from 1989 to 1993.

3.1. INDUCED MUTATION ADOPTING *IN VIVO* CULTURE

Three rose cultivars (plates 1 to 3) namely - 'Alliance' (white), 'Suraga'(pink) and 'Folklore'(light red) belonging to the hybrid tea group were selected for induced mutagenesis adopting *in vivo* culture technique.

3.1.1. Collection of materials

The material for irradiation was the budwoods collected at three different stages viz., on the day of flower opening and three and six days after flower opening. Buds were collected only from healthy shoots. Budwoods of eight to ten cm length having three to five dormant buds were used for irradiation. While collecting budwoods, three buds immediately below the flower were discarded. The dormancy of the selected buds were six days.

CULTIVARS (HYBRID TEAS) SELECTED FOR INDUCED MUTAGENESIS

PLATE 1. cv. ALLIANCE

PLATE 2. cv. SURAGA

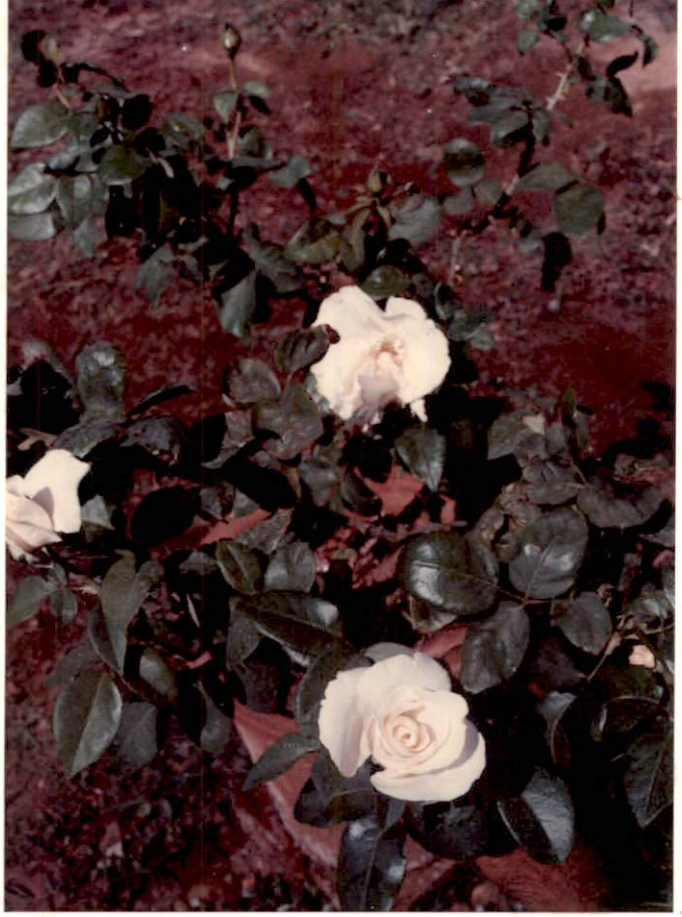


PLATE 3. cv. FOLKLORE

3.1.2. Treatment with gamma rays

The budwoods of the three different stages were grouped into six uniform lots, each having a minimum of 30 dormant buds suitable for budding. Each lot of buds was exposed to ^{60}Co gamma irradiation at doses of 0, 20, 30, 40, 50 and 60 GY at 0.1296 mR/hour at the gamma cell source available at the Radiotracer Laboratory, Kerala Agricultural University, Vellanikkara.

3.1.3. Method of budding

On the second day of irradiation the treated buds and control were separated and T-budded on rooted rootstocks raised in ploythene bags of 20 x 15 cm. Two months after establishment, they were transferred to earthen pots of 12 inches diameter and 14 inches height. Uniform cultural and management practices were adopted during the entire growth period. The genotype of the rootstock used is Rosa multiflora.

3.1.4. Experimental design and layout

A 3 x 3 x 6 factorial experiment with factors viz., varieties, bud stages and gamma rays was layed out in RBD with two replications. Each treatment contained 15 potted plants. As there was no budtake at 60 Gy, this treatment was omitted from the analysis of the data. One cultivar (cv. Folklore) was selected for *in vitro*

mutagenesis. A 5 x 5 factorial experiment was layed out in RBD with factors viz., bud stages and gamma rays.

3.1.5. Observation

In order to determine the effect of gamma rays on rose at different growth stages, observations were recorded from five plants selected at random from each treatment in vM_1 generation. The observations on flower characters were recorded on the basis of ten flowers per treatment.

3.1.5.1. Days to bud take

Observation on days to bud take was recorded at fortnightly intervals from the second to the tenth fortnight. The initial growth of buds (0.5 cm) was taken as the successful index of budtake. The buds remaining dormant or without activation were not considered.

3.1.5.2. Percentage of sprouting

The total number of buds sprouted per treatment was counted to calculate the percentage of sprouting. All the sprouted buds were taken into account irrespective of their survival.

3.1.5.3. Percentage of survival

The total number of plants that survived six months after budding was recorded for each treatment to calculate the percentage of survival.

3.1.5.4. Effective dose (ED_{50})

The dose of gamma rays to get 50 per cent survival of plants (ED_{50}) six months after budding was calculated. The relationship between survival percentage (Y) and gamma irradiation (X) is described by a modified exponential function of the form

$$Y = K + Ab^X$$

where, Y = survival percentage

X = the dose.

The survival percentage with respect to treatments were adjusted for the control survival percentage using Abbett's formulae (Finney, 1971).

3.1.5.5. Plant height (cm)

Plant height was measured from the bud union to the tip of the main shoot, six months after budding.

3.1.5.6. Primary branches per plant

The number of primary branches per plant was recorded one year after budding.

3.1.5.7. Prickle density

The number of well developed prickles was counted over a length of 10 cm in the middle portion of the stem and the prickle density expressed as the number of prickles per cm of shoot length.

3.1.5.8. Leaves per plant

The total number of leaves in the plant six months after budding was taken as leaves per plant.

3.1.5.9. Abnormalities in leaves and growth habit

The plants were regularly observed for recording the abnormalities in leaves and growth pattern. The plants showing variations in leaf size, shape or chlorophyll distribution were compared with the untreated ones with normal leaves.

3.1.5.10. Days taken to flowering

The days taken to flowering from the date of budding were recorded from 5 plants per treatment and the means worked out.

3.1.5.11. Neck length (cm)

The length from the terminal node to the base of the flower bud was measured and recorded as neck length.

3.1.5.12. Flower diameter (cm)

The diameter of ten fully opened flower was measured and the mean recorded. The largest diameter of each flower was taken for calculating the mean flower diameter.

3.1.5.13. Flower weight (g)

Fresh flower weight was taken on the day of flower opening immediately after excision to prevent water loss from the flowers.

3.1.5.14. Petal weight (g)

Fresh petals from each flower were weighed for calculating the mean petal weight of flower and expressed in g.

3.1.5.15. Petals per flower

The number of petals was counted from ten flowers in each treatment and the mean was calculated.

3.1.5.16. Flowers per plant

The total number of flowers produced in five sample plants of each treatment was recorded separately for a period of one year and the means calculated.

3.1.5.17. Abnormalities in flowers

Observations were made on flowers to assess induced

abnormalities if any, on variation in colour, shape, size or number of petals. Changes in flower colour were compared using Munsell soil charts (1954).

3.1.6. Observations in vM_2 generation

Plants or branches showing variation in the first vegetative generation were marked and buds from such branches were again budded on stock plants to observe their performance in vM_2 generation.

3.2. IN VITRO CULTURE OF ROSE

In general three main modes of *in vitro* propagation are practiced namely enhanced release of axillary buds, somatic organogenesis and somatic embryogenesis. In the present study also all these three methods were attempted to identify the most suitable and viable method for *in vitro* mutagenesis in rose.

3.2.1. Raising Mother plants

Budded plants of rose variety 'Folklore' were raised in large sized pots of 12" diameter and 16" height. A potting mixture consisting of soil, sand and cattle manure in 1:1:1 proportion was used for raising the plants. The plants were watered twice daily and sprayed with systemic fungicide (benlate, 0.1 per cent) at fortnightly intervals. However,

the explants were collected one week after spraying to reduce contamination subsequently in culture. The insect pests were controlled with nuvacron (0.05 per cent spray). Care was taken to maintain the plants in perfect healthy condition as far as possible.

3.2.2. Explant choice

The explants tried for the enhanced release of buds were shoot tip and axillary / lateral buds. Callus induction and subsequent somatic organogenesis and embryogenesis were tried with internodal segments and leaf discs. The internodal segments were collected from young healthy vegetative shoots after removing the shoot tip. The leaf discs were collected from young leaves on the day of its full opening. All the explants were immersed in distilled water immediately after collection and washed thoroughly in distilled water 3-5 times prior to surface sterilization.

3.2.3. Surface sterilization

Explants collected from field grown plants harboured a lot of fungi and microbes. Hence they were subjected to surface sterilization to avoid contamination in the medium. Surface sterilization was standardised using mercuric chloride as the sterilant with different concentrations and time intervals (Table 1).

Table 1. Trials on surface sterilization for different explants using mercuric chloride.

Explant	Concentration (per cent)			Duration (Minutes)		
Shoot tip	0.06	0.08	0.10	6	12	18
Axillary bud	0.06	0.08	0.10	6	12	18
Internodal segment	0.06	0.08	0.10	6	12	18
Leaf disc	0.06	0.08	0.10	6	12	18

The surface sterilization procedures were carried out under perfect aseptic conditions in a laminar air flow cabinet. After surface sterilization, the explants were immersed in sterile distilled water and subjected to intermittent shaking for five minutes and the procedure was repeated thrice to remove the sterilant thoroughly. The ends of the explants were sharply cut and removed using sterile surgical blade to avoid any chance of dead tissue due to surface sterilization. The explants were then inoculated into culture tubes containing 15ml of Murashige and Skoog

(MS) semi-solid medium (Table 2). The pH of the medium was adjusted to 5.8. The tubes were incubated at 26 ± 2^{o} and 70 per cent relative humidity under white fluorescent light, approximately 3000 lux intensity at 10/14 hour light-dark regime.

The percentage of explants which remained healthy and green without any contamination, after two weeks was recorded and this formed the measure to assess the effect of different treatments.

3.2.4. Size and type of explant

Inorder to fix the optimum size of different explants, a trial was also conducted using shoot tips and axillary buds of varying size. The experiment for enhanced release of axillary buds was tested with shoot tip and axillary buds and for callus induction, internodal segments and leaf discs were tried (Table 3).

Table 2. Composition of MS medium used for tissue culture of rose

Ingredients	Amount (mg/l)
KNO_3	1900.000
NH_4NO_3	1650.000
$KH_2PO_4.H_2O$	170.000
$MgSO_4.7H_2O$	370.000
$CaCl_2.2H_2O$	440.000
Micronutrients	
H_3BO_3	6.200
$MnSO_4.4H_2O$	22.300
$ZnSO_4.7H_2O$	8.600
$NaMoO_4.2H_2O$	0.250
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
KI	0.830
$FeSO_4.7H_2O$	27.800
Na_2EDTA	37.300
Vitamins	
Thiamine. HCl	0.10
Pyridoxine.HCl	0.50
Nicotinic acid	0.05
Others	
Glycine	2.00
Myo-inositol	100.00
Sucrose	30.00*
Agar	7.00*

* - g/l. Murashige and skoog (1962)

Table 3. Trials on size of the explants for *in vitro* culture of rose

Sl.No.	Method of multiplication	Type of the explant	Size of the explant (cm)
1.	Enhanced release of axillary bud	Shoot tip	0.5 and 1.0
2.	,,	Axillary bud	0.5 and 1.0
3.	Callus induction	Internodal segment	0.5 and 1.0
4.	,,	Leaf disc	
		(1) with petiole	1.0
		(2) Without petiole	1.0

The fully opened young leaves were collected from vegetative shoots and cut into small pieces of about 1cm, with and without petiole portion. The shoot tip explants of 0.5 and 1.0cm length and single noded axillary bud explants measuring 0.5 and 1.0 cm length were excised. The explants were washed thoroughly 3 to 5 times in distilled water prior to surface sterilization.

The shoot tip and axillary bud explants were surface sterilised at 0.08 per cent mercurie chloride for 12

minutes while internodal segments and leaf discs were sterilised at 0.06 per cent mercuric chloride for twelve minutes, based on the best performance in the preliminary experiment conducted.

The explants for the enhanced release of axillary buds were then inoculated into culture tubes containing 15 ml of basal MS medium supplemented with 2, 4 dichlorophenoxy acetic acid (2, 4-D) at 1.0 mg/l and the explants for callus induction were cultured on basal MS medium supplemented with 0.5 mg/l Benzyl amino purine (BAP), 1.0 mg/l Naphthalene acetic acid (NAA) and 0.5 mg/l 2, 4-D (Rout *et al.*, 1989).

Observations were recorded on the number of explants showing bud activation and establishment. This was assessed as the percentage of shoot tip and axillary bud explants showing an initial growth of about 1 cm three weeks after culture and the percentage of internodal segments and leaf disc explants which initiated callus growth four weeks after culture.

3.2.5. Stage of the explant

The most suitable stage of the axillary bud explant for *in vitro* culture establishment was standardised by conducting a trial with axillary buds collected at six defined physiological stages of the plant, viz., vegetative

shoot, flower bud stage, day of flower opening and two days, four days and six days after the flower opening.

The explants were collected from healthy shoots and single noded explants measuring 1.0 cm length were taken for inoculation. Axillary buds at the terminal and basal regions of the shoots were discarded. Six lots of axillary buds at the six different stages as explained above were excised and surface sterilised. The buds were then inoculated into culture tubes. The following observations were recorded to assess their comparative performance.

3.2.5.1 Days to bud break

The days taken for the buds to swell up and to separate the outer bud sheath were taken as the days to bud break. The number of days taken by each explant was recorded separately.

3.2.5.2. Bud break percentage

The number of explants showing bud break after three weeks of culturing was scored for each treatment and the average was worked out in percentage.

3.2.5.3. Shoot length

The shoot length was measured three weeks after culture.

3.2.6 Standardisation of medium for initial culture establishment

To find out the most suitable hormone supplements for initial culture establishment, a trial was conducted with BAP, 2,4-D, NAA and Gibberellic acid (GA_3) at different levels and their combinations, as presented in table. 4.

Table 4. Trials on initial culture establishment with different hormone supplements

Basal medium	Explant	Treatment combinations
MS	Axillary bud	1. BAP 4 levels (1.0,1.5,2.0 & 2.5 mg/l + 2,4-D 4 levels (0.25,0.50,0.75 & 1.0 mg/l)
		2. BAP 4 levels (1.0,1.5,2.0 & 2.5 mg/l) + NAA 4 levels (0.25,0.50,0.75 & 1.0mg/l)
		3. BAP 4 levels (1.0,1.5,2.0 & 2.5 mg/l) + GA_3 4 levels (0.5,1.0,1.5 & 2.0mg/l)

Standard procedure (Gamborg and Shyluck, 1981) was followed for the preparation of the media. Stock solutions of major and minor nutrients were prepared separately by dissolving the requisite quantity of the chemicals in double

glass distilled water and stored under refrigerated conditions. The stock solutions of nutrients were prepared fresh every four weeks and that of vitamins, amino acids and phytohormones were prepared fresh. Sucrose, inositol and calcium chloride were also added fresh at the time of preparation of medium.

The pH of the medium was adjusted to 5.8 before adding agar at 7.0 g/l. The solution was then melted in a water bath maintained at 90-95^{o} C. After melting agar, 15 ml of medium was dispensed to each culture tube which were autoclaved for 20 minutes at 15 psi pressure and 121^{o}C temperature.

The inoculation of the explants was done under perfect aseptic conditions in a laminar air flow cabinet to compare the efficacy of different treatments. The following observations were recorded to find out the most suitable hormone supplement for culture establishment.

3.2.6.1 Days to bud break

The days taken for the buds to swell up and to separate the outer bud sheath were taken as the days to bud break. The number of days taken by each treatment was recorded separately.

3.2.6.2 Bud break percentage

The number of explants showing bud break after three weeks of culturing was scored for each treatment and the percentage was worked out.

3.2.7 Standardisation of medium for shoot proliferation

In order to standardise the suitable hormone supplements for early and enhanced release of axillary buds a trial was conducted with four levels (0.5, 1.0, 1.5, and 2.0 mg/l) of BAP/kinetin alone and in combination with four levels of GA_3 (0.25, 0.50, 0.75 and 1.00 mg/l).

The explants used for this trial were the shoots measuring 1.5 - 2.0 cm. At the time of subculture the stem portion of the original explants was removed and only the elongated shoots were taken for the second stage of culture establishment. In the second stage, the cultures were maintained for a period of eight weeks and the following observations were recorded.

3.2.7.1 Days to initiate multiple shoot

The number of days taken from the date of subculture to initiate the multiple shoot form the days to multiple shoot formation and this was recorded.

3.2.7.2 Percentage of cultures with multiple shoot

Out of the total number cultured, number of cultures with multiple shoots was recorded upto eight weeks from the date of subculture and it was then expressed in percentage.

3.2.7.3 Number of healthy shoots per culture

The number of healthy and fairly elongated shoots per culture having approximately 3.0 cm and above length was scored at the end of eight weeks.

3.2.7.4 Shoots per culture

The number of shoots produced per culture was counted and the average number of shoots per culture calculated.

3.2.8 Standardisation of medium for *in vitro* rooting

Cultured shoots *in vitro* were not of uniform size. Two to four shoots per culture were fairly elongated and had a growth of 3.0 cm or above. Shoots of uniform growth (3.0 cm) alone were separated from the clump and utilized for root induction. Very small shoots were again subcultured in the proliferation medium for further growth.

Auxins, Indole acetic acid (IAA) and NAA at 0.5, 1.0, 1.5 and 2.0 mg/l alone and in combination and the same ~~concentration of each~~ in combination with 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/l) were attempted (Table 5). Observations on the following parameters were recorded.

3.2.8.1 Days to root initiation

The number of days taken for root initiation was recorded for each combination and the average for each treatment was worked out.

3.2.8.2 Rooting percentage

Out of the total number of shoots cultured, percentage of shoots showing root initiation was calculated.

Table 5. Trials on *in vitro* rooting of cultured shoots with different auxin supplements

Basal medium	Auxin supplements (mg/l)
MS	Without hormones
MS	IAA (0.5,1.0,1.5 & 2.0)
MS	NAA (0.5,1.0,1.5 & 2.0)
MS	IAA (0.5,1.0,1.5 & 2.0) + 2,4D(0.5,1.0,1.5 & 2.0)
MS	NAA (0.5,1.0,1.5 & 2.0) + 2,4D(0.5,1.0,1.5 & 2.0)
MS	IAA (0.5,1.0,1.5 & 2.0) + NAA(0.5,1.0,1.5 & 2.0)

3.2.8.3 Number of roots per shoot

The number of roots produced per shoot was recorded four weeks after culture and the mean was worked out.

3.2.8.4 Length of longest root

The length of longest root was measured in cm four weeks after culture for each treatment in each culture and the mean was worked out.

3.2.9 Effect of activated charcoal on *in vitro* rooting

In order to study the effect of activated charcoal on improving the rooting efficiency of cultured shoots an experiment was conducted by selecting four best combinations and supplementing them with activated charcoal. The different treatments were:

Basal medium	Treatments		
	IAA mg/l	NAA mg/l	Activated charcoal (mg/l)
MS	1.0	0.5	500
	1.0	0.5	1000
	1.0	1.0	500
	1.0	1.0	1000
	1.0	1.5	500
	1.0	1.5	1000
	1.0	2.0	500
	1.0	2.0	1000

The rooting efficiency was assessed by observing all the parameters as in the previous trials.

3.2.10 *Ex vitro* Establishment

The rooted plants having 5 to 10 roots at 3 to 4 weeks in the rooting medium were used to assess the *ex vitro* establishment.

3.2.10.1 Hardening and acclimatisation

The rooted plantlets were taken out without injury, from the culture vessels using forceps and put in a beaker containing distilled water and shaken thoroughly to remove adhering pieces of medium. Plantlets were planted out first in small plastic containers of 7.5 x 6.0 cm, containing sterilized sand. These plants, kept in shade, failed to establish after three days and gradually dried up.

3.2.10.2. Standardisation of potting media for planting out

In order to improve the hardening and acclimatization of rooted plantlets and to identify the growth stage at which plants are to be removed from culture media a trial was conducted with planting out at different stages (two, three and four weeks after transferring the shoots to rooting media) and six potting media viz., (i) sand, (ii) soilrite, (iii) vermiculate, (iv) sand :

soilrite (1:1), (v) sand : vermiculate (1:1) (vi) sand : peatmoss (1 : 1).

All the potting media were sterilized before use. The plantlets were kept in regulated humidity and temperature under perforated polythene cover. The plants were given fine intermittant water spray at 3 hour intervals to maintain temperature and humidity. Observations on survival rate at weekly interval were recorded for one month.

3.2.10.3 Nutrient requirement for *ex vitro* establishment

The *in vitro* plants after planting out showed very poor and slow growth rate when grown in river sand or any other potting media, without the supply of nutrients. Hence a trial was conducted with nutrient solutions of different concentrations to improve the growth and *ex vitro* establishment. Since river sand was found to be good for initial planting out, sterilized river sand alone was used in this trial. The frequencies and nutrient solutions tried were: (i) 5 ml water at 3 days interval (ii) liquid MS 1/10 sterngth (5 ml) at 3 days interval, (iii) liquid MS half strength (5 ml) at 3 days interval and (iv) liquid MS full strength (5 ml) at 3 days interval, all as basal application.

The plantlets were given additional water spray (mist) twice daily in the morning and evening and the plants

were covered with polythene cover and kept in the culture room for the first 3 days under fluorescent light at 3000 lux. The efficacy of nutrients supplied were tested based on :

1. Days taken for the first leaf to open in the potting media
2. Days taken for the second leaf to open
3. Shoot length three weeks after planting out

3.2.11 Effect of mycorrhizae on *ex vitro* establishment

An experiment was conducted to study the effect of vesicular arbuscular mycorrhizal colonisation on the growth and survival of plantlets. The plantlets were inoculated with three species of vesicular arbuscular mycorrhizae (VAM) viz., Glomus etunicatum, G. fasciculatum and G. constrictum at the time of panting out. The plantlets were inoculated with surface sterilised spores of above fungi obtained from the Department of Plant Pathology, College of Agriculture, Vellayani. The suspension containing 50 VAM spores were mixed with top 2 cm layers sand to which the plantlets were planted out.

3.2.12. Somatic organogenesis/embryogenesis

Induction of callus, callus multiplication and subsequent organogenesis/embryogenesis of calli were

attempted using internodal segments of 0.5 cm and leaf discs with a portion of petiole (1 cm) as explants.

3.2.13. Standardisation of medium

The details of different treatments tried to induce and multiply callus and their somatic organogenesis / embryogenesis are given in Table 6. The following observations were taken on callus induction and proliferation.

3.2.13.1 Percentage of callus induction

Eight weeks after culture, the number of cultures with calli were recorded for each treatment and the percentage of induction calculated.

3.2.13.2. Callus growth (G)

Growth of the calli was assessed based on visual rating (score 1 = less than 2 mm, score 2 = 2 to 4 mm, score 3 = 4 to 6 mm and score 4 = above 4 mm) eight weeks after culture. The mean score was expressed as growth score (G).

3.2.13.3 Callus index (CI)

The callus index (CI) was computed by multiplying

the percentage of explants initiated callus with the growth score (G).

Table 6. Trials on callus induction, callus multiplication, somatic organogenesis and somatic embryogenesis

Sl. No.	Observations	Explant	Treatments (mg/l)
1.	Callus induction	1.Leaf disc	BAP (0.5,1.0,1.5)+ NAA (0.5,1.0,1.5)
		2.Internodal segments	2, 4-D (0.5,1.0, 1.5)
2.	Multiplication of callus and Somatic organogenesis	Callus	1.BAP(0.5,1.0)+NAA(0.1,0.2)+ Ascorbic acid (5,10)
			2.BAP (0.5,1.0),NAA(2.0,2.5)+ 2, 4-D (0.5,1.0)
3.	Somatic embryo-genesis	Callus	1.BAP(0.5,1.0)+NAA(2.0,2.5)+ 2, 4-D (0.5,1.0)
			2.BAP(0.5,1.0),NAA(1.0,2.0)+ Ascorbic acid (100,150)+ Glutamine (500,600)

Somatic organogenesis/embryogenesis were tried with callus pieces of 0.5 cm diameter and above separated from the mother callus obtained from leaf disc and internodal explants.

The calli were subcultured three times, every eight weeks in fresh medium to induce further growth of callus. The sample cultures of each treatment were maintained for a period of six months and the cultures showing morphogenesis were recorded.

3.3. INDUCED MUTATION ADOPTING *IN VITRO* CULTURE

The experiments conducted on *in vitro* culture of rose using two explants namely shoot tip and axillary bud revealed that, axillary bud explant was the most successful one as it provided maximum release of shoots in the medium. Based on this observation, induced mutation in culture media was cunducted using axillary buds as explant materials. Axillary buds were collected at five different active stages of growth viz., from vegetative shoot, on the day of flower opening and two, four and six days after flower opening. The multiple shoots induced in the culture medium were also treated with the mutagen.

The mutagen used for inducing mutation in all the cases was 60 Cobalt (^{60}Co) gamma rays. Irradiation was done using the gamma cell source available at Radio Tracer Laboratory, Kerala Agricultural University Head Quarters, Vellanikkara.

3.3.1. Irradiation of axillary buds

The budwoods of uniform size (5 to 8 cm) each having 3 to 4 axillary buds at the five different growth stages were irradiated at doses of 20, 30, 40 and 50 gray (Gy) (1 Gray = 100 rads) units at 0.12 mR/hr. Thirty buds each were treated for each exposure. On the second day of irradiation, the irradiated buds and the control were inoculated into culture tubes containing 15 ml of the standardised initial culture medium. The contamination rate ranged from 10 to 30 per cent in different treatments. Because of this, observations were recorded only from 20 contamination-free cultures.

3.3.2. Effect of gamma rays on culture establishment

The direct effect of mutagen on *in vitro* culture establishment was studied by taking the following observations.

3.3.2.1. Bud break percentage

The number of explants showing bud break after three weeks of culturing was scored for each treatment and the percentage worked out.

3.3.2.2. Survival percentage

The survival rate of explants was scored three weeks after culture and the percentage was calculated.

3.3.2.3. Effective dose (ED_{50})

The effective dose of gamma rays to get 50 per cent survival of explants (ED_{50}) three weeks after culture was calculated in the data obtained when the explants collected six days after flower opening were used for the purpose.

The relationship between survival percentage (Y) and gamma irradiation (X) is described by a modified exponential function of the form

$$Y = K + Ab^X$$

where, Y = survival percentage

X = the dose.

The survival percentage with respect to treatments were adjusted for the control survival percentage using Abbett's formulae (Finney, 1971).

3.3.2.4. Morphological abnormalities (per cent)

The malformations induced by mutagen were scored three weeks after culture.

3.3.2.5. Days to bud break

The days taken for the buds to swell up and to separate from the outer bud sheath were taken as the days to bud break. The number of days taken by each explant was scored separately.

3.3.2.6. Days taken for the first leaf emergence

The number of days taken for emergence of first leaf was recorded for each culture and the mean calculated.

3.3.3. Effect of gamma rays on shoot proliferation

The cultured shoots were transferred to the proliferation medium after three weeks. The following observations were recorded to study the treatment effect on induction of proliferation.

3.3.3.1. Days to multiple shoot initiation

The number of days taken from the date of subculture to the developement of the mulitple shoots form the days to multiple shoot formation and this was recorded.

3.3.3.2. Percentage of cultures with multiple shoots

Out of the total number cultured, the number of cultures with multiple shoots were recorded upto eight weeks

from the date of subculture and was then expressed as percentage.

3.3.3.3. Shoots per culture

The number of shoots produced per culture was counted and the average number of shoots per culture was calculated.

3.3.3.4. Growth of main shoot at 2 weeks' interval

The length of main shoot of the multiple shoot was measured at two weeks' intervals for two months and the average worked out.

3.3.3.5. Growth of side shoot at 2 weeks' interval

The length of side shoot of the multiple shoot was measured at two weeks intervals for two months and the average worked out.

3.3.3.6. Number of leaflets at two weeks' interval

The number of leaflets produced by the multiple shoot was counted at two weeks' intervals for two months to work out the average.

3.3.3.7. Morphological variations

The number of cultures showing morphological variations in leaf or growth pattern was recorded for each treatment and the average worked out.

3.3.4. Effect of gamma rays on *in vitro* rooting

The elongated shoots from the multiple shoots were separated at each stage of subculturing and inoculated in root induction medium. The observations on the effect of *in vitro* rooting recorded from treated and control population were as follows.

3.3.4.1. Days to root initiation

The number of days taken for root initiation was recorded for each treatment and the average worked out.

3.3.4.2. Rooting percentage

Out of the total number of shoot cultures number of shoots showing root initiation was recorded and the percentage worked out.

3.3.4.3. Number of roots per shoot

The number of roots produced per shoot was reocrded four weeks after culture and the mean was worked out.

3.3.4.4. Length of longest root

The length of longest root was measured four weeks after culture for each treatment and the average was worked out.

3.3.5. Irradiation of microshoots

The multiple shoots having 4-8 shoots in the proliferation medium were subjected to gamma irradiation along with the culture vessels at doses of 10,20,30 and 40 gray at 0.093 mR/hr. On the second day of irradiation, the multiple shoots along with the untreated ones were subcultured in proliferation medium. The following observations were recorded to assess the effect of gamma irradiation.

3.3.5.1. Survival rate of explants four weeks after culture

The survival rate of explants four weeks after culture was recorded and expressed in percentage.

3.3.5.2. Days to multiple shoot initiation

The number of days taken from the date of subculture to the initiation of multiple shoots forms the days to multiple shoot formation and this was recorded.

3.3.5.3. Multiple shoot induction percentage

Out of the total number of shoots cultured, the number of cultures with multiple shoots were recorded upto eight weeks from the date of subculture and expressed as percentage.

3.3.5.4. Morphological variations

The percentage of cultures showing morphological variations was recorded for each treatment, eight weeks after culture.

3.3.5.5. Shoot production efficiency in subsequent stages of subcultures

The number of shoots produced per culture was counted at three stages of subculuring and the data were compared.

3.3.5.6. Rooting efficiency of gamma irradiated microshoots.

The rooting efficiency of shoots after gamma irradiation of the multiple shoots was assessed by recording observations on days taken for root initiation, rooting percentage, number of roots per shoot and length of the longest root as in the previous trial.

3.3.6. Statistical analysis

The data generated from various experiments were subjected to analysis of variance (Panse and Sukhatme, 1978). Angular transformation was done before analysis of the data wherever necessary.

RESULTS

4. RESULTS

The results of the present investigation on "Induced mutagenesis in rose under *in vivo* and *in vitro* culture" conducted at the Department of Agricultural Botany and at the Tissue Culture Laboratory attached to the Department of Horticulture are presented below in this chapter.

4.1. INDUCED MUTATION IN ROSE ADOPTING *IN VIVO* CULTURE

The experimental results of gamma ray irradiation of budwoods of three rose cultivars viz., Alliance (V_1), Suraga (V_2) and Folkore (V_3) at doses 0 (D_0), 20 Gy (D_1), 30 Gy (D_2), 40 Gy (D_3), 50 Gy (D_4) and 60 Gy (D5) at three different bud stages viz., on the day of flower opening (B_1), three days after flower opening (B_2) and six days after flower opening (B_3) are presented here under.

At highest dose of 60 Gy (D_5), none of the buds sprouted. At 50 Gy (D_4) the sprouting percentage was very low to provide sufficient number of plants for taking observations, hence it was included only in the analysis of bud take, sprouting and survival and excluded from other parameters studied. The mean values on sprouting, survival and morphological characters are presented in appendix (I - XIII).

vM_1 generation

4.1.1. Time taken for bud take

The observations on time for bud take are presented in Table 7. Time taken for bud take showed variation with respect to cultivars, budstages and doses of gamma rays. Bud take commenced from second fortnight irrespective of bud stages in control population of all the cultivars. In the second fortnight the highest bud take (46.67 per cent) was recorded in V_3 B_3 at 20 Gy gamma radiation followed by the untreated population of the same cultivar and bud stage. At highest dose of 50 Gy, no bud take was noticed in V_1B_1, V_1B_2, V_1B_3, V_3B_1 and V_3B_2. Bud take at 50 Gy was comparatively very low on the second fortnight and ranged from 3.34 to 6.67 per cent. The highest bud take of 6.67 per cent under 50 Gy on the second fortnight was recorded in V_2B_1 and V_2B_3.

In general, control and lowest dose of 20 Gy had the highest bud take in the second fortnight. In $V_1B_1D_4$ the bud take commenced only from the fifth fortnight. Gamma irradiation at higher doses reduced the bud take irrespective of bud stages. The same trend was observed in time of completion of bud take also. In control group, it took 4 to 9 fortnights, whereas in 20 Gy treatment it took 5 to 9, in 30 Gy 7 to 9, in 40 Gy 8 to 9 and in 50 Gy it took upto 10 fortnights. After tenth fortnight, there was no bud take at all.

Table. 7. Effect of gamma irradiation of budwoods of rose cvs. of different stages on time of bud take.

