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PARENTS

NANA AND AAI

..... BALU

INTERSPECIFIC HYBRIDIZATION IN *LYCOPERSICON* SPECIES

By

Balu Jagannath Shete

R.No.9926

A Thesis submitted to

MAHATMA PHULE KRISHI VIDYAPEETH

RAHURI-413 722, DIST. AHMEDNAGAR
MAHARASHTRA STATE (INDIA)

In partial fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

HORTICULTURE

DEPARTMENT OF HORTICULTURE
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI - 413 722, DIST. : AHMEDNAGAR

2003

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CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or other person to any other University or Institute for a Degree or Diploma.

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CERTIFICATE

This is to certify that the thesis entitled, "**INTERSPECIFIC HYBRIDIZATION IN *LYCOPERSICON* SPECIES**", submitted to Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **HORTICULTURE**, embodies the results of a *bona fide* research work carried out by **SHRI. BALU JAGANNATH SHETE**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance received by him during the course of investigation and source of literature have been duly acknowledged.

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CERTIFICATE

This is to certify that the thesis entitled, "**INTERSPECIFIC HYBRIDIZATION IN *LYCOPERSICON* SPECIES**", submitted to Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **HORTICULTURE**, embodies the results of a *bona fide* research work carried out by **SHRI. BALU JAGANNATH SHETE,** under the guidance and supervision of **Dr. R. S. Patil**, Professor of Horticulture, Department of Horticulture, MPKV, Rahuri-413 722, Dist. Ahmednagar (M. S.). It is sufficiently high standard to warrant its submission to the University for the award of the said degree. No part of the thesis has been submitted for any other degree or diploma.

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Place : Rahuri,

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(**B. J. Shete**)

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LIST OF ABBREVIATIONS

$AgCl_2$	:	Silver chloride
APS	:	Ammonium per sulphate
BA	:	Benzyl adenine
BAP	:	6-Benzyl amino-purine
BC	:	Back cross
°C	:	Degree celsius
C.D.	:	Critical difference
cm	:	Centimeter(s)
Cv.	:	Cultivar
d	:	day
DAT	:	Days after transplanting
DC	:	Direct current
Dha.	:	Dhanashree
E	:	Explant
EC	:	Electrical conductivity
Ed.	:	Editor
EDTA	:	Ethylene diamine tetracetic acid
e.g.	:	Exampli gratia (Example)
et al.	:	et allia (and others)
etc.	:	Etcetra
f	:	Fresh (Leaf explant)
Fig.	:	Figure
F_1		Sexual hybrid plant
g	:	Gramme (s)
G	:	Genotype(s)
GA_3	:	Gibberellic acid
g. f. wt.	:	gramme fresh weight
ha	:	hectare (s)
IAA	:	Indole acetic acid
IBA	:	Indole butyric acid
i.e.	:	id est (Latin : for instance)
kg	:	Kilogramme
Kinetin	:	6-furfuryl amino purine
l	:	Litre(s)
L	:	*Lycopersicon*
mg	:	Milligramme (s)

mg l^{-1}	:	Milligramme per litre
ml	:	Millilitre (s)
mm	:	Millimetre(s)
mol	:	Molecualr
MS	:	Murashige and Skoog medium (1962)
NAA	:	Naphthalene acetic acid
NaCl	:	Sodium chloride
NCP	:	Natural cross pollination
No(s).	:	Numbers
PAGE	:	Polyacrylamide gel electrophoresis
PEG	:	Polyethylene glycol
PGR	:	Plant growth regulator (s)
pH	:	Negative logarithm of hydrogen ion concentration
PI	:	Plant introduction
RAPD	:	Random amplified polymorphic DNA
RFLP	:	Restriction fragment length polymorphism
RH	:	Relative humidity
Rm	:	Relative migration value
rpm	:	Revolutions per minute
S.D.	:	Standard deviation
SDS	:	Sodium dodecyl sulphate
S.E.	:	Standard error of means
TEMED	:	N,N,N^{1},N^{1}-tetramethyle ethylene diamine
TLC	:	Tomato leaf curl virus
TMV	:	Tobacco mosaic virus
TSWV	:	Tomato spotted wilt virus
TT	:	Thousand tonnes
TYLC	:	Tomato yellow leaf curl virus
viz.	:	namely
v/v	:	Volume to volume ratio
Wt.	:	Weight
w/v	:	Weight to volume ratio
x	:	Basic set of chromosomes
µg	:	Micro gramme
2,4-D	:	2,4-dichlorophenoxy acetic acid
<	:	Less than
>	:	More than
%	:	Per cent

ABSTRACT
INTERSPECIFIC HYBRIDIZATION IN *LYCOPERSICON* SPECIES

By

BALU JAGANNATH SHETE

A candidate for the degree of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

Mahatma Phule Krishi Vidyapeeth,
Rahuri-413 722.

2003

Research Guide	:	Dr. R. S. Patil
Department	:	Horticulture

The major objective of interspecific hybridization is to incorporate resistance for biotic and abiotic stresses from wild taxa to cultivated type. Major crop improvement in tomato (*L. esculentum*) has been achieved for transfer of economic attributes from wild *Lycopersicon* species to commercial tomato cultivars, especially for disease resistance through interspecific hybridization. Eventhough wealth of nine species is available in genus *Lycopersicon* certain crossing barriers are observed for interspecific hybridization. In genus *Lycoperesicon*, "*peruvianum* complex" contained two species viz., *L. peruvianum* and *L. chilense* which distantly related with tomato (*L. esculentum*) and showed severe barriers for interspecific hybridization. But, "*peruvianum* complex" is the most important reservoir as a source of resistance for biotic and abiotic stresses.

Considering best source of resistance for biotic and abiotic stresses and its limited application in tomato breeding; *L. peruvianum* species was utilized in present investigation for tomato interspecific hybridization programme; which was carried out at MPKV, Rahuri during 2000-2003.

The study involved two tomato cultivars (viz., cv. Dhanashree and 85-1) were reciprocally crossed with six accessions of *L. peruvianum*. For development of *in vitro* interspecific F_1 hybrids, immature seeds from various eight fruit growth stages starting from 10 days after pollination with five days interval upto 49 DAP were cultured on 26 MS based media with various levels of cytokinins (BAP) and auxins (NAA). The *ex-vitro* F_1 plants grown under field conditions were used for morphological and biochemical studies for hybridity confirmation. The interspecific F_1 hybrids were also screened against biotic and abiotic stresses. Furthermore, plant regeneration studies were undertaken using 2 explants (leaf and cotyledon) of 3 genotypes (two tomato cultivars viz., Dhanashree and Bhagyashree and *L. peruvianum* accession EC 106294) on 20 culture media.

In *L. esculentum* x *L. peruvianum* hybridization, an unilateral compatibility was observed with use of tomato cultivar as a maternal parent and *L. peruvianum* as a pollen parent and 46 to 57.28% crossing index was recorded. Still decisive mechanism of post-zygotic sterility was observed for hybrid seed development; as immature seeds were observed at fruit ripening during interspecific hybridization.

Among eight fruit stages studied, the fruit growth stage of 30 to 34 DAP was found optimum which gave 0.77% seed germination for culture of immature seeds particularly in crosses involved tomato cv. Dhanashree as a maternal parent whereas for crosses involved tomato cv. 85-1 as a maternal parent, fruit growth stage of 25 to 29 DAP was optimal and gave 0.38% seed germination. Thus, fruit growth stage was an important parameter in culture of immature seed of tomato interspecific hybrids and it was based on involvement of tomato cv. as a maternal parent.

The best culture medium was MS based medium without supplement of plant growth regulators for culture of immature seed of *L. esculentum* x *L. peruvianum* (1.46% seed germination).

An interspecific cross of tomato cv. Dhanashree with *L. peruvianum* accessions EC 106294 or EC 252 showed lower crossing barrier and recorded the highest seed germination (0.43%). However, *L. peruvianum* accessions EC 34479 and EC 492 proved as recalcitrant pollen parents for seed germination. Overall, 0.18% seed germination i.e. recovery of *in vitro* interspecific F_1 hybrids was obtained.

In morphological characterization of interspecific F_1 hybrids, the most distinguishing feature was self-sterility (i.e. fruit set without mature seeds) while overall other morphological traits were dominated by wild pollen parent, *L. peruvianum*. Similarly, the hybridity of these F_1 hybrids were easily confirmed by protein electrophoretic analysis as hybrid specific and intermediate bands were amplified.

While screening interspecific F_1 hybrids against biotic and abiotic stresses, considerable lower pest incidence and complete disease resistance was observed under natural field conditions. Especially resistance was recorded against viral diseases like TSWV and TLCV.

For evaluating plant regeneration in genus *Lycopersicon*, 100 per cent shoot regeneration was recorded by leaf segment of 14 day old *in vitro* plants of all three genotypes (tomato cv. Dahanshree, Bhagyashree and *L. peruvianum* accession EC 106294) in 3 culture media containing MS salt supplemented with 1 mg l^{-1} NAA and 1.5-5.0 mg l^{-1} BAP. The root induction was seen on MS media devoid of hormones.

INTRODUCTION

1. INTRODUCTION

1.1 Preamble

The tomato (*Lycopersicon esculentum* Mill.) is a commercially important vegetable crop throughout the world, both for fresh fruit market and the processed food industry. It is grown under a wide range of climates (temperate to tropical) in the open field or under protection (e.g. in ventilated / heated poly or glasshouse).

Tomato, universally considered as a fruit vegetable crop is extensively grown as an annual crop all over the world. The growth habit is of both determinate and indeterminate type. Normally, the cultivated tomato is exclusively self pollinated crop but values of natural cross pollination (NCP) have been reported from 1.9 to 47.0% in various locations of California (Rick, 1949). Especially in wild self-incompatible types, cross pollination is very high (Rick, 1978).

The tomato, flower is normally perfect, having functional male and female parts. Flowers are easily emasculated and pollinated. Usually 4 to 8 flowers are born in each compound inflorescence and a single plant may produce as many as 20 or more inflorescence during its life cycle. In the favourable environmental conditions 50 to 200 seeds may be obtained from a single fruit. Under optimal conditions, the tomato completes its reproductive cycle within 95 to 115 days, depending upon the cultivar.

Self-incompatibility is a common feature of the wild relatives and is transmitted to hybrids with *L. esculentum*. It is the *Nicotiana* type, conditioned by a single locus which is controlled by multiple alleles of the 'S' gene.

Genetic male sterility has also been reported in tomato (Rick and Butler, 1956; Kaul, 1988).

Most probably, *Lycopersicon* originated in wild forms in Peru, Equador and Bolivia and the Andes in South America, which is the centre of diversity of wild tomato (Kalloo, 1988). However, according to Jenkins (1948) Mexico is the centre of diversity and domestication of the cultivated tomato.

The species of *Lycopersicon* evolved *via* gene substitution. The cultivated tomato exhibits spontaneous crossing with *L. pimpinellifolium*, which indicated a close relationship. Earlier, tomato fruits were considered poisonous because of association of the species with the family *Solanaceae*.

There is evidence to indicate that early work on tomato breeding was carried out in Europe and later in the United States. Before about 1925, tomato improvement was largely accomplished by selection of new genotypes within existing heterogenous cultivars or selection of chance variance originated from spontaneous mutations, natural outcrossing or recombination of pre-existing genetic variation. Later controlled hybridization with selection followed to develop new varieties. Development and utilization of several attributes have led to increasing yield, quality and resistance traits considerably, through interspecific hybridization.

Improved yield and quality are universal goals of most breeding programmes. The breeder must often define the production system and individual components that contribute to yield or quality and emphasize selection for those individual attributes. Plant breeder must be acquainted with the problems of commercial production and requirements of industry and consumers to establish relevant and realistic goals.

Tomato is grown on 3.74 million ha area with a production of 100 million metric tonnes (MT) in the world. Maximum area under tomato cultivation has been registered in China (7.8 lakh ha) followed by India (5.0 lakh ha), Egypt (1.89 lakh ha) and USA (1.64 lakh ha). While significant production recorded in China (20.13 million tonnes), USA (10.25 million tonnes), India (8.50 million tonnes) and Egypt (6.58 million tonnes). However, per ha yield was highest in the Netherlands (520.00 T) followed by France (107.50 T), USA (62.50 T) and Japan (61.54 T). These high yields are mainly due to the growing tomatoes under glasshouse conditions and using F_1 hybrids. A comparatively lower yield average in India (17.00 T) is primarily because of the growing of tomatoes on vast acreage in the field under wide range of climates and soils (Anonymous, 2001).

Lycopersicon is a relatively small genus within the extremely large and diverse family *Solanaceae*. All the species in the genus *Lycopersicon* are having an identical genome formula (2n=2x=24). There are total nine species namely, *L. esculentum*, *L. pimpinellifolium*, *L. cheesmanii*, *L. chemielewskii*, *L. parviflorum*, *L. hirsutum*, *L. pinnellii*, *L. chilense* and *L. peruvianum*.

Cultivated tomato is highly susceptible to several pathogens, insects and pests. An extensive survey of resistance made to identify source of resistance, which are mainly found in wild species. Generally, resistance is controlled by a single dominant gene and occasionally by oligogenes. Breeding for disease resistance is a continuous process because the resistance is not stable due to frequent evolution of new physiological races and strains of pathogens particularly viruses. Furthermore, resistance genes for abiotic stresses, such as salt and drought have been incorporated into cultivated from wild species (Kalloo, 1991).

1.2 Tomato interspecific hybridization

1.2.1 Objectives of interspecific hybridization

The wild taxa of *Lycopersicon* posses a large reservoir of economic attributes (Table 1). The importance of the wild taxa is apparent with the development of several resistant cultivars. Survival of tomato cultivars from biotic and abiotic stresses is largely due to the presence of resistance genes in the cultivars derived from the wild species. The main aim of distant crosses is to transfer resistance characteristics from wild species into commercial varieties.

The most urgent need for tomato improvement is incorporation of horizontal resistance against viral diseases, as viruses are not under chemical control and have poly races and therefore, resistance is polygenic character. At present the major threat for tomato cultivation under tropical climate is incidence of viral diseases i.e. Tomato Spotted Wilt Virus (TSWV) and Tomato Leaf Curl Virus (TLCV). To date, no commercial tomato cultivar is developed to offer horizontal resistance against both viral diseases. In this context, tomato interspecific hybridization can play a vital role; as sources of resistance for both these viral diseases are reported in distantly related wild species like *L. peruvianum* and *L. chilense* (Kalloo, 1991).

There are two types of barriers in inter-specific hybridization, one is prezygotic and second is postzygotic. The prezygotic mechanism operates through different ecological requirement, reproductive behaviour and allelic incompatibility. In postzygotic mechanism includes zygote degeneration, hybrid sterility and abnormality in an early segregating populations.

Table 1. Resistance and quality traits in the wild taxa of *Lycopersicon* (Kalloo, 1991a).

Sr. No.	Species	Resistance and quality traits
1.	*L. esculentum* var. *cerasiforme*	Leaf mold, grey leaf spot, anthracnose, bacterial wilt
2.	*L. pimpinellifolium*	Fusarium wilt, leaf spot, early and late blight, bacterial wilt, bacterial speak, bacterial canker, tomato spotted wilt virus (TSWV), tomato yellow leaf curl virus (TYLC), tomato leaf curl virus (TLC), high content of ascorbic acid and low pH tolerance
3.	*L. cheesmanii*	B gene, salt and drought resistance, high total soluble solids
4.	*L. chmielewskii*	High total soluble solids
5.	*L. hirsutum*	Collar rot, leaf mold, leaf spot, early blight, bacterial canker, TMV, TYLC, TLC, cold tolerance, spider mites, leaf miner
6.	*L. hirsutum* f. *glabratum*	Bacterial canker, corky roots, potato cyst nematodes, TLC, leaf miner, tobacco flee beetle, potato aphid, whitefly, fruit borer, tobacco born worm, tomato pin worm, spider mites, cold tolerance, frost resistance.
7.	*L. peruvianum*	*Septoria* leaf spot, leaf mold, root knot nematode, TMV, TSWV, TLC, TYLC, cucumber mosaic virus (CMV), curly top virus, tomato etch virus and resistance to salt, lower light and temperature.
8.	*L. chilense*	TMV, curly top virus, salt and drought resistance
9.	*L. pennellii*	Spider mite, aphids, drought resistance.

1.2.2 Crossability relationship among *Lycopersicon* species

Before undertaking any interspecific hybridization programme, the knowledge about crossability relationship between species is pre-requisite factor. The nine species of genus *Lycopersicon*, are reported and divided into two groups based on crossing compatibility with cultivated species *L. esculentum* (Rick, 1979). The "*esculentum complex*" consists of seven *Lycopersicon* species (*L. esculentum*, *L. pimpinellifolium*, *L. cheesmanii*, *L. chinielewskii*, *L. parviflorum*, *L. hirsutum* and *L. pennellii*) which can be easily crossed with cultivated tomato. The first three species displaying red fruit ripening and are reciprocally compatible with each other and thus showed closer similarly. While, the remaining four species of '*esculentum complex*' displaying green fruit ripening and are unilateral compatible with cultivated tomato especially when utilized as a pollen parent. The "*peruvinaum* complex" consist of only two species, namely *L. chilense* and *L. peruvianum*, which are distantly related with cultivated tomato and showed severe barriers in interspecific hybridization. Both of these species are green fruited and characterized by self-incompatibility and high polymorphism. Thus, intra- and interspecific breeding barriers have been noticed in *Lycopersicon* species (Table 2).

Table 2. Intra and interspecific breeding barriers in *Lycopersicon* (Tigchelaar, 1986).

Male / Female	*L. esculentum*	*L. pimpinellifolium*	*L. hirsutum*	*L. chilense*	*L. peruvianum*
L. esculentum	+	+	+	EA	EA
L. pimpinellifolium	+	+	+	EA	EA
L. hirsutum	+, UI	+, UI	+, SI, UI	?	EA
L. chilense	UI	UI	?	SI	EA
L. peruvianum	UI	UI	UI	SA	SI

UI = Unilateral incompatibility, SI = Self incompatibility
EA = embryo abortion, + = no serious barriers, ? = no data known.

1.2.3 Crossing barriers for tomato interspecific hybridization

The self-incompatibility is reported in *Lycopersicon* species like *L. hirsutum*, *L. pennelli*, *L. chilense* and *L. peruvianum*, which is gametophytically controlled by multiple alleles of the 'S' gene, preventing pollen tube growth in the style when both expressing same allele. Thus, self-incompatibility itself is a formidable barrier to interspecific hybridization preventing use of self-incompatible species as a maternal parent (Kalloo, 1988).

Similarly, unilateral incompatibility is also reported in above self-incompatible *Lycopersicon* species while crossing with *L. esculentum* (Hogenboom, 1979). For this type of interspecific incompatibility, the pollen growth of *L. esculentum* is inhibited in the styles of wild *Lycopersicon* species like *L. peruvianum*, which is controlled by one or more dominant genes (Hogenboom, 1972b). However, unilateral incompatibility is found as an independent phenomenon than the self-incompatibility and termed as "*incongruity*" (Hogenboom, 1975).

The prezygotic sterility is linked with unilateral incompatibility, when *L. esculentum* used as a pollen parent in interspecific hybridization and it result in flower drop upon pollination. Whereas post zygotic sterility is linked with unilateral compatibility when *L. esculentum* used as a maternal parent in interspecific hybridization and it result in normal fruit development with immature seeds. However, post zygotic sterility can be overcome through culture of embryos (Smith, 1944; Thomas and Pratt, 1981) and immature seeds (Imanishi *et al.*, 1985; Patil *et al.*, 1993) and leads into development of *in vitro* F_1 hybrids. However, interspecific sterility is further observed within F_1

hybrids (Thomas and Pratt, 1981; Wu, 1984). In spite of perfect chromosomal pairing, the tomato interspecific F_1 hybrids showed sterility due to involvement of genetic factors (Masum-Zade, 1973).

1.2.4 *L. peruvianum* Mill. : a donar parent for interspecific hybridization

L. peruvianum being as polymorphic in nature shows remarkable morphological variation within and between populations which resulted in number of fairly well defined racial types found at specific geographical locations. Towards the southern end of its distribution, a Maranon race of *L. peruvianum* was identified and characterized with thin wiry stem, shorter internodes, reduced leaves and unbranched inflorescences (Holle *et al.*, 1979). While, toward the northern end of its distribution, a race *L. peruvianum* var. *humifusum* Mull., is observed with typical characteristics of short, dense, non-glandular hair, thin stem, simplified leaves and inflorescences with relatively small bract. The remaining races of *L. peruvianum* broadly divided into two extreme categories i.e.coostal race and mountain race (Rick, 1963). The coastal races are distinctive but relatively few in number and distributed along with coastal strip of South America. In spite of morphological variation in coastal races, generally they produced elaborate leaves, complex pattern of branched inflorescence, relatively thick stem and long internode with typical sprawling plant growth. On the contrary, mountain races of *L. peruvianum* are distinctive but large in number, restricted in distribution and therefore, geographically isolated from each other. They have adapted to more extreme climates such as very high attitudes (>3000 m) and lower temperatures (<10°C). These races generally characterized with thin stem along with short dense glandular hairs and narrow leaflets (Muller, 1940).

In general, *L. peruvianum* can be characterized as perennial crop, sprawling and indeterminate growth with wiry (thin) stem and short dense trichomes, presence of bract at the base of axillary node, long flower trusses with complex pattern of branching, united petals with exerted stigma, which facilitates outbreeding. Eventhough, most of the plants are self-incompatible but in some cases self-compatibility is also noticed. Fruits are small cherry type (1 to 4 g fruit weight), greenish white fruit colour sometimes with a purple stripe. The seeds are tiny, hairless and light brown. Thus, *L. peruvianum* is one of the most variable species of the genus *Lycopersicon* and therefore offers greater opportunity for exploration of genetical sources of resistance for biotic and abiotic stresses.

