MICROPROPAGATIOIN IN PAPAYA VAR. RED LADY

A

THESIS
SUBMITTED TO
NAVSARI AGRICULTURAL UNIVERSITY
NAVSARI

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR

THE AWARD OF THE DEGREE

OF

MASTER OF SCIENCE
(HORTICULTURE)

IN

FRUIT SCIENCE

BY

PATEL JAIMINKUMAR RAMANBHAI
B. Sc. (Horticulture)

DEPARTMENT OF FRUIT SCIENCE
ASPEE COLLEGE OF HORTICULTURE AND FORESTRY
NAVSARI AGRICULTURAL UNIVERSITY
NAVSARI – 396 450
GUJARAT

SEPTEMBER– 2008

Registration No. 04-00264-2006

MICROPROPAGATIOIN IN PAPAYA VAR. RED LADY

Student's Name	Major Advisor
Mr. J. R. Patel	Dr. R. M. Patel

DEPARTMENT OF FRUIT SCIENCE
ASPEE COLLEGE OF HORTICULTURE AND FORESTRY
NAVSARI AGRICULTURAL UNIVERSITY
NAVSARI – 396 450
GUJARAT

ABSTRACT

The present investigation was carried out at Biotechnology and Plant Tissue Culture Laboratory, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, on Micropropagation in papaya var. Red Lady during the year 2006-2008.

1. The surface sterilization of explant, treatment involving $HgCl_2$ (0.1 %) for 3 minutes gave better sterilization of shoot tip and treatment $HgCl_2$ (0.1 %) for 10 minutes was found better for axillary buds. Length of shoot was higher at lower concentration and less duration of the treatment.
2. Maximum per cent establishment of shoot tip explant was observed on treatment MS medium supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA.
3. Maximum multiplication rate was observed in alternate sub culturing in MS basal and MS medium fortified with 0.5 mg/l BAP + 0.1 mg/l NAA exhibited for four sub culturing.
4. Maximum shoot proliferation, length of internodes and length of shoot were observed on treatment MS medium fortified with 0.1 mg/l NAA + 1.0 mg/l Kinetin at 3000 Lux Light intensity.

5. Maximum shoot growth was recorded at 3% of sucrose in the medium.
6. Media pH had much influence on number and growth of regenerated shoots. Among the different pH levels tested, 5.7 pH was found to be the best for maximum number of shoot and length of shoot.
7. Length of shoot and number of shoots and growth rate were increased as increased the level up to 160 mg/l Adenine sulphate in the proliferation medium.
8. *In vitro* rooting response in regenerated shoots was found to be the best with half strength MS medium supplemented with 1.0 mg/l IBA.
9. Acclimatization of plantlets showed better survival in FYM: soil: sand (1:1:1:: v/ v/v) in hardening procedure.

Dr. R.M. Patel
Associate Professor (Fruit crops)
ASPEE College of Horticulture and Forestry
Navsari Agricultural University
Navsari -396 450

CERTIFICATE

This is to certify that the thesis entitled, "**MICROPROPAGATION IN PAPAYA VAR. RED LADY**" submitted by **MR. JAIMINKUMAR RAMANBHAI PATEL** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (Horticulture)** in the subject **FRUIT SCIENCE** of Navsari Agricultural University is a record of bonafide research work carried out by him under my guidance and supervision and that the thesis has not been previously formed the basis for the award of any degree, diploma or has been published for other similar title. All the assistance and help received during the course of the investigation have been duly acknowledged by him.

Place: Navsari

(R. M. Patel)
Major Advisor

Date: 20th September 2008.

DECLARATION

This is to declare that the whole of the research work reported here in the thesis for the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **FRUIT SCIENCE** by the undersigned is the result of investigation done by him under direct guidance and supervision of **Dr. R. M. Patel**, Associate professor (Fruit crops), ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari and no part of the work has been submitted for any other degree so far.

Place: Navsari

Date: 20th September 2008.

(J. R. Patel)

Countersigned by

(R. M. Patel)
Associate Professor (Fruit Crops)
ASPEE College of Horticulture and Forestry
Navsari Agricultural University
Navsari -396 450

ACKNOWLEDGEMENT

Indeed the words at my command are not adequate to convey the debt of gratitude and indebtedness to my Major Advisor, Dr. R. M. Patel, Associate Professor (Fruit crops), ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari for his most valuable guidance, constant inspiration, personal interest, constructive criticism and sympathetic attitude right from the selection of research problem to the final shaping of this manuscript.

I take proud privilege to record my grateful thanks to the members of my Advisory committee Dr. R. R. Shah, Professor of Plant Physiology, ASPEE College of Horticulture and Forestry Dr. B.K. Dhaduk, Professor of Horticulture, ASPEE college of Horticulture and Forestry. Mr. H.N. Chatrola, Assistant Professor, Agricultural Statistics, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari for their constant attention, valuable suggestions and continuous co-operation throughout the period of investigation. My special thanks to Prof. J. R. Naik for statistical analysis.

I express my sincere thanks to Dr. N. L. Patel, Principal and Dean, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari for providing necessary facilities during my study.

I am also thankful to Prof. Taslim, Prof. Dipak Patel, Dr. Nitinbhai Patel Dr. Mahatma, Dr. Khandelwal, and Dr. S. K. Jha, for providing constant encouragement and help during the research

work. Also the invaluable timely help rendered by Mr. Ritesh, Mr. Girish, Mr. Arunaben, Mrs. Urvashi are duly acknowledged.

I would be unworthy, if I do not express my cordial thanks to Shinde Kiran, Jilen Patel, Hitesh Savani, Ketan Patel, Jaydev Parmar. I am also thankful to Minal Tandel, Mukesh Chamar, Jayesh Pathak, Maheta Abhishek, Rajmani Kumar, Vijay Anand, Kazi Aadil, Miss. Madura and Miss. Krishna Vyas for their help during this investigation.

My special thanks to Om Sai Zerox & Computer (Munna) for neat typing, excellent word processing and formatting of this manuscript.

My vocabulary fail to get words of expression of my respects and sense of gratitude to my father Shri Ramanbhai Patel, mother Smt. Kailashben, brother Sanjaybhai, Nishabhabhi, my niece Siddhi, nephew Damya and my loving wife Vedanti and members of the family for their everlasting love, constant encouragement, inspiration and prayer support and guidance.

Last but not least, I am writing this acknowledgement because of LORD SWAMINARAYANA's bless.

Place : Navsari.

Date: 20th September 2008.

(J. R. PATEL)

CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF PLATES

PLATE NO.	TITLE	AFTER PAGE
I	Mother plant and explants of papaya var. Red Lady	29
II	Establishment of shoot tip and axillary bud explants on MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA	43
III	Multiple shoots production by different treatment (A,B and C)	45
IV	Factors affecting on shoot proliferation	48
V	Rooted papaya plantlet on ½ strength MS medium 0.1 mg/l IBA	53
VI	Acclimatization of plantlets papaya var. Red Lady	54
VII	Acclimatized of plant in papaya	54
VIII	Protocol for micropropagation of seedling papaya var. Red Lady	55

ABBREVIATIONS

AC	Activated charcol
ANOVA	Analysis of variance
BAP	6-Benzyl amino purine
Conc.	Concentration
cv.	Cultivar
EDTA	Ethylene diamine tetra acetic acid
et al.	Et. alibi (and others)
FYM	Farm yard manure
gm	Gram
$HgCl_2$	Mercuric chloride
hr	Hour
IBA	Indole-3-butyric acid
mg/l	Milligram per litre
ml	milli Litre
Min.	Minutes
MS	Murashige and Skoog medium (1962)
NAA	a- Naphthalene acetic acid
NaOCl	Sodium hypochlorite
RT	Room temperature
Var	Variety
v/v	Volume / volume (concentration)
w/v	Weight / volume (concentration)
ZR	Zeatin riboside
%	Per cent
°C	Degree Celsius
µm	Micro molar
MT	Metric tone

I. INTRODUCTION

Fruits have been the men's food from time immemorial. There is tremendous scope for developing fruit industry in India. However, one of the bottlenecks in expanding fruit industry is non-availability of quality planting materials on large scale in case of most of the fruit plants. The fruit plants are highly heterozygous and their seed progenies are not true to type. Therefore, horticulturists have adopted the method of vegetative propagation for clonal multiplication of such cultivars. This method has been a long-standing practice, perhaps since the man started cultivating plants. However, it has certain limitations. The multiplication rate of most of the fruit crops is slow and in some cases difficult to propagate vegetatively on large scale. Similarly, in spite of careful realization of treatments against pest and diseases, bacterial and virus infections can not be prevented totally. The answer of these problems is expected through plant tissue culture techniques (Micro propagation).

The Papaya (*Carica papaya* L.) belongs to family Caricaceae which is, comparatively, a small family having only four genera and about 31 species. The edible fruits, however, are available only with Carica genus (Muthukrishnan and Irulappan, 1990). Papaya is a native of Tropical of North and South America (Litz, 1984). It is believed to have originated in Central America through natural hybridization (Purseglore, 1972). Though a large number of papaya cultivars are in cultivation throughout the world, but none of these can be considered a true cultivar since

parental characters are not reproduced in all the progenies (Muthukrishnan and Irulappan, 1990).

A ripe fruit of papaya is an excellent breakfast or dessert delicacy and the edible portion of fruit consists of water (86%) and carbohydrates (12.8%) besides, being a rich source of ascorbic acid and provitamin 'A' (Chen and Tang, 1979). Jam, soft drinks, crystallized fruits are the important products prepared from papaya. Unripe papaya is also used as a vegetable for cooking purpose. Another important commercial product obtained by extracting latex from unripe fruit is papain which serves as a proteolytic enzyme. It is also used as a tenderizer for meat clearing and in beer industries also as a tool for detecting stomach cancer.

Papaya is one the principal fruits crops of tropical and subtropical areas of the world. In India, production of papaya in the year 2005-06 was 23,17,200 tones obtained from an area of occupying 73,100 ha, having the productivity of 31.7 tones/ha. While Gujarat produced 3,23,000 tones papaya from an area of occupying 7,700 ha having the productivity of 41.8 tones/ha, which ranked second in the production in India (Anon; 2006).

Hence, it is important to extend the area under papaya cultivation in order to boost up the production. Production is also increased by adopting high yielding variety of papaya. Recently in Gujarat var. Red Lady of papaya is being cultivated on large scale due to following characteristics.

Characteristics of Red-Lady variety of papaya

- Early, vigorous, productive, and tolerant to papaya Ring Spot Virus.
- No male plant hence, all produced fruits.
- Fruit bears short-oblong on female plant and rather long- shaped on bisexual plants.
- Weighing about 1.5-2.0 kg. Flesh is thick, red with 13% sugars content, delicious taste and excellent aroma.
- Ideal for local market and for fruit processing.
- Good keeping quality of fruit.

Although, desirable characteristics of var. Red Lady, the growers are not able to adopt this variety due to the vary high cost of seed.

Normally, papaya is propagated through seed. It is a cross-pollinated crop, the plant raised from seeds have a mixed inheritance which make them highly variable in performance. The improvement of papaya is hindered by its heterozygosity, dioecious habit and susceptibility to diseases. The importance of these problems is evident the lack of true-to-type cultivars at present. Clonal propagation is an urgent necessity for improvement of papaya.

Attempts to multiply papaya by conventional clonal propagation *viz.* grafting and cutting methods were found to be slow, impracticable and less success (Jona and Menini, 1987).

The major advantages offered by *in vitro* propagation technique in papaya are as under.

- Rapid and efficient clonal propagation of selected papaya genotypes.
- Disease free uniform propagules.
- Easier transportation.
- Increase crop yield.
- Year round availability of planting material.
- Improve quality of fruit.
- Preservation of valuable germplasm and breeding lines for crop improvement programme.

The technique of *in vitro* cultures has been made clonal propagation a possibility in papaya. Litz and Conover (1978 a) and Rajeevan and Pandey (1983) demonstrated the feasibility of multiplication of papaya through tissue culture. However, considering the commercial point of view, their procedures require further improvement with respect to multiplication rate, rooting and plantlet development, establishment in soil etc. Moreover, *in vitro* response of plant tissue is dependent on a number of factors like physiological states of donor plant, genotype and explant, sex type, time of year and virus or systematic bacterial infection (Litz, 1984). The quality and type of nutrient media, plant growth regulators and sterilization treatment vary according to these conditions which

these conditions which heavily depend on local environment. Therefore, attempts have been made to study there aspects in the present investigation.

Various attempts have been made to propagate papaya *in vitro* through callus regeneration (Yie and Liaw, 1977; Arora and Singh, 1978c), somatic embryogenesis (Litz and Conover, 1982) and shoot proliferation (Litz and Conover, 1978a; 1978b; Rajeevan and Pandey, 1983). Research work on different aspects of papaya tissue culture has been reviewed by Litz (1984) and Muthukrishnan and Irulappan (1990).

The present investigation with this context aims to standardize the micro propagation technique for papaya making use of the existing research based information. Experiments were designed in order to meet following objectives with an aim to develop a complete protocol for papaya.

1) To standardize the media for culture establishment.

2) To standardize the optimum multiplication medium.

3) To standardize the light intensity for proliferation of shoots.

4) To standardize the medium for *in vitro*- rooting of regenerated shoots.

5) To standard technique for acclimatization of *in vitro* produced plantlets.

II. REVIEW OF LITERATURE

2.1 History of plant tissue culture research

Plant tissue culture has an important role to play in the production of horticultural plants and in the manipulation of plant for improved agronomic performance. Plant tissue culture research is a multi-dimensional science that offers exciting prospects for improvements in crop productivity. While most nurserymen have been introduced to the techniques of micropropagation, other dimensions of tissue culture research have been less publicized. For example, the potential for selecting pathogen-free or stress-resistant plant clones, the creation of novel genetic combinations through somatic hybridization, etc. are techniques that have been frequently transposed to the nursery industry.

Previous work carried out by various scientists in relation to plant tissue culture of different plant species is reviewed in this chapter.

The concept of totipotency was given by Haberlandt (1902) who was the first person to attempt the culture of plant cell *in vitro* on a nutrient media.

Some of the landmarks in the field of plant tissue culture during the 20^{th} century are reviewed in Table 2.1.

The rapid strides achieved in the field of micropropagation of fruit crops have been made possible by research accomplishment of mostly private laboratories and commercial nurseries. During 1993-94 about 680 million tissue

Table 2.1: Events in Plant tissue culture

Year	Events	Workers
1902	First attempt of plant tissue culture	Habelandt
1904	Embryo culture of selected crucifers attempted	Hanning
1922	Asymbiotic germination of orchid seeds *in vitro*	Knudson
1922	*In vitro* culture of root tips	Robbins
1925	Use of embryo culture technique in interspecific crosses of *Linum*	Laibach
1934	*In vitro* culture of the cambial tissue of a few trees and shrubs, although failed to sustain cell division	Gautheret
1934	Successful culture of tomato roots	White
1939	Successful establishment of continuously growing callus cultures	Gautheret, Nobecourt and White
1940	*In vitro* culture of cambial tissues of *Ulmus* to study adventitious shoot formation	Gautheret
1941	Use of coconut milk containing a cell division factor for the first time in *Datura*	Van Overbeek
1941	*In vitro* culture of crown gall tissues	Braun
1944	*In vitro* adventitious shoot formation in tobacco	Skoog
1946	Raising of whole plant of *Lupinus* and *Tropaeolum* by shoot tip culture	Ball
1950	Regeneration of organ from callus tissue of *Sequoia smpervirens*	Ball
1952	Use of meristem culture to obtain virus-free dahlias	Morel and Martin
1952	First application of micrografting	Morel and Martin
1953	Production of haploid callus of the gymnosperm *Ginkgo biloba from pollen*	Tulecke
1954	First plant from single cell	Muir *et al.*
1955	Discovery of kinetin, a cell division hormone	Muir *et al*
1957	Discovery of regulation of organ formation by changing the ratio of auxin: cytokinin	Skoog and Miller

Contd....

Year	Events	Workers
1958	Regeneration of somatic embryos *in vitro* from the nucles of *Citrus* ovules	Maheshwari and Rangaswamy
1959	Regeneration of embryos from callus clumps and cell suspensions of *Daucus carota*	Reinsert and Steward
1959	Publication of first handbook on plant tissue culture	Gautheret
1960	First successful test tube fertilization in *Papaver rhoeas*	Kanta
1960	Use of the micro culture method of growing single cells in hanging drops in a conditioned medium	Jones *et al.*
1960	Enzymatic degradation of cell walls to obtain large number of protoplasts	Cocking
1960	Filtration of cell suspensions and isolation of single cells by planting	Bergmann
1962	Development of Murashige and Skoog nutrition medium	Murashige and Skoog
1964	Production of first haploid plant from pollen grains of *Datura*	Guha and Maheshwari
1970	Selection of biochemical mutants *in vitro* by the use of tissue culture derived variation	Carlos
1970	First achievement of protoplast fusion	Power *et al.*
1970	Discovery of first restriction endonuclease from *Haemphillus influenzae* Rd. It was later purified and named *Hind*-II	Smith
1971	Regeneration of first plants from protoplasts	Takabe *et al.*
1972	First report of interspecific hybridization through protoplast fusion in two species of *Nicotiana*	Carlson *et al.*
1973	Cytokinin found capable of breaking dormancy in excised capitulum explants of Gerbera	Pierik *et al.*
1974	Regenation of haploid *Petunia hybrida* plants from protoplast	Binding
1976	Shoot initiation from cyropreserved shoot apices of carnation	Seibert
1978	Somatic hybridization of tomato and potato resulting in pomato	Melchers *et al.*
1981	Introduction of the term "Somaclonal variation"	Larkin and Scowcroft

(Chawala, 2000)

culture plants were produced worldwide. While India produced about 5 million tissues culture plants in 1990-91.

India, with its amazing diversity of horticultural crops offers tremendous scope for exploiting this technique for mass multiplication of large number of elite and rare plants. So, for micropropagation techniques, over 48 crops have been standardized to the extent that it can be utilized on commercial scale (Parthasarathy *et al.*, 2001). However, there are several problems and limitations which must be overcome a routing method of plant propagation. The key to successfully overcome the major problems lies with the standardization of correct combinations of medium composition, cultural and environmental condition, explants, acclimatization, etc. for local conditions.

Clonal propagation through tissue culture can be achieved in small space and short time. Rapid propagation is one of the benefits provided by this technique for economically important fruit crops, especially plants such as papaya that are so many problems to propagate conventionally.

Until three decades ago, plant tissue culture was regarded as merely an experimental tool for highly specialized botanical investigation; but with the progress in research, it has now emerged as dynamic and important field of endeavor. *In vitro* culture has found its best commercial application in Agriculture, Horticulture and Forestry.

In vitro culture has proved to be of immense practical value as an aid to plant breeding, plant propagation, raising and

maintenance of disease free plants, germplasm storage as well as creating novel types through plant genetic engineering. Considering the important and progress made during the last two decades, the National Biotechnology Board has chosen tissue culture as one of the area of immediate interest.

Using the above methods, innumerable plants species have been cloned which suggest that it is possible to multiply most of the plant species through micropropagation. Some horticultural plant species in which micropropagation is demonstrated are listed in Table 2.2.

Preview work carried out by various scientists in relation to plant tissue culture of different papaya species is reviewed and discussed in the subsequent pages.

2.2 General review on *in vitro* techniques of papaya

2.2.1 Embryo culture

Suksa- Ard *et al.* (1999) ovules, embryos and hypocotyls of papaya cv. Sunrise Solo, used as a explant for the induction of callus and somatic embryogenesis, required different concentration of 2,4-D and sucrose to enable these process to take place. Mature ovules did not produce callus or somatic embryos, but embryos and hypocotyls were good explants for the production of embryogenesis callus. 2,4-D was essential embryogenesis; Sucrose (>60 g/l) was important for embryogenesis. Hypocotyl callus formed embryos at a high rate, even after subculture on media containing only sucrose.

