SCREENING OF TOMATO SOMACLONES FOR RESISTANCE TO TOMATO LEAF CURL VIRUS

By

BIJU DANIEL

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

Faculty of Horticulture
Kerala Agricultural University

Department of Olericulture

COLLEGE OF HORTICULTURE

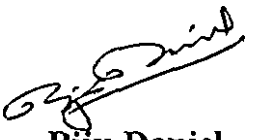
VELLANIKKARA THRISSUR-680 656

KERALA, INDIA

2003

DECLARATION

I hereby declare that the thesis entitled " **Screening of tomato somaclones for resistance to Tomato Leaf Curl Virus**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.



Vellanikkara,
29-09-2003

Biju Daniel

CERTIFICATE

Certified that the thesis entitled " **Screening of *tomato somaclones* for resistance to Tomato Leaf Curl Virus**" is a record of research work done independently by Mr. Biju Daniel under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

Vellanikkara
29-09-2003

Dr. S. Nirmaladevi
(Major Advisor, Advisory Committee)
Assistant Professor, AICVIP
Department of Olericulture
College of Horticulture

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Biju Daniel** a candidate for the degree of **Master of Science in Horticulture**, with major field in Olericulture, agree that the thesis entitled" **Screening of tomato somaclones for resistance to Tomato Leaf Curl Virus"** may be submitted by Mr. Biju Daniel in partial fulfilment of the requirement for the degree.

Dr. S. Nirmala Devi,
(Major advisor)
Assistant Professor ,AICVIP
Department of Olericulture
College of Horticulture
Vellanikkara.

Dr. T.R. Gopalakrishnan,
Associate Professor and Head
Department of Olericulture
College of Horticulture
Vellanikkara.
(Member)

27/01/03.

Dr. P.A. Nazeem,
Associate Professor,
Centre for Plant Biotechnology and
Molecular Biology (CPBMB)
College of Horticulture
Vellanikkara.
(Member)

27/9/03

Dr. Sally K. Mathew
Associate Professor,
AICVIP,
Department of Plant pathology,
College of Horticulture,
Vellanikkara.
(Member)

External Examiner
27/9/03

ACKNOWLEDGEMENT

I bow my head before the God Almighty, who enabled me to complete this venture successfully.

I express my profound sense of gratitude to my guide **Dr. S. Nirmaladevi,** *Assistant Professor (AICVIP), Department of Olericulture for her able guidance, valuable comments, ever willing help and constructive criticism rendered throughout the period of investigation and in the preparation of this manuscript. I am deeply obliged to her for the constant encouragement, understanding and wholehearted co-operation received during the period under her guidance.*

I thankfully acknowledge **Dr. T.. R,. Gopalakrishnan,** *Associate Professor and Head, Department of Olericulture and member of advisory committee for his valuable suggestions, support and critical analysis of the thesis.*

I am grateful to **Dr. P. A Nazeem** *Associate Professor, Centre for Plant Biotechnology and Molecular Biology and member of advisory committee for her guidance, warm and friendly nature and critical scrutiny of the manuscript of the thesis.*

I accord my sincere thanks to **Dr. Sally K.. Mathew,** *Associate Professor, AICVIP, Department of Plant Pathology and member of advisory committee for her wholehearted co-operation, help and valuable suggestions rendered during various stages of study and preparation of the manuscript.*

My profound sense of gratitude is due with **Mrs. Lissamma Joseph,** *Assistant Professor, Department of Plantation Crops and Spices for her ever-willing help extended to me throughout the period of investigation and also in the critical evaluation of the manuscript.*

I extend my sincere thanks to **Dr. P. G. Sadhankumar** *Assistant professor, AICVIP, Department of Olericulture for his critical suggestions and valuable advices throughout the course of my research work and preparation of the thesis.*

I am grateful to ***Dr. D. Girija*** *Assistant Professor, Centre for Plant Biotechnology and Molecular Biology, for her ever-willing help extended to me throughout the period of investigation.*

I express my deep sense of gratitude to all teachers and staff members of Department of Olericulture for their help and encouragement during the entire course of study.

I am deeply obliged to Josco studio, Mannuthy for the help in the preparation of good quality photographs.

My special thanks to ***Bhaskaran chettan, and Santha chechi, Lalitha chechi*** *for their valuable help and co-operation rendered to me during the conduct of research work.*

I am deeply indebted to my friends ***Rajesh, Shinoj, Nandan, Sanal, Anup, Sajnanath, Sajeesh, Sherin, Rajan, Sudeer Babu, Suresh, Allan, Vezhan, Binu, Gudi, Srevidya and Jamuna*** *for their sincere support, motivation and relentless help during the difficult times faced by me during the period of study.*

I am in dearth of words to express my deep sense of gratitude to my friends ***Pratheesh and Johnkutty*** *for their invaluable help and support without which the thesis would not have acquired the present form.*

A special word of thanks to ***Santhosh*** *and* ***Jeo*** *for their help in computer work.*

Lastly but not the least, I owe my profound sense of gratitude to my dearest, ***Roychan, Jaya, Shubha chechi, Saji aliyan, Irene, Christy*** *and all my near and dear ones for their boundless affection, constant prayers, moral support and unfailing inspiration all along my study. With respect and affection I dedicate my thesis to my loving parents.*

Biju Daniel

TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
1	Introduction	1-2
2	Review of Literature	3-21
3	Materials and Methods	22-32
4	Results	33-86
5	Discussion	87-92
6	Summary	93-95
	References	
	Abstract	

List of Tables

Table No.	Title	Page No.
1.	Chemical Composition of MS media	25
2.	Scoring of TLCV Disease in tomato.	31
3.	Scoring of leaf spot disease in tomato	32
4.	Effect of NAA and BA on callus induction and growth of leaf segments	35
5.	Effect of 2,4-D and BA on callus induction and growth of leaf segments	36
6.	Effect of 2,4-D and Kinetin callus induction and growth of leaf segments	37-38
7.	Effect of Auxins / Cytokinins on callus induction and growth of internodal segments	42
8.	Effect of NAA and BA on callus induction and growth of internodal segments.	43-44
9.	Effect of NAA and Kinetin on callus induction and growth of internodal segments	45-46
10.	Effect of 2,4-D and BA on callus induction and growth of internodal segments	47-48
11.	Effect of 2,4-D and Kinetin on callus induction and growth of internodal segments	49-50
12.	Effect of Auxins / Cytokinins on callus induction and growth of nodal segments	54
13.	Effect of NAA and BA on callus induction and growth of nodal segments	55-56
14.	Effect of NAA and Kinetin on callus induction and growth of nodal segments	57-58
15.	Effect of 2,4-D and BA on callus induction and growth of nodal segments	59-60
16.	Effect of 2,4-D and Kinetin on callus induction and growth of nodal segments	61-62

17.	Effect of Auxins and Cytokinins on organogenesis of leaf segment calli.	64-65
18.	Effect of Auxins and cytokinins on organogenesis of Internodal segment calli	65
19.	Effect of Auxins and Cytokinins on organogenesis of nodal segment calli	66
20.	Effect of different levels of Sucrose and Agar on organogenesis of leaf segment calli.	67
21.	Effect of different levels of MS, ½ MS, IAA and IBA combinations on root induction in 'Sakthi'	72
22.	Effect of different levels of MS, ½ MS, NAA and IBA combinations on root induction in 'Sakthi'	73
23.	Effect of different levels of MS, ½ MS, IAA and NAA combinations on root induction in 'Sakthi'	74
24.	Effect of different levels of IBA and IAA combinations of root induction in *L. pimpinellifolium*	75
25.	Grouping of somaclones	77
26.	Performance of somaclones (Group I)	80
27.	Performance of somaclones (Group II)	81
28.	Performance of somaclones (Group III)	82-83
29.	Performance of somaclones (Group IV)	84
30.	Performance of somaclones (Group V)	85

List of Figures

Fig.No.	Title	Page No.
Fig.1	Effect of NAA and BA on differentiation of leaf segment calli	68
Fig.2	Effect of NAA and BA on differentiation of internodal calli	68
Fig.3	Effect of sucrose and agar on differentiation of leaf segment calli	69
Fig.4	***Ex vitro*** survival of plantlets	69

List of Plates

Plate No.	Title	Between Page No.
Plate.1	Artificial inoculation of *Bemisia tabaci* in 'Sakthi' and *L. pimpinellifolium*	28-29
Plate.2	Scoring of callus growth rate in 'Sakthi' and *L. pimpinellifolium*	28-29
Plate.3	Response of explants to callusing in 'Sakthi' and *L. pimpinellifolium*	39-40
Plate.4	Response of media to callusing in'Sakthi' and *L. pimpinellifolium*	62-63
Plate.5	Response of media(NAA and BA) to organogenesis in 'Sakthi' and *L. pimpinellifolium* (internodal)	62-63
Plate.6	Response of media to rooting in 'Sakthi' and *L. pimpinellifolium* (internodal)	62-63
Plate.7	Variability in somaclones of 'Sakthi'	77-78
Plate.8	Reaction to TLCV disease in somaclones of tomato (Sakthi)	77-78

Dedicated to my beloved parents

Introduction

1. INTRODUCTION

China and India are the two largest vegetable producers in the world with an annual production of over 80 and 65 million tonnes respectively. Korea globally tops the per capita vegetable consumption at 150kg compared to 124kg of Thaiwan, 112kg of Japan and 65kg of India. (Tikoo, 2002) The total area under vegetable in India is 5866 thousand hectare and production is 87536 thousand metric tonnes. The productivity of vegetables 14.92 metric tonnes /ha.

Tomato is one of the most popular vegetables grown all over the world. In India, tomato has wider coverage compared to other vegetables. The leading tomato growing states are Uttar Pradesh, Karnataka, Maharashtra, Haryana, Panjab, Bihar and Tamil Nadu.The area under tomato is 466.3 thousand hectare and production is 8271 thousand metric tonnes. (Verma, *et al*, 2002). In the last two decades, significant progress has been made in tomato production with the development of new varieties and hybrids.

In Kerala, the tomato cultivation is limited due to the bacterial wilt disease caused by soil borne pathogen, *Ralstonia solanacearum.* The hot humid tropical climate coupled with high rainfall favour the incidence of the disease in the state. However, the release of bacterial wilt resistant varieties 'Sakthi' and 'Mukthi' has increased the area under tomato. But these varieties are susceptible to Tomato Leaf Curl Virus disease caused by Gemini group virus and transmitted by white fly (*Bemisia tabaci*). The disease incidence varied from 10.77% to 90.50%. (Annual Report, 1994). The yield loss due to TLCV disease is tremendous and may sometimes be as high as 100 percent. (Green and Kalloo, 1994) It varies with season and stage of the crop at which infection occurs (Saikia and Muniyappa, 1989). Over reliance on the use of conventional insecticides to control the white fly has resulted in the evolution of highly resistant biotypes of *Bemisia tabaci*. Hence, there is an urgent need to develop innovative, rational and environmentally safe technologies to reduce losses caused by the disease

Development of resistant variety is the only alternative for the production of tomato under disease prone condition. Attempts were made to identify sources of resistance and transfer the character to cultivated varieties. H-24 is a moderately leaf curl resistant variety of tomato (Kalloo and Banerjee, 2000). But this variety is susceptible to bacterial wilt disease caused by *Ralstonia solanacearum* race.1, biovar-3 and biovar-5 in Kerala. Unless the tomato variety resistant to TLCV disease is resistant to bacterial wilt disease also, it cannot be recommended for cultivation in the state.

Hence the project on 'Screening of tomato somaclones for resistance to Tomato Leaf Curl Virus' was conducted at the Department of Olericulture, College of Horticulture, Vellanikkara with the following objectives.

1. Standardisation of explants for callus induction
2. Standardisation of media for
    a. Callus induction
    b. Organogenesis/embryogenesis
    c. Rooting
3. Screening somaclonal variants for resistance to TLCV disease in bacterial wilt sick soil.

Review of Literature

2. REVIEW OF LITERATURE

Plant tissue culture can be defined as a technique of cultivating cells, tissues or organs of plants on artificial media under aseptic conditions. Tissue culture as a research field of importance started in the early 1900. Initial studies on tomato tissue culture started with the root tip culture as early as 19^{th} century.

Somaclonal variations are the variations detected in plants derived in any form of cell culture and has been described in sugarcane, potato, tomato etc. Somaclonal variation may be profitably utilised in crop improvement, since it reduces the time required for releasing the new variety by at least two years as compared to mutation breeding and by three years in comparison to back cross method of gene transfer. The use of somaclonal variation as a source of genetic variability for plant improvement has been examined extensively (Larkin and Scowcroft, 1981). Although conventional micro propagation has resulted to a large extent in clonal fidelity, it has become increasingly clear that under the appropriate culture conditions, a great deal of genetic variability can be recovered in regenerated plants. If cultures are established from explants that did not contain a pre organised meristem or if cultures are maintained as callus prior to plant regeneration, the regenerated plants will be quite variable.

Indications of somaclonal variations in several crop plants have stimulated interest in application of this method for crop improvement. There are several cases reported, were somaclonal variation have produced agriculturally useful changes in the progeny (eg. eye spot resistance or increase in sugar yield in sugar cane, late blight resistance in potato, resistance to *Fusarium* in tomato (Evans, 1989)). Today one of the most intensively studied areas of tissue culture is the *in vitro* induction of variability and selection for biotic/ abiotic stress tolerance.

The tomato cultivation in Kerala was hampered due to the incidence of bacterial wilt and several viral diseases. The release of bacterial wilt resistant varieties 'Sakthi' and

'Mukthi' has made it possible to cultivate tomato even in the acid soils. However, these varieties are susceptible to viral diseases like Tobacco Mosaic Virus (TMV) ,Tomato Spotted Wilt Virus (TSWV) and Tomato Leaf Curl Virus (TLCV). The disease incidence of TLCV varied from 0.0 to 22.6% in Rabi and 0.0 to 90.47% in summer in the bacterial wilt resistant variety Sakthi. The conventional methods of breeding using the resistant source *Lycopersicon hirsutum* f. *glabaratum* are time consuming and large segregating population has to be artificially screened. The work done on exploitation somaclonal variation for resistance to TLCV is very less. Therefore brief review is presented on the standardisation of explant source and media for callus induction, organogenesis and root induction in tomato. The works done in utilizing of somaclonal variations for resistance to viral disease of field crops.and vegetables are also presented.

2.I RESPONSE OF DIFFERENT EXPLANTS

2.1.1. Leaf segments

Padmanabhan *et al.* (1974) reported that morphogenesis of cultured leaf explants from three tomato genotypes varied in their response to 64 combinations of IAA and Kinetin. Callus proliferation began by eight to ten days and shoot formation occurred within 30 days.

Coleman and Greyson (1977) observed that shoot and root formation from leaf callus was totally dependent on some external factors, particularly auxins. After continuous subculturing Herman and Haas (1978) obtained regeneration of shoots from callus of leaf tissue. Meredith (1979) observed shoot regeneration at higher concentration of Zeatin coupled with lower auxins level, from established callus. But Behki and Lesley (1980) found that the shoot regeneration is rapid when the callus derived from leaf bits using 2,4-D and BA, were transferred to a medium containing Zeatin alone at higher concentration.

Kurtz and Lineberger (1983) evaluated 12 cultivars of tomato for regeneration using different combinations of IAA (0.0 to 2.0 mgl^{-1}) and BA (0.0 to 10 mgl^{-1}). They observed that morphogenic response were cultivar dependent and exhibited broad maxima over a range of growth regulator concentration. Genotypic responses were found to be very high in 'Star fire'.

Sukumar and Sree Rangaswamy (1988) reported the proliferation of tomato callus on MS medium containing coconut water (15 % v/v) and BAP 2 mgl^{-1}.Subsequently the calli differentiated into vigorous shoots. They observed that the presence of coconut water in the medium is useful for maximum regeneration. They obtained profuse rooting in the medium with the IAA where as calli were obtained in NAA medium.

Selvi and Khader (1993) reported that tomato cv. PKM-1 leaf disc when cultured on half or full strength MS medium supplemented with various growth regulators, the best callus and shoot formation responses were observed on MS medium with 0.2 mgl^{-1} IAA and 2.5 mgl^{-1} BA. The callus cultures were initiated from leaf let explants of 10 to 15 days old seedlings of tomato cv. CO-1 after two weeks of culture on MS medium supplemented with different combinations of NAA and BAP. The highest percent of shoot bud formation was obtained in the presence of 20 μ M of BAP and 1 μ M of NAA. But shoot bud differentiation and multiple shoot regeneration were observed on all media with BAP and NAA (Geetha *et al.*, 1998).

Mandal (1999) reported that in leaf disc explants of tomato, profuse callusing was observed within 10 to 12 days. Calli derived from leaf disc were greenish yellow and relatively less nodular in appearance. MS basal containing 2 mgl^{-1} BAP with 1 mgl^{-1} NAA showed maximum shoot regeneration via. organogenesis, followed by MS supplemented with 5 mgl^{-1} BAP. Maximum rooting was observed on half MS with 1 mg/l of NAA followed by 0.5 mgl^{-1} of IBA.

Chandel and Katiyar (2000) observed that 0.5 cm^2 leaf explants produced callus. MS medium with 1.5 mgl^{-1}BAP and 1.5 mgl^{-1} IAA was the most responsive for formation of shoot buds (organogenesis) from 8 to 12 week old callus culture. Soniya *et al.* (2001) reported that leaf explants of tomato var. Sakthi were aseptically inoculated on MS basal medium containing combinations of BA (4.2 to 17.7 μ M) and Picloram (4.13 to 8.26 μ M). Regeneration of calli was attempted on MS medium containing BA alone, Picloram alone and combinations of both. Green morphogenic calli were induced on leaf explants within three weeks of culture on MS medium supplemented with BA (8.9 μ M) and Picloram (4.13 μ M). Regeneration of multiple shoots (8-12 per callus) was noticed within two weeks following transfer to MS medium containing 17.7 μ M. BA alone. Regenerated shoots were rooted on MS medium supplemented with one mgl^{-1} IBA.

2.1.2. Internodal segments

de Langhe and de Bruijne (1970) reported shoot formation from stem-derived calli on LS medium with 20 % coconut water and 10 μ M Zeatin. The shoot forming ability of the explants increased greatly when the plants in the green house were pretreated with Chlormequat prior to taking the original explants. Later de Langhe (1973) reported regeneration of shoot from stem internodes of four cultivated species of *Lycopersicon esculentum* and one *L. peruvianum* strain using Linsmaier and Skoog (LS) medium supplemented with Zeatin and coconut water. For *L esculentum* optimal regenerants of 20 to 40 % was obtained with Zeatin. Shoot formation was stimulated by abscissic acid in some cases but was inhibited in others.

According to Herman and Haas (1978) the shoots and roots regenerates from stem internodes and leaf tissue by continuous sub culturing but not from callus in which all visible differentiation from stem and leaf tissue was absent. Vnuchkova (1977) developed medium composition with various levels of auxins and Kinetin that could be used to obtain callus from stem of young plants.

Cappadocia and Sree Ramulu (1980) succeeded in regenerating additional shoot from stem internode callus by changing the hormone content of the regeneration medium. They added a high level of adenine and coconut water and excluded auxin. The subcultured callus yielded an increasing percent of tetraploid plants with 50 % of the regenerants being tetraploid after 24 months in culture, while only 25 % of the initial regenerants were tetraploids.

Locy (1983) studied stem and hypocotyls explants of six *Lycopersicon sp.* for their ability to form callus, adventitious shoots and adventitious roots on media containing various levels of IAA and kinetin. Stem explants tended to form more callus than hypocotyls explants. Shoot formation by hypocotyl explants was superior to stem explants for all species and at nearly all hormonal levels. Selvi and Khader (1993) reported that in stem explants of tomato cv. PKM-1 when cultured on half or full strength MS medium supplemented with various growth regulators, the best callus, shoot and root formation responses were observed on MS medium with 0.2 mgl^{-1} IAA and 2.5 mgl^{-1} kinetin.

Mandal (1999) reported that within 7 to10 days after placement on the callus initiation medium, the tomato stem bits swelled up and calli started growing from the cut surface. MS medium supplemented with 2 mgl^{-1} BAP with 1 mgl^{-1} NAA showed maximum shoot regeneration via. organogenesis, followed by MS supplemented with 5 mgl^{-1} BAP. Maximum rooting was observed on half MS with 1 mgl^{-1} NAA followed by 0.5 mgl^{-1} of IBA. Later Chandel and Katiyar (2000) observed that one-centimeter long tomato shoot explants in MS medium with 1.5 mgl^{-1} BAP and 1.5 mgl^{-1} IAA was most responsive for the formation of shoot buds. MS with 1.5 mgl^{-1} BAP and 1 mgl^{-1} NAA produced somatic embryos from cultured shoot explants.

2.1.3. *Nodal segments*

Nodal explants of five tomato cvs. were cultured on various media, Seedat hip 1, Seedat hip91, VF-134-1-2, Peto-882 and Peto-86 were cultured on MS supplemented with

0.5 mgl^{-1} BA. Of these Peto-882 regenerated plantlets on MS medium supplemented with 2 mgl^{-1} Kinetin (Pongtongkam *et al.*, 1993).

Paranhos *et al.* (1996) reported that 0.5 cm long nodal segments of tomato cvs. Empire and Monte Carlo were culture in full or half strength MS medium supplemented with 0.05, 0.1 or 0.2 mgl^{-1} NAA in combination with 0.5, 1.0 or 2.0 mgl^{-1} Kinetin. Nodal segments of both cultivars regenerated plantlets.

2.2. STANDARDISTATION OF MEDIA

2.2.1. Callus induction

Behki and Lesley (1976) observed that the combination of NAA and BA induced callus and shoot formation. Later Selvi and Khader (1993) reported that when tomato cultivar PKM-1 leaf disc, stem and shoot tip explants were cultured on half or full strength MS, best results for callus induction were observed on MS with 0.2 mgl^{-1} IAA and 2.5 mgl^{-1} BA.

Ali and Li (1994) cultured leaf explants from terminal leaflets of Beijing Early, Floradel, Tropics and *L. pimpinellifolium* on MS medium in presence of growth regulators. They found that callus initiation was at greater rate and largest on MS medium supplemented with 3 mgl^{-1} Zeatin or 0.6 mgl^{-1} IAA and 2 mgl^{-1} BA in all genotypes and callus differentiated at the highest rate on MS medium containing 0.6 mgl^{-1} IAA and 2 mgl^{-1} BA. Similarly Plastira and Perdikaris (1997) reported culturing of leaf explants of cvs. ACE 55 VF, Pakmor, San Marzano nano, San Marzano, Principe Borghese and Toper. Leaf explants from six-week-old green house grown explants were cultured on agar solidified MS medium containing various concentrations and combinations of BA, Kinetin and Zeatin. Appropriate combinations of plant growth regulators promoted the formation of green, compact callus with numerous shoot primordia after 15 to 20 days.

Jawahar *et al.* (1998) observed that when tomato (cv. PKM-1) cotyledonary explants were cultured on MS medium supplemented with different concentrations of NAA, IAA or 2, 4-D or combination of NAA and Kinetin, white greenish and friable callus was induced on MS medium supplemented with NAA, IAA or 2,4-D alone. White green compact callus was obtained in the presence of NAA and Kinetin.

Geetha *et al.* (1998) observed that callus cultures were initiated from leaflet explants of 10 to15 day old seedlings of tomato cv. CO-1 after 2 weeks of culture on MS medium supplemented with different combinations of NAA (3 or 6 µM) and BA (2,4,6 or 8 µM). Ramiah and Rajappan (1998) reported that in tomato cv. CO-3, 2,4-D along with BA induced only callus. But IAA at low concentration in combination with BA induced callus as well as shoot regeneration without any root formation. NAA at low concentration in combination with BA induced callus formation and shoot regeneration, while NAA at high concentration in combination with BA induced callus and root formation. IAA alone at 0.2 mgl^{-1} induced callus formation.

The hypocotyl explants of *L. esculentum* cv. PKM were cultured on MS medium supplemented with different concentration and combination of various auxins and cytokinins. Among the combinations used, NAA (1 mgl^{-1}) and Kinetin (0.1 mgl^{-1}) were found to be the best highest frequency callus induction (Venkatachalam *et al.*, 2000). Soniya *et al.* (2001) reported that in *L. esculentum* Mill. cv. Sakthi calli were induced from leaf explants on MS medium supplemented with 8.88 µM BA and 4.13 µM Picloram.

2.2.2. Organogenesis.

Regeneration occur as a result of organogenesis and requires an initial period of callus proliferation which is stimulated by endogenously synthesized auxins and cytokinins or by exogenous addition of auxin and cytokinins to regeneration medium. Padmanabhan *et al.* (1974) reported shoot initiation from the tomato leaf callus in the medium containing IAA and Kinetin. For regeneration from leaf explant, Kartha *et al.* (1976) used 0.22 mgl^{-1}

Zeatin where as Behki and Lesley (1976) used higher concentration of cytokinin, 10.3 mgl^{-1} Zeatin. Similarly Dwivedi *et al.* (1990) used auxin concentration of 0.01 mgl^{-1} NAA where as Dhruva *et al.* (1978) used as high as 17.5 mgl^{-1} IAA.

For regeneration from shoot tips Kartha *et al.* (1977) used minimum concentration of auxin, 0.02 mgl^{-1} IAA and maximum 2.0 mgl^{-1} IAA. Similarly, minimum and maximum cytokinin used for regeneration from shoot tip were 0.02 mgl^{-1} BAP and 2.0 mgl^{-1} BAP. Taj *et al.* (1977) also reported tomato shoot regeneration with Zeatin and IAA combination.

For regeneration from stem bits Locy (1983) used auxin concentration ranging from 0.1 mgl^{-1} to 10.0 mgl^{-1} IAA. Cappadocia and Sree Ramalu (1980) used 0.05 mgl^{-1} BAP for regeneration where as Locy (1983) used a higher concentration of 10.0 mgl^{-1} Kinetin. This showed that regeneration from stem bits require slightly elevated levels of auxins than cytokinins.