Cultivar (V)	Bud stage (B)	Gamma ray (D)	Bud take at fortnightly intervals (%)								
			2	3	4	5	6	7	8	9	10
Alliance (V_1)	B_1	D_0	23.34	43.34	60.00	70.00	80.00	83.34	83.34	83.34	83.34
		D_1	10.00	30.00	36.67	53.34	56.67	76.67	76.67	76.67	76.67
		D_2	6.67	23.34	36.67	40.00	46.67	50.00	50.00	50.00	50.00
		D_3	0.00	10.00	20.00	23.24	30.00	36.67	40.00	43.34	43.34
		D_4	0.00	0.00	0.00	10.00	16.67	16.67	20.00	23.34	23.34
	B_2	D_0	16.67	43.34	60.00	70.00	73.34	73.34	73.34	73.34	73.34
		D_1	20.00	53.34	63.34	70.00	73.34	76.67	76.67	76.67	76.67
		D_2	3.34	30.00	36.67	36.67	43.34	50.00	56.67	60.00	60.00
		D_3	0.00	13.34	26.67	26.67	33.34	33.34	40.00	43.34	43.37
		D_4	0.00	6.67	6.67	13.34	13.34	16.67	16.67	20.00	20.00
	B_3	D_0	23.34	46.67	56.67	63.34	80.00	80.00	80.00	80.00	80.00
		D_1	23.34	36.67	43.34	60.00	73.34	83.34	83.34	83.34	83.34
		D_2	16.67	16.67	23.34	43.34	53.34	56.67	60.00	63.34	63.34
		D_3	3.34	6.67	13.34	13.34	20.00	23.34	26.67	33.34	33.34
		D_4	0.00	6.67	6.67	6.67	13.34	13.34	13.34	13.34	13.34
Suraga (V_2)	B_1	D_0	13.34	40.00	46.67	60.00	60.00	63.34	66.67	70.00	70.00
		D_1	20.00	40.00	46.67	56.67	60.00	63.34	70.00	73.34	73.34
		D_2	3.34	10.00	26.67	30.00	30.00	36.67	43.34	46.67	46.67
		D_3	10.00	10.00	20.00	20.00	23.34	26.67	33.34	40.00	40.00
		D_4	6.67	6.67	13.34	13.34	16.67	16.67	16.67	16.67	16.67
	B_2	D_0	30.00	50.00	70.00	83.34	83.34	83.34	83.34	83.34	83.34
		D_1	26.67	43.34	66.67	76.67	76.67	80.00	80.00	80.00	80.00
		D_2	26.67	30.00	43.34	56.67	60.00	60.00	60.00	60.00	60.00
		D_3	3.34	10.00	20.00	26.67	30.00	30.00	36.67	36.67	36.67
		D_4	3.34	3.34	6.67	6.67	6.67	6.67	6.67	6.67	6.67
	B_3	D_0	30.00	53.34	66.67	73.34	73.34	73.34	73.34	73.34	73.34
		D_1	40.00	56.67	70.00	73.34	73.34	73.34	73.34	73.34	73.34
		D_2	10.00	26.67	33.34	33.34	40.00	40.00	40.00	43.34	43.34
		D_3	6.67	13.34	20.00	23.34	23.34	30.00	30.00	36.67	36.67
		D_4	6.67	10.00	13.34	13.34	13.34	23.34	26.67	30.00	33.34
Folklore (V_3)	B_1	D_0	26.67	53.34	63.34	66.67	66.67	66.67	66.67	66.67	66.67
		D_1	36.67	50.00	56.67	63.34	73.34	73.34	73.34	73.34	73.34
		D_2	16.67	33.34	40.00	46.67	50.00	56.67	56.67	56.67	56.67
		D_3	3.34	10.00	20.00	26.67	30.00	36.67	43.34	43.34	43.34
		D_4	0.00	13.34	13.34	16.67	20.00	26.67	26.67	26.67	26.67
	B_2	D_0	30.00	70.00	83.34	90.00	90.00	90.00	90.00	90.00	90.00
		D_1	30.00	56.67	66.67	83.34	86.67	86.67	86.67	86.67	86.67
		D_2	20.00	20.00	46.67	53.34	60.00	63.34	63.34	63.34	63.34
		D_3	13.34	23.34	26.67	33.34	36.67	40.00	46.67	50.00	50.00
		D_4	0.00	6.67	10.00	13.34	13.34	20.00	20.00	20.00	23.34
	B_3	D_0	43.34	66.67	80.00	80.00	80.00	80.00	80.00	80.00	80.00
		D_1	46.67	60.00	80.00	83.34	83.34	83.34	83.34	83.34	83.34
		D_2	20.00	36.67	46.67	50.00	50.00	56.67	60.00	60.00	60.00
		D_3	3.34	20.00	23.34	30.00	30.00	40.00	46.67	46.67	46.67
		D_4	3.34	10.00	10.00	20.00	20.00	20.00	23.34	26.67	26.67

B_1 Day of flower opening
B_2 Three days after flower opening
B_3 Six days after flower opening

D_0 Control
D_1 20 Gy
D_2 30 Gy
D_3 40 Gy
D_4 50 Gy

4.1.2. Sprouting

Analysis of the data (Table 8) showed no significant variation on interaction among cultivars, budstages and gamma ray exposures on sprouting. The effect of cultivars and gamma rays were found to have significant influence on sprouting. On comparing the different cultivars, it was found that the cultivar V_3 was significantly superior to the other two (V_1 and V_2) varieties with respect to sprouting. Comparison of the main effect of Gamma rays on sprouting revealed that the 20 Gy exposure (62.80 per cent) and control population (62.45) were significantly superior and on par. At increasing exposures of gamma rays, the sprouting percentage was significantly reduced and was the lowest in the 50 Gy exposure (27.64 per cent).

4.1.3. Survival

Statistical analysis of the data (Table 9) revealed no significant interaction among cultivars, bud stages and gamma ray exposures. However, the main effect of cultivars and gamma rays showed significant differences with respect to survival. The mean values for cultivars ranged from 41.26 to 46.01 per cent. The highest survival was recorded by V_3 (46.01 per cent) which was significantly superior to V_1 and V_2.

Table 8. Interaction effect among cultivars, bud stages and gamma rays on sprouting* (%)

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	D_4	Mean
V_1	55.94 (48.41)	54.83 (47.77)	55.76 (48.31)	79.28 (62.92)	79.28 (62.92)	57.80 (49.49)	39.86 (39.15)	19.70 (26.35)	55.50 (48.16)
V_2	49.20 (44.54)	55.03 (47.89)	52.05 (46.18)	54.60 (66.88)	76.17 (60.78)	49.93 (44.96)	37.68 (37.87)	19.94 (26.52)	52.09 (46.20)
V_3	53.71 (47.13)	64.30 (53.31)	60.21 (50.89)	80.16 (63.55)	81.78 (64.73)	60.12 (50.84)	46.62 (43.06)	25.06 (30.04)	59.44 (50.44)
Mean	52.95 (46.69)	58.10 (49.66)	56.02 (48.46)	78.61 (62.45)	79.11 (62.80)	55.97 (48.43)	41.35 (40.02)	21.52 (27.64)	
D_0	73.95 (59.31)	83.37 (65.93)	78.11 (62.11)						
D_1	75.23 (60.15)	81.66 (64.64)	80.26 (63.62)						
D_2	51.05 (45.60)	61.26 (51.48)	55.57 (48.20)						
D_3	42.14 (40.48)	43.25 (41.12)	38.72 (38.48)						
D_4	21.90 (27.90)	18.01 (25.11)	24.85 (29.90)						

CD V = 3.36

D = 4.34

* The transformed values (angles) are given in paranthesis

Table 9. Interaction effect among cultivars, bud stages and gamma rays on survival* (%)

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	D_4	Mean
V_1	44.83 (42.03)	42.71 (40.81)	42.95 (40.95)	66.74 (54.78)	66.77 (54.80)	44.25 (41.70)	29.77 (33.07)	13.96 (21.94)	43.49 (41.26)
V_2	42.90 (40.92)	50.03 (45.02)	40.79 (39.69)	70.43 (57.06)	69.05 (56.20)	44.29 (41.71)	32.11 (34.52)	11.59 (19.90)	44.57 (41.88)
V_3	47.80 (43.74)	54.57 (47.62)	52.90 (46.66)	78.68 (62.48)	75.81 (60.54)	48.81 (44.32)	36.57 (37.21)	18.52 (25.49)	51.76 (46.01)
Mean	45.17 (42.23)	49.09 (44.48)	45.54 (42.43)	72.08 (58.10)	70.62 (57.18)	45.78 (42.58)	32.80 (34.94)	14.57 (22.44)	
D_0	66.87 (54.86)	78.49 (62.37)	70.48 (57.09)						
D_1	69.10 (56.23)	72.62 (58.45)	70.11 (56.86)						
D_2	40.91 (39.76)	51.08 (45.62)	45.38 (42.35)						
D_3	34.35 (35.88)	34.32 (35.86)	29.77 (33.07)						
D_4	17.08 (24.41)	11.83 (20.12)	15.02 (22.80)						

CD V = 3.02

D = 3.90

* The transformed values (angles) are given in paranthesis

The survival percentage ranged from 22.44 to 58.10 per cent in the different treatments. The control population recorded the highest survival of 58.10 per cent followed by 20 Gy treatment while these two were on par with each other. A significant reduction in survival was noticed with increasing doses of gamma rays. At the highest dose of 50 Gy, the survival percentage was reduced to 22.44 per cent.

4.1.4. Effective dose (ED_{50})

Dose (Gy)	Observed survival (%)	Adjusted survival (%)
0	72.08	100
20	70.62	98
30	45.78	64
40	32.80	46
50	14.57	20

$$Y = K + Ab^X$$

where Y is the survival percentage and X is the dose.

$$Y = 104.5 - 40.5 \times 1.444^X$$

For 50 per cent survival, the expected dose = 38 Gy.

4.1.5. Plant height (cm)

The analysis of the data (table 10) revealed that the interaction among cultivars, bud stages and gamma rays, (VxBxD) was not significant. Varietal interaction to bud stage (VxB) was also not significant. However, the interaction between cultivars and gamma ray exposures (V x D) and bud stages and gamma ray exposures (B x D) were significant. The mean values for interaction between bud stages and gamma rays ranged from 18.87 to 38.10 cm. The highest value was recorded in B_3D_0 (38.10 cm) followed by B_2D_1 (37.72 cm). The value for B_2D_1 was 36.17 cm and that of B_2D_0 35.63 cm. These four treatments were significantly superior and were on par with each other.

The interaction effect of cultivar x gamma rays (BxD) ranged from 17.65 cm in V_2D_3 to 46.53 cm in V_3D_1. The treatments V_3D_1 (46.53 cm) and V_3D_0 (44.67 cm) were on par and recorded significant interactions in all other treatment combinations.

The main effect of bud stages was not significant. However, it was significant in the case of cultivar and gamma rays. The main effect of cultivar on plant height ranged from 27.44 to 37.30 cm. The cultivar V_3 was significantly superior to V_1 and V_2. The effect of gamma rays showed significant difference between mean values. The control

Table 10. Interaction effect among cultivars, bud stages and gamma rays on plant height (cm)

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	28.60	26.99	26.73	30.87	30.08	27.53	21.27	27.44
V_2	24.76	25.63	27.48	31.57	31.27	23.33	17.65	25.95
V_3	36.65	37.45	37.80	44.67	46.53	34.13	22.87	37.30
Mean	30.00	30.02	30.67	36.03	35.03	28.33	20.59	
D_0	34.37	35.63	38.10					
D_1	34.00	37.22	36.17					
D_2	29.40	26.03	29.53					
D_3	22.22	20.70	18.87					

CD V = 1.82 V x D = 3.64

D = 2.10 B x D = 3.64

population had the maximum plant height of 36.03 cm followed by 35.96 cm in 20 Gy which were on par and was significantly superior to all other exposures.

4.1.6. Primary branches

The statistical analysis of the data (table 11) showed no significant interaction in VxBxD. The interaction of cultivars, with bud stages and also with gamma rays were found to be significant. The varietal variation and different exposures of gamma rays also showed significant difference.

The mean values for VxB interaction ranged from 1.75 to 2.40. The interaction between V_3B_3 (2.40) and V_3B_2 (2.30) were on par and found to be significantly superior to all other treatments. Mean values of interaction between cultivars and gamma ray exposures ranged from 1.30 to 3.03. The treatments V_3D_0 (3.03) and V_3D_1 (2.93) produced the highest numbers of primary branches, which were significantly superior to all other treatments.

The interaction effect of bud stages and gamma rays on primary branches ranged from 1.23 (B_3D_3) to 2.57 (B_3D_0). The most effective combination was found to be B_3D_0 (2.57), followed by B_1D_0 (2.40), B_2D_0 (2.27), B_2D_1 (1.83) and (B_2D_2) (1.83). These treatments were significantly superior and on par.

Table 11. Interaction effect among cultivars, bud stages and gamma rays on number of primary branches per plant

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	1.75	1.85	1.83	2.20	2.17	1.57	1.30	1.81
V_2	2.08	1.88	1.85	2.57	2.13	1.73	1.30	1.93
V_3	2.13	2.30	2.40	3.03	2.93	1.77	1.37	2.28
Mean	1.98	2.01	2.03	2.60	2.40	1.69	1.32	
D_0	2.40	2.27	2.57					
D_1	1.67	1.83	1.57					
D_2	1.67	1.83	1.57					
D_3	1.37	1.37	1.23					

CD V = 0.12 V x D = 0.21

D = 0.14 B x D = 0.24

The main effect of cultivars on primary branches varied significantly. The cultivar V_3 produced the highest number of primary branches and was significantly superior to V_2 and V_1. In respect to the main effect of gamma rays, the control group registered the maximum number of primary branches, which was significantly superior to higher doses of irradiation at which a reduction in number of primary branches were noticed.

4.1.7. Prickle density

The analysis of the data (table 12) indicated that none of the interactions were significant. However, the main effect of cultivars and doses of gamma rays were significant.

The main effect of cultivars on prickle density ranged from 0.72 to 1.57. The prickle density was significantly low in V_3 compared to V_1 and V_2. The effect of gamma rays on prickle density showed a significant reduction at the highest dose of 40 Gy.

4.1.8. Number of leaves

The interaction effect (VxBxD) was found to be significant (Table 13). The mean values for the number of leaves recorded significant and wide variation. It ranged from 8.5 to 24.40. Higher number of leaves were recorded in

Table 12. Interaction effect among cultivars, bud stages and gamma rays on prickle density (no. per cm length)

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	1.57	1.56	1.58	1.58	1.58	1.58	1.53	1.57
V_2	0.97	1.00	1.00	1.01	1.01	1.00	0.94	0.99
V_3	0.76	0.75	0.66	0.76	0.74	0.76	0.62	0.72
Mean	1.10	1.10	1.10	1.12	1.11	1.11	1.03	
D_0	1.10	1.13	1.12					
D_1	1.11	1.13	1.10					
D_2	1.11	1.11	1.12	CD	V = 0.067 B = 0.083 D = 0.067			
D_3	1.08	1.03	0.98					

Significant at 5 per cent level

Table 13. Interaction effect among cultivars, bud stages and gamma rays number of leaves per plant

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	15.46	17.53	16.06	17.83	18.45	15.60	14.20	16.35
V_2	15.71	15.23	15.30	16.73	16.40	15.55	12.97	15.41
V_3	19.71	18.70	20.18	17.93	22.17	20.87	17.13	19.53
Mean	16.96	17.15	17.18	17.27	19.01	17.34	14.77	
D_0	19.27	15.07	17.48					
D_1	19.22	19.53	18.27					
D_2	16.26	18.27	17.50					
D_3	13.10	15.73	15.47					

CD V = 1.10 V x D = 2.19 B x D = 2.19

D = 1.26 V x B x D = 3.79

$V_3B_1D_0$ and was on par with eight other treatments. The interactions of BxD and VxD were also significant. The main effects of V and D were found to be significant. The mean values of BxD interaction ranged from 13.10 (B_1D_3) to 19.53 (B_2D_1). The mean values of VxD interaction effect ranged from 12.97 (V_2D_3) to 22.17 (V_3D_1). V_3D_1 (22.17) and V_3D_2 (20.87) interactions were significantly superior to other treatments.

4.1.9. Days to flowering

Analysis on the factor interaction (Table 14) revealed that none of the interaction effects were significant, whereas the main effects of V, B and D were significant. The variation due to bud stages ranged from 108.89 (B_2) to 111.78 (B_3). The variation due to gamma rays ranged from 98.68 (D_0) to 125.0 (D_4). Minimum days to flowering was taken in control and this was significantly superior to the gamma ray treatments. At higher doses, flowering was significantly delayed. The three cultivars tested showed significant variation for days to flowering (104.69 in V_1 to 111.98 in V_3). Significantly early flowering was observed in V_1. V_2 and V_3 were on par for days to flowering.

4.1.10. Neck length (cm)

Statistical analysis of the data (Table 15)

Table 14. Interaction between cultivars, bud stages and gamma rays on days to flowering

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	101.78	104.36	107.93	92.20	98.70	108.18	119.67	104.69
V_2	113.25	112.10	114.73	105.23	105.97	115.03	127.20	113.36
V_3	113.08	112.20	112.68	98.60	105.07	116.13	128.13	111.98
Mean	109.37	108.89	111.78	98.68	103.24	113.12	125.00	
D_0	98.30	98.17	99.57					
D_1	102.57	102.40	104.77					
D_2	111.08	110.63	117.63					
D_3	125.52	124.35	125.13					

CD V = 2.40
B = 2.40
D = 2.77

Table 15. Interaction effect among cultivars, bud stages and gamma rays on neck length (cm)

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	mean
V_1	4.81	5.13	5.31	5.443	4.80	5.28	4.82	5.08
V_2	4.13	4.21	4.43	4.37	4.40	4.28	3.97	4.25
V_3	6.29	6.20	5.89	6.42	6.42	6.18	5.47	6.12
Mean	5.08	5.18	5.20	5.41	5.21	5.25	4.75	
D_0	5.67	5.35	5.40					
D_1	4.88	5.38	5.35	CD	V = 0.32			
D_2	5.33	5.22	5.20		D = 0.37			
D_3	4.62	4.77	4.87					

revealed significant differences in the main effect of cultivars and gamma rays. The interaction between different factors were not significant. The cultivar V_3 (6.12 cm) was significantly superior to V_2 (4.25 cm) and V_1 (5.08 cm). Comparison of the different doses of gamma rays indicated that the control was significantly superior (5.41 cm) and on par with D_2 (5.25 cm) and D_1 (5.21 cm).

4.1.11. Flower diameter (cm)

Analysis of the data (Table 16) revealed significant interaction between cultivars and bud stages for flower diameter. Comparison of main effects revealed that the cultivar V_1 was significantly superior (7.34 cm) to V_2 and V_3. Among the different bud stages, B_1 was significantly superior (6.88 cm) and on par with B_2 (6.70 cm). The effect of gamma rays revealed that 20 Gy exposed population had the maximum flower diameter (7.27 cm) and was on par with control group (7.08 cm)

4.1.12. Flower weight (g)

The treatment means for interaction between cultivars bud stages and gamma rays persented in table 17 indicated that the interaction between cultivars and gamma rays was significant. The treatment combinations viz., V_3D_1

Table 16. Interection effect among cultivars, bud stages and gamma rays on flower diameter (cm)

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	7.10	7.38	7.55	7.72	7.88	7.53	6.23	7.34
V_2	6.45	6.19	5.88	6.62	6.83	6.23	5.00	6.17
V_3	7.10	6.52	6.22	6.92	7.08	6.58	5.88	6.62
Mean	6.88	6.70	6.55	7.08	7.27	6.78	5.71	
D_0	7.17	7.18	6.90					
D_1	7.43	7.27	3.03	CD	V = 0.21			
D_2	7.12	6.62	6.62		B = 0.21			
D_3	5.82	5.72	5.58		D = 0.24			
					V x B = 0.37			

Table 17. Interaction effect among cultivars, bud stages and gamma rays on flower weight (g)

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	3.95	3.96	4.03	4.08	4.17	4.06	3.61	3.98
V_2	5.15	4.91	5.03	5.25	5.32	5.12	4.43	5.03
V_3	6.15	6.01	6.13	6.33	6.42	6.23	5.42	6.10
Mean	5.08	4.96	5.06	5.22	5.30	5.14	4.48	
D_0	5.25	5.19	5.23					
D_1	5.35	5.23	5.32					
D_2	5.22	5.05	5.15					
D_3	4.52	4.38	4.55					

CD V = 0.09

B = 0.09

D = 0.11

V x B = 0.19

(6.42 g), V_3D_0 (6.33 g) were significantly superior and were on par with each other.

Analysis of the main effect of cultivars, bud stages and gamma rays revealed that the difference are significant.

The cultivar V_3 (6.10 g) was significantly superior to V_1 and V_2. The bud stage B_1 was significantly superior to B_2 and B_3 for flower weight. The 20 Gy treatment was significantly superior (5.30 g) and was on par with control population (5.22 g) for flower weight.

4.1.13. Petal weight

Analysis of the data presented in table 18 revealed that none of the interactions were significant. The main effect of cultivars and gamma rays was found to be significant. The cultivar V_3 (4.02 g) was found to be significantly superior over V_1 (2.64 g) and V_2 (3,45 g). The effects of gamma ray exposures of 20 Gy (3.47 g) and 30 Gy (3.40 g) were significant and on par with control (3.46 g). Significant reduction in petal weight was observed in the highest dose of 40 Gy.

4.1.14. Petal number

Statistical analysis of the data (table 19) on petal number revealed significant interaction among cultivars

Table 18. Interaction effect among cultivars, bud stages and gamma rays on petal weight (g)

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	2.68	2.65	2.61	2.70	2.73	2.65	2.48	2.64
V_2	3.45	3.44	3.48	3.56	3.57	3.51	3.19	3.45
V_3	4.11	3.90	4.05	4.11	4.10	4.04	3.83	4.02
Mean	3.41	3.33	3.38	3.46	3.47	3.40	3.16	
D_0	3.49	3.46	3.45					
D_1	3.52	3.45	3.45					
D_2	3.46	3.33	3.40	CD	V = .06			
D_3	3.18	3.08	3.23		D = .08			

Table 19. Interaction effect among cultivars, bud stages and gamma rays on petal number

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	15.28	15.36	14.80	15.87	15.95	14.80	13.97	15.15
V_2	29.93	28.21	27.76	29.20	29.62	27.77	27.95	28.63
V_3	30.94	27.10	27.93	27.50	27.43	29.02	30.67	28.65
Mean	25.38	23.56	23.50	24.19	24.33	23.86	24.19	
D_0	24.2	24.43	23.93	CD	V = 0.98			
D_1	25.18	23.63	24.18		B = 0.98			
D_2	24.95	23.62	23.06		V x B = 1.13			
D_3	27.18	22.55	22.85		V x D = 1.96			
					B x D = 1.96			

into bud stages, cultivar into gamma rays and bud stages into gamma rays. The combination V_3 B_1 (30.94) and V_2 B_1 (29.93) were on par and were significantly superior. The interaction between bud stages and gamma rays revealed that four treatments viz., V_3 D_3 (30.67), V_2 D_1 (29.62), V_2 D_0 (29.20) and V_3 D_3 (29.02) were on par and significantly superior to all the other treatment combinations.

The interaction between bud stages and gamma rays revealed that B_1D_3 was significantly superior to all other treatment combinations. The main effect of cultivars indicated that V_3 (28.65) and V_2 (28.63) were on par and significantly superior to V_1. The first bud stage (B_1) was significantly superior to B_2 and B_3 for petal number.

4.1.15. Flowers per plant

Analysis of the data revealed no significant interaction among cultivars, bud stages and gamma rays for number of flower per plant. The main effect of cultivars and gamma rays showed significant variation on flower production. The cultivar V_3 (9.34) was significantly superior to V_1 (7.33) and V_2 (6.38). Among the different doses of gamma rays 20 Gy gamma exposure was significantly superior (8.79) and was on par with control population (8.58). The higher doses of gamma rays showed significant reduction in number of flowers produced per plant.

Table 20. Interaction effect among cultivars, bud stages and gamma rays on number of flowers

	B_1	B_2	B_3	D_0	D_1	D_2	D_3	Mean
V_1	6.80	7.69	7.50	7.95	8.44	7.89	5.04	7.33
V_2	6.34	6.12	6.68	7.21	7.34	6.93	4.05	6.38
V_3	9.52	9.07	9.42	10.59	10.60	9.75	6.41	9.34
Mean	8.95	8.20	5.18	8.58	8.79	8.19	5.17	
D_0	8.30	8.31	9.13					
D_1	8.55	8.88	8.95	CD	V = 0.46			
D_2	8.28	8.09	8.20		D = 0.53			
D_3	5.08	5.24	5.18					

SE

4.1.16. Morphological abnormalities

4.1.16.1. Foliar variations

Gamma irradiation of budwoods induced different types of abnormalities in leaf especially during the early period of growth. Exposure of budwood collected at six days after flower opening at 40 Gy induced production of leaf lets with yellow chlorophyll deficient patches (plate 4), deformed and miniatured leaves showing reduced number of leaflets (plate 5).

In the cultivar Suraga, 30 Gy gamma rays produced leaves with reduced size and number of leaf lets (plate 6) when buds were collected on the day of flower opening and also at six days after flower opening.

4.1.15.2. Floral variations

A variant with light pink coloured (plate 7) was observed from 20 Gy treated population in cv. Alliance (V_1) against the normal white colour. The budlings established from the buds collected from this variant failed to show the carryover effect.

One flower colour mutant with reddish yellow colour was observed under 30 Gy exposure in Floklore (V_3) against the normal light red colour (plate 8 & 9). The budlings

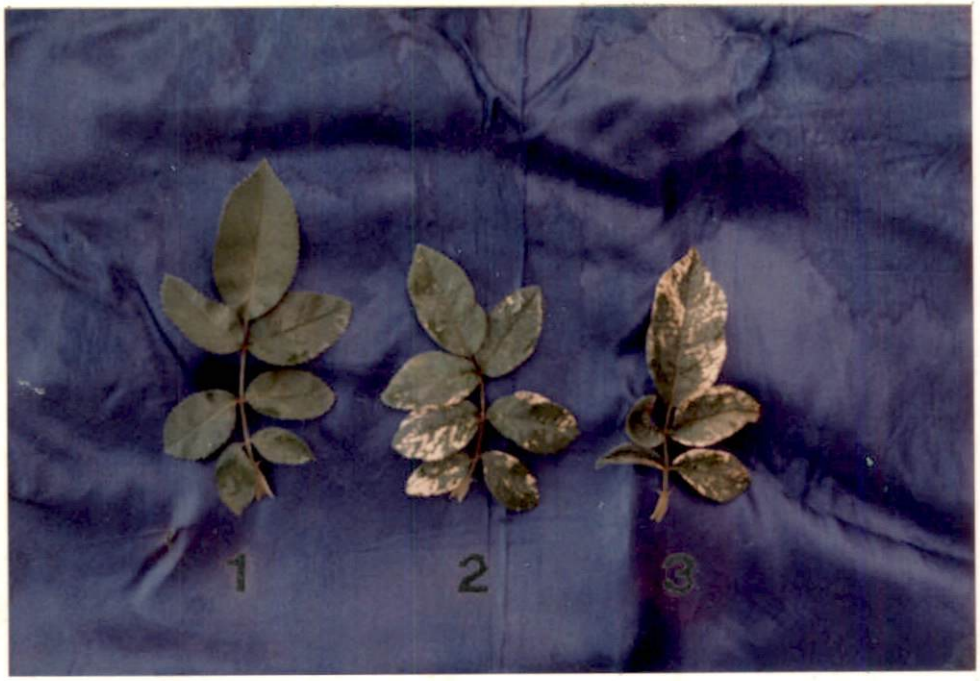
MORPHOLOGICAL VARIATIONS (FOLIAR)

PLATE 4. Chlorophyll variations (cv. Alliance)

1. Control
2 & 3. Leaflet showing yellow chlorophyll deficient patches

PLATE 5. Foliar deformities (cv. Alliance)

1. Control
2 & 3. Deformed leaves
4. Miniaturised leaf showing three leaflets only


1
2
3


1
2
3
4

PLATE 6. Foliar deformities (cv. Suraga)

1. Control
2 & 3. Leaves with reduced size and number of leaflets
4. Miniaturised leaf

MORPHOLOGICAL VARIATIONS (FLORAL)

PLATE 7. Variations in colour (cv. Alliance)

1. Variant with light pink colour
2. White flowered control


1
2
3
4

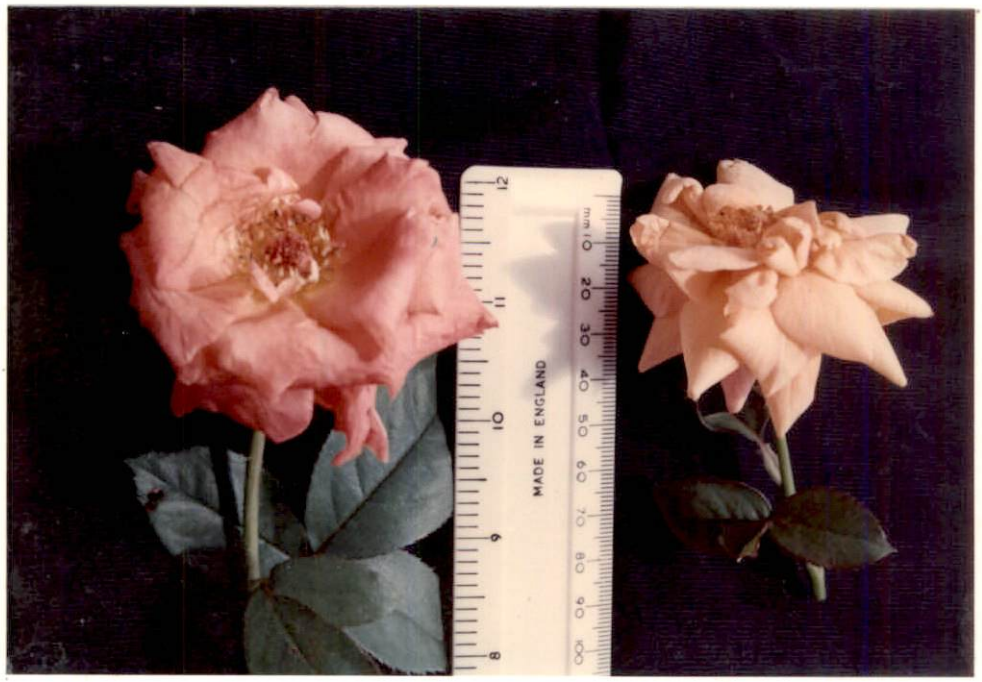

1
2

PLATE 8. Colour mutant (cv. Folklore)

1. Control — Light red
2. Mutant — Reddish yellow

PLATE 9

1. Separated petals of reddish yellow mutant
2. Control


MADE IN ENGLAND

established from this variant retained its altered flower colour. This important characteristic of the mutant are presented in table 21.

Table 21. Floral characters of the mutant obtained from cv. Folklore

Characters	cv. Folklore	Mutant of cv. Folklore
Colour	Light red	Reddish yellow
Diameter	6.60 cm	5.80 cm
Neck length	5.70 cm	5.10 cm
Flower weight	6.10 g	4.80 g
Petal weight	4.20 g	3.15 g
Petal number	22.00	23.50

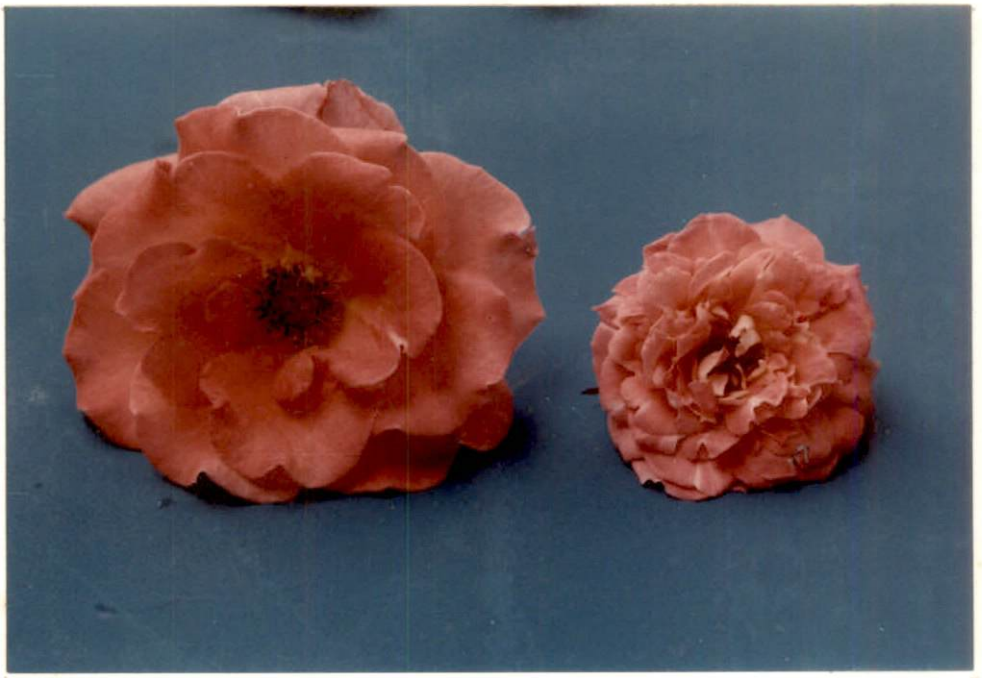
In 40 Gy exposed population of the same cv. Folklore, at B_1 bud stage one mutant having miniatured flower size with large number of petals (plate 10) compared to control was obtained. The budded plants from this particular variant failed to establish even after providing optimum conditions for budtake.