Table 2.2: Micropropagation of some horticultural plant species

Flower crops	Ornamental foliage plants
Amaryllis hybrid	*Anthurium*
Ammi majus	*Asparagus*
Anthurium andreanum	*Caladium*
Anthurium scherzerianum	*Coleus blumei*
Antirrhinum majus	*Dieffenbachia*
Begonia	*Dracaena sp.*
Chrysanthemum morifolium	*Ficus sp.*
Dianthus caryophyllus	*Monstera deliciosa*
Eschscholzia californica	*Scindapcus aureus*
Euphorbia pulcherrima	*Woodwardia fibriata*
Gerbera jamesonii	
Hemerocallis sp.	*Asclepias*
Iris hybrids	*Bouganvillea*
Linium species	*Clerodendrum*
Macleaya cordata	*Cycas*
Narcissus sp.	*Haworthia*
Pelargonium hortorum	*Jatropha*
Petunia hybrida	*Mamillaria*
Rosa sp.	*Passiflora*
Allium cepa	*Achrus sapota*
Allium sativum	*Actidinia chinensis*
Apium graveolens	*Aegle marmelos*
Brassica compestris	*Anonas comosus*
Brassica oleracea	*Carica papaya*
Brassica napus	*Citrus sp.*
Capsicum annuum	*Coffea arabica*
Carum carvi	*Musa cavendishii*

Contd---

Contd.. Table-2.2

Vegetable crops	Fruit and nut crops
Cichorium endivia	*Phoenix dactylifera*
Cichorbita intybus	*Punica granatum*
Cucumus pepo	*Prunus cerosus*
Cucumus melo	*Prunus hybrids*
Curcuma longa	*Rubus sp.*
Daucus carota	*Vitis viñifera*
Pisum sativum	*Zizyphus sps.*
Lactuca sativa	
Lycopersicon esculentum	
Phaseolus vulgaris	
Trigonella foenumgraecum	
Zinziber officinalis	

Prasad (1999)

2.2.2 Anther culture

Litz and Conover (1978 b) recovered low frequency of haploids by culturing anthers excised from 16-20 mm flower buds in liquid MS medium supplemented with 87.6 mM sucrose, 10 g/l activated charcoal, 8.8 µM BA and 2.7 µM NAA. They too have excised anthers from 8-16 mm long inflorescence and cultured them in stationary liquid MS medium fortified with 3 per cent sucrose, 1 per cent activated charcoal, 2.0 µM BA and 0.5 µM NAA for the recovery of haploids of late.

2.2.3 Protoplast culture

Chen and Chen (1992) isolated protoplasts from the immature hybrid embryos of the cross *C. papaya* ×*C. cauliflora* which, when cultured in KM8PS medium for 2 weeks and then plated in the same medium with 1 per sent agarose, resulted in somatic embryos. Protoplast-derived somatic embryos attain on MS medium with 1.0 mg/l ABA and developed into plantlets upon transfer to MS medium devoid of plant growth regulators.

2.2.4 Callus Culture

Fitch (1993) found that hypocotyls section required the presence of 2,4-D for callus induction. Hypocotyls segments of papaya lines CO-3 and Sun rise showed good callusing in the presence of IBA and BA. Mondal *et al.* (1994) initiated root callus on modified MS (half-strength) medium with 0.5 mg/l BAP and 2.0 mg/l IBA.

2.2.5 *In vitro* conservation

Drew (1992) has devised an important technique for *in vitro* propagation and germplasm storage of papaya. Multiple

shoots were obtained by culturing the single node sections in a modified De Fossard medium containing 0.5 µM IBA and 0.5 µM NAA. Shoot obtained were dissected and culture for three days in rooting medium with 10.0 µM IBA and transferred to hormone–free Drew and Smith medium, wherein the growth rate was significantly reduced by substitution of 1 per cent fructose for 2 per cent sucrose.

2.3 Micropropagation of papaya

It appears from the literature that the effort has been made on micropropagation of papaya. Reviews of work done on various aspects of *in vitro* propagation are dealt under the following major heads.

2.3.1 Review on *in vitro* clonal propagation in papaya

Mehdi and Hogan (1976) reported *in vitro* regeneration of single plantlet form shoot tip excised from seedling. The explants were established on MS medium supplemented with 4.7 µM kinetin and rooted on MS medium supplemented with 4.7 µM kinetin and 24.5 µM IBA.

Yie and Liaw (1977) established papaya seedling shoot tips and obtained multiple shoot growth on MS medium with 0.05 mg/l IAA and either 5 mg/l kinetin or 0.5 to 1 mg/l BAP.

The culture of shoot tip from mature field grown papaya clone propagation was reported by Litz and Conover (1978 a). Shoot tips of 2 to 3 mm in size were cultured on establishment medium consisting of MS medium with 87.6 µM sucrose, 47 µM kinetin and 10.8 µM NAA. Enlarged explants were subculture after

2 to 3 months on the shoot proliferation medium which consisted of MS medium with 2.2 µM BAP and 0.54 µM NAA. The rate of proliferation in general was 7 to 8 folds between subcultures. Rooting was induced by transferring the shoots to MS medium supplemented with 0.5 to 15 µM IBA or 1 to 5 µM NAA.

Litz and Conover (1981 a) collected explants from field grown plants of papaya shoot tip. Explants were cultured *in vitro* on establishment medium MS fortified with 50 µM kinetin and 10 µM NAA in a growth room 25 ^{0}C temperature and with 16 hr photoperiod at 1.5 K Lux. Established explants were transferred to proliferation medium consisting of MS with 2 µM 6-benezyl amino purine (BA) and 1 µM NAA. Establishment of explants was influenced by season, sex type and degree or type of microbial infection, proliferation rates varied for different clones and declined sharply after about 13 subcultures. Bacterial contamination rates were a severe problem; however the nearly ubiquitous *Pseudomonas sp*. Contaminat did not cause mortality. Explants could not be established from stock plants affected by a disease of unknown etiology that appeared in experimental planting. Frequency of rooting on MS medium with or without auxin declined with increased time in culture.

Litz *et al.* (1983) compared callus induction from cotyledon lamina explants with that from vein and midrib the lamina on MS medium supplemented with 0.3 to 0.5 mg/l BAP and 1.2 to 5.0 mg/l NAA. Adventitious shoots were obtained from both, lamina and midrib callus on MS medium supplemented with 0.04 to 0.88 mg/l BAP and 0 to 0.22 mg/l NAA. Adventitious root

formation was induced from midrib callus on MS medium supplemented with 0.1 to 15.0 mg/l NAA and 0.0 to 0.5 mg/l BAP.

Rajeevan and Pandey (1983) studied *in vitro* propagation by using shoot tips of papaya var. Coorg Honey Dew. Shoot tips of 5 mm in length were excised from seedlings and after surface sterilization; they were continues agitated in sterile water in a horizontal shaker at 160 rpm for 2 to 3 hours. The shoot tips were cultured successfully for establishment on MS medium supplemented with 0.5 to 1.0 µM NAA and 25 to 50 µM kinetin. For shoot multiplication, MS medium supplemented with 2 µM BAP and 0.5 µM NAA was used. Profuse *in vitro* rooting and normal planted formation was obtained by using MS medium with 10 µM IBA.

Drew and Smith (1986) had taken explants like apical and lateral bud of papaya both high concentration of minerals and addition 10 µM riboflavin to the culture medium reduced callus growth and quality. Best shoot growth occurred when 1.0 μmol^{-1} NAA and 1.0 μmol^{-1} BAP was added with to a modified De Fossard *et al.* (1974) basal medium.

Drew (1987) obtained the best *in vitro* growth from apical bud explants when cultured on De Fossard (1974) medium supplemented with NAA and BAP both at 1 µM. De winnaar (1988) described the shoot tip culture technique using apices of nursery and orchard trees of *Carica papaya* cv. Sunrise Solo. Explants were established with half strength inorganic salts and supplemented with 0.5 mg/l BAP and 0.2 mg/l NAA. Establishment shoot tips were transferred to proliferation medium which

consisted of MT medium with 0.5 mg/l BAP and 0.1 mg/l NAA, which caused extensive multiplication of shoots. Rooting was induced by sub culturing on a media with IBA.

Burikam *et al.* (1988) stated that establishment of cv. Khar Dum and solo was obtained in MS medium containing 0.5 mg/l BAP + 0.2 mg/l NAA. Proliferation was obtained in 15 per cent coconut water, 0.1 mg/l NAA and 120 mg/l adenine sulphate. For rooting, 2-3 cm in height of shoot could be rooted on MS medium with 1.5 mg/l IBA.

Drew (1988) reported that cultured axillary buds collected from matured field grown plants on Drew and Smith (DS) medium supplemented with 1.0 µM BAP + 1.0 µM NAA.

Drew and Miller (1989) studied the cultural factors affecting *in vitro* rooting of papaya. The highest rate of root initiation was obtained in actively growing shoots with the removal of 1 to 2 mm stem base. The optimum rooting occurred at 27°C temperature. Root initiation was significantly reduced in absence of sucrose. High concentrations of vitamins significantly reduced root initiation.

Mondal *et al.* (1990) observed that in papaya var. Honey Dew shoot buds were established successfully on MS medium containing 1.0 mg/l GA_3 and 2.0 mg/l Kinetin. Multiplication of shoots started on transfer to 0.1mg/l NAA and 0.5mg/l BAP media. Rooting was induced in MS medium with reduce salt concentration containing 2.0 mg/l IBA.

Rahaman *et al.* (1992) found that maximum frequency of survival and growth of shoot apices of explants was reported on MS medium fortified with 10.0 mg/l BAP and 1.0 mg/l NAA. After 6 weeks, the growing explants were transferred to 10.0 mg/l BAP and 1.0 mg/l NAA for shoot multiplication. Further, proliferation of shoots were obtained on MS medium supplemented 0.5 mg/l BAP + 0.1 mg/l GA. Root induction in shoot was noticed on half MS medium supplemented with 0.1 mg/l IBA.

Mondal *et al.* (1994) tried on cultivar 'Honey Dew for callus culture and found that callusing was maximum in root explants of *in vitro* grown plantlet, cultured in a modified MS (half strength) with 2.0 mg/l IBA and 0.5 mg/l BAP. Shoot regeneration was maximum in root derived callus, when cultured on full strength modified MS media supplemented with 0.5mg/l IBA and 1 to 2 mg/l Kinetin. Root formation was induced in shoots on half strength modified MS media containing 2 mg/l IBA. Rooted shoots were transferred successfully to the field.

Dhinesh Babu *et al.* (2000) observed that papaya genotype 9-1(D) auxiliary buds explants treated with 0.1 % mercuric chloride for 3 minutes were cultured on MS medium fortified with BAP (1.0 mg/l) + NAA (0.05 mg/l) which induced multiple shoots and elongation also. Further, higher rooting (71.25%) in micro shoot were reported on half MS medium supplemented with IBA (1.00 mg/l) with lesser number of days taken for rooting.

Bhattacharya and Khuspe (2001) studied various pre-sowing treatment *in vivo* and *in vitro* seed germinate .They found

that *in vitro* culture condition increase the percentage and rate of seed germination over *in vivo* in all cultivars. Maximum germination of 95.5% was obtained after 7-8 days for naked embryos, when culture in the light at 30 ^{0}C temperature on MS medium supplemented with TDZ (1.0µM).

Dhinesh Babu *et al.* (2002) studied the effect of age of mother plant on *in vitro* establishment buds of papaya genotype 9-1 D. The results revealed that the axillary buds collected from 10 months old papaya plant recorded minimum contamination (31.25%) and maximum bud break (68.25%) with less bud break time (14.30 days). Found that contamination is lower in 10 month old mother plant.

Suthamathi *et al.* (2002) observed that shoot tip of papaya var. CO-5 recorded the highest callus ability per cent within 25 days in MS medium supplemented with 1.0 mg/l IAA and 1.0 mg/l BA. When development of shoots was observed from shoot tip callus culture when callus cultured on MS medium supplemented with 4.5 mg/l BA compared to Kinetin. Rooting 1.0 mg/l IAA and they kept all explants under 2000-3000 Lux light intensity.

Reuveni *et al.* (2004) reported that maximum success in culture establishment was obtained on MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA. Addition of adenine sulphate 160 mg/l was improved multiplication and shoot growth. For elongation 1 mg/l Kinetin and 0.05 mg/l NAA was necessary before rooting. Rooting was obtained on half strength macro elements of MS medium supplemented with 1.0 mg/l IBA.

Beniwal *et al.* (2006) studded the effect of season on *in vitro* regeneration of papaya cultivars Ranchi Dwarf, Honey Dew and Farm Selection using MS medium supplemented with different concentrations of BAP, Kinetin, 2-4-D, IAA, NAA, IBA and activated charcoal. They observed that August and September month were most ideal for getting maximum regeneration percentage from *in vitro* shoot tip and axillary buds, irrespective of the cultures used in the study.

2.3.2 Review on Acclimatization and planting out of plantlets

Acclimatization is necessary in the case of *in vitro* produced plantlets because *in vitro* plant material is not adopted for *in vivo* conditions (Brainerd and Fuchigami, 1981). The success in acclimatization of *in vitro* produced plants is largely dependent upon not only the post-transfer growth conditions but also the pre-transfer culture conditions (Ziv, 1986). *In vitro* plantlets are very poorly adopted to resist the low humidity, high light levels and more variable temperatures prevailing outside (Wainwright, 1988). Thus, light, temperature and relative humidity are the major factors to be controlled during acclimatization to natural environment.

Litz and Conover (1980 a) observed that *in vitro* rooted plantlet papaya was well survival in soil-compost mixture.

Rajeevan and Pandey (1983) studied that papaya *in vitro* plant after rooting well survived in sand: soil: FYM potting mixture.

Rahaman *et al.* (1992) studied that rooted plantlets were planted in polyethylene bags filled with 1:1 non-sterile garden soil and compost, and successfully acclimated with natural condition through gradual increase of duration under sun-light and they eventually become suitable for final plantlets under sunlight.

Dinesh Babu *et al.* (2000) observed that papaya genotype 9-1(D) after *in vitro* rooting, plantlets were fairly good (33.33%) when planted in plastic cup containing potting medium of sand: soil: vermiculite (1:1:2) with 0.1% calcium sulphate.

Suthamathi *et al.* (2002) reported that after *in vitro* rooting, well rooted plantlets were hardened in a medium containing sand, soil and vermiculite(1:1:2 V/V), later transferred to earthen pots.

III. MATERIALS AND METHODS

The present investigation entitled "Micropropagation in papaya" was carried out at the Plant Tissue Culture and Biotechnology Laboratory, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat during the year 2006-2008. The chapter contains the details regarding the experimental materials used and methodology adopted during the course of investigation.

3.1 Source of explants materials

The experiment was conducted with two different sources of explant materials. Out of which shoot tip explants were collected from 4-6 weeks old nursery-raised papaya plants var. Red Lady grown at Horticulture Nursery, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, were used as mother (donor) plants.

Another source of explants (axillary bud) were collected from mature plant var. Red Lady (approximately 7-8 months old plant) grown at Soil and Water Management Unit Farm, Navsari Agricultural University, Navsari, used as mother (donor) plants.

3.2 Culture media

The most widely accepted MS medium (Murashige and Skoog, 1962) was used as basal medium. The composition of MS medium is presented in Table 3.1 containing only the basal salts (Macro and Micro nutrients), vitamins, sucrose and agar as reported in the original publication. This was supplemented with

Table 3.1: Composition of Murashige and Skoog (1962) medium

	Constituents	Conc. in stock solution (g/100ml)	Final concentration in medium (mg/l)
A)	**Macronutrients**		
	NH_4NO_3	33.00	1650.00
	KNO_3	38.00	1900.00
	$MgSO_4.7H_2O$	7.40	370.00
	KH_2PO_4	3.40	170.00
B)	**Micronutrients**		
	H_3BO_3	0.125	6.20
	KI	0.0166	0.83
	$Na_2MoO_4.2H_2O$	0.005	0.250
	$CoCl_2.6H_2O$	0.0005	0.025
	$MnSO_4.4H_2O$	0.446	22.30
	$ZnSO_4.7H_2O$	0.172	8.60
	$CuSO_4.5H_2O$	0.005	0.025
C)	**Iron sources**		
	$FeSO_4.7H_2O$	5.56	27.85
	Na_2EDTA	7.46	37.35
D)	$CaCl_2.2H_2O$	8.80	440.00
E)	**Vitamins**		
	Thiamine. HCl	0.100	0.5
	Pyridoxine.HCl	0.100	0.5
	Nicotinic acid	0.100	0.5
	Glycine	0.400	2.0
	Carbon sources		
	Sucrose-- 30.0gm/l		
	Myo-inositol -- 100.0mg /l		
	Gelling agent		
	Agar.agar-- 8.0gm/l		

cytokinins and / or auxin at various concentrations; the details of which are given at the appropriate places.

The $FeSO_4.7H_2O$ was dissolved in approximately 40 ml of double distilled water and heated (Table-3.1). The $Na_2EDTA.2H_2O$ was dissolved in approximately 40 ml of double distilled water, separately and mixed while heating (under continuous stirring) with $FeSO_4.7H_2O$ solution. After cooling, the volume was adjusted to 100 ml heating and stirring resulted in a more stable FeNaEDTA complex.

3.2.1 Chemicals

Analytical grade chemicals, obtained from Hi Media Laboratories, British Drug House (BDH), Sisco Research Laboratory (SRL) were used in the preparation of media.

3.2.1.1 Preparation of the medium

The media were prepared either from commercially prepared media package (Hi Media Laboratories) or from the stock solutions. Standard procedures were followed as described below:

3.2.1.2 Culture vessels

Bottles (250 ml) with screw caps and borosil glass test tubes (25 mm x 150 mm) of autoclavable glass were used as culture vessels.

The culture vessels and glass wares used in the preparation of media and the concomitant purposes were cleaned in chromic acid (Potassium dichromate in sulphuric acid). The acid was removed by prolonged and thorough washing in tap water. The

glass wares and culture vessels were then, washed with detergent (Teepol, BDH) followed by thorough washing with excess tap water. The glass wares and culture vessels were finally rinsed with double distilled water and dried in an oven at 70°C temperature. They were then used for experiments.

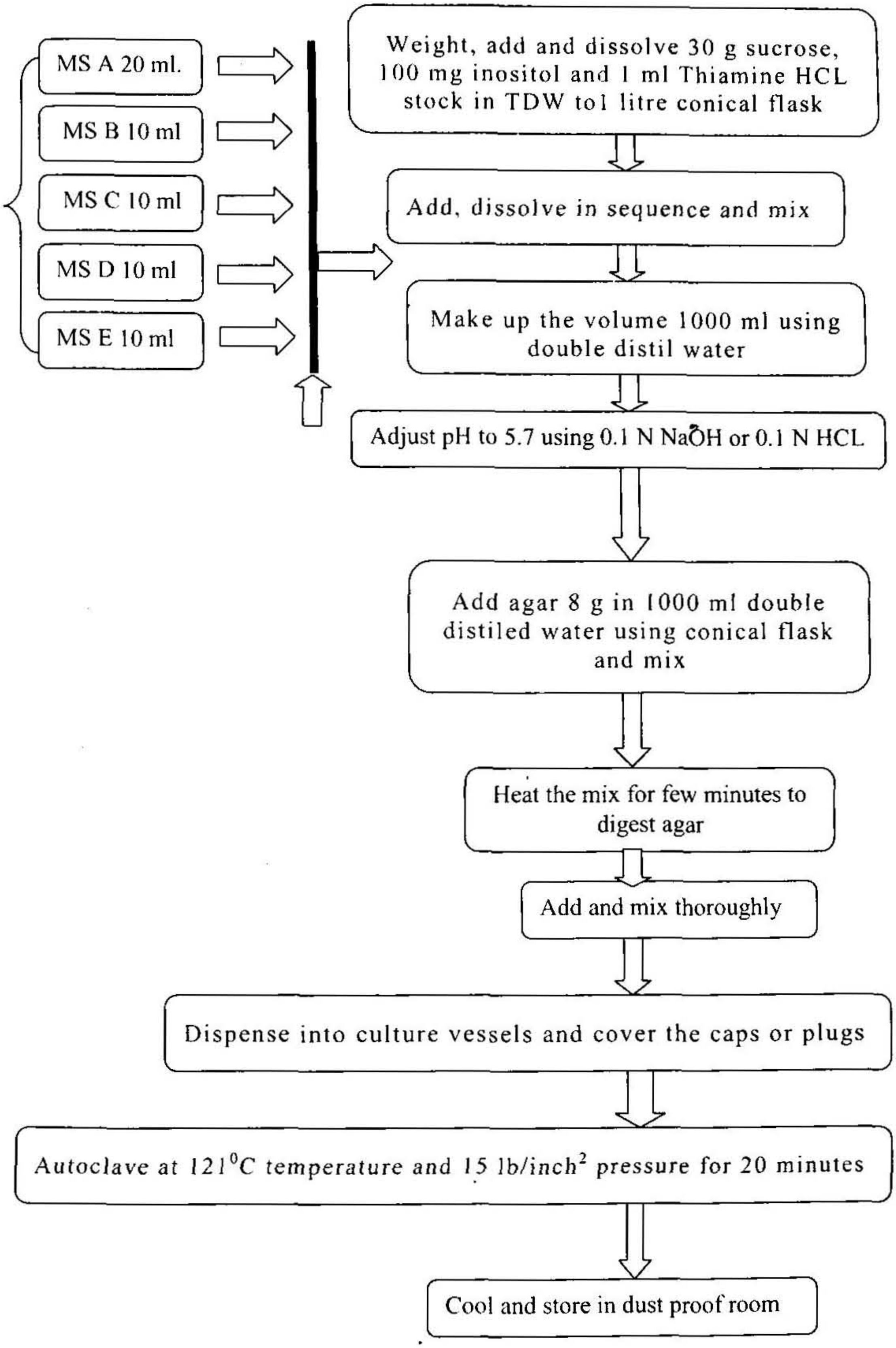
3.2.1.3 Preparation of the stock solutions

The stock solutions were prepared by dissolving the required quantity of chemicals in double distilled water and stored under refrigerated conditions. The stock solutions were prepared fresh after every 4 to 6 weeks and that of the plant growth regulators were prepared fresh after every week.