1.3 Aims of present investigation

The vast reservoir of genetic variability is available with the nine species of *Lycopersicon*. An almost inexhaustible supply of unexplored diversity exists within the wild taxa of *Lycopersicon*, which to date, have served as a primary source of resistance for biotic and abiotic stresses. Nevertheless, during tomato interspecific hybridization progamme due to unilateral incompatibility, sterility and abnormalities observed at earlier generations, an unlimited reserve of genetical resources from wild taxa has remained unexploited for tomato improvement programme.

Eventhough, some efforts were made to transfer economical traits from wild taxa to tomato cultivars, prominently they were utilized in tomato cultivars especially developed for polyhouse cultivation in temperate region. In this context, work on interspecific hybridization for improvement of tomato cultivars growing under tropical field conditions are very rare. In this regard,

to introgress resistance against biotic and abiotic stresses in Indian tomato cultivars, present work was undertaken with the following objectives.

1. To study crossing compatibility of wild *Lycopersicon* species (*L. peruvianum*) with tomato (*Lycopersicon esculentum* L.) cultivars.

2. To study the pattern of interspecific sterility (pre and post zygotic) during interspecific hybridization.

3. Standardization of tissue culture techniques (i.e. culture of immature seed at different fruit stages) for development of *in vitro* F_1 hybrids.

4. To study growth and development of *in vitro* and *in vivo* interspecific hybrids.

5. Morphological and biochemical characterization of interspecific F_1 hybrids.

6. Screening of interspecific F_1 hybrids alongwith their respective parents against biotic and abiotic stresses.

7. To assess *Lycopersicon* species (*L. esculentum* and *L. peruvianum*) for callus induction and plant regeneration.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The tomato plant is used in classical breeding and genetic material because of its relatively simple reproductive biology, its ease of culture and wealth of genetic variation in cultivated and wild forms.

An attempt has been made to review the available literature on interspecific hybridization in *Lycopericon* species under the appropriate heads.

2.1 The Genus *Lycopersicon*

Lycopersicon is a relatively small genus within the extremely large and diverse family *Solanaceae* which consists of around ninety genera (D'Arey, 1979). The family *Solanaceae* is divided in two subfamilies based on pattern of embryo development. The sub-family, *Solanoideae* has a coiled embryo of uniform size and basic chromosome number is n=12. While sub-family, *Cestroideae* consists of genera having typically straight embryo with more variable basic chromosome number. All the species in the genus *Lycopersicon* are typically of *Solanoideae* sub-family, each having an ideal genome constitution i.e. 2n=2x=24 (Rick and Butler, 1956).

The botanical name *Lycopersicon esculentum* was first proposed by Miller in 1968. Initially, the genus *Lycopersicon* divided into two subgenera namely the *Eulycopersicon* (red fruited) and *Eriopersicon* (green fruited) by Muller (1940) on the basis of fruit types. However, more meaningful subgenetic classification was carried out by Rick (1979) and Taylor (1986) based on involvement of crossing barriers in interspecific hybridization. According to this classification the species of genus *Lycopersicon* are divided into two groups.

***Esculentum* complex**

It consists of seven *Lycopersicon* species which easily crossed with cultivated tomato e.g. *L. esculentum, L. pimpinellifolium, L. cheesmanii, L. chimelewskii, L. parviflorum, L. hirsutum* and *L. pennellii*. Among these first three species are the red fruited type and are reciprocally compatible with each other, while the remaining four species are of green fruited type and are unilaterally compatible with cultivated tomato as a pollen parent.

***Peruvianum* complex**

This group consists of only two species, namely *L. peruvianum* and *L. chilense* which are isolated from *L. esculentum* by severe barriers to interspecific hybridization. Both of these species are green fruited and characterized by self-incompatibility and high polymorphism.

2.2 Barriers in interspecific hybridization in the genus *Lycopersicon*

Interspecific hybridization barriers in *Lycopersicon* species was updated by Tigchelaar (1986). In the genus *Lycopersicon* interspecific crosses resulted ranging from no fruit set to poor fruit set without seed maturity. Thus pre- and post-zygotic sterility is observed during interspecific hybridization in *Lycopersicon*. Pre-zygotic sterility was observed when wild tomato species used as a maternal parent where pollen tube growth of cultivated tomato is obstructed in style and resulted in flower drop. While post-zygotic sterility was observed in interspecific hybridization when wild tomato species used as a pollen parent and it resulted in normal fruit set but with immature seed development due to degeneration of embryo and endosperm (Kosova and Kiku, 1979). Furthermore, in such cases hybrid sterility was also observed controlled by genetic factors (Rick, 1979).

Self-incompatibility is the characteristics of *Lycopersicon* species viz., *L. hirsutum, L. pennellii, L. chilense* and *L. peruvianum* (Taylor, 1986) and controlled by multiple alleles of the 'S' gene. This prevents pollen tube growth in the style when the style and pollen tube express the same gene. Self-incompatibility itself is a formidable barrier to inter-specific hybridization in *Lycopersicon* (Hogenboom, 1973). Therefore, the self-incompatible species do not utilized as a female parent.

For interspecific hybridization in the genus *Lycopersicon* crossing relationship showed unilateral incompatibility exists between *L. esculentum* and species like *L. peruvianum, L. chilense.* The unilateral incompatibility between *Lycopersicon* species has been extensively documented by (Hogenboom, 1979; Kalloo, 1988) and found to be independent of self incompatibility i.e. particular alleles of the 'S' locus. For this type of interspecific incompatibility, Hogenboom (1975) described the term "*incongruity*".

When *L. peruvianum* used as a pollen parent it showed unilateral compatibility with *L. esculentum* (Kinsara *et al.*, 1986; Thomas and Pratt, 1981). *L. esculentum* when used as a pollen parent, pollen tube of *esculentum* is checked in the style of *L. peruvianum* (Hogenboom, 1972c). Unilateral interspecific incompatibility is mainly conditioned by the genes of the pistil or style, 'S' alleles of plants and cytoplasmic factors. Pollen tube inhibition in the style is the main reason for unilateral incompatibility (Hogenboom, 1979).

Demirel *et al.* (1997) reported reciprocal crosses between *L. esculentum* and *L. pimpinellifolium.* However, in interspecific crossing with *L. peruvianum*, they observed unilateral compatibility particularly in case of *L. esculentum* used as a maternal parent but fruit set was resulted with aborted embryos.

2.3 Development of tomato interspecific F_1 hybrids

Despite of interspecific crossing barriers, attempts were made to develop tomato interspecific F_1 hybrids through utilization of unilateral compatibility by use of wild genome as a donor parent and culture of embryos or immature seeds which resulted from post-zygotic sterility (Hogenboom, 1972a; Thomas and Pratt, 1981; Taylor and Al-kummer, 1982 and Segeren *et al.*, 1993).

Various approaches were tried to overcome interspecific hybridization barriers in the genus *Lycopersicon* including the use of chemicals (Kesicki, 1979; and Gradziel and Robinson, 1991), bridging lines (Taylor and Al-kummer, 1982) the repeated use of *L. peruvianum* as the maternal parent (Hogenboom, 1972b), embryo-derived callus (Thomas and Pratt, 1981). Immature hybrid seed culture (Imanishi *et al.*, 1985; Wu *et al.*, 1987), embryo rescue (Smith, 1944; Wolter *et al.*, 1994; Crino *et al.*, 1995; Chen Lanzhung *et al.*, 1996), development of tomato interspecific somatic hybrids through protoplast fusion (Kinsara *et al.*, 1986; San *et al.*, 1990; Ratushnyak *et al.*, 1991; Wijbrandi *et al.*, 1990 and Patil, 1994).

2.3.1 Medium for culture of immature seeds/embryoes of cross *L. esculentum* x *L. peruvianum*

Kosova and Kiku (1979) reported that failure of hybridization between *L. esculentum* x *L. peruvianum* was due to death of embryo through lack of nutrition caused by disturbances in development of endosperm and its subsequent degeneration.

Uralets (1984) cultured embryos of *L. esculentum* x *L. peruvainum* var. *minitum* F_1 hybrids on Linsmaier and Skoog medium with 5% sucrose and

3 mg kinetin/l at 5.8 pH. In this case, interspecific F_1 hybrids were recovered through initial callus induction followed by plant regeneration.

Imanishi *et al.* (1985) obtained tomato interspecific F_1 hybrid plantlets from culture of immature seeds (derived from mature fruits of cross *L. esculentum* x *L. peruvianum*) on MS medium without supplement of plant growth regulators.

Wu *et al.* (1987) in his experiment, immature hybrid seeds of *L. esculentum* x *L. peruvianum* were cultured on MS medium. On differentiation medium, the frequency of callus induction was 52% and bud formed as a frequency of 47%.

Lai *et al.* (1990) cultured hybrid embryos of *L. esculentum* x *L. peruvianum* on Smith (1944) medium and developed F_1 plantlets.

Cap *et al.* (1991) reported that immature seeds derived from cross between *L. esculentum* and *L. peruvianum* were cultured on culture medium described by Thomas and Pratt (1981).

Chen and Imanishi (1991) used MS medium for culture of hybrid seeds derived from *L. esculentum* x *L. hirsutum* and *L. esculentum* x *L. peruvinaum* showed high germination rate.

Patil *et al.* (1993) reported seed germination on MS medium without supplement of plant growth regulators or supplement with lower concentration of plant growth regulators (0.3 mg l^{-1} GA or BAP) while culturing embryos or immature seeds of backcross progenies (BC1, BC2 and BC3) of tomato-*peruvianum* somatic hybrids.

Segeren *et al.* (1993) used MS based culture media with 24 different combinations of auxins and cytokinins and addition of various complex organic supplements (i.e. coconut water, tomato and potato extract). The best treatment was found as MS medium fortified with 2.5 um IAA l^{-1} and 10 um BAP l^{-1}.

2.3.2 Evaluation of fruit growth stages for immature seed culture

Thomas and Pratt (1981) reported 25 to 30 days after pollination (DAP) was an optimal fruit growth stage for culture of immature hybrid seed of cross between *L. esculentum* x *L. peruvianum*.

Uralets (1984) used embryos extracted from the seed of 19 to 25 days old (after pollination) fruits of F_1 of *L. esculentum* x *L. peruvianum* hybrid and cultured *in vitro*.

Wu *et al.* (1987) cultured immature hybrid seeds of the cross *L. esculentum* x *L. peruvianum* 25 to 30 days after pollination (DAP) and cultured on MS medium.

Lai (1990) in his study on parameters influencing embryo rescue in interspecific (*L. esculentum* x *L. peruvianum*) hybridization found that the best time for embryo culture was 35 days after pollination (DAP). He found all embryos were aborted at 40 days after pollination.

Cap *et al.* (1991) reported 25 days after pollination for the *in vitro* culture of immature embryo of F_1 hybrid *L. esculentum* x *L. peruvianum* was an optimal fruit stage to get plantlets. While Segeren *et al.* (1993) noticed that at 25 days after pollination nearly all embryos had aborted. Therefore, they used 15 to 25 days old immature embryos for culture.

Chen Lanzhuang *et al.* (1996) observed globular-stage embryos at 13-15 days after pollination (DAP) in hybrid between *L. esculentum* x *L. peruvianum* and used for culture *in vitro*.

Demirel *et al.* (1997) revealed that 24 day old (after pollination) embryos cultured on modified MS medium showed maximum germination in cross, *L. esculentum* x *L. peruvianum*. They also observed unilateral incompatibility when *L. peruvianum* used as a female parent.

2.4 Confirmation of hybridity of tomato interspecific F_1 hybrids

2.4.1 Morphological characterization

In interspecific hybridization of *L. esculentum* x *L. hirsutum*, Makhalova (1972) reported that there was complete dominance of wild parent *L. hirsutum* for the morphological characters in the generations from F_1 to F_4.

Thomas and Pratt (1981) studied interspecific hybridization between cultivated tomato (*L. esculentum*) and wild species viz., *L. hirsutum*, *L. peruvianum*, *L. pimpinellifolium* and *L. cheesmanii* and observed that morphologically most of the sexual F_1 hybrids had large similarity to their wild parents, indicating dominance of morphological attributes of wild taxa over cultivated type.

Wu (1984) reported that F_1 plants generally exhibited variation in pollen fertility which varied from complete fertility to complete sterility in tomato interspecific hybridization depending on wild tomato species.

Imanishi *et al.* (1985) also noticed that sexually produced F_1 hybrids were always self sterile in tomato interspecific hybridization with *L. peruvianum*.

Chen and Imanishi (1991) studied interspecific hybridization between the cultivated tomato *L. esculentum* and wild species viz., *L. peruvianum*, *L. chilense* and reported that the morphology of both the putative F_1 plants was closely resembled with the male parents i.e. wild parents.

Kalloo (1991b) developed tomato interspecific F_1 hybrids from the cross, *L. esculentum* x *L. peruvianum* and observed that morphology of F_1 hybrids was similar to their wild, male parent and furthermore, hybrid sterility was noticed. In this case, self-incompatibility was recorded in F_1 generation.

Segeren *et al.* (1993) studied hybrid progenies (F_1 and F_2 generation) of *L. esculentum* x *L. peruvianum* and reported that all F_1 plants were morphologically similar to their pollen parent.

Nevertheless, Kinsara *et al.* (1986), Wijbrandi *et al.* (1990) and Patil (1994) reported that tomato somatic hybrids with *L. peruvianum* were also morphologically similar to their wild parents and exhibited reduced fertility or complete sterility.

2.4.2 Biochemical and molecular characterization

Palmer (1990) stated that modern chemical and molecular techniques such as isozyme, RFLP, RAPD analysis that are used in genetic and biochemical research and have assessed for cultivar identification and determination of genetic purity.

The analysis of protein composition as a mean of variety identification is now well established. The success of electrophoretic procedures depends on the wide ranging polymorphism of protein which represents primary gene production. Their expression and detection are unaffected by environmental

interactions and they can be conveniently used as gene markers (Cooke, 1988; Palmer, 1990). It provides rapid method for genomic discrimination. The criterion for distinctness between varieties is generally taken as the presence or absence of particular protein band.

Gel electrophoresis of seeds or vegetative proteins and enzymes is well documents and widely used technique for the identification of crop and horticultural varieties or cultivars (Cooke, 1993).

Patil (1994) used isozyme system of Acid phosphatase, Leucine amino peptidase, Amyl-esterase and total protein to confirm the hybridity of tomato somatic hybrids with *L. peruvianum* and *L. chilense.*

Hussain *et al.* (1986) developed electrophoretic procedure for seed protein which can discriminate cultivars of field bean using acid and SDS polyacrylamide gel electrophoresis.

Sujatha *et al.* (1991) studied various enzyme systems such as esterase (EST), peroxidase (PRX), glutamate oxalacetate transaminase (GOT), glutamate dehydrogenase (GDH) for discrimination of melon varieties and reported that isozyme variation in three out of the 14 loci were dimorphic with two alleles at each locus and 11 loci were monomorphic.

Sujatha and Seshadri (1991) observed 14 different isozymes in muskmelon and reported that 11 were monomorphic and 3 were polymorphic with two alleles at each locus.

Mishra *et al.* (2000) studied electrophoretic pattern analysed by SDS-PAGE method of seed proteins of different cultivated pea (*Pisum sativum* L.) along with its two wild species to establish genetic diversity among them.

2.5 Screening of tomato interspecific F_1 hybrids for biotic and abiotic stresses

2.5.1 Biotic stresses

According to Rick (1987), resistance to 30 different diseases has been detected in the wild taxa of tomato especially resistance to viral diseases like Tobacco Mosaic Virus (TMV), Tomato Spotted Wilt Virus (TSWV), Tomato Leaf Curl Virus (TLCV) and many other fungal diseases like anthracnose, collar rot, *Verticillium* wilt, early blight, etc. and to the root knot nematodes.

Kikuta and Frazier (1946) developed tomato cultivar, Pearl Harbor, from a cross of *L. esculentum* x *L. pimpinellifolium* which was resistant to tomato spotted wilt virus (TSWV) but it was strain specific.

Finlay (1953) identified source of resistance for five strains of TSWV in *L. peruvianum* as a polygenic character.

Nagai *et al.* (1992) reported *L. peruvianum* (LA 444-1) and *L. hirsutum* (PI 13447) as a source of resistance for TSWV. While, Sakata *et al.* (1991) through somatic hybridization showed that *L. peruvianum* could be exploited as source of resistance for biotic and abiotic stresses such as TMV, TSWV and cold resistance.

Furthermore, Sẽgeren *et al.* (1993) observed that tomato interspecific F_1 hybrid derived from *L. peruvianum* showed resistance to TSWV and leaf miner which was segregated in F_2 and F_3 generations.

Yamakawa (1977) studied tomato interspecific hybridization with *L. peruvianum* through use of gamma radiations for breeding for disease resistance. Intially at $BC2F_2$ generation, he noticed source of resistance for

TMV, races of *Fusarium oxysporum*, *Corynebacterium* and *Cladosporium fulvum*. Later on, 3 lines developed from $BC2F_4$ and $BC2F_6$ generations were released as resistant to TMV controlled by *TM2* gene in 1972.

Datar and Lonkar (1985) studied F_1 and F_2 progenies from *L. esculentum* x *L. hirsutum* interspecific hybridization and reported the resistance for fungal disease, *Alternaria solani*, as a monogenic dominant character.

Bonito and Ancora (1986) reported resistance to TMV through interspecific hybridization of *L. esculentum* and *L. peruvianum* (line EC 128650).

Saccardo and Monii (1987) reported resistance to fungal diseases at F_5 generation from tomato interspecific hybridization with wild taxa like *L. peruvianum*, *L. hirsutum* and *L. pimpinellifolium*.

While, Patil (1994) demonstrated resistance to TMV from tomato interspecific hybridization with *L. peruvianum* through backcrossing of tomato somatic hybrids.

Banerjee and Kalloo (1987) studied inheritance to TLCV resistance through tomato interspecific hybridization with *L. hirsutum*, *F. glabratum* (line B6013) and based on data of F_1, F_2, BC1 and BC2 generations, they concluded that resistance to TLCV was digenic character controlled by two epistatic genes. In this case, crossing compatibility was observed between two *Lycoperiscon* species, which included in "*esculentum complex*" of genus *Lycopersicon*.

However, for tomato yellow leaf curl virus (TYLCV) the resistance source was noticed in *L. pimpinelifoium* as a monogenic character (Laterrot *et al.*, 1990). Furthermore, Muniyappa *et al.* (1991) demonstrated resistance to TYLCV under field and laboratory conditions in *L. hirsutum* (PI 390658 and PI 390659) and *L. peruvianum* (PI 127830 and PI 127831).

Sakata *et al.* (1991) developed somatic hybrid between tomato *L. esculentum* x *L. peruvianum* and reported that the offsprings showed resistance to tobacco mosaic virus and tomato spotted wilt virus.

2.5.2 Abiotic stresses

2.5.2.1 Heat tolerance

In tomato, heat tolerance is measured by the ability of a plant to set fruits at high temperature when temperatures exceeds 32°C. Fruit set at higher temperatures (>38°C) is a major problem for summer tomato cultivation under sub-tropical and tropical climates. The adverse effect of high temperature on fruit set in tomato has been extensively studied by Iwahori (1966).

The adverse effect of high temperature (>40°C) on tomato fruit set was extensively documented e.g. splitting of anther cone, induction of pollen sterility, exerted stigma, ovule abortion, endosperm degeneration was noticed which resulted in flower and fruit drop at higher temperatures (Iwahori, 1965; Rana and Kalloo, 1992).

Arora (1977) reported heat tolerance in tomato cv. HS 102 with temperature range of 35 to 38°C under north Indian climatic conditions but the higher relative humidity may played important role in fruit set under sub-tropical climates.

More importantly, Villareal *et al.* (1978) observed that less than one per cent of world collection of tomato cultivars and its related species displayed a high level of heat tolerance based on fruit setting ability at high temperature.

Shete (1986) screened 40 tomato cultivars for hot set including heat tolerant lines from AVRDC, Taiwan and reported that; AVRDC lines L.4139, CL 246-0-3-B-9-00, CL 143-0-4B-1-0-0-0, CL 123-2-4, L.3960 and one Indian variety Pusa Early Dwarf were suitable for hot weather conditions. While, Raijadhav *et al.* (1996) reported that hybrid developed from L.4139 x CL 246-0-3-B-9-00 produced bigger fruits and gave high yield than other cultivars under study.

Rana and Kalloo (1989) studied various attributes of heat tolerance in tomato and concluded that *L. cheesmanii* displayed more number of fruits per cluster.

2.5.2.2 Salt tolerance

Sarangal *et al.* (1991) studied salt tolerance in tomato (*L. esculentum*) cultivars, *L. pennellii*, *L. peruvianum* accessions and their interspecific F_1 hybrids. It was noticed that *L. peruvianum*, *L. pennellii* and tomato interspecific F_1 hybrid with *L. pennellii* were the most effective sources for the highest salinity level (EC: = 10 and 20 dSm^{-1}).

Caro *et al.* (1991) compared normal fruited tomato (*L. esculentum*) cultivars with cherry fruited tomato (*L. esculentum* var. *cerasiforme*) cultivars for salinity threshold. In this investigation, it was observed that cherry tomato cultivars were more salt-tolerant (NaCl) than the other one.

2.6 Plant regeneration in tomato

It was with tomato roots that White (1934) first time demonstrated the possibility of growing plant tissues *in vitro*.

Norton and Boll (1954) reported first time shoot regeneration from tomato root derived callus of the wild tomato species, *L. peruvianum*. While shoot regeneration from stem-derived callus of the cultivated species *L. esculentum* was first reported by De Langhe and De Bruijhe (1973).

Padmanabhan *et al.* (1974) demonstrated effective plant regeneration in tomato through use of leaf segments.

Morgan and Cocking (1982) reported that plant regeneration of *Lycopersicon* species was both explant and genotypic dependent.

Kurtz and Lineberger (1983) studied genotypic differences in morphogenic capacity of cultured leaf explants of tomato by using 12 cultivars and 24 combinations of IAA and BA, and reported that morphogenic responses were cultivar dependent and exhibited broad maxima over a range of growth regulator concentrations. Media containing 0.2 mg l^{-1} IAA and 1.0 to 5.0 mg l^{-1} BAP were optimal for morphogenic callus induction. Genotypic differences were observed in the ability to regenerate shoots. Optimal shoot regeneration was observed on medium contained 0.2 to 1.0 mg l^{-1} IAA + 2.5 or 5.0 mg l^{-1} BAP.