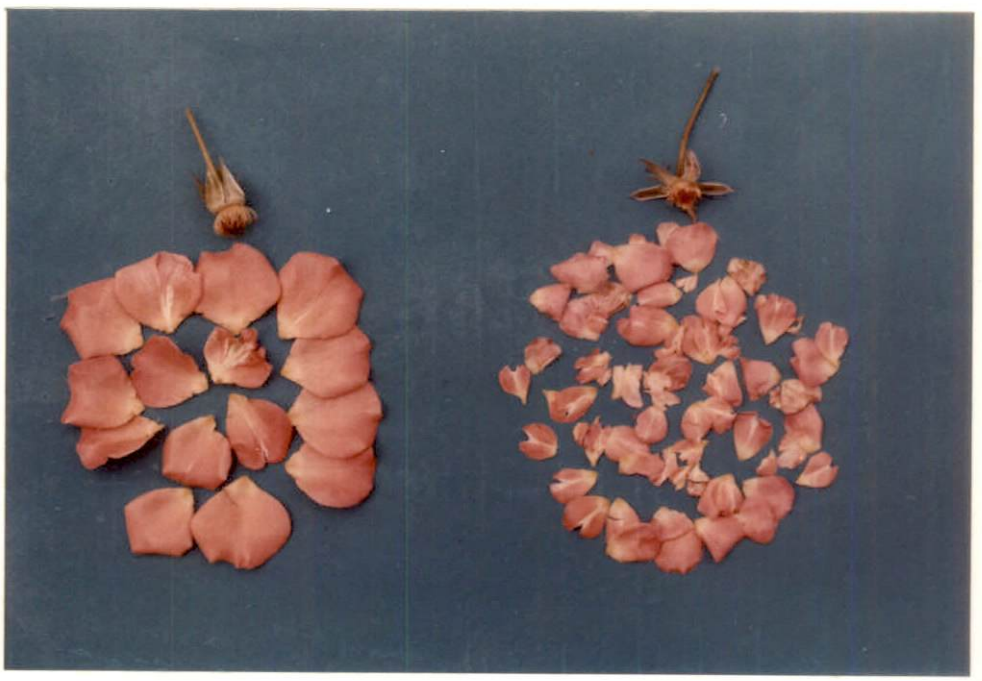
PLATE 10. Variation in size and number of petals (cv. Folklore)

1. Control (Large sized with few petals)
2. Mutant (Small sized with large number of petals)

PLATE 11. Separated petals

1. Control
2. Mutant





4.2. *IN VITRO* STUDIES

4.2.1. Surface sterilisation of the explant.

The results of the experiment conducted on surface sterilisation of shoot tip and axillary bud explants are presented in table 22 and internodal segments and leaf disc explants in table 23.

The results indicated that the treatment of mercuric chloride at 0.08 per cent for 12 minutes was the best showing highest survival of 83 percent for both shoot tip and axillary bud explants. At the highest concentration of 0.1 per cent tried for 18 minutes, none of the shoot tips survived but in the case of axillary buds there was 17 per cent survival.

With regard to internodal segments, a lower concentration of mercuric chloride (0.06 per cent for twelve minutes) gave the highest survival rate of 83 per cent. The treatment with 0.08 per cent for 18 minutes and 0.10 per cent for twelve minutes were on par and gave the same survival rate of 67 per cent. At the lowest concentration of 0.06 per cent, for six minutes all the explants showed contamination.

As far as the leaf disc explant was concerned, treatment of 0.10 per cent mercuric chloride for twelve minutes was the most effective treatment resulting in 67 per

explants-Shoot tip and axillary bud
Basal medium - MS.

Sl. No.	Sterilant	Explant	** Concentration(%)	Duration (Min)	Contamination(%)	* Survival (%)
1	Mercuric chloride	Shoot tip	0.06	6	83	17
2	"	"	0.06	12	50	50
3	"	"	0.06	18	33	67
4	"	"	0.08	6	50	50
5	"	"	0.08	12	17	83
6	"	"	0.08	18	33	67
7	"	"	0.10	6	33	67
8	"	"	0.10	12	83	17
9	"	"	0.10	18	100	0
10	"	Axillary bud	0.06	6	67	33
11	"	"	0.06	12	50	50
12	"	"	0.06	18	50	50
13	"	"	0.08	6	33	67
14	"	"	0.08	12	17	83
15	"	"	0.08	18	67	33
16	"	"	0.10	6	67	33
17	"	"	0.10	12	50	50
18	"	"	0.10	18	83	17

* Average of 6 observations ** Mercuric chloride on w/v basis
culture period - 15 days.

Table 23. Effect of surface sterilisation of the explants internodal segment and leaf disc (cv. Folklore) Basal medium - MS.

Sl. No.	Sterilant	Explant	** Concentration(%)	Duration (Min)	Contamination(%)	* Survival (%)
1	Mercuric chloride	Internodal segment	0.06	6	100	0
2	"	"	0.06	12	67	33
3	"	"	0.06	18	67	33
4	"	"	0.08	6	50	50
5	"	"	0.08	12	17	83
6	"	"	0.08	18	33	67
7	"	"	0.10	6	50	50
8	"	"	0.10	12	33	67
9	"	"	0.10	18	83	17
10	"	Leaf disc	0.06	6	100	0
11	"	"	0.06	12	83	17
12	"	"	0.06	18	67	33
13	"	"	0.08	6	83	17
14	"	"	0.08	12	83	17
15	"	"	0.08	18	67	33
16	"	"	0.10	6	50	50
17	"	"	0.10	12	33	67
18	"	"	0.10	18	83	17

* Average of 6 observations

** Mercuric chloride on w/v basis

Culture period - 15 days

cent survival. The explants did not survive in the treatment of 0.06 per cent for six minutes.

4.2.2. Size and type of the explant

The results of the experiment conducted to find out the most suitable size and type of the explants are presented in Table 24.

The results indicated that among the shoot tip explants of sizes 0.5 cm and 1.0 cm tried, best response of 50 per cent shoot elongation was obtained from shoot tips of 1.0 cm length. Among axillary bud explants, best response of 83 per cent shoot elongation resulted in axillary buds of 1.0 cm. The highest response of 75 per cent callus induction was noticed in the case of internodal segments of 0.5 cm size. Cent per cent of the explants with an intact portion of the petiole, responded to initial callusing in leaf disc explants. The callus formation initiated from the cut ends mainly from the petiole and mid rib region, was observed as small white globules which slowly spread and covered the entire explant.

Thus in all subsequent experiments for enhanced release of axillary buds, axillary buds of 1.0 cm length was used. For callus induction, internodal segments of 0.5 cm length and leaf disc with a portion of petiole intact were used.

Table 24. Standardisation of the size and type of explants on initiating shoot growth and callus induction (cv. Folklore)

Sl. No.	Observations	Type of explant	Size of explant (cm)	Culture* initiating shoot growth/callus (%)
1	Shoot growth	Shoot tip	0.5	33
2	Shoot growth	Shoot tip	1.0	50
3	Shoot growth	Axillary bud	0.5	67
4	Shoot growth	Axillary bud	1.0	83
5	Callus induction	Internodal segment	0.5	75
6	Callus induction	Internodal segment	1.0	50
7	Callus induction	Leaf disc with petiole	1.0	100
8	Callus induction	Leaf disc without petiole	1.0	67

* Average of 12 observations.

4.2.3. Stage of the explant

The effect of the different growth stages of the axillary bud explants on initial culture establishment was assessed based on days to bud break, bud break percentage and shoot length. The results are presented in table 25.

4.2.4. Days to bud break

The result showed significant variation among different growth stages of the axillary buds. Buds from shoots collected four days after flower opening showed early bud break within 4.44 days. This was significantly superior from the rest of the different physiological stages. Culturing after six days of flower opening took 5.38 days. Buds of vegetative shoots showed delayed bud break and took a maximum period of 8.93 days.

4.2.5. Percentage of bud break

The percentage of bud break ranged from 70 to 80. The explants from shoots at four and six days after flower opening recorded the highest percentage of bud break (80 per cent). The buds from vegetative shoots and two days after flower opening showed the lowest value of 70 per cent.

Table 25. Effect of bud stage on the *in vitro* culture establishment of rose (cv. Folklore)

Sl. No.	Treatment	Days to bud break	Bud break (%)	Shoot length (cm)
1.	Vegetative shoot	8.93(14)	70(20)	0.80(14)
2.	Flower bud stage	7.33(15)	75(20)	1.05(15)
3.	On the day of flower opening	7.13(15)	75(20)	1.42(15)
4.	2 days after flower opening	6.86(14)	70(20)	1.40(14)
5.	4 days after flower opening	4.44(16)	80(20)	1.95(16)
6.	6 days after flower opening	5.38(16)	80(20)	1.74(16)

Figures in paranthesis: number of replications Culture period: three weeks

4.2.6. Shoot length

The statistical analysis of the data showed significant variation among treatments. The buds from shoots collected four days after flower opening had the maximum shoot length of 1.95 cm, which was significantly superior to other bud stages. The buds from vegetative shoots recorded the lowest shoot length of 0.80 cm.

4.2.7. Standardisation of media for initial culture establishment

The results of the experiment conducted with auxins 2, 4-D or NAA at different concentrations in combination with cytokinin (BAP) and Gibberellin (GA_3) in combination with BAP on initial culture establishment are presented in Table 26. The effect of different treatments was assessed based on days to bud break and bud break percentage three weeks after culture.

4.2.7.1. Days to bud break

The number of days taken for bud break revealed significant difference with respect to different treatment combinations. The days to bud break ranged from 4 to 10 days. Among the different combinations, early bud break (four days) was observed in treatments containing BAP 2.5 mg/l + 2,

Table 26. Effect of different levels of cytokinin combination with auxin (2, 4-D or IAA) or GA_3 on initial culture establishment in rose (cv. Folklore)

Basal medium MS **Explant / axillary bud**

Sl. No.	Treatments				Days to bud break		Bud break percentage
	BAP (mg/l)	2, 4-D (mg/l)	NAA (mg/l)	GA_3 (mg/l)			
1	1.0	0.25	–	–	10.0	a	40
2	1.0	0.50	–	–	8.5	cdefghi	40
3	1.0	0.75	–	–	8.0	dehijklmnopqr	60
4	1.0	1.00	–	–	7.5	iklmnopqrstuvw	40
5	1.5	0.25	–	–	9.0	abcd	40
6	1.5	0.50	–	–	7.0	qrtvwxyzABCDE	60
7	1.5	0.75	–	–	7.3	opqrstuvwxyzA	70
8	1.5	1.00	–	–	7.3	opqrstuvwxyz	60
9	2.0	0.25	–	–	8.0	dehijklmnopqrs	50
10	2.0	0.50	–	–	7.0	qrtvwxyzABC	60
11	2.0	0.75	–	–	6.0	BDEFJKLM	60
12	2.0	1.00	–	–	5.0	JKLMNO	80
13	2.5	0.25	–	–	6.0	BCDEFGHJ	60
14	2.5	0.50	–	–	4.0	QRSTU	80
15	2.5	0.75	–	–	4.2	QRST	60
16	2.5	1.00	–	–	5.0	JLNQR	60
17	1.0	–	0.25	–	8.5	CDEFGHI	40
18	1.0	–	0.50	–	8.3	defghijklmn	40
19	1.0	–	0.75	–	8.0	dehijklmnopqrst	60
20	1.0	–	1.00	–	8.0	cehijklmno	50
21	1.5	–	0.25	–	8.0	dehijklmnopq	60
22	1.5	–	0.50	–	7.5	iklmnopqrstuvw	40
23	1.5	–	0.75	–	6.0	BDEFJK	60
24	1.5	–	1.00	–	6.0	BDEFJKL	60

Table 26. Contd.....

Sl. No.	Treatments				Days to bud break		Bud break percentage
	BAP (mg/l)	2, 4-D (mg/l)	NAA (mg/l)	GA_3 (mg/l)			
25	2.0	–	0.25	–	7.0	qrtvwxyzABCD	40
26	2.0	–	0.50	–	6.0	DEFJKLMN	80
27	2.0	–	0.75	–	4.0	QRSTU	60
28	2.0	–	1.00	–	5.0	JKLMNQ	50
29	2.5	–	0.25	–	8.3	cdefghijklm	60
30	2.5	–	0.50	–	6.0	BDEFJ	40
31	2.5	–	0.75	–	7.0	qrvwxyzABCDEFGH	20
32	2.5	–	1.00	–	7.0	opqrstuvwxyzAB	20
33	1.0	–	0.25	–	7.0	qrtvwxyzAB	60
34	1.0	–	0.50	–	9.0	abcdef	60
35	1.0	–	0.75	–	8.4	cdefghijk	70
36	1.0	–	1.00	–	6.0	dehijklmnopqrstu	50
37	1.5	–	–	0.50	7.0	qrtvwxyzABCDEF	60
38	1.5	–	–	1.00	7.0	qrtvwxyzABCDEFG	50
39	1.5	–	–	1.50	8.0	dehijklmno	60
40	1.5	–	–	2.00	8.4	cdefghijkl	50
41	2.0	–	–	0.50	6.0	DEFJKLMNO	80
42	2.0	–	–	1.00	7.4	iklmnopqrstuvwxy	70
43	2.0	–	–	1.50	8.0	dehijklmnop	50
44	2.0	–	–	2.00	9.2	abc	50
45	2.5	–	–	0.50	9.0	abcd	60
46	2.5	–	–	1.00	9.0	abcde	50
47	2.5	–	–	1.50	8.8	cdefgh	50
48	2.5	–	–	2.00	10.0	a	30

Number of cultures /treatment = 12

Means followed by a common letter are not significantly different at 5% level

4-D 0.5 mg/l and BAP 2.0 mg/l + NAA 0.75 mg/l followed by BAP 2.5 mg/l + 2,4-D 0.75 mg/l (4.2 days), BAP 2.0 mg/l + 2, 4-D 1.0 mg/l (5.0 days) and BAP 2.0 mg/l + NAA 1.0 mg/l (5.0 days). These treatments were significantly superior and on par with each other.

Two treatments viz., BAP 1.0 mg/l + 2, 4-D 2.25 mg/l and BAP 2.5 mg/l + GA_3 2.0 mg/l took the maximum of 10 days for bud break.

Among the different concentrations of BAP in combination with 2, 4-D at different concentrations days to bud break showed a wide variation from 4 (BAP 2.5 mg/l + 2, 4-D 0.5 mg/l) to 10 days (BAP 1.0 mg/l + 2, 4-D 0.25 mg/l) and in BAP and NAA treatment combinations the range was from 4 (BAP 2.0 mg/l + NAA 0.75 mg/l) to 8.5 days (BAP 1.0 mg/l + NAA 0.25 mg/l). In MS basal medium supplemented with different combinations of BAP and GA_3 the days to bud break ranged from 6 (BAP 2.0 mg/l + GA_3 0.5 mg/l) to 10 days (BAP 2.5 mg/l + GA_3 2.0 mg/l).

4.2.7.2. Bud break percentage

The bud break percentage three weeks after culture ranged from 20 to 80. The highest value of 80 per cent bud break recorded in four of the 48 treatments tried viz., BAP

2.0 mg/l + 2, 4-D 1.0 mg/l, BAP 2.5 mg/l + 2, 4-D 0.5 mg/l, BAP 2.0 mg/l + NAA 0.5 mg/l and BAP 2.0 mg/l + GA_3 0.5 mg/l. The lowest bud break percentage (20) was recorded at the highest concentration of both BAP (2.5 mg/l) and NAA (1.0 mg/l).

4.2.8. Standardisation of medium for shoot proliferation

The experimental data on the trial conducted to find out the most suitable hormone supplements for enhanced release of axillary buds are given in table 27. The parameters analysed for the induction and production of multiple shoots under different treatment combinations showed significant difference.

4.2.8.1. Days to initiate multiple shoot

The treatments showed significant differences. The number of days taken for multiple shoot production ranged from 23.4 to 44.3. MS medium supplemented with BAP alone (0.5 to 2.0 mg/l) took 35.2 to 43.0 days and kinetin (0.5 to 2.0 mg/l) recorded a range of 39.3 to 44.3 days. The days for multiple shoot induction ranged from 23.5 (BAP 2.0 + GA_3 0.75 mg/l) to 35 days (BAP 1.5 + GA_3 0.25 mg/l). The lowest level of BAP (0.5 mg/l) and GA_3 (0.25 mg/l) took 34.8 days.

Among kinetin and GA_3 combinations the time taken for induction of multiple shoot showed a wide variation

Table 27. Effect of BAP, kinetin and in combination with GA_3 on shoot proliferation (cv. Folklore)

Sl. No.	Treatments			Days to initiate multiple shoot	Cultures with multiple shoots(%)	Number of shoots per culture	Number of elongated shoots per culture
	BAP (mg/l)	Kinetin (mg/l)	GA_3 (mg/l)				
1	0.5	–	–	43.0 ab	58.3	1.6 JKLM	0.0 pqrstuvwxyzABCDEFGHIJKL
2	1.0	–	–	39.1 ghijkl	66.7	2.0 BCDEFGHIJK	0.0 pqrstuvwxyzABCDEFGHIJKLM
3	1.5	–	–	37.1 mn	58.3	3.0 nopqrstuvwxyzABCD	0.6 lmnopqrstuvwxyzAB
4	2.0	–	–	35.2 pq	41.7	3.4 lmnopqrstuvwxy	0.4 opqrstuvwxyzABCD
5	0.5	–	0.25	34.8 pqrs	33.3	3.0 nopqrstuvwxyzABCDEFG	0.3 pqrstuvwxyzABCDEFG
6	0.5	–	0.50	34.3 pqrstuvwx	33.3	3.5 jklmnopqrstuvw	0.8 hlmnopqrstuvwxyz
7	0.5	–	0.75	34.4 pqrstuvw	41.7	3.0 nopqrstuvwxyzABCD	0.2 pqrstuvwxyzABCDEFGHI
8	0.5	–	1.00	34.5 pqrstuv	33.3	3.0 nopqrstuvwxyzABCDE	0.8 hlmnopqrstuvwxy
9	1.0	–	0.25	33.4 rstuvwxy	41.7	2.0 DEFGHIJK	0.4 opqrstuvwxyzABCDE
10	1.0	–	0.50	31.6 yABCD	41.7	3.4 lmnopqrstuvwxy	1.0 efghijklmnop
11	1.0	–	0.75	31.7 yzABC	50.0	3.8 fghijklmnopqrst	0.8 efghijklmnopqrstu
12	1.0	–	1.00	31.6 yABCDE	58.3	4.0 efghijklmn	1.8 defghij
13	1.5	–	0.25	35.0 pqr	33.3	2.8 tuvwxyzABCDEFGHI	0.8 hlmnopqrstuvw
14	1.5	–	0.50	34.6 pqrstu	41.7	4.0 efghijklmnopq	1.6 defghijkl
15	1.5	–	0.75	32.3 xyzAB	66.7	4.8 cdef	2.0 cdef
16	1.5	–	1.00	29.7 CEFGHI	58.3	5.0 cde	2.4 bcd
17	2.0	–	0.25	31.3 BCDEF	58.3	4.6 defghij	1.0 efghijklmnop
18	2.0	–	0.50	29.0 GHIJ	58.3	4.8 cdefgh	1.8 defghi
19	2.0	–	0.75	23.5 LM	91.7	6.2 ab	3.6 a
20	2.0	–	1.00	24.8 L	75.0	5.8 abc	3.2 ab

Contd.....

Table 27. Contd.....

Sl. No.	Treatments			Days to initiate multiple shoot		Cultures with multiple shoots (%)	Number of shoots per culture		Number of elongated shoots per culture	
	BAP (mg/l)	Kinetin (mg/l)	GA_3 (mg/l)							
21	–	0.5	–	44.3	a	25.0	1.3	KLM	0.0	pqrstuvwxyzABCDEFGHIJ
22	–	1.0	–	43.0	ab	33.3	2.5	vwxyzABCDEFGHIJ	0.0	pqrstuvwxyzABCDEFGHIJ
23	–	1.5	–	42.6	abcd	41.7	3.2	mnopqrstuvwxyzAB	0.0	pqrstuvwxyzABCDEFGHIJK
24	–	2.0	–	39.3	ghijk	33.3	3.5	jklmnopqrstuvw	0.5	mnopqrstuvwxyzABC
25	–	0.5	0.25	40.2	fghi	41.7	3.2	mnopqrstuvwxyzAB	0.4	pqrstuvwxyzABCD
26	–	0.5	0.50	41.8	bcdef	50.0	4.0	efghijklmnopqr	1.0	efghijklmnopqr
27	–	0.5	0.75	40.5	efgh	50.0	3.6	fghijklmnopqrstuv	1.0	efghijklmnopqrs
28	–	0.5	1.00	42.3	bcde	33.3	3.3	mnopqrstuvwxyzA	0.8	hlmnopqrstuvwx
29	–	1.0	0.25	40.8	defg	33.3	3.0	nopqrstuvwxyzABCDEF	0.3	pqrstuvwxyzABCDEFG
30	–	1.0	0.50	40.0	fghij	50.0	4.0	efghijklmn	1.4	defghijklmno
31	–	1.0	0.75	38.8	ghijklm	41.7	4.4	defghijkl	1.6	defghijklm
32	–	1.0	1.00	34.7	pqrst	50.0	4.8	cdef	2.0	cde
33	–	1.5	0.25	36.8	mo	41.7	4.2	defghijklm	0.8	hijklmnopqrstu
34	–	1.5	0.50	35.8	nop	41.7	4.6	defghij	1.6	defghijkl
35	–	1.5	0.75	33.4	rstuvwxy	41.7	5.2	bcd	1.8	defgh
36	–	1.5	1.00	33.3	rstuvwxyzA	50.0	3.8	fghijklmnopqrst	1.8	defgh
37	–	2.0	0.25	30.0	CDEFGH	33.3	4.0	efghijklmno	0.8	hlmnopqrstuvw
38	–	2.0	0.50	30.4	BCDEFG	41.7	4.0	efghijklmnop	1.0	efghijklmnopq
39	–	2.0	0.75	27.6	JK	50.3	4.8	cdefg	2.0	cde
40	–	2.0	1.00	23.4	LM	83.3	6.8	a	3.0	abc

Number of cultures per treatment = 12

Means followed by a common letter are not significanlty different at 5% level.

Standardisation of culture medium for initial culture establishment (cv. Folklore)

PLATE 12

Shoot growth (3 weeks after culture) in best initial culture establishment medium (MS medium + BAP 2.5 mg / l + 2, 4-D 0.5 mg / l)

Standardisation medium for shoot proliferation (cv. Folklore)

PLATE 13

Multiple shoot (8 weeks after culture) in best shoot proliferation medium (MS medium + kinetin 2.0 mg / l + GA_3 1.0 mg / l)

ranging from 23.4 to 42.3 days. The best treatment for early multiple shoot induction (23.4 days) was found to be the MS medium supplemented with kinetin 2.0 mg/l + GA_3 1.0 mg/l and was on par with BAP 2.0 mg/l + GA_3 0.75 mg/l (plate 13). The maximum days (42.3) was taken by kinetin 0.5 + GA_3 1.0 mg/l). Percentage of cultures induced multiple shoots

The percentage of multiple shoot induction ranged from 25.0 to 91.7 per cent. The MS medium supplemented with BAP 2.0 mg/l + GA_3 0.75 mg/l recorded the best response (91.7 per cent) followed by MS + kinetin 2.0 mg/l + GA_3 0.75 mg/l (83.3 per cent). In treatments supplemented with BAP alone the percentage ranged from 41.7 (BAP 2.0 mg/l) to 66.7 (BAP 1.0 mg/l) per cent and in kinetin alone supplemented to the medium the percentage ranged from 25 (0.05 mg/l) to 41.7 (1.5 mg/l).

4.2.3.2 Number of shoots per culture

Significant differences were observed among treatments with respect to the number of shoots formed per culture. The maximum number of 6.8 shoots per culture was observed in the combination of MS + kinetin 2.0 mg/l + GA_3 1.0 mg/l which was on par with BAP 2.0 mg/l + GA_3 0.75 mg/l and BAP 2.0 mg/l + GA_3 1.0 mg/l. The lowest value (1.3) was recorded in MS medium supplemented with lowest level of

kinetin (0.5 mg/l) and was on par with lowest level of BAP (10.5 mg/l) alone. Treatments with low level of BAP/kinetin alone or in combination with lowest levels of GA_3 showed poor response.

4.2.8.3 Number of elongated shoots per culture

Statistical analysis of the data showed significant variation among treatments. The treatment combination of MS + BAP 2.0 mg/l + GA_3 0.75 mg/l recorded the maximum number of 3.6 shoots/ culture followed by MS + kinetin 2.0 mg/l + GA_3 1.0 mg/l (3.0 shoots/culture). These two treatments were significantly superior to all other treatment combinations.

4.2.8.4 Flower bud initiation

In one of the treatment combinations (BAP 2.0 mg/l + GA_3 0.5 mg/l) tried to induce multiple shoots flower bud initiation was observed (plate 14).

4.2.9. Standadardisation of medium for *in vitro* rooting

The result of the experiment conducted to find out the most suitable hormone supplements for *in vitro* rooting are presented in table 28. The effect of different treatments on *in vitro* rooting was assessed based on days taken for root initiation, percentage of rooting, number of roots per shoot and length of the longest root. A perusal of the

PLATE 14

Flower bud initiation 6 weeks after culture in cv. Folklore (MS medium + BAP 2.0 mg / l GA_3 0.5 mg / l)

Table 28. Effect of different levels of IAA, NAA and in combination with 2, 4-D on *in vitro* rooting

Sl. No.	Treatments			Days to root initiation	Rooting (%)	Number of roots per shoot	Length of longest root (cm)
	IAA (mg/l)	NAA (mg/l)	2, 4-D (mg/l)				
1	0.0	0.0	0.0	31.0 a	25.0	2.3 N	0.23 KLMN
2	0.5	–	–	22.0 cd	41.7	4.2 FGHIJK	0.54 wxyzABCDEFG
3	1.0	–	–	24.3 b	58.3	5.2 vwxyzABCDEF	0.94 ghijklmno
4	1.5	–	–	16.8 mnop	66.7	5.8 rstuvwx	1.12 defghi
5	2.0	–	–	11.0 GHIJKL	83.3	6.2 cd	1.46 b
6	–	0.5	–	19.2 fghij	50.0	3.6 HIJKLM	0.48 zABCDEFGHIJKL
7	–	1.0	–	19.5 efgh	75.0	5.4 uvwxyzABC	0.80 jklmnopqrstuvw
8	–	1.5	–	15.9 opqrst	75.0	6.4 jklmnopqrstu	1.02 ghijkl
9	–	2.0	–	15.0 pqrstuvw	91.7	6.6 ghijklmnopqrs	1.20 bcdefg
10	0.5	0.5	–	19.6 efg	91.7	4.4 CDEFGH	0.52 yzABCDEFGHIJ
11	0.5	1.0	–	20.0 ef	75.0	6.2 lmnopqrstuv	0.66 pqrstuvwxyzAB
12	0.5	1.5	–	19.1 fghijk	75.0	7.0 efghijklmnop	0.88 ijklmnopq
13	0.5	2.0	–	16.2 opqr	75.0	6.8 fghijklmnopqr	0.88 ijklmnopq
14	1.0	0.5	–	13.9 uvwxyzAB	66.7	7.6 cdefg	1.18 cdefgh
15	1.0	1.0	–	8.5 N	91.7	11.6 a	2.44 a
16	1.0	1.5	–	9.5 KLMN	66.7	9.4 b	1.36 bcd
17	1.0	2.0	–	10.9 GHIJKLM	66.7	8.0 cde	0.78 klmnopqrstuvwxy
18	1.5	0.5	–	11.4 FGHIJK	66.7	7.4 cdefghij	0.82 jklmnopqrstu
19	1.5	1.0	–	12.0 CDEFGHIJ	41.7	7.6 cdefg	1.40 bc
20	1.5	1.5	–	12.2 BCDEFGHI	41.7	5.6 stuvwxyzA	0.88 ijklmnopqrs

Contd.....

Table 28. Contd.....

Sl. No.	Treatments			Days to root initiation		Rooting (%)	Number of roots per shoot		Length of longest root (cm)	
	IAA (mg/l)	NAA (mg/l)	2, 4-D (mg/l)							
21	1.5	2.0	–	14.0	uvwxyzA	41.7	7.0	efghijklmn	0.82	jklmnopqrstu
22	2.0	0.5	–	16.2	opqrs	41.7	6.6	ghijklmnopqrs	0.96	ghijklmn
23	2.0	1.0	–	14.5	qrstuvwxy	33.3	7.3	defghijkl	1.00	ghijklm
24	2.0	1.5	–	14.3	tuvwxyz	33.3	7.8	cdef	0.80	jklmnopqrstuvw
25	2.0	2.0	–	15.0	pqrstuvw	33.3	7.5	cdefghi	0.85	jklmnopqrst
26	0.5	–	0.5	12.3	BCDEFGH	100.0	2.8	MN	0.54	wxyzABCDEFGH
27	0.5	–	1.0	15.7	opqrstu	75.0	4.4	CDEFGH	0.54	wxyzABCDEFG
28	1.0	–	0.5	13.1	wxyzABCDEF	58.3	5.4	uvwxyzABCD	0.66	pqrstuvwxyzAB
29	1.0	–	1.0	18.8	fghijkl	50.0	5.8	rstuvwx	0.70	mopqrstuvwxyz
30	1.5	–	0.5	13.4	vwxyzABCDE	41.7	7.0	efghijklm	0.68	opqrstuvwxyzA
31	1.5	–	1.0	18.0	hijklm	50.0	7.0	efghijklm	0.64	qrstuvwxyzABCD
32	2.0	–	0.5	13.9	uvwxyzABC	58.3	8.4	bc	1.30	bcdef
33	2.0	–	1.0	17.3	mno	33.3	5.5	uvwxyzAB	1.03	ghijk
34	–	0.5	0.5	12.9	wxyzABCDEFG	75.0	4.4	CDEFGHI	0.36	FGHIJKLM
35	–	0.5	1.0	16.3	nopq	50.0	5.4	uvwxyzABC	0.64	qrstuvwxyzABCD
36	–	1.0	0.5	13.5	vwxyzABCD	50.0	6.2	lmnopqrstuv	0.92	hijklmnop
37	–	1.0	1.0	19.4	efghi	41.7	7.0	efghijklmno	0.60	tuvwxyzABCDEF
38	–	1.5	0.5	15.2	pqrstuv	50.0	7.4	cdefghij	1.32	bcde
39	–	1.5	1.0	20.8	cde	33.3	5.8	rstuvwxyz	1.08	efghij
40	–	2.0	0.5	18.0	hijklm	41.7	5.2	vwxyzABCDEF	0.50	zABCDEFGHIJK
41		2.0	1.0	22.3	c	33.3	4.0	HIJKL	0.35	FGHIJKLMN

Number of cultures per treatment = 12

Means followed by a common letter are not significanlty different at 5% level.

data revealed that the treatments differed significantly with respect to different parameters studied on *in vitro* rooting.

4.2.9.1. Days to root initiation

The days taken for root initiation ranged from 8.6 to 31.0. earliest root initiation (8.6 days) was observed in the medium supplemented with IAA and NAA both at 1.0 mg/l followed by IAA 1.0 mg/l + NAA 1.5 mg/l which took 9.5 days. These two treatments were significantly superior to other treatments and were on par with each other. The maximum of 31.0 days was taken for root initiation in the medium devoid of any hormone (plate 15).

When IAA/NA alone was added to the medium the days to root initiation ranged from 11.0 to 24.3 and 15.0 to 19.5 respectively. In IAA and NAA combination treatments, the days to root initiation ranged from 8.5 to 20.0 days. With regard to IAA or NAA in combination with 2, 4-D the days to root initiation ranged from 12.3 to 22.3 days.

4.2.9.2. Rooting percentage

The rooting percentage of cultured shoots ranged from 25 to 100. Cent per cent rooting was observed in medium containing IAA and 2,4-D both at 0.5 mg/l followed by 91.7 per cent rooting in three of the treatments viz., NAA 2.0

Standardisation of culture medium for *in vitro* rooting (cv. Folklore)

PLATE 15

Culture showing absence of rooting at two weeks

PLATE 16

Root initiation in MS medium + IAA 1.0 mg/l + NAA 1.0 mg/l at two weeks

mg/l, IAA 0.5 mg/l + NAA 0.5 mg/l and IAA 1.0 mg/l + NAA 1.0 mg/l. The lowest rooting of 25 per cent was recorded in medium without any of the hormones.

4.2.9.3. Number of roots per shoot

The number of roots produced per shoot showed significant differences among different treatments and it ranged from 2.3 to 11.6. The highest number of 11.6 roots was produced in medium supplemented with IAA and NAA both at 1.0 mg/l which was significantly superior to all other treatments. The lowest number of roots (2.3) was produced in medium devoid of any hormone supplements. Addition of IAA or NAA alone to the medium produced roots ranging from 4.2 to 8.2 and 3.6 to 6.6 respectively. Better root production efficiency ranging from 4.6 to 11.6 was observed with the combined application of IAA and NAA. Incorporation of 2, 4-D into the medium supplemented with IAA or NAA did not improve the root production ~~did not improve the root production~~ efficiency and it ranged from 2.8 to 8.4.

4.2.9.4. Length of longest root (cm)

Analysis of the data revealed significant differences for root length four weeks after culture among the different treatments. The length of root showed a wide variation ranging from 0.23 cm to 2.44 cm. The longest root

(2.44 cm) was produced in medium containing IAA and NAA both at 1.0 mg/l which was significantly superior to other treatments. The shortest root of 0.23 cm was observed in medium lacking hormone supplements. When IAA or NAA alone was added to the medium the root length ranged from 0.54 to 1.46 cm and 0.48 to 1.20 cm respectively. Combined application of IAA and NAA produced roots with length ranging from 0.52 cm to 2.44 cm. Addition of 2, 4-D to the medium containing either IAA or NAA did not improve root length and in these treatments the root length ranged from 0.35 to 1.32 cm.

4.2.10. Effect of activated charcoal on *in vitro* rooting

The results on the effect of activated charcoal on *in vitro* rooting are given in table 29. Significant difference among treatment means were observed for different parameters.