3.2.1.4 Preparation of medium

The composition for preparing culture medium is given in Table 3.1. The basal medium was prepared from the concentrated stock solutions (Fig.3.1). Cytokinin and / or auxin to be incorporated into the basal medium were added before the final adjustment of the volume. Double distilled water was used for the preparation of media. The pH of the medium was adjusted at 5.7 with a systronic make pH meter using either 0.1 N NaOH or 0.1 N HCl and then, medium solidified with bacteriological agar (8 gm/l) boiled, dispensed into suitable culture vessels and autoclaved. Known volumes of media were distributed in culture vessels (30 ml in bottles and 20 ml in test tubes). The mouth of each bottles were closed with a screw cap to make it air tight. On the other hand the mouth of each glass test tubes were plugged with non-

Fig.3.1 : Schematic procedure for preparation of Murashige and skoog (1962) medium for one litre



absorbent cotton wrapped in gauze. Then, the autoclaved for sterilization.

3.2.1.5 Sterilization of media, culture vessels and instruments

The mouths of glass test tubes were further covered/ wrapped with double-layered ordinary paper to protect them from condensing water vapours while, this was not necessary in case of bottles. The culture vessels were then subjected to autoclave. Autoclaving was carried out at pressure of 15 lb/ $inch^2$ for 20 minutes at approximately 121°C temperature. After which the culture vessel containing autoclaved media were transferred to an air–conditioned room. They were stored for a minimum period of 4 days before use.

Instruments such as scalpels, scissors and forceps, petri dishes, beakers etc. were first wrapped in aluminum foil and then autoclaved as above. They were then, kept in dust free cabinet.

3.2.1.6 Equipments

The major equipments used in the present investigation are given in Table 3.2.

3.3 Aseptic techniques

All inoculations and manipulations involving sterile culture media were carried out under aseptic condition in a laminar air flow cabinet. The interior of the laminar flow was swabbed with 70 per cent ethanol. The instruments and other materials and the culture vessels containing medium were sprayed with 70 per cent ethanol and placed inside the laminar air flow cabinet. The

Table 3.2 Major equipments used in present investigation

Sr. No.	Equipment	Company
1.	Magnetic stirrer	MAC, India
2.	Temperature controlled oven	MAC, India
3.	Laminar air flow cabinet	MAC, India
4.	Analytical balance	Sartorious, Germany
5.	pH meter	Systronic
6.	Water purification system	Millipore, USA
7.	Autoclave	MAC, India

cabinet door was closed and UV light was switched on for 30 minutes prior to working. The plant material was not kept in the cabinet during the UV irradiation. The hands and arms were washed with soap and water and then, swabbed with 70 per cent ethanol before carrying out plant manipulations inside the cabinet. The instruments such as forceps, scalpels, blade handle etc. were sterilized by dipping them in 70 per cent absolute alcohol followed by flaming and cooling. This was done at the start of inoculation and also several times during the operation. During inoculation, first the cap or cotton plug of the culture vessel was removed and then immediately the neck of the vessel was flamed over a spirit lamp kept in the cabinet. The sterilized and trimmed explants were quickly transferred to the culture vessels containing suitable culture medium using sterilized forceps. The neck of the culture vessel was once again flamed and quickly closed up by cotton plug.

Care was taken to avoid any obstruction of the laminar air flow by placing nothing between the working area and the source of air flow. Further, crossing over of hands and arms was avoided. If any plant material fell on to the floor of the cabinet, it was discarded assuming that it was contaminated. After completion of work, the cabinet was sprayed with 70 per cent ethanol then, switched off.

3.4 Culture conditions

All the cultures were incubated in a culture room at a temperature of 26 $\pm$ 2°C with relative humidity at 55 $\pm$ 5 per cent.

Cultures were provided with light using fluorescent tubes with 16:8 hours light/dark cycle, kept 50 cm above bench surface (3000 Lux).

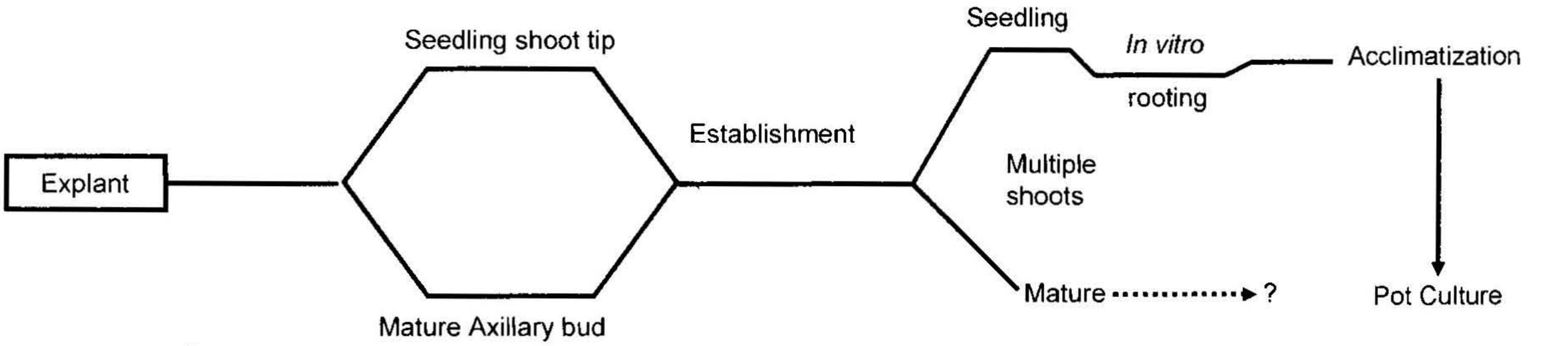
3.5 Micropropagation procedure

In general, micropropagation procedure adopted in the present investigation is illustrated in Fig. 2.

3.5.1 Establishment of explants

3.5.1.1 Preparation and inoculation of explants

Shoot tip were collected from 4-6 weeks old papaya seedling var. Red Lady, where as axillary buds were collected from 7-8 months old plant var. Red Lady grown in the field. Leaves were removed leaving the petioles. They were swabbed with cotton dipped in 70 per cent absolute alcohol and washed thoroughly in running tap water for 2-3 hours to remove traces of alcohol, dirt and latex. The nodal segments were then, kept in a solution of 0.05 per cent bavistin (carbendazim 50 per cent WP) and 0.01 per cent streptocyclin for two hours. The solution was then removed and nodal segments were treated with 10 per cent solution of detergent (Teepol) for 10 minutes. All traces of detergent were removed by repeated washing in double distilled water. Further, sterilization procedures were carried out under aseptic conditions in laminar air flow cabinet. The surface sterilization were made using 0.1 per cent mercuric chloride solution for 3 minutes in case of seedling explants (shoot tips) and that of mature explants (axillary buds) for 10 minutes. The details of surface sterilization treatments are given under separate heading (section 3.5.1.2). They were then,

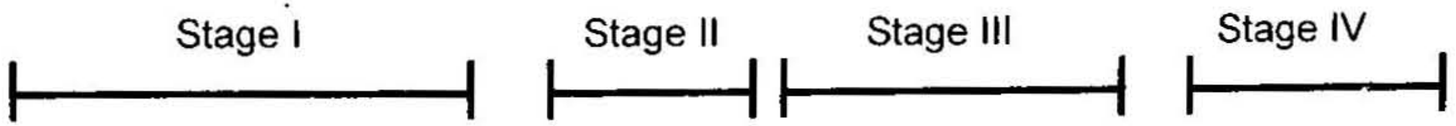


Stage-I = Culture establishment

Stage-II = Multiplication and proliferation

Stage-III = *In vtiro* rooting

Stage-IV = Planting out



3.2

Fig.-1 : *In vitro* cloning procedure in papaya

thoroughly rinsed at least three times with autoclaved de-ionized distilled water. The sterilized nodal segments were then cut and trimmed into small nodal explants of 1-2 cm length. Each explant was then, quickly inoculated on the nutrient medium Plate-I.

3.5.1.2 Standardization of surface sterilization method for the explants

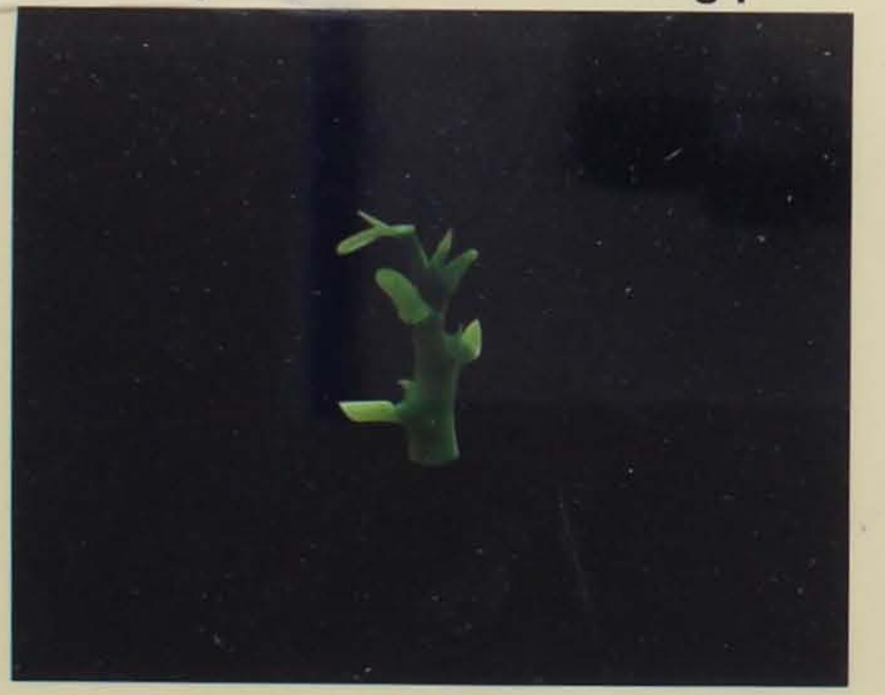
In order to standardize the most effective surface sterilization treatment to isolate contaminants free shoot tip and axillary bud explants of papaya var. Red Lady for culture establishment. A trial was conducted using different concentrations of sterilant as given below.

Treatment No.	Sterilant	Concentration (%)	Duration for treatment
T_1	$HgCl_2$	0.05	5 minutes
T_2	$HgCl_2$	0.05	10 minutes
T_3	$HgCl_2$	0.1	3 minutes
T_4	$HgCl_2$	0.1	5 minutes
T_5	$HgCl_2$	0.1	10 minutes
T_6	$HgCl_2$	0.2	2 minutes

Treatments were repeated four times.

The shoot tip and axillary bud explants after initial treatment as described in this chapter section 3.5.1.1 were subjected to surface sterilization using different concentrations of sterilant mentioned above. Thus, six treatments repeated for four times. The sterilization treatments were given under aseptic conditions in a laminar air flow cabinet. The traces of sterilant were washed out with sterilized double distilled water. Then, the

Shoot tip explant from seedling plant



Seedling plant (4-6 weeks)

Axillary bud of explant from mature female plant

Mature female plant (7-8 month old)

Plate-I : Mother plant and explant of papaya var. Red Leady

explants were inoculated on MS medium. The observations on per cent contamination, non responsive explant (per cent), length of shoot (cm) were recorded.

3.5.1.3 Standardization of establishment

Different growth regulators were tried to standardize the most suitable culture establishment medium for papaya var. Red Lady. The treatments details are given below.

Treatment No.	Treatment
E_1	MS + NAA 0.1 mg/l + BAP 0.5 mg/l
E_2	MS + NAA 0.1 mg/l + BAP 1.0 mg/l
E_3	MS + NAA 0.1 mg/l + BAP 2.0 mg/l
E_4	MS + NAA 0.5 mg/l + BAP 0.5 mg/l
E_5	MS + NAA 0.5 mg/l + BAP 1.0 mg/l
E_6	MS + NAA 0.5 mg/l + BAP 2.0 mg/l
E_7	MS + NAA 1.0 mg/l + BAP 0.5 mg/l
E_8	MS + NAA 1.0 mg/l + BAP 1.0 mg/l
E_9	MS + NAA 1.0 mg/l + BAP 2.0 mg/l
E_{10}	MS + NAA 0.1 mg/l + KN 0.5 mg/l
E_{11}	MS + NAA 0.1 mg/l + KN 1.0 mg/l
E_{12}	MS + NAA 0.1 mg/l + KN 2.0 mg/l
E_{13}	MS + NAA 0.5 mg/l + KN 0.5 mg/l
E_{14}	MS + NAA 0.5 mg/l + KN 1.0 mg/l
E_{15}	MS + NAA 0.5 mg/l + KN 2.0 mg/l
E_{16}	MS + NAA 1.0 mg/l + KN 0.5 mg/l
E_{17}	MS + NAA 1.0 mg/l + KN 1.0 mg/l
E_{18}	MS + NAA 1.0 mg/l + KN 2.0 mg/l

Treatments were repeated thrice.

3.5.1.4 Standardization of multiplication medium

MS medium was used in the trial. Different plant growth regulators were tried to standardize the most suitable culture multiplication medium for papaya var. Red Lady. The treatments details are given below.

Treatment No.	Treatment
A	Continues sub culturing on basal medium
B	Alternate subculturing on basal medium and the best treatment of establishment
C	Continuous sub culturing on the best treatment of establishment

Treatments were repeated seven times.

3.5.1.5.1 Standardization of proliferation medium

Different plant growth regulators were tried to standardize the most suitable culture proliferation medium for papaya var. Red Lady. The treatment details are as under.

W- Best NAA treatment for establishment

Light intensity : L_1 -1000 Lux, L_2- 2000 Lux, L_3- 3000 Lux

Treatment No.	Treatment
P_1	MS + W + L_1
P_2	MS + W + L_2
P_3	MS + W + L_3
P_4	MS + W + BAP 0.5 mg/l + L_1
P_5	MS + W + BAP 0.5 mg/l + L_2
P_6	MS + W + BAP 0.5 mg/l + L3
P_7	MS + W + BAP 1.0 mg/l + L_1

P_8	MS + W + BAP 1.0 mg/l + L_2
P_9	MS + W + BAP 1.0 mg/l + L_3
P_{10}	MS + W + BAP 2.0 mg/l + L_1
P_{11}	MS + W + BAP 2.0 mg/l + L_2
P_{12}	MS + W + BAP 2.0 mg/l + L_3
P_{13}	MS + W + KN 0.5 mg/l + L_1
P_{14}	MS + W + KN 0.5 mg/l + L_2
P_{15}	MS + W + KN 0.5 mg/l + L_3
P_{16}	MS + W + KN 1.0 mg/l + L_1
P_{17}	MS + W + KN 1.0 mg/l + L_2
P_{18}	MS + W + KN 1.0 mg/l + L_3
P_{19}	MS + W + KN 2.0 mg/l + L_1
P_{20}	MS + W + KN 2.0 mg/l + L_2
P_{21}	MS + W + KN 2.0 mg/l + L_3

Treatments were repeated thrice.

3.5.1.5.2 Standardization of optimum sucrose for shoot growth

Experiment was conducted to study the effect of different levels of sucrose on proliferation rate of shoot. MS medium supplemented with 1.0 mg/l Kinetin +0.1 mg/l NAA at 3000 Lux Light intensity and solidified with 0.8 per cent agar was used. The different levels of sucrose are as under.

Treatment No.	Sucrose (per cent)
S_1	1.0
S_2	2.0
S_3	3.0
S_4	4.0
S_5	5.0

3.5.1.5.3 Standardization of optimum pH for *in vitro* shoot growth

Experiment was conducted to study the effect of different pH of the medium on proliferation rate. The treatments details are given below.

Treatment No.	pH level
I_1	4.5
I_2	5.0
I_3	5.5
I_4	5.7
I_5	6.0
I_6	6.5

MS medium supplemented with 1.0 mg/l Kinetin 0.1 mg/l + NAA at 3000 Lux light intensity and solidified with 0.8 per cent agar was used. The pH was adjusted with 0.1N HCl or 0.1N NaOH as found necessary before autoclaving.

3.5.1.5.4 Standardization of optimum adenine sulphate in medium

A trial was conducted to study the effect of different levels of adenine sulphate for maximum shoot growth. The different levels of adenine sulphate tried are as under.

Treatment No.	Adenine sulphate (mg/l)
A_1	40
A_2	80
A_3	120
A_4	160

MS medium supplemented with 1.0 mg/l Kinetin +0.1 mg/l NAA at 3000 Lux light and solidified with 0.8 per cent agar was used.

3.5.1.6 Standardization of *in vitro* rooting medium

The trial on *in vitro* rooting was conducted on quadric, half and full MS medium gelled with 0.8 per cent agar. Each medium was supplemented with different concentration of IBA. The treatments details are as follow.

Treatment No.	Treatment
R_1	MS ¼ + 0.5 mg/l IBA
R_2	MS ¼ + 1.0 mg/l IBA
R_3	MS ¼ + 2.0 mg/l IBA
R_4	MS ½ + 0.5 mg/l IBA
R_5	MS ½ + 1.0 mg/l IBA
R_6	MS ½ + 2.0 mg/l IBA
R_7	MS + 0.5 mg/l IBA
R_8	MS + 1.0 mg/l IBA
R_9	MS + 2.0 mg/l IBA

Treatments were repeated four times.

3.5.1.7 Standardization of potting mixture for hardening of *in vitro* plants.

Rooted plantlets were taken out from the culture vessels with the help of forceps. The nutrient medium was gently removed and washed thoroughly in tap water ensuring that all agar particles were completely removed without damaging the roots. The rooted plantlets were then, dipped in 0.05 per cent bavistin, (carbendazim 50 per cent WP) and planted in earthen pots containing pretreated cocopeat. They were covered with glass baker continuously for 6-7

days and kept in air conditioned room. The cover was gradually removed after 7 days, initially for 3 hours followed by 6 hours and 12 hours in next 3 days. The cover was removed during night and lights put-off for next 3-4 days. Subsequently, the period of keeping the plantlets without any cover was gradually increased and after 15 days they were brought outside the room in shade. Within next 15 days by gradually exposing them to sun, they were acclimatized to natural environment.

Treatment No.	Treatment
H_1	Vermiculite
H_2	Cocopeat
H_3	FYM: soil: sand (1/1/1:: V/V/V)
H_4	Sand
H_5	Perlite

Treatments were repeated four times.

3.6 Observation recorded

3.6.1 Surface sterilization of nodal segment explants

Per cent contamination during incubation period of 4 weeks for calculation of contamination, the following formula was used.

$$\text{Contaminated explants (\%)} = \frac{\text{No. of contaminated explants}}{\text{Total no. of explants used}} \times 100$$

3.6.2 Explants culture establishment, multiplication and proliferation

Observations were recorded as under.

1. Contamination (Per cent)
2. Days taken for establishment
3. Number of shoots per explants
4. Length of shoot (cm)
5. Length of internodes per shoot (cm)
6. Number of internodes per explants
7. Multiplication of shoot in sub culturing
8. Number of shoots proliferation under different light intensity

3.6.3 *In vitro* rooting

1) Survival (per cent) of shoots
2) Days taken for root initiation
3) Length of root (cm)
4) Number of roots per shoot in rooting medium.
5) Length of shoot (cm)

3.6.4 Hardening

1) Survival (per cent) of plantlets
2) Days taken for new sprouting
3) Length of shoot (cm)

3.7 Statistical analysis

Statistical methods were used for comparison of treatment means during optimizing parameters for micropropagation. Completely randomized design (CRD) was used for all the experiment. The data were subjected to analysis of variance (ANOVA) and treatment means were compared (Panse and Sukhatme, 1985).

IV. EXPERIMENTAL RESULTS

Papaya is one of the few fruiting plants of commercial value to be propagated *in vitro* tissue culture. Micropropagation requires the organs and tissues to be passed through a sequence of steps in which different cultural and environmental conditions are provided. The stage I being physiological preconditioning of explant and culture establishment, stage II rapid multiplication of shoots, stage III *in vitro* rooting and stage IV acclimatization of *in vitro* raised plantlets and transplantation to field condition.

The result of experiments entitled, 'Micropropagation in papaya var. Red Lady' conducted at the Tissue Culture Laboratory, ASPEE College of Horticulture and Forestry, Navsari Agricultural university, Navsari during the period 2006-2008 are presented in this chapter.