Zorzoli *et al.* (1988) reported that shoot regeneration in cultivated tomato was best on MS medium supplemented with 1 μm IAA + 10 μm BA l^{-1}.

An efficient (100%) direct shoot regeneration was noticed in Indian tomato cultivars such as Pusa Ruby, Pusa Early Dwarf, Dhanashree and

Bhagyashree with leaf segments of 14 days *in vitro* plants cultured on MS media supplemented either with 0.5 mg l^{-1} NAA or IAA and 2.5 to 5.0 mg l^{-1} BAP or 1-2 mg l^{-1} Zeatin (Patil, 1994) which further used effectively in *Agrobacterium*-mediated genetic transformation (Patil *et al.*, 2002). Direct shoot regeneration in tomato was also reported by Dwivedi *et al.* (1990). Lech *et al.* (1996) reported that organogenesis was observed earlier in *L. peruvianum* than *L. esculentum*. Venkatachalam *et al.* (2000) studied tomato plant regeneration from hypocotyl explant and concluded that BAP was more suitable source of cytokinin than the kinetin for maximum shoot bud differentiation and multiple shoot induction. Patil (1994) demonstrated that Zeatin was the most effective cytokinin than the BAP and leaf was the best source of explant for plant regeneration in tomato.

MATERIAL AND METHODS

3. MATERIAL AND METHODS

The present investigation, "Interspecific hybridization in *Lycopersicon* species" was conducted in Tissue Culture Laboratory, Department of Horticulture and the Department of Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri. The crossing programme was carried out on the Instructional Farm of the Department of Horticulture, MPKV, Rahuri, during the year 2000-2002.

3.1 Interspecific hybridization of *Lycopersicon esculentum* and *Lyocpersicon peruvianum*

3.1.1 Source of plant material

Seeds of three tomato (*L. esculentum*) cultivars viz., Dhanashree, Bhagyashree and 85-1 were obtained from "Tomato Improvement Project", Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri. While seeds of six *L. peruvianum* accessions (EC 127774, EC 252, EC 34479, EC 106294, EC 486, EC 492) were obtained from Tomato Genetic Centre, University of California, USA.

3.1.2 Crossing programme

Two cultivated tomato cvs. Dhanashree and 85-1 were reciprocally crossed with six accessions of *L. peruvianum* viz., EC 127774, EC 106294, EC 252, EC 486, EC 34479 and EC 492. Thus total 24 crosses were attempted in following manner.

Two tomato cultivars x six *L. peruvianum* accesions x two reciprocal crosses, total number of crosses 24.

One hundred crosses for each cross combination were obtained while using cultivated tomato cv. as maternal parent. Whereas, fifty crosses for each cross combination were attempted when tomato cultivar used as a pollen parent. All sexual crosses were made during *rabi/kharif* 2000. Receptive stigma and viable pollen were identified at the time of anthesis.

3.1.3 Emasculation and pollination

Emasculation of flowers of maternal parent was carried out by removal of unmatured anthers using fine forcep, one day prior to anthesis and for pollination, the matured anther cone from protected donor parent was covered on stigma of emasculated flowers. To avoid outcrossing, the flowers were covered by perforated butter paper bags.

3.1.4 Per cent crossing index

Per cent crossing index was measured as per cent fruit set from crossed flowers. It was calculated with the following formula :

$$\frac{\text{No. of fruit set}}{\text{No. of flowers crossed}} \times 100$$

3.1.5 Study of different fruit stages for culture of immature seed

The fruit set was observed in tomato interspecific hybridization programme only in one way crossing (i.e. in 12 cross combinations) when tomato cvs. used as a maternal parent. In preliminary experiments, fruits of interspecific crosses were examined at fruit ripening stage to check viable seed Therefore, hybrid fruits, 10 days after pollination (DAP) onward collected at an interval of 5 days upto 49 DAP and immature ovules were cultured to get hybrid plants.

3.1.6 Culture of immature seeds

The fruits harvested at eight fruit growth stages (10 to 49 DAP) from 12 cross combinations were brought to tissue culture laboratory and washed thoroughly under tap water and surface sterilized with 70% ethyl alcohol for two minutes followed by three washing with sterilized distilled water. These fruits were once again surface sterilized with 0.1% silver chloride ($AgCl_2$) for 2 minutes followed by three rinsing with sterilized distilled water under laminar airflow. The immature seeds were cultured in each test tube (25 x 150 mm) containing 15 ml aliquots of MS based culture medium.

For culture of immature seed, the agar-solidified (0.8% w/v) MS medium (Murashige and Skoog, 1962) was used which further fortified with 5 levels each of NAA (0.2, 0.4, 0.6, 0.8 and 1.0 mg l^{-1}) and BAP (1, 2, 3, 4, 5 mg l^{-1}). Thus, total of 26 MS based culture media were evaluated.

For each culture medium, total of 960 immature seeds were cultured as per following formula.

No. of seeds cultured per culture medium	=	No. of seeds cultured/ replication (i.e. each test tube)	x	No. of replications (i.e. No. of test tubes)	x	No. of cross combinations	x	No. of fruit stages
960	=	5	x	2	x	12	x	8 ✓

Thus, for 26 culture media, all together 24,960 immature seeds were cultured from tomato interspecific hybridization programme.

3.1.7 Culture conditions

Cultures were incubated at 25±2°C with 16 hours photoperiod (1500-3000 Lux) supplied by cool white fluorescent tubes. The observations were recorded on seed germination, root and plant growth, any incidence of callus

induction and plant regeneration. The cultures were subcultured at interval of 21 days. Upon seed germination of interspecific F_1 hybrids, for further plant and root growth, MS medium without supplement of plant growth hormones (i.e. M_1 medium) was used.

3.1.8 Polyhouse conditions for hardening of *ex-vitro* putative interspecific F_1 hybrids

The *in vitro* developed F_1 hybrids without or with subculturing (once or twice at 21 days interval) were transferred to polyhouse having the 25 to 27°C temperature and 70 to 90% RH. Initially plants were cultured in soilless compost but upon survival, the plants were cultured in medium containing 1:1:1 ratio of compost, sand and alluvial soil.

3.1.9 Evaluation of *ex-vitro* F_1 hybrids grown under field conditions for morphological characterization and screening against biotic and abiotic stresses

The well developed putative F_1 hybrids under polyhouse conditions were transplanted along with their respective parents in the field and plant growth was evaluated under wide range of climatic conditions (temperature range of 25 to 45°C and RH 20 to 80%) without protection of fungicides and insecticides. The observations were recorded on growth habit and leaf, stem, floral and fruit morphological characters. After one month of transplanting, plant population was screened against major pests (i.e. leaf miner, mites, thrips and white fly) and diseases (e.g. early blight, late blight, powder mildew, TSWV and TLCV) at interval of 15 days.

The pollen viability was determined by staining freshly dehisced pollen in aceto-carmine stain under research microscope with photographic camera (x400) for deeply stained, fully rounded viable pollen. Two hundred pollen grains were examined for each replication. Pollen viability was counted from five different inflorescence and replicated three times.

3.1.10 Biochemical analysis to confirm hybridity of interspecific F_1 hybrids

3.1.10.1 Total protein analysis

The total protein system discriminates genetically unlike species by different protein profile pattern. Therefore, it allows confirmation of hybridity of plants by expressing summation of parental bands or in some cases, it might reveal additional or intermediate bands unique to hybrids. In present investigation, activities of protein system, "true protein" were analysed by polyacrylamide gel electrophoresis (PAGE) of leaf crude proteins.

3.1.10.2 Apparatus and chemicals for electrophoresis

The vertical slab gel electrophoresis apparatus from M/s Bangalore Genei was used for electrophoretic separations. Glass plates (16 x 14 cm), Spacers (1.0 mm) and 13 well combs were used.

The following chemicals/reagents (Analytical grade) were used.

1. Acrylamide / Bisacrylamide 30% 36:5:1 ratio
2. Alkaline copper reagent
3. Ammonium per sulphate (APS)
4. Brillant blue stain
5. Bromophenol blue
6. Destainer
7. Folinciocaltecis phenol reagent
8. Isobutanol
9. Phosphate buffer (pH 7.5)
10. Sample buffer
11. TEMED-N.N.N^1.N^1- Tetra methyl ethylene diamine
12. Tris-glycine – SDS buffer
13. 1.5 M Tris-SDS (pH 8.8)
14. 1.0 M Tris-SDS (pH 6.8)

All reagents were procured from M/s Bangalore Genei and were used as per their Instructional Manual (Appendix-I).

3.1.10.3 Extraction of leaf protein

Leaf (1.0 g fr. wt.) from polyhouse grown plants of putative F_1 hybrids and their parents was homogenized using a pre-chilled (4°C) mortar and pestle with 1.0 ml of extraction buffer (20% v/v) glycerol. Leaf extraction were transferred to 1.5 ml Eppendorf tubes. Fresh prepared phosphate buffer (0.5 ml) (pH 7.5) was added in each tube. The content of tubes were thoroughly mixed with the help of cyclo-mixer. The tubes was left overnight in refrigerator (4°C) for sedimentation of solid material. Then these tubes were centrifuged at 12000 rpm for 10 minutes in RC5B centrifuge machine. The supernants was filtered and used as protein source for electrophoresis.

3.1.10.4 Electrophoresis

The gel casting units, spacers (1.0 mm) and comb (1.0 mm) were cleaned thoroughly and dried in hot air oven at 50°C, the glass plates were fixed firmly in the gel casting unit with the help of clamps and tightened with the screws. Distilled water was poured in the gel cassette to check the leakages, if any.

For preparing 40 ml separating gel of 8% acrylamide concentration, 18.8 ml de-ionised water, 10.8 ml acrylamide mix (acrylamide and bisacrylamide in 36.5:1 ratio), 12.5 ml 1.5 M Tris (pH 8.8), 0.4 per cent SDS, 0.5 ml 10 per cent ammonium per sulphate (APS) and 0.02 ml TEMED were used. APS and TEMED were added after degassing the solution.

For preparation of 10 ml stacking gel of 5% acrylamide concentration, 13.8 ml de-ionised water, 3.4 ml acrylamide mix, 2.50 ml 1.0 M Tris (pH 6.8), 0.8% SDS, 0.2 ml 10% APS and 0.02 ml TEMED were mixed.

Firstly, the separating gel solution was poured quickly between the glass plate. One ml isobutanol was slowly added on it without disturbing the gel solution which was allowed to polymerise for half an hour. After polymerisation isobutanol layer was removed and thoroughly cleaned with distilled water to get rid of any unpolymerised acrylamide. A comb with 13 teeth was placed into the gel and allowed to polymerise for 10-15 minutes. After polymerisation of gel without disturbing the shape of wells, the sample wells were washed with distilled water and dried with blotting paper. The tank was filled with appropriate volume of tank buffer solution (Tris-glycine-SDS buffer).

Seventy µl protein extract and 30 µl sample buffer was added. These samples were then immersed in hot water (80°C) for denaturation of proteins. The gel plats were fixes over cathode chamber-cum-heat-exchanger with clamps and screws. The cathode chamber was then placed into the tank. The denatured protein samples were loaded separately with the help of micro-pipette into each well. High (molecular weight ranged from 29 to 200 kal) and low (molecular weight ranged from 3 to 43 kd) markers were also added in two separate wells for distinguishing the protein bands. In one of the wells, dye was added to observe the movement of proteins.

The electrodes were connected to the power supply. The anode was connected with negative plug and cathode with to the positive. Electrophoresis was conducted at a constant voltage of 150 volts and 40 amp for 6 hours.

The unit was disconnected from the power supply as soon as the sample (the thin blue line above the tracking dye) reached the bottom of the gel.

The gel cassette was removed from the tank, opened gently by unscrewing the tightened screws. The removed gel was then placed in a tray containing Brilliant blue stain for 15-16 hrs at room temperature. Care was taken so that the gel was completing submerged in the staining solution.

After staining, the gel was placed in the destainer for 14-16 hrs changing the destainer solution after every two hrs. When band become clear, the gel was removed from destainer solution and used for recording observations.

The band intensity was assessed visually and recorded as weak, light medium and dense. Observations were also recorded as absence or present bands (Cherry *et al.*, 1970). The banding pattern of protein bands (electrophoregrams) produced were considered as finger prints of the cultivars. The cultivars were separated into different groups according to the intensity and presence or absence of bands. The relative migration values (Rm) of each band was worked out using the following formula.

$$\text{Rm value} = \frac{\text{Distance migrated by the protein band from the origin (cm)}}{\text{Distance migrated by the tracking dye (cm)}}$$

The Rm value of bands of each cultivar was recorded separately. Similarly index between two varieties (all possible combinations) was worked out using the following formula.

$$\text{Similarity index} = \frac{\text{No. of similar bands in two varieties}}{\text{Total no. of bands in two varieties}} \times 100$$

Bands were categorized as follow :

1. Hybrid specific band : which is not present either of parent.
2. Intermediate band : value of F_1 hybrid plant in between parental bands.
3. Parent-1 specific band : value of F_1 hybrid band is equal to value of parent 1 band.
4. Parent 2 specific band : value of F_1 hybrid band is equal to value of parent band.
5. Common band : value of F_1 hybrid and both the parental bands are equal.

3.2 Callus induction and plant regeneration studies in *Lycopersicon* species

3.2.1 Source of plant material

To assess the callus induction and plant regeneration potential of *Lycopersicon* species, two tomato (*L. esculentum*) cultivars viz., Dhanashree and Bhagyashree and a *L. peruvianum* accession EC 106294 was used. The seed sources for *L. esculentum* and *L. peruvianum* were described in 3.1.1.

3.2.2 Development of *in-vitro* seedling for preparation of explants

The seeds of two tomato cultivars and *L. peruvianum* accessions were surface sterilized with 0.1% silver chloride for seven minutes followed by five rinses with sterilized distilled water. Five seeds were cultured aseptically on the surface of 40 ml aliquots of agar solidified MS (Murashige and Skoog, 1962) medium in 250 ml jar bottle. Cultures were incubated in the dark at 25±2°C until seed germination and later on cultures were transferred at 25±2°C with 16 hr. photoperiod (1500 Lux, fluorescent tubes).

3.2.3 Preparation and culture of explant

Fourteen day old seedlings of two tomato cultivars and a *L. peruvianum* accession were used from cotyledons (4 x 2 mm) and leaves (1 x 1 cm) and five segments were cultured on surface of 40 ml aliquots of agar solidified culture medium (refer 3.2.4) in 175 ml capacity screw capped bottles. Thus, two explants from three genotypes were cultured on 20 media (refer 3.2.4) and kept under illumination (1500 Lux fluorescent tubes)at 25±2°C.

3.2.4 Media for culture of explants

Twenty combinations using MS based culture medium supplemented with 4 levels of NAA (0.2, 0.5, 1.0 and 1.5 mg l^{-1}) and 5 levels of BAP (0.5, 1.0, 1.5, 2.5 and 5.0 mg l^{-1}) were used for culture of explants.

3.2.5 Statistical analysis

The effect of three factors (i.e. genotype, explant and culture medium) on callus induction and plant regeneration was analysed in a factorial C.R.D. design of 3 x 2 x 20 [i.e. 3 genotypes (2 tomato cvs. Dhanashree and Bhagyashree and a *L. peruvianum* accession EC 106294), two explants (cotyledon and leaf) and 20 culture media] with total of 120 treatments and three replications. The analysis of variance (ANOVA) and critical difference (CD) were calculated at 5% level of significance for individual main effects and their interactions.

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

The research findings obtained in the present investigation on "Interspecific hybridization in *Lycopersicon* species" are presented in this chapter with suitable headings.

4.1 Interspecific hybridization programme

4.1.1 Assessment of parents

In crossing programme of tomato interspecific hybridization, two tomato (*L. esculentum*) cultivars viz., Dhanashree and 85-1 and six accessions of *L. peruvianum* were selected. The plant growth of two *Lycopersicon* species was examined critically (Table 3; Plate 1). Distant plant morphology was noticed among two species of *Lycopersicon*. The plant growth habit of tomato (*L. esculentum*) cultivars was semi and indeterminate type including broad dark green, moderately serrated leaves, thick (12.3 to 13.6 cm) angular stem with trichomes showing upright growth, unbranched inflorescence with inserted stigma and semi-united petals, while fruits were big (50 to 75 g) with red ripening colour and containing bold and hairy seed.

While plant growth habit of *L. peruvianum* accessions was exclusively indeterminate with narrow, pale green and non-serrated leaves, presence of bract at axillary bud, thin (2.9 to 3.8 cm), round stem without trichomes showing typical sprawling growth, branched inflorescence with exerted stigma and united petals while fruits were small sized, typically of cherry type (4.1 to 4.8 g) with pale green ripening colour with purple stripe and containing tiny and non-hairy seeds.

Table 5: Morphological characterization of tomato genotypes (Parents) utilized in interspecific hybridization.

Character	*L. esculentum*		*L. peruvianum*					
	Dhanashree	85-1	EC 127774	EC106294	EC 252	EC 486	EC 34479	EC 492
Plant growth habit	Semi-indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate
Plant height	65±5.0	90±5.0	180±3.5	148±6.3	144±5.4	146±2.3	152±4.7	141±3.8
Leaf morphology								
Leaf colour	Dark green	Dark green	Light green	Light green	Light green	Light green	Light green	Light green
Leaf serration	Moderate	Moderate	Non-serrated	Non-serrated	Non-serrated	Non-serrated	Non-serrated	Non-serrated
Length (cm)	13.5±1.5	26.2±1.1	12.4±2.1	9.4±0.1	12.3±0.2	12.2±0.5	12.4±0.6	12.1±1.2
Breadth (cm)	10.6±1.5	15.4±1.1	7.1±0.9	6.4±0.2	5.8±0.8	6.5±0.6	6.3±0.5	6.5±0.5
Leaflet thickness	Thick	Thick	Thin	Thin	Thin	Thin	Thin	Thin
Stem morphology								
Main stem thickness (cm)	12.3 ± 1.1	13.6 ± 1.4	3.2±0.3	3.8±0.4	2.9±0.6	3.0±0.4	3.6±0.5	3.1±0.3
Bract	Absent	Absent	Present	Present	Present	Present	Present	Present
Trichome	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent
Stem type	Angular	Angular	Round	Round	Round	Round	Round	Round
Floral morphology								
Inflorescence	Unbranched	Unbranched	Branched	Branched	Branched	Branched	Branched	Branched
Stock length (cm)	6.2±0.8	6.9±1.2	12.4±0.6	13.0±0.4	10.4±0.8	10.8±1.0	11.8±1.2	9.1±0.9
Stigma exertion over anther cone	Inserted	Inserted	Exerted	Exerted	Exerted	Exerted	Exerted	Exerted
Flower petal	Semi-united	Semi-united	United	United	United	United	United	United

Table 3 (Contd...)

Character	*L. esculentum*		*L. peruvianum*					
	Dhanashree	85-1	EC 127774	EC106294	EC 252	EC 486	EC 34479	EC 492
Fruit morphology								
Fruit shape	Round	Oval round	Round	Round	Round	Round	Round	Round
Fruit size (dia., cm^2)	Big (5.1 x 4.9)	Big (5.5 x 5.9)	Cherry (1.2 x 1.1)	Cherry (1.5 x 1.6)	Cherry (1.4 x 1.4)	Cherry (1.3 x 1.1)	Cherry (1.0 x 0.8)	Cherry (1.3 x 1.2)
Fruit weight (g)	50±5	75±5	4.5±0.7	4±1.1	4.7±0.5	4.5±0.3	4.2±0.7	4.8±0.3
Ripening colour	Red	Red	Pale green with purple stripe	Pale green with purple stripe	Pale green with purple stripe	Pale green with purple stripe	Pale green with purple stripe	Pale green with purple stripe
No. of seeds / fruit	121-137	135-149	79-81	75-84	65-78	77-85	65-80	67-79
Seed type	Bold and hairy	Bold and hairy	Tiny and non-hairy	Tiny and non-hairy	Tiny and non-hairy	Tiny and non-hairy	Tiny and non-hairy	Tiny and non-hairy

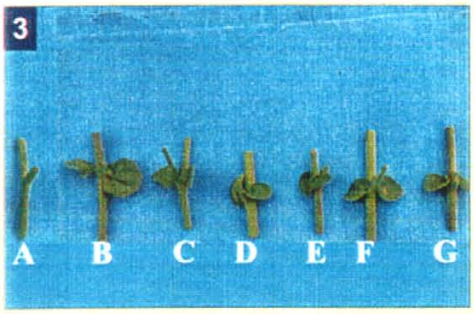
PLATE 1

Plate I : Morphological characterization of parents used in tomato interspecific hybridization

A.

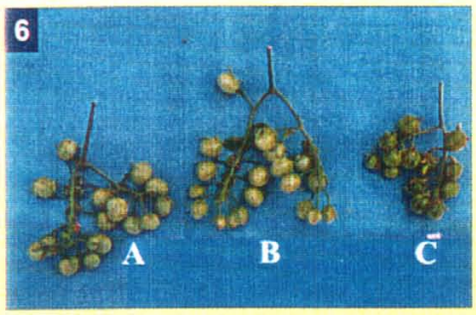
B.