Kurtz *et al.* (1983) stated that the amount of callus produced and shoot regeneration efficiency of tomato explants is dependent on types and concentration of growth regulators in the culture media, growth condition of donor plant, age of donor plant, explant of choice, portion and age of donor tissue and genotype of donor plant. Tomato cv. PKM-1 leaf disc, stem and shoot tip explants were cultured on half or full strength MS medium supplemented with various growth regulators. Shoot formation responses was high in MS medium supplemented with 0.2 mgl^{-1} IAA and 2.5 mgl^{-1} Kinetin (Selvi and Khader, 1993)

Pongtongkam *et al* (1993) observed that when excised leaf and stem explants of five tomato cvs. were cultured on various media, regeneration was obtained on MS medium supplemented with 1 mgl^{-1} or 2 mgl^{-1} Kinetin. Ye *et al.* (1994) reported that cotyledons from 10 to 12 day old seedlings produced shoots on MS medium with 1 mgl^{-1} IAA and 1 mgl^{-1} Zeatin. Cotyledon segments of tomato (*L. esculentum* Mill.) were cultured on the MS medium containing 0.1 mgl^{-1} IAA, 1.0 mgl^{-1} Zeatin, 3.0 % sucrose and agar, agarose or gellan gum as a gelling agent and supported on a polyester material. Shoot regeneration was

stimulated by a water extract from agar when explants were cultured on the polyester support (Ichimura and Oda, 1995).

Lich *et al.* (1996) reported the regeneration capacities of two tomato cvs. Potent and Rutgers and of there accessions of wild tomato sp. *L.peruvianum* PI 128650, *L. peruvianum* var. *dentatum* PI 128655 and *L.glandulosum*. Completely regenerated plants were obtained from all the tested species, but organogenesis occurred almost two weeks earlier in wild tomatoes than in the cultivated varieties of *L. esculentum*. Explants from seedlings grown on 6-benzyl adenine (BA) supplemented medium produced the highest frequency of regenerants when subcultured to media containing BA. In contrast, BA had an inhibitory effect on explants obtained from seedlings grown on media lacking BA (Newman *et al.*, 1996).

Plastira and Perdikaris (1997) reported regeneration of tomato leaf explants of cvs. ACE 55 VF, Pakmor, San Marzano nano, San Marzano, Principe Borghese and Toper. Green, compact callus with numerous shoot primordia was observed after 15 to 20 days. Callus growth continued and new shoot formation was observed for more than seven weeks. BA or Zeatin at 0.1 to 10 mgl^{-1} induced multiple shoot regeneration.

Jawahar *et al.* (1998) observed that when tomato (cv. PKM-1) cotyledonary explants were cultured on MS medium, \shoot regeneration was obtained only in the presence of combination of NAA and kinetin. The highest value of shoot regeneration frequency, number of multiple shoot and shoot length after five weeks of culture were achieved on MS medium supplemented with NAA at 2.5 mgl^{-1} and kinetin at 1.5 mgl^{-1}.

Ramiah and Rajappan (1998) observed shoot regeneration in 0.2 mgl^{-1} IAA and shoot multiplication and direct rooting after six weeks of culture in MS medium for tomato variety CO-3. The best results were obtained in MS medium supplemented with either 2.0 mgl^{-1} kinetin and 0.2 mgl^{-1} IAA or 2.0 mgl^{-1} 2 ip and 0.2 mgl^{-1} IAA. Geetha *et al.* (1998) reported that when leaf disc calli was kept on MS medium supplemented with different

combinations and concentrations of NAA and BAP shoot bud regeneration was observed after three weeks. The highest percentage of shoot bud formation was obtained in the presence of 20 μM BAP and 1.0 μM NAA, but shoot bud differentiation and multiple shoot regeneration were observed on all media with BAP and NAA.

Mandal (1999) reported that when somatic callus derived from stem and leaf explants of four tomato varieties viz. BWR-1, BWR-6, PKM-1 and culture 340 was cultured, MS basal medium supplemented with 2.0 mgl^{-1} BAP and 1.0 mgl^{-1} NAA was found to be the most efficient in generating maximum plantlets. Later Mandal (1999) reported that MS basal medium supplemented with 2 mgl^{-1} BAP (Benzyl Adenine) and 1.0 mgl^{-1} NAA was the most efficient in generating plantlets in stem and leaf explants.

Capote *et al.* (2000) observed the effect of different culture media on the regeneration of leaf tissues of five lines studied. The best results were obtained with MS medium supplemented with 0.175 mgl^{-1} NAA and 1.5 mgl^{-1} BA. Similarly Venkatachalam *et al.* (2000) reported that in *L. esculentum* cv. PKM-1, of the two cytokinins tested, BAP was found to be more suitable compared to kinetin for maximum shoot bud differentiation as well as multiple shoot induction. BAP was the most effective plant growth regulator in this study, indicating cytokinin specificity for shoot bud regeneration and multiple shoot induction in these tissues.

Fari *et al.* (2000) evaluate the regeneration capacity of the IPA-5 and IPA-6 Brazilian industrial tomato cultivars. In the case of IPA-5, the number of shoots was higher when the induction of shoot buds was accomplished in culture medium containing 2.5 mgl^{-1} BAP and 0.2 mgl^{-1} IAA followed by three subcultures on Zeatin (0.5 mgl^{-1}) containing medium.

Chandel and Katiyar (2000) standardized media and cultural conditions for plant regeneration from tomato via. embryogenesis and organogenesis. Effect of different concentration and combinations of phytohormones (2,4-D, Kinetin, BAP, NAA and IAA)

were examined and plantlets were regenerated. The optimum size of explant was found to be 0.5 cm^2 for leaf explants and one cm for shoot explants. Among the different combination of phytohormones tested, MS medium with 1.5 mgl^{-1} BAP and 1.5 mgl^{-1} IAA was most responsive for the formation of shoot buds (organogenesis) from 8 to12 week old callus culture of leaf explants. Soniya *et al.* (2001) obtained regeneration of *L. esculentum* var. sakthi after culturing freshly induced calli on MS medium containing 17.7 µM BA alone.

2.2.3. Rooting

Selvi (1993) obtained rooting of tomato cv. PKM-1 on medium containing half strength MS with B_5 vitamins and 2.0 mgl^{-1} IAA. According to Plastira and Pudikaris (1997) *in vitro* propagated shoots rooted within 7 to10 days on basal MS medium and grew into phenotypically normal plants when transferred to soil.

Jawahar *et al.* (1997) observed hypocotyl and cotyledon explants of tomato cv. PKM-1 when cultured regenerated shoots. They rooted on MS medium containing 2.0 c IBA. According to Geetha *et al.* (1998) elongated shoots of leaf bit explants produced shoots when subcultured on medium supplemented with IBA (5 µM).

Mandal (1999) reported half strength MS medium supplemented with 1.0 mgl^{-1} NAA was most effective in inducing rooting. Rooting of the regenerated shoots of PKM-1 was observed on half strength MS medium supplemented with IBA (0.1 to 0.5 mgl^{-1}) (Venkatachalam *et al.*, 2000). Soniya *et al.* (2001) reported that micro shoots rooted in the presence of 10.0 μMl^{-1} IBA on MS medium in 'Sakthi'.

2.3. SOMACLONAL VARIATION

Heinz and Mee (1969) were the first to give detailed cytological evidence of tissue culture induced chromosome variability in regenerated sugarcane plants. Culture induced

variation does occur in the regenerants from somatic explants involving an intervening callus phase (D'Amato, 1977). Similarly Ancora *et al.* (1977) reported that prolonged cultures of callus derived from anthers of self compatible genotypes S_1S_3 and $S_{12}S_{13}$ of *L. peruvianum* resulted in an increase in the ploidy levels of cells. They observed rapid growing and slow growing calli in $S_{12}S_{13}$ genotypes.

Somaclonal variation is of wide spread occurrence and has been described in a range of plant species including cereals, such as maize (Green *et al.*, 1977; Edallo *et al.*, 1981), oats (Cummings *et al.*, 1976), wheat (Larkins *et al.*, 1984; Jones *et al.*, 1984) and rice (Sun *et al.*, 1983). It has also been described in other seed crops such as tobacco (Barbius and Dulieu, 1980; Prul, 1983), tomato (Evans and Sharp, 1983) and alfaalfa (Pfeiffes and Bingham, 1984; Groose and Bingham, 1984).

Plants regenerated from somatic cells using tissue culture are not genetically uniform but show significant genetic variability termed as somaclonal variation. This variation is very high compared to spontaneous mutation (Evans and Sharp, 1983). Cappadocia and Sree Ramulu (1980) obtained increased percent of tetraploid regenerants from tomato stem callus, subcultured on media with high levels of adenine and coconut water. Regenerants generally display genetic modifications for a wide range of characters. Such variations are known as somaclonal variations (Larkin and Scowcroft, 1981). A number of factors including endomitosis, chromosome loss, polyploidy, aneuploidy, mutation, amplification and de amplification of genes, transposons etc. are reported to be responsible for the cause of somaclonal variation.

The occurrence of somaclonal variation has been reported in tomato, with a number of different types of traits appearing in regenerated plants. Evans and Sharp (1983) detected somaclonal variation even upto 15 % of the regenerated tomato plants and published a description of experiments designated to ascertain the genetic raising of somaclonal variation in tomato. They again stated that to exploit somaclonal variation properly, the somaclone should be produced in short time and in large scale. Evans *et al.*

(1984) concluded that the somaclonal variation in tomato were due to chromosome number variation, single gene mutation and organelle gene mutation. Similarly Colijn (1986) observed somaclonal variation and ploidy differences in the plants regenerated from the leaf disc of eight inbred tomato lines.

Zagorska *et al.* (1985) observed somaclonal variation in plants regenerated from leaf discs of *L. esculentum* Mill., *L. peruvianum* Mill., *L. chilense* Dun. and *L. cheesmanii* Riley. A high level of variability in morphological and cytolological properties and pollen fertility was observed in the regenerants.

Somasundar and Gostimsky (1992) regenerated plants (R_o) derived from long term callus tissue cultures induced from cotyledon explants of the Russian tomato variety 'Dabok'. They evaluated the morphological characters viz. violet cotyledon colour, potato leaf shape, dwarf habit, modification of first floral node, early flowering, chlorophyll deficiency and orange fruit colour. The recovery of such a high frequency of somaclonal variation for such agronomically important traits as early flowering and orange fruit colour demonstrated the importance of tissue culture as a method for improving tomatoes.

Zhuk (1993) observed somaclones obtained from callus derived leaf explants of four cultivars of tomato. The percent of somaclones resistant to Tobacco Mosaic Tobamo Virus (TMV) regenerated from TMV infected callus cultures was significantly higher than those from virus free ones. The use of repeatedly subcultured callus tissue for regeneration of somaclones promoted an increase in frequency of virus resistant forms, but the regeneration potential was much reduced in this tissue. Mandal (1999) reported that an efficient somaculture system involving somatic callus derived from stem and leaf explants of four tomato varieties viz. BWR-1, BWR-6, PKM-1 and culture 340 were evolved. Somaclones were simultaneously screened for bacterial wilt resistance caused by *Pseudomonas solanacearum* under sick plot condition. Marked variation in resistance profile was discernible in comparison to parental base population.

Hanus *et al.* (2000) reported the protocols developed with the objective of obtaining materials for selection of variance with virus resistance traits in tomato genotypes. Preliminary results are demonstrated in the testing for variability in somaclones obtained through indirect adventitious organogenesis initiated on leaf explants of cultivated tomato. Somaclones were grown in green house condition and symptoms upon infection with tomato mosaic virus (T_0 MV) or Cucumber Mosaic Virus (CMV) was observed. Desirable variance were selected from cultivars 'Money maker' 'Potantat' and 'Rutgers'. Some of the money maker somaclones exhibited increased tolerance to CMV, a few seemed to be even fully resistance though most were susceptible as donor plants.

Soniya *et al.* (2001) reported that DNA samples from the mother plant and eleven randomly selected regenerants obtained from a single callus were subjected to RAPD analysis for the detection of putative somaclones. Estimation of genetic similarity coefficient based on RAPD band was estimated. Ten regenerated plants were more than 95 % similar to mother plant, except one which was found to be distinctly different.

2.4. SOMACLONAL VARIATION AND RESISTANCE TO DISEASES

Somaclonal variation in many crop plants has exploited extensively for obtaining disease resistant lines. A single gene mutation and organelle gene mutation have been produced by somaclonal variation. It is possible to select new variants that retain all the favourable qualities of an existing variety while adding one additional trait, such as disease resistance. If the selected genes are recessive, no additional back crossing is necessary. If the selected genes are dominant only a few cycle of selfing- segregation and selection are needed to obtain homozygous plants.

The variation can contribute to the recovery of novel disease resistant genotypes, either through selection at the level of cultured cells or through selection of the plants regenerated from culture by screening directly against pathogen or pathotoxin either pure or enriched culture filtrate.

Behnke (1979) and Behnke (1980) reported that recovery of late blight resistant types may be enhanced by cell selection. Plants regenerated from resistant calli obtained from a population of 41000 calli of di-haploid potato, cultured in presence of unpurified *P.infestans.* Shepard *et al.* (1980) also screened a population of 500 somaclones for resistance to early blight (*Alternaria solani*) in potato. Five of these were more resistant than the parents and four displayed field resistance. Shepard (1981) found 0.2 % of protoclones displayed enhanced resistance to late blight (*Phytophthora infestans*) in potato cv. Rusel Barbank.

Out of 78 plants regenerated from selected tobacco calli resistant to *Pseudomonas syringe* pv. *tabaci* toxin, ten plants showed resistance to the pathogen in field condition (Thanutong *et al.*, 1983). Similarly out of 23 plants regenerated from selected *Alternaria alternata* pv. *tabaci* toxin resistant protoclones, five showed high degree of resistance to the pathogen.

Barden and Murakishi (1985) reported that over 200 rooted somaclones of the susceptible tomato lines (+/+) and 12 of resistant lines (TM-1/+1, TM-2/+1) obtained from leaf callus were rub inoculated with yellow strain of T_0 MV which has a host range similar to T_0 MV-$_0$-. Neither symptom expression nor virus multiplication (as detected by ELISA) occurred in somaclones of resistant lines. Also virus multiplication was not detected in several somaclones of the susceptible line one month after inoculation.

Shahin and Spivey (1986) regenerated fusarium wilt resistant plants from protoplast derived calli and studied the inheritance pattern of resistant somaclones. Similarly Paglinso and Rappoport (1987) reported that some of the regenerated celery plants showed increased tolerance to *Fusarium oxysporum* f.sp. apii in field as well as in green house condition.

Six out 370 tomato somaclones regenerated form a fully susceptible line (GCRI-26) were found to be resistant to viral infection after inoculation with Tobacco Mosaic Virus

(TMV) and Tomato Mosaic Virus (T_0 MV) as detected by ELISA and back inoculation to *N. gluctinosa* (Smith and Murakishi, 1987).

Bobisud *et al.* (1996) observed that when tomato cv. Healari somaclones were evaluated in a field infected with bacterial wilt pathogen (*Pseudomonas solanacearum*), 18 bacterial wilt resistant somaclones could be observed. Zhuk (1998) found that tomato somaclones regenerated from passivated callus tissue of different aged gamma-irradiated callus. Virus infected and virus free explants were studied for this susceptibility to Tobacco Mosaic Tobamo Virus (TMV). Virus resistant somaclones which exhibited fertility under self pollination have been selected.

Mandal (1999) reported an efficient *in vitro* culture system involving somatic callus derived from stem and leaf explants of tomato varieties BWR-1, BWR-6, PKM-1 and culture 340. Substantial somaclonal variation was observed for major agronomic traits including plant height, plant spread, number of primary branches, number of fruits and fruit yield per plant in the SC_2 generation. A few elite lines with bacterial wilt resistance were selected in order to develop superior wilt resistant lines for mass cultivation in the humid tropics of Andamans.

Sotirova *et al.* (1999) reported that the possibility of obtaining tomato plants resistant to *Clavibacter michiganense* sub.sp. *michganense* (Cmm) through anther and tissue culture. The regenerants differed in resistance to Cmm. All regenerants from anther culture of Roma ms and Roma ms x UC 82 A were susceptible to the disease, while those from Bella and Roma ms x L 31 varied from susceptible to be resistant. The highest number of regenerants obtained from somatic tissue culture lacking disease symptoms until the end of vegetative growth was observed in Cristy and L-24-13.

Hanus *et al.* (2000) observed that somaclones obtained through indirect adventitious organogenesis initiated on leaf explants of cultivated tomato (*Lycopersicon esculentum*) somaclones were grown in green house condition and symptom upon infection with T_0 MV

or CMV was observed. Desirable variants were selected from cultivars Money maker, Potentat and Rutgers. Some of the Money maker somaclones exhibited increased tolerance to CMV, a few seemed to be even fully resistant though most were susceptible as donor plants.

2. 4. TOMATO LEAF CURL VIRUS DISEASE.

Tomato is affected by 30 different viruses belonging to 16 different taxonomic groups. Among them, gemini virus group, which causes Tomato Leaf Curl diseases is more frequently found in subtropical and tropical environments. Hussain (1932) was the first to report the leaf curl disease in tomato. Thung (1932) reported that the leaf curl virus disease in tomato is caused by Tobacco Leaf Curl Virus. In India, the occurrence of leaf curl disease was first observed in Northern plains by Pal and Tandon (1937) and later by Pruthi and Samuel (1939).

In a green house study, TLCV reduced the yield by 63% when plants were inoculated three weeks after transplanting. The percentage of infection reached a peak of 58 and 78 respectively when planting in August and September, while it was six and four per cent with respect to planting during December and January respectively (Mazyad *et al.*, 1979).

The virus belongs to a monopartile gemini virus group and is transmitted by the tobacco white fly, *Bemisia tabaci* (Gennadius) which affects the crop during summer and autumn (Martelli and Quacquarelli, 1982). The yield loss due to Tomato Leaf Curl Virus (TLCV) is tremendous and may be some times as high as 90%. It varies with season and stages of the crop growth at which the infection occurs (Saikia and Muniyappa, 1989)

The vector of TLCV is a polyphagous insect with more than 300 hosts comprising of a lot of cultivated plants and weeds (Reddy *et al.*, 1986). The spread of TLCV was significantly correlated with population size of the vector, *Bemisia tabaci* (Cohen *et al.*,

1988 and Ratul and Bordoloi, 1988). It was observed that, the influence of TLCV in tomato ranged from 17-53 % during July to November and upto 100 % during February to May (Saikia and Muniyappa, 1989). According to Nainar (1996), the incidence of TLCV disease was the highest when tomato was transplanted during February.

2.5. SCREENING FOR RESISTANCE

An effective screening procedure, at large scale makes the TLCV breeding programme more efficient. Many successful screening programmes for TLCV resistance were carried out in the field, relying upon natural virus infection. However, it becomes reliable only when artificial method of virus inoculation is used.

Pilowsky and Cohen (1974) have conducted artificial inoculation using viruliferous white flies maintained on *Datura stamonium* plants for testing resistance to TLCV. Hayati (1978) suggested individual plant inoculation in which white flies were reared on immune egg plant, which were starved for one hour before transferring to TLCV infected tomato plants. After 48 hours of acquisition feeding, they were gently removed and allowed to feed on healthy tomato plants to be tested for their resistance to TLCV at three to four true leaf stages.

Bemisia tabaci is a thermophilic insect (Avidov, 1978), the fecundity of *which* is known to be influenced by higher temperature (Pruthi and Samuel, 1942). Butter and Rataul (1978) reported that, transmission of TLCV was 100 % at 33-39^{0}C, while it was 30 % at 44^{0}C or only 10 % at 10^{0}C. Mayee *et al.* (1974) reported that HS-110,HS-102,Nematex,T-1 and Nova are some of the TLCV tolerant varieties developed by conventional breeding methods.

Mishra *et al* (1998) reported resistance to tomato leaf curl virus in the tomato crosses of Anand T-1 X BT-12 and H-24XBT- 12. Resistance to leaf curl virus was also reported in tomato genotypes viz. H-11, H-22, H-106 and H-107 (Banerjee and Kalloo, 1998).

Kalloo and Banerjee (2000) reported the performance of H-24 with respect to yield and reaction to TLCV under field and artificial inoculation. They found that mean PDI values of H-24,Sel-7 and Panjab Chhuhara were 18.83%, 50.23% and 67.57% respectively. The symptom severity and co efficient of infection values were also very less in H-24 (0.28 and 1.51 respectively). On artificial inoculation also mean PDI, symptom severity and co efficient of infection were significantly less in H-24. The average yield of H-24 was maximum followed by Sel-7 and Panjab Chhuhara.

Rattan and Bindal (2002) screened two hundred fourteen genotypes of tomato against Tomato Leaf Curl Virus (TLCV). Seven lines viz., 620,646,672,742,761,765 and 776 were highly resistant (0.5% infection) where as seven lines were resistant (6-20% infection).

Materials and Methods

3. MATERIALS AND METHODS

The present investigation "Screening of tomato somaclones for resistance to Tomato Leaf Curl Virus (TLCV)"was carried out in the Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara during 2000-2002. The site is located at an altitude of 22.5m above MSL. and 10° 32'N latitude and 76°16' E longitude. The soil is sandy loam and the area enjoys a tropical warm humid climate. The study consisted of the following experiments.

3.1 STANDARDISATION OF EXPLANTS FOR CALLUS INDUCTION

The source materials selected for study were *L. esculentum* Mill. var. Sakthi and *L. pimpinellifolium.* 'Sakthi' evolved at the Department of Olericulture, Kerala Agricultural University, Vellanikkara, has semi determinate growth habit, flat round, medium sized, green shouldered fruits with jointed pedicel and resistance to bacterial wilt disease. It has duration of about four months and the average productivity is 32t/ ha.But it is susceptible to tomato leaf curl virus.

L. pimpinellifolium is found as a weed in the Peruvian coastal valleys and its fruits are red currant- like and small. The plants have a weak slender and profusely branched habit.

Seeds were sterilised with 1% Sodium hypo chlorite and 0.04% Teepol for twenty minutes in vacuum, washed in sterile water and cultured in 0.5 strength MS medium (Murashige and Skoog, 1962) containing $30gl^{-1}$ Sucrose and 0.8% Agar (pH 5.8). The seeds germinated after 15 to 20 days at 26 ± 1^0 c and 4000 lux. The seedlings were retained for a period of 15 to 20 days to obtain the explants.

The explants consisted of leaf segments (0.5 cm^2) from fully opened leaves, nodal segments (0.5cm) and internodal segments (1cm). The media used for the study consisted of

the basal MS and ½ MS media along with different auxins and cytokinins either singly or in combination. The auxins used were NAA (0, 1, 2 and 3 mgl^{-1}) and 2,4-D (0.5, 1, 1.5 and 2 mgl^{-1}) and cytokinins used were BA and kinetin (1, 2, 3, 4 and 5 mgl^{-1})

There were 20 tubes per treatment and observations were recorded at weekly intervals.

Observations

i. Number of test tubes inoculated

ii. Number of cultures established

iii. Percent contamination – fungal, bacterial, phenolics

3.2 STANDARDISATION OF MEDIA

3.2.1 Preparation of Media

The basal media, Murashige and Skoog(1962)(MS) consisted of inorganic nutrients, organic supplements, vitamins, and carbon source (Table 1). All the stock solutions of major, minor and micro nutrients, iron and vitamins were prepared separately in double distilled water taken in sterilised well stoppered bottles and kept in a refrigerator to reduce microbial contamination. Na_2EDTA and $FeSO_4.7H_2O$ were dissolved in separate beaker with approximately 200ml double distilled water. Both the beakers were placed on hotplate and brought to the point of almost boiling. Then the $FeSO_4.7H_2O$ was slowly added to the Na_2EDTA (never vice versa) over a five-minute period with continuous stirring. Then this 400ml solution was cooled to room temperature in the dark. After cooling to room temperature, the stock was made upto one litre with double distilled water. Clear yellow colour of the solution indicated correctness of preparation.

Naphthelene-3-acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D), each at 50mg were dissolved separately in a few drops of 0.1N NaOH and volume made upto 50ml with double distilled water to give final concentration of one $mgml^{-1}$ or 1000 ppm and stored at 4^0 C. Fifty mg. each kinetin and 6-benzly adenine (BA), were dissolved separately in a few drops of 0.1N HCl and volume was made upto 50ml with double distilled water to give final concentration of one $mgml^{-1}$ or 1000 ppm and stored at 4^0 C. This was calculated using the formula

$C_1V_1=C_2V_2$

Where

C_1 = Phytohormone concentration of stock solution

V_1 = Required volume of stock solution

C_2 = Required concentration of phytohormone in the medium

V_2 = Required volume of medium

The required volume of stock solutions of chemicals were pipetted in to a one litre glass beaker. The components like sucrose and myo-inositol were added and allowed to dissolve. The volume was made up to approximately 950 ml. with double distilled water. The pH was adjusted to 5.6 to 5.8 with few drops of 0.1 N. NaOH or 0.1 N. HCl. Agar agar at a concentration of $8gl^{-1}$ was dissolved in little amount of boiled water and added to the nutrient stock and volume was made up to one litre. The medium was thoroughly mixed following the procedures of Butenko (1967) and was dispensed in 20ml aliquots into clean sterilised culture tubes of 150x25mm size. Then the culture tubes were plugged with non-absorbent cotton wool as suggested by Bhojwani and Bhatnagar (1974). Test tubes containing media were autoclaved at $1.06kgcm^{-2}$ pressure at 121^0C for 20 minutes (Monaco *et al.*, 1977) in an autoclave.