4.1 Micropropagation technique

4.1.1 Surface sterilization of treatments for papaya explants var. Red Lady

The results of the surface sterilization of shoot tip of seedling and axillary bud explants are presented in Table 4.1 and 4.2 respectively.

A) Shoot tip explant

It is apparently seen from Table 4.1 that establishment, contamination, death of culture and growth of explants was significantly influenced by sterilization treatments. Maximum

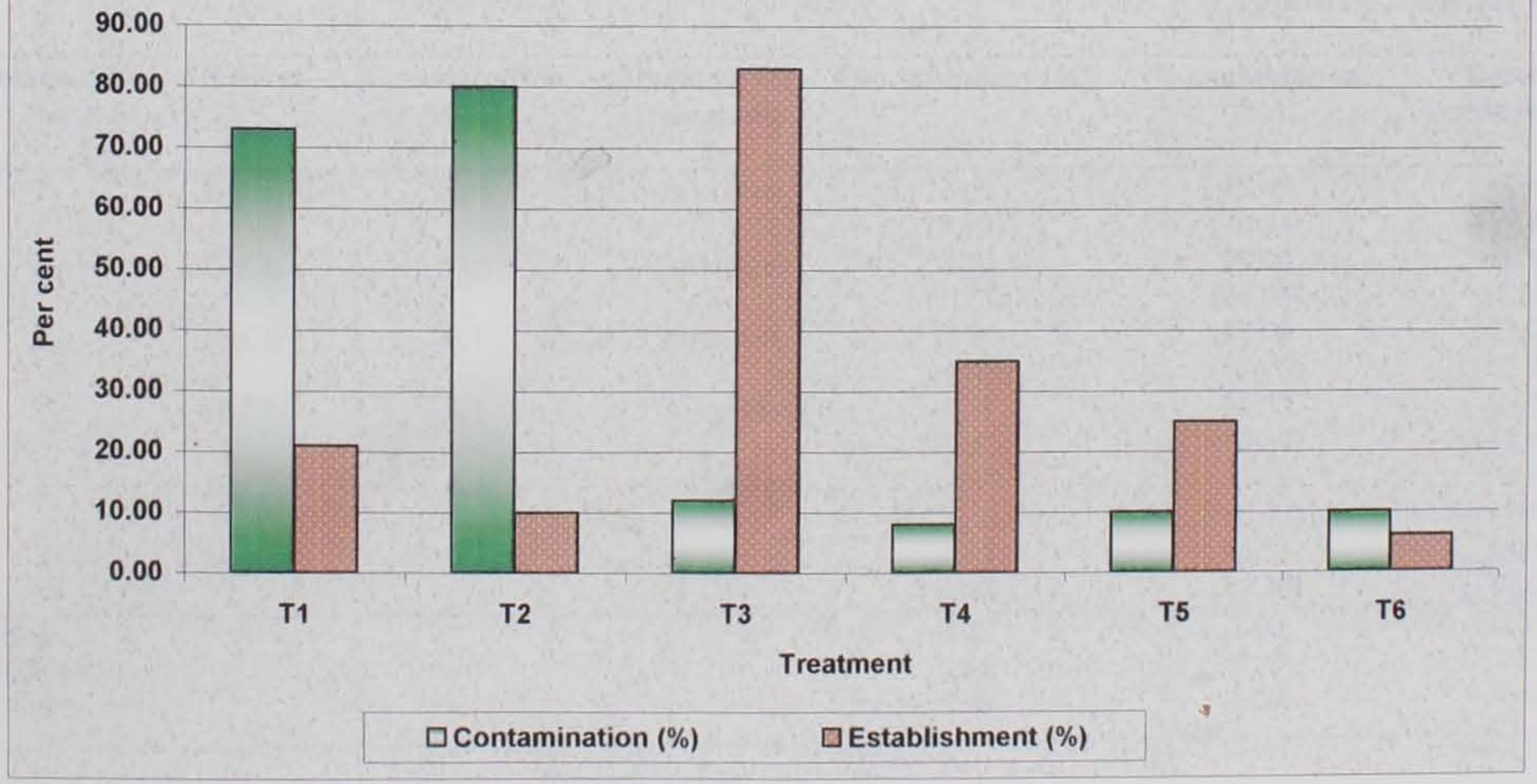
Table 4.1: Effect of surface sterilization agents on establishment, growth and contamination of papaya var. Red Lady

Medium : MS medium
Incubation : 4 weeks
Explants : Shoot tips

Treatment No.	Sterilant	Concentration (%)	Duration for treatments	Establishment (%)	Contamination (%)	Death of culture (%)	Length of shoot (cm)
T_1	$HgCl_2$	0.05	5 minutes	21.00 (27.27)	73.00 (85.70)	6.67 (14.95)	0.70
T_2	$HgCl_2$	0.05	10 minutes	10.00 (18.42)	80.00 (63.45)	10.00 (18.38)	0.50
T_3	$HgCl_2$	0.1	3 minutes	83.33 (65.92)	12.00 (20.26)	4.00 (11.48)	1.10
T_4	$HgCl_2$	0.1	5 minutes	35.00 (36.27)	8.00 (16.41)	49.67 (44.81)	0.90
T_5	$HgCl_2$	0.1	10 minutes	25.00 (29.98)	10.00 (18.42)	63.67 (52.94)	0.80
T_6	$HgCl_2$	0.2	2 minutes	6.00 (14.15)	10.00 (18.42)	83.67 (66.23)	0.60
SEm ±				0.69	0.58	0.86	0.02
CD 5%				2.14	1.80	2.71	0.08
CV %				3.77	3.12	4.39	5.95

Figure in paratheses are arc sine transformed value.

Fig. 3 : Effect of differetn sterilizing treatments on contamination and establishment in micropropagatio of shoot tip explant of papaya var. Red Lady



T1	$HgCl_2$	0.05	5 minutes
T2	$HgCl_2$	0.05	10 minutes
T3	$HgCl_2$	0.1	3 minutes
T4	$HgCl_2$	0.1	5 minutes
T5	$HgCl_2$	0.1	10 minutes
T6	$HgCl_2$	0.2	2 minutes

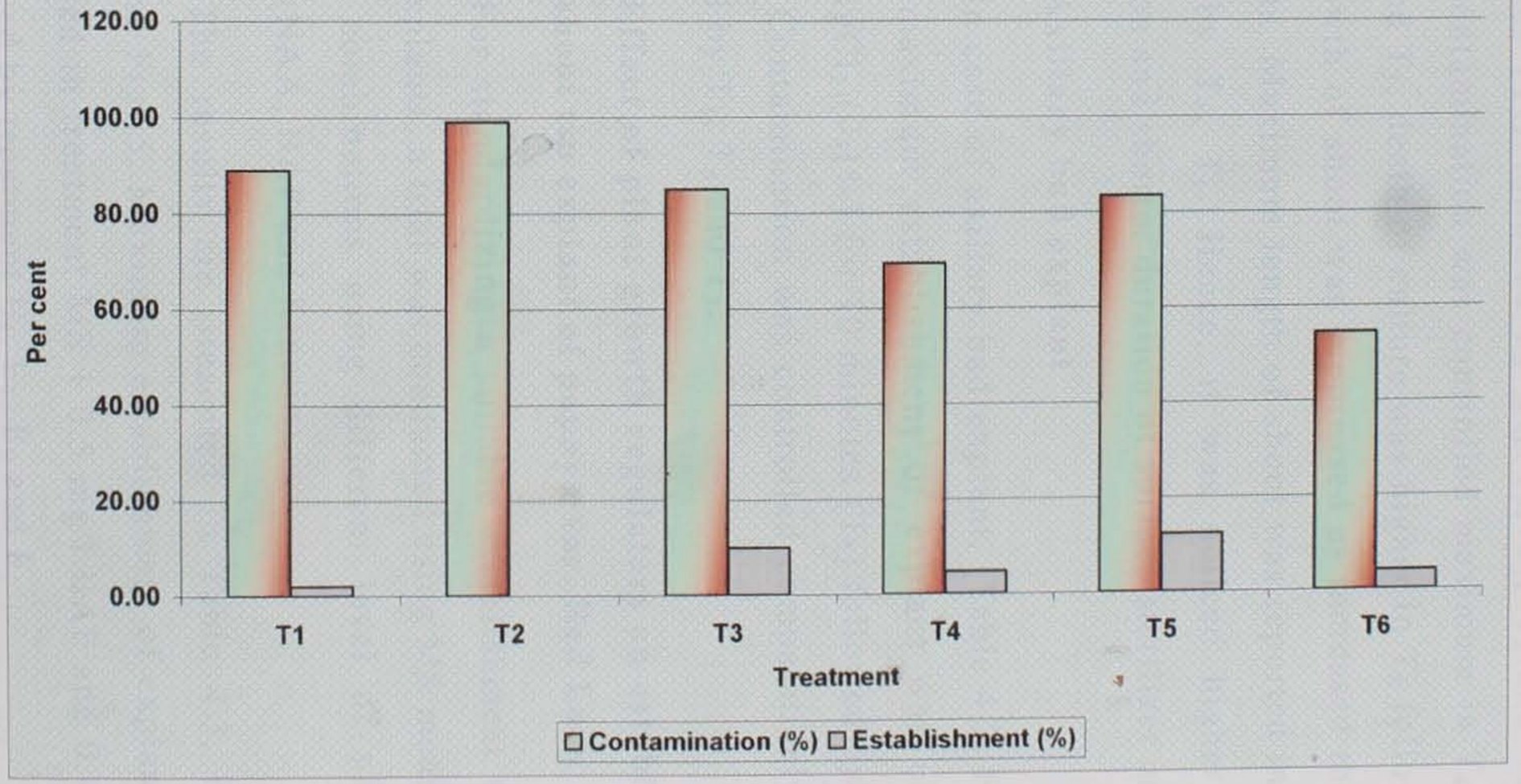
Table 4.2: Effect of surface sterilization agents on establishment, growth and contamination of papaya var. Red Lady

Medium : MS medium
Incubation: 4 weeks
Explants : Axillary buds

Treatment No.	Sterilant	Concentration (%)	Duration for treatments	Establishment (%)	Contamination (%)	Death of culture (%)	Length of shoot (cm)
T_1	$HgCl_2$	0.05	5 minutes	2.00 (8.13)	89.00 (70.83)	9.00 (17.44)	0.93
T_2	$HgCl_2$	0.05	10 minutes	0.00 (1.28)	99.00 (84.95)	0.00 (1.28)	0.67
T_3	$HgCl_2$	0.1	3 minutes	10.00 (18.43)	85.00 (67.55)	21.00 (27.27)	0.50
T_4	$HgCl_2$	0.1	5 minutes	4.73 (12.55)	69.33 (56.39)	49.00 (44.43)	0.80
T_5	$HgCl_2$	0.1	10 minutes	12.17 (20.41)	83.33 (66.02)	4.00 (11.48)	1.10
T_6	$HgCl_2$	0.2	2 minutes	4.00 (11.52)	54.33 (47.49)	58.00 (49.61)	0.60
SEm ±				0.27	2.00	0.59	0.03
CD 5%				0.85	6.17	1.83	0.11
CV %				3.98	5.29	4.08	8.70

Figure in paratheses are arc sine transformed value.

Fig. 4 : Effect of surface sterilization treatments on contamination and establishment in imcropropagatioin of axillary bud explants of papaya var. Red Lady



T1	$HgCl_2$	0.05	5 minutes
T2	$HgCl_2$	0.05	10 minutes
T3	$HgCl_2$	0.1	3 minutes
T4	$HgCl_2$	0.1	5 minutes
T5	$HgCl_2$	0.1	10 minutes

establishment of explants was recorded in treatment $HgCl_2$ (0.1 %) for 3 minutes (T_3) followed by T_4, T_5, T_1, T_2 and T_6 (Fig 3). Although, contamination was controlled maximum in T_4 followed by T_5, T_6 and T_3, death of culture was higher in T_6 followed by T_5 and T_4. Growth of shoot was suppressed as increased the duration of treatments. Maximum length of shoot was reported in treatment T_3 follow by T_4, T_5. Hence, it was noticed higher at lower concentration and shorter duration of surface sterilizer used.

B) Axillary bud explant

In case of axillary bud explant, (Table 4.2, Fig 4), it is clear that maximum establishment of explant was observed in treatment $HgCl_2$ (0.1%) for 10 minutes (T_5) followed by T_3, T_4, T_6, T_1 and T_2. Contamination was controlled comparatively higher in T_6 followed by T_4, T_5 and T_3.

4.2 Effect of plant growth regulators on establishment of shoot tip explant of papaya var. Red Lady

For standardizing a suitable establishment medium for shoot tip explants, a trial was conducted using MS medium with 18 treatments combinations using different level of plant growth regulators (NAA, BAP and Kinetin).

The results are presented in Table 4.3, illustrate in Plate: II and Fig: 5. Maximum establishment of explant (85.00 %) was recorded in treatment MS + 0.5 mg/l BAP and 0.1 mg/l NAA (E_1) followed by treatment E_4, E_7, E_8 and E_2.

Table 4.3: Effect of plant growth regulators on establishment of shoot tips of papaya var. Red Lady

Medium : MS medium
Incubation : 4 weeks
Explants : Shoot tips

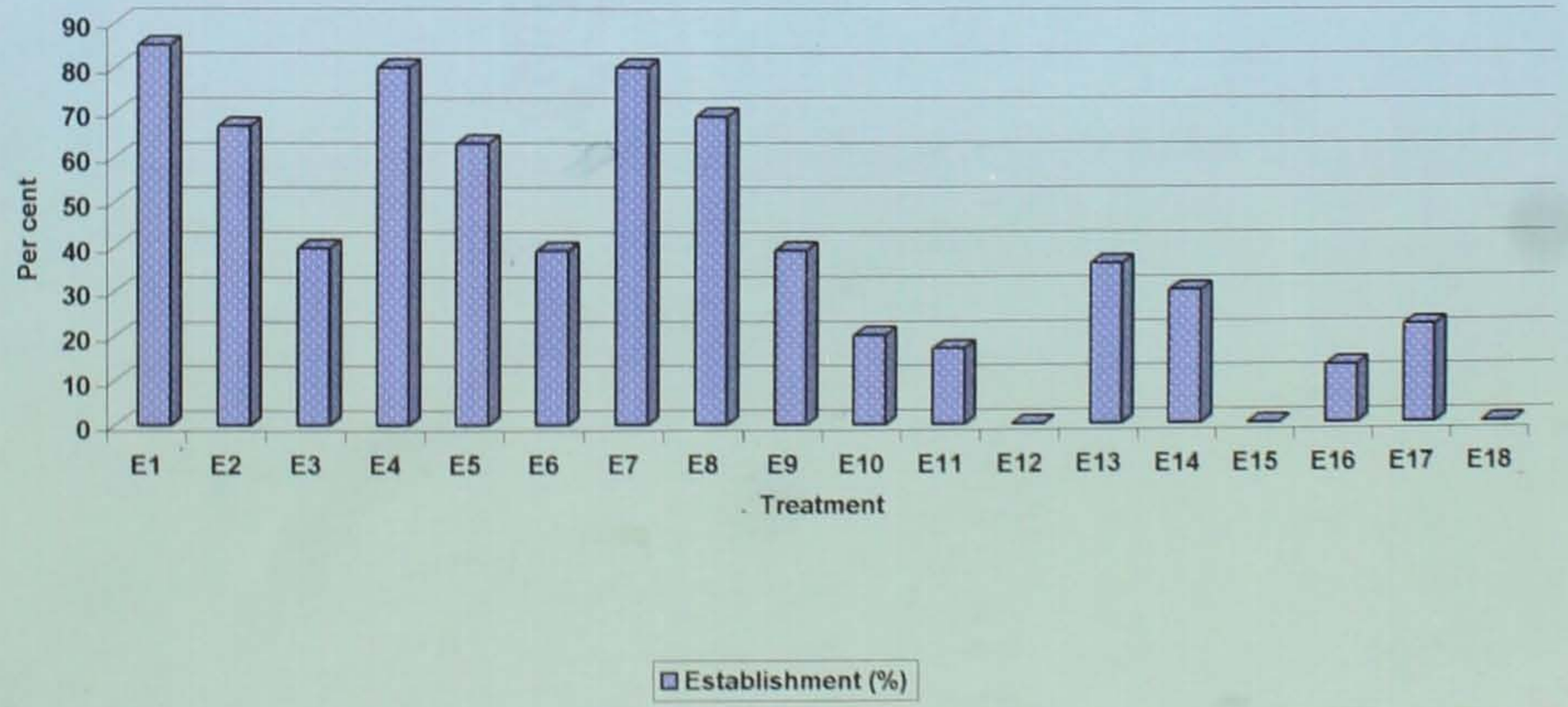
Treatment no.	Plant growth regulator (mg/l)			Establishment (%)	Days taken for establishment	Length of shoot (cm)
	NAA	BAP	Kinetin			
E_1	0.1	0.5	--	85.00 (67.38)	4.00	1.30
E_2	0.1	1.0	--	67.00 (54.95)	5.67	0.90
E_3	0.1	2.0	--	39.67 (39.03)	7.00	0.47
E_4	0.5	0.5	--	80.00 (63.45)	6.00	0.80
E_5	0.5	1.0	--	63.00 (52.54)	6.00	1.00
E_6	0.5	2.0	--	39.33 (38.84)	7.00	0.55
E_7	1.0	0.5	--	80.00 (63.44)	5.33	0.83
E_8	1.0	1.0	--	69.67 (56.59)	7.67	0.70
E_9	1.0	2.0	--	39.00 (38.64)	8.07	0.90
E_{10}	0.1	--	0.5	20.00 (26.55)	5.50	1.02

Contd...

E_{11}	0.1	--	1.0	17.00 (24.33)	7.67	0.70
E_{12}	0.1	--	2.0	0.00 (1.28)	---	0.00
E_{13}	0.5	--	0.5	36.00 (36.87)	6.00	0.90
E_{14}	0.5	--	1.0	30.00 (33.21)	7.00	0.80
E_{15}	0.5	--	2.0	0.00 (1.28)	---	0.00
E_{16}	1.0	--	0.5	13.00 (21.12)	9.83	0.30
E_{17}	1.0	--	1.0	22.00 (27.06)	14.83	1.20
E_{18}	1.0	--	2.0	0.00 (1.28)	---	0.00
S.Em. ±				0.79	0.18	0.02
CD 5%				2.27	0.51	0.06
CV %				3.81	5.24	5.75

Figure in paratheses are arc sine transformed value.

Fig.5 : Effect of establishment treatments on shoot tip explant of papaya var. Red Lady



E1- MS + NAA 0.1 mg/l + BAP 0.5 mg/l
E2- MS + NAA 0.1 mg/l + BAP 1.0 mg/l
E3- MS + NAA 0.1 mg/l + BAP 2.0 mg/l
E4- MS + NAA 0.5 mg/l + BAP 0.5 mg/l
E5- MS + NAA 0.5 mg/l + BAP 1.0 mg/l
E6- MS + NAA 0.5 mg/l + BAP 2.0 mg/l
E7- MS + NAA 1.0 mg/l + BAP 0.5 mg/l
E8- MS + NAA 1.0 mg/l + BAP 1.0 mg/l
E9- MS + NAA 1.0 mg/l + BAP 2.0 mg/l

E10- MS + NAA 0.1 mg/l + KN 0.5 mg/l
E11- MS + NAA 0.1 mg/l + KN 1.0 mg/l
E12- MS + NAA 0.1 mg/l + KN 2.0 mg/l
E13- MS + NAA 0.5 mg/l + KN 0.5 mg/l
E14- MS + NAA 0.5 mg/l + KN 1.0 mg/l
E15- MS + NAA 0.5 mg/l + KN 2.0 mg/l
E16- MS + NAA 1.0 mg/l + KN 0.5 mg/l
E17- MS + NAA 1.0 mg/l + KN 1.0 mg/l
E18- MS + NAA 1.0 mg/l + KN 2.0 mg/l

Establishment of shoot tip explants of seedling of papaya

Establishment of axillary bud of mature explant

Plate-II : Establishment of shoot tip axillary bud on MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA

Further, it was seen that lower levels of BAP and NAA concentrations were more responsive for establishment of explant. The establishment of explant was reduced as increased BAP level in the medium. Similarly a minimum day taken for establishment was observed at lower level of plant growth regulators. i.e. MS medium supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA (E_1) followed by E_7, E_{10}, and E_2. The trend for response of treatments was decreased as increased the levels of BAP, NAA and Kinetin in the medium. Maximum length of shoot was registered in treatment MS medium supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA (E_1) followed by E_{17} and E_{10}. Overall, treatment E_1 was found the most effective among all the treatments.