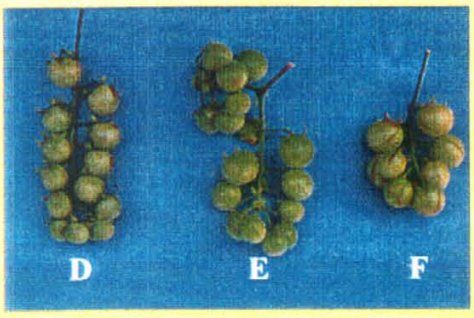
Overall plant growth



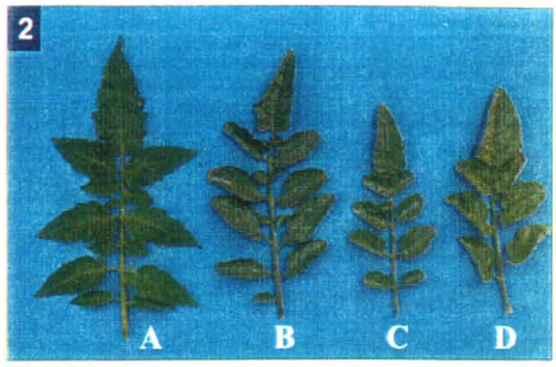
Presence of bracts at axillary bud

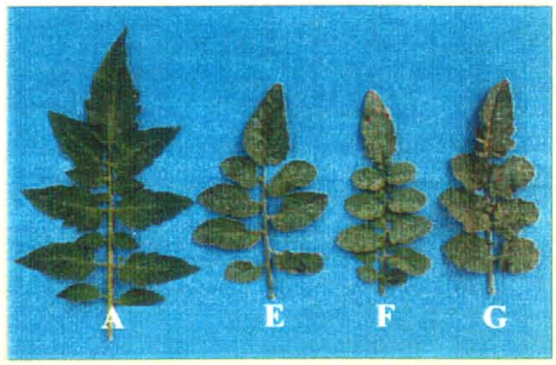
Anthercone with stigma position



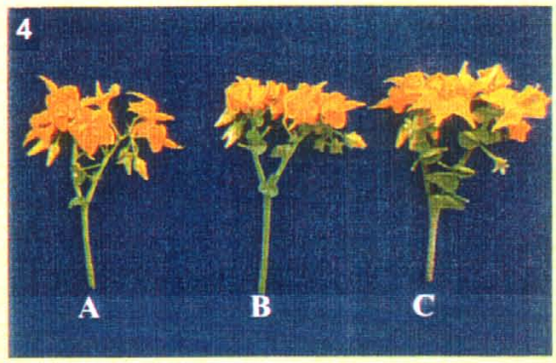


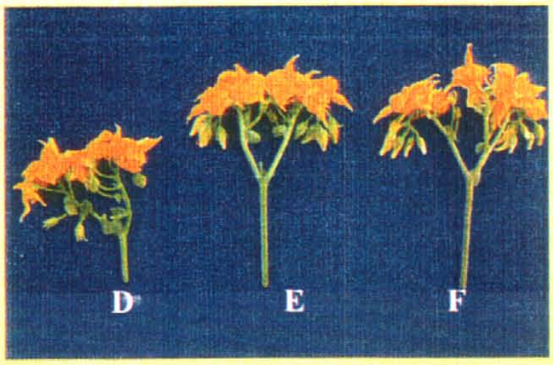
Cherry green fruits of *L.peruvianum*





Leaf characterization





Branched inflorescence in *L.Peruvianum*

Seed characterization

4.1.2 Evaluation of unilateral incompatibility and crossing index for interspecific hybridization, *L. esculentum* x *L. peruvianum*

4.1.2.1 Using wild tomato as a maternal parent

In crossing programme of interspecific hybridization, six accessions of *L. peruvianum* were used as a maternal parent while tomato cv. Dhanashree and 85-1 as a pollen parent. Total 600 crosses were made in twelve cross combinations. However, not a single cross showed any fruit set and every cross resulted into flower drop (Table 4). Thus, unilateral incompatibility was observed in tomato interspecific hybridization when wild tomato was used as a maternal parent and cultivated tomato as a pollen parent.

4.1.2.2 Using wild tomato as a pollen parent

In crossing programme of interspecific hybridization; cultivated tomato cvs. Dhanashree and 85-1 were used as a maternal parent while six accessions of *L. peruvianum* viz., EC 127774, EC 106294, EC 252, EC 486, EC 34479 and EC 492 as a pollen parent.

Overall 1209 crosses were made out of which 627 crosses recorded fruit set (51.86%). Results obtained are presented in Table 5A.

Among different crosses, per cent fruit set was ranged from 46.00 (Dhanshree x EC 486) to 5728 (Dhanashree x EC 106294) (Table 5A). Thus unilateral compatibility was observed during tomato interspecific crossing programme using tomato cultivars as a female parent.

For higher crossing index, among two maternal parents, tomato cv. Dhanashree was observed superior (53.52%) over cv. 85-1 (50.15%; Table 5B; Fig. 1).

Table 4. Evaluation of crossing index for interspecific hybridization of *Lycopersicon esculentum* x *L. peruvianaum* : Using wild tomato (*L. peruvianum*) as a maternal parent

Maternal parent	Pollen parent	No. of crosses made	No. of fruit set	Per cent fruit set (Crossing index)
EC 127774	Dhanashree	50	0	0
EC 106294		50	0	0
EC 252		50	0	0
EC 486		50	0	0
EC 34479		50	0	0
EC 492		50	0	0
	Total	300	0	0
EC 127774	85-1	50	0	0
EC 106294		50	0	0
EC 252		50	0	0
EC 486		50	0	0
EC 34479		50	0	0
EC 492		50	0	0
	Total	300	0	0
	Grand total	600	0	0

Table 5. Evaluation of crossing index for interspecific hybridization of *Lycopersicon esculentum* x *L. peruvianaum* : Using wild tomato (*L. peruvianum*) as a pollen parent

A. Performance of interspecific crosses

Maternal parent	Pollen parent	No. of crosses made	No. of fruits set	Per cent fruit set (Crossing index)	No. of mature seed per fruit at harvest	No. of immature seed per fruit at harvest
Dhanashree	EC 127774	105	59	56.20	-	46
	EC 106294	103	59	57.28	-	48
	EC 252	112	60	53.57	-	41
	EC 486	100	46	46.00	-	38
	EC 34479	89	49	55.06	-	48
	EC 492	100	53	53.00	-	45
	Total	**609**	**326**	**321.10**	**-**	**266**
	Mean	**101.5**	**54.33**	**53.52**	**-**	**44.33**
85-1	EC 127774	115	58	50.43	-	40
	EC 106294	100	55	55.00	-	45
	EC 252	104	51	49.04	-	39
	EC 486	90	42	46.67	-	33
	EC 34479	100	50	50.00	-	42
	EC 492	91	45	49.45	-	43
	Total	**600**	**301.00**	**300.59**	**-**	**242**
	Mean	**100**	**50.17**	**50.15**	**-**	**40.33**
	Grand total	**1209**	**627**	**621.59**	**-**	**508**
	Grand mean	**100.75**	**52.25**	**51.86**	**-**	**42.33**

Table 5B. Performance of maternal parent during interspecific hybridization

Sr. No.	Maternal parent	Per cent fruit set (Crossing index)
1	Dhanashree	53.52
2	85-1	50.15
	S.E.±	1.06
	C.D.	3.11

Table 5C. Performance of pollen parent during interspecific hybridization

Sr. No.	Pollen parent	Per cent fruit set (Crossing index)
1	EC 127774	53.31
2	EC 106294	56.14
3	EC 252	51.31
4	EC 486	46.34
5	EC 34479	52.53
6	EC 492	51.23
	S.E.±	1.84
	C.D.	5.39

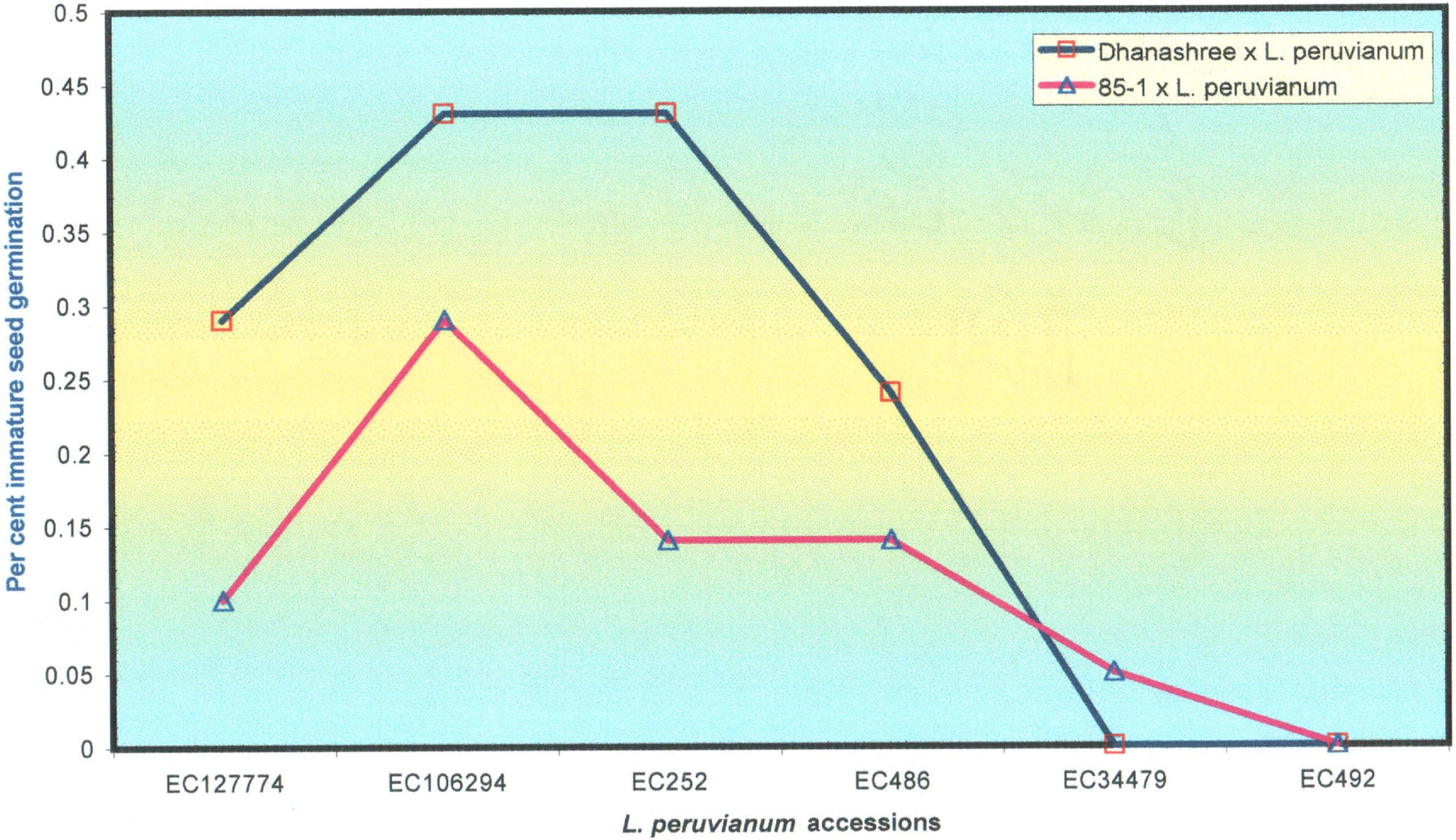


Fig. 1. Effect of maternal parent on germination of cultured immature seeds of interspecific (*L. esculentum* x *L. peruvianum*) F_1 hybrids

While among six pollen parents fruit set ranged from 46.34 to 56.14 per cent. The highest crossing index was registered by the *L. peruvianum* accession, EC 106294 while, the lowest by EC 486 (Table 5C).

When wild tomato used as a pollen parent, eventhough normal fruit development was noticed in interspecific hybridization; seeds were not completely developed and matured at fruit maturity stage (Table 5A). Thus, it showed post-zygotic sterility in this particular case. Therefore, tissue culture technique was employed for the embryo rescue and further development of interspecific hybrid plants. In this regard experiment was conducted to standardize the culture medium for immature seed culture.

4.2 Development of *in vitro* interspecific F_1 hybrid plants

4.2.1 Standardization of medium for immature seed culture

In present investigation immature seed of interspecific crosses (12 cross combinations) at different fruit development stages (8) were cultured in *in vitro* on Murashige and Skoog (1962) based media with 26 different combinations of auxin and cytokinin (i.e. NAA and BAP, respectively). Results are presented in Table 6. Data of Table 6A represents numbers while data of Table 6B and Fig. 2, the percentages of immature seed germination.

While assessing 26 culture media for development of *in vitro* interspecific F_1 hybrid through culture of immature seed, it was noticed that maximum overall seed germination (0.94%) was observed in MS based medium (i.e. M_1 medium; Table 6B) medium without supplement of plant growth regulators. While in particular, M_1 medium also showed the highest seed germination for crosses involving maternal parents cv. Dhanashree (1.46%) and cv. 85-1 (0.42%). Among the specific cross combination, the

Table 6A.Effect of culture media on germination of cultured immature seeds of interspecific (*L. esculentum* x *L. peruvianum*) tomato hybrids : No. of immature seed germination

Culture media	MS Medium		No. of cultured immature seed germinated													
	NAA mg l^{-1}	BAP mg l^{-1}	Dhanashree x *L. perivianum*							85-1 x *L. peruvianum*						
			EC 127774	EC 106294	EC 252	EC 486	EC 34479	EC 492	Total	EC 127774	EC 106294	EC 252	EC 486	EC 34479	EC 492	Total
M_1	MSO		2	2	2	1	-	-	7	-	1	-	1	-	-	2
M_2	0.2	1.0	-	1	-	1	-	-	2	-	-	1	-	-	-	1
M_3	0.2	2.0	1	1	2	-	-	-	4	-	1	-	-	1	-	2
M_4	0.2	3.0	-	1	-	2	-	-	3	-	1	-	1	-	-	2
M_5	0.2	4.0	-	-	1	-	-	-	1	-	-	1	-	-	-	1
M_6	0.2	5.0	-	-	1	-	-	-	1	1	-	-	-	-	-	1
M_7	0.4	1.0	1	-	-	1	-	-	2	-	2	-	-	-	-	2
M_8	0.4	2.0	-	-	1	-	-	-	1	-	-	-	-	-	-	-
M_9	0.4	3.0	-	-	-	-	-	-	-	-	-	1	-	-	-	1
M_{10}	0.4	4.0	-	2	-	-	-	-	2	-	-	-	1	-	-	1
M_{11}	0.4	5.0	-	-	-	-	-	-	-	1	-	-	-	-	-	1
M_{12}	0.6	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{13}	0.6	2.0	-	-	1	-	-	-	1	-	-	-	-	-	-	-
M14	0.6	3.0	1	-	-	-	-	-	1	-	-	-	-	-	-	-
M_{15}	0.6	4.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6A (Contd..)

Culture media	MS Medium		No. of cultured immature seed germinated													
	NAA mg l^{-1}	BAP mg l^{-1}	Dhanashree x *L. perivianum*							85-1 x *L. peruvianum*						
			EC 127774	EC 106294	EC 252	EC 486	EC 34479	EC 492	Total	EC 127774	EC 106294	EC 252	EC 486	EC 34479	EC 492	Total
M_{16}	0.6	5.0	-	1	-	-	-	-	1	-	1	-	-	-	-	1
M_{17}	0.8	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{18}	0.8	2.0	-	-	1	-	-	-	1	-	-	-	-	-	-	-
M_{19}	0.8	3.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{20}	0.8	4.0	-	1	-	-	-	-	1	-	-	-	-	-	-	-
M_{21}	0.8	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{22}	1.0	1.0	1	-	-	-	-	-	1	-	-	-	-	-	-	-
M_{23}	1.0	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{24}	1.0	3.0	-		-	-	-	-	-	-	-	-	-	-	-	-
M_{25}	1.0	4.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{26}	1.0	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Total		6	9	9	5	-	-	29	2	6	3	3	1	-	15

hybrids : Per cent immature seed germination

Culture media	MS Medium		Per cent cultured immature seed germinated													
	NAA mg l^{-1}	BAP mg l^{-1}	Dhanashree x *L. peruvianum*							85-1 x *L. peruvianum*							Grand mean
			EC 127774	EC 106294	EC 252	EC 486	EC 34479	EC 492	Mean	EC 127774	EC 106294	EC 252	EC 486	EC 34479	EC 492	Mean	
M_1	0	0	2.50	2.50	2.50	1.25	-	-	1.46	-	1.25	-	1.25	-	-	0.42	0.94
M_2	0.2	1.0	-	1.25	-	1.25	-	-	0.42	-	-	1.25	-	-	-	0.21	0.31
M_3	0.2	2.0	1.25	1.25	2.50	-	-	-	0.83	-	1.25	-	-	1.25	-	0.42	0.62
M_4	0.2	3.0	-	1.25	-	2.50	-	-	0.63	-	1.25	-	1.25	-	-	0.42	0.52
M_5	0.2	4.0	-	-	1.25	-	-	-	0.21	-	-	1.25	-	-	-	0.21	0.21
M_6	0.2	5.0	-	-	1.25	-	-	-	0.21	1.25	-	-	-	-	-	0.21	0.21
M_7	0.4	1.0	1.25	-	-	1.25	-	-	0.42	-	2.50	-	-	-	-	0.42	0.42
M_8	0.4	2.0	-	-	1.25	-	-	-	0.21	-	-	-	-	-	-	-	0.10
M_9	0.4	3.0	-	-	-	-	-	-	-	-	-	1.25	-	-	-	0.21	0.10
M_{10}	0.4	4.0	-	2.50	-	-	-	-	0.42	-	-	-	1.25	-	-	0.21	0.31
M_{11}	0.4	5.0	-	-	-	-	-	-	-	1.25	-	-	-	-	-	0.21	0.10
M_{12}	0.6	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{13}	0.6	2.0	-	-	1.25	-	-	-	0.21	-	-	-	-	-	-	-	0.10
M14	0.6	3.0	1.25	-	-	-	-	-	0.21	-	-	-	-	-	-	-	0.10
M_{15}	0.6	4.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6B (Contd..)

Culture media	MS Medium		Per cent cultured immature seed germinated														
	NAA mg l^{-1}	BAP mg l^{-1}	Dhanashree x *L. peruvianum*							85-1 x *L. peruvianum*							Grand mean
			EC 127774	EC 106294	EC 252	EC 486	EC 34479	EC 492	Mean	EC 127774	EC 106294	EC 252	EC 486	EC 34479	EC 492	Mean	
M_{16}	0.6	5.0	-	1.25	-	-	-	-	0.21	-	1.25	-	-	-	-	0.21	0.21
M_{17}	0.8	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{18}	0.8	2.0	-	-	1.25	-	-	-	0.21	-	-	-	-	-	-	-	0.10
M_{19}	0.8	3.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{20}	0.8	4.0	-	1.25	-	-	-	-	0.21	-	-	-	-	-	-	-	0.10
M_{21}	0.8	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{22}	1.0	1.0	1.25	-	-	-	-	-	0.21	-	-	-	-	-	-	-	0.10
M_{23}	1.0	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{24}	1.0	3.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{25}	1.0	4.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M_{26}	1.0	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean %		0.29	0.43	0.43	0.24	-	-	0.23	0.1	0.29	0.14	0.14	0.05	-	0.12	0.18

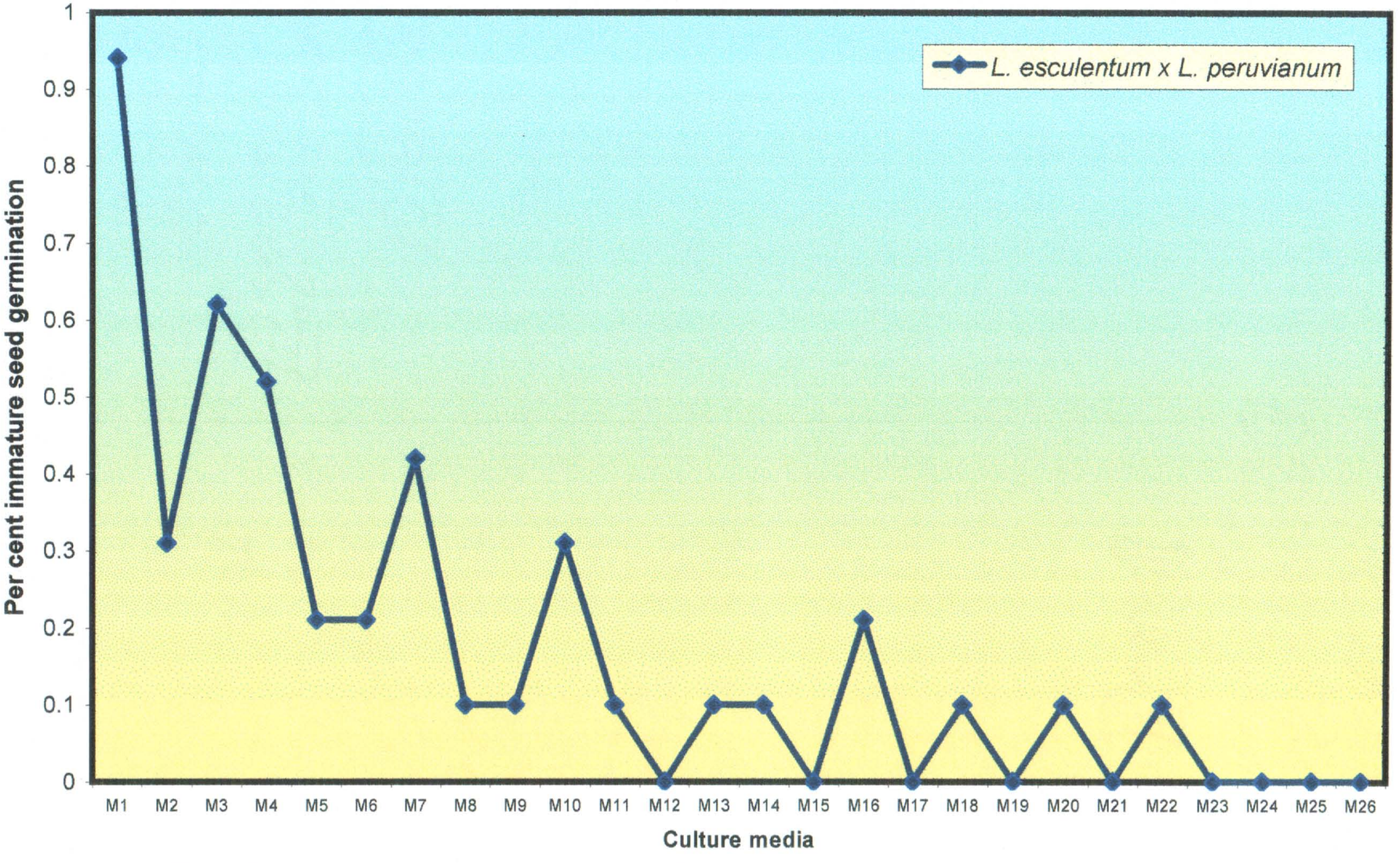


Fig. 2. Effect of culture media on germination of cultured immature seeds of interspecficic (*L. esculentum* x *L. peruvianum*) F_1 hybrids

highest seed germination (2.50%) was recorded in three crosses when cultured in M_1 medium.