4.2.10.1 Days to root initiation

Number of days to root initiation was the minimum (7.5) in MS medium + IAA and NAA 1.0 mg/l each + sucrose 30 g/l + agar 7 g/l and charcoal 500 mg/l followed by IAA and NAA at 1.0 mg/l each + sucrose 30 g/l + agar 7 g/l and charcoal 1000 mg/l. Statistical analysis showed that these two treatments were on par and were significantly superior to

Table 29. Effect of activated charcoal in combination with IAA, NAA on *in vitro* rooting of cultured shoots (cv. Folkore) Basal medium MS.

Sl No	Treatments			Days to root initiation	Rooting (%)	Number of roots per shoot	Length of longest root (cm)
	IAA (mg/l)	NAA (mg/l)	Activated charcoal (mg/l)				
1	1.0	0.5	500	12.8 a	75.0	8.2 ef	3.24 bcde
2	1.0	0.5	1000	12.4 ab	75.0	9.0 cde	2.94 cdefg
3	1.0	1.0	500	7.5 g	91.7	12.0 a	5.20 a
4	1.0	1.0	1000	8.1 fg	91.7	11.6 ab	4.14 b
5	1.0	1.5	500	8.8 ef	83.3	9.6 cd	3.58 bc
6	1.0	1.5	1000	9.9 e	75.0	9.8 c	3.48 bcd
7	1.0	2.0	500	11.5 bcd	75.0	8.0 efg	3.10 cdef
8	1.0	2.0	1000	11.6 bc	75.0	7.8 fg	2.90 cdefg
'F value'				25.71**		17.88**	5.30**

Number of cultures per treatment = 12

** Significant at 5 per cent level

Means followed by a common letter are not significanlty different at 5% level.

other treatments. Root initiation was delayed (12.8 days) in treatment involving MS + sucrose 30 g/l + agar 7 g/l added with IAA 1.0 mg/l and NAA 0.5 mg/l and activated charcoal 500 mg/l followed by the same levels of hormone supplemented medium with 1000 mg/l activated charcoal.

4.2.10.2. Rooting percentage

The rooting percentage ranged from 75 to 91.7 per cent. Maximum rooting of 91.7 per cent was recorded in two treatments involving MS basal medium both containing same hormone supplements of IAA and NAA at 1.0 mg/l sucrose 30 g/l + agar 7 g/l + activated charcoal at 500 and 1000 mg/l.

4.2.10.3. Number of roots per shoot

The MS medium + IAA and NAA 1.0 mg/l each + sucrose 30 g/l + agar 7 g/l and activated charcoal 500 mg/l produced the highest number of 12 roots followed by same medium with the same hormone supplements with activated charcoal at 1000 mg/l (11.6 days). These two combinations were significantly superior to other treatment combinations tried.

4.2.10.4. Length of longest root

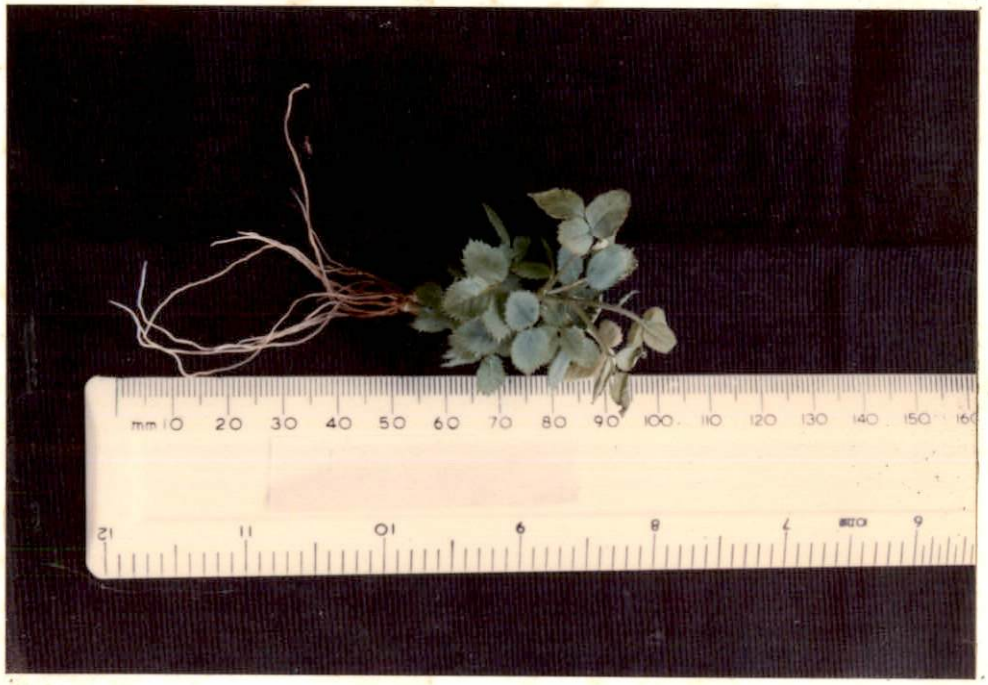
A significant difference in root length (5.20 cm) was recorded in MS basal medium with auxin supplements IAA

PLATE 17

Root growth in MS medium + IAA 1.0 mg / l + NAA 1.0 mg / l at 4 weeks (Mean root length 2.44 cm)

PLATE 18

Best root growth in MS medium + IAA 1.0 mg / l + NAA 1.0 mg / l + activated charcoal 500 mg / l at four weeks (Mean root length 5.20 cm)



and NAA 1.0 mg/l each + sucrose 30 g/l + agar 7 g/l + activated charcoal at 500 mg/l (plate 18). The root growth showed a significant reduction at higher levels of auxins with activated charcoal at both levels of 500/1000 mg/l.

4.2.11. Acclimatization and *ex vitro* establishment

Plantlets regenerated by *in vitro* technique under high humidity and temperature could not survive by direct planting out in small pots. The plantlets exhibited desiccation and wilting on planting out from the cultured vessels. None of the *ex vitro* plantlets under diffused light in small pots (7.5 x 6 cm) survived. Therefore it was found that hardening was highly essential for *ex vitro* establishment of rose.

4.2.11.1. Standardisation of potting medium

In order to assess the suitability of different potting media on acclimatization and survival a trial with six different potting media and three periods of root induction was conducted. The results are presented in table 30.

A critical analysis of the data revealed that survival rate was dependent on both the media used for planting out and the duration to which it was kept in the

Table 30. Effect of rooting period and potting mixture on survival of plantlets (cv. Folklore)

Sl. No.	Potting mixture	Rooting period (weeks)	Survival percentage		
			one week	two weeks	three week
1	Sand	Two	87.50	62.50	25.00
		Three	100.00	87.50	62.50
		Four	100.00	50.00	50.00
2	Soilrite	Two	87.50	37.50	00.00
		Three	100.00	87.50	62.50
		Four	87.50	37.50	37.50
3	Vermiculate	Two	50.00	25.00	00.00
		Three	75.00	25.00	00.00
		Four	75.00	50.00	12.50
4	Sand:soiltrite (1:1)	Two	100.00	75.00	25.00
		Three	87.50	50.00	25.00
		Four	100.00	75.00	37.50
5	Sand:vermiculate (1:1)	Two	87.50	25.00	00.00
		Three	100.00	37.50	12.50
		Four	87.50	60.00	12.50
6	Sand:peatmoss (1:1)	Two	37.50	12.50	00.00
		Three	50.00	50.00	12.50
		Four	25.00	25.00	00.00

Number of plants per treatment = 8

root induction medium. Survival rate was cent per cent one week after planting out where plantlets were planted out in i) sand medium after keeping them in the root induction medium for 3 weeks ii) sand medium after keeping them in the root induction medium for 4 weeks iii) soilrite after keeping them in the root induction medium for 3 weeks iv) sand: soilrite (1 : 1) for 2 weeks in the root induction medium and sand vermiculate (1 : 1) for 3 weeks. Potting medium containing peat moss recorded high mortality during the first week itself irrespective of the time kept in rooting medium.

At second week after planting out (plate 20), survival rate showed a reducing trend (12.5 to 87.5 per cent). The highest rate of 87.5 per cent survival was noticed in sand and in soilrite when planted out after three weeks in rooting medium followed by sand : soilrite (1:1) 75 per cent.

The survival rate of plantlets three weeks after planting out drastically reduced. In six out of eighteen treatments, complete mortality was recorded. A better response of 50 per cent survival was noticed in sand, when plantlets were grown for three weeks or four weeks in rooting medium. In sand : soilrite (1:1) 50 per cent survival observed when the period of rooting was four weeks. Among the different potting media sand and sand : soilrite (1:1) were

found to give comparatively good survival after three weeks. However, further growth was arrested and plantlets started desiccation and wilting resulting in complete mortality by fourth week.

4.2.11.2. Control of temperature and relative humidity

Satisfactory maintenance of relative humidity could be achieved by using an improvised structure made of polythene sheet and wooden frames. (Plate 19). Spraying of cold water (12 $\pm$ 2^{o}c) fine spray at an interval of three hours during the day time using a hand sprayer with fine mist nozzle enabled to maintain high humidity (90 to 100 per cent). During the first three days, the plantlets under polythene covers were maintained inside the culture room under 3000 lux light intensity. Three days after planting out, the plantlets were kept outside the culture room during day time. The relative humidity was progressively lowered five days after planting out by reducing the frequency of water spray and by providing perforations in the polybag.

4.2.11.3. Supply of nutrients

The results of the trial conducted with nutrient solution of different concentrations on the growth of plant lets are presented in table 31. The mean number of days taken for the emergence of first leaf ranged from 5.6 to 6.0,

143

Ex vitro (Field) establishment of cv. Folklore

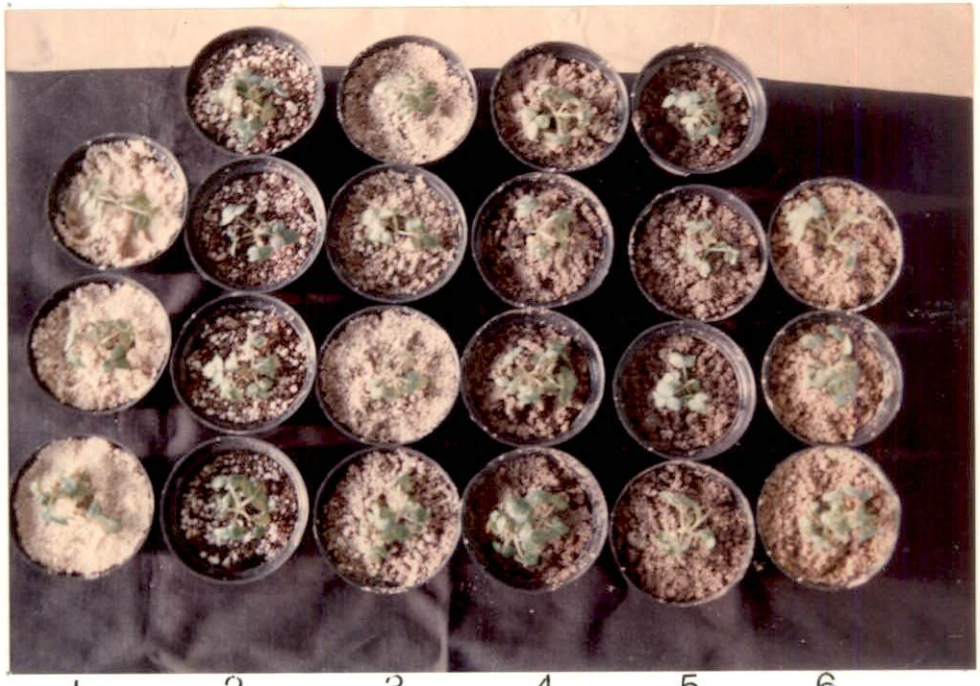
PLATE 19

Improvised hardening structure consisting of wooden frame and polythene cover

PLATE 20

Effect of different potting mixtures on hardening and establishment (2 weeks after planting out)

1. Sand 2. Soilrite 3. Vermiculate
4. Sand : Soilrite (1 : 1) 5. Sand : Vermiculate (1 : 1)
6. Sand : Peatmoss (1 : 1)



1 2 3 4 5 6

Table 31. Effect of supply of nutrient solution on growth and survival of plantlets (cv. Folklore)

Sl. No.	Treatments	Qty. (ml)	Frequency (days)	Days to emergence of		Plant height (cm)		Mean increase in plant height	Survival (%) after three weeks
				first leaf	second leaf	Time of planting out	Three weeks planting out		
1	Water	5	3	5.8	11.8	2.96	3.60	0.64	25.00
2	MS one tenth strength	5	3	5.6	10.4	2.92	3.84	0.92	37.50
3	MS half strength	5	3	5.8	11.5	3.02	3.90	0.88	12.50
4	MS full strength	5	3	6.0	12.0	3.00	3.80	0.80	12.5

Number of plants per treatment - 8

and second leaf 10.4 to 12.0. Supply of MS inorganic salts in liquid form at one tenth concentration at a dose of 5 ml each at 3 days interval recorded early leaf production in 10.4 days whereas supply of MS full strength solution delayed leaf emergence and took 12.0 days for the same.

The mean increase in plant height three weeks after planting out ranged from 0.64 to 0.92 cm. The lowest growth rate (0.64) was recorded in treatment in which water alone was supplied. The maximum growth rate was recorded by the addition of MS at one tenth concentration and a dose of 5 ml per plant at three days interval. The same treatment also recorded the highest survival rate of 37.50 per cent. Survival percentage was reduced at higher concentration of nutrient solution applied.

However, three weeks after planting out the plantlets started dehydration and wilting and resulted in cent per cent mortality by fourth week.

4.2.12. The effect of mycorrhizae on *ex vitro* establishment

The results of the experiment conducted with the inoculation of three spp. of mycorrhizae on *ex vitro* establishment of plantlets cultured *in vitro* are presented in table 32.

Table 32. Effect of mycorrhizae on *ex vitro* establishment of rose (cv. Folklore)

Treatments	Days to emergence		Plant hight (cm)					Survival (%) after planting out at weekly intervals						Days to flowering
	First leaf	second leaf	Time of planting out	3 weeks after planting out	increase in plant hight	6 weeks after planting out	increase in plant hight	1	2	3	4	5	6	
Control	5.4	10.8	3.9	4.5	0.60	0.0	0.0	100	86.67	46.67	00.00	00.00	00.00	000
G. entunicatum	5.8	11.0	3.5	4.2	0.70	7.5	3.3	100	93.33	66.67	66.67	66.67	66.67	105
G. fasciculatum	4.8	10.6	3.9	4.4	0.50	7.1	2.7	100	86.67	73.33	73.33	53.33	53.33	108
G. constrictum	5.2	10.3	3.8	4.6	0.80	7.2	2.6	100	100.00	66.67	66.67	53.33	53.33	130

Number of plants per treatment 15

The result indicated that the days required for the emergence of first leaf ranged from 4.8 to 5.8 and second leaf 10.3 to 11.0. The mean increase in plant height three weeks after planting out recorded was in the range of 0.5 to 0.8 cm. The inoculation with G. constrictum had the highest growth rate (0.8 cm) and lowest with G. fasiculatum (0.5 cm). Six weeks after planting out G. etunicatum had the highest growth rate of 3.3 cm.

The survival of plantlets were cent per cent in all the treatments one week after planting out, however, it was reduced in the second week except in the treatments inoculated with G. constrictum. Three weeks and four weeks after planting out highest survival rate of 73.33 per cent was observed in treatment inoculated with G. fasciculatum (plate 22) and lowest 46.67 per cent survival in control population. Four weeks after planting out, cent per cent mortality was observed in non-mycorrhizae inoculated plants (plate 21). Four weeks after planting out, the plantlets were transferred to large earthen pots (16 x 12 cm). A potting mixture (unsterilised) consisting of soil, sand and cattle manure in 1:1:1 proportion was used, The plantlets inoculated with G etunicatum had the highest survival rate of 66.67 per cent. Six weeks after planting out, there was no mortality of plantlets. The plate, 23 shows the growth and appearance of the Plantlets eight weeks after planting out.

EFFECT OF MYCORRHIZAE ON PLANTLET ESTABLISHMENT

PLATE 21

Control (plantlets in mycorrhizae free sand)
4 weeks after planting out

PLATE 22

Plantlets on sand inoculated with mycorrhizae (VAM)
4 weeks after planting out

PLATE 23

Plantlets 8 weeks after planting out
(inoculated with mycorrhizae)

PLATE 24

Plantlets 16 weeks after planting out
(inoculated with mycorrhizae)

The *ex vitro* established mycorrhizal inoculated plants started flowering from 105 days onwards. However, there was much variation in the number of days required for flowering (plate 24). Plantlets inoculated with G etunicatum was the earliest to flower (105 days) followed by G fasiculatum (108 days). Plantlets inoculated with G constrictum took as high as 130 days for flowering.

4.2.13. Somatic organogenesis/embryogenesis

4.2.13.1. Standardisation of basal medium

The response of internodal segments and leaf disc explants in inducing callus and growth are given in Table 33. The callus induction was initiated from the cut ends of the internodal explants and slowly spread the entire explants. In the case of leaf disc explants, the callus initiation started from the cut end of the midrib in the form of small globules and then spread over the entire surface.

4.2.13.1.1. Percentage of cultures induced callus

The percentage of cultures inducing callus in leaf explants ranged from 33.3 to 83.3. Three treatments BAP 0.5 mg/l + NAA 1.0 mg/l, BAP 0.5 mg/l + NAA 2.0 mg/l and BAP 1.0 mg/l + NAA 1.0 mg/l with the lowest level of 2, 4-D, 0.5 mg/l recorded 83.3 per cent callus induction. At higher levels of

Table 33. Effect of Cytokinin (BAP) in combination with auxin (NAA or 2, 4-D) on the induction and growth of callus (cv. Folklore)
Explants : internodal segment and leaf disc.
Basal medium MS half strength.

Sl. No.	Treatments BAP (mg/l)	NAA (mg/l)	2,4-D (mg/l)	Leaf disc cultures induced callus(%)	Growth score (G)	callus index (CI)	Internodal segment cultures induced callus(%)	Growth score (G)	Callus index (CI)
1	0.5	1.0	0.5	83.3	1.2	99.96	66.7	1.3	86.71
2	0.5	1.0	1.0	50.0	1.3	65.00	66.7	1.0	66.70
3	0.5	2.0	0.5	83.3	3.0	249.90	83.3	2.4	199.92
4	0.5	2.0	1.0	50.0	1.7	85.00	66.7	1.0	66.70
5	1.0	1.0	0.5	83.3	1.8	149.94	50.0	1.3	65.00
6	1.0	1.0	1.0	50.0	1.3	65.00	50.0	1.0	50.00
7	1.0	2.0	0.5	66.7	1.7	113.39	50.0	1.0	50.00
8	1.0	2.0	1.0	33.3	1.0	33.30	50.0	1.0	50.00

Number of cultures / treatment - 8 Culture period 8 weeks.

auxin and cytokinin, the percentage of culture that induced callus reduced to 33.7 per cent.

With regard to internodal segments, the highest percentage was observed at low levels of BAP (0.5 mg/l) and 2, 4-D (0.5 mg/l) and higher level of NAA (2.0 mg/l). Four out of eight treatments recorded only 50 per cent callus induction.

4.2.13.1.2. Growth score

The scores for the growth of callus ranged from 1.0 to 3.0 in the case of leaf disc explants and it ranged from 1.0 to 2.4 in the internodal segments. The best response was observed at low level of BAP (0.5 mg/l) and 2, 4-D (0.5 mg/l) + NAA (2.0 mg/l) for both the explants (plate 25 & 26). In one of the cultures direct rhizogenesis from leaf disc callus was observed (plate 27).

4.2.13.1.3. Callus index (CI)

The callus index recorded a wide variation from 33.30 to 249.90 for leaf disc explants and 50.0 to 199.92 for internodal segments. The highest value was recorded in the treatment MS medium in combination with BAP (0.5 mg/l), 2,4-D 0.5 mg/l and NAA 2.0 mg/l for both the explants.

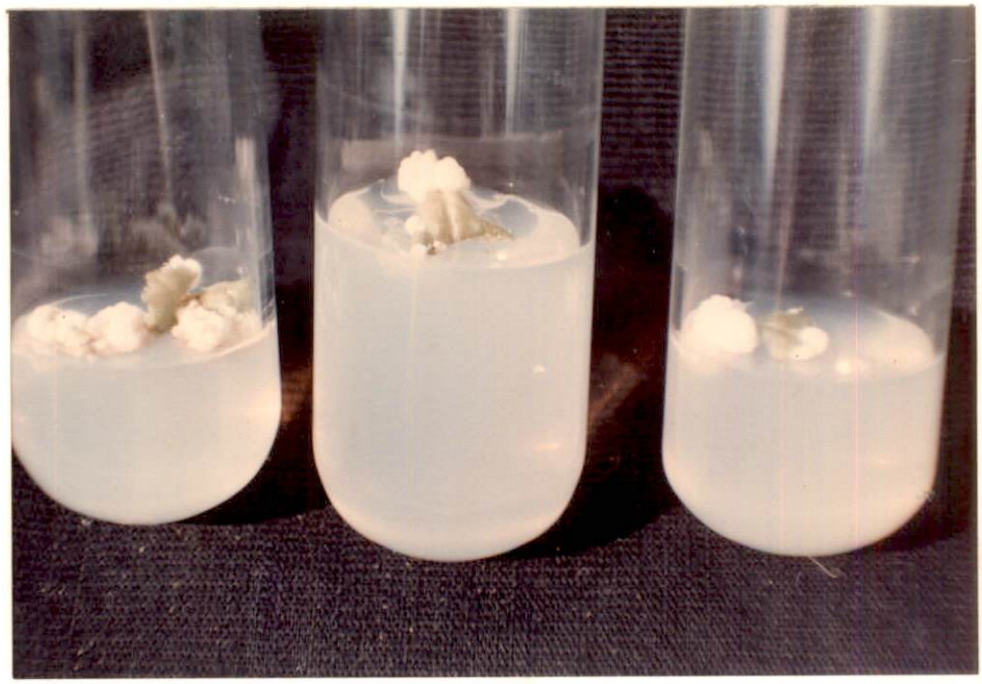
Standardisation of medium for callus induction (cv. Folklore)

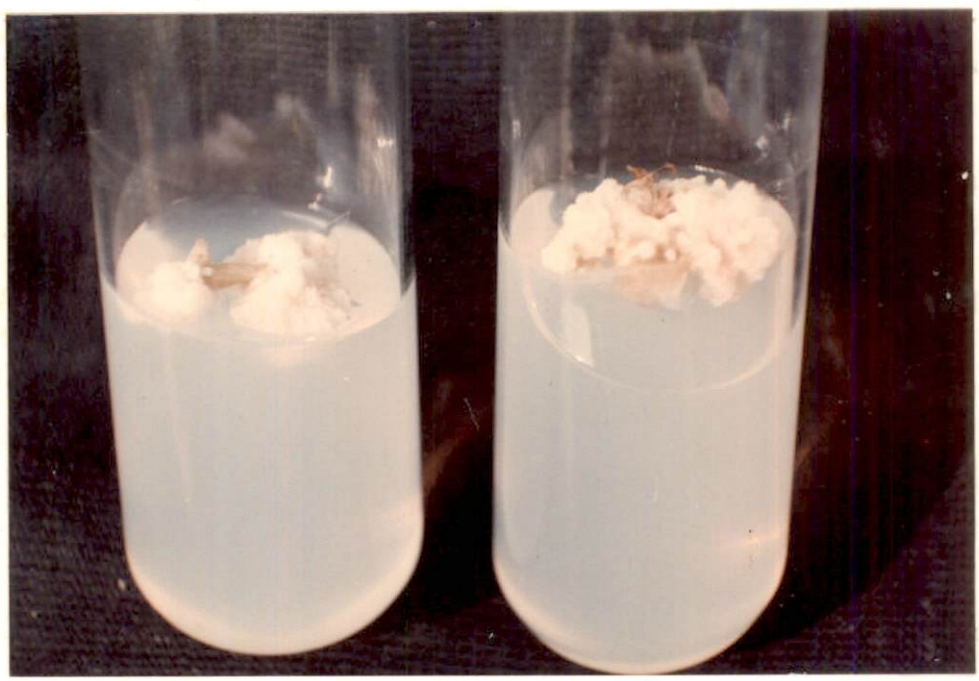
PLATE 25

Best callus induction using leaf disc explants (MS medium + BAP 0.5 mg / l + NAA 2.0 mg / l + 2, 4-D 0.5 mg / l) at 6 weeks after culture

PLATE 26

Best callus induction using leaf disc explants (MS medium + BAP 0.5 mg / l + NAA 2.0 mg / l + 2, 4-D 0.5 mg / l) at 8 weeks after culture





4.2.13.2. Standardisation of medium for somatic organogenesis

The results obtained for standardisation of the medium for somatic organogenesis is presented in Table 34.

4.2.13.2.1. Callus proliferation (percentage)

The percentage of callus exhibiting callus proliferation ranged form 33.3 to 100 for leaf disc explants and 16.7 to 83.3 for internodal callus. In both the cases, addition of BAP (0.5 mg/l) + NAA (0.1 mg/l) + and Ascorbic acid 5 mg/l was found to be the most effective.

4.2.13.2.2. Growth score (G)

The maximum score of 2.1 for leaf disc explant was noticed in the treatment MS + BAP 0.5 mg/l + NAA 0.1 mg/l + ascorbic acid 5 mg/l. The same treatment was also found to be the best in the case of internodal segment with a score of 2.9

4.2.13.2.3. Callus Index (CI)

Callus index for leaf disc explants ranged from 33.3 to 210.0 and for internodal segments from 16.7 to 241.6. The least response was recorded in combination MS + BAP 0.5 mg/l, NAA 0.1 mg/l and ascorbic acid 5 mg/l.

Table 34. Effect of different levels of BAP and NAA in combination with 2, 4-D or ascorbic acid on callus prolifraton and organogenesis. (cv. Folklore) Basal medium - MS half strength

Sl.	Treatments				Leaf disc				Indernodal segment			
No.	BAP (mg/l)	NAA (mg/l)	2,4-D (mg/l)	A.A (mg/l)	callus prolife-ration(%)	growth score (G)	callus index (CI)	cultures with roots (%)	callus prolife-ration(%)	growth score (G)	callus index (CI)	cultures with roots (%)
1	0.5	0.1	-	5	100.0	2.1	210.0	0	83.3	2.9	214.6	0
2	0.5	0.1	-	10	83.3	1.4	116.7	0	83.3	1.9	158.3	0
3	0.5	0.2	-	5	83.3	1.2	100.0	0	83.3	1.4	116.6	0
4	0.5	0.2	-	10	66.7	1.1	73.4	0	66.7	1.2	80.0	0
5	1.0	0.1	-	5	66.7	1.0	66.7	0	66.7	1.0	66.7	0
6	1.0	0.1	-	10	66.7	1.1	73.4	0	66.7	1.3	86.7	0
7	1.0	0.2	-	5	50.0	1.0	50.0	0	50.0	1.3	65.0	0
8	1.0	0.2	-	10	66.7	1.0	66.7	0	33.3	1.0	33.0	0
9	0.5	2.0	0.5	-	66.7	1.1	73.4	0	50.0	2.0	100.0	0
10	0.5	2.0	1.0	-	50.0	1.0	50.0	0	33.3	1.7	56.6	0
11	0.5	2.5	0.5	-	66.7	1.5	100.1	33.3	50.0	2.5	125.0	33.3
12	0.5	2.5	1.0	-	50.0	1.3	65.0	0	50.0	2.3	115.0	0
13	1.0	2.0	0.5	-	33.3	1.1	36.6	0	66.7	1.1	73.4	0
14	1.0	2.0	1.0	-	50.0	1.0	50.0	0	50.0	1.1	55.0	0
15	1.0	2.5	0.5	-	33.3	1.0	33.3	0	33.3	2.1	69.9	0
16	1.0	2.5	1.0	-	33.3	1.5	33.3	0	16.7	1.0	16.7	0

Number of cultures / treatment - 6 Culture period - 12 weeks

4.2.13.2.4. Rhizogenesis from callus cultures

Among the different treatment combinations tried to induce somatic organogenesis from callus cultures, shoot induction was not observed either in cultures which were transferred to fresh medium every six weeks or in the cultures which were observed for six months without subculturing.

However, 33.3 per cent of the leaf and internodal callus cultures induced rhizogenesis (Plates 27 & 28) and produced thick and fleshy roots in MS medium supplemented with BAP and 2,4-D 0.5 mg/l and NAA 2.5 mg/l. The cultures were again transferred to the fresh medium to intiate the fresh growth of callus for morphogenesis but it was not successful.

4.2.13.3. Standardisation of medium for somatic embryogenesis

Attempts made to induce somatic embryogenesis were not successful. Different treatment combinations were tried, but no positive results were obtained.

4.3. INDUCED MUTATION ADOPTING *IN VITRO* CULTURE

4.3.1. Irradiation of budwood

The direct effect of gamma rays on percentage

PLATE 27

Rhizogenesis from leaf callus (MS medium + BAP 0.5 mg / l + NAA 2.0 mg / l + 2, 4-D 0.5 mg / l) at 6 weeks after culture

PLATE 28

Rhizogenesis from internodal callus (MS medium + BAP 0.5 mg / l + NAA 2.0 mg / l + 2, 4-D 0.5 mg / l) at 8 weeks after culture

sprouting, survival and malformed leaves are presented in table 35.

4.3.1.1 Bud break percentage

The percentage of bud break in control and 20 Gy treated population ranged from 60 to 90 at different stages of axillary bud explants. In 30 Gy, it ranged from 45 to 70 and in 40 Gy 10 to 30 percent. The bud break percentage range at different doses of gamma rays was as follows. At B_1 it was 20 to 60, at B_2 it was 30 to 70 at B_3 it was 20 to 80 at B_4 it was 10 to 90 and at B_5 it was 10 to 85. The lowest bud break percentage was recorded in 50 Gy followed by 40 and 30 Gy. No difference in bud break was observed in the control and in 20 Gy treatment when the explants were excised from B_1, B_2 and B_4 bud stages. However, in B_3 and B_5, 20 Gy treatment reduced the percentage of bud break. The irradiation with gamma rays at 30 Gy in B_3 bud stage resulted in 10 per cent increase in bud break over the 20 Gy exposure and 10 per cent decrease when compared to the control.

4.3.1.2. Survival percentage

Survival of the cultures three weeks after inoculation ranged from 0 to 90 per cent. At 50 Gy treatment, none of the cultures survived after three weeks. The survival percentage showed a progressive decline at higher

Table 35. Effect of gamma rays on percentage of bud break, survival and malformed leaves (cv. Folklore)

Treatments		Percentage of		
Bud stage (B)	Gamma ray(Gy) (D)	Bud break (Two weeks)	Survival (Three weeks)	Malformed leaves
Vegetative shoot (B_1)	D_0 00	60	60	00.00
	D_1 20	60	60	16.70
	D_2 30	45	40	37.50
	D_3 40	20	10	50.00
	D_4 50	00	00	00.00
Day of flower opening (B_2)	D_0 00	70	60	00.00
	D_1 20	70	70	21.43
	D_2 30	50	40	37.50
	D_3 40	30	15	33.33
	D_4 50	00	00	00.00
Two days after flower opening (B_3)	D_0 00	80	80	6.25
	D_1 20	60	55	27.27
	D_2 30	70	55	27.27
	D_3 40	30	10	50.00
	D_4 50	20	00	50.00
Four days after flower Opening (B_4)	D_0 00	90	90	00.00
	D_1 20	90	80	5.56
	D_2 30	70	55	6.25
	D_3 40	40	20	36.36
	D_4 50	10	00	00.00
Six days after flower Opening (B_5)	D_0 00	85	85	00.00
	D_1 20	80	60	13.33
	D_2 30	65	40	33.33
	D_3 40	30	10	50.00
	D_4 50	10	00	00.00

Number of cultures / treatment - 20

doses of gamma rays irrespective of the physiological stage of the bud. In the untreated popuation, 60 to 90 per cent survival was obtained as against 60 to 80 per cent in 20 Gy, 30 to 55 per cent in 30 Gy and 10 to 20 per cent in 40 gy.

4.3.1.3. Effective dose (ED_{50})

Dose (Gy)	Observed survival (%)	Adjusted survival (%)
0	85	100
20	60	71
30	40	59
40	10	12
50	0	0

$$Y = K + Ab^X$$

where Y is the survival percentage and X is the dose.

$$Y = 75.1143 - 4.1143 \times 3.9167^X$$

For 50 per cent survival the expected dose = 33 Gy.

4.3.1.4. Percentage of cultures with malformed leaves.

The percentage of survived plants showing mal formation of leaves ranged from 0 to 50. Among the untreated population only in B_2 bud stage 6.25 percent of cultures showed malformation of leaves.

The percentage of cultures with malformed leaves ranged from 5.56 to 27.27 in 20 Gy (plate 30), 6.25 to 37.50 in 30 Gy, (plate 31) 33.73 to 50 per cent in 40 Gy (plate 32) treatments respectively. The differences in percentage of malformed leaves were also noticed at different bud stages. At B_1 it was 0 to 50, B_2 0 to 37, B_3 6.25 to 50 and at B_4 5.56 to 36.36.

4.3.1.5. Days to bud break

The statistical analysis of the data revealed that the effect of gamma ray exposure varied, depending on the bud stage. ~~The number of~~ (table 36). The main effect of bud stages and gamma rays were also significant. The mean values of days to bud break ranged from 3.72 to 10 days. Early bud break of 3.72 days was observed in gamma irradiation of buds of B_4 bud stage at 20 Gy, which was significantly superior to other treatments.