4.3 Effect of serial subculturing on multiplication of shoot tip of papaya var. Red Lady

For standardizing a suitable multiplication medium and serial subculturing (upto 4 subcultures) for shoot tip explants was examined with three treatment combinations i.e. A- Continues sub culturing on basal medium, B- Alternate subculture on basal medium and best of establishment treatment (E1- MS medium + 0.5 mg/l BAP + 0.1 mg/l NAA) and C - Continuous sub culturing on treatment (E1) . The results are presented in Table 4.4 and depicted in Plate- III and Figure- 6 and 7.

The culture obtained on the best establishment treatment E_1 were transferred to continuously on basal MS medium (A); alternate on basal MS medium and E_1 treatment (B) and continues on E_1 treatment (C) for four subcultures. When the shoots obtained on E_1 were transferred on the same medium

Table 4.4: **Effect of serial subculturing on multiplication rate of seedling shoot tip of papaya var. Red Lady**

Medium : MS medium,
Incubation : 4 weeks
Explants : Shoot tips

Group of trial	1st sub culturing		2nd sub culturing		3rd sub culturing		4th sub culturing	
	Average no. of shoot per explants	Length of shoot (cm)	Average no. of shoot per explants	Length of shoot (cm)	Average no. of shoot per explants	Length of shoot (cm)	Average no. of shoot per explants	Length of shoot (cm)
A	3.00	1.30	1.49	1.00	1.00	1.20	1.01	0.81
B	4.50	1.40	4.00	1.30	3.50	1.30	3.41	1.17
C	5.00	1.27	3.61	1.00	2.60	1.00	2.51	1.10
S.Em. ±	0.08	0.03	0.04	0.02	0.05	0.02	0.04	0.01
CD 5%	0.24	0.09	0.12	0.06	0.15	0.75	0.12	0.55
CV %	5.31	6.52	3.62	5.25	5.81	5.71	4.48	4.74

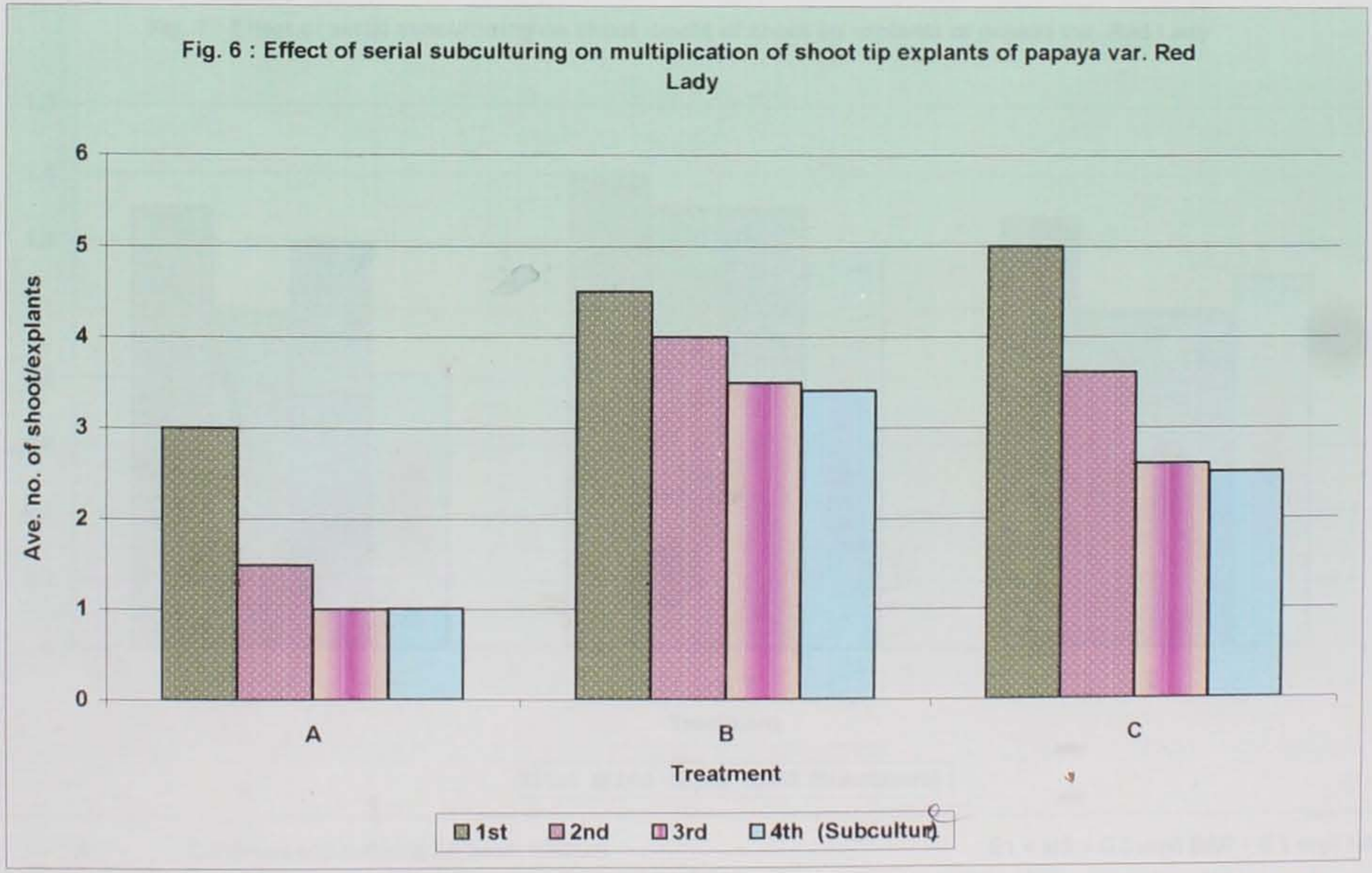
A- Continues sub culturing on basal medium.

B- Alternate subculture on basal medium and best of establishment treatment (E1).

C - Continuous sub culturing on treatment (E1).

E_1- MS medium + 0.5 mg/l BAP + 0.1 mg/l NAA.

Fig. 6 : Effect of serial subculturing on multiplication of shoot tip explants of papaya var. Red Lady



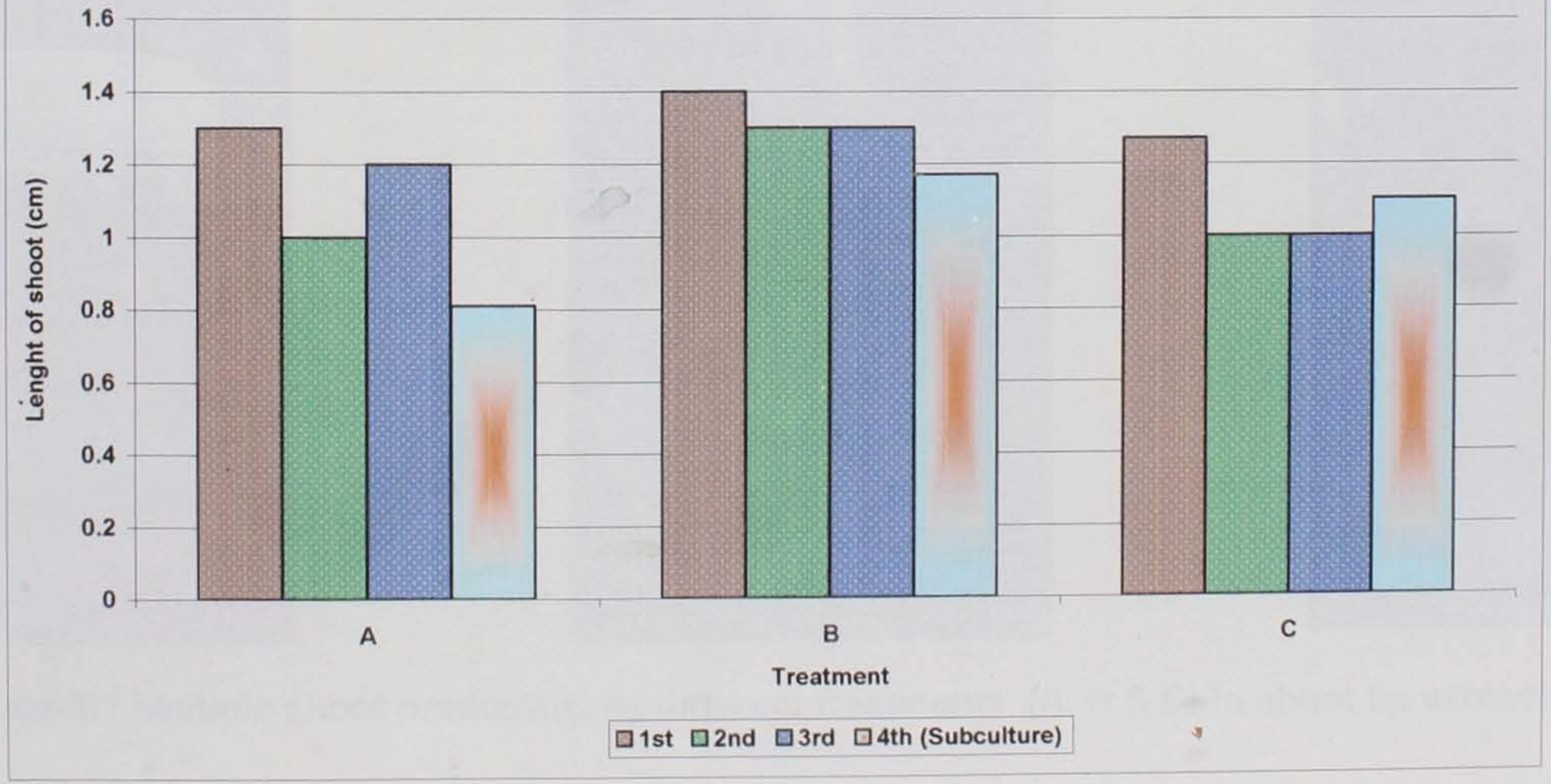
A Continues sub culturing on basal medium

B Alternate subculture on basal medium and treatment (E1)

C Continuous sub culturing on (E1) treatment

E1 = MS + 0.5 mg/l BAP + 0.1 mg/l NAA

Fig. 7 : Effect of serial subculturing on shoot lenght of shoot tip explants of papaya var. Red Lady



A	Continues sub culturing on basal medium	E1 = MS + 0.5 mg/l BAP + 0.1 mg/l NAA
B	Alternate subculture on basal medium and treatment (E1)	
C	Continuous sub culturing on (E1) treatment	

A

B

C

Plate-III : Multiple shoot production by different treatments (A, B & C) in shoot tip explant of papaya

A = Continous subculturing on basal MS medium

B = Alternate subculturing on basal MS medium and treatment E_1

C = Continous subculturing on treatment E_1

E_1 = MS + 0.5 mg/l BAP +0.1 mg/l NAA

continuously for four subculture, the multiplication rate and length of shoot declined considerably. Similar trend was seen in all the treatments. However, maximum multiplication rate and growth of shoots were reported higher in treatment B in all the subcultures as compared to other treatments. The third set of treatment (A) i.e. continuously subculturing the shoot to basal medium practically failed to support the multiplication of shoot.

4.4 Standardization of proliferation medium

4.4.1 Effect of BAP, Kinetin and NAA with different light intensity on shoot proliferation of papaya var. Red Lady

The data on proliferation response to different level of BAP, Kinetin and NAA supplemented in MS medium in combination of different light intensity are presented in Table 4.5, Fig-8 and Plate-IV (A). It was noticed that maximum proliferation (67 %) was recorded in treatment MS medium + 1.0 mg/l Kinetin + 0.1 mg/l NAA at 3000 Lux light intensity (P_{18}) followed by P_6 and P_{17}, which was 62.00 and 59.00 per cent respectively. Maximum length of internodes recorded in P_{18} (0.60 cm) followed by treatment P_9 (0.55 cm). Similarly, length of shoot was significantly higher in treatment P_{18} (2.00 cm) followed by treatment P_9 (1.90 cm).

4.4.2 Effect of level of sucrose in medium on shoot proliferation papaya var. Red Lady

From the perusal of data given in Table 4.6. It is evident that the proliferation.was directly affected by the different concentrations of sucrose in the medium. Maximum number of

Table 4.5: Effect of, BAP, KN, and NAA with different light intensity on shoot proliferation of papaya var. Red Lady

Medium : MS medium.
Incubation : 4 weeks
Explants : Shoot tips

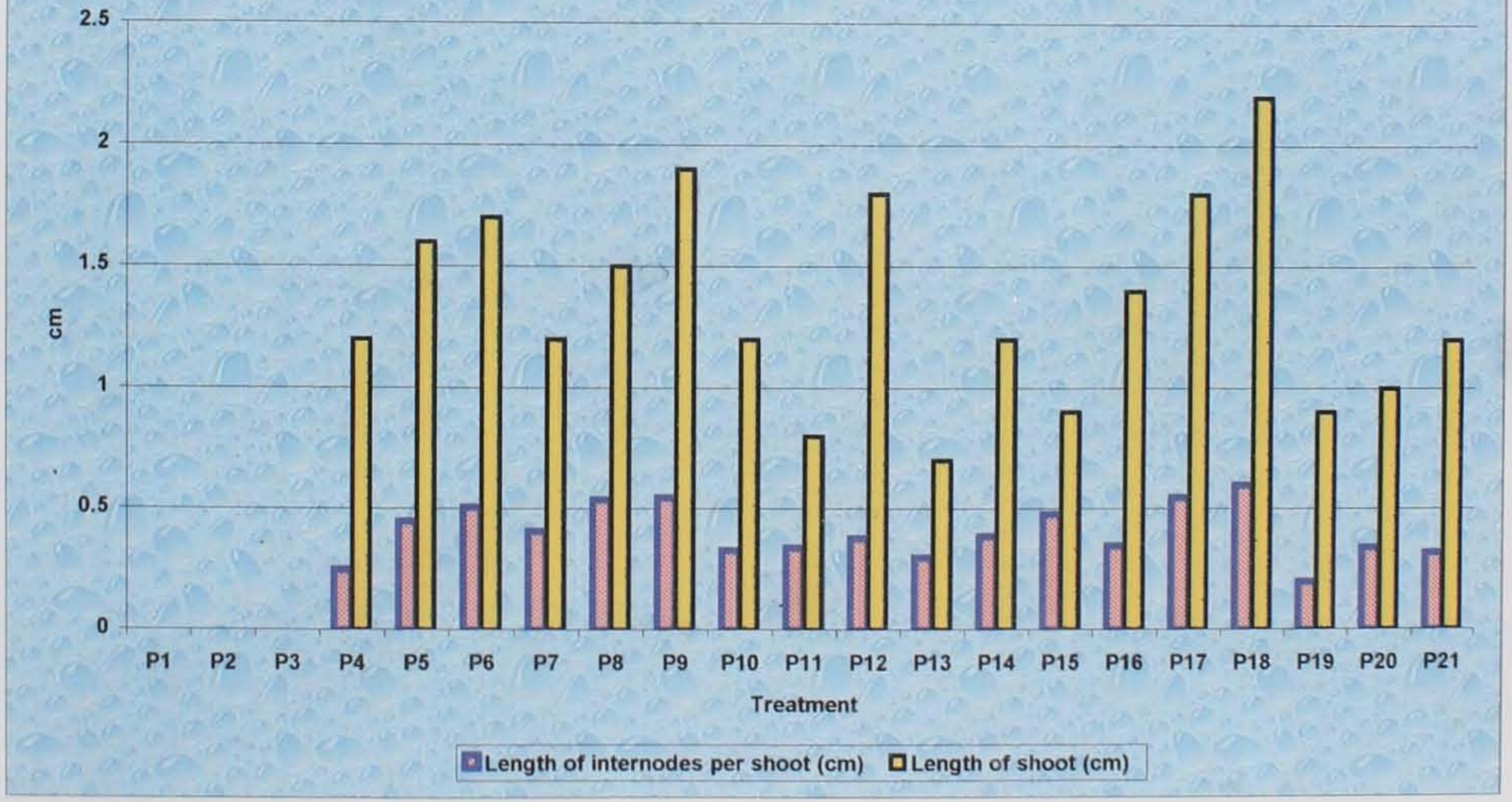
Treatment no.	Plant growth regulator			Light intensity (Lux)	Proliferation (%)	Length of internodes per shoot (cm)	Length of shoot (cm)
	NAA (mg/l)	BAP (mg/l)	KN (mg/l)				
P_1	0.1	--	--	1000	0.00 (1.28)	0.00	0.00
P_2	0.1	--	--	2000	0.00 (1.28)	0.00	0.00
P_3	0.1	--	--	3000	0.00 (1.28)	0.00	0.00
P_4	0.1	0.5	--	1000	40.83 (39.72)	0.25	1.20
P_5	0.1	0.5	--	2000	44.67 (41.94)	0.45	1.60
P_6	0.1	0.5	--	3000	62.00 (51.96)	0.51	1.70
P_7	0.1	1.0	--	1000	46.00 (42.70)	0.41	1.20
P_8	0.1	1.0	--	2000	45.00 (42.13)	0.54	1.50
P_9	0.1	1.0	--	3000	49.00 (44.43)	0.55	1.90
P_{10}	0.1	2.0	--	1000	30.00 (33.21)	0.33	1.20
P_{11}	0.1	2.0	--	2000	57.00 (49.03)	0.34	0.80

Contd...

P_{12}	0.1	2.0	--	3000	52.00 (46.15)	0.38	1.80
P_{13}	0.1	--	0.5	1000	36.00 (36.87)	0.30	0.70
P_{14}	0.1	--	0.5	2000	38.00 (38.05)	0.39	1.20
P_{15}	0.1	--	0.5	3000	44.00 (41.55)	0.48	0.90
P_{16}	0.1	--	1.0	1000	38.00 (38.05)	0.35	1.40
P_{17}	0.1	--	1.0	2000	59.00 (50.19)	0.55	1.80
P_{18}	0.1	--	1.0	3000	67.00 (54.95)	0.60	2.20
P_{19}	0.1	--	2.0	1000	45.00 (42.13)	0.20	0.90
P_{20}	0.1	--	2.0	2000	52.00 (46.15)	0.34	1.00
P_{21}	0.1	--	2.0	3000	59.00 (50.19)	0.32	1.20
SEm ±					0.68	0.01	0.03
CD 5%					1.94	0.32	0.11
CV %					3.12	5.60	5.48

Figure in paratheses are arc sine transformed value.

Fig. 8 : Effect of shoot prolifaration treatment



P1	MS + W + L1	P12	MS + W + BAP 2.0 mg/l + L3
P2	MS + W + L2	P13	MS + W + KN 0.5 mg/l + L1
P3	MS + W + L3	P14	MS + W + KN 0.5 mg/l + L2
P4	MS + W + BAP 0.5 mg/l + L1	P15	MS + W + KN 0.5 mg/l + L3
P5	MS + W + BAP 0.5 mg/l + L2	P16	MS + W + KN 1.0 mg/l + L1
P6	MS + W + BAP 0.5 mg/l + L3	P17	MS + W + KN 1.0 mg/l + L2
P7	MS + W + BAP 1.0 mg/l + L1	P18	MS + W + KN 1.0 mg/l + L3
P8	MS + W + BAP 1.0 mg/l + L2	P19	MS + W + KN 2.0 mg/l + L1
P9	MS + W + BAP 1.0 mg/l + L3	P20	MS + W + KN 2.0 mg/l + L2
P10	MS + W + BAP 2.0 mg/l + L1	P21	MS + W + KN 2.0 mg/l + L3
P11	MS + W + BAP 2.0 mg/l + L2		

W = 0.1 mg/l NAA
L1= 1000 Lux light intensity
L2= 2000 Lux light intensity
L3=3000 Lux light intensity

A

MS medium supplimented with
1.0 mg/l Kinetin & 0.1 mg/l NAA
at 3000 Lux light intensity

B

Sucrose (3%)

C

pH 5.7

D

Adenine shalphate 160 mg/l

Plate-IV : Factor affecting on shoot proliferation

shoot per explant and length of longest shoot was observed in 3 per cent sucrose level (S_3) that is 2.2 and 2.0 cm respectively (Fig. 9). In general, maximum proliferation was seen at S_3 treatment; moreover, it was reduced gradually at either increased or decreased levels of sucrose from S_3, Fig 9 and Plate – IV (B).