In general, it was observed that MS based media without or with lower concentration of plant growth regulators (PGR) were more effective than MS medium than higher concentration of PGR. In this context, upon pure MS medium, other effective culture media were MS supplemented with 0.2 mg l^{-1} NAA and 2 and 3 mg l^{-1} BAP (i.e. M_3 and M_4 respectively) as 0.62% and 0.52% seed germination was recorded, respectively by these two media (Table 6B). In crosses involving maternal parent cv. Dhanashree, the concern values of seed germination for M_3 and M_4 media were 0.83 and 0.63% as compared to 0.42% in crosses involving maternal parent cv. 85-1. Thus culture media M_1, M_3 and M_4 were found more effective for germination of immature seed of tomato interspecific hybrid (Plate 2).

However, it was important to note that even upon attempting different culture media, fruit stages and various cross combinations; very limited success was noticed in tomato interspecific hybridization as 26 media recorded seed germination within range of 0 to 1.46%. While 12 specific cross combinations recorded 0 to 2.50% seed germination range. The overall mean for seed germination was 0.18%. Thus, it showed severe interspecific crossing barriers in tomato.

4.2.2 Performance of parents in development of tomato interspecific F_1 hybrid

While comparing performance of parents in interspecific crosses, it was observed that among tomato (*L. esculentum*) cultivars cv. Dhanashree showed its superiority over cv. 85-1 as 0.23% immature seed germination was recorded by crosses involved cv. Dhanashree than cv. 85-1 (0.12%; Table 6Ci).

Table 6C : Parental performance in interspecific hybridization for immature F_1 seed germination

Table 6C (i). Performance of *L. esculentum* as a maternal parent

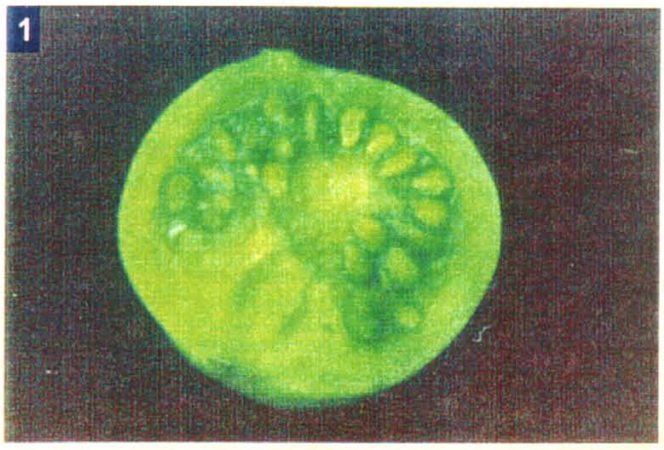
Sr. No.	Maternal parent	Per cent seed germination
1	Dhanashree	0.23
2	85-1	0.12
	Total percentage seed germination	0.18

Table 6C(ii). Performance of *L. peruvianum* as a pollen parent

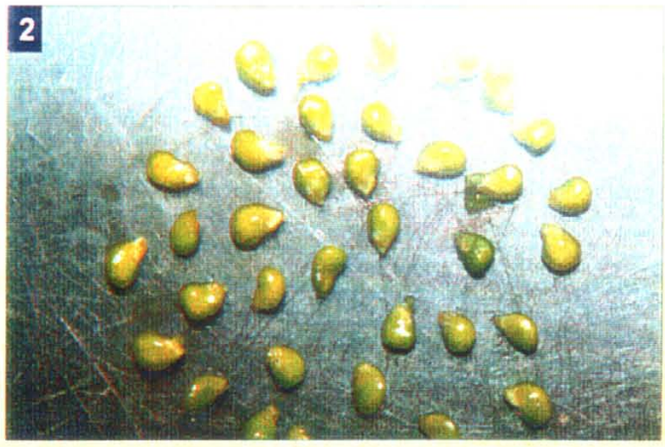
Sr. No.	Pollen parent	Per cent seed germination
1	EC 127774	0.19
2	EC 106294	0.36
3	EC 252	0.29
4	EC 486	0.19
5	EC 34479	0.02
6	EC 492	0.00

PLATE 2

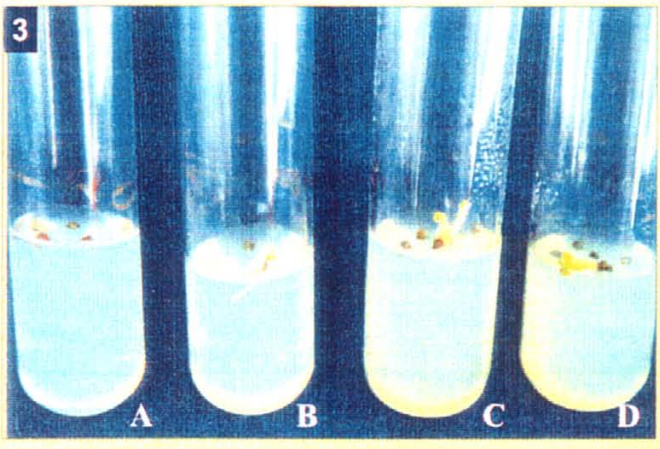
Plate 2 : - Culture of immature seeds and seeds development of *in-vitro* interspecific F_1 hybrids



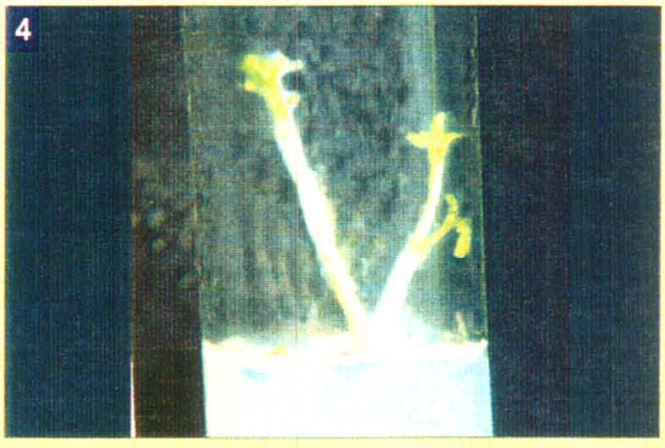
Immature fruit (30-35 DAP)



Immature seed



Seed germination in culture medium



In-vitro growth of F_1 hybrid

Complete growth of *in-vitro* F_1 hybrids

Among six accessions of *L. peruvianum,* the accession EC 106294 was the most effective pollen parent with 0.36% seed germination followed by EC 252 (0.29%) and EC 127774 and EC 486 (0.19%) (Table 6Cii).

While, *L. peruvianum* accessions EC 492 and EC 34479 showed severe crossing barriers with 0 and 0.02% seed germination. Thus, variability was observed for interspecific breeding barriers within two species of *Lycopersicon* i.e. *L. esculentum* and *L. peruvianum.*

4.2.3 Effect of fruit growth stages on culture of immature seed and seed germination of interspecific F_1 hybrid

In twelve cross combinations (2 tomato cvs. Crossed with 6 accessions of *L. peruvianum*) eight fruit growth stages were assessed for culture of immature seeds, i.e. from 10 to 14 days upto 45 to 49 days after pollination (DAP) at interval of 5 days.

Data of Table 7 revealed that in general early (10 to 14 DAP) as well as late (40 to 49 DAP) fruit development stages were not suitable for culture of immature seed as no germination was observed.

Since 15 to 24 DAP fruit growth stages seed germination was initiated (0.16%) which showed progressive upward trend at 25 to 29 DAP fruit growth stage (0.35%) reached peak at 30 to 34 DAP fruit growth stage (0.48%) then showed progressive downward trend at 35 to 39 DAP fruit growth stage (0.26%).

In particular, with crosses involved maternal parent cv. Dhanashree shown upward trend from 15 to 19 DAP to 25 to 29 DAP (0.26 to 0.32%)

Table 7. Significance of fruit growth stages in culture of immature hybrid seed of *L. esculentum* x *L. peruvianum*

Sr. No.	DAP levels	Per cent mean seed germination		
		Dhanashree x *L. peruvianum*	85-1 x *L. peruvianum*	Total mean
1.	10-14	0.00	0.00	0.00
2.	15-19	0.26	0.06	0.16
3.	20-24	0.26	0.06	0.16
4.	25-29	0.32	0.38	0.35
5.	30-34	0.77	0.19	0.48
6.	35-39	0.26	0.26	0.26
7.	40-44	0.00	0.00	0.00
8.	45-49	0.00	0.00	0.00

followed by sudden peak rise at 30 to 34 DAP (0.77%) and then progressive downward trend at 35 to 39 DAP (0.26%) (Fig. 3) .

While for crosses involved maternal parent cv. 85-1 showed megre seed germination (0.06%) during initial fruit growth stages i.e. 15 to 24 DAP, which followed by the highest seed germination (0.38%) at 25 to 29 DAP, then sudden downward trend (0.19%) at 30 to 34 DAP and again slightly upward trend (0.26%) at 35 to 39 DAP fruit growth stages.

In brief, for crosses involved with maternal parent cv. Dhanashree, the most ideal fruit stage was 30 to 34 DAP whereas for crosses involved with cv. 85-1 it was 25 to 29 DAP.

The detail information of per cent immature seed germination of particular cross combination was given in Table 8.

4.2.4 Plant growth of *in vitro* interspecific F_1 hybrids during culture

From culture of immature seed from the cross *L. esculentum* and *L. peruvianum*, total 44 *in vitro* plantlets were recovered in tissue culture laboratory (Plate 3), Among these 9 were obtained in pure MS medium i.e. M_1 medium (Table 6A) which showed normal plant growth and rooting on the same medium. Similarly, 32 plantlets obtained in MS culture media supplemented with lower concentration of napthalene acetic acid (i.e. 0.2 to 0.6 mg l^{-1}) showed normal seed germination but later on plant and root growth was observed ceased and therefore, these plants were subcultured in pure MS medium (i.e. M_1 medium) for further growth. Furthermore, 3 plantlets were recovered in culture media containing napthalene acetic acid within the range of 0.8-1.0 mg l^{-1}. In these cases, initial seed germination was followed by

Table 8. Effect of fruit growth stages on culture of immature hybrid (*L. esculentum* x *L. peruvianum*) seed germination (%)

A. With maternal parent Dhanashree

Sr. No.	DAP levels	Dhan.x EC 127774	Dhan.x EC 106294	Dhan.x EC 252	Dhan.x EC 486	Dhan.x EC 34479	Dhan.x EC 492	Total	Mean
1.	10-14	0.00	0.00	0.00	0.00	0.00	0 00	0.00	0.00
2.	15-19	0.77	0.00	0.38	0.38	0.00	0 00	1.54	0.26
3.	20-24	0.00	0.77	0.00	0.77	0.00	0 00	1.54	0.26
4.	25-29	0.38	0.77	0.77	0.00	0.00	0 00	1.93	0.32
5.	30-34	0.77	1.54	1.54	0.77	0.00	0 00	4.62	0.77
6.	35-39	0.38	0.38	0.77	0.00	0.00	0 00	1.54	0.26
7.	40-44	0.00	0.00	0.00	0.00	0.00	0 00	0.00	0.00
8.	45-49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	2.30	3.46	3.46	1.92	0.00	0 00	11.17	1.86
	Mean	0.29	0.43	0.43	0.24	0.00	0 00	1.46	0.23

Dhan. = Dhanashree

B. With maternal parent 85-1

Sr. No.	DAP levels	85-1 x EC 127774	85-1 x EC 106294	85-1 x EC 252	85-1 x EC 486	85-1 x EC 34479	85-1 x EC 492	Total	Mean
1.	10-14	0.00	0.00	0 00	0.00	0.00	0.00	0.00	0.00
2.	15-19	0.00	0.38	0.00	0.00	0.00	0 00	0.38	0.06
3.	20-24	0.00	0.00	0.00	0.38	0.00	0.00	0.38	0.06
4.	25-29	0.38	1.15	0.00	0.38	0.38	0.00	2.31	0.38
5.	30-34	0.00	0.00	0.77	0.38	0.00	0 00	1.16	0.19
6.	35-39	0.38	0.77	0.38	0.00	0.00	0.00	1.54	0.26
7.	40-44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.	45-49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.77	2.30	1 15	1.15	0.38	0.00	5.77	0.95
	Mean	0.09	0.29	0 14	0.14	0.05	0 00	0 720	0.12

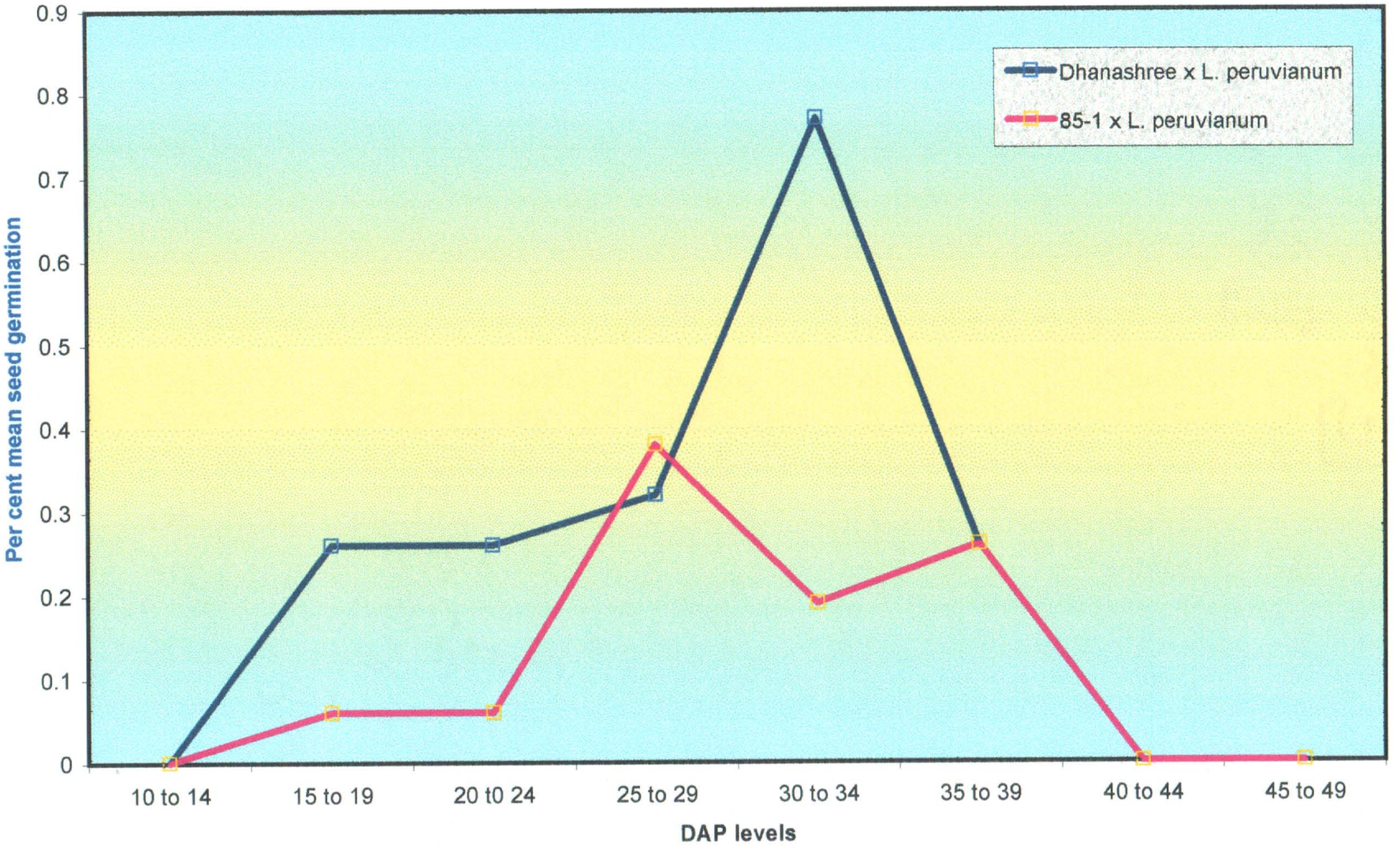


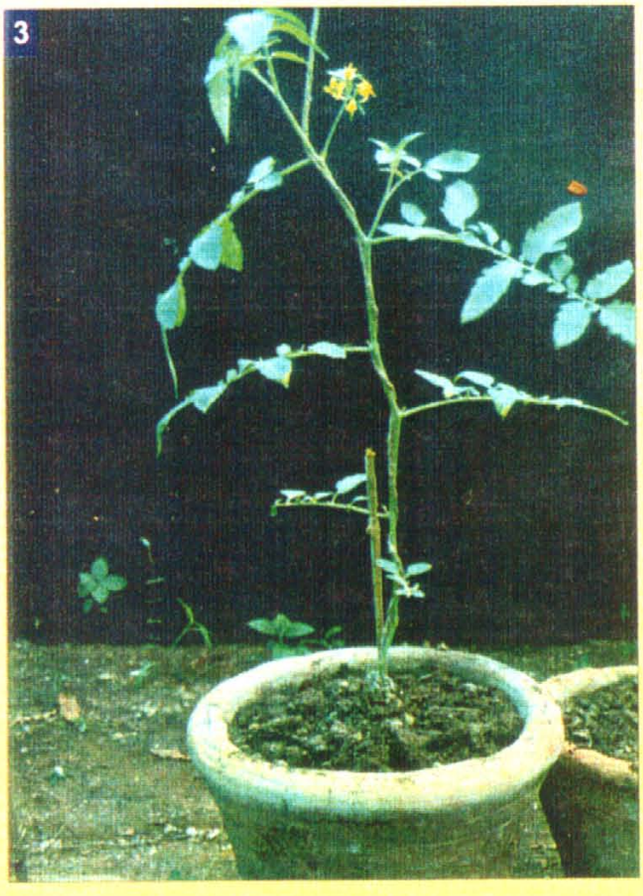
Fig. 3. Significance of fruit growth stages in culture of immature hybrid seed of *L. esculentum* x *L. peruvianum*

PLATE 3

Plate 3 : Growth of *in-vitro* interspecific F_1 hybrids

F_1 hybrid plantlets ready for transplanting

Growth of F_1 hybrid plantlets under polyhouse condition



Growth of F_1 hybrid plantlets under field condition

Full grown plant of interspecific F_1 hybrid

callusing and plant regeneration of 2-3 plantlets were observed from each embryo-derived callus. These plantlets were subcultured in pure MS medium (i.e. M_1 medium) for rooting.

4.3 Hardening of interspecific tomato F_1 hybrids (*in vivo* plants)

Well rooted fresh hybrid seedlings were transferred in March, 2002 from culture room to polyhouse in plastic pots (2 x 3") having sterilized soilrite with controlled conditions (25±2°C temperature and 70% R.H.). However, severe mortality was observed within a six days (Table 9).

In first attempt of hardening, old 24 (21 days) seedlings were transplanted in polyhouse. But complete mortality was observed in first three days of hardening.

To minimise the mortality of F_1 hybrid seedlings, an experiment was conducted for subculturing of *in vitro* plants. Ten plantlets were subcultured once and another ten plantlets were subcultured twice at interval of 21 days before transplanting in polyhouse. It was observed that plantlets those subcultured once (42 days old) also did not survive for more than six days under polyhouse conditions in the month of April 2002. Whereas from ten plantlets those subcultured twice (63 days old), two plantlets could survive and showed development of interspecific hybrid plants.

Thus, out of 44 F_1 *in vitro* hybrids, only 2 F_1 hybrids were survived. These two F_1 hybrids were transplanted again in polybags (6 x 9") with soil, sand and F.Y.M. (1:1:1 v/v) and placed in partial shade for another 15 days for hardening. After 30 days of hardening these two F_1 hybrids were transplanted under field conditions for further study.

Table 9. Hardening response of putative interspecific hybrids under polyhouse and field conditions.

Sr. No.	Cross combinations of interspecific parents	No. of *in vitro* interspecific hybrid plants developed	No. of plants survived								
			Under polyhouse conditions						Under field conditions		
			3 d	6 d	9 d	12 d	15 d	30 d	60 d	90 d	120 d
1	Dhanashree x EC 127774	6	3	1*	1	0	0	0	0	0	0
2	Dhanashree x EC 106294	9	4	2**	1	1	1	1	1	1	1**
3	Dhanashree x EC 252	9	3	1**	1	1	1	1	1	1	1**
4	Dhanashree x EC 486	5	2	1*	0	0	0	0	0	0	0
5	Dhanashree x EC 34479	0	0	0	0	0	0	0	0	0	0
6	Dhanashree x EC 492	0	0	0	0	0	0	0	0	0	0
7	85-1 x EC 127774	2	0	0	0	0	0	0	0	0	0
8	85-1 x EC 106294	6	3	2*	1	0	0	0	0	0	0
9	85-1 x EC 252	3	0	0	0	0	0	0	0	0	0
10	85-1 x EC 486	3	1	0	0	0	0	0	0	0	0
11	85-1 x EC 34479	1	0	0	0	0	0	0	0	0	0
12	85-1 x EC 492	0	0	0	0	0	0	0	0	0	0
	Total	44	16	7	3	2	2	2	2	2	2

* Sub-cultured once 21 days

** Sub-cultured twice at interval of 21 days.

4.4 Confirmation of hybridity of putative interspecific F_1 hybrid plants

For confirmation of hybridity of putative tomato interspecific hybrids between *L. esculentum* x *L. peruvianum*, morphological characterization and biochemical (isozyme) analysis was carried out.