Generally, higher doses of gamma rays delayed bud break. However, 20 Gy gamma irradiation induced early bud break except at B_3 bud stage. A significant difference in days to bud break was recorded in B_1 bud stage when irradiated at 40 Gy. Comparison of the main effects revealed that B_4 bud stage showed significant superiority over other stages to induce early bud break, and comparison of the dose

Effect of Gamma irradiation on initial culture establishment in cv. Folklore (three weeks after culture)

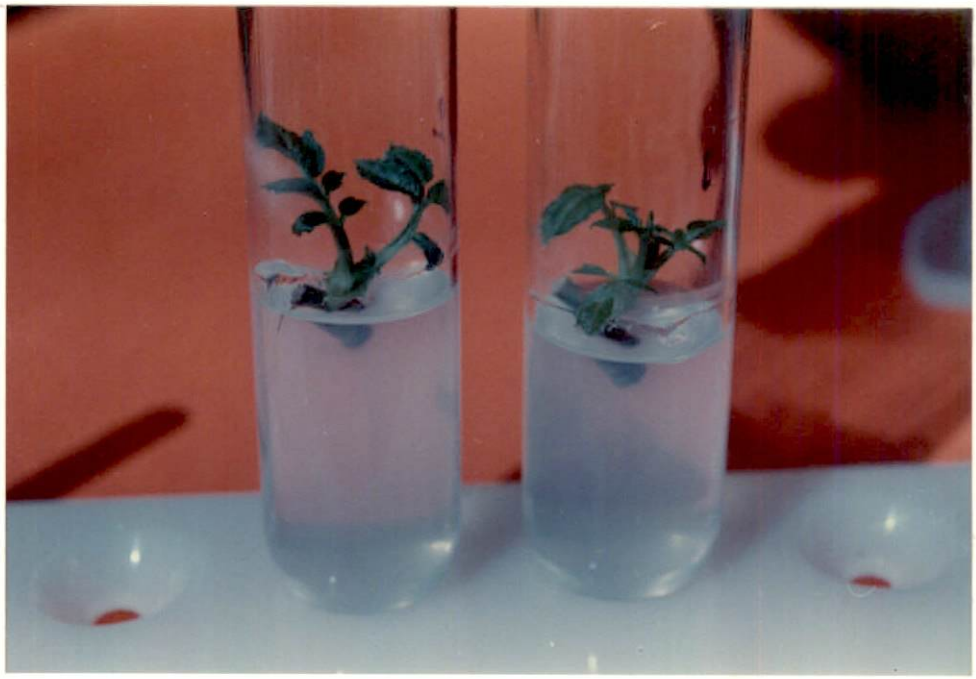
PLATE 29

Control

PLATE 30

20 Gy

1. Control **2 & 3. Treated**



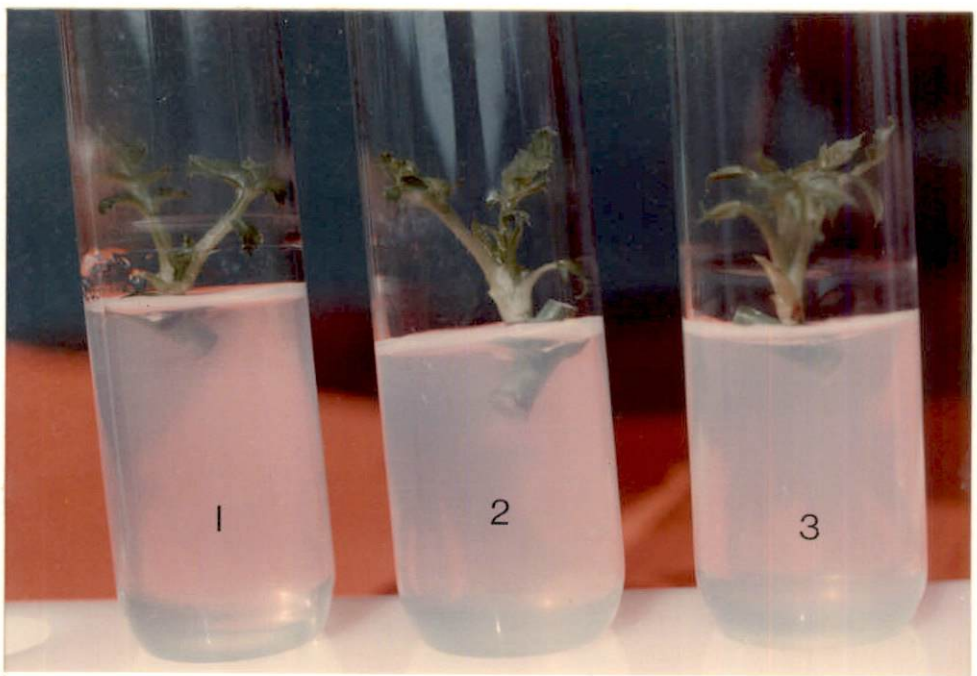

1
2
3

Table 36. Effect of gamma rays on days to bud break (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)				Mean
	0(D_0)	20(D_1)	30(D_2)	40(D_3)	
B_1	7.83	7.33	8.89	10.00	8.51
B_2	5.79	5.71	6.55	7.83	6.42
B_3	5.00	5.50	6.31	7.67	6.12
B_4	4.39	3.72	4.50	8.00	5.15
B_5	4.88	4.44	5.46	7.67	5.61
Mean	5.58	5.74	6.34	8.23	

'F' value 'B'= 141.16* D = 128.07* BxD = 5.71*

* Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

effect showed control and 20 Gy treatment were on par with each other for early bud break and significantly superior to other treatments.

4.3.1.6. Days to first leaf emergence

The data on days to first leaf emergence presented in table 37 revealed significant interaction between bud stages and gamma rays. The main effects of bud stages and doses of gamma rays were also found to be significant.

Early leaf emergence was noticed in 20 Gy gamma rays at B_4 bud stage. At all the bud stages highest dose of gamma rays (40 Gy) delayed leaf emergence whereas the lowest dose (20 Gy) stimulated early emergence except in B_3 bud stage. The bud collected in the B_4 stage showed significant superiority for earliness in leaf emergence (10.53 days). In the case of dose effect it was found that 20 Gy gamma ray irradiation and control plants were on par and significantly superior to other treatments.

4.3.1.7. Percentage of cultures inducing multiple shoot

The effect of gamma rays on induction of multiple shoot are presented in table 38. The percentage of cultures with multiple shoot ranged from 33.33 to 94.44. The highest percentage was noticed at B_4 bud stage when irradiated at 20 Gy gamma rays (94.44). The highest percentage of multiple

Table 37. Effect of gamma rays on days to first leaf emergence. (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)			
	0(D_0)	20(D_1)	30(D_2)	40(D_3)
B_1	12.50	11.75	13.00	15.50
B_2	10.93	10.71	13.09	15.67
B_3	10.00	10.58	11.77	14.50
B_4	9.44	8.44	11.00	13.25
B_5	10.06	10.00	12.00	14.33
Mean	10.59	10.30	12.17	14.65

'F' value 'B'= 57.64* D = 197.15* BxD =3.56*

* Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flowe

B_5 - Six days after flower opening

Table 38. Effect of gamma rays on induction of multiple shoots (%) (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)			
	0(D_0)	20(D_1)	30(D_2)	40(D_3)
B_1	83.33	83.33	66.67	50.00
B_2	85.71	78.57	81.82	50.00
B_3	87.50	83.33	76.92	50.00
B_4	88.89	94.44	85.71	62.50
B_5	82.35	81.25	69.23	33.33

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

shoot induction was recorded in control except in B_4 bud stage. At the highest dose of 40 Gy, the highest multiple shoot was observed at B_4 (62.50) and the lowest at B_5 (33.32) bud stage.

4.3.1.8. Days to induce multiple shoots.

The statistical analysis of data on days to induce multiple shoot presented in table 39 revealed that interaction of bud stage and gamma ray treatment was significant.

Early induction of multiple shoot was noticed under 20 Gy gamma rays at B_4 bud stage which was significantly superior. Among the different treatments the days to multiple shoot induction ranged from 21.41 to 60 days. Irradiation of gamma rays at 20 Gy induced early multiple shoot at B_1, B_3 & B_4 bud stages. Higher doses of gamma rays delayed multiple shoot induction. Comparison of the main effects of the bud stages revealed that for early multiple shoot induction B_4 bud stage was significantly superior to other stages and took only 27.75 days whereas 40 gy treatment took as high as 50.06 days.

4.3.1.9. Number of shoots per culture

The data on number of shoots produced per culture are presented in table 40. The results revealed significant

Table 39. Effect of gamma rays on days to induce multiple shoots (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)				Mean
	0(D_0)	20(D_1)	30(D_2)	40(D_3)	
B_1	33.10	22.60	34.50	60.00	37.55
B_2	29.67	31.45	32.78	49.00	37.73
B_3	28.07	26.20	30.20	45.00	32.37
B_4	26.19	24.41	27.42	38.80	29.21
B_5	33.71	34.08	35.67	57.50	40.24
Mean	30.15	27.75	32.11	50.06	

'F' value 'B'= 157.10* D = 415.19* BxD = 18.32*

* Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

Table 40. Effect of gamma rays on number of shoots per culture (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)				Mean
	0(D_0)	20(D_1)	30(D_2)	40(D_3)	
B_1	3.40	3.50	3.30	2.50	3.18
B_2	4.58	4.73	3.67	1.67	3.66
B_3	5.21	5.00	4.40	2.67	4.32
B_4	5.31	5.59	4.33	2.80	4.51
B_5	4.50	4.31	3.78	1.50	3.52
Mean	4.60	4.67	3.90	2.23	

'F' value 'B'= 20.65* D = 41.16* BxD = 1.86*

* Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

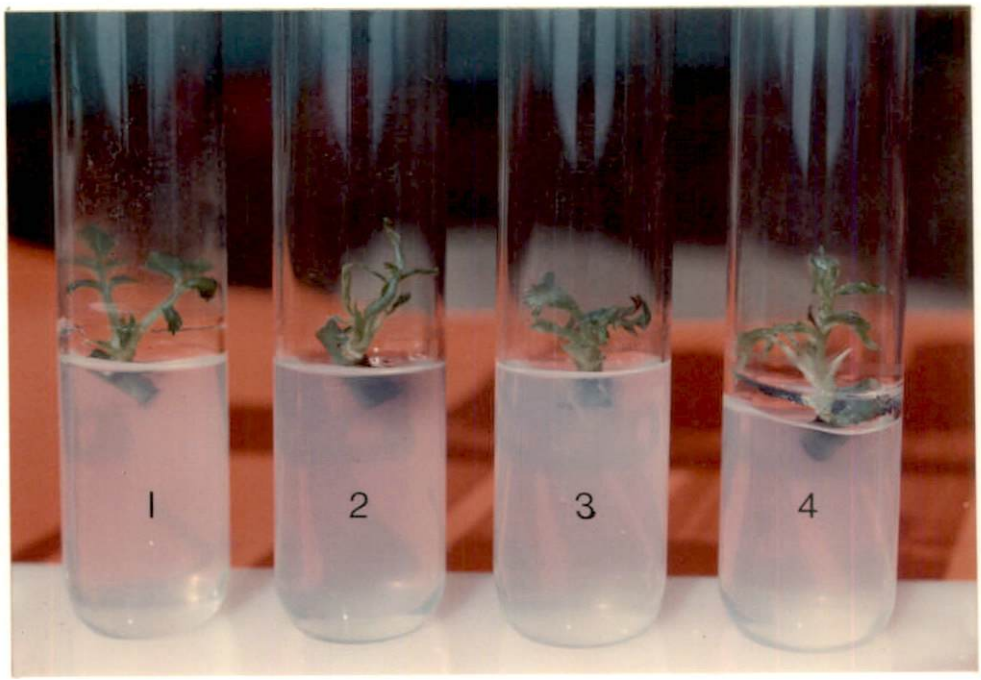
PLATE 31

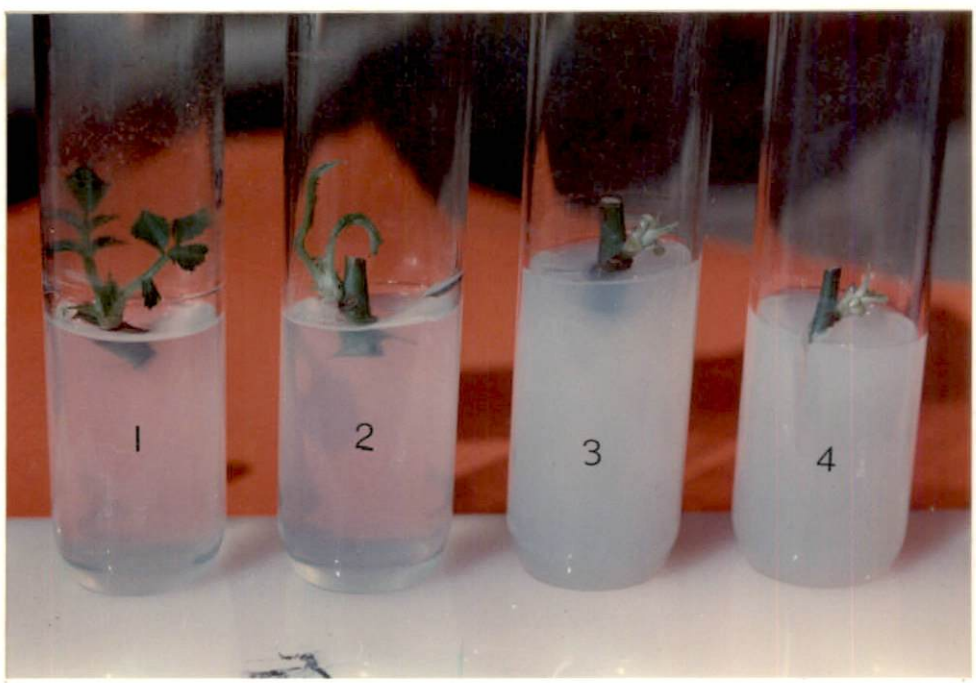
1. Control 2, 3 & 4. Treated

PLATE 32

40 Gy

1. Control 2, 3 & 4. Treated


1
2
3
4


1
2
3
4

difference in interaction between bud stages and gamma rays. The main effects of bud stages and gamma rays were also significant. The maximum number of shoots per culture were produced in the 20 Gy treated population at B_4 bud stage.

4.3.1.10. Cultures with morphological variation

The observations recorded on cultures with morphological variations are presented in table 41. The results indicated that percentage of morphological variations ranged from 0 (B_1 D_0) to 50 (B_1 D_3). Control population also showed slight variation ranging from 0 (B_1 B_5) to 7.14 per cent (B_2). But gamma rays exposure induced higher variation ranging from 8.33 (B_1 D_1) to 50 (B_1 D_3). The type of variations observed were shoots with yellowing of leaves (plate 33), enlarged leaf blade (plate 34), deformed leaflets (plate 35), and chlorophyll deficient leaves (plate 36). Except at B_5 bud stage maximum variation was observed in the highest dose. Highest variation was recorded in 30 Gy at B_5 bud stage. No variation was noticed in the untreated population at B_1 and B_5 bud stages.

4.3.1.11. Growth analysis of microshoots

i) Main shoot

Growth measurements recorded at two weeks interval are presented in Appendix XIV. The interaction between bud

Table 41. Effect of gamma rays on inducing morphological variations in culture (%) (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)			
	0(D_0)	20(D_1)	30(D_2)	40(D_3)
B_1	0.00	8.33	22.22	50.00
B_2	7.14	21.43	18.18	33.33
B_3	6.25	16.66	15.38	33.33
B_4	5.56	16.66	14.29	25.00
B_5	0.00	12.50	23.07	16.66

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

Effect of Gamma irradiation on shoot proliferation (cv. Folklore)

Morphological variations

PLATE 33

Yellowing of leaf

PLATE 34

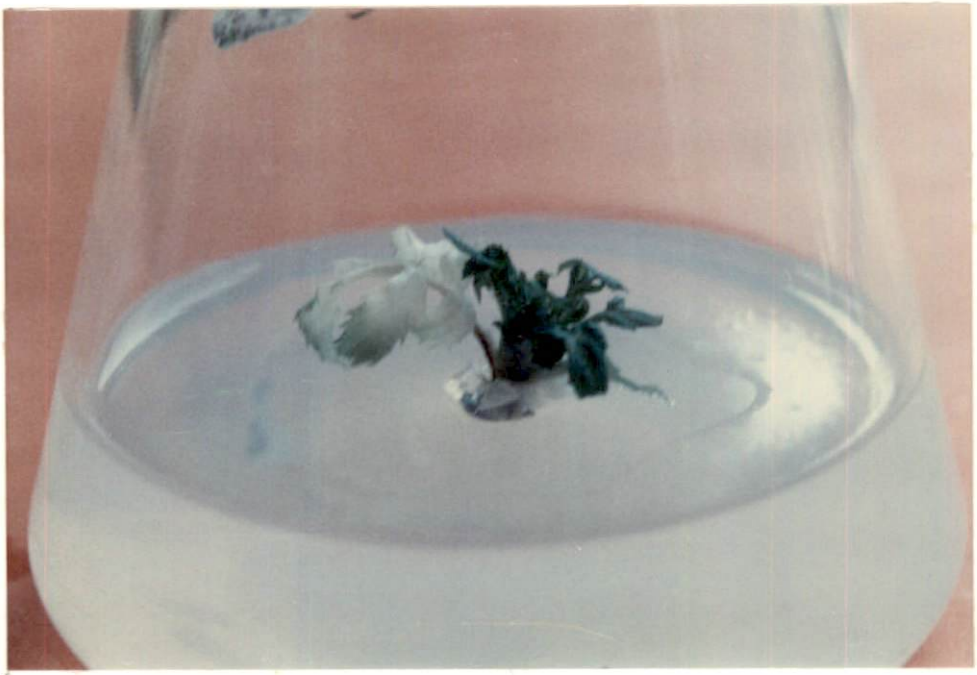
Enlarged leaf blade

PLATE 35

Deformed Leaflets

PLATE 36

Chlorophyll deficiency of leaf



stages and gamma rays at different stages of growth showed significant differences between them (Table 42).

The growth of the mainshoot at two weeks and four weeks after culture showed significant difference in B_5 (1.98 cm), and B_4 (1.90 cm) among the untreated group. Six and eight weeks after culture maximum shoot length (3.36 and 4.04 cm) recorded in B_4 bud stage of control population which was significantly superior to all other treatments.

The interaction between bud stages and gamma rays indicated significant differences with respect to growth of the main shoot. The control population at B_4 bud stage (2.99 cm) was found to be significantly superior and on par with 20 Gy treated population (2.87 cm) of same bud group followed by control and 20 Gy treated population of B_5 bud stage (2.81 cm).

Comparison of gamma rays and growth period interaction on growth of main shoot revealed significant difference. The shoot length was significantly superior, eight weeks after culture in 20 Gy treatment (3.12 cm) and on par with control group (3.10 cm).

The main effect of bud stages on growth of main shoot revealed that B_4 bud stage (2.42 cm) was significantly superior and on par with B_5 bud stage (2.33 cm). It was

Table 42. Interaction effect between bud stages and gamma rays on growth at two weeks interval (cv. Folklore) i Main shoot

Bud stages (B)	Gamma rays (Gy) 0	20	30	40	Culture period (weeks) 2	4	6	8	Mean
B_1	1.44	1.63	1.45	1.22	0.87	1.34	1.64	1.89	1.43
B_2	2.23	2.32	2.21	1.56	1.33	1.89	2.35	2.75	2.08
B_3	2.59	2.50	2.52	1.28	1.52	2.07	2.50	2.80	2.22
B_4	2.99	2.87	2.47	1.38	1.62	2.19	2.70	3.19	2.42
B_5	2.81	2.81	2.44	1.25	1.57	2.26	2.61	2.87	2.33
Mean	2.41	2.42	2.22	1.34	1.38	1.95	2.36	2.70	
Culture period									
2	1.62	1.60	1.46	0.84	CD	B 0.11	BxD = 0.22		
4	2.22	2.24	2.07	1.26		D 0.10	BxP = 0.07		
6	2.69	2.73	2.48	1.52		P 0.03	DxP = 0.06		
8	3.10	3.12	2.85	1.72			BxDxP = 0.14		

Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

evident that 20 Gy gamma rays had a stimulatory effect on growth of mainshoot (2.42 cm) which was significantly superior and on par with control (2.41 cm)

ii) Side shoot

The analysis of the data (table 43) on growth rate of the side shoots recorded at two weeks interval revealed significant interaction between bud stages and gamma rays at different stages of growth.

Two weeks after culture, the growth of side shoot was significantly superior in B_5 bud stage of untreated group (1.32 cm) where as in four weeks after culture, both control and 20 Gy treated population of B_4 and B_5 bud stages were significantly superior and on par, compared to other treatments. At the sixth week, B_5 bud stage of untreated population alone (2.62 cm) was found to be significant, however, the eight weeks growth of side shoot in the B_4(3.10) and B_5 (3.06) bud stages of untreated population were found to be superior and on par with each other. Growth of side shoot recorded maximum value in B_3 bud stage at eight weeks after culture (2.67cm) which was significantly superior to other bud stages.

Comparison of the effect of gamma rays on growth of side shoot at different periods indicated the maximum growth

Table 43. Interaction effect between bud stages and gamma rays on growth at two weeks interval (cv. Folklore)

Bud stages (B)	Gamma rays (Gy) 0	20	30	40	Culture period (weeks) 2	4	6	8	Mean
B_1	1.39	1.47	1.36	0.93	0.77	1.16	1.48	1.74	1.29
B_2	1.64	1.69	1.68	1.07	0.82	1.40	1.73	2.14	1.52
B_3	1.87	1.95	1.56	1.08	0.86	1.43	1.90	2.67	1.61
B_4	2.04	1.93	1.46	0.81	0.84	1.40	1.77	2.22	1.57
B_5	2.26	1.95	1.79	0.76	0.93	1.49	1.93	2.40	1.69
Mean	1.84	1.80	1.57	0.93	0.84	1.37	1.76	2.15	
Culture period (weeks)									
2	0.98	0.92	0.90	0.57	CD	B 0.09	B x D 0.20		
						D 0.09	B x P 0.10		
4	1.65	1.62	1.42	0.81		P 0.04	D x P 0.08		
6	2.10	2.07	1.78	1.10					
8	2.62	2.58	2.16	1.24					

Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

in eight weeks after culture in untreated population (2.62cm) followed by in 20 Gy treatment (2.58 cm). These were significantly superior to other treatments.

Significant differences were observed with regard to the main effect of bud stages. B_5 bud stage (1.69 cm) was significantly superior to other bud stages. The main effect of gamma rays revealed significant difference in maximum length of side shoot (1.84 cm) which was on par with 20 Gy treated population (1.80 cm). Significant reduction in shoot length was recorded at higher doses of gamma rays.

iii) Leaf production

Statistical analysis of the data (table 44) on the number of leaves produced in the multiple shoots at two weeks interval indicated significant interaction between bud stages and gamma rays.

The observations recorded two weeeks after culture showed significant difference in seven treatments. The maximum number of leaves were produced in B_4 stage at 20 Gy treatment (5.8) followed by B_3 bud stage (5.6), control population of B_3 bud stage (5.6), B_4 bud stage (5.6) B_5 bud stage (5.4) and finally B_5 bud stage of 20 Gy treated population (5.2), which were significantly superior and on par with each other.

Table 44. Interaction effect between bud stages and gamma rays on leaf production at two weeks interval (cv. Folklore)

Bud stages (B)	Gamma rays (Gy)				Culture period (weeks)				Mean
	0	20	30	40	2	4	6	8	
B_1	6.70	7.10	6.60	3.65	3.40	5.50	7.30	7.85	6.01
B_2	8.55	6.40	6.60	4.45	3.90	6.30	7.50	8.30	6.50
B_3	8.60	8.55	7.45	3.95	4.55	6.95	8.15	8.90	7.41
B_4	8.95	8.90	8.15	4.25	4.80	7.25	8.65	9.55	7.56
B_5	8.75	8.70	7.30	3.75	4.35	7.10	8.10	8.95	7.13
Mean	8.31	7.93	7.22	4.01	4.20	6.62	7.94	8.71	
Culture period (weeks)									
2	5.16	5.00	4.12	2.52					
4	7.88	7.56	7.08	3.96					
6	9.64	9.28	5.12						
8	10.56	9.88	9.28	5.12					

B = 0.50 B x D = 1.00
D = 0.45 B x P = 0.38
P = 0.17 D x P = 0.34
B x D x P = 0.76

Significant at 5 per cent level

B_1 Vegetative shoot
B_2 Day of flower opening
B_3 Two days after flower opening
B_4 Four days after flower opening
B_5 Six days after flower opening

Four weeks after culture, the treatments viz., 20 Gy treated population of B_2 (8.6), B_4 (8.6) and B_5 (8.4) bud stages were significantly superior and on par. Six weeks after culture, the 20 Gy treated population of B_4 and B_5 bud stages (10.2), control population of B_4 (10.2) and B_5 (10.0) bud stages, 20 Gy treated population of B_3 bud stage, control population of B_2 and B_3 bud stages (9.8) and 30 Gy treated population of B_4 bud stage (9.6) were significantly superior and on par with each other.

Comparison of the means for interaction between bud stages and gamma rays revealed that in the control group all the stages of bud except B_1 bud stage, in 20 Gy treated population except B_1 and B_2 bud stages and in 30 Gy treated population B_4 bud stage were significantly superior and on par with each other for leaf production. Significant difference in leaf production was observed in B_4 bud stage (10.56) of control group in eight weeks after culture.

The main effect of bud stages on leaf production indicated that the bud stages B_4 (7.56), B_3 (7.14) and B_5 (7.13) were significantly superior and on par with each other. The dose effect of gamma rays clearly indicated a reducing trend in leaf production. The control population recorded 8.31 and it was on par with value of 7.93 in 20 Gy treatment.

At higher doses of 30 Gy and 40 Gy, a progressive decline in rooting was observed. Gamma rays at 40 Gy reduced the rooting percentage to as low as 20 per cent at B_1, bud stage and it was 40 per cent in B_5 and 60 per cent in B_2, B_3 and 66 per cent in B_4 bud stages respectively.

4.3.1.10. Percentage of culture induced rooting.

The variations induced on rooting percentage by gamma rays are presented in table 45. Among the different treatments, the rooting percentage varied between 20 and 83.33 per cent. There was no statistical difference in rooting percentage in 20 Gy treatment and control except in B_3 bud stage. At B_3 bud stage, the 20 Gy treated population had a higher rooting percentage than the control.

4.3.1.11. Days to induction of rooting.

The effect of gamma rays on days to rooting of cultured shoots is detailed in table 46. Statistical analysis of data revealed significant differences for interaction between bud stages and gamma rays. The main effects of bud stages and gamma rays also showed significant differences. Early root induction observed at B_1 bud stage was significant and on par with B_4 and B_3 bud stages.

Table 45. Effect of gamma rays on rooting percentage of cultured shoots (cv. Folklore)

Bud stages (B)	Gamma rays (Gy)			
	0(D_0)	20(D_1)	30(D_2)	40(D_3)
B_1	66.67	66.67	50.00	20.00
B_2	75.00	75.00	66.67	60.00
B_3	75.00	83.33	75.00	60.00
B_4	83.33	83.33	66.67	66.00
B_5	83.33	83.33	75.00	40.00

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

Table 46. Effect of gamma rays on days to initiate rooting of cultured shoots (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)				Mean
	0(D_0)	20(D_1)	30(D_2)	40(D_3)	
B_1	15.50	15.00	18.83	28.00	15.83
B_2	13.44	13.00	16.00	31.00	18.36
B_3	10.78	13.20	15.78	28.00	16.94
B_4	10.60	11.00	13.63	32.00	16.81
B_5	10.50	10.40	16.78	33.00	17.60
Mean	12.16	12.52	16.20	30.40	

'F' value 'B'= 9.71* D = 240.10* BxD =3.93*

* Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

Untreated population induced rooting significantly early and on par with 20 Gy treatment. The best dose for early induction of rooting was 20 Gy at B_5 bud stage. Root induction was noticed in B_1, B_2 and B_5 bud stages at 20 Gy gamma irradiation. The higher doses of gamma rays delayed rooting upto 33 days in B_5 bud stage and 32 days at B_4 bud stage.

4.3.1.12. Number of roots per shoot.

The data on the number of shoots per culture presented in table 47 revealed that the difference in treatment means for dose, bud stages and their interactions were significant. The number of roots per shoot ranged from 2.5 to 7.44. The maximum roots were recorded in untreated buds at B_3 bud stage. The root number was enhanced in 20 Gy gamma radiation at B_2 and B_5 bud stages. Exposure to the buds at 40 Gy reduced root number irrespective of stages of bud.

Comparison of bud stages revealed significant difference in number of roots produced at B_3 bud stage which was on par with B_4, B_5 and B_2 bud stages. Analysis of effect of gamma rays indicated that there was a progressive decline in number of roots with increasing doses of gamma rays. Significantly high number of roots was produced in control and was on par with 20 Gy treatment.

Table 47. Effect of gamma rays on number of roots per shoot (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)				Mean
	0(D_0)	20(D_1)	30(D_2)	40(D_3)	
B_1	5.25	3.50	5.17	3.00	4.23
B_2	5.89	6.33	4.78	3.00	4.90
B_3	7.44	6.70	4.89	2.67	5.43
B_4	6.40	5.70	4.50	3.33	4.98
B_5	6.30	6.40	4.44	2.50	4.91
Mean	6.26	5.73	4.68	2.90	

'F' value 'B'= 4.03* D = 25.25* BxD =2.41*

* Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

4.3.1.13. Length of the longest root

The data on the length of the longest roots are presented in table 48. The statistical analysis of data indicated that there was no significant difference in the interaction between bud stages and the gamma rays with respect to the root length. The main effects of bud stages and gamma rays were found to be significant. Among the different bud stages, the root length ranged from 1.62 to 1.97 cm. The best bud stage to produce longest roots was found to be B_2 bud stage which was significantly superior and on par with B_4 and B_1 bud stages.

In the different doses of gamma ray treatment, the root length ranged from 1.24 to 2.09 cm. Maximum root length was recorded in the untreated plants (2.09 cm) which was on par with the root length of 20 and 30 Gy treated materials. At the highest dose of 40 Gy, significant reduction in root length was noticed.

4.3.2. Irradiation of mircoshoots (Multiple shoot)

The effect of gamma irradiation of multiple shoots on percentage of survival, multiple shoot induction and morphological variations on subculturing after irradiation are given in table 49.

Table 48. Effect of gamma rays on the length of root (cv. Folklore)

Bud stage (B)	Gamma rays (Gy)				Mean
	0(D_0)	20(D_1)	30(D_2)	40(D_3)	
B_1	2.26	2.24	1.83	0.80	1.78
B_2	2.09	2.09	2.04	1.67	1.97
B_3	2.08	2.05	1.58	1.10	1.70
B_4	2.02	1.92	1.74	1.47	1.79
B_5	1.98	1.73	1.60	1.15	1.62
Mean	2.09	2.01	1.76	1.24	

'F' value 'B'= 3.21* D = 19.61*

* Significant at 5 per cent level

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

Table 49. Effect of gamma irradiation of multiple shoots on survival induction of multiple shoot and morphological abnormalities on subculturing (cv. Folklore)

Sl. No.	Gamma Ray (Gy)	Number of cultures	Survival (Four weeks after culture		Cultures with Multiple shoots		Morphological abnormalities	
			Number	Percentage	Number	Percentage	Number	Percentage
1	00	15	15	100.00	15	100.00	0	00.00
2	10	24	23	95.83	22	91.67	4	17.39
3	20	22	19	86.36	13	59.09	6	31.59
4	30	26	16	61.53	8	30.77	7	43.75
5	40	28	11	39.29	5	17.86	5	45.45

The survival rate of treated multiple shoots on subculturing ranged from 39.29 to 100 per cent. The control population recorded cent per cent survival whereas the higher doses of gamma rays reduced the survival rate and the highest dose of 40 Gy recorded the lowest survival rate of 39.29 per cent.

The same trend was noticed regarding the multiple shoot also. The percentage of cultures inducing multiple shoots ranged from 17.86 to cent per cent. The lowest being in highest dose of gamma rays and the highest being in the control.

The percentage of cultures having morphological abnormalities ranged from 17.39 to 45.45 per cent. The untreated plants had no morphological abnormalities. The highest percentage was recorded in the highest dose of gamma ray. At the highest dose of gamma rays, multiple shoots were produced with narrow leaves (plate 37), deformed striated leaves (plate 38) deformed leaves with prominant striations, (plate 39) deformed leaves with foliar petiole (plate 40) and deformed leaves with chlorophyll deficiency.

The influence of gamma irradiation on microshoots in terms of days to multiple shoot induction, number of shoots per culture in three subsequent stages of subculturing are presented in table 50.