Table 1. Chemical Composition of MS media

Murashige and Skoog medium (MS)				
Stock	**Chemical**	**mg l^{-1}**	**Stock concentration**	**Stock**
I	NH_4NO_3	1,650	50X	82.5 g l^{-1}
	KNO_3	1,900		95.0 g l^{-1}
	KH_2PO_4	170		8.5 g l^{-1}
	$MgSO_4.7H_2O$	370		18.5 g l^{-1}
II	$CaCl_2$	440	50X	22.0 g l^{-1}
III	Na_2EDTA	37.3	100X	3.7 g l^{-1}
	$FeSO_4.7H_2O$	27.8		2.8 g l^{-1}
IV	$MnSO_4.4H_2O$	22.3	100X	2.23 g l^{-1}
	$ZnSO_4.7H_2O$	8.6		860 mg l^{-1}
	H_3BO_3	6.2		620 mg l^{-1}
	KI	0.83		83.0 mg l^{-1}
	$Na_2MoO_4.2H_2O$	0.25		25.0 mg l^{-1}
	$CuSO_4.5H_2O$	0.025		2.5 mg l^{-1}
	$CoCl_2.6H_2O$	0.02		2.5 mg l^{-1}
V	Glycine	2.0	100X	200 mg l^{-1}
	Nicotinic acid	0.5		50 mg l^{-1}
	Pyridoxine acid-HCl	0.5		50 mg l^{-1}
	Thiamine-HCl	0.1		10 mg l^{-1}
100 mg l^{-1} myo-inositol 30 g l^{-1} sucrose 8 g l^{-1} Agar semisolid pH - 5.6-5.8				

3.2.2 Callus induction

The culture medium for callus formation consisted of inorganic salts of Murashige and Skoog (MS) full and half concentration supplemented with 30g/litre sucrose and varying levels of NAA (0, 1, 2, 3 mg/l), 2,4-D (0.5, 1, 1.5, 2 mgl^{-1}), BA (1, 2, 3, 4, 5 mg l^{-1}) and Kinetin (1, 2, 3, 4, 5 mg l^{-1}) added singly and in combinations. The medium was solidified with 0.8% agar.

The transfer of explants for callus induction was done under aseptic condition. For this, the laminar flow chamber was thoroughly wiped with ethyl alcohol and the UV light was put on for 20 minutes for sterilisation. The surgical instruments were sterilised with 70% alcohol and flamed to red hot over a spirit lamp (Bhojwani and Bhatnagar, 1974). There was a minimum of 18 culture tubes per treatment combination. The explant was placed on the culture medium in each tube under aseptic condition in the laminar flow.

The racks containing cultures were transferred to growth chamber where the temperature, light and humidity were controlled. The cultures were incubated at 25 ± 1^0C in dark for one week for better callus induction. The cultures were then kept under 16hr. light (2500 lux) and 8 hr dark (Gresshoff and Doy, 1972). The relative humidity was maintained above 60%.

Observations

i. Number of days for callus induction

The number of days taken from inoculation to callus initiation was recorded.

ii. Percentage callusing (P)

Of all the inoculated tubes, those, which showed signs of callusing, were counted and expressed as percentage of total number of inoculated tubes

iii. Growth rate (G) (scale of 0, 1, 2, 3, 4)

At 30 days after inoculation visual grades were awarded i.e. no response (0), poor callus initiation (1), average callus initiation (2), profuse callus initiation (3) and excellent callus initiation (4) for volume of callus observed. (Plate 1.)

iv. Callus index (P x G)

This was computed to get an idea of the initiation and proliferation of callus as influenced by any medium. At the end of the culture period, based on the growth of the callus in each tube, the tubes in each treatment were classified into five categories based on the extent of surface of media covered by the calli.

Callus index was computed by the formula

Callus index (C.I.) = Percentage callusing (P) x Growth rate (G)

3.2.3 Organogenesis/Embryogenesis

The culture media used for induction of organogenesis from callus also consisted of MS medium and 1/2MS medium supplemented with NAA (0, 1, 2 mg l^{-1}), 2,4 – D (0.5, 1, 1.5 mg l^{-1}) BA (3,4,5,6 mg l^{-1}) and Kinetin (3, 4, 5, 6 mg l^{-1}) either singly or in combination. Twenty to twenty five day old calli, obtained from explants of tomato variety 'Sakthi' cultured in MS medium containing NAA 1 mgl^{-1} and BA (leaf), NAA 2mgl^{-1} and BA1 mgl^{-1} (internode) and 2,4-D 1mgl^{-1} and BA 1 mgl^{-1} (node) were cut in to 2 to 4 pieces according to their size and transferred to regeneration media.

The calli produced by explants of . *L. pimpinellifolium* in basal medium containing NAA 2 mgl^{-1} and BA 1 mgl^{-1} was used for shoot induction.

Observations

i. Number of cultures differentiated – shoot, root, embryo

The number of cultures differentiated from fifteenth day onwards was recorded

ii. Number of shoots / culture

The number of shoots produced per test tube for each treatment was recorded 35 days after inoculation.

iii. Number of roots / culture

The number of roots produced per test tube for each treatment was recorded after inoculation.

iv. Number of embryos / culture

The number of embryos produced per test tube for each treatment was recorded.

3.2.4 Root induction

The multiple shoots derived from callus transferred to regeneration media were subcultured on MS media supplemented with IAA, NAA and IBA at different levels of 0.5, 1, 1.5, 2 mg l^{-1} either singly or in combination for rooting and incubated at 26± 1^0C.

Observations

i. Number of days for root initiation

The number of days required for root initiation was recorded in each treatment.

ii. Percentage culture rooted

The number of test tubes that produced roots, in each treatment were counted and expressed as percentage

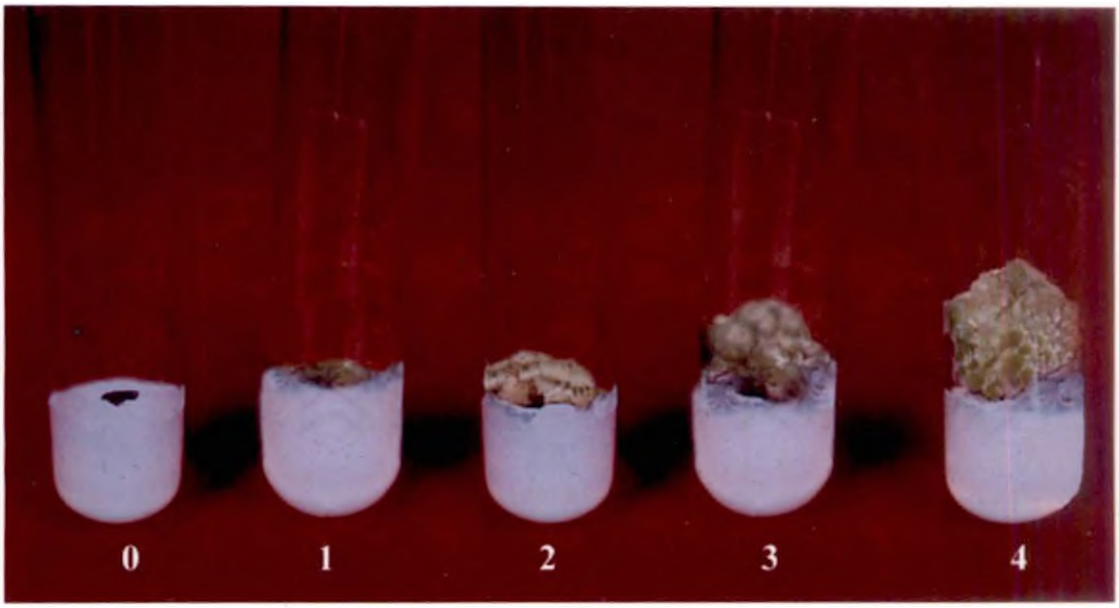


Plate 1. Scoring of callus growth rate in 'Sakthi and *L.pimpinellifolium* (30 days after inoculation)

Plate 2. Artificial inoculation of *Bemisia tabaci* in 'Sakthi and *L.pimpinellifolium*

iii. Number of roots / culture

The number of roots produced in each tube was recorded and average was worked out

iv. Presence of root hairs

The presence of root hairs was recorded for all the treatments

v. Presence of intervening callus

The presence of intervening callus in each tube was recorded

3.2.5 HARDENING AND PLANTING OUT

The agar adhering to the root zones of whole regenerated plants were thoroughly cleaned with sterile distilled water in laminar airflow chamber. Then they were transferred to mist chamber for hardening. The regenerants were planted in net pots containing sterile sand, soil, coir pith compost and coco pith. After 10 days of hardening the plants were transferred to earthen pots (capacity of 5 kg) containing the potting mixture with wilt sick soil. Simultaneously the parental lines *L.esculentum* var. Sakthi and *L. pimpinellifolium* were also planted in pots and kept in between rows of somaclones as check

3.3 SCREENING SOMACLONAL VARIANTS FOR RESISTANCE TO TLCV DISEASE

The somaclones along with the parental materials were screened for resistance to TLCV by artificial inoculation through the vector, whitefly, *Bemisia tabaci*. Whiteflies collected from tomato fields were reared on healthy tomato plants in the insect proof cages. After preacquisition fasting for 30 minutes ten whiteflies were released on each diseased plants for acquisition of virus using a micro cage (Plate.2). After an hour of acquisition feeding micro cage along with the viruliferous white flies were removed carefully from the diseased plants and fixed on the healthy test plants to be screened for resistance. After 12 hours of inoculation feeding period, white flies were removed and the inoculated plants sprayed with Rogor0.05% (Dimethoate) and observations were recorded.

Observations

a. *Ex vitro* survival percent

The number of regenerants that survived hardening were counted and expressed as percentage in each treatment

b. Reaction to TLCV disease

The reaction of somaclones and parental lines to TLCV disease was scored in the scale of 0 to 4 (Table 2)

c. Biometric characters

i. Plant height (cm)

The plant height at 60 days after planting was recorded for each somaclone and the parental lines

ii. Plant spread (cm)

The plant spread at 60 days after planting was recorded for each somaclone and the parental lines

iii. Primary branches / plant

The number of primary branches in each regenerant and the parental lines were counted.

iv. Number of fruits / plant

The total number of fruits in each plant was recorded.

v. Yield / plant (g)

The fruit yield in each plant was recorded separately.

d. Reaction to bacterial wilt

The response of each regenerant and the parental line to bacterial wilt disease was recorded.

a. Incidence of other diseases

The severity of leaf spot disease observed was scored using 0 to 5 scale (Table 3)

Table 2. Scoring of TLCV disease in tomato

Disease grade	Symptoms	Category
0	Symptom absent	Resistant
1	Very mild curling (upto 25 % leaves)	Moderately resistant
2	Curling and puckering of 26 – 50 % leaves	Moderately susceptible
3	Curling and puckering of 51 – 75 % leaves	Susceptible
4	Severe curling and puckering of > 75 % leaves	Highly susceptible

Table 3. Scoring of leaf spot disease in tomato

Disease grade	Per cent area infected
0	No infection
1	<10% of leaf area infected
2	<10-25% of leaf area infected
3	<25-50% of leaf area infected
4	<50-75% of leaf area infected
5	>75% of leaf area infected

Results

4. RESULTS

The results generated from the study on the "Screening of tomato somaclones for resistance to tomato leaf curl virus (TLCV)" are presented in this chapter.

4.1 CALLUS INDUCTION FROM DIFFERENT EXPLANTS

4.1.1 Leaf segments

Callus induction in leaf segments of tomato var. 'Sakthi' and *L. pimpinellifolium* as influenced by various levels of salt concentration (Full MS and ½ MS) and growth regulators singly and in combinations are presented (Table 4, Table 5 and Table 6) and (Plate.3). Callus formation and growth varied considerably at different combinations of auxins (NAA or 2,4– D) and cytokinins (BA or Kinetin) in both the species.(Plate.4)

4.1.1.1 Effect of auxins on callus induction and growth of leaf segments

There was no callus induction in tomato variety 'Sakthi' and *L. pimpinellifolium* when the leaf segments were cultured in MS medium and ½ MS medium without growth regulators. NAA alone at different concentrations (1, 2, 3 mg l^{-1}) induced rooting directly from the cultured leaf segments in both the basal media. But in *L. pimpinellifolium,* full MS with NAA at 3 mg l^{-1} gave average callus growth and other combinations showed the same results as of Sakthi. The leaf segments of both the species did not induce callus in presence of 2,4- D in both the basal media tried.

4.1.1.2 Effect of cytokinin on callus induction and growth of leaf segments

BA and Kinetin alone at different concentrations showed no response to callusing of leaf segments in both the basal media.

4.1.1.3 Effect of combinations of auxins and cytokinins on callus induction and growth of leaf segments

4.1.1.3.1 Effect of NAA and BA

The combination of MS + NAA (2 mg l^{-1}) + BA (1 mg l^{-1}), NAA (2 mg l^{-1}) + BA (2 mg l^{-1}) and NAA (2 mg l^{-1}) + BA (3 mg l^{-1}) recorded excellent callus growth in both the species (Table 4.). The callusing of tomato variety 'Sakthi' was poor with other combinations of growth regulators in both the media. In *L. pimpinellifolium,* MS + NAA (2 mg l^{-1}) + BA (1 mg l^{-1}), NAA (2 mg l^{-1}) + BA (2 mg l^{-1}) and NAA (2 mg l^{-1}) + BA (3 mg l^{-1}) gave excellent callus growth and development. In both the species callus induction started 18 to 28 days after inoculation (Table 4). The colour of calli obtained from the combination of NAA + BA was light greenish white and the texture was compact.

4.1.1.3.2 Effect of NAA and Kinetin

The callusing in 'Sakthi' and *L. pimpinellifolium* was poor when NAA (1 mg l^{-1}) + Kinetin (1 mg l^{-1}) and NAA (1 mg l^{-1}) + Kinetin (2 mg l^{-1}) was used in both the basal media. The colour of the calli was greenish white. There was no callusing when other concentrations of NAA and Kinetin were used.

4.1.1.3.3 Effect of 2,4 – D and BA

Effect of 2,4 – D and BA on callus induction from leaf segments of both species were observed. In case of leaf segments MS + 2,4 – D (2 mg l^{-1}) + BA (1 mg l^{-1}) and 2,4 – D (2 mg l^{-1}) + BA (2 mg l^{-1}) showed excellent callusing in all the test tubes of both the species. Profuse callusing was observed in various other combinations such as MS + 2, 4 – D (1.5 mg l^{-1}) + BA (1 mg l^{-1}), MS + 2, 4 – D (2 mg l^{-1}) + BA (3 mg l^{-1}) for 'Sakthi'. In *L.. pimpinellifollium* MS + 2,4 – D (1.5 mg l^{-1}) + BA (2, 3, 4 mg l^{-1}), MS + 2,4 – D (2 mg l^{-1}) + BA (3 mg l^{-1}) induced profuse callusing (Table 5). In 'Sakthi' callus induction started 18 to

Table 4 Effect of NAA and BA on callus induction and growth of leaf segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + NAA + BA (mgl^{-1})					MS + NAA+ BA (mgl^{-1})				
1+1	-	-	-	-	1+1	-	-	-	-
1+2	22	70.00	1	70.00	1+2	25	70.00	1	70.00
1+3	-	-	-	-	1+3	24	52.38	1	52.38
1+4	-	-	-	-	1+4	-	-	-	-
1+5	24		1	70.00	1+5	-	-	-	-
½ MS+NAA +BA (mgl^{-1})					½ MS+NAA +BA (mgl^{-1})				
1+1	-	-	-	-	1+1	28	50	1	50
1+2	28	50.00	1	50.00	1+2	18	40	1	40
1+3	28	40.00	1	40.00	1+3	-	-	-	-
1+4	-	-	-	-	1+4	-	-	-	-
1+5	-	-	-	-	1+5	-	-	-	-
MS+NAA+ BA (mgl^{-1})					MS+NAA+ BA (mgl^{-1})				
2+1	18	90.00	4	360.00	2+1	18	85.71	4	342.84
2+2	18	80.00	4	320.00	2+2	18	90.48	4	361.90
2+3	18	80.00	4	320.00	2+3	18	90.00	4	360.00
2+4	-	-	-	-	2+4	21	47.60	2	95.24
2+5	-	-	-	-	2+5	24	57.14	2	114.29
½ MS+NAA +BA (mgl^{-1})					½ MS+NAA + BA (mgl^{-1})				
2+1	22	40.00	1	40.00	2+1	19	50.00.	2	100.00
2+2	-	-	-	-	2+2	21	52.38	1	52.38
2+3	-	-	-	-	2+3	26	57.14	2	114.29
2+4	22	90.00	1	90.00	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-

Table 5 Effect of 2,4-D and BA on callus induction and growth of leaf segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate (G) (Scale of 0,1,2,3,4)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + 2,4-D + BA (mgl^{-1})					MS +2,4-D + BA (mgl^{-1})				
1.5+1	18	70.00	3	210.00	1.5+1	12	76.19	2	152.38
1.5+2	18	60.00	2	110.00	1.5+2	10	85.71	3	257.14
1.5+3	18	50.00	2	100.00	1.5+3	11	90.47	3	271.42
1.5+4	-	-	-	-	1.5+4	10	57.14	3	171.42
1.5+5	-	-	-	-	1.5+5	12	66.66	2	133.32
½ MS+2,4-D +BA (mgl^{-1})					½ MS+2,4D +BA (mgl^{-1})				
1.5+1	-	-	-	-	1.5+1	11	57.14	1	57.14
1.5+2	-	-	-	-	1.5+2	12	66.66	1	66.66
1.5+3	-	-	-	-	1.5+3	10	61.90	1	61.90
1.5+4	-	-	-	-	1.5+4	13	80.00	1	80.00
1.5+5	-	-	-	-	1.5+5	14	76.19	1	76.19
MS+2,4-D +BA (mgl^{-1})					MS+2,4-D +BA (mgl^{-1})				
2+1	19	80.00	4	320.00	2+1	11	76.19	4	304.76
2+2	19	70.00	4	280.00	2+2	12	85.71	4	342.85
2+3	19	60.00	3	180.00	2+3	11	57.14	3	171.42
2+4	-	-	-	-	2+4	12	50.00	2	100.00
2+5	-	-	-	-	2+5	13	52.38	1	52.38
½ MS+2,4-D +BA (mgl^{-1})					½ MS+2,4-D +BA (mgl^{-1})				
2+1	-	-	-	-	2+1	16	57.14	2	114.28
2+2	-	-	-	-	2+2	14	42.85	2	85.71
2+3	-	-	-	-	2+3	18	85.71	1	85.71
2+4	-	-	-	-	2+4	19	57.14	1	57.14
2+5	-	-	-	-	2+5	21	66.66	1	66.66

Table 6 Effect of 2,4-D and Kinetin on callus induction and growth of leaf segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of ,1,2,3,4.)	Callus index (PXG)
MS +2,4-D + Kinetin (mgl^{-1})					MS + 2,4-D + Kinetin (mgl^{-1})				
0.5+1	11	70.00	2	140.00	0.5+1	11	70.00	2	140.00
0.5+2	11	90.00	2	180.00	0.5+2	10	80.00	2	160.00
s0.5+3	11	90.00	2	180.00	0.5+3	12	57.14	2	114.20
0.5+4	14	60.00	2	120.00	0.5+4	14	61.90	2	123.80
0.5+5	14	60.00	2	120.00	0.5+5	13	42.85	2	85.71
½ MS+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D + Kinetin (mgl^{-1})				
0.5+1	-	-	-	-	0.5+1	14	38.09	1	38.09
0.5+2	-	-	-	-	0.5+2	16	33.33	2	66.66
0.5+3	-	-	-	-	0.5+3	18	30.00	1	30.00
0.5+4	-	-	-	-	0.5+4	19	40.00	1	40.00
0.5+5	-	-	-	-	0.5+5	20	42.85	1	42.85
MS+2,4-D + Kinetin (mgl^{-1})					MS+2,4-D + Kinetin (mgl^{-1})				
1+1	-	-	-	-	1+1	11	76.19	2	152.38
1+2	-	-	-	-	1+2	12	57.14	2	114.20
1+3	-	-	-	-	1+3	16	61.90	1	61.90
1+4	-	-	-	-	1+4	14	66.66	1	66.66
1+5	-	-	-	-	1+5	13	95.23	2	190.47
½ MS+2,4-D + Kinetin (mgl^{-1})					½ MS+2,4-D + Kinetin (mgl^{-1})				
1+1	-	-	-	-	1+1	14	38.09	2	76.19
1+2	-	-	-	-	1+2	16	42.85	2	85.71
1+3	-	-	-	-	1+3	19	52.38	1	52.38
1+4	-	-	-	-	1+4	21	70	1	70
1+5	-	-	-	-	1+5	14	60	1	60

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + 2,4-D + Kinetin (mgl^{-1})					MS + 2,4-D + Kinetin (mgl^{-1})				
1.5+1	16	100.00	2	200.00	1.5+1	16	57.14	2	114.28
1.5+2	16	100.00	2	200.00	1.5+2	17	52.38	2	104.76
1.5+3	14	80.00	1	80.00	1.5+3	14	42.85	1	42.85
1.5+4	14	70.00	1	70.00	1.5+4	18	74.19	1	74.19
1.5+5	19	90.00	1	90.00	1.5+5	20	66.66	1	66.66
½ M+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D +Kinetin (mgl^{-1})				
1.5+1	13	80.00	1	80.00	1.5+1	13	19.04	1	19.04
1.5+2	-	-	-	-	1.5+2	14	19.04	2	38.08
1.5+3	-	-	-	-	1.5+3	15	27.27	1	27.27
1.5+4	-	-	-	-	1.5+4	-	-	-	-
1.5+5	-	-	-	-	1.5+5	-	-	-	-
MS+2,4-D + Kinetin (mgl^{-1})					MS+2,4-D + Kinetin (mgl^{-1})				
2+1	12	60.00	2	120.00	2+1	12	80.00	2	160.00
2+2	12	80.00	2	160.00	2+2	13	50.00	2	100.00
2+3	25	80.00	1	80.00	2+3	22	38.09	1	38.09
2+4	24	80.00	1	80.00	2+4	24	66.66	1	66.66
2+5	14	80.00	2	160.00	2+5	14	70.00	2	140.00
½ MS+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D +Kinetin (mgl^{-1})				
2+1	-	-	-	-	2+1	-	-	-	-
2+2	-	-	-	-	2+2	-	-	-	-
2+3	-	-	-	-	2+3	-	-	-	-
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-

19 days after inoculation whereas in *L. pimpinellifolium* it was recorded 11 to 19 days after inoculation. The colour of calli was creamy white and texture was compact.

4.1.1.3.4 Effect of 2,4 – D and Kinetin

The combinations of 2,4- D and Kinetin showed average callusing in 'Sakthi' and *L. pimpinellifolium.* However, percentage callusing and callus index were higher in MS medium supplemented with 2, 4 – D (1.5 mg l^{-1}) + Kinetin (1 mg l^{-1}) and 2, 4 – D(1.5 mg l^{-1}) + Kinetin (2 mg l^{-1}), (Table 6). The callus induction started 11 to 25 days after inoculation in both the species. The calli was light greenish in colour.

4.1.2 Internodal segments

4.1.2.1Effect of auxins on callus induction and growth of internodal segments

4.1.2.1.1 Effect of NAA

The different levels of NAA in MS medium induced average callusing in both the species. The callusing started 13 days after inoculation. The callusing was poor when various levels of NAA were used in ½ MS medium. The callus was initiated 20 to 22 days after inoculation (Table 7).

4.1.2.1.2 *Effect of 2,4 – D*

In both the species, average callusing response was observed in MS medium supplemented with various levels of 2,4 - D. Callusing was observed 14 days after inoculation. The callusing was poor in ½ MS medium supplemented with various concentrations of 2,4 - D. The time required for callus initiation ranged from 18 to 20 days (Table 7).

a. Leaf segment

b. Internodal segment

c. Nodal segment

Plate 3. Response of explant to callusing in 'Sakthi' and *L.pimpinellifolium*

4.1.2.2 *Effect of cytokinins on callus induction and growth of internodal segments*

4.1.2.2.1 *Effect of BA*

Profuse callusing was observed in combination of MS + BA (2 mg l^{-1}) in Sakthi and MS + BA (1, 3 mg l^{-1}) in *L. pimpinellifolium*. In Sakthi callus induction started 7 days after inoculation whereas in *L. pimpinellifolium* it ranged from 9 to 17 days after inoculation. Other combinations of MS and ½ MS showed negative response (Table 7).

4.1.2.2 .2 Effect of Kinetin

Kinetin had no significant role in callus induction and growth in 'Sakthi' and *L. pimpinellifolium* (Table 7).

4.1.2.2 Effect of auxins and cytokinins in callus induction and growth of Internodal segments (Plate. 3 and 4)

4.1.2.2.2 *Effect of NAA and BA*

The percentage callusing was highest and growth rate was profuse in MS medium supplemented with NAA (1 mg l^{-1}) and BA (3, 4 and 5 mg l^{-1}) in both the species. In all other combinations average callusing was recorded. In 'Sakthi' callus induction started 8 to 14 days after inoculation whereas in ½ MS medium 10 to 18 days was required for callus induction. In case of *L. pimpinellifolium*, callus induction started 10 to 21 days after inoculation in both the media (Table 8).

4.1.2.2.3 Effect of NAA and Kinetin

The combination of MS + NAA (1 mg l^{-1}) + Kinetin (1, 2, 3 and 4 mg l^{-1}) and NAA (3 mg l^{-1}) + Kinetin (1, 2, 3 and 4 mg l^{-1}) induced excellent calli in 'Sakthi' whereas in *L.*

pimpinellifolium MS + NAA (1 mg l^{-1}) + Kinetin (1and 2 mg l^{-1}), NAA (3 mg l^{-1}) + Kinetin (4 mg l^{-1}) gave excellent calli within 10 to 14 days after inoculation. In 'Sakthi', MS + NAA (3 mg l^{-1}) + Kinetin (3 and 5 mg l^{-1}) gave profuse callusing. In *L. pimpinellifolium*, MS + NAA (1mg l^{-1}) + Kinetin (3 and 4 mg l^{-1}), MS + NAA (3 mg l^{-1}) + Kinetin (1, 2, 3 and 5 mg l^{-1}) showed profuse callusing. The half strength MS media supplemented with different combinations of growth regulators induced average or poor callusing in both the species. (Table 9)

4.1.2.2.4 Effect of 2, 4 – D and BA

The basal medium containing 2,4 – D (2 mg l^{-1}) + BA (1 and 2 mg l^{-1}) induced excellent callus in 'Sakthi'. Profuse callusing was observed when 2, 4 – D (1 mg l^{-1}) + BA (1, 2, 3, 4 and 5 mg l^{-1}) and 2, 4 – D (2 mg l^{-1}) + BA (3 mg l^{-1}) was added in the medium. In case of *L. pimpinellifolium*, MS + 2, 4 – D (1 mg l^{-1}) + BA (1, 2, 3, 4 and 5 mg l^{-1}) and MS + 2, 4 – D (2 mg l^{-1}) + BA (1 and 2 mg l^{-1}) recorded profuse callusing. The number of days for callus induction ranged from 7 to 13 days in 'Sakthi' and 9 to 21 days in *L. pimpinellifolium* (Table 10). The colour of calli was creamy white and the texture was friable.