4.4.1 Morphological characterization of F_1 hybrids and their parents

Two F_1 hybrid plants (derived from cultivated tomato cv. Dhanshree x *L. peruvianum* accession EC 106294 and cv. Dhanshree x *L. peruvianum* accession EC 252) showed intermediate and parent specific morphological characters for plant growth habit, leaf, stem, floral and fruit morphology. However, overall morphological traits of F_1 hybrids were inclined by wild pollen parent. Thus, it proved hybridity status of both interspecific F_1 hybrids (Table 10; Plate 4).

The growth habit of both F_1 hybrid plants was indeterminate type similar to their wild parents, while leaf colour of F_1 hybrids was green (intermediate) as compared to dark green leaf colour of tomato cvs. and pale green foliage of wild parents. Leaflet size was also intermediate. Leaf serration and leaf thickness was similar to that of wild parents. For stem morphology, trichomes were not present on stems of F_1 hybrids and their wild parents. Stem type of F_1 hybrids was round (like wild parent) with moderate thickness (intermediate).

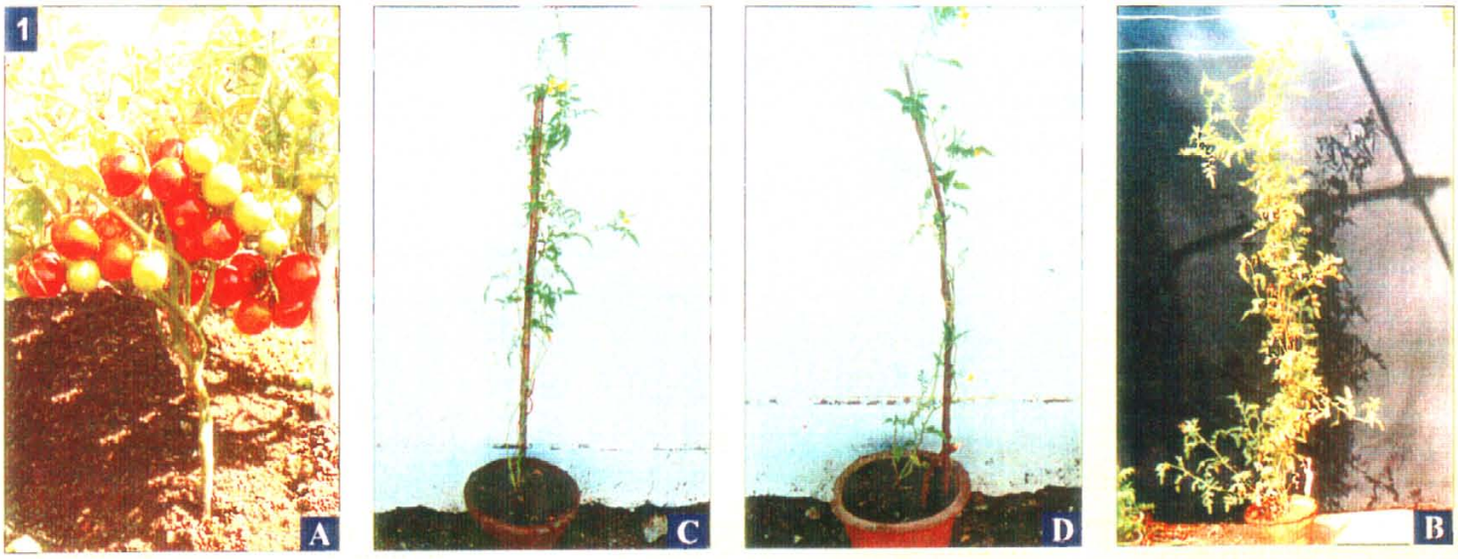
The floral morphology of F_1 hybrids was with unbranched inflorescence similar to that of cultivated tomato while exerted stigma and united floral petals were observed like wild parents (Plate 5). While assessing for pollen viability of F_1 hybrids along with their parents, it was noticed that remarkable reduction was observed in both F_1 hybrids (43.29 to 46.23%;

Table 10. Morphological characterization of *in vivo* F_1 hybrids of *L. esculentum* and *L. peruvianum* in comparison to their parents

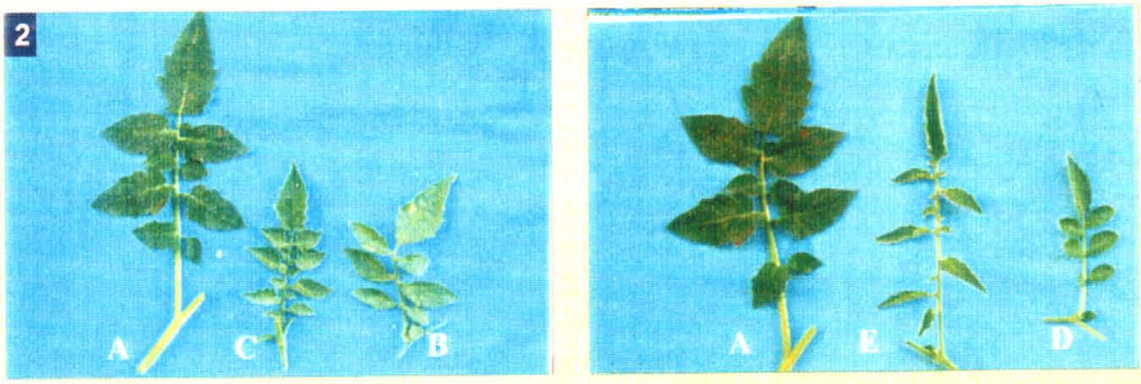
Character	*L. esculentum*	*L. peruvianum*		Interspecific hybrid	
	Dhanashree (P1)	EC 106294 (P2)	EC 252 (P3)	P1 x P2	P1 x P3
Plant growth habit	Semi-indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate
Plant height (cm)	65±5.0	148±3.5	144±5.4	132±4.0	127±3.8
Leaf morphology					
Leaf colour	Dark green	Light green	Light green	Green	Green
Leaflet length (cm)	13.5±1.5	9.4±0.1	12.0±0.2	10.8±0.7	12.5±0.6
Leaflet breadth (cm)	10.6±1.5	6.4±0.2	5.8±0.8	8.2±0.3	7.0±0.5
Leaf serration	Moderate	Non-serrated	Non-serrated	Non-serrated	Non-serrated
Leaflet thickness	Thick	Thin	Thin	Thin	Thin
Stem morphology					
Main stem thickness (cm)	12.3±1.1	3.8±0.4	2.9±0.6	4.1±0.2	3.3±0.5
Trichome	Present	Absent	Absent	Absent	Absent
Stem type	Angular	Round	Round	Round	Round
Floral morphology					
Inflorescence	Unbranched	Branched	Branched	Unbranched	Unbranched
Stock length(cm)	6.2±0.8	13.0±0.4	10.4±0.8	12.3±0.6	10.0±0.5
Stigma type	Inserted	Exerted	Exerted	Exerted	Exerted
Flower petal	Semi-united	United	United	United	United
Pollen viability	96.75±3.5	89.20±4.27	81.76±3.95	46.23±4.07	43.29±5.23
Fruit morphology					
Fruit shape	Round	Round	Round	Round	Round
Fruit size (cm)	Big (5.1 x 4.9)	Cherry (1.5 x 1.6)	Cherry (1.4 x 1.4)	Cherry (1.6 x 1.4)	Cherry (1.5 x 1.3)
Fruit weight (g)	50±5	4±1	4.7±0.5	4.6±0.4	4.9±0.8
Blossom end	Non-nipped	Non-nipped	Non-nipped	Non-nipped	Non-nipped
Ripening colour	Red	Pale green with purple stripe	Pale green with purple stripe	Pale green with purple stripe	Pale green with purple stripe
No. of seeds per fruit	121-137	75-84	65-78	Nil	Nil
Seed type	Bold and hairy	Tiny and non-hairy	Tiny and non-hairy	Nil	Nil

PLATE 4

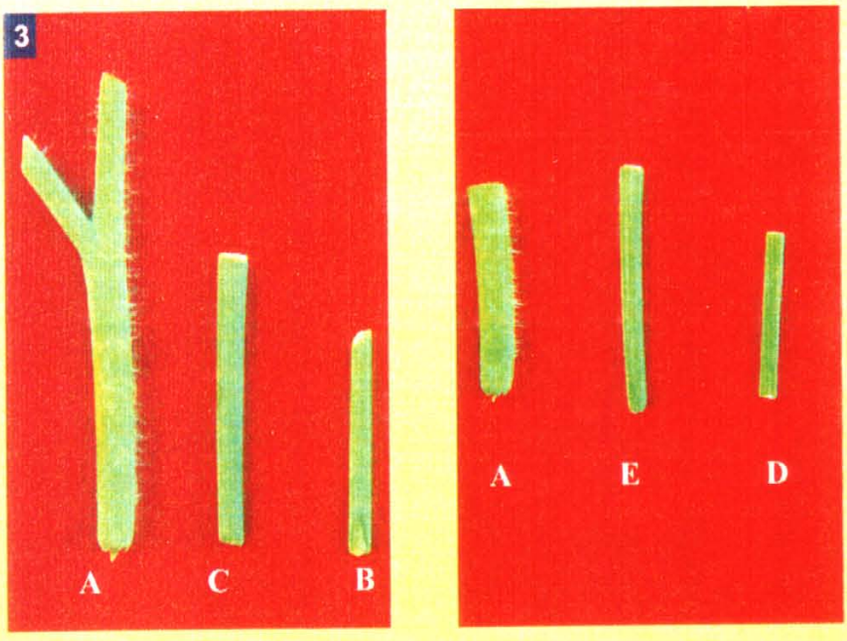
Plate 4 : Morphological characterization of inter-specific F_1 hybrids



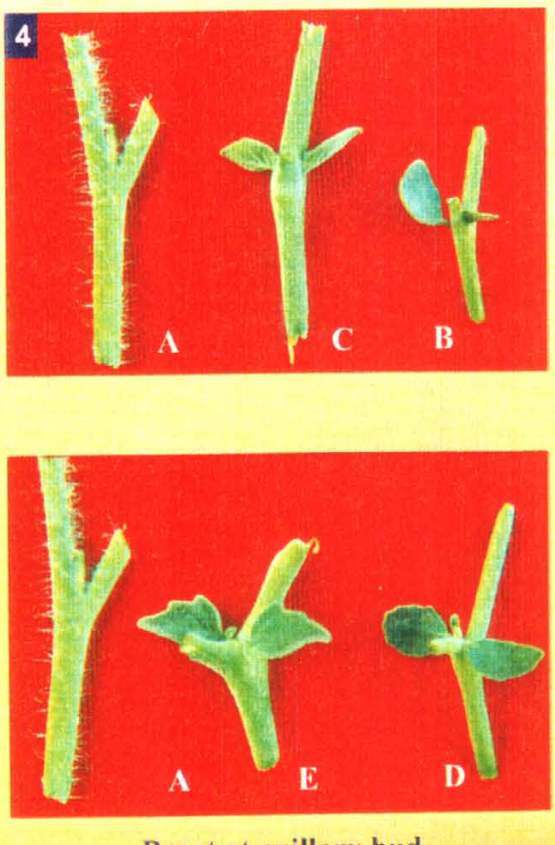
Plant growth



Leaf morphology



Stem morphology

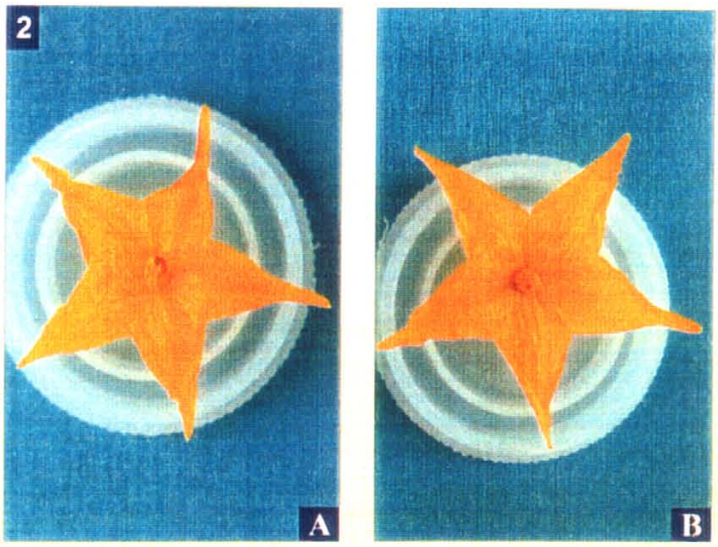


Bract at axillary bud

PLATE 5

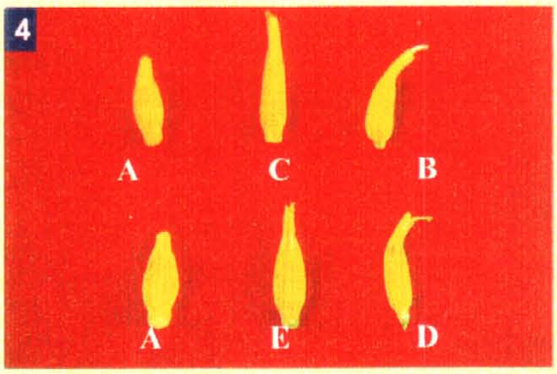
Plate 5 : Morphological characterization of interspecific F_1 hybrids

Unbranched inflorescence of F_1 hybrids

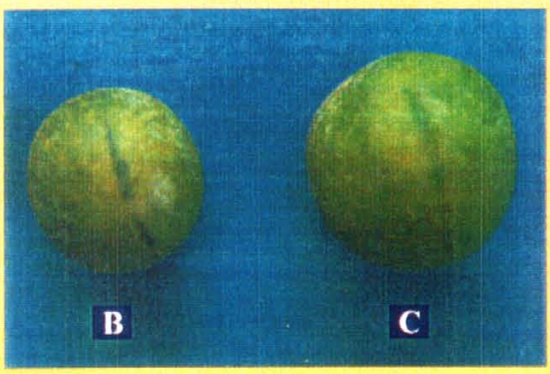


Petal arrangement of flowers of F_1 hybrids

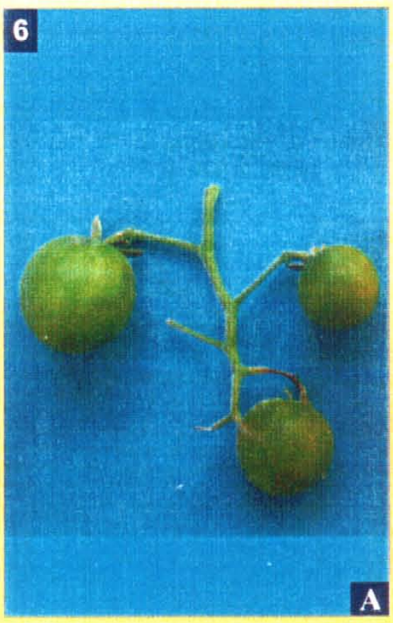
Petal arrangement of *L.esculentum*

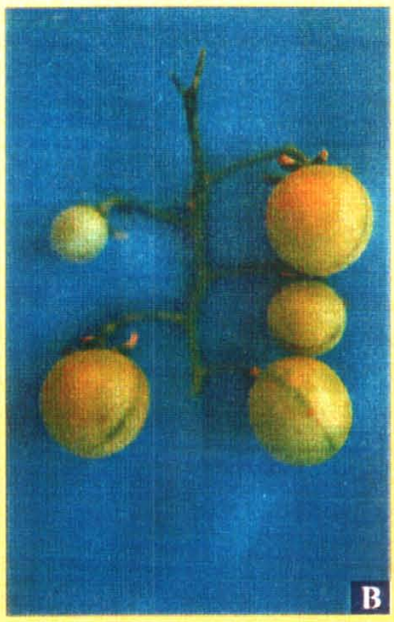


Anthercone with stigma position



Fruit characterization





Fruits of F_1 hybrids

(Dha x EC 252) (Dha x EC 106294)

Table 10) as compared to their parents (81.76 to 96.75%). The significant reduced pollen viability in F_1 hybrids showed severe interspecific breeding barriers and may be contributed in hybrid-sterility displaying by F_1 hybrids.

For fruit morphology, round cherry fruit type with non-nipped blossom end and pale green fruit colour at maturity was noticed. These characters were more like to that of pollen parents (*L. peruvianum*). Eventhough, fruit morphology of F_1 hybrid displayed close similarity with *L. peruvianum* genotypes, it showed distinguish differences with self fertility. The both pollen parents of F_1 hybrids i.e. EC 106294 and EC 252 were self fertile (Table 10) but both F_1 hybrids were self-sterile i.e. fruit development without seed.

Thus, severe interspecific breeding barriers were noticed in both F_1 hybrids which was an obvious evidence of interspecific hybridity.

In general both F_1 plants of interspecific hybrids had a close morphological similarity to their pollen parents, *L. peruvianum*.

4.4.2 Biochemical characterization with total protein analysis

Total proteins were extracted from both parents and hybrid plant and checked the electrophoratically by SDS-PAGE method. The variation (existence or presence) of total protein bands were analysed and studied to confirm the hybridity of putative hybrids between *L. esculentum* x *L. peruvianum*.

The schematic diagram of SDS-PAGE denaturing electrophoretic pattern of soluble leaf protein is presented in Fig. 4 and Plate 6. While intensity of bands and banding pattern for specific cultivar is given in Table 11 and 12, respectively.

Table 11. R. M. values of parent and F_1 plants of tomato interspecific (*L. esculentum* x *L. peruvianum*) hybrids

Sr. No.	Dhana-shree	EC 252	F_1	Band type of F_1	Dhana-shree	EC 106294	F_1	Band type of F_1
1	0.20	0.20	0.22	5	0.20	0.25	0.27	2
2	0.25	0.25	0.24	5	0.25	0.31	0.36	5
3	0.31	0.31	0.28	1	0.31	0.36	0.40	1
4	0.36	0.36	0.32	5	0.36	0.45	0.43	2
5	0.53	0.54	0.35	5	0.53	0.50	0.48	1
6	0.56	0.56	0.37	1	0.56	0.55	0.52	1
7	0.69	0.59	0.43	1	0.69	0.59	0.56	3
8	0.78	0.65	0.47	1	0.78	0.66	0.59	4
9	0.80	0.70	0.51	1	0.80	0.69	0.63	1
10	0.81	0.76	0.59	4	0.81	0.74	0.65	4
11	0.85	0.81	0.61	1	0.85	0.78	0.68	5
12	0.95	0.92	0.67	2	0.95	0.82	0.71	1
13	-	0.95	0.70	4	-	0.85	0.75	4
14	-	-	0.75	4	-	0.93	0.81	3
15	-	-	0.89	3	-	0.97	0.85	4
16	-	-	0.93	4	-	-	0.94	2
17	-	-	0.97	5	-	-	0.97	4
18	-	-	0.98	1	-	-	-	-
19	-	-	-	-	-	-	-	-
	12	13	18		12	15	17	-

Band types : 1 = Hybrid specific band
2 = Intermediate band
3 = Parent 1 Specific band
4 = Parent 2 Specific band
5 = Bands common for both the parents.