Effect of Gamma irradiation on multiple shoots (cv. Folklore)

Foliar deformities

PLATE 37

Narrow leaves

PLATE 38

Deformed striated leaves

PLATE 39

Deformed leaves with prominant striations

PLATE 40

Deformed leaves with foliar petiole

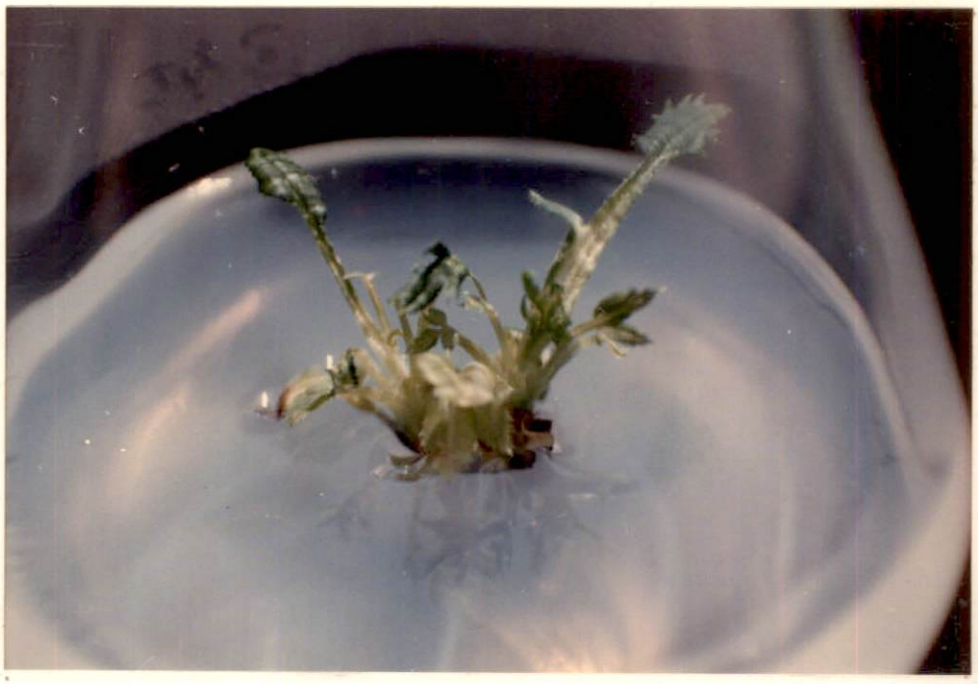


PLATE 41

Deformed leaves with chlorophyll deficiency

PLATE 42

Plantlet showing altered pigmentation on leaf

1. Mutant 2. Control

Table 50. Effect of gamma rays on days to multiple shoot induction and number of shoots per culture on subculturing (cv. Folklore)

Sl. No.	Dose (Gy)	Days to initiate multiple shoots	Shoots/subcultures		
			Subculture 1	Subculture 2	Subculture 3
1	00	27.0	5.0	5.4	5.2
2	10	26.6	5.4	5.8	5.6
3	20	29.0	3.2	3.0	2.0
4	30	35.2	2.4	1.8	1.4
5	40	44.6	1.6	1.4	1.0
CD		3.30	1.33	1.82	1.5

Number of cultures per treatment 5

Significant at 5 per cent level

Significant differences were found between the treatments with respect to the days to initiate multiple shoots. The early induction of multiple shoot (26.6 days) was recorded in the 20 Gy gamma treatment which was on par with the control and significantly superior to other treatments.

At each stage of subculturing, the number of shoot per culture recorded significant difference in respect of different doses of gamma rays. In the first subculture treatment of 10 Gy, highest number of 5.4 shoots/culture was recorded which was followed by control. These two treatments were on par and significantly superior to higher doses of gamma rays.

The same trend was observed in both the second and third subcultures. 10 Gy irradiation produced the maximum number of shoots per culture.

Gamma rays induced variation in the rooting efficiency of microshoots. The results are presented in Table 51.

The rooting behaviour was analysed in terms of days to root initiation, number of roots produced per shoot and length of the longest root.

Table 51. Effect of gamma irradiation of multiple shoot on rooting efficiency (cv. Folklore)

Sl. No.	Gamma ray (Gy)	Days to initiate roots	Number of roots per shoot	Length of longest root (cm)
1	00	10.2	8.2	3.20
2	10	9.4	7.2	3.14
3	20	9.6	6.2	4.50
4	30	11.6	6.0	3.18
5	40	14.2	3.0	2.02
CD		1.59	2.99	0.71

Significant at 5 per cent level

Significant differences were observed between treatments with respect to the number of days taken for initiation of roots. The 10 Gy gamma irradiation treatment recorded earliest initiation of roots, followed by 20 Gy treatment and control. They were on par and were significantly superior.

The number of roots per shoot showed significant difference and ranged from 3.0to 8.2. Lowest number of '3' was observed in 40 Gy treatment and was significantly inferior to all other treatments.

Analysis on growth rate of irradiated microshoots are presented in table 52.

Statistical analysis of the data revealed significant interaction between dose of gamma rays and growth at diffetent stages. The growth rate at two weeks after culture ranged from 1.04 to 1.94 cm. The growth rate was significantly reduced at higher doses of gamma ray exposure. Maximum growth rate was observed in control group (1.94) which was significantly superior and on par with lower doses of treatment 10 Gy (1.90) and 20 Gy (1.74).

Significant differences were observed under the diferent doses of gamma ray treatments for growth at four weeks after culture. It ranged from 1.60 to 2.58 (cm). The

Table 52. Growth analysis of irradiated micro shoots at two weeks intervals (cv. Folklore)

Sl. No.	Gamma rays (Gy)	Shoot length (cm) after culture				Mean
		2 weeks	4 weeks	6 weeks	8 weeks	
1	00	1.94	2.44	3.22	4.14	2.94
2	10	1.90	2.58	3.22	4.22	3.00
3	20	1.74	2.40	2.78	3.52	2.61
4	30	1.52	2.06	2.82	3.24	2.41
5	40	1.04	1.60	2.08	2.68	1.85
	Mean	1.63	2.22	2.84	3.56	
	CD	D=0.24	P=0.12	PxD=0.27		

Significant at 5 per cent level

highest shoot growth (2.58 cm) was recorded at the lowest dose of gamma rays (10 Gy) followed by control and 20 Gy exposure. Three treatments were on par and significantly superior to higher doses.

Six weeks after culture, the shoot length was uniform (3.22 cm) in both control and 10 Gy population. Those two treatments were significantly superior to other treatments. A reduction in shoot length was observed at the highest dose.

The same trend was observed eight weeks after culture. Highest shoot length (4.22 cm) was noticed in control followed by 10 Gy (4.14 cm) treatment. These two treatments were on par and significantly superior to other treatments. Significant differences in growth rate were recorded between 20, 30 and 40 Gy exposures.

DISCUSSION

5. DISCUSSION

5.1. INDUCED MUTATION ADOPTING *IN VIVO* CULTURE

Roses are less amenable to conventional breeding methods due to high heterozygosity and complex genetic constitution. Hence induced mutation assumes special significance in the improvement of rose as in other seed/vegetatively propagated plants. Besides in an ornamental crop like rose any change that gives a novel flower colour or form is more acceptable to growers. As in majority of ornamental crops mutations of interest, once recovered can be maintained by means of vegetative propagation in this crop.

5.1.1. Time taken for bud take

One of the direct effects of gamma rays was inhibition of sprouting of buds. Irrespective of bud stages and cultivars, irradiated buds took longer period to sprout compared to the control. Lowest dose of 20 Gy did not have much effect on inhibition of sprouting. Irradiation at 20 Gy had a stimulatory effect on early sprouting in two of the cultivars viz., 'Suraga' and 'Folklore' tested in the present investigation. commenced from the second fortnight in 'Suraga' and 'Folklore' at all the doses of exposures while

in cv. Alliance at 50 Gy irradiation sprouting initiated from third fortnight. Time taken for the bud take was dependent upon the dose of gamma rays. The radiation not only inhibited sprouting but also delayed the attainment of maximum sprouting. In 'Folklore' irradiation at 50 Gy in B_2 bud stage attained the maximum sprouting on 10th fortnight, but in B_1 and B_3 bud stages, the maximum sprouting was attained on 7th and 8th fortnight respectively. At 50 Gy level, irrespective of bud stages and cultivars, bud take was delayed.

The delay in sprouting of buds following gamma irradiation had been reported in rose by several workers including Gupta and Shukla 1970; Kaicker and Swarup, 1972; Lata, 1980; Gupta *et al.*, 1982 and Datta, 1986 a.

The sprouting of buds largely depends on the union of stock and scion tissue. The effective union is determined by the cambial activity of stock and scion. Snow (1933) demonstrated that indole acetic acid (IAA) was very effective in stimulating the meristematic activity of cambium. Auxins which are important for the meristematic activity of cells show a reduced concentration immediately after irradiation (Skoog, 1935; Gordon, 1954 and 1957). Radiations are known to hamper the auxin synthesis and may even lead to its complete destruction (Skoog, 1935; Smith and Kersten, 1942;

Sparrow *et al.*, 1952; Gordon, 1954, 1956,1957). Ionising radiations also inhibit cell division due to chromosome damage (Sparrow *et al.*, 1952 and 1961). The delayed sprouting obtained in this study may be due to either destruction of auxins or chromosomal aberrations induced by gamma rays or both.

Two cultivars showed higher percentage of sprouting compared to control in the second fortnight particularly in B_1 and B_3 bud stages by 20 Gy exposure. Lower doses of radiation might have produced certain chemical substances which are comparable to certain physiologically active substances as reported by Sax, 1955 and Gordon, 1956. The stimulatory effect observed in this study may be due to the activity of these substances which are released as a result of gamma irradiation. The physiological activity of the bud stages also might have influenced the stimulatory effect, as it was observed in B_1 and B_3 bud stages of both the cultivars. Gupta and Shukla (1970) also reported the stimulatory effect in sprouting at lower doses of gamma rays.

5.1.2. Sprouting

Varietal response to sprouting was evident due to the significant differences observed among cultivars. The bud stages had no recognisable role to play either with cultivars or with gamma rays, since there was no significant

interaction between them. Significant reduction in sprouting may be caused by irradiation irrespective of cultivars and bud stages. Drastic reduction in sprouting at highest dose of 50 Gy was observed in the present investigation as reported by several workers, Gupta and Shukla, 1970; Kaicker and Swarup, 1972; Gupta and Datta, 1982; Gupta et al., 1982 and Datta, 1985, 1986 a, 1987, 1988.

There was no significant interaction among cultivars and doses of gamma rays which indicated similar response of cultivars towards different doses of gamma rays. Increased bud break also was observed at 20 Gy treatment in some of the bud stages of all the three cultivars. The bud stages did not have any uniformity in the response. The direct effect of gamma rays on inhibiting sprouting of buds clearly indicated in the present investigation.

5.1.3. Survival

There was significant reduction in survival of plants at higher doses of gamma rays (30, 40 & 50 Gy) 6 months after budding. Percentage of survived plants decreased with increasing dose of gamma rays. Bud stages had no significant interaction either with cultivars or with gamma ray exposures in determining the survival of the plants. Mortality of irradiated buds may occur before

sprouting or at later stages of growth. Reduction in survival after irradiation has been reported in rose cultivars by Gupta and Datta, 1982; Gupta *et al.*, 1982; Guo *et al.*, 1983, Datta, 1985 and Mathew, 1989. It was also reported that the reduction in survival rate may be attributed to the change in auxin concentration and chromosomal aberrations caused by Gamma irradiation (Gordon, 1957; Sparrow, 1961)

5.1.4. Effective dose (ED_{50})

The effective dose of gamma rays for getting 50 per cent survival of plants six months after budding was estimated as 38 Gy. LD_{50} for various rose cvs. had been reported as 10 to 15 Gy (Heslot, 1964) 50 to 60 Gy (Gupta, 1966) 35.2 to 42.5 (Mathew, 1989). This shows the varietal sensitivity of rose cultivars to different gamma ray exposures.

5.1.5. Effect of gamma ray exposures on morphological characters

The gamma irradiation was found to have pronounced effect on the morphological characters of rose. The variations induced varied depending on cultivar, physiological stage of bud, dose rate and radio sensitivity. The effect of radiation was manifested in the form of inhibition, stimulation or morphological abnormalities.

The inhibition in growth was expressed in the form of reduction in plant height, number of branches, number of leaves and prickle density. The physiological activity of buds treated also showed interaction with cultivars for the variations showed in plant height, and number of branches. Reduction in plant height due to gamma irradiation had been reported by many workers (Gupta and Shukla, 1970; Kaicker and Swarup, 1972, 1978; Lata, 1980; Gupta and Datta, 1982; Gupta *et al.*, 1982; Datta, 1986, 1987 and 1988; Mathew, 1989).

The increase in plant height observed in cv. Suraga in B_1 bud stage (on the day of flower opening) bud stage and cv. Folklore in B_1 and B_2 (three days after flower opening) bud stages at 20 Gy gamma irradiation may be due to the stimulatory effect of the radiation at specific physiological stage of the buds. This sort of stimulatory effect was also observed with respect to number of branches, number of leaves and prickle density at 20 Gy. At higher doses of 30 and 40 Gy, there was significant reduction in the number of primary branches and plant height.

There was significant difference among cultivars for prickle density also. Gamma ray affected reduction in prickle density was confined to the highest dose of 40 Gy in cv. 'Suraga' and 'Folklore'. But in 'Alliance', it was observed only in B_2 bud stage. This indicated the influence

of bud stage and gamma rays on the expression of this character.

The gamma irradiation at 30 and 40 Gy reduced the number of leaves produced in all the three cultivars. The sensitivity of the cultivars to gamma rays was significant. Bud stages as such did not contribute to the variations. But the bud stages in combination with different doses of gamma rays had significant influence.

The reduction in growth after irradiation has been considered due to chromosome damage and mitotic inhibition (Gray, 1956). Reduction in growth rate can be explained as differential killing of meristematic cells resulting in reduction in cell multiplication. Growth inhibition was reported to be not only due to radiation effects on mitosis but also due to induced physiological changes (Sparrow, 1951 and Quastler *et al*, 1952). Auxin destruction (Skoog, 1935) or inhibition of auxin synthesis also leads to reduced growth (Gordon, 1957).

5.1.6. Abnormalities in leaves

Radiation induced various types of morphological abnormalities of varied types were more frequent during the early growth period. Such abnormalities in leaves had also been reported in rose by various other workers including

Kaicker and Swarup, 1972; Desai and Abraham, 1978 and Datta, 1982. The abnormalities are reported to be mainly due to the effect of radiation on cell division and auxins (Gordon, 1957).

Abnormalitites in leaves were observed in all the cultivars at 40 Gy. The induced abnormalities included leaves with smaller and narrow leaflets (Suraga, plate 6) and fused leaflets (Suraga, plate 6 and Alliance, plate 5). Unequal development of lamina (Alliance, plate 5) were observed in some plants at lower frequency. Chlorophyll deficiency was also observed in some leaves of cv. Alliance, plate 4.

5.1.7. Days to flowering

The variability among cultivars and physiological stages of the buds had significant influence on the days taken for flowering. Irrespective of these two factors gamma irradiation in higher doses delayed flowering. Delayed flowering as a result of radiation had been reported by Nishida *et al.* (1967) and Kaicker and Swarup (1972). The delay in flowering may be due to the delayed sprouting and inhibition of growth during the early period.

5.1.8. Flowers per plant

There was significant variation in the flower production capacity which was contributed by the differences

in varieties and various exposures of gamma rays. Higher doses of gamma rays, 30 and 40 Gy, significantly reduced the flower production. Maximum flower production was recorded at the lowest dose (20 Gy) followed by the control. The stimulatory effect at lower doses had also been reported in roses by many workers including Gupta and Shukla, 1970; Irulappan and Rao, 1981 and Mathew, 1989.

5.1.9. Variations in Floral characters

Among the different floral characters studied, there was no significant interaction between cultivars, bud stages and gamma rays for necklength. Neck length showed a significant reduction at the highest dose of 40 Gy. There was significant interaction between cultivars and bud stages for flower diameter. The bud stages, B_1 and B_2, were significantly superior to B_3 with respected to flower diameter. The higher doses of gamma rays, 30 and 40 Gy showed significant reduction. With regard to flower weight, the interaction between cultivars and gamma rays was significant. Exposures of 30 and 40 Gy significantly reduced flower weight. The petal weight was not significantly influenced by the interaction of different factors. Significant reduction in petal weight was observed at the highest dose (40 Gy).

The interaction between cultivars with bud stages and with gamma rays and bud stages with gamma rays were significant for petal number. Reduction in flower size as a result of radiation has also been reported in rose by other workers (Gaul, 1970; Gupta and shukla, 1970; Datta and Gupta, 1982 a, b and Mathew, 1989). However, Irulappan and Rao, (1981) and Desai and Abraham (1978) reported an increase in flower size at lower dose of gamma irradiation. In the present study, petal weight was on par among exposure of 30 and 20 Gy and the control.

5.1.10. Morphological abnormalities

Floral

The type of floral abnormalities observed in this study include flowers with altered colour and number of petals. A variant with light pink colour observed in 20 Gy treated population of cv. Alliance (plate 7) was not stable. However, a stable mutant with light red colour (plate 8) was detected among 30 Gy treated population of cv Folklore. A mutant with increased number of petals with reduced size was obtained from 40 Gy exposed population of the same cultivar (plate 10). In both the cases, budwoods were collected on the day of flower opening. Occurrence of abnormal flowers in rose following irradiation had been reported by several workers (Gupta and Shukla, 1970; Kaicker and Swarup, 1972; Gupta and Datta, 1982; Gupta *et al.*, 1982 and Mathew, 1989).

5.2. *IN VITRO* STUDIES IN ROSE

In vitro culture of higher plants under sterile conditions has shown spectacular development over the past three decades resulting in the production of viable plants of many species. In addition, since 1980 there has been an explosion in the genetic manipulation and bio-technology. Earliest report on *in vitro* propagation of rose cv. 'Forever Yours' was made by Skirvin and Chu (1979 b). Although the stages involved are the same, response of the cultivars to the nutrient and hormone components of the medium varies. *In vitro* culture coupled with induced mutagenesis is a novel approach for the production of desirable mutants. In the present investigations, carried out in the College of Agriculture, Vellayani, during 1989-93 attempts were made to standardise the *in vitro* culture methods viz., enhanced release of axillary buds, somatic organogenesis and somatic embryogenesis for rose cv. 'Folklore'. The most viable system was identified as enhanced release of axillary buds, and was used for induced mutagenesis. The results of the investigation are discussed in the following pages.

5.2.1. Standardisation of explants

The concentration as well as duration of treatment of the sterilant were highly dependent on the microbial status and sensitivity of the explant to the sterilant.

Mercuric chloride 0.1 per cent resulted in browning and high rate of the tissue, probably due to the toxicity of the sterilant at higher concentration. Rout *et al.* (1989 a, 1991) could successfully disinfect axillary bud, leaves and internode explants of rose ev. Landora with mercuric chloride 0.1 per cent for 25 minutes. This may probably be due to the varietal sensitivity to the sterilant. However, the surface sterilization of explants with ethanol (25 per cent) for 25 seconds followed by mercuric chloride (0.1 per cent) for 2 minutes, resulted in 90 per cent contamination free cultures (Bhat, 1992).

Among the different sizes of shoot tip and axillary bud explants tried for enhanced release of axillary buds, 1.0 cm length was found suitable to attain the highest response of 50 and 83 per cent respectively. The size of explants used were different by different workers. Shoot tips of 0.5 to 1.0 cm (Hasegawa, 1979) 1.5 to 2.5 (Khosh khui and Sink, 1982 c) and 1.0 to 1.5 cm (Podwysznka and Hempel, 1988) were used for initial culture establishment. Monaco *et al.* (1977) observed difficulty in the survival of explants when the explant size was small and cut surface : volume ratio is high.

For callus induction, internodal segments of 0.5 cm had the best response of 75 per cent and leaf discs of 1.0 cm, with petiole portion intact, produced cent per cent

callus induction. Rout *et al.* (1991) used internodal segments of 0.3 to 0.5cm and leaf discs of 0.5 x 1.0 cm size.

The physiological stage of the axillary bud explants was found to have a significant role in the initial culture establishment in terms of days to bud break, percentage of bud break and length of shoot three weeks after culture. When explants of shoots were taken four days after flower opening early bud break (4.44 days), the highest percentage bud break (80) and longest shoots (1.95 cm) resulted. Explants of vegetative shoot when used resulted in delayed bud break (8.93 days), reduced percentage of bud break (70) and reduced length of shoot (0.80cm). This may be because the buds below the apex that are suppressed due to apical dominance, get stimulated with the harvest of flower, and attain the maximum physiological activity by fourth day. The influence of the position of buds on the *in vitro* response was also reported by Bressan *et al.* (1982). However, Meredos and Rodriguez (1987) reported early shoot growth in buds excised from vegetative shoots and shoots of flower bud stage.

5.2.2. Standardisation of medium for initial culture esablishment

After standardising the surface sterilization procedures for obtaining a reasonably low percentage of

contamination rates and identifying optimum size and physiological stage of the axillary explants, efforts were made to standardise the culture medium.

Four main stages have been identified for the *in vitro* propagation of rose, namely culture establishment, multiplication of cultures, *in vitro* rooting and hardening and *ex vitro* establishment.

Although in a number of plants it was established that cytokinin can induce shoot formation auxin root formation, the mechanism of action of these two regulators at molecular level is completely unknown. The favourable effects of cytokinin in axillary bud breaking and multiple shoot production had been demonstrated by Murashige (1974). But at higher levels of cytokinin were proved to have deleterious effect on shoot growth. Auxins added to the medium help to nullify the suppressive effects of high cytokinin concentration (Lundergan and Janick, 1980).

Although shoots grown *in vitro* are capable of synthesising a small quantity of cytokinin, roots are the principal site of cytokinin biosynthesis (Koda and Okazawa, 1980). It is unlikely that shoot tip and axillary buds have sufficient endogenous cytokinin to support growth and development.

In the case of auxins, when large shoot tip explants of actively growing plants are used, exogenous auxins are not required in the initial establishment phase (Kusey *et al.*, 1980: Lundergan and Janick, 1980; Lane, 1979). However when explants of inactive stage or meristems of 0.4 cm or less are used exogenous auxin need to be supplied to the medium (Ziv, 1979; Evans, 1981; Jones *et al.*, 1977).

Four levels of cytokinin, BAP (1.0, 1.5, 2.0, 2.5 mg/l) were tried in combination with four levels of auxins (2, 4-D or NAA 0.25, 0.50, 0.75, 1.00 mg/l) on MS basal medium for initial culture establishment. Among the different hormone supplements, the best treatment identified was MS basal medium supplemented with BAP 2.5 mg/l + 2, 4-D 0.5 mg/l. This combination was most suitable to induce early bud break in four days and had highest percentage of bud break (80). At lowest concentration of BAP (1.0 mg/l) and 2, 4-D (0.25 mg/l), the days to bud break drastically increased upto 10 days. The bud break percentage (40) was also not encouraging. Early bud break was observed with increasing concentration of both BAP and 2, 4-D. However, in the highest concentration of 2,4-D along with highest concentration of BAP 2.5 mg/l bud break was delayed (5.0) and bud break percentage was also reduced to 60 per cent.

The stimulatory effect of 2, 4-D and cytokinin had been reported in other crop species also viz., Dactylics

glomerata and Festuca spp. (Dale, 1977 b), and Lolium multiflorium (Dale, 1977 a). The most potent auxin was 2, 4-D, which stimulates callus induction but at the same time strongly antagonised organised development. In the present study also, basal callusing was observed at lower levels of 2, 4-D. Hill (1967), and Engvild (1978) reported that higher levels of auxins, particularly 2, 4-D tended to suppress morphogenesis. The present results indicated that at higher level of BAP (2.5 mg/l) increasing the 2, 4-D concentration upto 0.5 mg/l had a favourable effect on initial culture establishement.

Among the BAP, NAA combinations BAP 2.0 mg/l + NAA 0.75 mg/l could induce early bud break in 4 days but budbreak percentage was reduced to 60. Considering both its earliness in bud break and bud break percentage, BAP 2.5 mg/l + 2, 4-D 0.5 mg/l holds good.

The favourable effect of IBA or NAA in combination with BAP had also been reported in Rosa hybrida (Skirvin and Chu, (1979 b) and Rose cultivar 'Joyfulness' (Pittet and Mancousin, 1982).

Cytokinin (BAP) tried in combination with Gibberellin (GA_3) did not give any favourable results compared to auxin cytokinin combinations. The best BAP and

GA_3 combination (BAP 2.0 mg/l + GA_3 0.5 mg/l) took 6 days for bud break. According to Krishnamoorthy (1981), GA_3 treatment enhanced the level of endogenous auxins in the plant, which might have suppressed the early release of axillary bud.

It can be concluded that cytokinin had a favourable effect on axillary bud release, which in combination with auxin had a stimulatory effect to induce early bud break. The concentration of cytokinin may vary with respect to the type and concentration of auxin added to the medium. When BAP 2.5 mg/l was supplemented to the medium 0.5 mg/l was the optimum concentration of the auxin 2,4-D to get the best response. When the auxin NAA was used BAP, 2.0 mg/l + NAA 0.75 mg/l was found to be most suitable combination to induce early bud break.

5.2.3. Standardisation of medium for shoot proliferation

Cytokinin is utilized to overcome the apical dominance of shoots and to enhance 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. BAP appeared to be the most effective cytokinin for stimulating shoot proliferation. The same results were also reported in other cultivars of rose by Hasegawa, 1979; Davies, 1980 and Skirvin and Chu, 1979 a.

At the highest concentrations of BAP/Kinetin (2.0 mg/l), early multiple shoot induction and higher number of shoots per culture was observed. Addition of BAP alone resulted in high proliferation rate in Rosa indica (Avramis, 1982a b). Similar observations were also reported in other crops viz., Anthurium andreanum (Kunisaki, 1980) Strawberry (Kartha *et al.*, 1980), Gypsophila paniculata (Kusey *et al.*, 1980), Golden Delicious apple (Lundergan and Janick, 1980), Phaseolus vulgaris (Kartha *et al.*, 1981) and Santalum sp (Barlass *et al.*, 1980).

The addition of GA_3, to medium supplemented with BAP induced early multiple shoot formation particularly in combination with BAP 2.0 mg/l + GA_3 0.75 mg/l which recorded 91.7 per cent cultures with multiple shoots in 23 days. The same treatment also produced the maximum number of shoots (6.2) and elongated shoots per culture (3.60). This treatment was on par with kinetin 2.0 mg/l + GA_3 1.0 mg/l, which induced multiple shoot, in 23.4 days and produced 6.8 shoots and 3.0 elongated shoots per culture.

Cytokinin in combination with GA_3 was found to have pronounced effect on shoot proliferation compared to the individual application of any one of the cytokinin. When cytokinin alone was applied, the number of shoots per culture was reduced, in comparison to the combined effect. The shoot length was also reduced (less than 3 cm).

Rout *et al.* (1990) observed that the cultivars and species showed considerable variation with respect to the growth regulator requirement and rate of multiplication. Valles and Boxus (1987) obtained enhanced axillary branching by the addition of GA_3 1.0 mg/l. Hasegawa (1980) reported inhibition of shoot proliferation in rose with the addition of GA_3 at different concentrations. The better results obtained in the present study indicated an additive or synergistic effect of cytokinin and GA_3 on shoot multiplication. Improved shoot proliferation was also reported in combination with kinetin and GA_3 in rose (Barve *et al.*, 1984). However, Rout *et al.* (1989 a) in *Rosa hybrida* cv. Landora observed the highest number of shoots per culture in lower concentrations of BAP (0.5 mg/l) + GA_3 (0.25 mg/l), Cai *et al.* (1984) also observed effective shoot proliferation when a combination of BAP and GA_3 was used. A combination of BAP and GA_3 was also successful in other crops such as *Beta vulgaris*, *Fragaria virginiana* x *Fchiloensis*, *Malus sp*, *Phlox* x *subulata* and raspberry (Atanassov, 1980; James, 1979; Jones *et al.*, 1977; Sehnabelrauch and Sink, 1979).

Efficient shoot proliferation was reported by several workers when a combination of BAP and auxins at low concentration was used (Hasegawa, 1979; Skirvin and Chu, 1979 and Damiano *et al.*, 1987). However, Bhat (1992) reported

improved shoot production efficiency by the addition of NAA (0.1 mg/l) in combination with GA_3 (0.5 mg/l).

5.2.4. Standardisation of meduium for *in vitro* rooting

Stage 3 involves de novo regeneration of adventitious roots from shoots obtained in Stage 2. The role of auxins on root formation has been demonstrated in a number of trials. In the present studies, MS basal medium supplemented with auxins either alone or in combination was tried to standardise the most suitable combination for in vitro rooting. The trial was conducted with MS basal medium supplemented with either IAA or NAA at four diifferent levels (0.5, 1.0, 1.5, 2.0 mg/l). Twenty five per cent rooting was observed in MS basal medium without hormones. However, this resulted in delayed root initiation (31 days) and reduced number (2.0 to 2.3) and length (0.20 to 0.23) of roots compared to the medium supplemented with auxins. Hasegawa (1980) also observed rooting on MS basal medium without hormones. Skirvin and Chu (1979 a,b) obtained on half strength MS medium without hormones. This may be due to the presence of sufficient levels of endogenous auxin in the microshoots.

The auxins IAA /NAA was found to have remarkable influence on root induction in the present study. Improved

rooting efficiency was obtained in medium supplemented with auxins compared to the medium devoid of any auxin supplements. Among the different levels of IAA, 2.0 mg/l was found to have the best efficiency in terms of days to rooting (11.0), percentage of rooting (83.3), number of roots per shoot (8.2) and length of the longest root (1.46 cm).

Addition of NAA to the basal medium also helped in the *in vitro* rooting of rose microshoots particularly at 2.0 mg/l. With this treatment root initiation occured in 15 days with 91.7 per cent rooting an average of 6.6 roots per shoot. The length of the longest root was 1.2 cm. Compared to NAA, IAA was found to be more efficient for early root induction, number of roots, and length of root. In many instances although rooting could be induced on the medium without hormones, rooting efficiency increased with the addition of auxins (Hasegawa, 1980). Root induction on half strength MS basal medium supplemented with IAA 2 mg/l was also reported (Sauer *et al.*, 1985).

The rooting media supplimented with 2, 4-D in combination with either NAA or IAA were not favourable in general for the *in vitro* rooting of rose microshoots although the rooting percentage was found to be 100, in one of the combinations. However, Rout, *et al.* (1989 a) obtained good

rooting on MS medium supplemented with NAA 0.25 mg/l + 2, 4-D 0.1 mg/l in Rosa hybrida cv. Landora.

IAA and NAA had an additive/synergistic effect on rooting *in vitro*. The synergism was evident in the root initiation (8.5 days), rooting percentage (91.7), number of roots per shoot (11.6) and length of the longest root (2.44 cm). The treatment IAA 1.0 mg/l + NA 1.0 mg/l was superior to those of IAA 2.0 mg/l or NAA 2.0 mg/l. Kosh-Khui and Sink (1982 c) also observed an additive effect on *in vitro* rooting, using NAA and IAA at most of the concentrations they tested. Good rooting of rose cv. 'Crimson Glory' was reported by Barve *et al.* (1984) using a combination of the auxins - IAA, IBA and IPA (each at 0.5 mg/l).

Activated charcoal (AC) added to the culture medium had a remarkable influence on improving the rooting efficiency of cultured rose microshoots. Incorporation of AC 500 mg/l to the basal rooting medium containing IAA and NAA each at 1.0 mg/l was found to be the best treatment in which the days for root initiation was reduced to 7.5 days, and the rooting percentage (91.7) and the number of roots (12) per shoot were increased. The favourable effect of AC in rooting had been reported by many workers (Wang and Huang, 1976; Banks and Hackett, 1978). The capacity of AC to adsorb inhibiting compounds and excessive concentrations of plant growth hormones and the property to darken the medium which

mimics the soil conditions are the favourable factors proposed in favour of ~~the favourable effects of~~ AC in rooting (Proskauer and Berman, 1970; Wang and Huang, 1976; Fridborg *et al.*, 1978).

5.2.5. Acclimatization and *ex vitro* establishment

The critical part of acclimatization is the initial step of inducing the formation of fully functional roots in a potting mixture, while ensuring that the delicate root system is protected against desiccation. The main cause of mortality on transplantation is due to desiccation since the *in vitro* cultures are maintained at high relative humidity of around 100 per cent. A period of humidity acclimatization was considered necessary for the newly transferred plantlets to adapt to the outside environment during which the plantlets undergo morphological and physiological adaptations enabling them to develop typical terrestrial plant water control mechanism (Grout and Aston, 1977; Sutter *et al.*, 1985).

In the present investigations, high relative humudity (90 to 100 per cent) was maintained during the initial period of planting out with the help of polythene covers large enough to accomodate twenty five potted plants at a time on a wooden frame, with fine mist spray at an interval of three hours. Phased planting out of rooted

plantlets, at two, three and four weeks interval and trial with different potting mixtures also did not help the survival of plantlets after three weeks. The general vigour and appearance of the plantlets upto three weeks indicated that, ~~three to four weeks~~ keeping the cultured shoots in root induction medium was best for getting better establishment. and among different potting mixtures tried sand and sand: soilrite (1:1) were found to be equally effective in maintaining the plants without desiccation upto three weeks of planting out.

Addition of inorganic nutrients to the potting mixture had been reported to be essential for the normal growth of potted plants (Brown and Sommer, 1982; Amerson *et al.*, 1985). In the present studies, application of MS salts in liquid form at $1/_{10}$, $1/_{2}$ and full strength, 5 ml each along with water as control, at an interval of three days resulted in the production of two fresh leaves in all the treatments. The application of MS salts at $1/_{10}$ concentration resulted in early leaf emergence within 10.4 days as against 12 days when full strength MS salts was applied and 11.8 days when water alone was applied. It was also observed that application of MS salts at $1/_{10}$ concentration recorded increased growth rate of 0.92 cm, against 0.64 cm when water was applied. However, complete mortality was observed by fourth week.