Table 4.6: Effect of different level of sucrose on *in vitro* shoot proliferation of papaya var. Red Lady

Incubation: 4 weeks

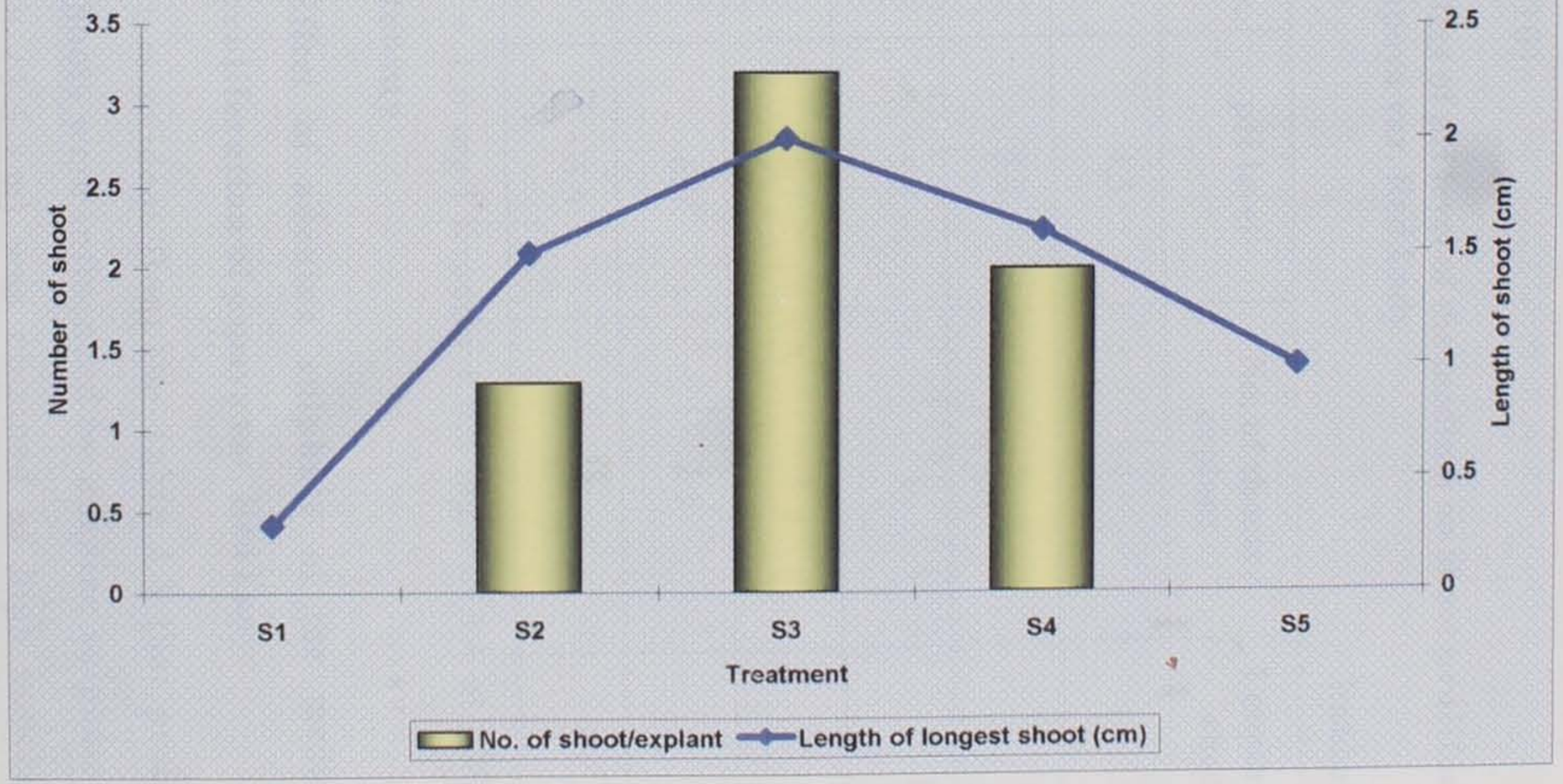
Treatment No.	No. of shoots/explant	Length of longest shoot (cm)
S_1 - Sucrose (1 %)	0	0.3
S_2 - Sucrose (2 %)	1.3	1.5
S_3 - Sucrose (3 %)	2.2	2.0
S_4 - Sucrose (4 %)	2	1.6
S_5 - Sucrose (5 %)	0	1

Medium- MS + 1.0 mg/l kinetin + 0.1 mg/l NAA at 3000 Lux light intensity

4.4.3 Effect of pH on shoot proliferation of papaya var. Red Lady

The influences of pH on proliferation rate data are presented in Table 4.7. Different pH levels tested were 4.5, 5.0, 5.5, 5.7, 6.0 and 6.5. Out of all pH levels tested, maximum number of shoots per explant (3.0) and length of longest shoot (2.75 cm) were recorded with pH 5.7 among all the treatments. The trends of

Fig. 9 : Effect of different levels of sucrose on number of shoots and lenght of shoot in proliferation medium of papaya var. Red Lady



S1	0 % sucrose
S2	2 % sucrose
S3	3 % sucrose
S4	4 % sucrose
S5	5 % sucrose

number of shoots per explant and length of longest shoot increased as pH level increased up to 5.7, then, subsequently declined (Plate – IV(C), Fig 10).

Table 4.7: Effect of pH level of the medium on shoot proliferation of papaya var. Red Lady

Incubation: 4 weeks

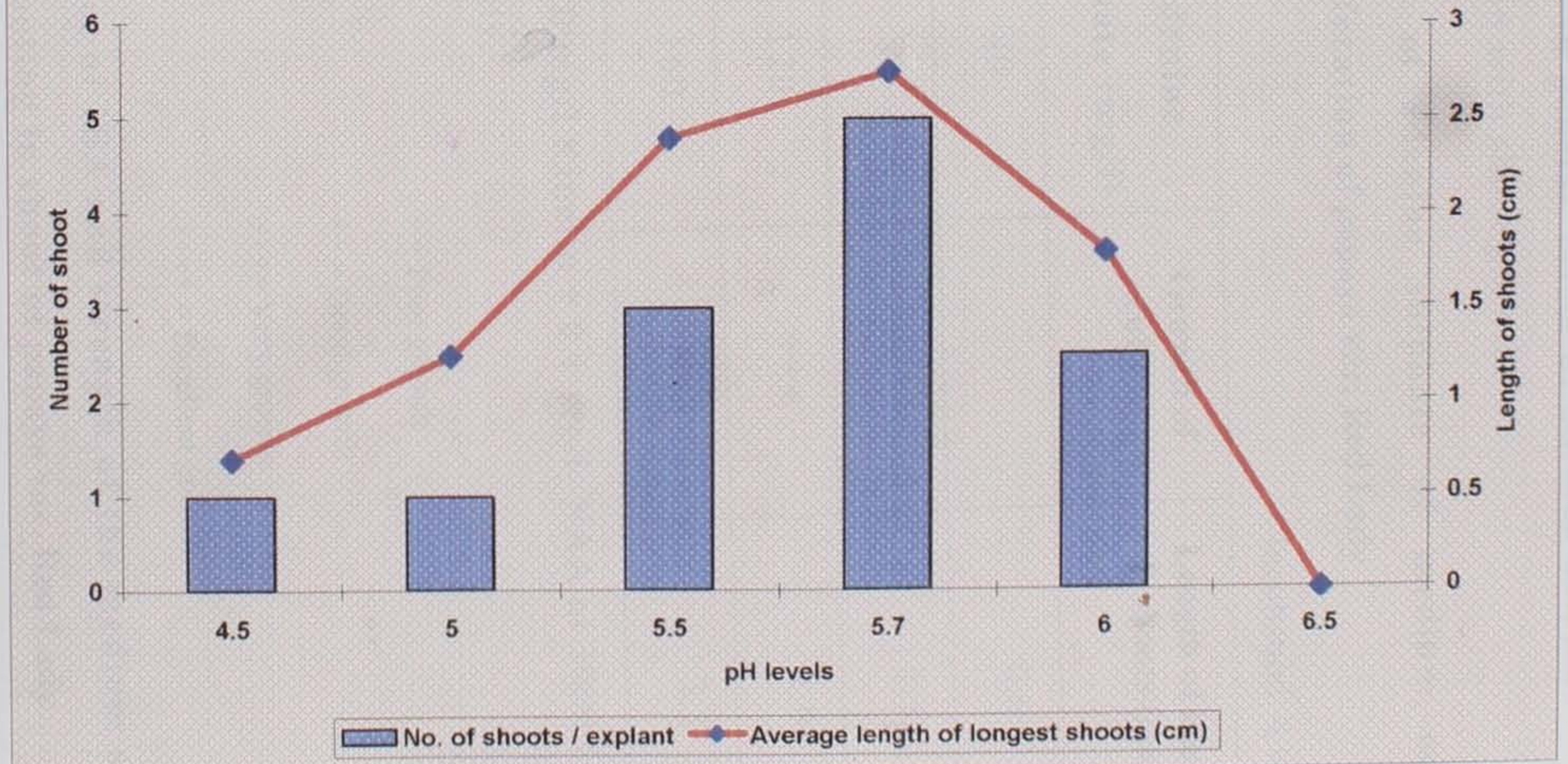
Treatment No.	pH level	No. of shoots / explant	Length of longest shoots (cm)
I_1	4.5	1	0.7
I_2	5.0	1	1.25
I_3	5.5	3	2.4
I_4	5.7	3	2.75
I_5	6.0	2.5	1.8
I_6	6.5	0	0

Medium- MS + 1.0 mg/l Kinetin + 0.1 mg/l NAA at 3000 Lux light intensity

4.4.4 Effect of adenine sulphate of the medium on shoot proliferation of papaya var. Red Lady

It is evident from the Table 4.8 that proliferation and growth of shoots were influenced by adenine sulphate. The longest shoot length (3.5 cm) was obtained at high concentration (160 mg/l) adenine sulphate in medium while lowest level (40 mg/l) of

Fig. 10 : Effect of different pH levels on number of shoots and lenght of shoot in proliferation medium of papaya var. Red Lady



adenine sulphate registered minimum shoot length (0.6cm). (Plate – IV (D) Fig 11). In general, effect of adenine sulphate was seen positive correlation with shoot growth.

Table 4.8: Effect of adenine sulphate of medium on shoot proliferation of papaya var. Red Lady

Incubation: 4 weeks

Treatment No.	Adenine sulphate (mg/l)	Growth of culture	Length of longest shoots (cm)
A_1	40	+	0.6
A_2	80	++	1.1
A_3	120	+++	1.9
A_4	160	++++	3.5

Medium- MS + 1.0 mg/l Kinetin + 0.1 mg/l NAA at 3000 Lux light intensity

\+ = Poor growth

++ = Good growth

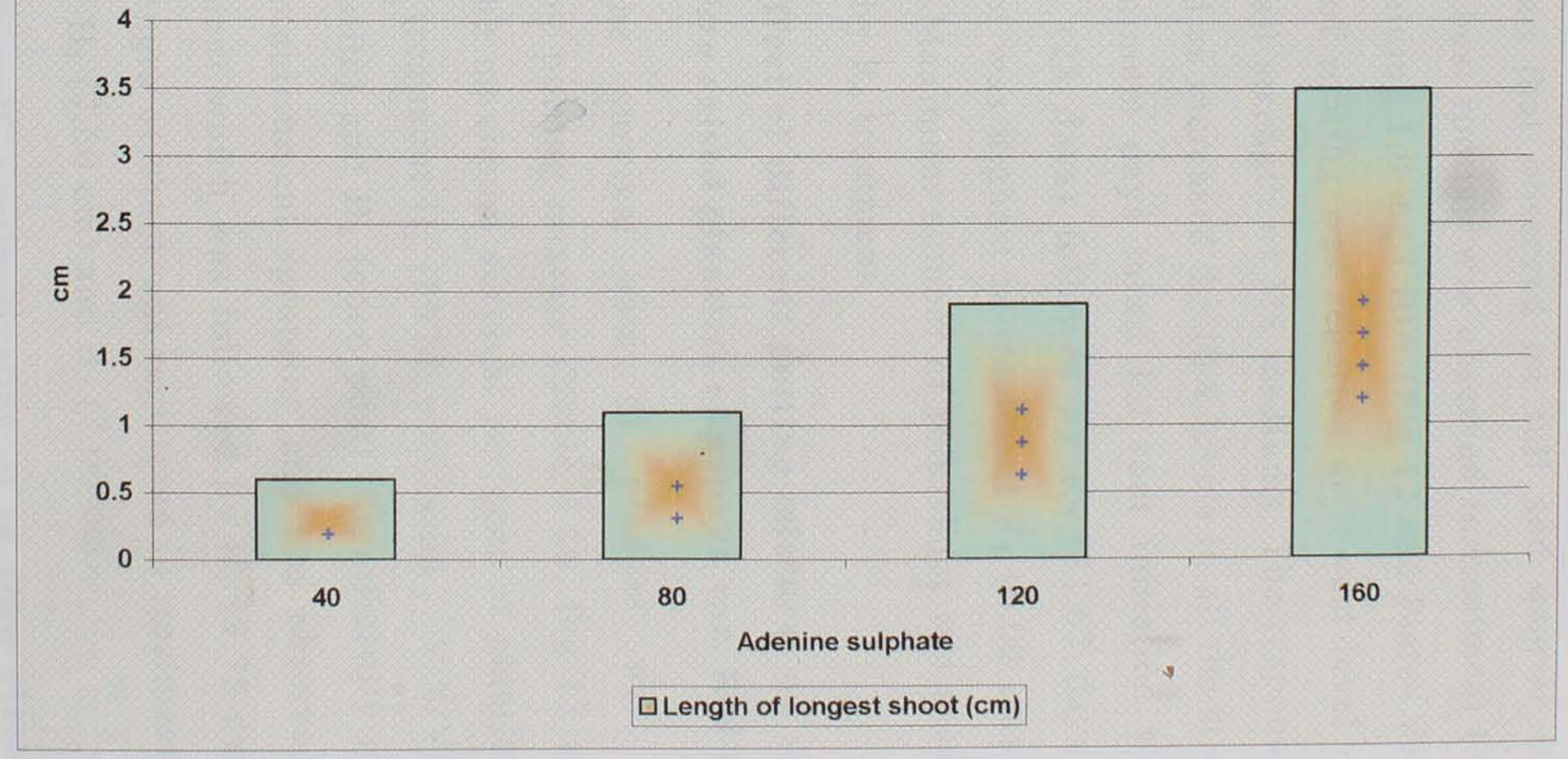
+++= Very growth

++++ = Excellent growth

4.5. Effect of IBA and strength of the medium on *in vitro* of rooting in shoot of papaya var. Red Lady

The data on rooting response to different levels of IBA supplemented in quarter, half and full strength of MS medium is

Fig.-11 : Effect of adenine sulhpate in proliferation medium on shoot lenght and growth of papaya var. Red Lady



+ = Poor growth
++ = Good growth
+++= Very growth
++++ = Excellent growth

presented in Table 4.9. It was noticed that rooting of *in vitro* shoot on half strength MS medium was better in respect to all the rooting characters than that observed on full and quarter strength MS medium. The best rooting was observed in treatment (R_5) half MS medium containing 1.0 mg/l IBA (Fig 12 and Plate- V). Moreover, no response of rooting was reported at lowest level of IBA (0.5 mg/l) in the all strength of MS mediums. The response of rooting was increased as increased the IBA levels in all the strength of MS medium. Minimum days taken for root initiation was found in treatment R_5 (28 days) followed by R_2 (31.67 days). Similarly, length of root was highest in treatment R_5 (6.00 cm) followed by R_8 (4.5 cm). Maximum number of root/shoot was also observed in R_5 followed by R_6 treatment.

4.6 Effect of different potting mixtures on survival of *in vitro* raised plantlets of papaya var. Red Lady

The survival rate of plantlet was significantly influenced by potting mixture (Table 4.10, Fig 13 and Plate –VI and VII). The maximum survival per cent of plantlet (70 %) was observed in treatment H_3 (FYM: soil: sand :: V/V/V) followed by H_1 (Vermiculite) and H_2 (coco peat). Significantly minimum days taken for establishment sprouting and was observed in H_3 (8.00 days) treatment which was followed by H_1 (9.75 days). Similarly maximum length of shoot was registered in treatment H_3 (8.30 cm) followed by H_1 (7.60 cm) and H_2 (7.00 cm).

Overall, acclimatization of *in vitro* plantlet was found better in H_3 among all the potting mixtures.

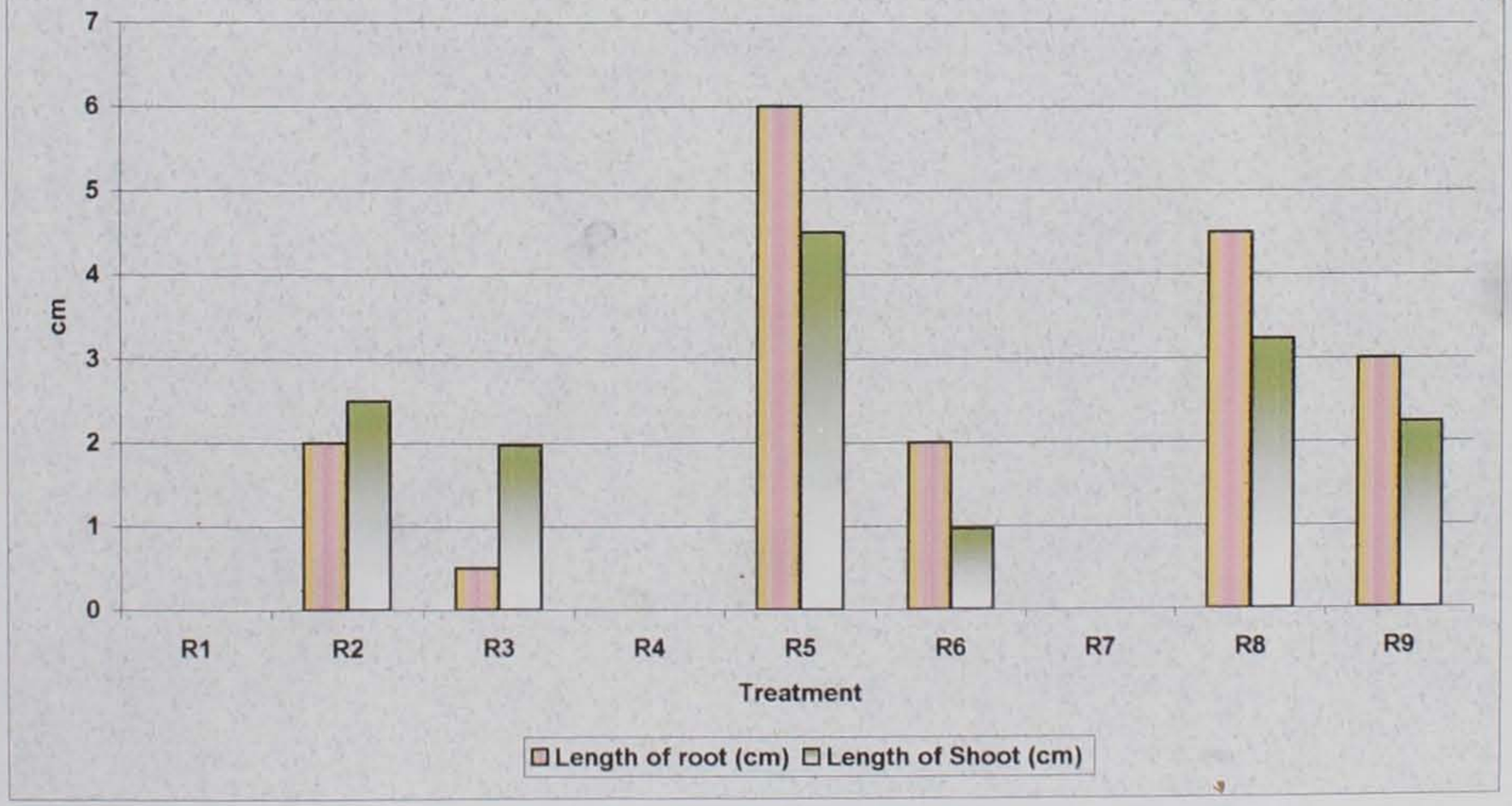
Table 4.9: Effect of IBA and strength of medium on induction of rooting of papaya var. Red Lady

Incubation: 6 weeks

Treatment No.	Rooting (%)	Days taken for root initiation	Length of root (cm)	No. of root / shoot	Length of shoot (cm)
R_1	0.00 (1.28)	---	0.00	0.00	0.00
R_2	65.00 (53.73)	31.67	2.00	2.00	2.50
R_3	69.00 (56.17)	36.00	0.50	2.00	1.97
R_4	0.00 (1.28)	---	0.00	0.00	0.00
R_5	78.00 (62.03)	28.00	6.00	5.00	4.50
R_6	70.00 (56.79)	32.67	2.00	4.00	0.97
R_7	0.00 (1.28)	---	0.00	0.00	0.00
R_8	60.00 (50.77)	35.33	4.50	3.00	3.23
R_9	50.33 (45.19)	37.33	3.00	2.00	2.23
S.Em. ±	0.57	1.63	0.05	0.05	0.04
CD 5%	1.70	4.86	0.16	0.16	0.13
CV %	2.73	12.69	4.71	4.71	4.50

Figure in paratheses are arc sine transformed value.