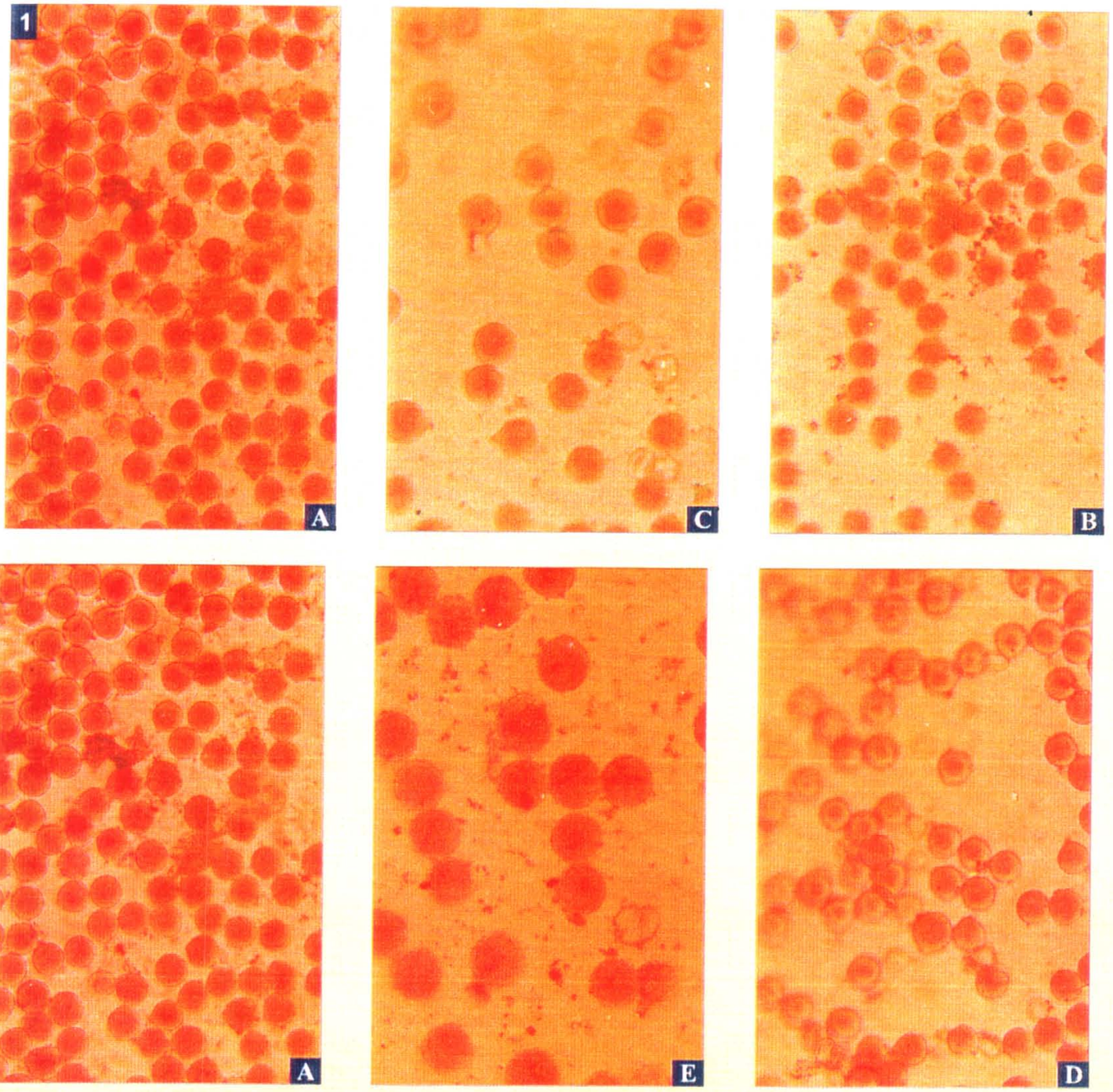
Table 12. Characteristics of protein banding pattern of tomato interspecific (*L. esculentum* x *L. peruvianum*) F_1 hybrids and their parents

Sr. No.	Genotypes	Category	Banding numbers
1.	Dhanashree	P	1, 4, 6, 10, 18, 20, 29, 37, 38, 39, 40, 45
		A	2, 3, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 34, 35, 36, 41, 42, 43, 44
2.	EC 252	P	1, 4, 6, 10, 19, 20, 22, 26, 31, 35, 39
		A	2, 3, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 21, 23, 24, 25, 27, 28, 29, 30, 32, 33, 34, 36, 37, 38
3.	F_1 (Dhanashree x EC 252)	P	1, 2, 5, 7, 8, 9, 11, 13, 16, 21, 22, 26, 29, 32, 37, 38, 41, 42
		A	3, 4, 6, 10, 12, 14, 15, 17, 18, 19, 20, 23, 24, 25, 27, 28, 30, 31, 33, 34, 35, 36, 39, 40
4.	EC 106294	P	3, 6, 8, 12, 15, 19, 21, 25, 28, 31, 34, 36, 37, 38, 41
		A	1, 2, 4, 5, 7, 9, 10, 11, 13, 14, 16, 17, 18, 20, 22, 23, 24, 26, 27, 29, 30, 32, 33, 35, 39, 40
5.	F_1 (Dhanashree x EC 106294)	P	4, 8, 10, 11, 14, 17, 19, 21, 23, 25, 27, 30, 32, 35, 37, 39, 41
		A	1, 2, 3, 5, 6, 7, 8, 9, 12, 13, 15, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 34, 36, 38, 40

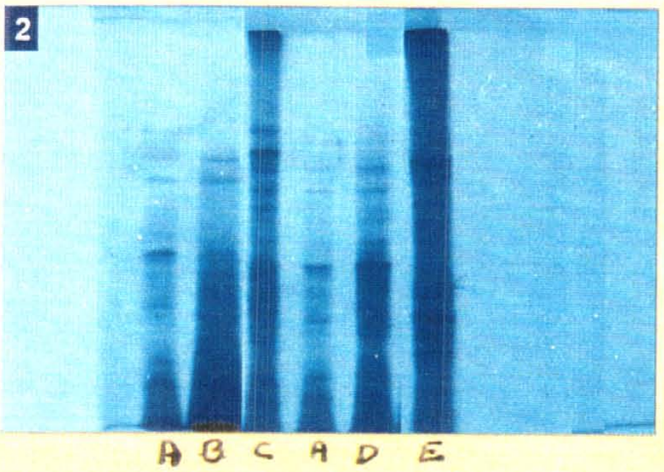
P = Present, A = Absent.

PLATE 6

Plate 6 : Characterization of interspecific F_1 hybrids



Pollen viability



Total protein analysis

Flower drop at high temperature

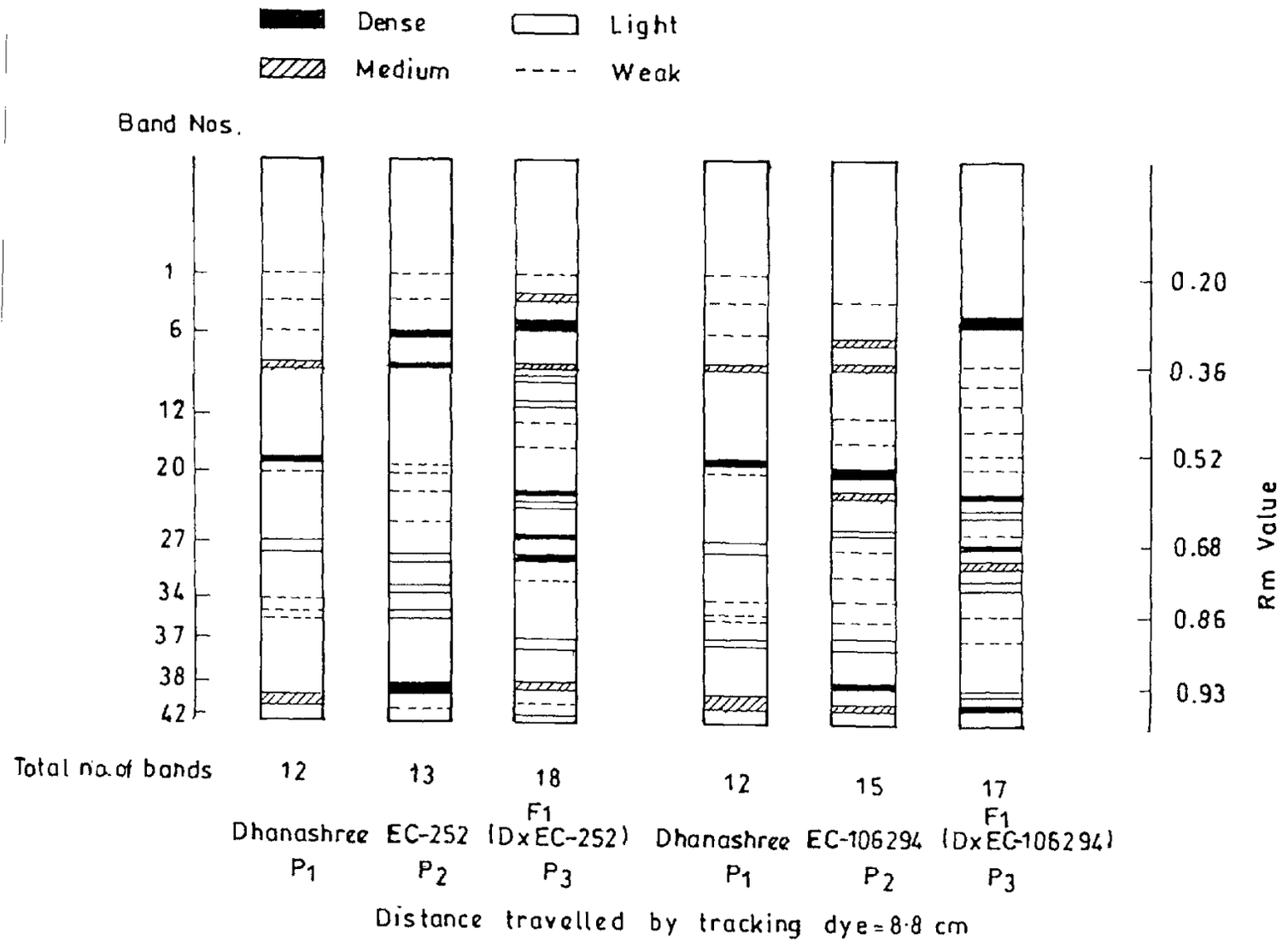


Fig. Electrophoragrams of leaf protein of tomato interspecific hybrids and their parents

In total protein analysis, hybridity of F_1 plants was confirmed with banding pattern of electrophorogram on the basis of presence or absence of parent specific bands, intermediate bands and emergence of new band as a hybrid originated bands (Table 12). A total 47 bands were resolved in the gel material of the leaf protein in different genotypes under study. The overall banding pattern revealed a variation in the number and intensity of bands in all the genotypes from 12 to 18. The qualitative and quantitative variations were observed in the banding pattern of soluble leaf protein of tomato genotypes.

The Rm values of different bands are given in Table 11. The hybrid cv. Dhanashree x EC 252 exhibited the maximum number of bands (18) followed by other hybrid (17) cv. Dhanashree x EC 106294, than their respective parents.

4.4.2.1 Confirmation of hybridity for F_1 hybrid, Dhanashree x EC 252

In this particular hybrid out of 18 bands, 5 bands were common for both the parents, 4 bands were parent 2 specific and 1 band each as intermediate with both the parents and parent 1 specific band. Additionally, 7 bands were noticed as unique hybrid bands. Thus, most of the bands of F_1 hybrids were intermediate to their both the parents rather than summation of parent specific bands.

4.4.2.2 Confirmation of hybridity for F_1 hybrid, Dhanashree x EC 106294

The bands with Rm values out of 17 bands, 5 bands were observed parent 2 specific, 3 bands were intermediate with both the parents and 2 bands each were observed common for both the parents and parent 1 specific bands. Additionally, 5 bands were noticed as unique hybrid bands. Thus, most of the

bands of F_1 hybrid were intermediate to their both the parents rather than summation of parent specific bands.

4.5 Screening of interspecific tomato F_1 hybrids for biotic and abiotic stresses

4.5.1 Biotic stresses

4.5.1.1 Pest incidence

Both tomato interspecific F_1 hybrids and their parents were screened against important pests like leaf miner, mites, whitefly and thrips under field conditions (Table 13). Observations were recorded at an interval of 15 days from 30 to 75 days after transplanting under field conditions. The cv. Dhanashree showed highest incidence for all pest as compared to wild parents (i.e. *L. peruvianum* accessions) and their hybrids. While F_1 hybrids recorded intermediate incidence for all pests in comparison to their parents but it was almost similar with their wild parents. Especially, these observations were important for virus vectors i.e. whiteflies and thrips.

4.5.1.2 Disease incidence

The observations were recorded on important fungal diseases like early and late blight, powdery mildew and viral diseases like tomato leaf curl virus (TLCV) and tomato spotted wilt virus (TSWV) during *kharif* 2002. However, it was observed that only cultivated tomato (*L. esculentum*) cv. Dhanashree showed disease symptoms for all diseases while F_1 hybrids and their wild parents were free from any disease symptoms (Table 14).

4.5.2 Abiotic stresses

The major abiotic stress in tomato is fruit set and development under high temperatures (i.e. >40°C) especially during summer season. Therefore,

Table 13. Screening of interspecific tomato F_1 hybrids and their parents for pest infestation during *kharif* 2002 under field conditions

Sr. No.	Pest	DAT	Per cent pest infestation				
			Dhanashree (P1)	EC 106294 (P2)	EC 252 (P3)	F_1 hybrid (P1+P2)	F_1 hybrid (P1+P3)
1.	**Leaf miner** (No. of mines per leaflet)	30	1.67±0.47	0.33±0.47	0.33±0.47	0.67±0.47	0.33±0.47
		45	2.33±0.94	1.33±0.47	0.67±0.47	1.00±0.82	1.33±0.94
		60	2.67±1.24	1.00±0.82	1.33±0.47	1.33±0.94	1.67±0.82
		75	3.00±1.67	1.33±0.47	1.33±0.47	1.67±0.94	2.00±0.47
2.	**Mites** (No. of mites per leaf)	30	3.33±1.25	1.33±0.47	1.33±0.47	1.67±0.47	1.00±1.41
		45	5.33±1.70	2.67±0.47	2.67±0.94	2.33±0.47	1.67±0.94
		60	8.67±1.70	4.00±0.81	3.67±0.94	4.33±0.94	2.67±1.25
		75	9.67±2.00	4.67±1.25	5.00±0.81	4.67±1.25	3.00±0.82
3.	**Whitefly** (No. of flies per leaf)	30	1.33±0.94	0.00±0.00	0.33±0.47	0.33±0.47	0.67±0.47
		45	2.67±1.25	0.33±0.47	0.67±0.47	1.00±0.47	1.00±0.80
		60	3.00±1.63	1.33±1.25	1.33±0.94	1.67±0.82	1.67±0.94
		75	4.00±1.41	1.67±0.94	1.67±0.82	2.00±0.81	2.33±1.24
4.	**Thrips** (No. of thrips per leaf)	30	4.00±1.41	1.33±0.47	1.67±0.82	1.33±0.94	1.67±0.94
		45	4.67±1.88	2.33±0.47	2.00±0.47	2.67±1.25	2.33±0.47
		60	3.67±1.69	2.67±0.47	2.33±0.47	2.67±0.94	2.33±0.94
		75	5.67±0.94	2.67±1.25	2.67±0.94	2.67±0.94	3.00±0.81

DAT = Days after transplanting.

Table 14. Screening of interspecific tomato F_1 hybrids and their parents for per cent disease incidence during *kharif* 2002 under field conditions

Sr. No.	Pest	DAT	Per cent disease incidence				
			Dhanashree (P1)	EC 106294 (P2)	EC 252 (P3)	F_1 hybrid (P1+P2)	F_1 hybrid (P1+P3)
1.	Early blight	30	26.67±6.79	-	-	-	-
		45	39.33±10.78	-	-	-	-
		60	53.67±18.35	-	-	-	-
		75	55.67±8.65	-	-	-	-
2.	Late blight	30	10.33±2.05	-	-	-	-
		45	14.00±1.69	-	-	-	-
		60	16.33±4.64	-	-	-	-
		75	18.67±3.77	-	-	-	-
3.	Powdery mildew	30	9.33±2.87	-	-	-	-
		45	17.33±8.73	-	-	-	-
		60	29.67±4.03	-	-	-	-
		75	33.00±8.00	-	-	-	-
4.	Total leaf curl virus (TLCV)	30	5.00±4.08	-	-	-	-
		45	10.33±8.67	-	-	-	-
		60	27.67±4.19	-	-	-	-
		75	30.00±8.04	-	-	-	-
5.	Spotted wilt virus (SWV)	30	7.67±8.22	-	-	-	-
		45	15.33±4.78	-	-	-	-
		60	22.00±6.16	-	-	-	-
		75	28.33±8.33	-	-	-	-

DAT = Days after transplanting.

flowering and fruiting behaviour of F_1 hybrids and their parents was assessed especially in the months of April and May 2003 when maximum temperatures were more than 40°C and relative humidity lower than 50%. However, all plants under study including F_1 hybrids and their parents showed severe flower drop without fruit setting.

4.6 Tissue culture technique for plant regeneration studies in *Lycopersicon* species

Plant regeneration studies are of prime important in tomato for tissue culture and other biotechnological studies such as genetic transformation, somatic hybridization, etc. In this view, plant regeneration studies in *L. esculentum* and *L. peruvianum* accessions were undertaken in present investigation. In this studies standardization of culture media and responsiveness of various plant segments of different tomato genotypes were assessed for plant regeneration.

To study the plant regeneration in genus *Lycopersicon*, 2 explants (leaf and cotyledon) of 3 genotypes (two tomato cultivars viz., Dhanashree and Bhagyashree and one accession of *L. peruvianum* viz., EC 106294) and 20 culture media were assessed for callus induction and plant regeneration in factorial completely randomized design (FCRD).

4.6.1 Number of days required for callus induction

For callus induction effect of three factors (i.e. genotype, explant and culture media) were studied and significant differences were observed for all three factors (Table 15).

Among three genotypes, *L. peruvianum* accession EC 106294 recorded the most early callus induction (7.55 days) which was at par with cv.

Table 15. Three-way analysis of variance for the effect of culture medium, genotype and explant on number of days required for callus induction in genus *Lycopersicon*

Culture media	MS medium with		Dhanashree		Bhagyashree		EC 106294 (*L. peruvianum*)	
	NAA mg l^{-1}	BAP mg l^{-1}	Leaf segment	Coty-ledon segment	Leaf segment	Coty-ledon segment	Leaf segment	Coty-ledon segment
M_1	0.2	0.5	6.00	7.67	6.67	8.33	5.00	7.00
M_2	0.2	1.0	6.67	8.00	7.33	7.33	5.67	6.67
M_3	0.2	1.5	6.00	6.33	6.33	8.00	5.00	7.00
M_4	0.2	2.5	7.00	6.67	6.33	7.00	6.67	9.00
M_5	0.2	5.0	5.33	6.33	7.67	7.67	6.33	6.67
M_6	0.5	0.5	6.33	7.33	6.67	7.33	6.00	7.67
M_7	0.5	1.0	7.00	8.33	5.33	6.67	7.00	7.00
M_8	0.5	1.5	6.67	8.33	8.67	9.00	6.67	7.00
M_9	0.5	2.5	7.33	7.67	7.00	8.67	6.33	8.00
M_{10}	0.5	5.0	5.67	9.33	6.67	8.00	7.00	9.00
M_{11}	1.0	0.5	7.33	8.67	9.00	8.33	7.00	7.00
M_{12}	1.0	1.0	7.00	6.33	7.67	8.33	5.67	7.00
M_{13}	1.0	1.5	7.67	8.67	9.00	8.67	7.00	7.33
M_{14}	1.0	2.5	7.33	8.67	6.67	9.67	7.33	9.33
M_{15}	1.0	5.0	7.67	8.33	7.67	12.00	7.00	8.33
M_{16}	1.5	0.5	7.33	10.33	10.33	11.67	6.67	9.33
M_{17}	1.5	1.0	7.67	10.00	10.00	10.00	8.33	9.67
M_{18}	1.5	1.5	8.33	10.33	8.67	10.67	8.33	10.67
M_{19}	1.5	2.5	9.67	11.00	10.33	11.00	8.00	11.00
M_{20}	1.5	5.0	9.00	10.33	9.00	11.33	10.33	10.00

Genotypic mean (G) : Dhanashree = 7.74, Bhagyashree = 8.42, EC 106294 (*L. peruvianum*) = 7.55

Explant mean (E) : Leaf segment = 7.26, Cotyledon segment = 8.55

Medium mean (M) : M_1 = 6.78, M_2 = 6.94, M_3 = 6.44, M_4 = 7.11, M_5 = 6.67, M_6 = 6.89, M_7 = 6.89, M_8 = 7.72, M_9 = 7.50, M_{10} = 7.61, M_{11} = 7.89, M_{12} = 6.78, M_{13} = 8.00, M_{14} = 8.11, M_{15} = 8.50, M_{16} = 9.28, M_{17} = 9.28, M_{18} = 9.50, M_{19} = 10.17, M_{20} = 10.00

SE for mean : Genotype (G) = 0.14, Explant (E) = 0.12, Medium (M) = 0.36, G x E = 0.19, G x M = 0.63, E x M = 0.51, G x E x M = 0.89

C.D. at 5% level : Genotype (G) = 0.39, Explant (E) = 0.32, Medium (M) = 1.02, G x E = N.S., G x M = 1.8, E x M = N.S., G x E x M = N S.

Dhanashree (7.74 days), while significant late callus induction was observed in cv. Bhagyashree (8.42 days).

Among two explants, leaf segment showed significantly early callus induction (7.26 days) than the cotyledon explant (8.55 days).

While evaluating 20 callus media for callus induction the significant early callus induction was recorded on medium M_3 (6.44 days) which was at par with M_1, M_2, M_4, M_5, M_6 and M_7 culture media. The late callus induction was observed with increasing concentration of NAA (0.5 to 1.5 mg l^{-1}) coupled with BAP (2.5 to 5.0 mg l^{-1}).

For interaction effect on callus induction except genotype x media, all other interaction effects for two and three factors were non-significant. However, the most early callus inductions (5.0 days) was recorded by leaf segments of *L. peruvianum* accessions on M_1 and M_3 culture media. While the most late callus induction (12.00 days) was recorded by cotyledon segment of cv. Bhagyashree on M_{15} culture medium.

4.6.2 Callus induction

Among three factors (genotype, explant and culture media) studied significant differences were observed only for two factors explant and culture media. While non-significant differences were observed for their interactions (Table 16).

Eventhough non-significant differences were observed for genotypes, the highest callus induction was noticed in cv. Dhanashree (47.83%) followed by EC 106294 (47.08%) and cv. Bhagyashree (43.92%).