4.1.2.2.5 Effect of 2, 4 –D and Kinetin

In 'Sakthi, MS medium with 2, 4 –D (0.5 mg l^{-1}) + Kinetin (1, 2, 3 and 4 mg l^{-1}), 2, 4 – D (1 mg l^{-1}) + Kinetin (1 and 2 mg l^{-1}), 2, 4 – D (1.5 mg l^{-1}) + Kinetin (1 mg l^{-1}) and 2, 4 – D (2 mg l^{-1}) + Kinetin (1, 2 and 3 mg l^{-1}) produced profuse callusing. The percentage callusing ranged from 90 to 100 %. In *L. pimpinellifolium* MS medium containing 2, 4 – D (0.5 mg l^{-1}) + Kinetin (1, 2, 3 and 4 mg l^{-1}), 2, 4 – D (1 mg l^{-1}) + Kinetin (3 and 4 mg l^{-1}), 2, 4 – D (1.5 mg l^{-1}) + Kinetin (1 mg l^{-1}) and 2, 4 – D (2 mg l^{-1}) + Kinetin (1, 2 and 3 mg l^{-1}) recorded profuse callusing. In 'Sakthi' number of days for callus induction ranged from 7 to 17 and in *L. pimpinellifolium*, it varied from 8 to 17 (Table 11). The callusing index of 'Sakthi' varied from 80 to 300 in basal MS medium supplemented with different levels of

Table 7 Effect of Auxins / Cytokinins on callus induction and growth of internodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + NAA (mgl^{-1})					MS + NAA (mgl^{-1})				
0	-	-	-	-	0	-	-	-	-
1	13	50.00	2	100.00	1	13	50.00	2	100.00
2	13	60.00	2	120.00	2	13	60.00	2	120.00
3	13	70.00	2	140.00	3	13	70.00	2	140.00
½ MS+NAA (mgl^{-1})					½ MS+NAA (mgl^{-1})				
0	-	-	-	-	0	-	-	-	-
1	22	50.00	1	50.00	1	22	50.00	1	50.00
2	20	60.00	1	60.00	2	20	60.00	1	60.00
3	20	40.00	1	40.00	3	20	40.00	1	40.00
MS+2,4-D (mgl^{-1})					MS+2,4-D (mgl^{-1})				
0.5	14	70.00	2	140.00	0.5	14	42.85	2	85.70
1.0	14	70.00	2	140.00	1.0	14	47.62	2	95.24
1.5	14	70.00	2	140.00	1.5	14	57.14	2	114.29
2.0	14	80.00	2	160.00	2.0	14	66.66	2	133.33
½ MS+2,4-D (mgl^{-1})					½ MS+2,4-D (mgl^{-1})				
0.5	18	80.00	1	80.00	0.5	19	80.00	1	80.00
1.0	18	70.00	1	70.00	1.0	20	70.00	1	70.00
1.5	18	70.00	1	70.00	1.5	19	70.00	1	70.00
2.0	18	70.00	1	70.00	2.0	18	70.00	1	70.00
MS + BA (mgl^{-1})					MS + BA (mgl^{-1})				
1	-	-	-	-	1	17	61.90	3	185.70
2	7	70.00	3	210.00	2	-	-	-	-
3	-	-	-	-	3	9	70.00	3	210..00
½ MS + BA	nil	nil	nil	nil	½ MS + BA	Nil	nil	nil	nil
MS + Kinetin	nil	nil	nil	nil	MS + Kinetin	Nil	nil	nil	nil
½ MS + Kinetin	nil	nil	nil	nil	½ MS +Kinetin	Nil	nil	nil	nil

Table 8 Effect of NAA and BA on callus induction and growth of internodal segments.

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + NAA + BA (mgl^{-1})					MS + NAA + BA (mgl^{-1})				
1+1	14	90.00	2	180.00	1+1	14	85.71	2	171.42
1+2	14	90.00	2	180.00	1+2	15	80.95	2	161.90
1+3	8	100.00	3	300.00	1+3	18	100.00	3	300.00
1+4	8	100.00	3	300.00	1+4	19	100.00	3	300.00
1+5	8	100.00	3	300.00	1+5	10	90.48	3	271.42
½ MS+NAA+ BA (mgl^{-1})					½ MS+NAA+ BA (mgl^{-1})				
1+1	18	60.00	1	60.00	1+1	21	60.00	1	60.00
1+2	18	60.00	1	60.00	1+2	21	55.00	1	55.00
1+3	18	80.00	1	80.00	1+3	21	70.00	1	70.00
1+4	18	60.00	1	60.00	1+4	21	60.00	1	60.00
1+5	18	80.00	1	80.00	1+5	21	70.00	1	70.00
MS+NAA+ BA (mgl^{-1})					MS+NAA+ BA(mgl^{-1})				
2+1	16	80.00	2	160.00	2+1	10	80.00	2	160.00
2+2	14	100.00	2	200.00	2+2	14	100.00	2	200.00
2+3	14	100.00	2	200.00	2+3	15	100.00	2	200.00
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-
½ MS+NAA+ BA (mgl^{-1})					½ MS+NAA+ BA (mgl^{-1})				
2+1	-	-	-	-	2+1	-	-	-	-
2+2	-	-	-	-	2+2	-	-	-	-
2+3	-	-	-	-	2+3	-	-	-	-
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + NAA + BA (mgl^{-1})					MS + NAA + BA (mgl^{-1})				
3+1	8	70.00	2	140.00	3+1	12	70.00	2	140.00
3+2	-	-	-	-	3+2	13	65.00	2	130.00
3+3	8	100.00	2	200.00	3+3	12	66.66	2	133.33
3+4	8	100.00	2	200.00	3+4	14	100.00	2	200.00
3+5	-	-	-	-	3+5	18	26.66	2	53.33
½ MS+NAA +BA (mgl^{-1})					½ MS+NAA +BA (mgl^{-1})				
3+1	-	-	-	-	3+1	-	-	-	-
3+2	-	-	-	-	3+2	14	55.00	1	55.00
3+3	10	100.00	1	100.00	3+3	14	50.00	1	50.00
3+4	10	80.00	1	80.00	3+4	14	40.00	1	40.00
3+5	10	90.00	1	90.00	3+5	14	33.33	1	33.33

Table 9 Effect of NAA and Kinetin on callus induction and growth of internodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS+NAA+ Kinetin (mgl^{-1})					MS+NAA+ Kinetin (mgl^{-1})				
1+1	14	80.00	4	320.00	1+1	14	60.00	4	320.00
1+2	14	70.00	4	280.00	1+2	14	65.00	4	260.00
1+3	14	80.00	4	320.00	1+3	15	68.75	3	206.25
1+4	14	90.00	4	360.00	1+4	13	77.77	3	233.31
1+5	-	-	-	-	1+5	-	-	-	-
½ MS+NAA+ Kinetin (mgl^{-1})					½ MS+NAA+ Kinetin (mgl^{-1})				
1+1	13	70.00	2	140.00	1+1	9	60.00	2	120.00
1+2	13	50.00	2	100.00	1+2	10	70.00	2	140.00
1+3	13	80.00	2	160.00	1+3	12	80.00	1	80.00
1+4	13	80.00	1	80.00	1+4	14	66.66	1	66.66
1+5	13	50.00	1	50.00	1+5	16	57.89	1	57.89
MS + NAA+ Kinetin (mgl^{-1})					MS +N AA+ Kinetin (mgl^{-1})				
2+1	10	50.00	1	50.00	2+1	10	57.14	1	57.14
2+2	10	70.00	1	70.00	2+2	11	70.00	1	70.00
2+3	10	60.00	1	60.00	2+3	10	71.42	1	71.42
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
½ MS+NAA+ Kinetin (mgl^{-1})					½ MS+NAA+ Kinetin (mgl^{-1})				
2+1	10	80.00	1	80.00	2+1	11	60.00	1	60.00
2+2	10	30.00	1	30.00	2+2	14	66.66	1	66.66
2+3	-	-	-	-	2+3	10	33.33	1	33.33
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-
MS+NAA+ Kinetin (mgl^{-1})					MS+NAA+ Kinetin (mgl^{-1})				
3+1	12	70.00	4	280.00	3+1	12	76.19	3	226.57
3+2	12	70.00	4	280.00	3+2	13	47.61	3	142.85
3+3	12	60.00	3	180.00	3+3	12	60.00	3	180.00
3+4	12	60.00	4	240.00	3+4	14	61.90	4	247.61
3+5	12	90.00	3	270.00	3+5	11	90.00	3	270.00
½ MS+NAA + Kinetin (mgl^{-1})					½ MS+NAA + Kinetin (mgl^{-1})				
3+1	10	90.00	3	270.00	3+1	14	60.00	2	120.00
3+2	10	80.00	3	240.00	3+2	13	60.00	2	120.00
3+3	10	80.00	1	80.00	3+3	10	52.38	1	52.38
3+4	10	80.00	1	80.00	3+4	11	60.66	1	66.66
3+5	10	70.00	1	70.00	3+5	14	40.00	1	40.00

Table 10 Effect of 2,4-D and BA on callus induction and growth of internodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate (G) (Scale of 0,1,2,3,4)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + 2,4-D + BA (mgl^{-1})					MS +2,4-D + BA (mgl^{-1})				
0.5+1	10	90.00	3	270.00	0.5+1	11	85.71	3	257.14
0.5+2	10	90.00	3	270.00	0.5+2	12	76.19	3	228.57
0.5+3	10	80.00	3	240.00	0.5+3	13	60.00	2	120.00
0.5+4	10	60.00	1	60.00	0.5+4	12	85.71	1	85.71
0.5+5	10	90.00	1	90.00	0.5+5	10	66.66	1	66.66
½ MS+2,4-D +BA (mgl^{-1})					½ MS+2,4D +BA (mgl^{-1})				
0.5+1	13	80.00	1	80.00	0.5+1	14	76.19	1	76.19
0.5+2	13	80.00	1	80.00	0.5+2	15	80.95	1	80.95
0.5+3	13	70.00	1	70.00	0.5+3	13	57.14	1	57.14
0.5+4	13	90.00	1	90.00	0.5+4	16	70.00	1	70.00
0.5+5	13	70.00	1	70.00	0.5+5	18	60.00	1	60.00
MS+2,4-D +BA (mgl^{-1})					MS+2,4-D + BA (mgl^{-1})				
1+1	13	100.00	3	300.00	1+1	18	94.73	3	284.21
1+2	13	70.00	3	210.00	1+2	18	76.19	3	228.57
1+3	13	60.00	3	180.00	1+3	14	95.23	3	313.50
1+4	13	70.00	3	210.00	1+4	16	60.00	3	180.00
1+5	13	90.00	3	270.00	1+5	20	70.00	3	210.00
½ MS+2,4-D +BA (mgl^{-1})					½ MS+2,4-D +BA (mgl^{-1})				
1+1	7	80.00	1	80.00	1+1	12	60.00	1	60.00
1+2	7	70.00	1	70.00	1+2	13	70.00	1	70.00
1+3	-	-	-	-	1+3	14	76.19	1	76.19
1+4	-	-	-	-	1+4	11	80.95	1	80.95
1+5	-	-	-	-	1+5	14	70.00	1	70.00

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + 2,4-D + BA (mgl^{-1})					MS + 2,4-D + BA (mgl^{-1})				
1.5+1	10	70	2	140	1.5+1	11	57.14	2	114.28
1.5+2	10	100	2	200	1.5+2	12	66.66	2	133.22
1.5+3	10	60	2	120	1.5+3	14	76.19	1	76.19
1.5+4	10	70	1	70	1.5+4	16	66.66	1	66.66
1.5+5	10	60	1	60	1.5+5	12	50.00	1	50.00
½ MS+2,4-D +BA (mgl^{-1})					½ MS+2,4-D +BA (mgl^{-1})				
1.5+1	10	80	1	80	1.5+1	16	33.33	1	33.33
1.5+2	10	90	1	90	1.5+2	12	38.09	1	38.09
1.5+3	10	100	1	100	1.5+3	13	57.14	1	57.14
1.5+4	10	100	1	100	1.5+4	10	50.00	1	50.00
1.5+5	10	90	1	90	1.5+5	14	52.63	1	52.63
MS+2,4-D +BA (mgl^{-1})					MS+2,4-D +BA (mgl^{-1})				
2+1	8	80	4	320	2+1	9	47.36	3	142.10
2+2	8	70	4	280	2+2	10	76.19	3	228.57
2+3	8	80	3	240	2+3	12	57.14	2	114.28
2+4	8	60	2	120	2+4	14	57.14	1	57.14
2+5	8	90	1	90	2+5	11	55.00	1	55.00
½ MS+2,4-D + BA (mgl^{-1})					½ MS+2,4-D +BA (mgl^{-1})				
2+1	7	100	2	200	2+1	21	57.14	2	114.28
2+2	7	70	2	140	2+2	14	70.00	2	140.00
2+3	7	70	2	140	2+3	13	80.00	1	80.00
2+4	7	70	2	140	2+4	14	66.66	1	66.66
2+5	7	60	1	60	2+5	15	63.15	1	63.15

Table 11 Effect of 2,4-D and Kinetin on callus induction and growth of internodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS +2,4-D + Kinetin (mgl^{-1})					MS + 2,4-D + Kinetin (mgl^{-1})				
0.5+1	7	100	3	300	0.5+1	9	38.09	3	114.28
0.5+2	7	100	3	300	0.5+2	11	90.00	3	270.00
0.5+3	7	100	3	300	0.5+3	12	68.75	3	206.25
0.5+4	7	100	3	300	0.5+4	14	57.14	3	171.42
0.5+5	7	60	1	60	0.5+5	13	76.19	2	152.38
½ MS+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D +Kinetin (mgl^{-1})				
0.5+1	10	100	2	200	0.5+1	11	52.38	2	104.76
0.5+2	10	60	2	60	0.5+2	12	57.14	2	114.28
0.5+3	10	60	1	60	0.5+3	13	66.66	2	133.33
0.5+4	11	80	1	80	0.5+4	14	57.14	1	57.14
0.5+5	11	70	1	70	0.5+5	10	66.66	1	66.66
MS+2,4-D +Kinetin (mgl^{-1})					MS+2,4-D +Kinetin (mgl^{-1})				
1+1	10	90	3	270	1+1	11	100.00	1	100.00
1+2	10	90	3	270	1+2	12	87.50	1	87.50
1+3	10	100	1	100	1+3	11	52.48	3	157.14
1+4	10	100	1	100	1+4	12	47.82	3	143.47
1+5	10	80	2	160	1+5	10	75.00	2	150.00
½ MS+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D +Kinetin (mgl^{-1})				
1+1	12	80	2	160	1+1	12	61.90	1	61.90
1+2	12	80	2	160	1+2	12	42.85	2	85.71
1+3	10	70	1	70	1+3	12	55.55	3	166.66
1+4	9	70	1	70	1+4	10	61.11	2	122.22
1+5	11	60	1	60	1+5	11	78.57	1	78.57

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + 2,4-D + Kinetin (mgl^{-1})					MS + 2,4-D + Kinetin (mgl^{-1})				
1.5+1	8	100	3	300	1.5+1	8	45.45	3	136.36
1.5+2	7	80	1	80	1.5+2	9	47.82	1	47.82
1.5+3	9	80	1	80	1.5+3	11	55.00	2	110.00
1.5+4	8	60	2	120	1.5+4	12	62.50	1	62.50
1.5+5	8	60	2	120	1.5+5	13	75.00	2	150.00
½ M+2,4-D + Kinetin (mgl^{-1})					½ MS+2,4-D + Kinetin (mgl^{-1})				
1.5+1	7	100	1	100	1.5+1	9	61.90	1	61.90
1.5+2	7	100	1	100	1.5+2	11	57.14	1	57.14
1.5+3	9	60	1	60	1.5+3	14	52.38	2	104.76
1.5+4	9	60	1	60	1.5+4	9	47.61	1	47.61
1.5+5	9	90	1	90	1.5+5	8	50.00	1	50.00
MS+2,4-D + Kinetin (mgl^{-1})					MS+c2,4-D + Kinetin (mgl^{-1})				
2+1	9	100	3	300	2+1	9	57.14	3	171.42
2+2	9	100	3	300	2+2	10	61.90	3	185.70
2+3	9	90	3	270	2+3	11	40.00	3	120.00
2+4	-	-	-	-	2+4	14	50.00	2	100.00
2+5	-	-	-	-	2+5	15	47.36	1	47.36
½ MS+2,4-D + Kinetin (mgl^{-1})					½ MS+2,4-D + Kinetin (mgl^{-1})				
2+1	17	90	1	90	2+1	17	28.57	1	28.57
2+2	17	90	1	90	2+2	13	38.09	1	38.09
2+3	13	80	1	80	2+3	14	52.38	1	52.38
2+4	-	-	-	-	2+4	13	76.19	2	152.38
2+5	-	-	-	-	2+5	16	85.71	1	85.71

2,4- D and Kinetin. The callus index (CI) of *L. pimpinellifolium* ranged from 62.50 to 270 in MS medium (Table 11). The colour of callus was light greenish and texture was friable.

4.1.3 Nodal segments

4.1.3.1 *Effect of auxins on callus induction and growth of nodal segments*

4.1.3.1.1 Effect of NAA

The tomato variety 'Sakthi' showed average callusing in MS medium containing NAA (1 and3 mg l^{-1}). MS medium with NAA initiated callusing 11 days after inoculation in both the species whereas in ½ MS it was 18 – 20 days after inoculation (Table 12).

4.1.3.1.2Effect of 2, 4 – D

The MS medium with different levels of 2, 4 – D gave average callusing in both the species after 10 to 13 days of inoculation. The callusing was poor in both the species when ½ MS medium with different concentrations of 2,4- D was used. The callusing initiated 18 to 20 days after inoculation (Table 12).

4.1.3.2 Effect of cytokinin on callus induction and growth of nodal segments

4.1.3.2.1 *Effect of BA*

In both the species MS + BA (1 mg l^{-1}) recorded profuse callusing. In 'Sakthi' callusing observed after 7 days of inoculation, which was 17 days after inoculation in *L. pimpinellifolium*. In both the species ½ MS medium was not suitable for callus induction (Table 12).

4.1.3.2.2 Effect of Kinetin

Kinetin had no significant role in nodal callusing (Table 12).

4.1.3.3 Effect of auxins and cytokinins on callus growth and development of nodal segments (Plate. 3 and 4)

4.1.3.3.1 Effect of NAA and BA

Among all the combinations MS + NAA (1 mg l^{-1}) + BA (4, 5 mg l^{-1}) recorded excellent callusing in both the species. A highly significant profuse callusing was recorded for MS + NAA (1 mg l^{-1}) + BA (3 mg l^{-1}), ½ MS + NAA (1 mg l^{-1}) +BA (4 and 5 mg l^{-1}), MS + NAA (2 mg l^{-1}) + BA (2 and 4 mg l^{-1}) and MS + NAA (3 mg l^{-1}) + BA (5 mg l^{-1}) in 'Sakthi' whereas in *L. pimpinellifolium* it was for MS + NAA (1 mg l^{-1}) + BA (3 mg l^{-1}) and MS + NAA (2 mg l^{-1}) + BA (2 and 3 mg l^{-1}).All other combinations of MS and ½ MS recorded average and poor callusing in both the species. The number of days for callus induction ranged from 7 to 26 days in both the species (Table 13). The callusing was 100 % and CI was 400 for both the species when MS medium with NAA (1 mg l^{-1}) + BA (4 and 5 mg l^{-1}) was used. When callusing was profuse, the percentage ranged from 90 to 100 and CI ranged from 270 to 300 in 'Sakthi'. In *L. pimpinellifolium* the percentage callusing ranged from 85.71 to 100 and CI from 171.42 to 300, when the calli produced was profuse (Table 13).

4.1.3.3.2 Effect of NAA and Kinetin

In 'Sakthi' and *L. pimpinellifolium* effective excellent calli were produced when MS medium supplemented with NAA (1 mg l^{-1}) + Kinetin(1 and 2 mg l^{-1}) and NAA (3 mg l^{-1}) + Kinetin (1 and 2 mg l^{-1}) were used. Profuse callusing was observed in medium containing MS + NAA (1 mg l^{-1}) +Kinetin (3 mg l^{-1}), NAA (3 mg l^{-1}) + Kinetin(3, 4 and 5 mg l^{-1}) and ½ MS + NAA (3 mg l^{-1}) + Kinetin(1 and 2 mg l^{-1}) in 'Sakthi'. In *L. pimpinellifolium*

MS + NAA (1 mg l^{-1}) +Kinetin (3 mg l^{-1}) and MS + NAA (3 mg l^{-1}) + Kinetin (3 mg l^{-1}) induced profuse callusing. The number of days for callus induction ranged from 10 to 18 in both the species (Table 14).

4.1.3.3.3 Effect of 2, 4 –D and BA

In 'Sakthi' basal MS medium supplemented with 2, 4 – D (0.5 mg l^{-1}) + BA (1 and 2 mg l^{-1}), 2, 4 – D (1 mg l^{-1}) + BA (1 and 2 mg l^{-1}) and 2, 4 – D (2 mg l^{-1}) + BA (1 and 2 mg l^{-1}) induced profuse callusing (Table 15). In *L. pimpinellifolium* basal medium containing 2, 4 – D (2 mg l^{-1}) + BA (1 mg l^{-1}) produced excellent callus. Profuse callusing was observed when 2, 4 – D (0.5 mg l^{-1}) + BA (1 and 2 mg l^{-1}), 2, 4 – D (1 mg l^{-1}) + BA (1 and 2 mg l^{-1}) and 2, 4 – D (2 mg l^{-1}) + BA (2 mg l^{-1}) were added to the medium. In both the species, callus initiation started 7 to 18days after inoculation (Table 15).

4.1.3.3.4 *Effect of 2, 4 – D and Kinetin*

In 'Sakthi' and *L. pimpinellifolium* early response to callusing (7 days) and excellent callusing was observed in MS + 2, 4 – D (0.5 mg l^{-1}) + Kinetin (1 and 2 mg l^{-1}). In 'Sakthi' highly significant profuse callusing was observed when MS medium containing 2, 4 – D (0.5 mg l^{-1}) + Kinetin (3 mg l^{-1}), 2, 4 – D (1 mg l^{-1}) + Kinetin (3 and 4 mg l^{-1}), 2, 4 – D (1.5 mg l^{-1}) + Kinetin (2 mg l^{-1}), 2, 4 – D (2 mg l^{-1}) + Kinetin (1 and 2 mg l^{-1}) and ½ MS + 2, 4 – D (1 mg l^{-1}) + Kinetin (5 mg l^{-1})was used. In *L. pimpinellifolium* basal medium with 2, 4 – D (0.5 mg l^{-1}) + Kinetin (3 mg l^{-1}), 2, 4 – D (1 mg l^{-1}) + Kinetin (3 mg l^{-1}) and 2, 4 – D (1.5 mg l^{-1}) + Kinetin (2 mg l^{-1}) induced profuse callusing. The number of days for callus induction ranged from 7 to 14 in both the species. (Table 16).

The callus index in 'Sakthi' varied from 70 to 360 when different concentrations of 2,4- D and kinetin was added to the basal medium. In *L. pimpinellifolium,* it varied from 33.30 to 280.