Some of the established aberrant features were reported as characteristics of *in vitro* raised plantlets. Leaves were with poor or no development of cuticular wax on leaf surfaces, poor development of palisade and pronounced mesophyl air spaces. (Grout and Aston, 1978; Leshem, 1983; Donnelly and Vidaver, 1984). Impaired stomatal mechanism with non-closure of stomata (Brainerd and Funchigami, 1982; Capellades *et al.*, 1990; Ghashghaie *et al.*, 1992). *In vitro* plantlets had poor photosynthetic ability due to the availability of sucrose in the medium and were not said to be truely photoautotrophic, but mixo or heterotrophic. Poor organisation of grana in the chloroplasts of *in vitro* plantlets along with etiolated effect produced by ethylene in the culture vessels greatly contributed to their reduced photosynthetic activity. (Grout and Aston, 1977; Lee *et al.*, 1985). Vitrification of shoots to different extent was associated with poor vascular differentiation both in leaves and stem. Poor vascular connection between root and shoot, very often, due to intervening callus, and lack of root hairs, in general, were the problems in *ex vitro* establishment. The failure of plantlets to establish under *ex vitro* condition may be due to these aberrant morphological and physiological characteristics imparted to *in vitro* plantlets by the unnatural environment obtained *in vitro*.

The favourable effect of mycorrhizae on *ex vitro* establishment had been reported by many workers. Glomus species are reported to be very useful for improving transplant success of Rubus idaeus and Paxillus involutus (Pierik, 1987) and jack plantlets (Ramesh, 1990). In the present studies also, attempts were made to improve transplant success using three species of VAM viz., Glomus fasciculatum, G. constrictum and G. etunicatum.

Among the three species of Glomus tested, inoculation of plantlets with G. etunicatum had the highest survival rate (66.67 per cent). It also took the minimum number of days (105) for flowering. There was no survival at all among the untreated plantlets. The mycorrhizal association might have increased the nutrient uptake and their effective utilization and increased stress tolerance might have contributed to the successful establishment and survival of plantlets. Higher photosynthetic efficiency (Sivaprasad and Rai, 1984) and phytochrome production (Allen *et al.*, 1980) have been suggested as the beneficial effects of mycorrhizae in plants.

5.2.6. Somatic Organogenesis/Embryogenesis

As a preliminary step to induce somatic organogenesis/embryogenesis, callus induction was achieved

from internodal segments and leaf disc explants. Even though callus induction was obtained in all the hormone supplemented media (BAP + NAA + 2, 4-D) the best result of 83.3 per cent callus induction and highest growth score (3.0) and highest callus index of 249.90 for leaf disc explants, and 83.3 per cent callus induction, highest growth score (2.4) and highest callus index of 199.92 for internodal segments were obtained in MS basal medium in which inorganic salts reduced to half strength containing BAP 0.5 mg/l, NAA 2.0 mg/l and 2, 4-D 0.5 mg/l. At highest level of auxins and cytokinin tested, callusing was comparatively poor. The callus initiation started from the cut ends of the internodal segments. In leaf disc explants it started from the cut ends of the mid-rib region in small white globules and gradually spread to cover the entire explant. In one of the cultures, direct rhizogenesis from the callus was noticed.

The rate of callus proliferation and organogenetic potential of the calli were studied with modified medium supplements incorporating ascorbic acid in some of the combinations. Callus proliferation was cent percent in leaf callus, 83.3 per cent in internodal callus, at lowest levels of BAP 0.5 mg/l + NAA 0.1 mg/l + ascorbic acid 5 mg/l. The same treatment also had the highest growth score and callus index. In a medium containing ascorbic acid, increased concentration of auxin (NAA) or cytokinin (BAP), callus

proliferation efficiency of both leaf and internodal callus was reduced. Replacement of ascorbic acid with another auxin 2, 4-D and increasing the concentration of BAP or NAA or both did not give any improved result with respect to callus proliferation.

In one of the treatment combinations with lowest level of BAP 0.5 mg/l + 2, 4-D 0.5 mg/l and highest level of NAA 2.5 mg/l, 33.3 per cent of the leaf and internodal calli induced rhizogenesis resulting in well developed thick and fleshy roots. But shoot formation was not obtained in any of the cultures or in any of the media combinations tested.

Attempts were made by different workers to induce rose callus and organogenesis (Hill, 1967; Jacobs *et al.*, 1968; Kireeva *et al.*, 1977; Khosh Khui and Sink, 1982b; Li, 1983; Tweddle *et al.*, 1984; Lloyd *et al.*, 1988; Gavish *et al.*, 1986; Ziestin *et al.*, 1987; and Rout *et al.*, 1992).

Hill (1967) reported formation of shoot primordia in long term stem cultures of hybrid Tea rose. The addition of IBA 1.0 mg/l promoted bud formation in pith callus cultures (Jacobs *et al.*, 1968). They also observed that callus growth was dependent on the presence of IAA. The present results also enlightened the role of NAA on callus induction and proliferation. Kireeva *et al.* (1977) observed MS medium supplemented with 2, 4-D (1.0 to 4.0 mg/l) and kinetin

(0.05 to 0.10 mg/l) to be the best for optimum callus formation from petal, leaf, sepal and embryo of a rose variety 'Krymskaya Krasnaya'. Khosh Khui and Sink (1982 b) observed faster callus initiation in the dark than in light. Improved callus formation and bud differentiation was achieved by treatment with phloridzin (Li, 1983). Shoot formation from callus was also reported by Tweddle *et al.*, (1984) and Lloyd *et al.*, (1988). Formation of shoot primordia, bud differentiation and shoot formation had been reported, however, no reports are available on direct rhizogenesis from callus cultures as observed in the present investigation.

Successful case of adventitious shoot bud differentiation from leaf and internodal calli, and rooting of regenerated shoots were reported (Rout *et al.*, 1992). They also developed the protocol for the induction of somatic embryogenesis in callus cultures of Rosa Hybrida cv. Landora Somatic embryos were initiated on half strength MS basal medium supplemented with BAP 0.5 mg/l, NAA 0.01 mg/l and GA_3 0.1 mg/l and various concentrations of l-proline (200 - 800 mg/l). However, they reported that somatic embryos failed to develop into plantlets.

In the present studies, attempts made to induce somatic embryogensis were not successful. This may be due to the fact that varietal response of a species to *in vitro*

culture is never synonymous, since the factors which control are numerous.

5.3. INDUCED MUTATION ADOPTING *IN VITRO* CULTURE

5.3.1. Irradiation of budwood

There was a progressive reduction in the percentage of bud break with increasing doses of gamma rays, irrespective of the physiological stage of the bud. Radiations are known to hamper the auxin synthesis, leading to even its complete destruction under *in vivo* culture as reported by Sparrow *et al*. 1952 and Gordon, 1957. Cell division may also be inhibited after exposure to ionising radiation on account of chromosome damage and depending upon the severity of exposure, mitotic process may completely stop. Walther and Sauer (1986 a) reported reduction is survival rate of explants as a result of X-radiation in rose.

At highest dose of 50 Gy, complete mortality was recorded three weeks after culture, irrespective of bud stages. This may be due to the failure of mitotic process as a result of chromosome damage. The effective dose of gamma rays for 50 per cent survival of explants was estimated as 33 Gy, where as under *in vivo* culture, the ED_{50} was 38 Gy.

The percentage of malformed leaves was maximum under 40 Gy. Malformation of leaves was absolutely nil in

control except in B_3 bud stage, that too at the lowest level of 6.24 per cent as against 50 per cent in 40 Gy. Leaf abnormalities as a consequence of radiation had been reported in rose by many workers (Kaicker and Swarup, 1972; Desai and Abraham 1978; gupta and Datta 1982 and Datta, 1986 a) adopting *in vivo* culture.

There was significant interaction between bud stages and gamma ray exposures for days to bud break. the exposure of gamma rays at 30 Gy and 40 Gy significantly delayed bud break, whereas 20 Gy stimulated bud break except in B_4 bud stage. There was no significant difference between control and 20 Gy treatment for days to bud break.

When doses of gamma rays were enhanced, percentage of cultures with multiple shoots was reduced. This may be due to the inhibition of mitotic activity in the meristematic region. Significant interaction between bud stages and gamma rays was observed for days to multiple shoot induction. The results clearly indicated delay in multiple shoot induction with increasing doses of gamma rays.

The shoot production efficiency of cultured shoots was significantly reduced at highest dose of 40 Gy. Walther and Sauer (1986 a) also reported inhibition in shoot production following X-irradiation with increasing doses. They also suggested that radiosensitivity of cultivars can be

estimated by determining the survival rate of explants, productivity of axillary buds, and inhibition of shoot development on the first date of cut off.

The highest percentage of cultures with morphological variations was recorded at 40 Gy except in B_5 bud stage. The induced morphological variations include shoots with yellowing of leaves, enlarged leaf blade, deformed leaflets and chlorophyll deficient leaves. Walther and Sauer (1986 b) observed different types of leaf variations as a result of X-irradiation in rose.

5.3.2. Growth analysis of microshoots

Growth analysis was conducted by recording the growth of mainshoot, side shoot and number of leaves produced at two weeks interval upto 8 weeks. The results indicated significant interaction between bud stage and gamma rays. In general, gamma irradiation significantly reduced the growth rate and leaf production efficiency at 30 and 40 Gy irrespective of the stage of the explants irradiated. The lowest dose of 20 Gy had minimum effect on retarding growth and in same cases had a stimulater effect also. inhibition of mitotic process with increasing doses of gamma rays (Smith and Kersten 1942) may be the possible cause for the reduced growth recorded.

5.3.3. Effect of gamma rays on *in vitro* rooting

The lowest percentage of rooting was observed in exposure of gamma rays at 40 Gy although it depended upon the stage of the explant. Buds collected from vegetative shoots were found to be the maximum affected by highest dose and buds of 4 days after flower harvest were the least affected. Radiation is known to hamper auxin synthesis (Sparrow *et al.* 1952, Gordon 1957).

There was significant interaction between bud stages and gamma rays for days to root initiation and number of roots per culture. Gamma irradiation at higher doses of 30 and 40 Gy significantly delayed root initiation at all stages of buds exposed. The number of roots per culture was also reduced at higher exposures. Root length also recorded a significant reduction with increasing exposures. The reduced rooting efficiency of the gamma ray exposed materials may be due to inhibition of cell division on account of chromosome damage.

5.3.4. Irradiation of multiple shoots

Gamma irradiation of multiple shoots drastically reduced the survival rate at increasing exposures. The same trend was observed with respect to the percentage of cultures with multiple shoots. Maximum morphological abnormalities

were recorded at highest exposure (40 Gy). The types of abnormalities induced at highest exposures were shoots with narrow leaves, striated leaves, deformed leaves with prominent striations and deformed leaves with chlorophyll deficiency. Walther and Sauer (1986 a) induced mutants with double serrated leaf edge, and chlorophyll deficient leaves by X-irradiation on the basal segments of *in vitro* derived microshoots.

The days taken for multiple shoots production was significantly delayed at 30 and 40 Gy. The shoot production efficiency in the subsequent three stages of sub culture was significantly reduced at 30 and 40 Gy. Walther and Sauer (1986 a) also observed reduction in shoot production efficiency with increasing the X-ray doses. At higher doses of 30 and 40 Gy, the days to root initiation was significantly delayed. The root number was progressively reduced with increasing the exposure and at the highest dose of 40 Gy the difference was significant. The root length also had significant reduction at highest dose.

The growth rate of irradiated micro shoots showed a reducing trend except at the lowest dose of 10 Gy. The same trend was observed at all the four stages of observations at two weeks intervals.

SUMMARY

6. SUMMARY

Investigations were carried out at the Department of Agricultural Botany and Tissue Culture Laboratory attached to the Department of Horticulture, College of Agriculture, Vellayani during the period from 1989-1993 on "Induced mutagenesis in rose under *in vivo* and *in vitro* culture". The main objectives were to study the effect of gamma rays on morphological attributes in rose, to isolate beneficial mutants if any, to standardise the explant materials and culture conditions for *in vitro* establishment of rose and to study the variations induced by gamma rays under *in vitro* culture.

Three rose cvs. of Hybrid Tea group viz., Alliance, Suraga and Folklore were used for *in vivo* studies and cv. Folklore alone was selected for *in vitro* studies. Budwoods of three maturity stages were exposed to gamma rays at doses 20, 30, 40, 50 and 60 gray. Observations on sprouting, survival, morphological characters and induced variations on other characters were made in vM_1 generation.

In vitro culture conditions and *ex vitro* establishment conditions were standardised for rose cv. Folklore. Budwoods and multiple shoots were exposed to gamma rays at different doses to study their *in vitro* variations.

One of the direct effects of gamma rays was inhibition of sprouting of buds. Irrespective of bud stages, irradiated buds took longer time to sprout than the control. Lowest dose of 20 Gy did not have much effect on inhibition of sprouting and two cvs. viz., Suraga and Folklore induced early sprouting.

Significant reduction in sprouting and survival was recoreded at higher exposures of 30, 40 and 50 Gy, irrespective of stage of the bud. There was no significant interaction between bud stages and gamma ray exposures on sprouting and survival. The exposure of bud woods at 38 Gy gamma rays was estimated to be the optimum dose to get 50 per cent survival of plants six months after budding.

The morphological variations induced were influenced by the cultivars, physiological stages of the bud and exposures of gamma rays. The effect of radiation was manifested in the form of inhibition, stimulation or morphological abnormalities. The growth retardation was expressed in the form of reduction in plant height, number of branches and number of leaves. The physiological stages of buds treated also showed interaction with cultivars for the variations induced in plant height and number of branches. Gamma ray induced maximum reduction in plant height confined to highest dose of 40 Gy.

Radiation induced various types of morphological abnormalities were more frequent during the early period of growth. Abnormal leaves were observed at 40 Gy in all the cultivars. The major abnormalities observed were leaves with united leaflets, deformed leaves with united leaflets, and miniaturised leaves (Alliance and Suraga). In cv. Alliance leaves with chlorophyll deficient patches were also observed.

Gamma ray induced variations in flower colour were obtained in cvs. Alliance and Folklore. One variant with light pink colour flower was observed in 20 Gy treated population of cv. Alliance. One reddish yellow mutant was isolated from 30 Gy treated population of cv. Folklore. One mutant with reduced size and large number of petals was also isolated from 40 Gy exposure in folklore.

The maximum number of contamination free cultures were obtained when shoot tip and axillary buds were suface sterilized with mercuric chloride at 0.08 per cent for 12 minutes. For internode and leaf disc explants, treatment of mercuric chloride 0.06 per cent for 12 minutes was effective. Axillary buds of 1.0 cm, internodes of 0.5 cm and leaf disc of 1.0 cm with a portion of petiole were found to give best response. The most suitable age of the axillary bud explant was four days after flower opening.

Among the 48 treatments tested for culture establishment, MS basal medium supplemented with BAP 2.5 mg/l 2,4-D 0.5 mg/l had the highest bud break (80 per cent) and induced early bud break (4.0 days).

The medium supplemented with kinetin 2.0 mg/l + GA_3 1.0 mg/l took the minimum number of days (23.4) for induction of multiple shoot and also produced highest number of shoots/culture (6.87). Whereas, highest percentage of cultures induced multiple shoots in medium containing BAP 2.0 mg/l + GA_3 0.75 mg/l, flower bud initiation was observed in combination of BAP 2.0 mg/l + GA_3 0.5 mg/l.

The most suitable hormone supplements for *in vitro* rooting was identified as IAA and NAA at 1.0 mg/l each. This treatment induced early root initiation (8.5 days) in 91.7 per cent of the cultures producing an average of 11.6 roots/shoot, with the root length of 2.44 cm. Incorporation of activated charcoal to the same medium improved the rooting efficiency in terms of reducing the days to root initiation (7.5), increasing the number of roots (12.0) and root length (5.20 cm).

Hardening and *ex vitro* establishment of plantlet was the most difficult part of the *in vitro* propagation. An improvised structure made of wooden frame and polythene cover helped to maintain high humidity to *ex vitro* plantlets.

Trials with different potting media did not give any positive results on *ex vitro* establishment. Supply of MS salts at different concentrations also failed to provide any successful results. In both of the above trials, complete mortality was recorded after three weeks.

Successful hardening and *ex vitro* establishment of plantlets were achieved by surface inoculation of germinated spores of mycorrhizae (VAM) in liquid suspension. The highest survival rate of 66.67 per cent (six weeks after culture) was obtained by inoculation with Glomus etunicatum, against no plants in the untreated lot. The ... plantlets inoculated with G. etunicatum took the minimum number of days for flowering (105).

Best callus induction from leaf disc and internodal segments were obtained in MS medium supplemented with BAP 0.5 mg/l + 2,4-D 0.5 mg/l + NAA 2.0 mg/l and best callus proliferation obtained in medium supplemented with BAP 0.5 mg/l + NAA 0.1 mg/l + ascorbic acid 5 mg/l. Rhizogenesis was obtained from leaf and internodal calli in MS medium supplemented with BAP 0.5 mg/l + 2,4-D 0.5 mg/l + NAA 2.5 mg/l.

Gamma irradiation delayed bud break and induced reduction in bud break and survival in *in vitro* culture. None of the cultures survived at the highest dose of 50 Gy.

The estimated value for ED_{50} was 33 Gy. Cultures with abnormal leaves increased with increasing doses.

The shoot proliferation efficiency declined progressively with the gamma exposures of higher levels. There was significant delay in multiple shoot induction and reduction in number of shoots. The same trend was observed with respect to rooting efficiency also.

The radiation effect manifested in the form of morphological abnormalities increased with exposures of higher levels. The abnormalities recorded were cultres with deformed leaflets, enlarged leaf blades, chlorophyll deficient patches and yellowing of leaf.

The irradiation of multiple shoots significantly reduced the poliferation rate, rooting efficiency and increased the morphological variations. The induced variaions were cultures with narrow leaves, deformed and striated leaves, deformed leaves with foliar petiole and leaves with chlorophyll deficiency.

REFERENCES

REFERENCES

Alderson, P.G., McKinless, J. and Rice, R.D. (1988). Rooting of cultured rose shoots. Acta Hort., 226: 175-182

Aldrufeu, A., Pages, M., Messeguer, J. and Mele, E. (1983). *In vitro* rhizogenesis of Rosa species in different substrates. Acta Hort., 150: 315 - 323.

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

Alekhno, G.D. and Vysotskii, V.A. (1987). Effect of nutrient medium on the micropropagation of roses. In Sortoizuchenie i Razmnozhenie Kul' tur 57 - 61 from Referativnyi Zhurnal 6: 855 - 858.

*
Allen, M.F., Moore, T.S. and Christensen, M. (1980). Phytohormones changes in Bouteloua gracilis infested by vesicular-arbuscular mycorrhiza. I. Cytokinin increase in the host plant. Can. J. Bot., 58: 371-372.

Amerson, H.V., Frampton, Jr. L.J., Mc Keand, S.E., Mott, R.L. and Weir, R.J. (1985). Loblolly pine tissue culture : Laboratory green house and field studies. Henke, R.R., Hughes, K.W., Constantin, M.J. and Hollaender, A. (Ed.), Tissue Culture in Forestry and Agriculture. Plenum Press, New York, pp. 271-287.

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: 91 - 95.

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

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

Auerbach, C. and Robson, J.M. (1944). Production of mutation by alkyl isothiocynate. Nature, 154: 81.

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

Avramis, T., Hugard, J. and Jonard, R. (1982 b). 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. Science de la vie., 294: 679 - 682.

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

Banks, M.S. and Hackett, W.P. (1978). Differentiation from Hedera helix. Pl. Physiol. Suppl., 61: 245.

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. Australian J. Bot., 28: 405-409.

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

Ben Jaacov, J. and Langhans, R.W. (1968). A tissue culture technique for rapid multiplication of Chrysanthemum morifolium. Proc. Int. Hort. Congr., 18: 211 - 255.

Ben Jaacov, J. and Langhans, R.W. (1970). A tissue culture technique for rapid multiplication of Chrysanthemum morifolium. Proc. Int. Hort. Congr., 18: 211 - 255.

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

Bhat, M.S. (1992). Micropropagation in Rose. Indian Horticulture, 2: 17 - 19.

Binding, H. (1977). Organogenesis in callus of Petunia hybrida Z. Pflanzenphysiol., 65: 359 - 364.

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

Brainerd, K.E. Fuchigami, L.J. (1982). Stomatal functioning of *in vitro* and greenhouse apple leaves in darkness, mannitol, ABA and CO_2. J. Exp. Bot., 33: 388-392.

Bressan, P.H., Kim, Y.J., Hyndman, S.E., Hasegawa, P.M. and Bressan, R.A. (1982). Factors affecting *in vitro* propagation of rose. J. Amer. Soc. Hort. Sci., 107: 979 - 990.

Broertjes, C. Haccius, B. and Weidlich, S. (1968). Adventitious bud formation on isloated leaves and its significance for mutation breeding. Euphytica., 17: 321 - 344.

Broertjes, C., Roest, S. and Bokelmann, G.S. (1976). Mutation breeding in Chrysanthemum morifolium using *in vivo* and *in vitro* adventitious bud technique. Euphytica, 25: 11 - 19.

Brown, C.L. and Sommer, H.E. (1982). Vegetative propagation of dicotyledonous trees. Bongo, J.M. and Durzan, D.J. (Ed.) Tissue culture in forestery. Martinus

Nijhoff Dr. W. Junk Publishers, London, pp. 109-149.

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: 37 - 38.

Campos, P.S. and Salome S. Pais, M. (1990). Mass propagation of the dwarf rose cultivar 'Rosamini'. Scientia Hortic., 43: 321 - 330.

Capellades, M., Fontarnau, R., Carulla, C. and Debergh,P. (1990). Environment influences anatomy of stomata and epidermal cells in tissue cultured Rosa multflora. J. Am. Soc. Hort., 115: 141-145.

Chan, A.P. (1966). Chrysanthemum and rose mutations induced by X-rays. Proc. Am. Hort. Sci., 88: 613 - 620.

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: 101 - 106.

Curir, P., Damiano, C. and Cosmi, T. (1985). *In vitro* propagation of some rose cultivars. Annali. dell' Instituto Sperimentale. Per la. Floricultura, 16: 67 - 73.

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

Dale, P.J. (1977 b). Meristem tip culture in Lolium, Festuca, Phleum and Dactylis. Plant Sci. Lett., 9: 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: 43-45.

Datta, S.K. (1985). Radiosensitivity of garden roses J. Nuclear Agric. Biol., 14: 133 - 135.

Datta, S.K. (1986 a). Improvement of miniature roses by gamma irradiation. The Indian Rose A., 5: 36 - 40.

Datta, S.K. (1986 b). Effect of recurrent gamma irradiation on rose cv. Contempo. J. Nuclear Agric. Biol., 15: 125 - 127.

Datta, S.K. (1986 c). Thin layer chromatographic studies and evaluation of somatic mutations in roses. The Indian Rose A., 5: 41 - 47.

Datta, S.K. (1987). Sensitivity of garden roses to gamma radiation. The Indian Rose A., 6: 121 - 126.

Datta, S.K. (1988). Sensitivity of seven more H T roses to gamma irradiation. The Indian Rose A., 7: 101-103.

Datta, S. K. and Gupta, M.N. (1982 a). Gamma ray induced white flower mutant in rose cv. Junior 'Miss' J. Nuclear Agric. Biol., 11: 32 - 33.

Datta, S.K. and Gupta, M.N. (1982 b). Gamma ray induced white flower mutant in rose cv. 'Contempo' Am. Rose A., 36: 36 - 37.

Datta, S.K. and Gupta, M.N. (1983). Effect of gamma rays on pigment synthesis of rose cv. 'Contempo' - J. Nuclear Agric. Biol., 12: 79 - 80.

Datta S.K. and Gupta M.N. (1984). 'Saroda and 'Sukumari' - new rose cultivars evolved by gamma irradiation. Sci. Cul., 50: 200 - 201.

Davies, D.R. (1980). Rapid propagation of roses *in vitro* Scientia Hortic., 13: 385 - 389.

Desai, B.M. (1973). Report on mutation experiments on ornamentals at BARC, Trombay. Second Workshop on Floriculture, Agric - Hort Society Culcutta: 29-31.

Desai, B.M. and Abraham, V. (1978). Use of radiations in rose improvement. First All India Rose Convention, Bombay Rose Society. 42 - 44.

*
Dommergues, P. (1976). Induced mutagenesis in roses Hort. Fr., 74: 13 - 15.

Dommergues, P., Heslot, H., Gillot, J. and Martin, C. (1967). L' induction de mutations chez lesp rosiers. In: Induced mutations and their utilization. (H. Stubbe, ed.) Gatersleben 1966, Academic - Verbag, Berlin, 319 - 348.

Donnelly, D.J. and Vidaver, W.E. (1984). Leaf anatomy of red raspberry transferred from culture to soil. J. Amer. Soc. Hort. Sci., 109: 172-176.

Doreswamy, D.R., Rao, N.K.S. and Chacko E.K. (1983) Tissue culture propagation of banana. Sci. Hort., 18: 247 - 252.

Dubois, L.A.M., Roggmans,J., Soyeurt, G and de Vries, D.P. (1988). Comparison of the growth and development of dwarf rose cultivars propagated *in vitro* and *in vivo* by softwood cuttings. Scientia Hortic., 35: 293 - 299.

Duron and Decourtye, L (1986). Biological effect of gamma irradiation applied to Weigelacv Bristol Ruby plants cultured *in vitro*. In nuclear techniques and *in vitro* culture for Plant Improvement IAEA, pp. 301 - 311.

Duskova, J., Sovova, M., Dusek, J. and Jamodar, L. (1988). The effect of ionising radiation on the tissue culture of Artostaphylas uva - ursi 43: 518 - 519.

Elliot, R.F. (1970). Axenic culture of meristem tips of Rosa multiflora. Planta, 95: 183 - 186.

Engivild, K.C. (1978). Substituted indol acetic acid tested in tissue cultures. Physiol. Plant., 44: 345.

Evans, D.A., Sharp, W.R. and Flinck, C.E. (1981). Growth and behaviour of cell cultures: Embryogenesis and organogenesis. In: Plant Tissue Culture : Methods and Applications in Agriculture (T.A. Thorpe, ed.) pp. 45-114. Academic Press, New York, Ist Ed.

Finney, D.J. (1971). Probit Analysis. (3rd ed.) Schand and Co. Ltd., New Delhi. p. 327.

Fletcher, J.S. (1974). Metabolism of Glutamate in suspension cultures of Paul's Scarlet rose cells. Plant Physiol., 54: 303 - 305.

Fridborg, G., Pedersen, M., Landstorm, L. and Eriksson, T. (1978). The effect of activated charcoal on tissue cultures. Absorption of metabolites inhibiting morphogenesis. Physiol. Plant., 43: 104-106.

Gamborg, O.L. and Shyluk, J.P. (1981). Nutrition, media and characteristics of plant cell and tissue cultures. Thorpe, J.A. (Ed.), Plant Tissue Culture, Methods and Application in Agriculture. Academic press, New York, pp. 21 - 24

Gaul, H. (1970). Mutagen effects observable in the first generation. Plant injury and lethality. Cytological effects and sterility. In IAEA, Vienna Manual on mutation breeding. PP 237.

Gavish, H., Zieslin, N. and Ziv, M. (1986). Growth interaction between calli of rose cultivars *in vitro*. Acta Hort., 189: 47 - 50.

*
Gelin, O. (1955). Studies on the X - rays mutation. Stral. Pea Agr. Hort. Genet., 13: 189 - 193.

Ghashghaie, J., Brenckmann, F. and Saugier, B. (1992). Water relations and growth of rose plants cultured *in vitro* under various relative humidities. Plant Cell Tissue Organ Cul., 30: 51-57.

Gordon, S.A. (1954). Occurrence, formation and inactivation of auxins. A. Rev. Plant Physiol., 5: 341-378.

Gordon, S.A. (1956). The Biogenesis of natural auxins. In: The chemistry and mechanism of action of plant growth substances. Butterworth Scientific Pub., London, pp. 65-75.

Gordon, S.A. (1957). The effect of ionising radiation on plants: biochemical and physiological aspects. Quart. Rev. Biol., 32: 3-14.

Goud, J. V., Nayar, K.M. and Rao, N.G. (1970). Mutagenesis in sorghum. Indian J. Genet., 30: 81 - 90.

Graifenberg, A. (1973). The *in vitro* culture of embryos and seed parts in Rosa canina. Italiana, 57: 374 - 380.

Gray, L.H. (1956). Biological damage from ionising radiations. Proc. Int. Conf. on the peacefull uses of atomic energy, Geneva, 1955, pp. 209-212.

Grout, B.W.W. and Aston, M.J. (1977). Transplanting cauliflower plants regenerated from meristem culture. 1. Waterloss and water transfer related to changes in leaf wax and to xylem regeneration. Hort. Res., 17: 1-7.

Grout, B.W.W. and Aston, M.J. (1978). Modified leaf anatomy of cauliflower plantlets regenerated from meristem culture. Ann. Bot., 42: 993-995.

Gunckel, J.E. and Sparrow, A.H. (1961). Ionising radiations: Biochemical, physiological and morphological aspects of their effects on plants. Encycl. Plant Physiol., 16: 555-611.

*
Guo, J., Liu, Y., Tang, J and Liu, C (1983). Mutation in roses induced by r-rays. J. of Nan. Tech. Coll. Forest Prod.,2: 68 - 74.

Gupta, M.N. (1966). Induction of somatic mutations in some ornamental plants. Proc. All India Symp. Hort. Calcutta, pp. 107-114.

Gupta, M.N. and Datta, S.K. (1982). Effect of gamma rays on roses: sprouting, growth and induction of somatic mutations. The Indian Rose A., 42 - 54.

Gupta, M.N., Nath, P. and Datta, S.K. (1982). Effect of gamma irradiation on Rosa damascena Mill. The Indian Rose A., 2: 55 - 61.

Gupta, M.N. and Shukla, R.C. (1970). Mutation breeding of garden rose: Studies on induction of somatic mutations after ɣ. irradiation of budwood. Prog. Hort., 2: 69 - 83.

Gupta, M.N. and Shukla, R. (1971 a). Mutation breeding of garden roses. Effect of gamma irradiation on some scented roses. Jap. J. Breed., 21: 129 - 136.

Gupta, M.N. and Shukla, R.C. (1971 b) Mutation breeding of garden roses: recovery of somatic mutation after three years of gamma irradiation of budwood. Recent Adv. in Hort. Kanpur pp. 81 - 86.

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

Harney, P.M. (1976). Breeding of Floricultural crops in canada. Acta Hort., 63: 19 - 26.

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

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

Heslot, H. (1964). L' induction experimentale de mutations chez les plantes florales. C.R. Acad. Seances Agric. Fr., 1281 - 1308.

Heslot, H. (1968). Mutation research done in 1967 on barley, roses and marigold. Mutation in plant Breeding, 11: 153 - 159. IAEA, Vienna.

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

Holdgate, D.P. (1977). Propagation of ornamentals by tissue culture. In Reinert, J. and Bajaj Y.P.S. (ed.) Plant Cell Tissue and Organ cult., Springer - Verlag, Berlin. 18 - 43.

Huang, S. and Chen, Y. (1986). Mutation breeding in rose. Mutation Breeding News Lett., 27: 14.

Hurst, C.C. and Breeze, M.S.G. (1922). Notes on the origin of moss rose. J. Roy. Hort. Soc., 47: 26 - 42.

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

Irulappan, I. and Rao, V.N.M. (1981). Induced mutation in Edward rose (Rosa bourboniana Desp.) II. Mean performance in vM_1 and vM_2 generations. South Indian Hort., 29: 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. ii. Gibberellin effects. Agroplantae, 1: 179 - 187.

Jacobs, G., Allan, P. and Bornman, C.H. (1970). Tissue culture studies on rose: Use of shoot tip explants iii. Auxin: Gibberellin effects. Agroplantae, 2: 45 - 49.

Jacobs, G., Bornman, C.H. and Allan, P. (1968). Tissue culture studies on rose: Use of pith explants. South Afr. J. Agric. Sci., 11: 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: 273-277.

Johnson, R.T. (1980) Gamma irradiation and *in vitro* induced separation of chimeral genotypes in carnation. Hort. Sci., 15: 605 - 606.

Jones, O.P., Hopgood, M.E. and O'Farrel, D. (1977). Propagation *in vitro* of M.26 apple rootstocks. J. Hort. Sci., 52: 235-238.

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

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

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

Kaicker, U.S. (1988). Stable and unstable mutants of rose cv. Folklore. Genome, 30: Suppl (1): 335.

Kaicker, U.S. and Dhyani, D. (1986). Induced mutations in roses through gamma irradiation. Indian J. Hort., 43: 287-290.

Kaicker, U.S. and Pandey, R.M. (1973). Florachrome in control and mutants. Indian J. Hort., 30: 435-439.

Kaicker, U.S. and Swarup, V. (1972). Induced mutation in roses. Indian J. Genet., 32: 257 - 265.

Kaicker, U.S. and Swarup, V. (1978). Induced mutation in the rose cv. Gulzar and effect of chemical and physical mutagens on plant growth. Acta Agronomica Academiae Scientiarium, Hungaricae, 27: 43 - 48.

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

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

Khosh-Khui, M. and Sink, K.C. (1982 a). Micropropagation of new and old world rose species. J. Hort. Sci., 57: 315 - 319.

Khosh-Khui, M and Sink, K.C. (1982 b). Callus induction and culture of Rosa. scientia Hortic., 17: 361 - 370.

Khosh-Khui, M. and Sink, K.C. (1982 c). Rooting enchancement of Rosa hybrida for tissue culture propagation. Scientia Hortic., 17: 371-376.

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

Kleffel, B., Walther. F. and Preil, W. (1986). X-ray induced mutability in embryonic suspension cultures of Euphorbia pulcherrima. Nuculear techniques and in vitro cultiure for plant improvement, IAEA, pp. 113 - 129.

Klimenko, Z.K., Chemarin, N.G. and Golumbivskaya, L.L. (1974). The effect of 'r' - rays from a ^{137}Ce source on pollen viability in garden roses. Byulleten Gosdarstvennoyo Nikitskogo, Botanicheskogo Sada, 1: 59 - 62.

*
Klimenko, Z.K., Chemarin, N.G. and Golumbivskaya, L.L. (1975). Using r-irridation seeds for garden rose breeding. Genetike i Selektsii, 54: 292 - 294.