Fig. 12 : Effect of strength of medium and IBA concentrations on rooting in shoot of papaya var. Red Lady



R1	MS ¼ + 0.5 mg/l IBA	R6	MS ½ + 2.0 mg/l IBA
R2	MS ¼ + 1.0 mg/l IBA	R7	MS + 0.5 mg/l IBA
R3	MS ¼ + 2.0 mg/l IBA	R8	MS + 1.0 mg/l IBA
R4	MS ½ + 0.5 mg/l IBA	R9	MS + 2.0 mg/l IBA
R5	MS ½ + 1.0 mg/l IBA		

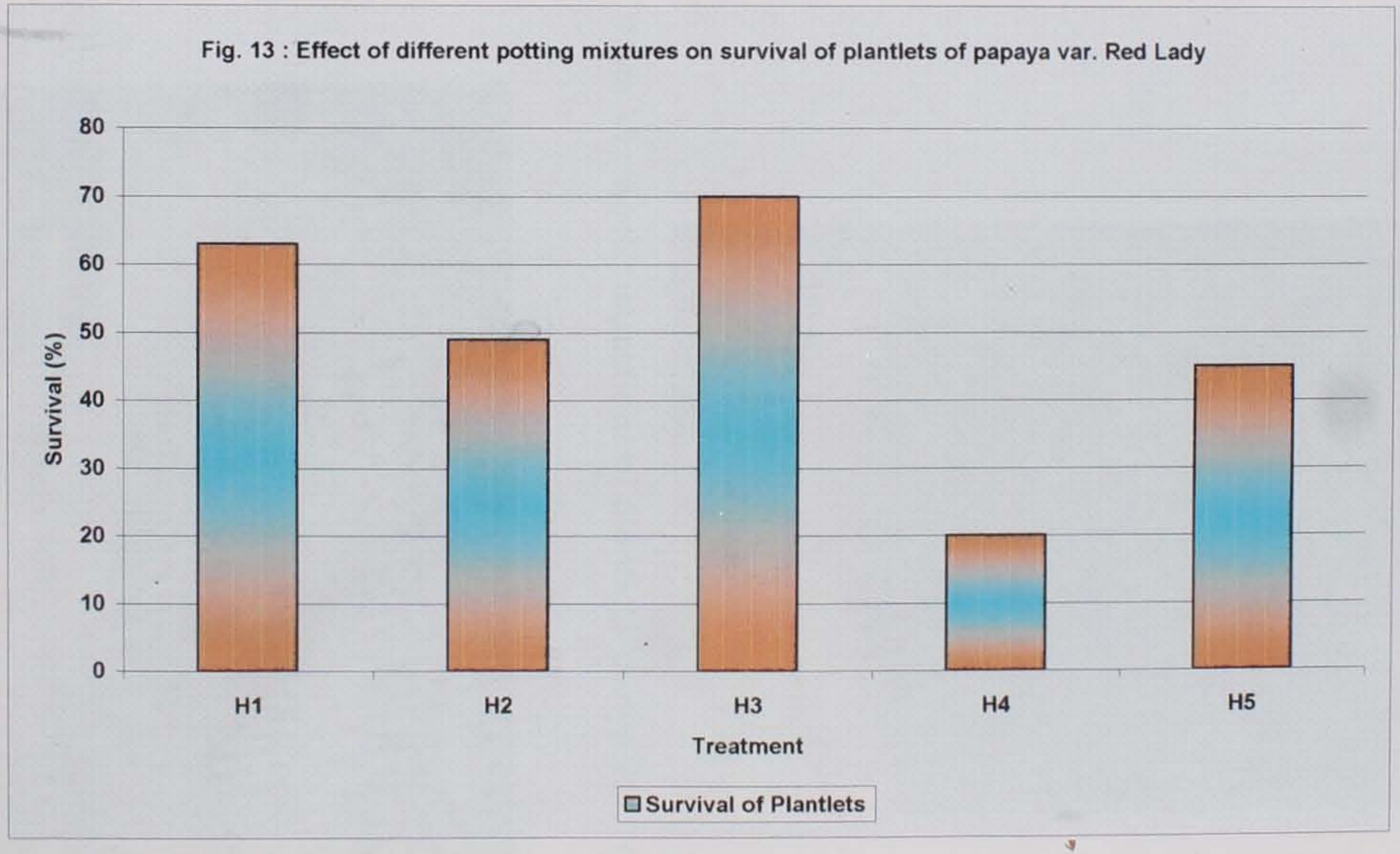
Plate-V : *In vitro* rooting in shoot of papaya on 1/2 strength MS medium supplimented with 0.1 mg/l IBA

Table 4.10 Effect of different potting mixtures on hardening of papaya var. Red Lady

Treatment No.	Potting mixture	Survival of Plantlets (%)	Days taken for establishment	Length of shoot (cm)
H_1	Vermiculite	63.00 (52.44)	9.75	7.60
H_2	Cocopeat	49.00 (44.43)	13.00	7.00
H_3	FYM: Soil: Sand (1:1:1 :: V/V/V)	70.00 (56.80)	8.00	8.30
H_4	Sand	20.00 (26.54)	16.00	6.80
H_5	Perlite	45.00 (42.14)	11.00	6.50
S.Em. ±		0.84	1.01	0.14
CD 5%		2.55	0.33	0.45
CV %		3.82	5.81	4.13

Figure in paratheses are arc sine transformed value.

Fig. 13 : Effect of different potting mixtures on survival of plantlets of papaya var. Red Lady



H1	Vermiculite
H2	Cocopeat
H3	FYM: soil: sand (1/1/1:: V/V/V)
H4	Sand
H5	Perlite

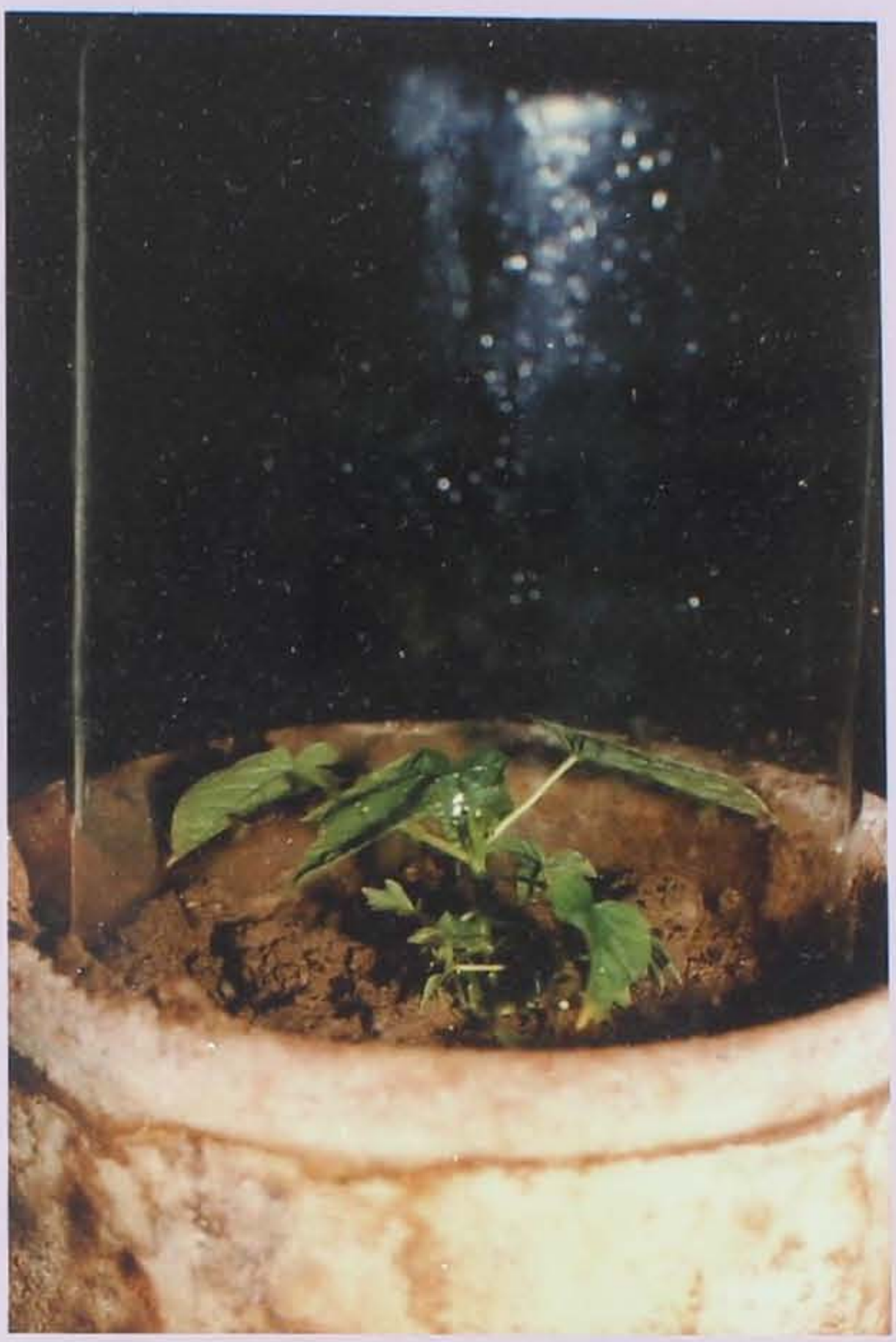


Plate-VI : Acclimatizationi of plantlets papya var. Red Leady

Plate-VII : Acclimatized of plant in pot

4.7 Protocol developed for micropropagation in papaya var. Red Lady

The protocol developed in the present study on "Micropropagation in papaya var. Red Lady" using shoot tip bud explants is given below (Plate – VIII).

A) In working laboratory

1. Shoot tip (2-3 cm) were collected from four to six weeks old seedling of papaya. Remove the leaves with stainless steel knife then, swabbed with cotton dipped in absolute alcohol and washed thoroughly in running tap water for 2-3 hours to remove traces of alcohol, dirt and latex.

2. Treat the segment with 0.05 per cent bavistin and 0.01 per cent Streptocyclin for 2 hours.

3. Remove the solution and treat explant with 10 per cent solution of detergent (Teepol) for 10 minutes.

4. Remove all the trace of detergent by repeated washing in double distilled glass water.

B) In sterile room (Laminar air flow chamber)

1. Surface sterilizes the explant using (0.1 per cent w/v) $HgCl_2$ for 3 minutes. Remove traces of $HgCl_2$ by thorough rinsing with doubled distilled glass water. Trim the surface sterilized shoot pieces and prepare nodal explant of 1.0-2.0 cm.

2. Inoculate the explant in culture bottles containing MS medium gelled with 0.8 per cent agar and supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA; adjust the pH 5.7 and 30 gm/l

Plate-VIII : Protocol for micropropagation of seedling papaya var. Red Leady using shoot tip culture

sucrose. The basal end of the explant should be inserted in the medium in such away that the tip of explant remains above the surface of the medium.

C) Incubation

1. Incubate the culture bottles in an air condition culture room at 26 ±2 ^{0}C temperature with relative humidity 55 ± 5 per cent and 16 hours photoperiod in which light is supplied with 40-w cool white fluorescent tubes at intensity of approximately 3000 Lux.
2. After four weeks of incubation transfer the *in vitro* established explant for further continues sub culturing in Basal MS and then MS medium + 0.5 mg/l BAP + 0.1 mg/l NAA, alternatively.
3. After 4th sub culturing, transfer on the proliferation medium MS medium supplemented with 0.1 mg/l NAA + 1.0 Kinetin at 3000 Lux light intensity.
4. After four weeks, separate the individual shoot for *in vitro* root induction; transfer the shoot on ½ MS medium containing 1.0 mg/l IBA.
5. Approximately after six weeks, the well rooted plantlets are ready for acclimatization.

D) Acclimatization

1. Carefully take out the plantlets from culture bottles, remove the adhering agar from roots by thorough washing in tap water.

2. After four weeks, transplant well developed plantlets to earthen pot containing FYM: sand: soil (1:1:1:: V/V/V). Drench potting mixture with 0.05 per cent bavistin at weekly interval.

3. Acclimatize the plantlets to natural environment, in air condition room by covering them individually with glass beaker for initial three days. The plantlets should then be kept in net house for a week for further hardening.

V. DISCUSSION

The present investigation was done for protocol development of micropropagation in papaya var. Red Lady. The investigations were carried out at the Plant Tissue Culture and Biotechnology Laboratory, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari during 2006-2008.

At present, Plant Tissue Culture technique is being successfully employed for rapid production of uniform and superior quality planting material. Rapid and large scale clonal production of many horticultural species is now possible, outstanding example being large scale cloning of orchids, banana, rose, anthurium, gerbera, oil palm, date palm, etc. producing unlimited number of planting material. A wide range of tissues can be used as explants from different crops as source for micropropagation. The regeneration can take place as extension or proliferation of explant.

Papaya is essentially a cross pollinated crop and propagated through seeds. Seed price of var. Red Lady is very high. The improvement of papaya is hindered by its heterogeneous nature, dioecious habit and susceptibility to viruses. Conventional vegetative propagation methods are not found to be successful for papaya due to the limited branching even after the decapitation. The technique of tissue culture has made clonal propagation a possibility in papaya as reported by many workers (Drew and Smith, 1986; Drew and Miller, 1989; Mondal *et al.*, 1990; Rahaman *et al.*, 1992;

Castillo et al., 1997; Naik., 1997; Dhinesh Babu *et al.*, 2000; Suthamathi *et al.*, 2002; Dhinesh Babu *et al.*,2002; Reuveni *et al.*, 2004; Beniwal *et al.*, 2006).

In *in vitro* propagation, the organs and tissues are carried out through a sequence of steps in which differential cultural and environmental conditions are provided.

Murashige (1974) grouped this sequence of steps into different stages as under.

Stage: I : Explant establishment including selection of mother plant and collection of explant.

Stage: II : Rapid multiplication of shoots through increased axillary branching/ somatic organogenesis

Stage: III : *In vitro* rooting

Stage: IV : Acclimatization and planting out

The explants collected from field grown stock harbour fungi and bacteria in addition to adhered soil and dust particles. Thus, it is necessary to go through an effective surface sterilization procedure for the explants before culturing. The results on effectiveness of surface sterilization of seedling (4-6 weeks) shoot tip explants and axillary buds from mature female plants (7-8 months old) are presented in Table 4.1 and Table 4.2, respectively (chapter IV section 4.1.1, A and B). For the conditions prevailing in the area of mother plants from which explants were collected for shoot tip, $HgCl_2$ (0.01 %) for 3 minutes and for axillary bud $HgCl_2$ (0.1 %) for 10 minutes were found to be

very effective for sterilization and survival of explant. Naik (1997) was also able to sterilize effectively papaya cv. Honey Dew explants shoot tip of seedling (4-6 weeks) and mature female plants (7-8 months old) using 0.1 per cent $HgCl_2$ for 5 minutes and 10 minutes, respectively. Similarly, Rajeevan and Pandey (1993) were reported satisfactorily sterilization of papaya explants using 0.1 per cent $HgCl_2$ for 5 to 15 minutes.

It is very clear from the presented findings, that response of seedling explants and mature explant to surface sterilization treatments was different, the time needed to sterilize mature explant was being more than seedling explants. This may be possible due to difference in intensity of contamination presenting these explants. The intensity of contamination might be more in mature explants as they were collected from field grown plants which might be due to the endogenous microbial infection which are less in seedling explants (Dhinesh Babu *et al.*, 2002). Therefore, required more time of treatment for their effective control. The research work of our department on Furcraea (Patel. 2006), anthurium (Sunila Kumari, 2006), citrus (Desai, 1994), rose (Patel, 1995), papaya (Naik, 1997) and tuberose (Thosar, 1997) also supported this view where all together a new procedure is reported or effective sterilization of explant suitable to local conditions.

In present investigation, establishment of axillary bud of female mature plant was greatly hampered due to leaching of latex released from the cut end of the explants which form a thin

layer at the cut end of the explant thereby checking the movement of chemical from medium to the tissue, resulting in death of tissue. Similar, finding was also reported by Arora and Singh (1978 c).

Such, problem was not noticed in case of seedling explants. For encountering the problem of latex exudation from mature explants, the explants were washed in running tap water for three hours was also not very effective for latex control. The results are in conformity with findings of Naik (1997) in papaya.

Further, axillary bud from female mature plant almost fail to establish in medium, as they were found to be less amenable for *in vitro* culture. Pandey *et al.* (1986) also reported that the establishment of lateral bud was very low. Yadav *et al.* (1990 a) and Jordan and Oyanedel (1992) also reported that the juvenility of material has better potentiality to express totipotency under *in vitro* condition than the explant taken from mature adult phase of development.

The basic phenomenon involved in explant establishment, multiplication and subsequent plantlet formation *in vitro* are reported to be due to the action of plant hormones. The requirement of growth regulators varies with the species as well as the mode of development desired i.e. organogenesis or embryogenesis. However, there is one universally accepted concept that morphogenetic response is regulated by the relative concentrations of auxins and cytokinins in the medium as pointed out by Skoog and Miller (1957) and it should serve as guidelines in plant tissue culture work. The data on establishment of explant on

different hormonal treatments are given in chapter 4 (section 4.1, and Table 4.2). BAP and NAA supported maximum establishment. It was observed that stunted shoot growth with increasing concentration of BAP. This may be due to the fact that cytokinins at higher concentrations led to oxidation of polyphenols at a rapid rate. The role of cytokinins in shoot organogenesis is well established by Skoog and Miller (1957) and Evans *et al.* (1981). Cytokinins are used in combination with auxin for initiation as well as maintenance of shoot development. During the present investigation cytokinins were found to be essential for establishment. Among the various treatments tried, low level of BAP and NAA gave higher establishment. These findings are supported by that of Litz and Conover, 1978 a; Rajeevan and Panday, 1983; Naik, 1997.

Drew (1988), working with mature papaya explants, demonstrated that shoot growth deteriorated if multiplying shoots were continually subculture on medium containing BAP, irrespective of auxin concentration, resulted in subsequent subculturing growth of the shoots in terms of length. When basal medium and medium with BAP were used alternately in subsequent subcultures such no deteoration was noticed. Reuveni *et al.* (2004) used adenine sulphate in addition to BAP to improve growth and rate of multiplication. Similarly, Rajeevan and Pandey (1986) tried zeatin in place of BAP for the same purpose. However, the multiplication rate declined at each subculturing. It was suggested that this problem might be solved by varying the cytokinin level used at successive subcultures. The testing of alternate media, i.e.

basal medium in the subculture and so on, in the present investigation have clearly demonstrated that up to four subcultures, decline in the rate of multiplcation was improved as compared to other treatments. The possible reason for such results may be that varying cytokinin cocentration of endogenous growth subcultures, particularly cytokinin, at favourabale level to support better growth and rate of shoot multiplication. These results are in agreement with the work of Naik (1997) in papaya.

Higher level of cytokinins to auxin favoured shoot bud induction where as, an opposite condition i.e. an increased concentration of auxin and low cytokinins promoted roots (Skoog and Miller, 1957). In general, the "Skoog –Miller Model" holds good for many species. In most of the studies of *in vitro* culture of papaya, basal media derived from the MS (Beniwal *et al.* 2006, Reuveni *et al.* 2004, Suthamathi *et al.* 2002, Rahaman *et al.*, 1992, Dhinesh Babu *et al.* 2000, Burikam *et al.* 1988). The result on shoot proliferation in chapter 4 (section 4.4.1, Table 4.5). The highest rate of proliferation was recorded in treatment P_{18} (MS + 1.0 mg/l Kinetin + 0.1 mg/l NAA at 3000 Lux light intensity). Siriwaan *et al.* (1988) reported that papaya shoot were best grown on MS medium containing 15% coconut water, 0.1 mg/l NAA and 120 mg/l adenine sulphate. Coconut water is substitute of Kinetin. In our study also the Kinetin (1.0 mg/l) in proliferation medium (P_{18}) give the best growth of shoot. Light intensity exhibited more effective role on the proliferation of shoot in papaya by earlier workers (Suthamathi *et al.*, 2002; Reuveni *et al.*, 2004).

Proliferation of shoot was increased as increased the light intensity in our study also.

The culture growth is influenced by the source of carbon energy, which is inheritable in any culture medium. Tissue very considerably in their ability to utilize different carbohydrates. Sucrose is usually the carbohydrates of choice in most of the cell culture media. Out of five levels of sucrose te,,,,,,,,sted, sucrose 3 per cent gave high shoot proliferation (Chapter 4,"Section 4.4.2 and Table 4.6). Kumar and Kumar (1998) reported that level of sucrose was maintained between 2 to 3 per cent in majority of medium. Further, in papaya Glucose 1.5 per cent + Fructose 1.5 per cent in medium gave the best response to growth of shoot (Naik, 1997). The requirement may be related to the specific carbohydrates metabolism through which water relations and endogenous phytohormones are regulated.