Table12 Effect of Auxins / Cytokinins on callus induction and growth of nodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + NAA (mgl^{-1})					MS + NAA (mgl^{-1})				
0	-	-	-	-	0	-	-	-	-
1	11	70	2	140	1	11	70	2	140
2	11	60	1	60	2	11	60	2	120
3	11	80	2	160	3	11	80	2	160
½ MS+NAA (mgl^{-1})					½ MS+NAA (mgl^{-1})				
0	-	-	-	-	0	-	-	-	-
1	18	40	1	40	1	-	-	-	-
2	19	70	1	70	2	20	38.09	1	38.09
3	19	80	1	80	3	20	66.66	1	66.66
MS+2,4-D (mgl^{-1})					MS+2,4-D (mgl^{-1})				
0.5	10	80	2	160	0.5	11	76.19	2	152.38
1	10	80	2	160	1	11	61.90	2	123.80
1.5	10	60	2	120	1.5	12	52.38	2	104.76
2	10	80	2	160	2	13	76.19	2	152.38
½ MS+2,4-D (mgl^{-1})					½ MS+2,4-D (mgl^{-1})				
0.5	18	80	1	80	0.5	18	40.00	1	40.00
1.0	18	60	1	60	1.0	19	42.86	1	42.86
1.5	18	60	1	60	1.5	20	55.00	1	55.00
2.0	18	80	1	80	2.0	19	80.00	1	80.00
MS + BA (mgl^{-1})					MS + BA (mgl^{-1})				
1	7	60	3	180	1	17	40.00	3	120.00
2 to 5	nil	nil	nil	nil	2 to 5	Nil	nil	nil	nil
½ MS + BA	nil	nil	nil	nil	½ MS + BA	Nil	nil	nil	nil
MS + Kinetin	nil	nil	nil	nil	MS + Kinetin	Nil	nil	nil	nil
½ MS + Kinetin	nil	nil	nil	nil	½ MS + Kinetin	Nil	nil	nil	nil

Table13 Effect of NAA and BA on callus induction and growth of nodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + NAA + BA (mgl^{-1})					MS + NAA + BA (mgl^{-1})				
1+1	-	-	-	-	1+1	-	-	-	-
1+2	-	-	-	-	1+2	-	-	-	-
1+3	14	90	3	270	1+3	14	85.71	3	171.42
1+4	7	100	4	400	1+4	14	100.00	4	400.00
1+5	7	100	4	400	1+5	13	100.00	4	400.00
½ MS+NAA +BA (mgl^{-1})					½ MS+NAA+ BA (mgl^{-1})				
1+1	20	80	1	80	1+1	20	66.66	1	66.66
1+2	20	90	1	90	1+2	20	76.19	1	76.19
1+3	20	80	2	160	1+3	20	76.19	1	76.19
1+4	20	90	3	270	1+4	20	85.71	2	171.42
1+5	20	100	3	300	1+5	20	100.00	2	200.00
MS+NAA +BA (mgl^{-1})					MS+NAA +BA (mgl^{-1})				
2+1	-	-	-	-	2+1	-	-	-	-
2+2	16	100	3	300	2+2	16	100.00	3	300.00
2+3	-	-	-	-	2+3	14	100.00	3	300.00
2+4	14	100	3	300	2+4	18	57.14	2	114.24
2+5	-	-	-	-	2+5	-	-	-	-
½ MS+NAA +BA (mgl^{-1})					½ MS+NAA + BA (mgl^{-1})				
2+1	15	70	2	140	2+1	26	60.00	1	60.00
2+2	-	-	-	-	2+2	24	20.00	1	20.00
2+3	19	60	2	120	2+3	23	23.81	1	23.81
2+4	19	90	2	180	2+4	-	-	-	-
2+5	19	60	2	120	2+5	-	-	-	-

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + NAA + BA (mgl^{-1})					MS + NAA + BA (mgl^{-1})				
3+1	9	80	2	160	3+1	11	80.00	2	160.00
3+2	9	90	2	180	3+2	12	80.95	2	161.90
3+3	9	-	-	-	3+3	11	52.38	2	104.76
3+4	9	100	2	200	3+4	14	38.10	1	38.10
3+5	9	100	3	300	3+5	13	40.00	1	40.00
½ MS+NAA +BA (mgl^{-1})					½ MS+NAA +BA (mgl^{-1})				
3+1	11	90	1	90	3+1	12	90.48	1	90.48
3+2	11	80	1	80	3+2	12	76.19	1	76.19
3+3	11	80	1	80	3+3	12	66.66	1	66.66
3+4	12	90	1	90	3+4	12	95.24	1	95.24
3+5	13	100	1	100	3+5	12	100.00	1	100.00

Table 14 Effect of NAA and Kinetin on callus induction and growth of nodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS+NAA+ Kinetin (mgl^{-1})					MS+NAA +Kinetin (mgl^{-1})				
1+1	14	90	4	360	1+1	14	60.00	4	240.00
1+2	14	60	4	240	1+2	15	55.00	4	220.00
1+3	14	100	3	300	1+3	16	90.00	3	240.00
1+4	14	80	2	160	1+4	14	60.00	2	120.00
1+5	14	100	2	200	1+5	14	95.23	1	95.23
½ MS+NAA+ Kinetin (mgl^{-1})					½ MS+NAA+ Kinetin (mgl^{-1})				
1+1	12	50	2	100	1+1	11	70.00	2	140.00
1+2	12	80	2	160	1+2	10	76.19	2	152.38
1+3	12	80	2	160	1+3	18	85.71	2	171.42
1+4	12	70	1	70	1+4	11	70.00	1	70.00
1+5	12	40	1	40	1+5	12	35.00	1	35.00
MS + NAA+ Kinetin (mgl^{-1})					MS +N AA+ Kinetin (mgl^{-1})				
2+1	11	80	1	80	2+1	11	80.00	1	80.00
2+2	11	70	1	70	2+2	12	70.00	1	70.00
2+3	11	50	1	50	2+3	12	52.38	1	52.38
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
½ MS+NAA +Kinetin (mgl^{-1})					½ MS+NAA +Kinetin (mgl^{-1})				
2+1	10	50	1	50	2+1	11	60	1	60
2+2	-	-	-	-	2+2	-	-	-	-
2+3	-	-	-	-	2+3	-	-	-	-
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-		2+5	-	-	-	-
MS+NAA+ Kinetin (mgl^{-1})					MS+NAA+ Kinetin (mgl^{-1})				
3+1	12	100	4	400	3+1	12	100.00	4	400.00
3+2	12	90	4	360	3+2	13	90.00	4	360.00
3+3	12	60	3	180	3+3	14	76.19	3	228.77
3+4	12	80	3	240	3+4	13	80.95	2	161.90
3+5	12	80	3	240	3+5	14	76.19	1	76.19
½ MS+NAA + Kinetin (mgl^{-1})					½ MS+NAA +Kinetin (mgl^{-1})				
3+1	10	90	3	270	3+1	12	70.00	2	140.00
3+2	10	70	3	210	3+2	13	57.14	2	114.28
3+3	10	80	1	80	3+3	10	90.47	1	90.47
3+4	10	80	1	80	3+4	11	25.00	1	25.00
3+5	10	70	1	70	3+5	14	66.66	1	66.66

Table 15 Effect of 2,4-D and BA on callus induction and growth of nodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate (G) (Scale of 0,1,2,3,4)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + 2,4-D + BA (mgl^{-1})					MS +2,4-D + BA (mgl^{-1})				
0.5+1	10	70	3	210	0.5+1	11	70.00	3	210.00
0.5+2	10	90	3	270	0.5+2	12	90.00	3	270.00
0.5+3	10	70	1	70	0.5+3	13	70.00	2	140.00
0.5+4	10	80	1	80	0.5+4	14	42.85	1	42.85
0.5+5	10	80	1	80	0.5+5	12	50.00	1	50.00
½ MS+2,4-D +BA (mgl^{-1})					½ MS+2,4D +BA (mgl^{-1})				
0.5+1	14	70	1	70	0.5+1	14	77.77	1	77.77
0.5+2	14	60	1	60	0.5+2	16	66.66	1	66.66
0.5+3	14	80	1	80	0.5+3	17	73.68	1	73.68
0.5+4	14	70	1	70	0.5+4	14	55.00	1	55.00
0.5+5	14	80	1	80	0.5+5	15	54.54	1	54.54
MS+2,4-D +BA (mgl^{-1})					MS+2,4-D +BA (mgl^{-1})				
1+1	17	70	3	210	1+1	11	90.00	3	270.00
1+2	17	80	3	240	1+2	14	38.09	3	114.28
1+3	17	80	1	80	1+3	16	76.19	1	76.19
1+4	17	60	1	60	1+4	13	57.14	1	57.14
1+5	17	80	1	80	1+5	18	80.00	1	80.00
½ MS+,4-D +BA (mgl^{-1})					½ MS+2,4-D+ BA (mgl^{-1})				
1+1	7	80	1	80	1+1	10	52.38	1	52.38
1+2	7	80	1	80	1+2	14	55.50	1	55.50
1+3	-	-	-	-	1+3	15	87.50	1	87.50
1+4	-	-	-	-	1+4	16	80.00	1	80.00
1+5	-	-	-	-	1+5	18	76.19	1	76.19

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + 2,4-D + BA (mgl^{-1})					MS + 2,4-D + BA (mgl^{-1})				
1.5+1	9	90	2	180	1.5+1	10	50.00	2	100.00
1.5+2	9	100	2	200	1.5+2	11	76.19	2	152.38
1.5+3	9	60	1	60	1.5+3	10	85.71	1	85.71
1.5+4	9	80	1	80	1.5+4	11	62.50	1	62.50
1.5+5	9	80	1	80	1.5+5	10	60.00	1	60.00
½ MS+2,4-D +BA (mgl^{-1})					½ MS+2,4-D +BA (mgl^{-1})				
1.5+1	10	100	1	100	1.5+1	10	66.66	1	66.66
1.5+2	10	70	1	70	1.5+2	11	57.14	1	57.14
1.5+3	10	40	1	40	1.5+3	11	33.33	1	33.33
1.5+4	10	90	1	90	1.5+4	12	57.14	1	57.14
1.5+5	10	100	1	100	1.5+5	11	66.66	1	66.66
MS+2,4-D +BA (mgl^{-1})					MS+2,4-D +BA (mgl^{-1})				
2+1	8	50	3	150	2+1	10	78.57	4	314.28
2+2	8	60	3	180	2+2	12	66.66	3	199.98
2+3	8	80	2	160	2+3	11	57.14	2	114.28
2+4	8	70	2	140	2+4	14	14.28	1	14.28
2+5	8	90	1	90	2+5	14	33.33	1	33.33
½ MS+2,4-D + BA (mgl^{-1})					½ MS+2,4-D +BA (mgl^{-1})				
2+1	7	70	2	140	2+1	10	61.11	3	183.33
2+2	7	70	1	70	2+2	12	57.14	1	57.14
2+3	7	80	2	160	2+3	11	57.14	2	114.28
2+4	7	70	1	70	2+4	14	52.38	1	52.38
2+5	7	70	1	70	2+5	14	52.38	1	52.38

Table 16 Effect of 2,4-D and Kinetin on callus induction and growth of nodal segments

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% Callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS +2,4-D + Kinetin (mgl^{-1})					MS + 2,4-D + Kinetin (mgl^{-1})				
0.5+1	7	90	4	360	0.5+1	10	57.14	4	228.56
0.5+2	7	80	4	320	0.5+2	8	70.00	4	280.00
0.5+3	7	100	3	300	0.5+3	9	70.00	3	210.00
0.5+4	7	70	1	70	0.5+4	11	76.19	2	152.38
0.5+5	7	80	1	80	0.5+5	14	43.75	2	87.50
½ MS+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D +Kinetin (mgl^{-1})				
0.5+1	9	90	2	180	0.5+1	11	61.90	2	123.80
0.5+2	9	80	1	80	0.5+2	12	66.66	1	66.66
0.5+3	12	90	1	90	0.5+3	9	61.90	1	61.90
0.5+4	11	70	1	70	0.5+4	12	61.90	1	61.90
0.5+5	12	80	1	80	0.5+5	11	57.14	1	57.14
MS+2,4-D + Kinetin (mgl^{-1})					MS+2,4-D + Kinetin (mgl^{-1})				
1+1	12	70	2	140	1+1	12	66.66	2	133.32
1+2	12	90	2	180	1+2	13	76.19	2	152.38
1+3	10	80	3	240	1+3	10	66.66	3	199.98
1+4	12	90	3	270	1+4	11	57.14	2	114.28
1+5	10	90	1	90	1+5	12	66.66	2	133.32
½ MS+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D +Kinetin (mgl^{-1})				
1+1	13	100	1	100	1+1	13	57.14	1	57.14
1+2	9	90	2	180	1+2	14	54.54	2	109.09
1+3	10	80	2	160	1+3	10	61.90	2	123.80
1+4	14	70	2	140	1+4	9	80.00	2	160.00
1+5	14	70	3	210	1+5	14	47.61	3	142.85

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	Number of days for callus induction.	% Callusing (P)	Growth rate. (G)(Scale of 0,1,2,3,4.)	Callus index (PXG)	Media	Number of days for callus induction.	% callusing (P).	Growth rate (G) (Scale of 0,1,2,3,4.)	Callus index (PXG)
MS + 2,4-D +Kinetin (mgl^{-1})					MS + 2,4-D +Kinetin (mgl^{-1})				
1.5+1	7	90	2	180	1.5+1	7	66.66	2	133.32
1.5+2	7	70	3	210	1.5+2	8	47.61	3	142.85
1.5+3	7	80	1	80	1.5+3	9	33.33	1	33.33
1.5+4	8	80	2	160	1.5+4	11	40.90	2	81.81
1.5+5	8	90	2	180	1.5+5	14	15.00	1	15.00
½ MS+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D +Kinetin (mgl^{-1})				
1.5+1	8	80	1	80	1.5+1	8	68.75	1	68.75
1.5+2	8	90	1	90	1.5+2	11	75.00	2	150.00
1.5+3	7	100	1	100	1.5+3	10	66.66	1	66.66
1.5+4	7	90	1	90	1.5+4	11	47.61	1	47.61
1.5+5	7	80	1	80	1.5+5	11	52.38	1	52.38
MS+2,4-D +Kinetin (mgl^{-1})					MS+c2,4-D +Kinetin (mgl^{-1})				
2+1	8	70	3	210	2+1	8	57.14	2	114.28
2+2	8	80	3	240	2+2	10	52.38	2	104.76
2+3	12	80	2	160	2+3	13	80.95	1	80.95
2+4	12	70	1	70	2+4	14	80.95	1	80.95
2+5	16	80	1	80	2+5	13	47.61	1	47.61
½ MS+2,4-D +Kinetin (mgl^{-1})					½ MS+2,4-D +Kinetin (mgl^{-1})				
2+1	8	70	1	70	2+1	17	76.19	1	76.19
2+2	9	70	1	70	2+2	13	33.33	2	66.66
2+3	-	-	-	-	2+3	14	66.66	1	66.66
2+4	-	-	-	-	2+4	13	85.75	1	85.75
2+5	-	-	-	-	2+5	12	95.00	1	95.00

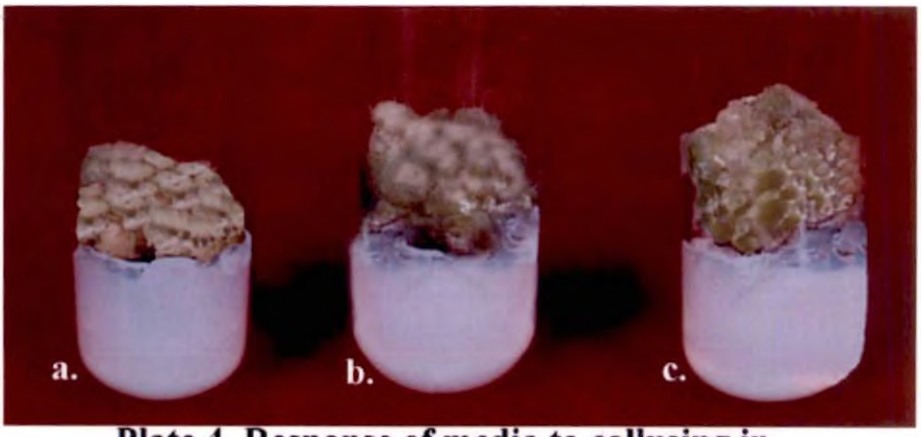


Plate 4. Response of media to callusing in 'Sakthi' and *L.pimpinellifolium*

a. Leaf (NAA 2mg/l + BA 1mg/l) b. Internode (NAA 1mg/l + BA 3mg/l) c. Node (2,4-D 0.5mg/l + BA 2mg/l)

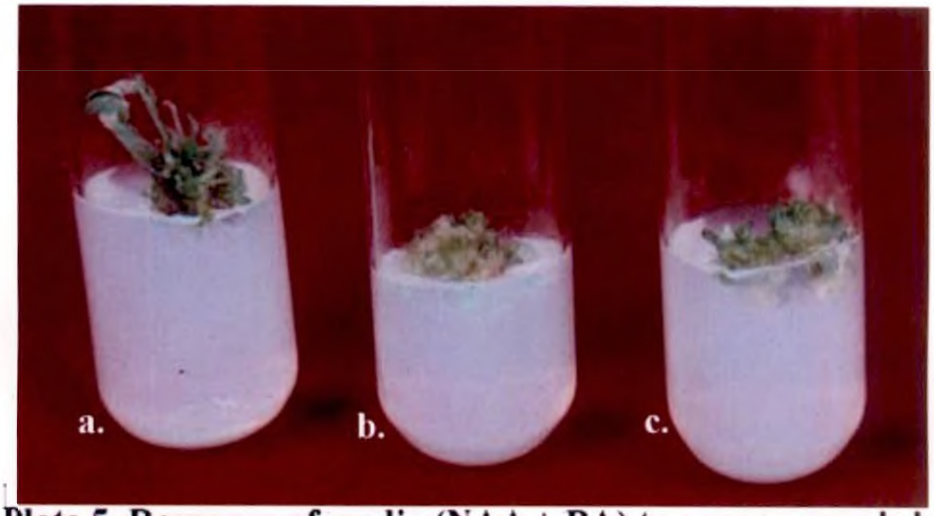


Plate 5. Response of media (NAA + BA) to organogenesis in 'Sakthi' (internodal calli)

a. 2+3 mg/l b. 0+3 mg/l c. 1+3 mg/l

Plate 6. Response of media (MS+IBA (0.5 mg/l)) to rooting (internodal)

The percentage callusing in 'Sakthi' ranged from 70 to 100 and in *L. pimpinellifolium* it ranged from 33.33 to 95.

4. 2 ORGANOGENESIS

The shoot regeneration frequency of the callus obtained from three explants of species *L. esculentum* var. Sakthi and *L. pimpinellilfolium* was studied by culturing them in MS and ½ MS medium containing various concentrations of auxins [NAA (0, 1, 2, 3 mg l^{-1}), 2, 4 – D (0.5, 1, 1.5, 2 mg l^{-1})] and cytokinins [BA (3, 4 mg l^{-1}), Kinetin (3, 4, 5, 6 mg l^{-1})] and the results are presented (Table 17, Table 18 and Table 19).

Healthy compact calli, from leaf explants induced in basal medium supplemented with NAA (2 mg l^{-1}) and BA (1 mg l^{-1}); from internodal segments cultured in basal medium containing NAA (2 mg l^{-1}) + BA (1 mg l^{-1}) and from nodal segments cultured in basal medium with 2,4 - D (0.5 mg l^{-1}) and BA (2 mg l^{-1}) were transferred to regeneration media containing various levels of auxins and cytokinin. The shoot primordia were initiated 15 to 20 days after sub culturing. The shoot primordial elongation started 35 to 40 days after sub culturing.

The percent response of callus to regeneration ranged from 0 to 95 in different calli (leaf, internode and node) in both the species. In 'Sakthi', percent regeneration was higher in internodal calli (95 %) (Table 18) (Fig.2) (Plate5) followed by leaf segment wwwcalli (88.88 %)(Fig.1) (Table 17) on MS medium supplemented with NAA (2 mg l^{-1}) + BA (3 mg l^{-1}). The nodal calli exhibited 52.38 % regeneration in the media containing NAA (2 mg l^{-1}) and kinetin (3 mg l^{-1}) (Table 19)

The number of shoots produced during regeneration of leaf calli varied with the level of growth regulators. It ranged from 3.80 to 4.60 per tube. It was observed that when concentration of NAA was increased roots were produced in some test tubes (Table 17)

Table 17 Effect of Auxins and Cytokinins on organogenesis of leaf segment calli.

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	% Differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos/ Culture	Media	% Differentiation	Number of shoots/ Culture	Number of roots / Culture	Number of embryos / Culture
MS+NAA +BA (mgl^{-1})					MS+NAA +BA (mgl^{-1})				
0+3	50.00	4.2	-	-	0+3	66.66	4.3	-	-
0+4	55.00	4.6	-	-	0+4	-	-	-	-
1+3	60.00	4.3	-	-	1+3	77.70	4.7	2.5	-
1+4	72.20	4.1	-	-	1+4	-	-	-	-
2+3	88.80	4.5	4.1	-	2+3	38.10	3.8	5.1	-
2+4	63.10	3.8	3.5	-	2+4	28.60	3.5	2.0	-
Control (MS)	-	-	-	-	Control (MS)	-	-	-	-
1/2MS+NAA +BA (mgl^{-1})					1/2MS+NAA +BA (mgl^{-1})				
0+3	-	-	-	-	0+3	-	-	-	-
0+4	-	-	-	-	0+4	-	-	-	-
1+3	-	-	-	-	1+3	-	-	-	-
1+4	-	-	-	-	1+4	-	-	-	-
2+3	-	-	-	-	2+3	-	-	-	-
2+4					2+4				
Control (MS)	-	-	-	-	Control (MS)	-	-	-	-

Contd.....

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	% Differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos / Culture	Media	% Differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos / Culture
MS+2,4-D+ BA (mgl^{-1})					MS+2,4-D +BA (mgl^{-1})				
0.5+3	19.04	3.9	-	-	0.5+3	-	-	-	-
0.5+4	20.00	4.4	-	-	0.5+4	-	-	-	-
1.0+3	-	-	-	-	1.0+3	-	-	-	-
1.0+4	-	-	-	-	1.0+4	-	-	-	-
1.5+3	-	-	-	-	1.5+3	-	-	-	-
1.5+4	-	-	-	-	1.5+4	-	-	-	-
2.0+3	-	-	-	-	2.0+3	-	-	-	-
2.0+4	-	-	-	-	2.0+4	-	-	-	-

Table 18 Effect of Auxins and cytokinins on organogenesis of internodal segment calli

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	% Differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos/ Culture	Media	%Differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos/ Culture
MS+NAA +BA (mgl^{-1})					MS+NAA +BA (mgl^{-1})				
0+3	45.00	4.6	-	-	0+3	-	-	-	-
0+4	44.40	3.8	-	-	0+4	-	-	-	-
1+3	89.47	5.1	-	-	1+3	-	-	-	-
1+4	40.00	4.6	-	-	1+4	-	-	-	-
2+3	95.00	5.2	6.8	-	2+3	-	-	-	-
2+4	35.00	3.9	8.2	-	2+4	-	-	-	-
Control (MS)	-	-	-	-	Control (MS)	-	-	-	-

Table 19 Effect of Auxins and Cytokinins on organogenesis of nodal segment calli

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	% Differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos /Culture	Media	% Differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos /Culture
MS+NAA+ Kinetin (mgl^{-1})					MS+NAA +Kinetin (mgl^{-1})				
0+3	44.40	4.3	-	-	0+3	-	-	-	-
0+4	-	-	-	-	0+4	-	-	-	-
0+5	-	-	-	-	0+5	-	-	-	-
0+6	-	-	-	-	0+6	-	-	-	-
1+3	47.60	3.7	-	-	1+3	-	-	-	-
1+4	47.60	4.9	-	-	1+4	-	-	-	-
1+5	-	-	-	-	1+5	-	-	-	-
1+6	-	-	-	-	1+6	-	-	-	-
2+3	52.38	3.5	4.1	-	2+3	-	-	-	-
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-
2+6	-	-	-	-	2+6	-	-	-	-
1/2MS+NAA +Kiinetin ($mgl^{-1)}$					1/2MS+NAA +Kinetin (mgl^{-1})				
0+3	-	-	-	-	0+3	-	-	-	-
0+4	-	-	-	-	0+4	-	-	-	-
0+5	-	-	-	-	0+5	-	-	-	-
0+6	-	-	-	-	0+6	-	-	-	-
1+3	-	-	-	-	1+3	-	-	-	-
1+4	-	-	-	-	1+4	-	-	-	-
1+5	-	-	-	-	1+5	-	-	-	-
1+6	-	-	-	-	1+6	-	-	-	-
2+3	-	-	-	-	2+3	-	-	-	-
2+4	-	-	-	-	2+4	-	-	-	-
2+5	-	-	-	-	2+5	-	-	-	-
2+6	-	-	-	-	2+6	-	-	-	-

Table 20 Effect of different levels of Sucrose and Agar on organogenesis of leaf segment calli.