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

Krishnamoorthy, H.N. (1981). Plant Growth Substances. Tata Mc Graw-Hill Pub. Co. Ltd., New Delhi. pp. 79.

*
Krussmann, G. (1974). Rosen, Rosen, Rosen Parey Verl. Berlin - Hamburg.

Kunisaki, J.T. (1980). *In vitro* propagation of Arthurium andreanum Lind. HortScience, 15: 508-509.

Kusey, W.E., Jr. Jammer, P.A. and Weiler, T.C. (1980). *In vitro* propagation of Gypsophila paniculata L. 'Bristol Fairy' HortScience, 15: 600-601.

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

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

Lata, P. (1980). Effect of ionising radiations on roses. Induction of somatic mutations. Env. Exp. Bot., 20: 325 - 333.

Lata, P. and Gupta, M.N. (1971). Mutation breeding of graden rose. Effect of gamma rays on essential oil content in some scented roses. Perfum. Kosmet., 52: 267 - 270.

Lata, P. and Gupta, M.N. (1975). Effect of ionising radiation on roses. Meiotic studies in control and gamma -ray induced mutants of rose cultivar Montezuma. Cytologia, 40: 213 - 219.

Lauwaars, N.P. (1984). Propagation of roses *in vitro* is not without problems. Vakblad voor de Bloemisterij., 39: 49.

Lee, N., Wetzstein, H.Y. and Sommer, H.E. (1985). Effects of quantum flux density on photosynthesis and chloroplasts ultr structure in tissue cultured plantlets and seedlings of Liquidambar stryraciflua L. towards improved acclimatization and field survival. Plant Physiol., 78: 637-641.

Lefering, L. (1985). *In vitro* propagation of roses. Ornamental Hort., 40: 35.

Leshem, B. (1983). Growth of carnation meristems *in vitro*. Anatomical structure of abnormal plantlets and effect of agar concentration in the medium on their formation. Ann. Bot., 42: 413-415.

Li, Z.P. (1983). The effect of phloridzin on *in vitro* cultures of Begonia feasti, Rosa hybrida and Cedrus deodara. Plant Physiology communications, 4: 37 - 39.

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

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

Lundergan, C.A. and Janick, J. (1980). Regulation of apple shoot proliferation and growth *in vitro*. Hort. Res., 20: 19-24.

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

Malik, R.S. and Singh, A.P. (1980). Some new Indian roses. Indian Hort., 25: 24.

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

Mathew, V. (1989). Mutational studies in garden roses. Ph.D thesis, Indian Agricultural Research Institute. New Delhi.

Mc Farland, (1958). Modern roses V. The Mc Farland Company Harrisburg, Pennsylvania.

Mederos, S. and Rodriguez, M.J. (1987). *In vitro* propagation of 'Golden Times' roses. Factors affecting shoot tips and axillary bud growth and morphogenesis. Acta Hort., 11: 619 - 624.

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

Monaco, L.C., Sondahl, M.R., Carvalho, A., Crocomo, O.J. and Sharp, W.R. (1977). Application of tissue culture in the improvement of coffee. In: fundamental and applied aspects of plant cell, Tissue and Organ culture (J. Reinert and Y.P.S. Bajaj, eds). Springer-Verlag, New York Ist Ed. pp. 109-120.

Morey, D. (1954). Observations on the genetics of the mutant climbing factor in Hybrid Tea roses. Am. rose A., 33: 89 - 97.

Muller, H.J. (1927). Artificial transmutation of the gene. Science, 66: 84 - 87.

Murashige, T. (1974). Plant propagation through tissue culture. Ann. Rev. Pl. Physiol., 25: 135-166.

Murashige, T. (1977). Manipulation of organ initiation in plant tissue cultures. Bot. Bull. Acad. Sinica., 18: 1 - 24.

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

Nakajima, K. (1965). Induction of sports in roses by gamma ray irradiation. Gamma Field Symp., 4: 55-70.

Nakajima, K. (1970) Gamma irradiation induced mutants of the rose cv. Peace. Tech. News No. 4 : 2 Institution of Radiation Breeding.

Nakajima, K. (1977). Studies on effective methods of inducing mutation in vegetatively propagated plants by gamma irradiation. Acta Radiobotanica et Genetica, 49: 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: 75 - 91.

Nesius, K.K. and Fletcher, J.S. (1973). Carbondioxide and pH hydrogen ion concentration requirements of non-photosynthetic culture cells. Physiol. Plant., 28: 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: 173 - 176.

Nishida, T., Nakajima,K., Ohba, K. and Takato, S. (1967). Radiosensitivity and induction of somatic mutations in woody perennials under chronic gamma ray irradiations. Gamma Field Symp., 6: 19 - 42.

Panse, V.G. and Sukhatme, P.V. (1978). Statistical Methods for Agricultural Workers 3rd ed. I.C.A.R., New Delhi.

Pearce, R.S. and Cocking, E.C. (1973). Behaviour in culture of isolated protoplasts from "Paul's Scarlet" rose suspension culture cells. Protoplasma, 77: 165-180.

Pierik, R.L.M. (1987). In vitro culture of Higher Plants. Martinus Nijhoff publishers, Boston pp. 344.

Pittet, H. and Mancousin, C. (1982). Rose in vitro micropropagation. Revue Horticole Suisse, 55: 67 -

Podwyszynska, M. and Hempel, M. (1988). The factors influencing acclimatization of Rosa hybrida plants multiplied *in vitro* to green house conditions. Acta Hort., 226: 639 - 642.

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

Proskauer, K. and Berman, R. (1970). Agar culture medium modified to approximate soil conditions. Nature, 227: 1161.

*

Quastler, H., Schertiger, A.M. and Stewart, W.N. (1952). Inhibition of growth by irradiation. IV. Growth arrest vs. effects on mitotic activity. J. Cellular comp physiol., 39: 357-369.

Rajmohan, (1985). Standardisation of tissue/meristem culture techniques in improvement of horticultural crops. Ph.D. thesis, Kerala Agricultural univeristy.

Ramesh, B. (1990). *Ex vitro* establishment of jack (Artocarpus heterophyllus Lam.) plantlets. M.Sc. (Hort.) Thesis Kerala Agricultural University.

Reghunath, B.R. (1989). *In vitro* studies on the propagation of cardamom (Elettaria cardamomum Maton) Ph.D. thesis, Kerala Agricultural University.

Roest, S. (1977). Vegetative propagation *in vitro* and its significance for mutation breeding. Acta Hort., 78: 349-359.

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

Rout, G.R. Debata, B.K. and Das, P. (1989 a). *In vitro* mass scale Propagation of Rosa hybrida cv. Landora. Current Sci., 58: 876 - 878.

Rout, G.R., Debata, B.K. and Das, P. (1989 b). Micropropagation of Rosa hybrida L. cv. Queen Elizabeth through *in vitro* culture of Axillary buds. Orissa J. Hort., 16: 1 - 9.

Rout, G.R., Debata, B.K. and Das, P (1989c). Induction of somatic embryogenesis in Rosa hybrida cv. Landora. Orissa J. Hort., 17: 46 - 49.

Rout, G.R., Debata, B.K and Das, P. (1990). *In vitro* clonal multiplication of roses. Proc. Nat. Acad. Sci., 15: 311-319.

Rout, G.R., Debata, B.K. and Das, P. (1991). Somatic embryogenesis in callus cultures of Rosa Hybrida L. cv. Landora Pl. Cell Tissue Organ Cult., 27: 65-69.

Rout, G.R., Debata, B.K and Das, P. (1992). *In vitro* regeneration of shoots from callus cultures of Rosa hybrida L. cv. Landora. Indian J. Exp. Biol., 30: 15 - 18.

Saakow, S.G. (1960). Die sortenbildung bei Rosen durch Knospen mutation en. Arch. Gartenbau, 8: 595 - 629.

Sauer, A. and Walther, F. (1985). Different suitability for *in vitro* propagation of rose cultivars. Gartenbauwissenschaft, 50: 133 - 138.

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

Schnabdrauch, L.S. and Sink, K.C. (1978). *In vitro* propagation of Phlox subulata and Phlox paniculata. Hort. Sci., 14: 607-608.

Shahare, M.L and Shastry, S.V.S. (1963). Meiosis in garden roses. Chromosoma, 13: 702 - 724.

Simonsen, J. and Hildebrandt, A.C. (1971). *In vitro* growth and differentiation of gladiolus plants from callus cultures. Canadian J. Bot., 49: 1817 - 1819.

Sivaprasad, P. and Rai, P.V. (1984). Photosynthesis and competition for photosynthate in tripartite symbiosis. Curr. Sci., 54: 468-469.

Skirvin, R.M. and Chu, M.C. (1979 a). Propagation of roses with tissue culture. Illinois Research, 21: 3.

Skirvin, R.M. and Chu, M.C. (1979 b). *In vitro* propagation of 'Forever Yours' rose. HortScience, 14: 608 - 610.

Skirvin, R.M. and Chu, M.C. (1979 c). Tissue culture propagation of rose. Illinois State Florists Association Bulletin, 384: 22 - 23.

Skoog, F. (1935). The effect of X-radiation on auxin and plant growth. J. Cellular Comp. Physiol., 7: 227-270.

Smith, G.F. and Kersten, H. (1942). Auxins and calines in seedlings from X-rayed seed. Am. J. Bot., 29: 189-192.

Snow, R. (1933). The nature of cambial stimulus. New physiol., 32: 285-296.

Sparrow, A.H. (1951). Some cytological and morphological changes induced in plants by ionising radiation. Science, 114: 488.

Sparrow, A.H., Cuany, R.L., Miksche, J.P. and Schairer, L.A. (1961). Some factors affecting the responses of plants to acute and chronic radiation. Rad. Bot., 1: 10-34.

Sparrow, A.H., Moses, M.J. and Steele, R. (1952). A cytological and cytochemical approach to an understanding of radiation damage in dividing cells. Brit. J. Radiol., 25: 182-188.

Spiegel Roy, P. and Kochba. (1976). Use of ionising radiation for mutagenesis in tissue culture. In Proc. Workshop Eur. Domm. Israel, 434 - 444.

Stadler, C.J. (1928). Mutation in barley induced by X-ray and radium. Science, 68: 186 - 187.

Strauss, A. and Potrykus, I. (1980). Callus formation from protoplasts of cell suspension cultures of rose "Paul's Scarlet". Physiol. Planta. 48: 15-20.

Streitberg. H. (1964). Abschlussbeticht zur forschengsarbeit : Scuhaffung wirtzchaftlich wertvoller muattionen bei zierpflanzen (Rhododendron - und rosa Arten) durch Behandlung mit Rotgenstrahlen Teil b. Muationen bei Rosen, Dtsch. Akad. Land Wirtsch. Wiss. Berlin.

Streitberg, H. (1966 a). Neue Rosen - und-Azaleen - Sorten mit Hilfe der Rontgenbestrahlung. Dtsch. Gartenbau., 13: 267 - 268.

Streitberg, H. (1966 b). Rose breeding with the help of X - rays. Arch. Gartenbau., 14: 81 - 88.

Streitberg, H. (1967). Production of economically valuable variation in roses and azaleas by means of X-irradiation. In (Stubbe, H ed) Induced mutations and their utilization. Gatersleben, Akademic Verlag, Berlin PP 359 - 362.

Streitberg, H. (1977). List of released or approved varieties produced with induced mutations. Mutat. breed. Newsl., AEA, 9: 16.

Sunnino, A., Ancora, G. and Locardi, C.H. (1984). *In vitro* mutation breeding of potato. Mutat. breed. Newsl., 24: 9-10.

Sunnio, A., Ancora, G. and Locardi, Ch. (1986). In vitro mutation breeding of potato. The use of propagation by microcuttings and in vitro cultures for plant improvement. Proc. Symp., Vienna IAEA pp. 385 - 394.

Sutter, E.G., Fabbri, A. and Dunston, S. (1985). Morphological adaptation of leaves of strawberry plants grown *in vitro* after removal from culture. Henke, R.R., Hughes, K.W., Constantin, M.J. and Hollaender, A. (Ed.), Tissue Culture in Forestry and Agriculture. Plenum press, New York, pp. 358-359.

Swaminathan, M.S. Siddiq, E.A., Singh, C.R. and Pai, R.A. (1970). Mutation breeding in rice in India II IAEA, Vienna 25 - 43.

Swarup, V., Kaicker, U.S. and Gill, H.S. (1971). Induced mutations in French bean and roses (Abst.) Int. sym. on the use of Isotopes and Radiation in Agricultural and Animal Husbandry Research. 30 Nov-2 Dec., New Delhi, pp. 13-25.

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

Tackholm, G. (1920). On the cytology of the genus Rosa. Svensk. Bot. Tidskr., 14: 301 - 311.

Tulecke, W. and Nickel, L.G. (1959). Production of large amounts of plant tissues by submerged culture. Science, 130: 363 - 364.

Tweddle, D., Roberts, A.V. and Short, K.C. (1984). In: Plant Tissue and cell culture Application to crop Improvement. Novak, F.J., Havel, L and Dolezel, J. (Ed.) pp. 529-530.

Usenbaev, E.K. and Iman Kulova, K.I. (1974). Radiation mutant of roses. Proc. Int. Hort. Congr., 19: 109.

Valles, M. and Boxus, P. (1987). Micropropagation of several Rosa hybrida L. cultivars. Acta Hort. 11: 611-617.

Vasil, I.K., Ahuja, M.R. and Vasil, V. (1979). Plant tissue culture in plant breeding. Adv. Genet., 20: 127 - 215.

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

Walther, F, and Sauer, A. (1986 a). Analysis of radiosensitivity a basic requirement for *in vitro* somatic mutagenesis III Rose cultivars. Gartenbauwissenschaft, 51: 40 - 43.

Walther, F. and Sauer, A. (1986 b). *In vitro* mutagenesis in roses., Acta Hort., 189: 37 - 46.

Walther, F. and Sauer, A. (1986 c). Analyisis of radiosensitivity a basic requirement for *in vitro* somatic mutagenesis II. Gerbera Jamesonii. in Nuclear and *in vitro* culture for plant improvement. IAEA, Vienna 155 - 597.

Wang, P.J. and Huang, L.C. (1976). Beneficial effects of activated charcoal on tissue and organ culture. *In. Vitro* 12: 26

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

Wylie, A.P (1955 a). The histroy of garden roses part II. J. Roy. Hort. Soc., 80: 8 - 24.

Wylie, A.P. (1955 b). The history of garden roses part III. J. Roy.Hort. Soc., 80: 77 - 87.

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

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

---

* - Originals not seen

INDUCED MUTAGENESIS IN ROSE UNDER *IN VIVO* AND *IN VITRO* CULTURE

BY

D. WILSON

Abstract of the Thesis
submitted in partial fulfilment of the requirement
for the Degree of
DOCTOR OF PHILOSOPHY
Faculty of Agriculture
Kerala Agricultural University

Department of Agricultural Botany
COLLEGE OF AGRICULTURE
Vellayani, Thiruvananthapuram

1993

ABSTRACT

Investigations were carried out at the Department of Agricultural Botany and Tissue Culture Laboratory attached to the Department of Horticulture, College of Agriculture, Vellayani during the period from 1989-1993 on "Induced mutagenesis in rose under *in vivo* and *in vitro* culture.

Induced mutagenesis adopting *in vivo* method was carried out with three rose cvs. Alliance, Suraga and Folklore belonging to Hybrid Tea group. The cv. Folklore alone was utilized for induced mutagenesis adopting *in vitro* culture.

The budwoods of three selected cultivars were collected at three different stages of growth and exposed to Gamma rays at 20, 30, 40, 50 and 60 Gy, and budded on rooted stock plants and effect of gamma rays on morphological attributes were recorded.

In vitro culture conditions were standardised for cv. Folklore. Budwoods were collected at five different growth stages and exposed to gamma rays at 20, 30, 40 and 50 Gy, before culturing. The *in vitro* variations in terms of culture establishment, shoot proliferation and rooting efficiency were studied. Multiple shoots were also subjected to gamma irradiation to study their *in vitro* variations.

Gamma irradiation of bud woods induced inhibition and reduction in sprouting and survival. Growth retardation exhibited in the form of reduction in plant height and number of branches. The cultivars showwed no significant interaction with different doses of gamma rays for sprouting and survival. The ED_{50} was estimated as 38 Gy.

One reddish yellow mutant was isolated from cv. Folklore from 30 Gy treated population and one mutant for increased number of petals from 40 Gy treated population of the same cultivar. In addition, gamma exposure induced variation in size and shape of leaves at 30 and 40 Gy.

The treatment of mercuric chloride 0.08 per cent for 12 minutes had the minimum contamination rate for shoot tip and axillary bud explants, and 0.06 per cent for 12 minutes was most effective in the case of internodal segments and leaf disc explants.

Axillary buds of 1.0 cm length for enhanced release of axillary bud, internodal segments of 0.5 cm and leaf discs of 1.0 cm with a petiole portion for callus induction were identified as the most suitable explants.

Axillary buds excised 4 days after flower opening had the best response in culture establishment.

MS basal medium supplemented with BAP 2.5 mg/l + 2,4-D 0.5 mg/l recorded bud break percentage of 80 per cent within 4 days.

Early multiple shoot induction and highest number of shoots/culture observed in medium supplemented with kinetin 2.0 mg/l + GA_3 1.0 mg/l. Addition of BAP 2.0 mg/l + GA_3 0.75 mg/l was the best for getting highest percentage of cultures with multiple shoots. Flower bud initiation was observed in combination of BAP 2.0 mg/l + GA_3 0.5 mg/l.

The best medium for *in vitro* rooting was found to be IAA and NAA 1.0 mg/l each, along with activated charcoal 500 mg/l.

Successful hardening and *ex vitro* establishment of plantlets were achieved by surface inoculation of germinated spores of mycorrhizae (VAM) in liquid suspension. Highest survival rate of 66.67 per cent was observed by inoculation with Glomus etunicatum against no plants in the untreated lot. Minimum number of days to flowering (105) was taken in plantlets inoculated with G. etunicatum.

BAP 0.5 mg/l + NAA 2.0 mg/l + 2,4-D 0.5 mg/l was the best combination for callus induction and BAP 0.5 mg/l + NAA 0.1 mg/l + ascorbic acid 5 mg/l had the highest callus proliferation.

In vitro rhizogenesis obtained from internodal and leaf calli in MS medium supplemented with BAP 0.5 mg/l + NAA 2.5 mg/l + 2,4-D 0.5 mg/l.

Gamma irradiation of axillary buds delayed bud break, reduced percentage of bud break, multiple shoot production and rooting efficiency and also induced morphological variations in leaf and growth pattern. The estimated value for ED_{50} was 33 Gy under *in vitro* culture.

Exposure of multiple shoots to gamma rays induced several morphological abnormalities and reduced the shoot production and rooting efficiency.

APPENDICES

APPENDIX I

Effect of gamma irradiation of budwoods of different stages on sprouting*

Cultivar (V)	Bud stage (B)	Gamma rays (Gy)				
		D_0	D_1	D_2	D_3	D_4
Alliance (V_1)	B_1	83.47 (65.98)	76.75 (61.15)	50.00 (44.98)	43.32 (41.14)	23.25 (28.82)
	B_2	73.60 (59.06)	77.47 (41.64)	60.00 (50.75)	43.32 (41.14)	19.57 (26.24)
	B_3	80.43 (63.72)	83.47 (65.98)	63.38 (52.73)	33.17 (35.15)	16.53 (23.98)
Suraga (V_2)	B_1	70.05 (56.80)	74.51 (59.65)	46.54 (43.00)	39.91 (39.16)	16.53 (23.98)
	B_2	84.70 (66.94)	80.43 (63.72)	60.09 (50.80)	36.63 (37.23)	12.57 (20.76)
	B_3	73.33 (58.88)	73.33 (58.88)	43.19 (41.07)	36.63 (37.23)	32.62 (34.81)
Folklore (V_3)	B_1	67.38 (55.15)	74.51 (59.65)	56.68 (48.82)	43.32 (41.14)	26.39 (30.90)
	B_2	90.25 (71.78)	86.67 (68.56)	63.63 (52.89)	50.00 (44.98)	22.53 (28.33)
	B_3	80.43 (63.72)	83.47 (65.98)	60.09 (50.80)	46.63 (43.05)	26.39 (30.90)

* The transformed values (angles) are given in paranthesis

C.D. V = 3.36 , D = 4.34

APPENDIX II

Effect of gamma irradiation of budwoods of different stages on survival*

Cultivar (V)	Bud stage (B)	Gamma rays (Gy)				
		D_0	D_1	D_2	D_3	D_4
Alliance (V_1)	B_1	66.67 (54.72)	70.05 (56.80)	33.17 (35.15)	36.63 (37.23)	19.57 (26.24)
	B_2	66.83 (54.82)	63.63 (52.89)	50.00 (44.98)	29.95 (33.16)	9.75 (18.18)
	B_3	66.83 (54.82)	66.67 (54.72)	50.00 (44.98)	23.25 (28.82)	13.33 (21.40)
Suraga (V_2)	B_1	66.67 (54.72)	66.83 (54.82)	39.91 (39.16)	33.17 (35.15)	12.57 (20.76)
	B_2	80.43 (63.72)	73.61 (59.06)	56.81 (48.89)	33.33 (35.25)	9.75 (18.18)
	B_3	63.38 (52.73)	66.67 (54.72)	36.37 (37.07)	29.95 (33.16)	12.57 (20.76)
Folklore (V_3)	B_1	67.38 (55.15)	70.50 (57.08)	50.00 (44.98)	33.33 (35.25)	19.57 (26.24)
	B_2	86.67 (68.56)	80.00 (63.41)	46.54 (43.00)	39.91 (39.16)	16.53 (23.98)
	B_3	80.43 (63.72)	76.75 (61.15)	50.00 (44.98)	36.63 (37.23)	19.57 (26.24)

* The transformed values (angles) are given in paranthesis CD. V = 3·02, D = 3·90

APPENDIX III

Effect of gamma irradiation of budwoods of different stages on plant height (cm)

Cultivar (V)	Bud stage (B)	Gamma rays (Gy)			
		D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	33.0	28.5	30.5	22.4
	B_2	28.1	31.5	26.9	21.5
	B_3	31.5	30.3	25.2	19.9
Suraga (V_2)	B_1	29.0	30.2	22.2	17.7
	B_2	32.5	31.2	20.5	18.3
	B_3	33.2	32.4	27.3	17.0
Folklore (V_3)	B_1	41.1	43.3	35.6	26.6
	B_2	46.3	50.5	30.7	22.3
	B_3	49.6	45.8	36.1	19.7

B_1 - Day of flower opening

B_2 - Three days after flower opening

B_3 - Six days after flower opening

CD : V = 1.82, V × D = 3.64

D = 2.10 B × D = 3.64

APPENDIX IV

Effect of gamma irradiation of budwoods of different stages on number of primary branches per plant

Cultivar (V)	Bud stage (B)	Gamma rays (Gy)			
		D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	2.2	2.1	1.4	1.3
	B_2	2.3	2.1	1.7	1.3
	B_3	2.1	2.3	1.6	1.3
Suraga (V_2)	B_1	2.6	2.3	1.9	1.5
	B_2	2.5	2.0	1.7	1.3
	B_3	2.6	2.1	1.6	1.1
Folklore (V_3)	B_1	2.7	2.8	1.7	1.3
	B_2	2.9	2.7	2.1	1.5
	B_3	3.5	3.3	1.5	1.3

CD : V = 0·12 V × D = 0·21
D = 0·14 B × D = 0·24

APPENDIX V

Effect of gamma irradiation of budwoods of different stages on prickle density

Cultivar (V)	Bud stage (B)	Gamma rays (Gy)			
		D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	1.55	1.60	1.57	1.57
	B_2	1.61	1.61	1.58	1.42
	B_3	1.57	1.53	1.60	1.61
Suraga (V_2)	B_1	0.99	0.99	0.98	0.92
	B_2	1.03	1.03	1.00	0.94
	B_3	1.02	1.02	1.01	0.96
Folklore (V_3)	B_1	0.77	0.73	0.77	0.76
	B_2	0.74	0.75	0.76	0.74
	B_3	0.77	0.75	0.74	0.36

CD V = 0.067 B = 0.093 D = 0.067

APPENDIX VI

Effect of gamma irradiation of budwoods of different stages on days to flowering

Cultivar (V)	Bud stage (B)	Gamma rays (Gy)			
		D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	90.50	95.80	105.95	114.85
	B_2	90.90	97.80	106.00	112.75
	B_3	95.20	102.50	112.60	121.40
Suraga (V_2)	B_1	103.80	105.70	112.20	131.30
	B_2	105.50	106.50	111.50	124.90
	B_3	106.40	105.70	121.40	125.40
Folklore (V_3)	B_1	100.60	106.20	115.10	130.40
	B_2	90.10	102.90	114.40	125.40
	B_3	97.10	106.10	118.90	128.60

CD V = 2.40 B = 2.40 D = 3.77

APPENDIX VII

Effect of gamma irradiation of budwoods of different stages on number of leaves per plant

Cultivar (V)	Bud stage (B)	Gamma rays (Gy)			
		D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	16.30	18.25	14.20	13.10
	B_2	19.70	20.40	15.90	14.10
	B_3	15.45	16.70	16.70	15.40
Suraga (V_2)	B_1	17.10	16.80	15.85	13.10
	B_2	17.00	16.00	16.20	11.70
	B_3	16.10	16.40	14.60	14.10
Folklore (V_3)	B_1	24.40	22.60	18.72	13.10
	B_2	8.50	22.20	22.70	21.40
	B_3	20.90	21.40	21.20	16.90

CD V = 1.10 V x D = 2.19 V x B x D = 3.79
D = 1.26 B x D 2.19

APPENDIX VIII

Effect of gamma irradiation of budwoods of different stages on neck length (cm)

Cultivar (V)	Budstage (B)	Gamma rays (Gy)			
		D_o	D_1	D_2	D_3
Athance (V_1	B_1	5.55	3.65	5.35	4.70
	B_2	5.30	5.35	4.25	4.60
	B_3	5.45	5.40	5.25	5.15
Suraga (V_2)	B_1	4.20	4.25	4.15	3.90
	B_2	4.35	4.25	4.30	3.95
	B_3	4.55	4.70	4.40	4.05
Folklore (V_3)	B_1	6.65	6.75	6.50	5.25
	B_2	6.40	6.55	6.10	5.75
	B_3	6.20	5.95	5.95	5.40

CD V = 0·32 D = 0·37

APPENDIX IX

Effect of gamma irradiation of budwoods of different stages on flower diameter (cm)

Cultivar (V)	Bud stage B	Gamma ray (B) D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	7.50	7.75	7.15	6.00
	B_2	7.70	7.90	7.75	6.15
	B_3	7.95	8.00	7.70	6.65
Suraga (V_2)	B_1	6.75	7.10	6.90	5.05
	B_2	6.85	6.80	5.75	5.35
	B_3	6.25	6.60	6.05	4.60
Folklore (V_3)	B_1	7.25	7.45	7.30	6.40
	B_2	7.00	7.01	6.35	5.65
	B_3	6.50	6.70	6.10	5.60

C.D. V = 0.21, B = 0.21 D = 0.24 V x B = 0.34

APPENDIX X

Effect of gamma irradiation of bud woods of different stages on flower weight (g)

Cultivar (V)	Bud stage	Gamma ray (B)			
	B	D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	4.13	4.20	4.04	3.45
	B_2	4.03	4.12	3.98	3.73
	B_3	4.10	4.20	4.18	3.65
Suraga (V_2)	B_1	5.25	5.33	5.26	4.75
	B_2	5.26	5.26	4.92	4.22
	B_3	5.25	5.37	5.19	4.31
Folklore (V_3)	B_1	6.36	6.53	6.38	5.35
	B_2	6.28	6.33	6.25	5.20
	B_3	6.35	6.40	6.08	5.71

C.D. V. 0.09, B 0.09, D 0.11 V×B = 0.19

APPENDIX XI

Effect of gamma irradiation of budwoods of different stages on petal weight (g)

Cultivar (V)	Bud stage B	Gamma ray (B) D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	2.72	2.74	2.73	2.45
	B_2	2.72	2.78	2.62	2.51
	B_3	2.68	2.69	2.60	2.47
Suraga (V_2)	B_1	3.51	3.60	3.53	3.15
	B_2	3.65	3.54	3.50	3.07
	B_3	3.52	3.56	3.50	3.34
Folklore (V_3)	B_1	4.17	4.21	4.14	3.93
	B_2	4.00	4.03	3.89	3.68
	B_3	4.16	4.05	4.09	3.59

CD V = 0.06, D 0.08,

APPENDIX XII

Effect of gamma irradiation of budwoodS of different stages on petal number

Cultivar (V)	Bud stage	Gamma ray (Gy)			
	B	D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	15.80	16.50	14.15	14.35
	B_2	16.45	15.85	15.35	13.80
	B_3	15.35	15.20	14.90	13.75
Suraga (V_2)	B_1	29.45	31.25	29.30	29.70
	B_2	30.10	28.75	27.25	26.75
	B_3	28.05	28.85	26.75	27.40
Folklore (V_3)	B_1	27.35	27.50	31.40	37.50
	B_2	26.75	26.30	28.25	27.10
	B_3	28.40	28.50	27.40	27.40

CD V = 0.98, B. 0.98, V x B 1.13, V x D 1.96, B x D = 1.96

APPENDIX XIII

Effect of gamma irradiation of budwoods of different stages on number of flowers

Cultivar (V)	Bud stage	Gamma ray (B)			
	B	D_0	D_1	D_2	D_3
Alliance (V_1)	B_1	7.48	8.03	7.42	4.26
	B_2	8.01	8.81	7.91	6.02
	B_3	8.35	8.48	8.33	4.84
Suraga (V_2)	B_1	7.05	7.21	7.05	4.05
	B_2	7.04	7.36	6.55	3.55
	B_3	7.53	7.46	7.18	4.56
Folklore (V_3)	B_1	10.38	10.40	10.36	6.94
	B_2	9.88	10.46	9.80	6.15
	B_3	11.50	10.93	9.10	6.15

CD V-0.46, D 0.53

APPENDIX XIV

Effect of gamma rays on growth rate of multiple shoots cultured *in vitro* i. Main shoot

Bud stage (B)	Length of shoot (cm)															
	0(D_0)				20Gy(D_1)				30Gy(D_2)				40Gy(D_3)			
	Culture period (weeks)				Culture period (weeks)				Culture period (weeks)				Culture period (weeks)			
	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
B_1	0.86	1.30	1.66	1.92	1.04	1.50	1.84	2.12	0.82	1.34	1.64	2.00	0.74	1.22	1.40	1.52
B_2	1.56	2.08	2.44	2.82	1.40	2.22	2.64	3.00	1.34	1.92	2.52	3.06	1.02	1.32	1.78	2.10
B_3	1.80	2.34	2.90	3.30	1.74	2.22	2.80	3.22	1.64	2.44	2.84	3.14	0.88	1.26	1.44	1.52
B_4	1.90	2.64	3.36	4.04	1.94	2.46	3.20	3.86	1.80	2.26	2.70	3.10	0.84	1.38	1.54	1.76
B_5	1.98	2.74	3.10	3.42	1.86	2.82	3.16	3.38	1.70	2.38	2.72	2.96	0.74	1.10	1.46	1.70

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

APPENDIX XV

Effect of gamma rays on growth rate of multiple shoots (cv. Folklore) ii. Side shoot

Bud stage (B)	Length of shoot (cm)															
	0(D_0)				20Gy(D_1)				30Gy(D_2)				40Gy(D_3)			
	Culture period (weeks)				Culture period (weeks)				Culture period (weeks)				Culture period (weeks)			
	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
B_1	0.84	1.26	1.56	1.90	0.84	1.30	1.68	2.04	0.82	1.24	1.54	1.82	0.56	0.84	1.12	1.20
B_2	0.80	1.54	1.84	2.38	0.82	1.46	1.94	2.52	0.88	1.62	1.88	2.32	0.76	0.96	1.24	1.32
B_3	0.90	1.66	2.24	2.66	0.98	1.70	2.32	2.80	0.96	1.38	1.72	2.16	0.60	0.95	1.32	1.44
B_4	1.04	1.80	2.24	3.06	0.96	1.84	2.26	2.66	0.86	1.22	1.66	2.08	0.48	0.74	0.92	1.08
B_5	1.32	1.98	2.62	3.10	0.98	1.80	2.14	2.88	0.98	1.64	2.08	2.44	0.44	0.54	0.88	1.16

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening

APPENDIX XVI

Effect of gamma rays on leaf production (cv. Folklore)

Bud stage (B)	Number of leaves															
	0(D_0)				20Gy(D_1)				30Gy(D_2)				40Gy(D_3)			
	Culture period (weeks)				Culture period (weeks)				Culture period (weeks)				Culture period (weeks)			
	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
B_1	3.8	5.6	8.4	9.0	4.4	7.0	8.4	8.6	3.2	6.2	8.2	8.8	2.2	3.2	4.2	5.0
B_2	5.4	8.6	9.8	10.4	4.0	5.6	7.6	8.4	3.6	6.4	7.8	8.6	2.6	4.6	4.8	5.8
B_3	5.6	8.2	9.8	10.8	5.6	8.2	10.0	10.4	4.4	7.4	8.4	9.6	2.6	4.0	4.4	4.8
B_4	5.6	8.6	10.2	11.4	5.8	8.0	10.2	11.6	5.0	8.2	9.6	9.8	2.8	4.2	4.6	5.4
B_5	5.4	8.4	10.0	11.2	5.2	9.0	10.2	10.4	4.4	7.2	8.0	9.6	2.4	3.8	4.2	4.6

B_1 - Vegetative shoot

B_2 - Day of flower opening

B_3 - Two days after flower opening

B_4 - Four days after flower opening

B_5 - Six days after flower opening