The result obtained on influence of pH on proliferation and growth of explant .Though the importance of pH in tissue culture studies was reported by Gauthert as early as 1947 by observing drift in pH during the growth of a culture. Tissue culture of majority of fruit crops are grown satisfactory at pH 5.6 to 5.8 (Conger, 1987). In the present investigation, maximum proliferation rate and best growth observed at pH 5.7, and decreased at lower as well as higher pH value. This work is supported by work carried out in our laboratory on number of plant cultures such as anthurium (Sunila Kumari, 2006); citrus (Desai, 1994); rose (Patel, 1995); papaya (Naik, 1997) and tuberose

(Thosar, 1997); Patel *et al.* (2005). The different effects of pH of culture medium on the development of *in vitro* culture as obtained in present investigation may be due to the well known effect of pH on the availability of nutrients from the medium. Such effect of pH on the availability of nutrients has been reported by many workers using sand water culture technique (Wallihan, *et al.*; 1997 and Harley *et al.*, 1981). According to Street (1966), the ambient pH could be desire for absorption of various nitrogen sources: pH growth response curves indicated that nitrate N when pH is acidic (4.7-4.9 pH approx.), ammonical N at neutral pH (7.0-7.2 pH approx.) and nitrate at pH 5.0 to 6.0 supported maximum growth. The result obtained with in present investigation may be considered in ligth of presence of nutrients for growth; growth at pH 5.7 suggesting favourable effect of nitrite N rather than ammonical N (Chapter 4, Section 4.4.3and Table 4.7).

The adenine sulphate has also a role of culture growth. In present study, adenine sulphate (160 mg/l) enhanced the shoot growth (Chapter 4, Section 4.4.4 and Table 4.8). This finding is in accordance with those of Reuveni *et al.* (2004).

It is observed that auxin like IBA and NAA were used commonly for inducing rooting of *in vitro* shoots (Bhojwani and Razdan, 1992). It was also stated by Prasad (1999) that IBA, IAA as well as NAA were effective in inducing rooting in shoot. The result clearly is indicated from the present findings (Chapter 4, Section 4.5 and Table 4.9) that rootings of *in vitro* shoots of papaya required lower nutrient status (half MS medium) and

optimum level of IBA (1 mg/l). The response of rooting was decrease with either increased or decreased the level of IBA. Bannok *et al.*(1989) in pear and Yadav *et al.*(1990 a) in black plum observed reduction in rooting response at higher concentration of auxin. The present finding is supported by those of Burikam *et al.* (1988); Mondal *et al.* (1990); Rahaman *et al.* (1992); Mondal *et al.*, (1994); Teo and Chan. (1994); Dhinesh Babu *et al.*, (2000); Suthamathi *et al.*, (2002); Beniwal *et al.*, (2006); in papaya .

In vitro plantlets are delicate as they are raised in controlled conditions of temperature, humidity and light intensity. They require being hardened before transplanted in the field. For most of the plant species, there is a set procedure for acclimatization such as maintaining high humidity initially and gradually reducing it to natural environmental conditions. However, the survival of *in vitro* plantlets also depends upon the potting mixture used for raising *in vitro* plants under green house conditions. The survival rate of plantlets as influenced by potting mixtures is illustrated in chapter 4, Section 4.6 and Table 4.10. The potting mixtures used in the present investigation helped in giving better grip for the roots and ample aeration, in FYM: Soil: Sand (1:1:1 :: v/v/v) mixture, which gave maximum per cent of survival in papaya. These observations are supported by various earlier workers (Rahaman *et al.*1992; Dinesh Babu *et al.* 2000; Suthamathi *et al.* 2002; The hardened plants were successfully transplanted in the field where they are growing equally or better than conventionally propagated material (visual observation).

The foregoing discussion clearly leads to the possibilities of efficient micropropagation system in papaya var. Red Lady using shoot tip culture. However, before the protocol developed is utilized as commercial method, there is need to examine the performance of *in vitro* propagules in the field.

VI. SUMMARY AND CONCLUSION

The present investigation on "Micropropagation in papaya var. Red Lady" was carried out at Biotechnology and Plant Tissue Culture Laboratory, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, during the year 2006-2008. The results obtained from the present study can be summarized and concluded as following.

1) The surface sterilization treatments involving shoot tip explants required $HgCl_2$ (0.1 %) for 3 minutes (T_3) and $HgCl_2$ (0.1 %) for 10 minutes (T_5) was found better for shoot tips and axillary buds explants, respectively. Though, higher concentration or longer duration of treatment was more effective in controlling the contamination, they were found toxic resulting in death or suppression in shoot growth.

2) Higher establishment of explants was noticed satisfactorily on lower concentration of BAP and NAA tested, and maximum establishment (83.33%) was observed in treatment MS medium supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA.

3) In multiplication study, the maximum shoot multiplication was observed in alternate sub culturing in basal medium and MS medium + 0.5 mg/l BAP + 0.1 mg/l NAA and gave highest number of shoots per explants and also longest shoot upto four sub cultures.

4) Out of 21 proliferation treatments combinations tried with different concentrations of BAP and Kinetin along with 0.1

mg/l NAA and with different light intensity, maximum shoot growth was obtained on MS medium supplemented with MS + 0.1 mg/l NAA + 1.0 mg/l Kinetin at 3000 Lux intensity light for 16 hours.

5) Sucrose 30 gm/l in medium was found to be more favourable for maximum number of shoot and length of longest shoots.

6) Out of various pH level tested, pH 5.7 recorded maximum numbers of shoots (3) and maximum length of longest shoots (2.75 cm).

7) In proliferation medium the length of shoot, numbers of shoots and growth rate were increased as increased the adenine sulphate level in the medium. Maximum proliferation was reported on 160 mg/l adenine sulphate in the medium.

8) *In vitro* rooting occurred on shoot regeneration medium; however, it was a slow process. Rooting treatment consisting of half MS medium supplemented with 1.0 mg/l IBA was found to be the best for early induction of roots (28 days), maximum number of root/shoot and length of root also.

9) Keeping the plantlets in the air conditioned at 26 ± 2^0 C and covered individually with glass beaker resulted 70 per cent survival of plantlets. Potting mixture containing soil: sand: FYM (1/1/1:: V/V/V) was found to be suitable for hardening *in vitro* raised papaya plantlets.

CONCLUSION

The present investigation on "Micropropagation in papaya var. Red Lady" has clearly demonstrated its potentiality for rapid clonal propagation. It estimated that using the present protocol of micropropagation, large number of plantlets can be produced in a year starting from a single shoot tip explant. This protocol may be made commercially viable provided some work is intensified to increase the vigor and growth of the plantlets in the initial stage after transplanting and testing the plantlets in field conditions, besides some scaling up techniques for large scale production. The results obtained would be very much useful for mass multiplication of papaya var. Red Lady using shoot tips under local condition and proved guidelines for setting commercial unit for propagation of papaya in Gujarat.

REFERENCES

Arora, I.K. and Singh, R.N. (1978c). *In vitro* plant regeration in papaya. *Curr. Sci.* **47**: 867-868.

Anonymous (2005). Report on area and production of fruits and vegetables in India. NHB, New Delhi.

Anonymous (2006). Report on area and production of fruits and vegetables in India. NHB, New Delhi.

*Bannok,: Mayashi, S. and Tanabe, K.(1989). *In vitro* propagation of Japanese pear cultivars. *J. Jap. Soc. Hort. Sci.*, **58**: 37-42

Bhaskaran, S. and Prabhudesai, V.R. (1989). Tissue culture of plantation crops. In: Applications of Biotechnology in Forestry and Horticulture (Dhawan V Ed), Plenum Press, New York, pp. 88-108.

Bhattacharya, J. and Khuspe, S. S. (2001). *In vitro* and *in vivo* germination of papaya (*Carica papaya* L.) seeds. *Sci. Hort.*, **91**: 39-49.

Bhojawali, S. S. and Rozdan, M. K. (1992). Plant Tissue Culture Theory and Practice. Elsevier Science Publishers, Amsterdam, The Netherlands.

Beniwal, V. S; Sehrawat, S. K.; Dahiya, D. S. Benival, L. S. and Singh, S. (2006). Effect of season on *In vitro* regeneration of papaya. *Haryana J. hortic. Sci.*, **53** (1 & 2): 35-37.

Brainerd, K.E. and Fuchigami, L.H. (1981). Acclimatization of aseptically cultured apple plant to low relative humidity. *J. Am. Soc. Hort. Sci.*, **106**: 515-518.

Burikam, S.; Chommalee, V. and Attathom, S. (1988) Effect of Plant Growth Regulators Papayas (*Carica papaya*) Cultured *In Vitro*. *Kesetsart J.(Nat. Sci. Suppl.)* Vol. **22**: 1-6.

Chawala, H.S. (2000). Introduction to Plant Biotechnology. Oxford and IBH Publishing Co. Pvt. Ltd., pp 7-8.

Chen, H.T. and Tang. C.S. (1979). The chemistry and biochemistry of papaya. In: Tropical Foods, Vol. I, (Inglett G E & Charolambous G Eds), Academic Press, New York, pp. 33-53.

Chen, M.H. and Chen, C.C. (1992) Plant regeneration from Carica protoplast. *Plant Cell Report.*, **11**, 404-407.

*Conger, B. V. (1987). *Cloning Agriculture Plants viz. in vitro* techniques CRC press. Florida.

Desai, R.A. (1994). *In vitro* propagation studies on citrus-acid lime var. Kagzi Lime. M. Sc. (Hort.) thesis submitted to the Gujarat Agricultural University, S. K. Nagar, Gujarat.

*De Fossard, R.A. (1974). Tissue culture for plant propagators. In: Conger, B,V. (Ed). Cloning Agriculture Plants Via *In vitro* techniques. CRC Press, Florida.

*De Winner, W. (1988). Clonal propagation of papaya *in vitro*. *Pl. Cell Tissue Organ Cult.* **12**(3): 305-310.

Dinesh Babu, Sathiamoorthy, and Chezhiyan. (2000). Rooting *In vitro*and hardening of papaya (*Carica papaya* L.) plantelets. *South Indian Hort.*, **48**(1-6): 23-25.

Dinesh Babu, Sathiamoorthy, and Chezhiyan. (2002). Effect of age mother trees on *In vitro* establishment of axillary buds of papaya. (*Carica papaya* L.). *South Indian. Horti.* **48**(1-6): 23-25.

Drew, R. A. and Smith, N. G. (1986). Growth of apical and lateral buds of papaya (*Carica papaya* L.) as affected by nutritional and hormonal factors. *J. Hort. Sci.*, **61** (4): 535-543.

Drew, R. A. (1987). The effect of medium composition and cultural condition on *In vitro*rooting initiation and growth of papaya (*Carica papaya* L.) . *J. Hort. Sci.*, **62** (4): 551-556.

Drew, R. A. (1988). Rapid clonal propagation of papaya *In vitro* from mature field grown trees. *Hort. Sci.*, **23** (3): 565-575.

Drew, R.A. (1992) Improved techniques for *In vitro* propagation and germplasm storage of papaya. *Hort Science* **27**, 1122-1124.

Drew, R. A. and Miller, R. M. (1989). Nutritional and cultural factors affecting rooting of papaya (*Carica papaya* L.) *in vitro*. *J Hort. Sci.*, **64** (6): 767-773.

*Evans, D.A.; Sharp, W.R. and Flick, C.E. (1981). Growth and behavior of cell cultures. Embryogenesis and

organogenesis. In. Thorpe, T.A., (Ed.). Plant tissue culture: Method and applications in agriculture. Academic press, New york. pp 45-144.

Fitch, M.M.M. (1993). High frequency somatic embryogenesis and plant regeneration from papaya hypocotyls callus. *Plant Cell Tissue and Organ Culture.*, 32, 205-212.

*Haberlandt, G. (1902). Culturversuchemit isolierten Pflanzenzellen. *Sitzungsber. Math. Naturwiss. Kl. Kais. Akad. Wiss. Wien.*, 111: 69-92.

Hurley, N. K.; Einert, A. E. And Hileman, L. E. (1981). Effect of pH on growth and quality of *Iris Germanic* L. *Hort. Sci.*, 16 (5) : 672-673.

*Jona, R. and Menini, U. G. (1987). Tissue culture of selected tropical fruit plants. FAO *Plant Production and Protection Paper.*, 79, Rome.

*Jordan, M. and Oyanedel, E. (1992). Regeneration of Pomaria Plants *in vitro*. *Pl. Cell. Tiss. Org. Culture.*, 31: 249-52

Kumar, A. and Kumar, V.A. (1998). Clonal tissue culture of important fruit crops. International book distribution co.. pp.35.

Litz, R.E. and Conover, R.A. (1978a). Recent advances in papaya tissue culture. *Proc. Fla. State Hort. Soc.*, 91: 180-182.

Litz, R.E, and Conover, R.A. (1978b) Recent advances in papaya tissue culture. Proceedings of the Florida State Horticultural Society ..90. 180-182

Litz, R.E. and Conover, R.A. (1980a). Somatic embryogenesis in cell culture of *Carica stipula*. *Hort. Science*, **15** : 735.

Litz, R.E. and Conover, R.A. (1981a). Effect of sex type, season and other factor on *In vitro* establishment and culture of Carica papaya L. explants. *J. Amer. Soc. Hort. Sci.*, **106**: 792-794.

Litz, R, E. and Conover, R.A. (1981b). *In vitro* polyembryony in *Carica papaya* L. Ovules. *Z. Pflanzenphysiol*., 104: 285-288.

Litz, R.E. and Conover, R.A. (1982). *In vitro* somatic embryogenesis and plant regeneration from *Carica papaya* L. ovular callus. *Plant Sci. Letters*, **26: 153-158.**

Litz, R.E.; O'Hair, S. K. and Conover, R. A., (1983). *In vitro* growth of *Carica papaya* L. cotyledons. *Scientia Hort.*, **19**:287 -293.

Litz, R. E. (1984). Papaya. In: Handbook of Plant Cell Culture (Sharp, W. R., Evans, D. A., Ammirato, P. V. and Yamada, Y. Eds.) Macmillon Publishing Co., New York, pp. 349-368.

Mehdi, A.A. and Hogan, L. (1976). Tissue Culture of *Carica papaya* L. *Horti Science*, 11: 311.

Mondal, M.; Gupta, S. and Mukherjee, B. B. (1990). *In vitro* propagation of shoot buds of *Carica papaya* L. (Caricaceae) Var. Honey Dew. *Plant Cell Rpt.*, 8: 609-612.

Mondal, M.; Gupta, S. and Mukherjee, B. B. (1994). Callus Culture and plantlet production in *Carica papaya* L. (Var. Honey Dew). *Plant cell Rpt.*, **13**: 390-393

*Muthukrishnan, C.R. and Irrulappan, I. (1990). Papaya. In: Fruits: Tropical and Subtropical (Bose, T.K. and Mitra, S.K. Eds), Naya Prakash Calcutta, pp. 304-355.

Murashige, T. (1974). Plant propagation through tissue culture. *A. Rev. Pl. Phyiol.*, **5**: 135-136.

*Murashige, T. and Skoog, F. (1962). A revised medium for rapied growth and bioassays with tobacco tissue cultures. *Physiol. Plant,* **15**: 473-497.

Naik, U.I. (1997), Micropropagation of papaya (*Carica papaya* L.) var. Honey Dew. M. Sc. (Agri.) thesis submitted to Gujarat Agricultural University, S. K. Nagar, Gujarat.

Panse, V.G. and Sukhatme, (1985). Statistical methods for agricultural workes. Ed., ICAR, New Delhi.

Pandey, R.M., Kishore, D.K. and Arumozhi, K. (1986). Effect of seasons, plant type and some pre-excision treatments on in vitro behavior of explants of *Carica papaya* L. *Ind. J. Hort.*, **43** (3-4) : 174-179.

Parthasarathy, V.A.; Bose, T.K.; Deka, P.C.; Das, P.; Mitra, S.K. and Mohandas, S. (2001). Biotechnology of Horticultural crops. (**1**): 206-237.

Patel, H.D. (1995). Micropropagation of rose (*Rosa hybrida* L.) var. Red Masterpiece. M. Sc. (Plant Physiol.) a thesis

submitted to Gujarat Agricultural University, Navsari.

Patel, R.M. (2006). *In vitro* studies of furcraea and examining the possibility of extraction of fibres for value addition. Ph.D. (Hort.) a thesis submitted to the Navsari Agricultural University, Navsari, Gujarat.

Patel, R.M.; Dhaduk, B.K. and Shah, R.R. (2005). Factors affecting *In vitro* growth and proliferation of furcraea var. Green. *Crop productivity and quality improvement through physiological intervantions.* pp 256-260.

Patel, R.M. (2006). *In vitro* studies of furcraea and examining the possibility of extraction of fibres for value addition. Ph.D. (Hort.) a thesis submitted to the Navsari Agricultural University, Navsari, Gujarat.

Prasad, S. (1999). Impact of plant Biotechnology on Horticulture. 2nd Ed., *Agro Botanica*, Bikaner., pp.183.

*Purseglore, J.W. (1972). Musaceae. In: Tropical Crops, Monocotyledonous, John Wiley & Sons, New York, pp. 343-384.

Rahaman, S. M.; Hossain, M., Joarden, O. I. and Islam, R. (1992). Rapid clonal propagation of papaya through Cultivar of shoot apices. *Indian J. Hort.*, **49** (1): 18-22.

Rajeevan, M.S. and Pandey, R.M. (1983). Propagation of papaya through tissue culture. *Acta Hort.*, **131**: 131-139.

Rajeevan, M.S. and Pandey, R.M. (1986). Lateral bud culture of

Papaya (*Carica papaya* L.) for clonal propagation.

Reuveni, O.; Shlesinger, D.R. and Lavi, U. (2004). *In vitro*clonal propagation of dioecious *Carica papaya. Pl. Cell Tissue Organ Cult.*, **20(1)**: 41-46.Suksa-Ard, P.; Kataoka, I.; Fujime, Y. and Suranit, S. (1990) Requirement of 2,4-D and sucrose for somatic embryogenesis of papaya .

*Skoog, F.C. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. *Symp. Sec. Exp. Biol.*, **11**: 118-130.

Street, H. E. (1966). The nutrition and metabolism of plant tissue and organ culture. In Cells and Tissues in culture : Methods Biology and physiology (E. N. Willmer Ed.) vol. 3 Academic Press, London Pp. 533-629.

Sunila Kumari, (2006). *In vitro*studies in *Anthurium andreanum* Hort. M. Sc. (Hort.) a thesis submitted to the Navsari Agricultural University, Navsari, Gujarat.

Suthamathi, S. J.; Haripya, K. and Kamalakannan, S. (2002). Micropropagation cv. CO-5. *Indian J. Hort.*, **59** (1): 13-16.

Suksa-Ard, P., Kataoka, I, Fujime, Y. amd Suranit, S (1999). Requirement of 2, 4-D and sucrose for somatic embryogenesis of papaya. *Japanese Journal of Tropical Agriculture*, **43**. 1-4

Teo, C. K. H. and Chan, L.K. (1994). The effect of agar content, nutrient concentration, genotype and light intensity on the *in-vitro* rooting of papaya micro cuttings., *J. of Hort.* Sci.,

69 (2): 267-273. *Japanese journal of Tropical Agriculture.*, 4, 1-4

Thosar, A. P. (1997). Micropropagation of tuberose (*Polianthes tuberose* L.) var. Local Double. M. Sc. (Agri.) thesis submitted to Gujarat Agricultural University, S. K. Nagar, Gujarat.

*Wainwright, H. (1988). Overcoming problems in establishing micropropagules- guidelines for growers. *Prog. Hort.*, 2(3): 67-72.

Wallihan, E. F.; Sharpless, R. G. and Printy W. L. (1977). Effect of pH on yield and leaf composition of Hydroponic Tomatoes. *Hort. Sci.*, 12 (4) : 316-317.

Yadav, V.; Lal, M. and Jaiswal, V. S. (1990a). In vitro micropropagation of tropical fruit tree. *Syzigium cumini* L. *Plant Cell Tissue and Organ Culture.* 21: 87-92.

*Yie, S. and Liaw, S.I. (1977). Plant regeneration from shoot tips and callus of papaya. *In vitro*, 13: 564-567.

*Ziv, M. (1986). *In vitro* hardening and acclimatization of tissue culture plants. In: withers, L.A. and Alderson P.G. (Ed.) Plant Tissue Culture and its Agricultural applications. Butter worths, London pp. 187-196.

* Original not seen

CERTIFICATE

This is to certify that I have no objection for supplying to any scientist only one copy or any part of this thesis at a time through reprographic process, if necessary for rendering reference services in a library or documentation centre.

Place: Navsari

Date: 20/09/2008.

(J. R. Patel)