Lycopersicon esculentum var. Sakthi					*Lycopersicon pimpinellifolium*				
Media	% Shoot differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos/ Culture	Media	% Shoot differentiation	Number of shoots/ Culture	Number of roots/ Culture	Number of embryos/ Culture
Sucrose+ Agar (%)					Sucrose+ Agar (%)				
0.0+0.0	-	-	-	-	0.0+0.0	-	-	-	-
0.0+0.5	21.42	0	3.1	-	0.0+0.5	-	-	-	-
0.0+0.7	28.57	0	2.5	-	0.0+0.7	-	-	-	-
0.0+1.0	-	-	-	-	0.0+1.0	-	-	-	-
1.0+0.0	-	-	-	-	1.0+0.0	-	-	-	-
1.0+0.5	44.44	2.3	1.0	-	1.0+0.5	-	-	-	-
1.0+0.7	33.33	2.6	1.2	-	1.0+0.7	-	-	-	-
1.0+1.0	28.57	1.9	3.5	-	1.0+1.0	-	-	-	-
3.0+0.0	-	-	-	-	3.0+0.0	-	-	-	-
3.0+0.5	66.66	3.2	1.2	-	3.0+0.5	-	-	-	-
3.0+0.7	80.95	4.6	2.4	-	3.0+0.7	-	-	-	-
3.0+1.0	57.14	2.3	2.9	-	3.0+1.0	-	-	-	-
5.0+0.0	-	-	-	-	5.0+0.0	-	-	-	-
5.0+0.5	52.38	2.5	3.0	-	5.0+0.5	-	-	-	-
5.0+0.7	52.38	3.9	2.2	-	5.0+0.7	-	-	-	-
5.0+1.0	14.28	1.2	4.6	-	5.0+1.0	-	-	-	-
Control	-	-	-	-	Control	-	-	-	-

Fig. 1 Effect of NAA and BA on differentiation of leaf segment calli

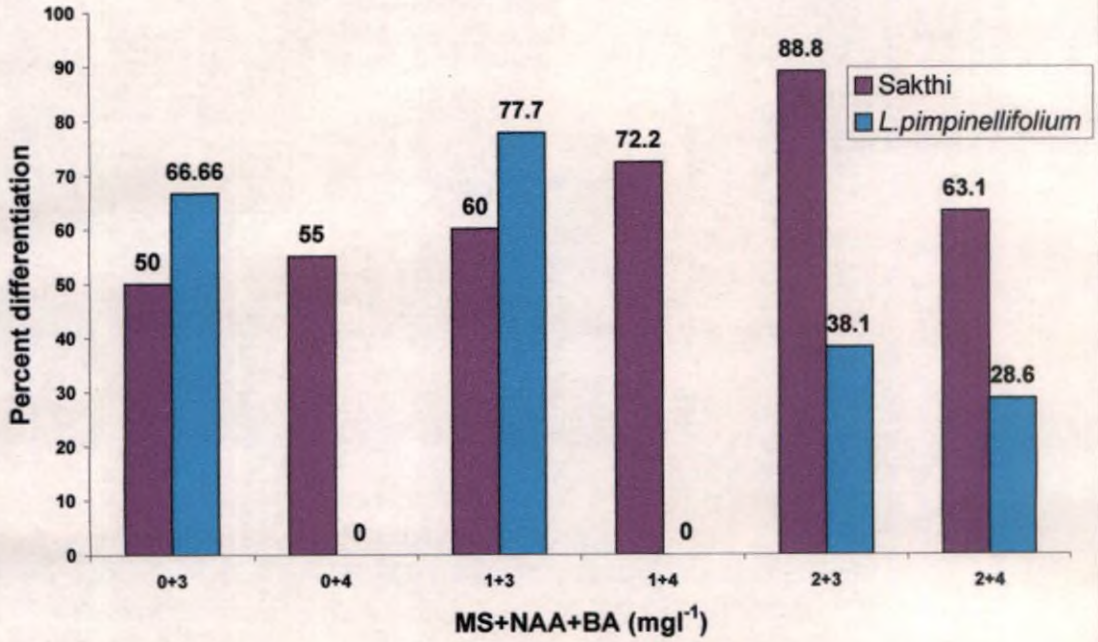
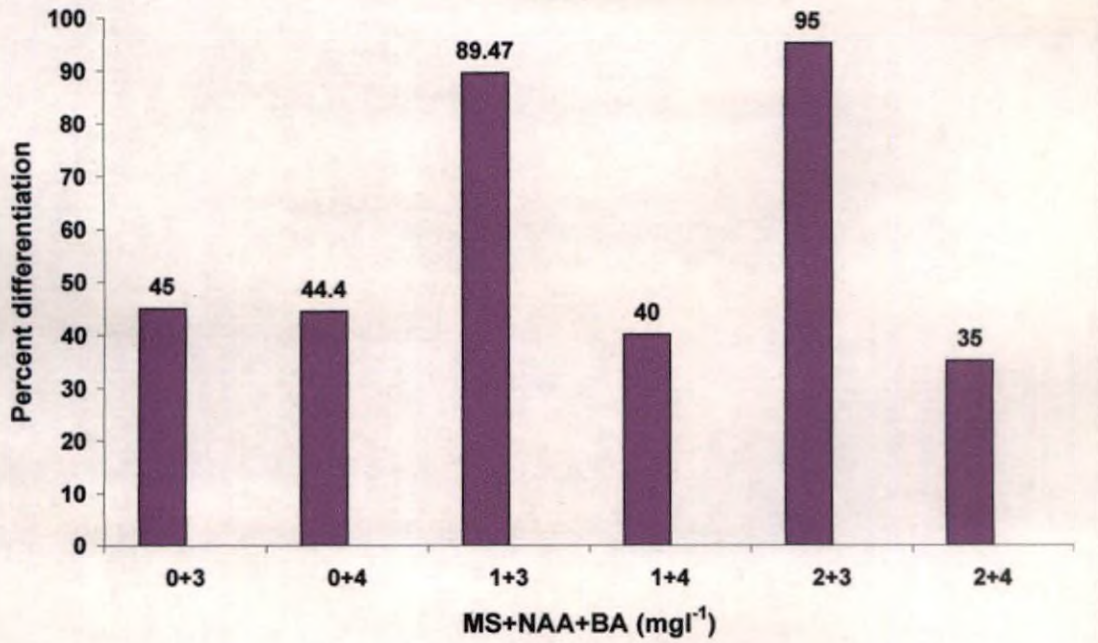


Fig. 2 Effect of NAA and BA on differentiation of internodal calli in 'Sakthi'



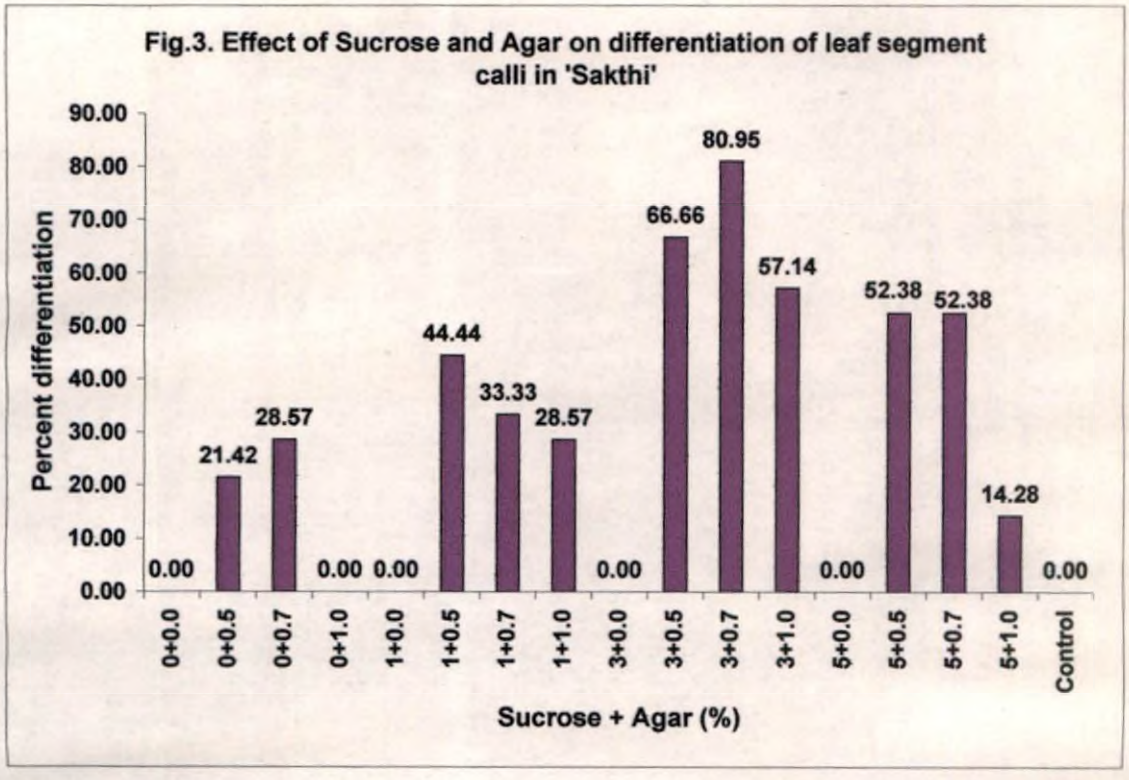


Fig.3. Effect of Sucrose and Agar on differentiation of leaf segment calli in 'Sakthi'
Percent differentiation
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.00
21.42
28.57
0.00
0.00
44.44
33.33
28.57
0.00
66.66
80.95
57.14
0.00
52.38
52.38
14.28
0.00
0+0.0
0+0.5
0+0.7
0+1.0
1+0.0
1+0.5
1+0.7
1+1.0
3+0.0
3+0.5
3+0.7
3+1.0
5+0.0
5+0.5
5+0.7
5+1.0
Control
Sucrose + Agar (%)

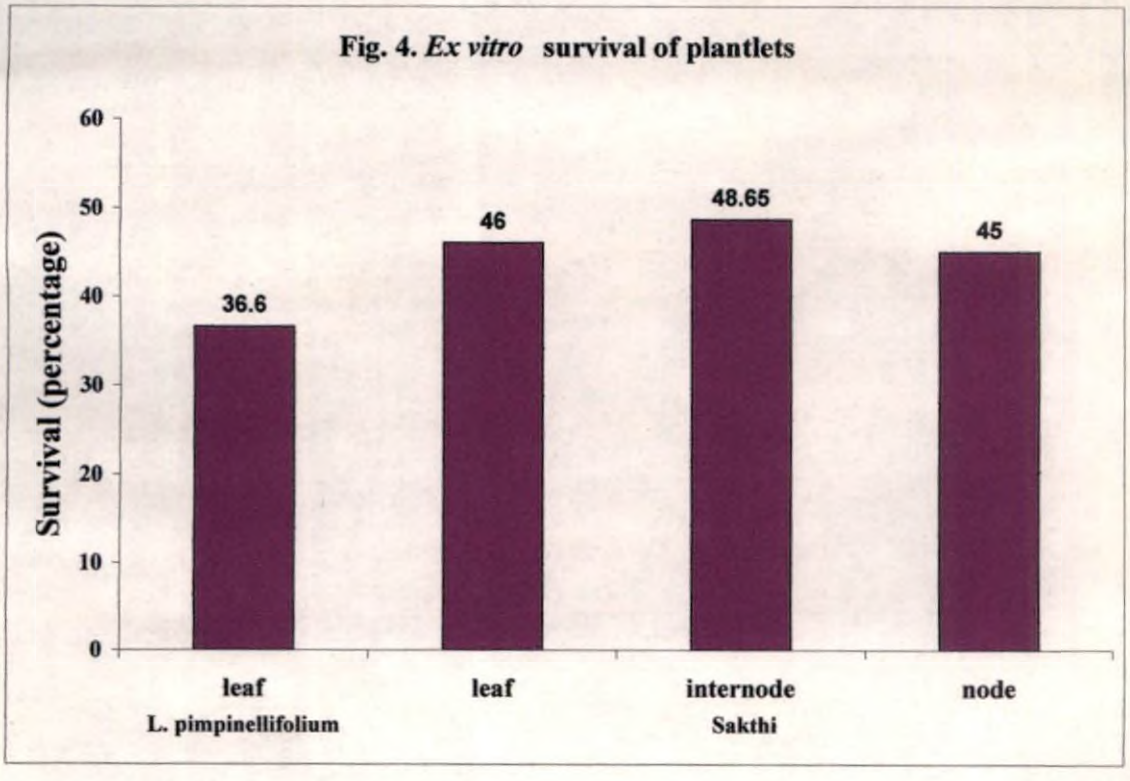


Fig. 4. *Ex vitro* survival of plantlets
Survival (percentage)
60
50
40
30
20
10
0
36.6
46
48.65
45
leaf
L. pimpinellifolium
leaf
internode
Sakthi
node

The number of shoots produced during regeneration of inter nodal calli ranged from 3.80 to 5.20 and that of nodal calli ranged from 3.50 to 4.90 per tube depending on the levels of growth regulators (Table 18 and Table 19)

The calli obtained by culturing leaf segments of *L. pimpinellifolium* in MS + NAA (2 mg l^{-1}) + BA (1 mg l^{-1}) was transferred to the regeneration media. It was observed that MS + NAA (1 mg l^{-1}) + BA (3 mg l^{-1}) produced 77.7 % regenerants (Table 17). The inter nodal and nodal calli did not regenerate in the media used (Table 18 and Table 19).

4.2.1 Effect of different concentrations of sucrose and agar on shoot regeneration

Effect of different concentrations of sucrose and agar on shoot regeneration of leaf segment calli of 'Sakthi' obtained from basal medium containing NAA (2 mg l^{-1}) and BA (1 mg l^{-1}) was studied and result is presented in Table 20.

Leaf calli were subcultured on the regeneration media containing different concentration of sucrose (0, 1, 3 and 5 %) and agar (0, 0.5, 0.7 and 1 %). The regeneration and number of shoots per tube was maximum when subculturing was done in the media containing NAA (2 mg l^{-1}) and BA (3 mg l^{-1}), sucrose 3% and agar 0.7% (Table 20). *L. pimpinellifolium* did not regenerate in the media where sucrose and agar was introduced in different concentrations (Table 20)(Fig.3).

4. 3 ROOT INDUCTION

In the tomato variety 'Sakthi', the number of days for rooting varied from 8 to16 (Table 21)(Plate.6) when different levels of IBA and IAA were used in the medium. Root induction was delayed in the medium where no hormone was used. The rooting was 100 % in basal medium supplemented with IBA (1 mg l^{-1}), IBA (1 mg l^{-1}) + IAA (0.5 mg l^{-1}), IBA (1.5 mg l^{-1}) + IAA (0.5 mg l^{-1}) and IBA (1.5 mg l^{-1}) + IAA (1.5 mg l^{-1}). The number of roots per culture tube ranged from 1.20 to 4.60. Maximum numbers of roots were

produced when IBA and IAA were added at 1 mg l^{-1} in the medium. In the case of ½ MS the number of days for rooting varied from 6 to 12 (Table 21) when different levels of IBA and IAA were used in the medium. The rooting was 100% in half strength basal medium supplemented with IBA (1 mg l^{-1}) + IAA (1 mg l^{-1}) and IBA (1 mg l^{-1}) + IAA(1.5 mg l^{-1}). The number of roots per tube ranged from 1.2 to 4.0. Maximum number of roots were produced when IBA (1.5 mg l^{-1}) + IAA (1.5 mg l^{-1}) were added in the medium.

In 'Sakthi', the number of days for rooting varied from 10 to20 when MS medium with different levels of NAA and IBA were used. The percentage culture rooted ranged from 37.50 to 100. The rooting was maximum when basal medium supplemented with NAA (1.5 mg l^{-1}) and NAA (1.5 mg l^{-1}) + IBA (0.5 mg l^{-1}). Maximum numbers of roots were observed in the combination of NAA (1.5 mg l^{-1}) + IBA (0.5 mg l^{-1}). Presence of root hairs was observed in some combinations of NAA and IBA. Half strength medium with different levels of NAA and IBA did not give 100 % rooting. The number of days for root induction and number of roots per culture ranged from 10 to 16 and 1.5 to 4.2 respectively (Table 22). Presence of root hairs was observed in most of the combinations and presence of intervening callus was observed when NAA (1 mg l^{-1}) and IBA (1 mg l^{-1}) were used.

In tomato variety 'Sakthi', MS medium supplemented with different levels of IAA and NAA, the number of days for root induction varied from 10 to 20. The percentage culture rooted ranged from 33.33 to 100. The rooting was 100 per cent when basal medium supplemented with IAA (1.5 mg l^{-1}) and IAA (1.5 mg l^{-1}) + NAA (0.5 mg l^{-1}) was used. The number of rooted cultures ranged from 1.1 to 4.60. Presence of intervening callus was observed in the medium containing IAA (1 mg l^{-1}) + NAA (1 mg l^{-1}) (Table 23). The ½MS medium with different levels of IAA and NAA singly and in combination did not show 100 per cent rooting efficiency. The number of days for root induction ranged from 8 to 16 and presence of root hairs was observed in some of the combinations. Presence of intervening callus was observed when IAA (1 mg l^{-1}) + NAA (1 mg l^{-1}) were added in the medium.

Table 21 Effect of different levels of MS, ½ MS, IAA and IBA combinations on root induction in 'Sakthi'

Media	No. of days for root induction	% Culture rooted	No. of roots/ Culture	Presence of root hairs	Presence of intervening callus	Media	No. of days for root induction	% Culture rooted	No. of roots/ Culture	Presence of root hairs	Presence of intervening callus
MS+IBA+ IAA (mgl^{-1})						1/2MS+IBA +IAA (mgl^{-1})					
0.5+0.0	8	75.00	2.6	Y	N	0.5+0.0	6	77.70	3.9	Y	N
0.5+0.5	9	83.30	3.1	N	Y	0.5+0.5	9	83.30	1.8	Y	N
0.5+1.0	12	57.10	2.9	Y	N	0.5+1.0	10	75.00	3.2	Y	Y
0.5+1.5	10	75.00	3.2	Y	N	0.5+1.5	9	37.50	3.1	N	N
0.5+2.0	9	37.50	3.3	Y	N	0.5+2.0	8	85.70	3.5	N	N
1.0+0.0	8	100.00	4.0	Y	N	1.0+0.0	8	77.70	3.7	Y	N
1.0+0.5	9	100.00	3.2	Y	N	1.0+0.5	8	33.33	2.9	Y	N
1.0+1.0	10	77.70	4.6	N	N	1.0+1.0	9	100.00	2.2	N	N
1.0+1.5	9	33.30	2.9	Y	N	1.0+1.5	8	100.00	2.5	Y	N
1.0+2.0	8	25.00	2.1	N	N	1.0+2.0	7	66.66	3.3	Y	N
1.5+0.0	9	42.80	3.0	Y	N	1.5+0.0	8	66.66	2.4	Y	N
1.5+0.5	10	100.00	1.5	N	Y	1.5+0.5	9	83.30	1.4	Y	N
1.5+1.0	8	66.66	3.3	Y	N	1.5+1.0	7	66.66	3.1	N	N
1.5+1.5	9	100.00	1.8	Y	N	1.5+1.5	8	66.66	4.0	N	N
1.5+2.0	10	33.33	3.4	N	N	1.5+2.0	9	57.10	2.2	N	N
2.0+0.0	8	57.10	1.2	Y	N	2.0+0.0	8	33.33	1.6	Y	N
2.0+0.5	8	66.66	2.3	N	N	2.0+0.5	9	66.66	3.2	Y	N
2.0+1.0	9	33.33	1.6	N	N	2.0+1.0	8	57.10	2.3	N	N
2.0+1.5	9	66.66	2.9	Y	N	2.0+1.5	8	66.66	1.2	N	N
2.0+2.0	12	75.00	1.5	N	N	2.0+2.0	10	66.66	3.0	Y	N
Control (MS)	16	33.33	18	N	N	Control (1/2 MS)	12	42.60	2.6	N	N

Table 22 Effect of different levels of MS, ½ MS, NAA and IBA combinations on root induction in 'Sakthi'

Media	Number of days for Root induction	% Culture rooted	Number of roots / Culture	Presence of root hairs	Presence of intervening callus	Media	Number of days for Root induction	% Culture rooted	Number of roots/ Culture	Presence of root hairs	Presence of intervening callus
MS+NAA+ IBA (mgl^{-1})						1/2MS+NAA +IBA (mgl^{-1})					
0.5+0.0	16	83.30	4.1	N	N	0.5+0.0	14	66.66	4.2	Y	N
0.5+0.5	14	57.10	2.5	Y	N	0.5+0.5	13	75.00	2.6	N	N
0.5+1.0	19	75.00	2.6	N	N	0.5+1.0	-	-	-	-	-
0.5+1.5	18	37.50	2.0	N	N	0.5+1.5	14	40.00	1.8	Y	N
0.5+2.0	10	85.70	3.1	Y	N	0.5+2.0	8	20.00	1.5	N	N
1.0+0.0	18	50.00	3.7	N	N	1.0+0.0	16	75.00	3.2	N	N
1.0+0.5	13	66.66	5.1	Y	N	1.0+0.5	13	86.30	3.3	Y	N
1.0+1.0	14	83.30	4.2	N	N	1.0+1.0	14	66.66	4.0	N	Y
1.0+1.5	16	75.00	3.0	N	N	1.0+1.5	11	20.00	2.7	Y	N
1.0+2.0	11	66.66	2.2	N	N	1.0+2.0	10	40.00	2.8	Y	N
1.5+0.0	20	100.00	1.9	N	N	1.5+0.0	-	-	-	-	-
1.5+0.5	14	100.00	6.1	Y	N	1.5+0.5	14	66.66	4.1	N	N
1.5+1.0	11	66.66	2.8	N	N	1.5+1.0	11	66.66	2.8	N	N
1.5+1.5	14	60.00	1.9	Y	N	1.5+1.5	13	20.00	2.6	N	N
1.5+2.0	12	83.30	1.5	Y	N	1.5+2.0	11	66.66	3.3	N	N
2.0+0.0	-	-	-	-	-	2.0+0.0	11	66.66	3.4	N	N
2.0+0.5	-	-	-	-	-	2.0+0.5	12	83.30	2.8	Y	N
2.0+1.0	10	60.00	3.2	Y	N	2.0+1.0	10	60.00	3.0	N	N
2.0+1.5	11	83.30	2.3	N	N	2.0+1.5	11	40.00	2.2	N	N
2.0+2.0	12	66.66	2.7	N	N	2.0+2.0	11	16.00	2.6	Y	N
Control (MS)	18	37.50	2.8	N	N	Control (1/2 MS)	16	66.66	2.7	Y	N

Table 23 Effect of different levels of MS, ½ MS, IAA and NAA combinations on root induction in 'Sakthi'

Media	Number of days for Root induction	% Culture rooted	Number of roots/ Culture	Presence of root hairs	Presence of intervening callus	Media	Number of days for Root induction	% Culture rooted	Number of roots/ Culture	Presence of root hairs	Presence of intervening callus
MS+IAA+ NAA (mgl^{-1})						1/2MS+IAA +NAA (mgl^{-1})					
0.5+0.0	16	83.30	4.1	Y	N	0.5+0.0	14	66.66	4.1	Y	N
0.5+0.5	14	57.10	2.3	N	N	0.5+0.5	13	75.00	2.5	N	N
0.5+1.0	19	75.00	3.3	N	N	0.5+1.0	-	-	-	-	-
0.5+1.5	18	37.50	1.1	Y	N	0.5+1.5	14	40.00	1.3	Y	N
0.5+2.0	10	85.70	1.3	N	N	0.5+2.0	8	20.00	1.6	N	N
1.0+0.0	18	50.00	3.3	N	N	1.0+0.0	16	75.00	3.9	N	N
1.0+0.5	13	66.66	3.8	Y	N	1.0+0.5	13	83.30	3.2	Y	N
1.0+1.0	14	83.33	4.1	N	Y	1.0+1.0	14	66.66	4.4	N	Y
1.0+1.5	16	75.00	2.8	Y	N	1.0+1.5	12	20.00	2.2	Y	N
1.0+2.0	11	66.66	2.5	Y	N	1.0+2.0	16	40.00	2.4	Y	N
1.5+0.0	20	100.00	1.2	N	N	1.5+0.0	-	-	-	-	-
1.5+0.5	14	100.00	4.6	N	N	1.5+0.5	11	66.66	4.3	N	N
1.5+1.0	11	66.66	2.3	N	N	1.5+1.0	10	66.66	2.5	N	N
1.5+1.5	14	60.00	2.4	N	N	1.5+1.5	11	20.00	2.4	N	N
1.5+2.0	12	83.30	2.4	N	N	1.5+2.0	13	66.66	3.2	N	N
2.0+0.0	-	-	-	-	-	2.0+0.0	11	66.66	3.2	N	N
2.0+0.5	-	-	-	-	-	2.0+0.5	12	83.30	2.2	Y	N
2.0+1.0	10	60.00	2.2	N	N	2.0+1.0	10	60.00	3.1	N	N
2.0+1.5	11	83.30	2.2	N	N	2.0+1.5	11	40.00	2.2	N	N
2.0+2.0	12	66.66	3.4	Y	N	2.0+2.0	11	16.00	2.3	Y	N
Control (MS)	15	33.33	3.2	N	N	Control (1/2MS)	15	33.33	2.4	N	N

Table 24 Effect of different levels of IBA and IAA combinations of root induction in *L. pimpinellifolium*

Media	No. of days for root induction	% Culture rooted	No.of roots/ Culture	Presence of root hairs	Presence of intervening callus
MS+IBA+ IAA (mgl^{-1})					
0.5+0.0	7	50.00	3.9	Y	N
0.5+0.5	9	33.33	4.3	Y	N
0.5+1.0	11	100.00	1.4	N	N
0.5+1.5	8	100.00	1.9	N	N
0.5+2.0	12	66.66	2.2	N	N
1.0+0.0	7	66.66	2.4	Y	N
1.0+0.5	8	83.30	3.5	N	N
1.0+1.0	9	66.66	2.1	N	N
1.0+1.5	11	57.10	2.3	N	N
1.0+2.0	10	37.50	2.6	N	N
1.5+0.0	8	83.30	3.8	Y	N
1.5+0.5	9	66.66	2.5	N	N
1.5+1.0	8	66.66	1.9	Y	N
1.5+1.5	12	33.33	1.1	N	N
1.5+2.0	11	66.66	2.2	N	N
2.0+0.0	7	66.66	1.0	Y	N
2.0+0.5	10	83.30	1.3	N	N
2.0+1.0	7	14.20	2.9	N	N
2.0+1.5	10	33.33	2.3	N	N
2.0+2.0	10	57.10	2.2	N	N
Control (MS)	18	33.33	2.1	N	N

In *L. pimpinellifolium* rooting was initiated 7 to 18 days after culturing in the basal media containing different concentrations of IBA and IAA. Rooting was 100% in the media supplemented with IBA (0.5 mg l^{-1}) and IAA (1and 1.5 mg l^{-1}). The rooting varied from 14.2% to 100%. The number roots ranged from 1.0 to 4.3 per culture (Table 24).

4.4 SCREENING SOMACLONAL VARIANTS FOR RESISTANCE TO TLCV DISEASE

The somaclones were grouped based on the explant and media used (Table 25). The rooted somaclones were transferred to hardening unit and after hardening somaclones were planted in pots and observations were recorded for *exvitro* survival percent, reaction to TLCV, biometric characters, reaction to bacterial wilt and incidence of other diseases.

4.4.1 *Ex vitro* survival of plantlets (%)

In 'Sakthi' *exvitro* survival percentage varied with explant (Fig.4). Survival was maximum (48.65%) in internodes. There was no significant variation between the different explants for percent survival. In *L. pimpinellifolium*, the survival was 36.6% for the plantlets from leaf calli. The survival of plants obtained from leaf calli and regenerated in the media containing NAA (2 mg l^{-1}) + BA (3 mg l^{-1}) + sucrose (3%) and agar (0.7%) was 37.5%. (Fig.4)

4.4.2 Reaction to TLCV

The Tomato Leaf Curl Virus (TLCV) disease grade varied from 0 to 4 in the somaclones of group I. The plant number 6 did not show any symptom (Plate.8) while plant number 1, 2 and 5 exhibited very mild curling of the leaves. Other plants were susceptible to the disease (Table 25). The plant number 2 in group II (Table 26) did not exhibit any symptom of the disease. Other plants were either moderately resistant or moderately susceptible or susceptible or highly susceptible to the disease. None of the plants in group III (Table 27) were resistant to TLCV disease. The plants were either moderately susceptible or susceptible

or highly susceptible to the disease. The plants of group IV (Table 28) were either susceptible or highly susceptible. The disease grade varied from 2 to 4. The control plants of the variety 'Sakthi' were also susceptible to the disease. The somaclones of *L.pimpinellifolium* as well as the control did not exhibit symptoms of TLCV disease (Table 29).

Table 25. Grouping of somaclones

Group	Explant	Media		
		Callusing	Organogenesis	Rooting
Group I ('Sakthi')	Leaf segments	MS + NAA (2mg l^{-1}) + BA (1 mg l^{-1})	MS + NAA (2 mg l^{-1}) + BA (3 mg l^{-1})	MS + IBA (0.5 mg l^{-1})
Group II ('Sakthi')	Leaf segments	MS + NAA (2mg l^{-1}) + BA (1 mg l^{-1})	MS + NAA (2 mg l^{-1}) + BA (3 mg l^{-1}) + agar 0.7% and sucrose 3%	½ MS + IBA (1 mg l^{-1}) + NAA (1 mg l^{-1})
Group III ('Sakthi')	Internodal segments	MS + NAA (1 mg l^{-1}) + BA (3mg l^{-1})	MS + NAA (2 mg l^{-1}) + BA (3 mg l^{-1})	MS + IAA (1 mg l^{-1})
Group IV ('Sakthi')	Nodal segments	MS + 2,4 - D (0.5 mg l^{-1}) + BA (2 mg l^{-1})	MS + NAA (2 mg l^{-1}) + kinetin (3 mg l^{-1})	½ MS + IAA (0.5 mg l^{-1}) + IBA (1 mg l^{-})
Group V (*L. pimpinellifolium*)	Leaf segments	MS + NAA (2 mg l^{-1}) + BA (1 mg l^{-1})	MS + NAA (2 mg l^{-1}) + BA (3 mg l^{-1})	MS + IBA (0.5 mg l^{-1})

Plate 7. Variability in somaclones of 'Sakthi'

a. Control b. Potato leaved

Plate 8. Reaction to TLCV disease in somaclones of tomato (Sakthi)

a. Control b. Tolerant plant

4.4.3 Biometric characters

The biometric characters of the somaclones of *L.esculentum* var. Sakthi and *L. pimpinellifolium* are presented. (Tables 25 to 29).

4.4.3.1 Plant height

The plant height in the somaclones of group I (Table 25) varied from 29.10 to 59.20 cm. In group II (Table 26) the plant number 2 had maximum height (62.30 cm.). The height of plants in group III (Table 27) varied from 26.2 to 54.60 cm and that in group IV (Table 28) ranged from 28.30 to 64.40cm. The height of *L. pimpinellifolium* somaclones in group V (Table 29) varied from 68.20 to 98.10 cm.

4.4.3.2 *Plant spread*

The plant spread in the somaclones of group I (Table 25) varied from 14.10 to 45.20 cm. Maximum plant spread with potato leaf shaped plants were observed in group I (Plant no. 5 and 6)(Plate.7). In group II (Table 26) the plant spread varied from 19.00 to 40.20 cm. The spread of plants in group III (Table 27) varied from 11.20 to 38.20 cm and that in group IV (Table 28) ranged from 16.40 to 45.00cm. The plant spread of *L. pimpinellifolium* somaclones in group V (Table 29) varied from 21.40 to 36.40 cm.

4.4.3.3. *Primary branches / plant*

The number of primary branches per plant of the somaclones of group I (Table 25) varied from 1 to 7 and that of group II (Table 26) varied from 2 to 6.The number of primary branches of group III (Table 27) varied from 1 to 4 and that of group IV (Table 28) varied from 1 to 4. The number of primary branches of *L. pimpinellifolium* somaclones varied from 1 to 3 (Table 29).

4.4.3.4 *Number of fruits / plant*

The number of fruits per plant of the somaclones of group I (Table 25) varied from 0 to 16; that of group II (Table 26) varied from 0 to 14; that of group III (Table 27) varied from 0 to 14 and that of group IV (Table 28) varied from 0 to 8. The number of fruits per plant of *L. pimpinellifolium* somaclones varied from 9 to 33 (Table 29). The fruit obtained from leaf regenerants of group II (Plant no. 1) was observed to be small and free from cracking (Table 26). The fruits obtained from internodal regenerants of group III (Plant no. 11 and 14) were found to be free from cracking. The Plant no. 14 showed green shoulder character and Plant no. 11 was free from green shoulder (Table 27).

4.4.3.5 Yield / plant

The fruit yield per plant in the somaclones of group I (Table 25) varied from 0 to 456.50 g. The Plant no. 6 in group I had maximum yield per plant (456.50 g). The yield per plant in group II (Table 26) varied from 0 to 364.80 g. In the case of group III (Table 27) yield per plant ranged from 0 to 360.80 g and that in group IV (Table 28) ranged from 0 to 112.40 g. The yield per plant of *L. pimpinellifolium* somaclones in group V (Table 29) varied from 12.30 to 169.60 g.

4.4.3.6 Reaction to bacterial wilt

The somaclones and the control plants ('Sakthi' and *L. pimpinellifolium*) did not exhibit symptoms of bacterial wilt disease (Tables 25 to 29).

4.4.3.7 Incidence of other diseases

Incidence of *Cercospora* leaf spot was observed. The plant number 1, 7 and 14 of group I (Table 25) were susceptible to the disease. The infection of the leaf area varied from 10 to 50%. In group II (Table 26) the incidence of *Cercospora* leaf spot was observed only

Table 26 Performance of somaclones (Group I)

Group I	Reaction to TLCV (score)	*Biometric Characters*					Reaction to Bacterial Wilt	Reaction to Cercospora leaf spot
		Plant Height (cm)	Plant Spread (cm)	Primary Branches / Plant	No. of Fruits / Plant	Yield / Plant (g)		
Plant no.1	1	40.00	28.60	4	10	325.00	0	1
Plant no.2	1	39.50	19.40	3	13	318.00	0	0
Plant no.3	4	32.50	15.00	1	00	00.00	0	0
Plant no.4	3	59.50	24.00	7	02	49.60	0	0
Plant no.5	1	30.00	25.40	3	12	335.40	0	0
Plant no.6	0	71.50	45.20	3	16	456.50	0	0
Plant no.7	3	59.20	29.81	4	03	72.80	0	3
Plant no.8	3	42.10	20.00	3	02	48.20	0	0
Plant no.9	4	31.20	14.20	3	00	00.00	0	0
Plant no.10	4	39.40	17.20	3	00	00.00	0	0
Plant no.11	4	41.10	20.50	4	00	00.00	0	0
Plant no.12	3	40.10	19.60	5	03	62.40	0	0
Plant no.13	4	32.30	16.10	3	00	00.00	0	0
Plant no.14	3	38.10	18.00	3	01	28.20	0	1
Plant no.15	4	36.40	14.00	3	00	00.00	0	0
Plant no.16	4	56.40	35.00	4	00	00.00	0	0
Plant no.17	4	41.20	30.00	3	00	00.00	0	0
Plant no.18	3	39.80	35.00	3	03	88.20	0	0
Plant no.19	4	32.60	14.10	3	00	00.00	0	0
Plant no.20	4	42.10	26.20	2	00	00.00	0	0
Plant no.21	3	31.10	19.60	3	01	18.20	0	0
Plant no.22	3	29.10	22.00	3	02	38.20	0	0
Plant no.23	4	29.20	16.00	3	00	00.00	0	0
Plant no.24	4	33.40	18.00	3	00	00.00	0	0
Plant no.25	4	41.30	28.20	4	00	00.00	0	0
Plant no.26	3	45.60	26.20	3	01	22.80	0	0
Control 'Sakthi'	3	38.64	22.40	3	10	240.50	0	3

Table 27 Performance of somaclones (Group II)

Group II	Reaction to TLCV (Score)	Biometric Characters					Reaction to Bacterial Wilt	Reaction to Cercospora leaf spot
		Plant Height (cm)	Plant Spread (cm)	Primary Branches / Plant	No. of Fruits / Plant	Yield / Plant (g)		
Plant no.1	3	39.20	19.00	4	02	48.20	0	0
Plant no.2	0	62.30	40.20	3	14	364.80	0	0
Plant no.3	3	57.30	38.20	2	02	38.30	0	0
Plant no.4	2	29.20	18.40	3	10	288.30	0	1
Plant no.5	1	33.20	30.00	3	12	342.50	0	0
Plant no.6	2	26.80	19.20	4	08	172.30	0	0
Plant no.7	4	34.20	21.20	6	00	00.00	0	0
Plant no.8	4	41.20	34.60	4	00	00.00	0	0
Plant no.9	4	24.20	19.40	2	00	00.00	0	0
Control 'Sakthi'	3	38.64	22.40	3	10	240.50	0	3

Table 28 Performance of somaclones (Group III)

Group III	Reaction to TLCV (Score)	Biometric Characters					Reaction to Bacterial Wilt	Reaction to Cercospora leaf spot
		Plant Height (cm)	Plant Spread (cm)	Primary Branches / Plant	No. of Fruits / Plant	Yield / Plant (g)		
Plant no.1	4	29.40	16.00	4	0	00.00	0	0
Plant no.2	4	31.60	20.00	3	0	00.00	0	0
Plant no.3	3	41.40	21.40	3	2	62.30	0	0
Plant no.4	3	46.40	28.20	2	3	54.20	0	0
Plant no.5	2	54.60	34.60	2	8	204.20	0	3
Plant no.6	3	36.20	22.00	1	3	69.80	0	0
Plant no.7	3	31.40	16.00	2	2	38.20	0	0
Plant no.8	2	53.10	38.20	1	7	190.40	0	0
Plant no.9	4	26.20	14.00	2	0	00.00	0	0
Plantno.10	3	38.50	19.60	1	1	18.20	0	0
Plant no.11	`2	55.20	36.20	2	09	320.68	0	2
Plant no.12	2	34.50	19.40	2	11	318.20	0	0
Plant no.13	1	57.30	38.20	1	14	360.80	0	0
Plant no.14	3	38.00	19.20	2	04	85.50	0	0
Plant no.15	2	24.20	11.20	1	06	128.30	0	0
Plant no.16	2	34.60	16.20	4	08	184.50	0	0
Plant no.17	4	38.40	21.00	2	00	00.00	0	0
Plant no.18	4	34.50	24.00	2	00	00.00	0	0
Plant no.19	3	52.50	33.00	1	03	72.40	0	0

Contd.....

Group III	Reaction to TLCV (Score)	Biometric Characters					Reaction to Bacterial Wilt	Reaction to Cercospora leaf spot
		Plant Height (cm)	Plant Spread (cm)	Primary Branches / Plant	No. of Fruits / Plant	Yield / Plant (g)		
Plant no.20	3	38.40	28.20	1	02	33.60	0	0
Plant no.21	3	26.20	14.20	2	02	41.20	0	2
Plant no.22	4	34.60	17.80	2	00	00.00	0	0
Plant no.23	4	37.20	28.00	1	00	00.00	0	0
Plant no.24	4	26.40	11.00	2	00	00.00	0	0
Plant no.25	3	31.60	18.20	2	01	18.60	0	0
Plant no.26	3	32.60	17.10	1	02	36.40	0	0
Plant no.27	3	41.60	24.20	2	02	40.50	0	0
Plant no.28	3	51.40	31.20	2	03	54.20	0	0
Contrrol 'Sakthi'	3	38.64	22.40	3	10	240.50	0	3

Table 29 Performance of somaclones (Group IV)

Group IV	Reaction to TLCV (Score)	Biometric Characters					Reaction to Bacterial Wilt	Reaction to Cercospora leaf spot
		Plant Height (cm)	Plant Spread (cm)	Primary Branches / Plant	No. of Fruits / Plant	Yield / Plant (g)		
Plant no.1	3	58.60	38.00	1	4	62.40	0	0
Plant no.2	3	45.30	16.40	1	3	26.30	0	0
Plant no.3	2	51.50	31.20	1	6	90.80	0	3
Plant no.4	2	64.40	40.10	2	8	112.40	0	0
Plant no.5	3	48.30	38.20	3	4	62.30	0	0
Plant no.6	3	52.10	45.00	4	2	34.20	0	0
Plant no.7	3	53.10	38.20	3	4	71.20	0	0
Plant no.8	4	48.30	24.00	1	0	00.00	0	0
Plant no.9	4	46.40	29.00	2	0	00.00	0	0
Plant no.10	4	28.30	19.02	1	0	00.00	0	0
Control 'Sakthi'	3	38.64	22.40	3	10	240.50	0	3

Table 30 Performance of somaclones (Group V)

Group V	Reaction to TLCV (Score)	Biometric Characters					Reaction to Bacterial Wilt	Reaction to Cercospora leaf spot
		Plant Height (cm)	Plant Spread (cm)	Primary Branches / Plant	No. of Fruits / Plant	Yield / Plant (g)		
Plant no.1	0	82.40	31.20	3	26	121.30	0	0
Plant no.2	0	78.60	28.30	3	21	102.31	0	0
Plant no.3	0	81.20	34.20	2	30	128.39	0	0
Plant no.4	0	94.20	28.00	2	31	148.70	0	0
Plant no.5	0	98.10	21.40	2	16	72.30	0	0
Plant no.6	0	82.30	31.20	3	14	82.10	0	0
Plant no.7	0	79.20	28.20	3	12	56.90	0	0
Plant no.8	0	78.20	36.40	3	09	35.40	0	0
Plant no.9	0	68.20	30.20	1	18	79.25	0	0
Plantno.10	0	78.30	36.00	2	33	169.60	0	0
Control *L. pimpinellifolium*	0	78.40	30.10	2	18	124.20	0	0

on plant number 4. Other plants were not infected. In group III (Table 27) 4 plants were susceptible to the Cercospora leaf spot. The infection varied from 10 to 50% of the leaf area. The plant number 3 in the group IV ((Table 28) was susceptible to the disease. The control plants of the tomato variety 'Sakthi' also exhibited symptoms of Cercospora leaf spot disease. The somaclones of *L. pimpinellifolium* were free from infection of the Cercospora leaf spot disease (Table 29).

Discussion

5. DISCUSSION

Somaclonal variation doesn't appear to be species or organ specific and many of the plant traits for which genetic variability is generated during tissue culture, provided a valuable adjunct to plant improvement (Bhaskaran, 1989). A wide range of variability among the regenerated plants from cell and callus cultures have stimulated interest in utilising this variation in plant breeding (Shepard, 1981).

In the last three decades significant achievements were made in inducing and utilising the genetic variability through tissue culture technique, for improvement of various crops by Barbier and Dulieu (1980) and Prat (1983) in tobacco; Evans and Sharp (1983) in tomato; Pfeiffer and Bingham (1984), Groose and Bingham (1984) in alfa alfa and Paglinso and Rappoport (1987) in celery plants. In tomato only little work has so far been done to produce disease resistant plants through biotechnological methods. The results of the work done on screening of tomato somaclones for resistance to TLCV disease are discussed here in the light of work done earlier.

5.1 STANDARDISATION OF MEDIA

5.1.1 Callus induction

5.1.1.1 Leaf segments - effect of auxins and cytokinins

NAA (0, 1, 2, 3 mg l^{-1}) and 2,4-D (0.5, 1, 1.5, 2 mg l^{-1}) were tested for their potential to produce callus from the leaf segment explants of *L. esculentum* var. sakthi and *L. pimpinellifolium*. It was found that response to 2,4-D was very little in both the species, and NAA induced only rooting. This may be because of very little movement of 2,4-D in the plant tissue as reported by Goldsmith (1977). As a result, it remains accumulated where it was applied and triggers growth in these areas. Kurtz and Lineberger (1983) also had reported that leaf explants rarely callused in the absence of growth regulators whereas, NAA alone at various levels produced roots directly from the leaf explants.

The cytokinins, BA and Kinetin did not give any response to callusing. However, the combination of auxins and cytokinins resulted in increased callus initiation, higher proliferation, higher callus index and faster growth rate. The highest callus index was obtained in MS media when combinations of NAA (2 mg l^{-1}) + BA (1 mg l^{-1}), NAA (2 mg l^{-1})+BA (2 mg l^{-1}) and NAA (2 mg l^{-1}) +BA (3 mg l^{-1}) were used. This was obtained because of the combined effect of auxins to induce callus induction and cytokinins to induce cell division resulting in enhanced growth rate. Ramiah and Rajappan (1998) also had reported that NAA at high concentration in combination with BA induced callus.

5.1.1.2 Internodal segments – Effect of auxins and cytokinins

The auxins (NAA and 2,4-D) gave average callusing and the cytokinin (BA) gave profuse callusing of the internodal segments in *L esculentum* var. Sakthi and *L pimpinellifolium.* In Sakthi excellent calli was produced in the MS media when NAA (1 mg l^{-1}) + Kinetin (1,2,3 and 4 mg l^{-1}) and NAA (3 mg l^{-1}) + Kinetin (1,2 and 3 mg l^{-1}) were used. Profuse callusing was observed in *L pimpinellifolium* in the same combinations of media, auxins and cytokinins. The use of 2,4-D and BA in the MS media also resulted in the production of excellent calli in 'Sakthi' and *L pimpinellifolium.* 2,4-D alone and in combination with BA reduced the time required for callus initiation in the media. Similar observations were reported by Goldsmith (1977). The time required for callus initiation and proliferation was less when 2,4-D was used in the medium because it provided continuous and sustained stimulus to the explants. Similar results were reported in cotyledon explants of tomato by Jawahar *et al.* (1998) and hypocotyl explants by Venkatachalam *et al.* (2000).

5.1.1.3 Nodal segments - effect of auxins and cytokinins

Auxins alone (NAA or 2,4-D) gave average callus in nodal segments whereas, cytokinin (BA) gave profuse callusing and kinetin had no significant role in callusing. Nodal segments recorded excellent callusing when combinations of auxins and cytokinin were supplemented in the media. This is probably because auxins induced callusing and cytokinin induced cell division in the media. Similar results were reported by

Paranhos *et al.* (1996) when NAA and Kinetin were used.

5.1.2 Organogenesis

5.1.2.1 Effect of auxins and cytokinins

Regeneration via callus into plantlets was achieved in *L. esculentum* var. Sakthi and *L. pimpinellifolium*. Both the species exhibited maximum frequency of shooting when NAA (2 mg l^{-1}) +BA (3 mg l^{-1}) were used in the MS medium. The percent regeneration capacity was higher in the calli of internodal segments (95%) produced in the basal medium containing NAA (2 mg l^{-1}) and BA (1 mg l^{-1}) followed by leaf segments (88.8%) in 'Sakthi'. The regeneration capacity of nodal segment was average (52.38%). In *L. pimpinellifolium* regeneration was obtained only from the leaf segments (97.7%) cultured in basal medium containing NAA (2 mg l^{-1}) and BA (3 mg l^{-1}). Similar results were reported by Geetha *et al.* (1998) and Mandal (1999) in BWR-1, BWR-6, PKM-1 and Culture-340.

It was observed that relatively high concentration of cytokinins in the media resulted in enhanced regeneration probably because of the inherent effect of cytokinins in promoting shoot bud differentiation. However Ma *et al.* (1987) reported that the ability to differentiate plant from callus was heritable and controlled by two dominant genes in tomato.

The morphogenic response of a genotype is reported to be controlled by physiological and development stage of the explant (Ohki *et al.*, 1978) and environment of source plant (Frankenberger, 1981). The regeneration efficiency varied widely with the type of explants, callus induction media and time taken for regeneration as reported by Padmanabhan *et al.* (1974), Coleman and Greyson (1977) and Selvi and Khader (1993) in leaves; de Langhe and de Bruijne (1970), Herman and Haas (1978) and Mandal (1999) in internodal segments and Pongtongkam *et al.* (1993), Paranhos *et al.* (1996) in nodal segments.

5.1.3 Effect of sucrose and agar

Maximum regeneration and number of shoots was observed from leaf calli when 3% sucrose and 0.7% agar was used in the medium. Tan *et. al* (1987) reported that the sucrose concentration is important for shoot regeneration and both high and low concentration of sucrose resulted in fewer number of shoots with poor shoot growth in tomato. Fowleer (1978) reported that sucrose is the chief mobile carbohydrate and most commonly used energy source and osmoticum. But according to Hackett (1976) high sucrose concentration promoted maturation and senescence.

5.1.4 Rooting

Effect of auxins (IAA, IBA, NAA either singly or in combination) in MS and ½ MS media for root was studied . Among all, MS + IBA (mg l^{-1}) gave 100% rooting with excellent root growth and root hairs in Sakthi. In *L. pimpinellifolium* ½ MS + IBA (0.5 mg l^{-1}) + IAA(0.5 mg l^{-1}) showed excellent root growth. Similar results were observed by Jawahar *et al* (1997) using IBA (2 mg l^{-1}) and Geetha *et al* (1998) using IBA (5 µM) in tomato. This is because auxins at high concentration promote rooting. IBA is an internationally accepted rooting hormone. Synthetic auxins such as NAA and IBA are usually more effective than IAA, apparently because they are not destroyed by IAA oxidising enzymes or other enzymes in the media.

5.2 SCREENING OF TOMATO SOMACLONAL VARIANTS FOR RESISTANCE TO TLCV DISEASE

The mean *ex vitro* survival was 46.2% in 'Sakthi' and 36.6% in *L. pimpinellifolium*. The somaclones were grouped to five groups based on the explant and the media used. The somaclones along with 'Sakthi' and *L. pimpinellifolium* were evaluated for resistance to TLCV, reaction to bacterial wilt and other diseases and various biometrical characters. Among the somaclones two plants did not show symptoms of TLCV disease. The other plants exhibited symptoms varying from mild curling of leaves to severe curling and puckering of more than 75% of leaves. The

resistance of somaclones is probably due to the *in vitro* culture conditions as suggested by Hanus *et al* (2000). They had observed increased tolerance of somaclones to Cucumber Mosaic Virus in tomato. Mandal (1999)reported that somatic callus derived plants showed resistance to bacterial wilt in four tomato varieties viz.BWR-1,BWR-2,PKM-1 and Culture-340.Barden and Murakishi(1985)reported that susceptible lines of tomato somaclones showed resistance to TMV one month after inoculation of TMV virus.Zhuk(1998) found that tomato somaclones showed TMV resistance ,when the plants regenerated from passivated callus tissue of different aged gamma irradiated callus.

To study the extent of tissue culture induced variation a few horticultural characters were documented in the somaclones as well as in the control. There was wide variation for plant height in the somaclones 'Sakthi' and *L.pimpinellifolium*. Mandal *et al* (1996) had reported occurrence of somaclonal variants for plant height in rice. Similar observations were reported by Mandal (1999) in leaf and internodal derived somaclones. The plant spread also showed a great deal of variation in Sakthi when compared to *L. pimpinellifolium*. Plant spread may precisely offer more selection opportunity to choose suitable plant type for optimum yield. Two somaclones exhibited gigantism with broader leaves (Potato leaf shape). These finding are in agreement with the reports of Somasundar and Gostimsky (1992) in cotyledonary explants of the Russian tomato Dabok, and de Langhe and de Bruijne (1970) in the progenies of plants regenerated from stem internode of *L. esculentum* var. Sakthi and *L. pimpinellifolium*.

Two somaclones were observed free from fruit cracking and another two somaclones yielded higher than the control. These morphological variations may be due to explants materials, length of culture period, growth regulators and nutritional stress in the medium.

Overall, it can be concluded that somaclonal variation expressed as wide range of variation in fruit yield and resistance to TLCV disease may have far reaching potential in identifying TLCV resistant line(s) in tomato. Creating such useful traits in the high yielding bacterial wilt resistant background of Sakthi will be off immense importance in

enhancing the productivity. The study also indicated the possibility of using somaclonal variation in genetic upgradation of tomato as feasible proposition in contrast to conventional breeding approaches

Summary

6. SUMMARY

Experiment entitled "Screening of tomato somaclones for resistance to tomato leaf curl virus (TLCV) was conducted in the Dept. of Olericulture, College of Horticulture, Vellanikkara, Thrissur with the following objectives.

1. Standardisation of explants of *L. esculentum* var. Sakthi and *L. pimpinellifolium* for callus induction
2. Standardisation of media for
    a. Callus induction
    b. Organogenesis
    c. Rooting

3. Screening of somaclones for resistance to TLCV diseases in bacterial wilt sick soil

In the first two experiments explants used were leaf segments, internodal segments and nodal segments. These were cultured on MS and ½ MS medium supplemented with various concentrations and combinations of growth regulators viz. auxins (NAA and 2,4 – D) and cytokinins (BA and kinetin). Observations were recorded on number of days for callus induction, Percentage callusing,growth rate and callus index.

Among the treatment combinations, the leaf segment exhibited high callus index in the MS media when combinations of NAA (2 mg l^{-1}) + BA (1 and 2mg l^{-1}) were used for Sakthi and *L. pimpinellifolium.*

Regarding internodal segments, MS with NAA (1 mg l^{-1}) + Kinetin (4 mg l^{-1}) recorded maximum callus index in Sakthi whereas MS medium with NAA (1 mg l^{-1}) + kinetin (1mg l^{-1}) gave excellent calli in *L. pimpinellifolium.*

In the case of nodal segments MS medium with NAA (1 mg l^{-1}) + BA (4 and 5 mg l^{-1}) recorded early callusing, highest percent callusing and highest callus index in both 'Sakthi' and *L. pimpinellifolium*.

With the progress in the experiment, both 'Sakthi' and *L. pimpinellifolium* exhibited maximum frequency of shooting in leaf and internodal calli when NAA (2 mg l^{-1}) + BA (3 mg l^{-1}) were used in the MS medium. The percent morphogenic capacity was highest in the internodes (95 %) followed by leaf segments (88.8 %) in Sakthi. The morphogenic capacity of nodal segment was average (52.38 %) in Sakthi. Whereas in *L. pimpinellifolium* regenerants was obtained only from the leaf segments (97.7 %).

Regarding effect of sucrose and agar in the regeneration medium, maximum regeneration and number of shoots was obtained from leaf calli when basal medium supplemented with NAA (2 mg l^{-1}) + BA (3 mg l^{-1}) + 3 % sucrose and 0.7 % agar was used in the media.

In 'Sakthi' highest percent root growth and maximum number of root hairs was obtained in MS medium with IBA (1 mg l^{-1})and in *L. pimpinellifolium*, it was recorded in the MS medium with IBA (0.5 mg l^{-1}) + IAA (1.5 mg l^{-1}).

In the third experiment screening of somaclonal variants resistant to TLCV disease, the mean *ex vitro* survival was 46.2 % in Sakthi and 36.6 % in *L. pimpinellifolium*.

Among the'Sakthi' somaclones from different explants two plants were free from TLCV disease and 5 plants showed only mild curling and puckering . The somaclones obtained from *L. pimpinellifolium* were found to be free from TLCV disease.

Plant height and plant spread showed a wide variation in 'Sakthi'somaclones when compared to *L.pimpinellifolium*. Two somaclones exhibited gigantism with broader leaves (Potato leaf shape).

In the case of fruit character and yield two somaclones were found to be free from fruit cracking and another two somaclones yielded higher than the control.

The future line of work include advancing the S_1 generation of 'Sakthi' and *L. pimpinellifolium* and screening for resistance to TLCV disease in wilt sick field. The plants having combined resistance to bacterial wilt and TLCV disease and good horticultural characters will be selected. Similar work can be extended to other bacterial wilt resistant varieties which are susceptible to TLCV disease in Kerala.

References

Reference

AICVIP (1994) Status Report; 1985 - 1995. All India Co-ordinated Vegetable Improvement Project Kerala Agricultural University, Vellanikkara, Thrissur, India.p.31

Ali, Y. and Li, S.J.1994. Effect of various growth regulators on the initiation, size, colour and differentiation of callus in different genotypes of tomato. *Sarhad J. Agric.*10: 399-405

*Ancora, K., Sree Ramulu, K.and Devreux, M.1977. *In vitro* culture of anther and stem internodes of *L.peruvianum*: nuclear DNA determination in calli and cytological analysis of regenerated plants. *Z. Pflanzenphysiol.*82: 377-388.

Avidov, Z. 1978. Bionomics of tobacco white fly in Israel. *Nature* 7: 25-41

Banerjee, M. K. and Kalloo,G. 1998. Multiple disease resistant haploid plant of tomato (*L.* esculentum.Mill.) from anther culture. National Symposium on Emerging Scenario in Vegetable Research and Development. December. 12-14, Project Directorate of Vegetable Research, Varanasi *Abstract* 193.

*Barbier, M. and Dulieu, H.L.1980. Genetic changes observed in tobacco plants regenerated from cotyledons by *in vitro* culture. *Ann. Amelior Pl.*30: 321-344.

Barden, K.A. and Murakishi, H.H.1985.Leaf regeneration and virus screening of somaclones of isogenic lines of tomato.*Phytopathology.*75: 1359.

Behki, R.M. and Lesley, S.M.1976.*In vitro* plant regeneration for leaf explants of *Lycopersicon esculentum* (tomato). *Can.J.Bot.*54: 2409-2414.

*Behki, R.M., Lesley, S.M.1980.Shoot regeneration from leaf callus of *L. esculentum* Z. *Pflanzenphysiol.* 98:83-87.

Behnke, M. 1979. Selection of potato callus for resistance to culture filtrate of *Phytophthora infestans* and regeneration of resistant plants. *Theor. appl. Genet.* 55: 66-71

Behnke, M. 1980. General resistance to late blight of *Solanum tuberosum* plants regenerated from callus resistant to culture filtrate of *Phytophthora infestans*. *Theor. appl. Genet.* 56: 151-152

Bhaskaran, S. 1989. Somaclonal variation. *Biotechnology in Agriculture* (eds. S. Natesh, V. L. Chopra and S. Ramachandran), Oxford and IBH Pub. Co. Pvt. Ltd., New Delhi, p: 137

Bhojwani, S.S. and Bhatnagar, S.P.1974.*The Embryology of Angiosperm.* Vikas Publishing House Pvt. Ltd., Bombay, India p: 368

*Bobisud, C.A, Martin, S.P. and Sekioka, T.T.1996. Field-testing bacterial wilt resistant tomato somaclones. *J. Am. Soc. Hort. Sci.* 121: 384-387.

*Butenko, P.G..(ed.). 1967. *Plant Tissue Culture and Plant Morphogenesis.* Israel programme for scientific translation. Jerusalem.

Butter, N. S. and Rataul, H. S. 1978. Influence of temperature on the transmission efficiency and acquisition threshold of white fly (*Bemisia* tabaci Gem.) in the transmission of tomato leaf curl virus. *Sci. Cult.* 44: 168-170

*Capote, R. A., Fundora, M. and Perez, D.O.2001.Effect of different factor on the *in vitro* plant regeneration from leaflets of five genotypes of tomato (*Lycopersicon esculentum* Mill) *Revista del Jardin Botanico Nacional.* 21: 71-76

Cappadocia, M. and Sree Ramulu, K.1980.Plant regeneration from *in vitro* cultures of anther and stem internodes in an interspecific hybrid, *Lycopersicon esculentum*. L x *L. peruvianum*. Mill. and cytogenetic analysis of the regenerated plants. *Pl. Sci.Lett.* 20:157-166.

Chandel, G. and Katiyar, S.K.2000.Organogenesis and somatic embryogenesis in tomato (*L esculentum* Mill) *Adv. Pl. Sci.* 13:11-17

*Cohen, S., Kern, J., Harpaz, I. and Brn. Joseph, R. 1988. Epidemiological studies of tomato yellow leaf curl virus in Jordan valley, Israel. *Phytoparasitica* 16: 259-270

Coleman, W.K. and Greyson, R.I.1977.Analysis of formation in leaf discs of *L. esculentum* Mill cultured *in vitro*. *Ann. Bot.* 41:307-320.

Colijn, H. T.1986. *Lycopersicon esculentum* regeneration from leaf discs and protoplasts. International symposium on Plant molecular biology. Strasbury, France. *Pl. Breed Abstr.* 56: 11142.

Cummings, D.P., Green, C.E. and Struthman, D.D.1976. Callus induction and plant regeneration in oats. *Crop Sci.*16: 465-470.

D'Amato, F.1977.Cytogenetics of differentiation in tissue and cell culture. *Applied and Fundamental Aspects of Plant cell, tissue and organ culture,* pp.343-357.J.Reinert and Y.P.S.bajaj (Eds.) Springer-verlag, Berlin.

de Langhe, E.1973.Vegetative multiplication *in vitro*. *int. Ass. Pl. Tissue Cult. Newsl.* 9:7

de Langh.E.and de Bruijne, E.1970. Continuous propagation of tomato by means of callus culture. *HortScience* 4:221-227.

Dhruva, B., Ramakrishnan, T.and Vaidyanathan, C.S.1978. Regeneration of hybrid tomato plants from leaf callus. *Curr. Sci.* 47:458-460

Dhruva, B., Ramakrishnan, T.and Vaidyanathan, C.S.1978. Regeneration of hybrid tomato plants from leaf callus. *Curr. Sci.* 47:458-460

Dwivedi, K., Srivastava, P., Verma, H.N. and Chadurvedi, H.C.1990.Direct regeneration of shoots from leaf segments of tomato (*Lycopersicon esculentum*) cultured *in vitro* and production of plants. *Indian J.exp.Biol.* 28:32-35.

Edallo, S., Zuchinali, C., Perenzin, M.and Salamin, F.1981.Chromosomal variation and frequency of spontaneous mutation associated with *in vitro* culture and plant regeneration in maize. *Maydica*.26: 39-56.

ElEnamy, A.E.1997.Shoot regeneration and protein synthesis in tomato tissue cultures. *Biologia Plantarum* 39: 303-308

Evans, D.A., and Sharp, W.R.1983.Single gene mutation in tomato plants regenerated from tissue culture. *Science.*221: 949-951.

Evans, D.A., Sharp, W.R. and Medina-Filha, H.P.1984.Somaclonal and gametoclonal variation *Am. J. Bot.* 71: 759-774.

Evans, D.A.1989.Somaclonal variation-genetic basis and breeding applications. *Trends in Genet.*5: 46-50

*Fari, M., De Resende, G.M. and De Nelo, N.F. 2000. Evaluation of the *in vitro* regeneration capacity in Brazilian industrial tomato. *Perquisa Agroperiuaria Brasileira.* 35:1523-1529

*Fowleer, M. W. 1978. Regulation of carbohydrate metabolism in cell suspension culture. In: *Frontiers of plant tissue culture* (Thorpe, T.A.), Calgary Univ, Calgary Printing Services, Calgary, pp: 443-452

*Frankenberger, E. A., Hasegawi, P. M. and Tigcheller, E. C. 1981. Influence of environment and developmental state on the shoot forming capacity of tomato genotypes. *Z. Pflanzenphysiol.* 102: 221-232

Geetha, N., Venkatachalam, P., Reddy, P.S. and Rajaseger, G.1998.*In vitro* plant regeneration from leaf callus cultures of tomato (*Lycopersicon esculentum.* Mill). *Adv. Pl. Sci.* 11: 253-257

Goldsmith, M.H.M.1977. The polar transport of auxins. *A. Rev.Pl.Physiol.*28: 439

Green, S. K., and Kalloo. G. 1994. Leaf curl and yellowing virus of pepper and tomato: An overview. Technical Bulletin No.21 AVRDC, Taiwan

Green, C.E., Philips, R.L. and Wang, A.S.1977. Cytological analysis of plants regenerated from maize tissue cultures. *Maize Genetic Cooperative Newsl.*51: 53-54.

Gresshoff, P.M. and Doy, C.H.1972.Development and differentiation of haploid *Lycopersicon esculentum* Mill. *Planta.*107: 161-170

Groose, R.W. and Bingham, E.T.1984.Variation in plants regenerated from tissue culture of tetraploid alfalfa heterozygous for several traits. *Crop Sci.*24: 655-658.

Hackett, W. P. 1976. Control phase change in plants. *Acta Horticulturae.* 56: 143-154

*Hanus, F. E., Lich, M., Pindel, A. and Miczynki, K. 2000. Selection of virus resistance in tomato exposed to tissue culture procedures. *Acta Physiologiae Pl.* 22: 317-324

Hayati, J. 1978. Some investigation on control of tomato leaf curl virus in Haryana. M.Sc. thesis, Haryana Agricultual University, Hissar, p: 113

Heinz, D.J. and Mee, G.W.P.1969.Plant differentiation from callus tissue of *Saccharum spp. Crop Sci.*9: 346-348

*Herman, E.B. and Haas, G.J.1978.Shoot formation in tissue cultures of *Lycopersicon esculentum* Mill. *Z. Pflanzenphysiol.*84: 467-470.

Hussain, M.A. 1932. Leaf curl in cotton and other plants. *Nature 130*: 132

*Ichimura, K. and Oda, M.1995.Stimulation of shoot regeneration from cotyledon segment of tomato (*Lycopersicon esculentum* Mill) by agar and its extract. *J. Jap. Soc. hort. Sci.* 64: 135-141.

Jawahar, M.; Mohamed, S.V. and Jayabalan.N.1997.*In vitro* callus culture and plant regeneration from different explants of *Lycopersicon esculentum* Mill. *J. Physiol. Res* .10: 75-78

Jawahar, M.; Mohamed, S.V. and Jayabalan.N.1998.*In vitro* induction of plantlets regeneration from cotyledonary callus of tomato (*Lycopersicon esculentum* Mill.) *Adv. Pl. Sci.* 11: 199-204

Jones, M.G.K., Maddock, S.E., Karp, Nelson, R.S., Creissen, G.P., Foulger, D. and Bright, S.W.J.1984.Tissue and protoplast culture- the way to new crop plant varieties. *The World Biotech Report.*1984.Vol.1.Europe.pp.443-454.

Kalloo,G.and Banerjee,M.K.2000. H-24: Moderately leaf curl resistant variety of tomato (*Lycopersicon esculentum* Mill.).*Veg.Sci.*27: 117-120

Kartha, K.K., Champoux, S., Gamborg, O.L. and Pahl, K. 1977. *In vitro* propagation of tomato by shoot apical meristem culture. *J. Am. Soc. hort. Sci.* 102: 346-347.

*Kartha, K.K., Gamborg, O.L., Shyluk, J.P. and Constabel, F.1976.Morphogenetic investigations on *in vitro* leaf culture on tomato (*Lycopersicon esculentum* Mill.cv.Starfire.) and high frequency plant regeneration. *Z. Pflanzenphysiol.*77: 292-301

Kurtz, S.A. and Lineberger, R.D.1983.Genotypic differences in morphogenic capacity of cultured leaf explants of tomato.*J.Am.Soc.hort.Sci.*108 : 710-714.

Larkin, P.J. and Scowcroft.1981.Somaclonal variation: a novel source of variability from cell culture for plant improvement.*Theor.appl.Genet.* 60:197-214.

Larkin, P.J., Ryan, S.A., Brettell, R.I.S. and Scowcroft, W.R.1984.Heritable somaclonal variation in wheat. *Theor.appl.Genet.* 67:443-455.

Lercari, B., Moscatelli, S., Ghiradi, E.Nicelora, R. and Berkram, L. 1999. Photomorphogenic control of shoots regeneration from etiolated and light grown hypocotyls of tomato. *Pl.Sci.*140: 53-62

Lich, M., Miczynski, K. and Pindel, A.1996 Comparison of regeneration potential in tissue cultures of primitive and cultivated tomato species (*Lycopersicon species*) *Acta Soc. Botanicorum Poloniae,* 65:53-56.

Locy, R.D.1983.Callus formation and organogenesis by explants of six *Lycopersicon species. Can.J.Bot.61: 1072-1079*

Ma, H. T., Gui, M. H. and Liong, G. H. 1987. Plant regeneration from cultured immature embryos of *Sorghum bicolor* (L.) Moench. *Theor. appl. Genet.* 73: 389-394

Mandal, A. B., Prashant, M. and Bandyopadhyay, A. K. 1996. Assessment of somaclonal variation induced in an *indica* rice. *Oryza* 33: 89-94

Mandal, A.B.1999.Efficient somaculture system and exploitation of somaclonal variation for bacterial wilt resistance in tomato. *Indian J.Hort.*56: 321-327

Martelli, G. P. and Quacquarelli, A. 1992. The present status of tomato and pepper viruses. *Acta Horticulturae.* 127: 36-64

Mascarenhas, A. F. 1991. *Hand book of Plant Tissue Culture,* ICAR Publications, New Delhi, India, p: 182

Mayee, C.D., Kanwar, J.S. and Nandpuri, K.S.1974. The comparative performance of different genotypes of tomato vis-vis leaf curl and mosaic. *P.A.U.J.Res.*11 : 362-364

Mazyad, H. M., Omar, F. and Tahen, K. Al. 1979. Observations on the epidemiology of tomato yellow leaf curl disease on tomato plants. *Pl. Dis. Rep.* 63:695-698

*Meredith, C.P. 1979. Shoot development in established callus cultures of cultivated tomato (*L.esculentum* Mill.) *Z.Pflanzenphysiol.* 95:405-411.

Mishra, A. K., Narayan, M. and Latif, H.1998. Reaction of tomato Leaf Curl Virus in certain tomato genotypes. Silver Jubilee National.Symposium. December. 12-14, Project Directorate of Vegetable Research, Varanasi *Abstract*193.

Monaco, L. C., Sondhal, M. R.,Carvalho, A., Crocomo, O. J. and Sharp, W.R.1977. Application of tissue culture for improvement of Coffee. In: *Plant Cell, Tissue and Organ Culture* (eds. Reinert, J. and Y .P .S. Bajaj) pp: 109-129

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Pl. Physiol.* 15: 473-497

Nainar, P. 1996. Studies on breeding of tomato (*Lycopersicon esculentum* Mill.) for resistance to leaf curl virus disease. *Ph.D. thesis,* Tamilnadu Agricultural University, Coimbatore, and p: 187

Newman, P.G., Krishnaraj.S. and Saxena, P.K.1996. Regeneration of tomato (*Lycopersicon esculentum* Mill.) somatic embyogenesis and shoot organogenesis from hypocotyls explants induced with 6-benzyladenine. *Int. J. Pl. Sci.* 157:554-560.

Ohki, S., Bigot, C. and Mousseau, J. 1978. Analysis of shoot forming capacity *in vitro* in two lines of tomato (*Lycopersicon esculentum* Mill.) and their hybrids. *Pl. Cell Physiol.* 19: 27-42

Padmanabhan, V., Paddock, E.F., Sharp, W.R.1974. Plantlet formation from *Lycopersicon esculentum* leaf callus. *Can.J.Bot.* 52:1429-1432.

Pal, B. F. and Tandon, R. N. 1937. Types of tobacco leaf curl in Northern India. *Indian J. agric. Sci.* 7: 368-393

*Paranhos, J.T., Perrando, E., Franco, E.T.H. and Aita, A.1996.*In vitro* regeneration of tomato cultivars Empire and Monte Carlo. *Horticultura Brasileira* 14: 203-206

Pfeiffer, T.W. and Bingham, E.T.1984.Comparison of alfalfa somaclonal and sexual derivatives from the same genetic source.*Theor.appl.Genet.* 67:263-266.

Phillips, R. L., Kaeppler, S. M. and Olhaft, P. 1994. Genetic instability of plant tissue cultures - Breakdown of normal controls. *Proceedings of the National Academy of Sciences of United States of America* 91: 5222-5226

Pilowsky, M. and Cohen, S. 1974. Inheritance of resistance to tomato yellow leaf curl virus in tomato. *Phytopathology.* 64: 632-635

Plastira, V.A., and Perdikaris, A.K.1997.Effect of genotype and explant type in regeneration frequency of tomato *in vitro. Acta Horticulturae.*447: 231-234.

Pongtongkam, P,; Ratisoontorn,P.; Suputtitada,S.;Priyachoknagal,S.;Ngirnsiri,L. and Thonpan,A.1993.Tomato propagation by tissue culture. *Kasetsart J. nat. Sci.*27 : 269-277.

Prat, D.1983.Genetic variability induced in *Nicotiana sylvestris* by protoplast culture. *Theor.appl.Genet.*64:223-230.

Pruthi, H. S. and Samuel, C. K. 1939. Entomological investigation on leaf curls disease of tobacco in Northern India. *Indian J. agric. Sci.* 11: 389-409

Pruthi, H. S. and Samuel, C. K. 1942. Entomological investigations on the leaf curl disease of tomato in Northern India - Biology and population of white fly. *Indian J. agric. Sci.* 12: 35-37

Puglinso, H. S., and Rappoport, C. 1987. Somaclonal variation: selection for disease resistance in celery. *In vitro* 23:494

Ramiah, M.and Rajappan, K.1998.Direct shoot regeneration from excised cotyledonary leaf of tomato. *S. Indian Hort.* 44 : 101-102

Ratan, R.S. and Bindal,A. 2002. Reaction of tomato (*Lycopersicon esculentum* Mill.) genotypes against leaf curl virus. *Veg.Sci.*29 : 176-177

Ratul, K. B. and Bordoloi, D. K. 1988. Influence of planting time on the incidence of leaf curl virus disease and white fly population on tomato. *Indian J. Virol.* 14 : 71-73

Reddy, A. S., Rosaiah, B., Bhaskara Rao, T., Rama Rao, B. and Venugopal Rao, V. 1986. The problem of white fly on cotton. *Proceeding of the seminar on 'Problem of White Fly on Cotton', Pune,* 16th March 1986

Saikia, A. K. and Muniyappa, V. 1989. Epidemiology and control of tomato leaf curl virus in Southern India. *Trop. Agric.* 66: 350-354

Selvi, D.T. and Khader, M.A.1993. *In vitro* morphogenetic capacity of tomato (*Lycopersicon esculentum* Mill) var. PKM.1 *S. Indian Hort.* 41: 251-58

Shahin, E.A. and Spivey, R.1986.A single dominant gene for Fusarium wilt resistance protoplast-derived tomato plants. *Theor.appl.Genet.*73: 164-169.

Shepard, J.F.1980.Mutant selection and plant regeneration from potato mesophyll protoplasts. In: *Genetic improvement of crops/emergent techniques* (edn.) Univ. of Minnesota Press. Minneapolis. pp 185-219

Shepard, J. E.1981. Protoplast as source of disease resistance in plant. *A. Rev. Phytopath.* 19: 145-166

Smith, S.S and Murakishi, H.H. 1987. Nature of resistance of tomato somaclones to tomato mosaic virus (T_0MV)- Somaclonal variation. *Phytopathology.* 78: 1536

Somasundar, M. and Gostimsky, S.A.1992. Somaclonal variation in tomato tissue culture. *Crop Improv.* 19 : 92-96.

Soniya, E.V., Banerjee, N.S. and Das, M.R.2001.Genetic analysis of somaclonal variation among callus derived plants of tomato. *Curr. Sci.* 80: 1213-1215.

Sotirova, V., Shtereva, L., Zagorska, N., Demitrov, B. and Bogatsevska, N. 1999. Resistance responses of plants regenerated from tomato anther and somatic tissue cultures to *Clavibacter michiganense* Ssp. *michiganense, Israel J. Pl. Sci.* 47 : 237-243

Sukumar, S. and Sree Rangaswamy, S.R. 1988. Response of *in vitro* leaf callus cultures for regeneration and evaluation of the regenerents in tomato. *Curr. Sci.* 57: 890-892.

Sun, Zong-Xiu, Chen-Zhang, Z., Kang-le, Z., Xiu-Fang, Q. and Ya-Ping, Fu. 1983. Somaclonal genetics of rice, *Oryza sativa.* L. *Theor. appl. Genet.* 67: 67-73

Taj, M., Dehan, K. and Heikin, H.1977.Morphogenic potential of cultured leaf sections of cultivated and wild species of tomato. *Ann .Bot.* (London) 41:937-941

Tan, M. M. C., Collijn-Hooymans, C. M., Lindhout, W. H. and Kool, A. J. 1987. A comparison of shoot regeneration from protoplasts and leaf discs of different genotypes of cultivated tomato. *Theor. appl. Genet.* 75: 105-108

Thanutong, P., Furusawa, L. and Yamato, M. 1983. Resistant tobacco plants from protoplast derived callus selected for their resistance to *pseudomonas* and *Alternaria* toxins. . *Theor. appl. Genet.*66: 209-215

Thung, T. H. 1932. De krulen kroepockziekten van tabak en de corzsken van har ver breeding. Proeft. vor stenlandsche Tabal Meded 72. *Rev. Appl. Mycol.* 11: 478-479

Tikoo, S. K.,2002. Vegetable breeding in Asia-important milestones and future prospects. International conference on vegetable 11-14, November 2002. Dr.Premnath Agricultural Science Foundation, Bangalore.*Abstract* 42-45

Venkatachalam, P., Geetha, N., Priya, P., Rajasegar, G. and Jayabalan.N.2000.High frequency plantlet regeneration from hypocotyls explants of tomato(*Lycopersicon esculentum* Mill) via organogenesis. *Pl. Cell Biotech. molec. Biol.*1 : 95-100.

Verma, A., Kalloo, G., Singh, K.P. and Banerjee,M.K.2002.Production,Productivity and export of vegetables. Technical Bulletein No.7. Indian Institute of Vegetable Research, Varanasi, p.56

Vnuchkova, V.A.1977.Development of a method for obtaining regenerant tomato plant under tissue culture condition. Soviet *Pl. Physiol.* 24: 884-889

*Ye, Z.B.; Li, H.X. and Zhou, G.L.1994. *In vitro* culture of tomato cotyledons and regenerated plants. *J. Huazhong agric. Univ.*13 : 1291-95

*Zagorska, N., Abadjceva, M., Chalukova, M., Achkeva, Z. and Nikova, V. 1985. Somaclonal variation in tomato plants regenerated from tissue cultures.Proceedings of a syposium organised jointly by the IAEA and FAO,19-23 August *Pl. Breed. Abstr.* 56 : 9338

*Zhuk, I.P. 1993. Resistance of tomato somaclones to tobacco mosaic virus. *Mikrobiologichnii-zhurmal.* 55 : 41-46.

*Zhuk, I.P. 1998. Production of tomato clones resistant to TMV using somaclonal variability. *Mikrobiologichnii- Zhurmal.* 60 : 38-42.

*Original not seen

SCREENING OF TOMATO SOMACLONES FOR RESISTANCE TO TOMATO LEAF CURL VIRUS

By

BIJU DANIEL

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

Faculty of Horticulture
Kerala Agricultural University

Department of Olericulture

COLLEGE OF HORTICULTURE

VELLANIKKARA THRISSUR-680 656

KERALA, INDIA

2003

ABSTRACT

An investigation on "Screening of tomato somaclones for resistance to Tomato Leaf Curl Virus" was undertaken in the Department of Olericulture, College of Horticulture during 2000 – 2002 to standardize the explants of *L.esculentum* var. 'Sakthi' and; to standardize the media for callus induction, organogenesis and shoot induction in tomato and to screen the somaclones of bacterial wilt resistant 'Sakthi' and *L. pimpinellifolium* for resistance to Tomato Leaf Curl Virus.

The leaf segments exhibited high callus index in the MS medium supplemented with NAA (2 mgl^{-1}) and BA (1 mgl^{-1}, 2 mgl^{-1} and 3mgl^{-1}) for 'Sakthi' and *L. pimpinellifolium.* The basal medium containing NAA (1 mgl^{-1}) and kinetin (4 mgl^{-1}) gave maximum callusing in internodal segments of 'Sakthi'. In the case of nodal segments MS medium supplemented with NAA (1 mgl^{-1}) and BA (4 and 5 mgl^{-1}) recorded early callusing and highest callus index in both 'Sakthi' and *L. pimpinellifolium.*

The percent morphogenic capacity was highest in internodal calli (95%) followed by leaf segment calli (88.8%) in 'Sakthi' when basal medium supplemented with NAA (2 mgl^{-1}) and BA (3 mgl^{-1}).

In 'Sakthi' the highest percent root growth and maximum number of root hairs was obtained in MS medium with IBA (1 mgl^{-1}). In *L. pimpinellifolium* percent root growth was maximum in the medium containing IBA (0.5 mgl^{-1}) and IAA (1.5 mgl^{-1}).

Screening of somaclones of 'Sakthi ' and *L. pimpinellifolium* for resistance to TLCV disease, transmitted by white fly *Bemisia tabaci* has revealed that two somaclones of 'Sakthi' were free from the disease and five plants exhibited mild symptoms. Two somaclones yielded higher than control and another two plants were free from fruit cracking.

The somaclones of *L. pimpinellifolium* were free from TLCV disease. However the resistance to the disease improvement in horticultural traits can be confirmed only in subsequent generations.