Table 16. Three-way analysis of variance for the effect of culture medium, genotype and explant on per cent callus induction

Culture media	MS medium with		Dhanashree		Bhagyashree		EC 106294 (*L. peruvianum*)	
	NAA mg l^{-1}	BAP mg l^{-1}	Leaf segment	Coty-ledon segment	Leaf segment	Coty-ledon segment	Leaf segment	Coty-ledon segment
M_1	0.2	0.5	36.67	20.00	20.00	23.33	30.00	26.67
M_2	0.2	1.0	56.67	43.33	56.67	36.67	53.33	33.33
M_3	0.2	1.5	50.00	40.00	60.00	30.00	63.33	40.00
M_4	0.2	2.5	43.33	50.00	36.67	43.33	40.00	46.67
M_5	0.2	5.0	36.67	30.00	36.67	26.67	43.33	36.67
M_6	0.5	0.5	40.00	40.00	30.00	33.33	33.33	36.67
M_7	0.5	1.0	56.67	46.67	53.33	40.00	56.67	46.67
M_8	0.5	1.5	63.33	46.67	66.67	46.67	60.00	50.00
M_9	0.5	2.5	56.67	43.33	50.00	40.00	46.67	36.67
M_{10}	0.5	5.0	40.00	36.67	43.33	36.67	43.33	33.33
M_{11}	1.0	0.5	30.00	40.00	33.33	20.00	26.67	26.67
M_{12}	1.0	1.0	50.00	40.00	50.00	50.00	43.33	30.00
M_{13}	1.0	1.5	63.33	53.33	56.67	46.67	66.67	50.00
M_{14}	1.0	2.5	73.33	66.67	60.00	53.33	76.67	60.00
M_{15}	1.0	5.0	90.00	76.67	80.00	66.67	86.67	63.33
M_{16}	1.5	0.5	40.00	26.67	40.00	36.67	43.33	30.00
M_{17}	1.5	1.0	43.33	33.33	40.00	30.00	40.00	46.67
M_{18}	1.5	1.5	50.00	46.67	46.67	36.67	56.67	46.67
M_{19}	1.5	2.5	53.33	50.00	50.00	43.33	63.33	50.00
M_{20}	1.5	5.0	56.67	53.33	50.00	56.67	70.00	50.00

Genotypic mean (G) : Dhanashree = 47.83, Bhagyashree = 43.92, EC 106294 (*L. peruvianum*) = 47.08

Explant mean (E) : Leaf segment = 50.56, Cotyledon segment = 42.00

Medium mean (M) : M_1 = 26.11, M_2 = 46.67, M_3 = 47.22, M_4 = 43.33, M_5 = 55.00, M_6 = 35.00, M_7 = 50.00, M_8 = 55.56, M_9 = 45.56, M_{10}= 38.89, M_{11}= 29.44, M_{12}= 43.89, M_{13}= 56.11, M_{14}= 65.00, M_{15}= 77.22, M_{16}= 36.11, M_{17}= 38.89, M_{18}= 47.22, M_{19}=51.67, M_{20}= 56.11

SE for mean : Genotype (G) = 1.89, Explant (E) = 1.55, Medium (M) = 4.89, G x E = 2.68, G x M = 8.46, E x M = 6.91, G x E x M = 11.97

C.D at 5% level : Genotype (G) = N.S., Explant (E) = 4.35, Medium (M) = 13.75, G x E = N.S., G x M = N.S., E x M = N.S., G x E x M = N.S.

Among the explants, leaf segment induced callus significantly higher (50.56%) than cotyledon segment (42.00%).

Among culture media, medium M_{15} was significantly superior (77.22%) over other media except medium M_{14} (65.00%) which was at par with each other.

4.6.3 Shoot regeneration

Effect of three factors (i.e. genotype, explant and culture media) was assessed on shoot regeneration. The range for shoot regeneration was observed 0 to 100%. The significant differences were recorded for three factors and interactions, genotype x explant and explant x media (Table 17).

Among the genotypes, *L. peruvianum* accession EC 106294 recorded significantly higher shoot regeneration rate (45.58%) over tomato cv. Bhagyashree (41.00%) but it was at par with cv. Dhanashree (44.58%).

Among the explants, the leaf segment proved to be the best explant source as significantly higher shoot regeneration (52.89%) was recorded than cotyledon segment (34.56%) (Plate 7).

Among the culture media, medium M_{14} had a significantly superior morphogenic potential (88.33%) over other media except medium M_{15} (85.00%) which was at par with each other. Other effective culture media were M_{13} (78.33%), M_{20} (70.00%) which were at par with each other. However, media M_1 and M_2 did not exhibit morphogenesis while medium M_3 showed meagre shoot regeneration (3.33%).

Among interactions of genotype x explants, the significantly higher shoot regeneration was recorded by *L. peruvianum* accession, EC 106294 with

Table 17. Three-way analysis of variance for the effect of culture medium, genotype and explant on percentage explants giving direct shoot regeneration

Culture media	MS medium with		Dahanshree		Bhagyashree		*L. peruvianum* EC 106294	
	NAA mg l^{-1}	BAP mg l^{-1}	Leaf segment	Cotyledon segment	Leaf segment	Cotyledon segment	Leaf segment	Cotyledon segment
M_1	0.2	0.5	0.00	0.00	0.00	0.00	0.00	0.00
M_2	0.2	1.0	0.00	0.00	0.00	0.00	0.00	0.00
M_3	0.2	1.5	0.00	0.00	0.00	0.00	20.00	0.00
M_4	0.2	2.5	13.33	16.67	10.00	10.00	20.00	10.00
M_5	0.2	5.0	26.67	16.67	30.00	13.33	36.67	13.33
M_6	0.5	0.5	20.00	0.00	30.00	0.00	26.67	0.00
M_7	0.5	1.0	40.00	30.00	33.33	16.67	50.00	23.33
M_8	0.5	1.5	43.33	36.00	53.33	36.67	50.00	30.00
M_9	0.5	2.5	56.67	50.00	50.00	40.00	63.33	46.67
M_{10}	0.5	5.0	63.33	53.00	56.67	46.67	60.00	60.00
M_{11}	1.0	0.5	33.33	26.67	23.33	30.00	40.00	23.33
M_{12}	1.0	1.0	70.00	56.67	70.00	30.00	93.33	46.67
M_{13}	1.0	1.5	100.00	60.00	100.00	56.67	100.00	53.33
M_{14}	1.0	2.5	100.00	80.00	100.00	73.33	100.00	76.67
M_{15}	1.0	5.0	100.00	73.33	100.00	66.67	100.00	70.00
M_{16}	1.5	0.5	66.67	30.00	53.33	23.33	50.00	30.00
M_{17}	1.5	1.0	70.00	50.00	70.00	43.33	80.00	40.00
M_{18}	1.5	1.5	73.33	50.00	70.00	46.67	83.33	46.67
M_{19}	1.5	2.5	76.67	56.07	73.33	50.00	86.67	50.00
M_{20}	1.5	5.0	76.67	66.67	73.33	60.00	86.67	56.67

Genotypic mean (G) : Dhanashree = 44.58, Bhagyashree = 41.00, EC 106294 (*L. peruvianum*) = 45.58

Explant mean (E) : Leaf segment = 52.89, Cotyledon segment = 34.56

Medium mean (M) : M_1 = 0.0, M_2 = 0.0, M_3 = 3.33, M_4 = 13.33, M_5 = 22.78, M_6 = 12.78, M_7 = 32.22, M_8 = 41.67, M_9 = 51.11, M_{10}= 56.67, M_{11}= 29.44, M_{12}= 61.11, M_{13}= 78.33, M_{14}= 88.33, M_{15}= 85.00, M_{16}= 42.22, M_{17}= 58.89, M_{18}= 61.67, M_{19}= 65.56, M_{20}= 70.00

SE for mean : Genotype (G) = 1.31, Explant (E) = 1.07, Medium (M) = 3.39, G x E = 1.85, G x M = 5.87, E x M = 4.79, G x E x M = 8.31

C.D. at 5% level : Genotype (G) = 3.69, Explant (E) = 3.01, Medium (M) = 9.54, G x E = 5.22, G x M = N.S., E x M = 13.49, G x E x M = N.S.

Table 18. Two-way table for interaction between genotype and explant for per cent explant giving one or more shoots

Sr. No.	Genotype	Explant	
		Leaf segment	Cotyledon segment
1.	Dhanashree	51.50	37.67
2.	Bhagyashree	49.83	32.17
3.	EC 106294 (*L. peruvianum*)	57.33	33.83

Genotypic mean (G) : Dhanashree = 44.58, Bhagyashree = 41.00, EC 106294 (*L. peruvianum*) = 45.58

Explant mean (E) : Leaf segment = 52.89, Cotyledon segment = 34.56

Interaction mean (G x E) : 43.72

Table 19. Two-way table for interaction between medium and explant for per cent explant giving one or more shoots

Culture media	MS medium with		Explant	
	NAA mg l^{-1}	BAP mg l^{-1}	Leaf segment	Cotyledon segment
M_1	0.2	0.5	0.00	0.00
M_2	0.2	1.0	0.00	0.00
M_3	0.2	1.5	6.67	0.00
M_4	0.2	2.5	14.44	12.22
M_5	0.2	5.0	31.11	14 44
M_6	0.5	0.5	25.56	0.00
M_7	0.5	1.0	41.11	23.33
M_8	0.5	1.5	48.89	34.44
M_9	0.5	2.5	56.67	45.56
M_{10}	0.5	5.0	60.00	53.33
M_{11}	1.0	0.5	32.22	26.67
M_{12}	1.0	1.0	77.78	44.44
M_{13}	1.0	1.5	100.00	56.67
M_{14}	1.0	2.5	100.00	76.67
M_{15}	1.0	5.0	100.00	70.00
M_{16}	1.5	0.5	56.67	27.78
M_{17}	1.5	1.0	73.33	44.44
M_{18}	1.5	1.5	75.56	47.78
M_{19}	1.5	2.5	78.89	52.22
M_{20}	1.5	5.0	78.89	61.11

Explant mean (E) : Leaf segment = 52.89
Cotyledon segment 34.56

Interaction mean (G x E) : 43.72

PLATE 7

Plate 7 : Plant regeneration studies in tomato cv. Dhanshree

Morphogenesis from cotyledon & leaf segment

Plant regeneration from cotyledon & leaf segment

leaf segments (57.33%). It was followed by cv. Dhanashree and cv. Bhagyashree with leaf segment (i.e. 51.50 and 49.83%, respectively) which were at par with each other (Table 18).

Among interaction of explant x medium, culture media M_{13}, M_{14} and M_{15} with leaf segment showed 100% shoot regeneration in all three genotypes (Table 19).

DISCUSSION

5. DISCUSSION

The tomato cultivation is susceptible to number of biotic and abiotic stresses. In the tomato cultivars, the resistance for these stresses has been introgressed from wild taxa through interspecific hybridization. Thus, wild tomato species serve as an important source of genetic resistance to biotic and abiotic stresses. The tomato is classical breeding and genetic material because of wealth of genetic variation in cultivated and wild forms. The genus *Lycopersicon* consist of nine species. Rick (1979) and Taylor (1986) divided genus *Lycopersicon* into two groups based on the involvement of barriers to interspecific hybridization. Those *Lycopersicon* species which can be easily crossed with cultivated tomato (*L. esculentum*) are grouped under "*esculentum complex*". This group consists of seven *Lycopersicon* species such as *L. esculentum*, *L. pimpinellifolium*, *L. cheesmanii*, *L. chmielewskii*, *L. parviflorum*, *L. hirsutum* and *L. pennellii*. Among these *Lycopersicon* species, first three species are the red fruited type and are reciprocally compatible with each other; while the remaining species are of green fruited type and are unilaterally compatible with cultivated tomato when used as a pollen parent in interspecific hybridization programme.

While, "*peruvianum complex*" consists of only two species, namely *L. peruvianum* and *L. chilense* which are isolated from *L. esculentum* (cultivated tomato) severe barriers to interspecific hybridization. Both these species are green fruited and characterized by self-incompatibility and high polymorphism.

In this context, eventhough *L. peruvianum* is one of the most variable species of genus *Lycopersicon* and source of resistance for most of biotic and abiotic stresses; it is the least exploited in tomato breeding due to severe barriers in interspecific hybridization. In interspecific hybridization of *L. esculentum* and *L. peruvianum*, a decisive factor of failure of hybridization is post zygotic sterility mechanism which includes degeneration of the embryo at all stages of development, death of embryo through lack of nutrition caused by disturbances in the development of endosperm and its subsequent degeneration and zygote degeneration. Furthermore, hybrid sterility and abnormality is observed in early segregating populations (Kosava and Kiku, 1979).

Thus, in present investigation, the species *L. peruvianum* was selected for interspecific F_1 hybrid development, eventhough presence of severe breeding barriers in interspecific hybridization but more importantly it is a major source of resistance for abiotic and biotic stresses. In present studies, interspecific hybridization was carried out through involvement of two tomato cultivars and six lines of *L. peruvianum* in the crossing block as a maternal and pollen parents, respectively and problem of post zygotic sterility was circumvented with the aid of tissue culture technique viz., culture of immature seeds (Patil *et al.*, 1993) at eight fruit stages with use of 26 culture media.

5.1 Morphological characters of parents used in interspecific hybridization

In the present study, two tomato cultivars (viz., Dhanashree and 85-1) and six accessions of *Lycopersicon peruvianum* (viz., EC 127774, EC 106294, EC 252, EC 486, EC 34479 and EC 492) were used (Table 3). For morphological characterization, parent plants were assessed for plant growth, leaf, stem, floral and fruit morphological characters. Distinct differences were noticed in total plant morphology among two species of *Lycopersicon* used for

hybridization. The salient feature of *L. esculentum* were semi-indeterminate growth habit, up-right growth due to thick angular stem with trichome, leaves with broad leaf lamina, dark green colour and moderately serrated, unbranched inflorescence with inserted stigma and big fruits with red fruit ripening containing bold, hairy seeds while plant morphology of *L. peruvianum* represented indeterminate sprawling plant growth due to thin, round stems without trichomes presence of bract at axillary node, pale green, narrow leaves without serration, branched inflorescence with exerted stigma and more distinctly cherry fruits with green ripening fruits containing tiny, non-hairy seeds. These results were in agreement with reports of earlier workers (Rick, 1963; Thomas and Pratt, 1981; Taylor and Al-Kummer, 1982 and Patil *et al.*, 1993).

5.2 Evaluation of unilateral incompatability and crossing index for interspecific hybridization

In crossing block of interspecific hybridization, the wild tomato *L. peruvianum* was involved as a female, and as a male parent. But when it was used as a female parent; from 600 crosses it showed total flower drop (i.e. prezygotic sterility; Table 4) without any fruit set. Thus, unilateral incompatibility was observed when *L. peruvianum* used as a maternal parent in tomato interspecific hybridization. On the other hand, in interspecific crossing block where *L. peruvianum* involved as male parent, the crossing index was observed from 46.00 to 57.28% in twelve cross combinations (Table 5). Hence, in tomato interspecific hybridization programme, when *L. peruvianum* used as a pollen parent, it showed unilateral compatibility with *L. esculentum*. These results confirmed the findings of Hogenboom (1975), Kinsara *et al.* (1986), Thomas and Pratt (1981) and Kalloo (1988).

In interspecific hybridization, although *L. peruvianum* showed unilateral compatibility with *L. esculentum*, but it resulted fruit development without mature seeds. Thus post-zygotic sterility was observed in present investigation. Similar observations were recorded by Hogenboom (1972c), Thomas and Pratt (1981), Taylor and Al-Kummer (1982) and Segeren *et al.* (1993).

5.3 Development of tomato interspecific F_1 hybrid through standardization of culture media and fruit growth stages

5.3.1 Standardization of medium for immature seed culture

As post-zygotic sterility was observed during tomato interspecific hybridization programme, the immature hybrid seed from eight different fruit stages were cultured in 26 different MS (Murashige and Skoog, 1962) culture media containing various combinations of naphthalene acetic acid (0.2 to 1.0 mg l^{-1}) and 6-benzylaminopurine (1 to 5 mg l^{-1}) as a source for auxin and cytokinin, respectively.

In present investigation, the maximum overall seed germination (0.94%) was observed on MS medium without any supplement of plant growth regulators i.e. M_1 medium (Table 6B). It was followed by culture media M_3 (0.62%) and M_4 (0.52%) where MS salt was supplemented with 0.2 mg l^{-1} NAA and 2 and 3 mg l^{-1} BAP, respectively. Thus, culture of immature seed of tomato interspecific F_1 hybrid was the most effective in pure MS medium followed by lower concentration of plant growth substances. It clearly indicates that post-zygotic sterility of tomato interspecific F_1 hybrid seeds can be overcome to certain extent by providing necessary nutrients to cultured immature seed. Kosova and Kiku (1979) also reported that failure of

hybridization between *L. esculentum* and *L. peruvianum* was due to death of embryo through lack of nutrition caused by disturbances in development of endosperm (which act as a reserve food reservoir for embryo) and its subsequent degeneration.

Still with standardization of culture media, the success in seed germination of tomato interspecific F_1 hybrid was very limited as seed germination was observed within range of 0 to 1.46% with overall mean of 0.18%. It showed severe interspecific crossing barriers in tomato while crossing *L. esculentum* with *L. peruvianum*.

The present results were in agreement with the findings of earlier reports. As Imanishi *et al.* (1985) and Wu *et al.* (1987) noticed that MS medium without supplement of plant growth regulator was the best medium for culture of immature seed of interspecific F_1 hybrid of *L. esculentum* and *L. peruvianum*. Patil *et al.* (1993) also reported seed germination on MS medium either without supplement of plant growth regulators or with lower concentration of plant growth regulators (0.3 mg l^{-1} GA or BAP) while culturing embryos and immature seeds of backcross progenies (BC1, BC2 and BC3) of tomato-*peruvianum* somatic hybrids. However, Uralets (1984) observed recovery of tomato interspecific F_1 hybrid through embryo culture on Linsmaier and Skoog medium with 5% sucrose and 3 mg l^{-1} Kinetin.

In present investigation the overall recovery of interspecific F_1 hybrid plants through culture of immature seed was 0.18% which was very well compared with the findings of Cap *et al.* (1991) who recorded 0.13% recovery of interspecific F_1 hybrid between *L. esculentum* and *L. peruvianum* through embryo culture.

5.3.2 Performance of parent in development of tomato interspecific F_1 hybrids

An interspecific hybridization programme was undertaken in twelve cross combinations involving two tomato (*L. esculentum*) cultivars and six accessions of *L. peruvianum*.

Among crosses involving two tomato cultivars as a maternal parent, the higher seed germination of interspecific F_1 hybrids was observed with cv. Dhanashree (0.23%, Table 6B & 6C) than that of cv. 85-1 (0.12%). Thus, tomato cv. Dhanashree proved as a promising maternal parent for interspecific hybridization.

While among six accessions of *L. peruvianum* used as a pollen parent, wide variability was observed for seed germination of interspecific F_1 hybrids (0 to 0.36%). The accession EC 106294 and EC 252 proved promising pollen parents while accessions EC 34479 and EC 492 noticed as recalcitrant pollen parent for tomato interspecific hybridization.

Thus, genetic variability was observed within *L. esculentum* and *L. peruvianum* species for compatibility to interspecific hybridization. These results confirmed the findings of Hogenboom (1972d) who observed variable breeding barriers among *L. esculentum* and *L. peruvianum* species. Thus, in future, tomato interspecific programme can be implemented efficiently by exploiting variability among the parents for minimum interspecific breeding barriers.

5.3.3 Standardization of fruit growth stages for immature seed culture

From twelve cross combinations, eight fruit growth stages were assessed for culture of immature seed starting from 10 to 14 days upto 45 to 49

days after pollination (DAP) with 5 days interval (Table 7). Generally early (10 to 14 DAP) and late (40 to 49 DAP) fruit development stages were not found suitable for culture of immature seed as no germination was observed among these stages. In general, seed germination was initiated (0.16%) from 15 to 24 DAP fruit growth stages reached peak at 30 to 34 DAP fruit growth stage (0.48%) then showed progressive downwards trend at 35 to 39 DAP fruit growth stage (0.26%). However, slightly different trend was observed among crosses involving different tomato cultivar as a maternal parent.

When maternal parent cv. Dhanashree was involved in crosses showed upward tend from 15 to 19 DAP (0.26%) and attained peak rise at 30 to 34 DAP (0.77%) and then progressive downward trend was observed at 35 to 39 DAP (0.26%).

When maternal parent cv. 85-1 was involved in crosses, it showed very low seed germination during initial fruit growth stages (i.e. 15 to 19 and 20 to 24 DAP) followed by the highest seed germination (0.38%) recorded at 25 to 29 DAP fruit stage. Then after, sudden downward trend (0.19%) was noticed at 30 to 34 DAP which again showed slightly upward trend (0.26%) at 35 to 39 DAP fruit growth stages. In brief, for crosses involved maternal parent Dhanashree, the most ideal fruit development stage was 30 to 34 DAP, whereas for crosses involved with cv. 85-1 it was 25 to 29 DAP (Table 8).

In this context, similar results were reported by earlier scientists. Cap *et al.* (1991) observed 25 DAP level was best for immature seed culture in development of interspecific tomato F_1 hybrid. While Wu *et al.* (1987) reported that in interspecific hybridization between *L. esculentum* x *L. peruvianum* 20 to 30 days after pollination was best stage for culture of immature hybrid seed.

Whereas, in tomato *peruvianum* interspecific hybridization programme, the Chen Lanzhuang (1996) reported 13 to15 DAP fruit stage while Uralets (1984) recorded 19 to 25 DAP fruit stage and Lai *et al.* (1990) noticed 35 DAP fruit stage were crucial for culture of embryos. Thus, present results were in agreement with earlier reports as range of 15 to 39 DAP fruit stage was observed effective in recovery of tomato interspecific F_1 hybrid.

5.4 Development of *ex-vitro* interspecific F_1 hybrids

In present studies, eventhough 44 *in vitro* F_1 hybrid plants were obtained, very high mortality of *ex-vitro* F_1 hybrid plants were noticed under polyhouse conditions (Table 9). Eventually, only two F_1 hybrid plants were successfully recovered for field conditions. To control mortality of *ex-vitro* plants, the efforts were made by subculturing of *in-vitro* plants but limited success was achieved. It indicates that hardening of *in vitro* tomato plants should be done with the most care under well-equipped polyhouse conditions avoiding summer season. In this context, it is suggested that proper hardening procedure for tomato *in vitro* plants needs to be standardized under tropical conditions.

5.5 Confirmation of hybridity of tomato in interspecific F_1 hybrid

For confirmation of hybridity of interspecific hybrids between *L. esculentum* x *L. peruvianum*, morphological characterization and biochemical (total protein) analysis were undertaken.

5.5.1 Morphological characterization

Distinct morphological features are of prime importance to distinguish hybrid nature of plants. Plant morphology of both interspecific F_1 hybrids

showed closer similarity with their wild parent, *L. peruvianum*. It clearly indicates dominance of wild morphological attributes over cultivated type.

Similar results were recorded by Thomas and Pratt (1981), Makhalova (1972) and Kalloo (1991) for characterization of tomato interspecific F_1 hybrids and Kinsara *et al.* (1986), Wijbrandi *et al.* (1990) and Patil (1994) for characterization of tomato interspecific somatic hybrids.

The *L. peruvianum* specific dominant morphological characters in F_1 hybrids were indeterminate plant growth, round stem without trichome, presence of bract at axillary node, united petals with exerted stigma, cherry fruit with green ripening colour. While the only *L. esculentum* specific dominant morphological feature expressed in F_1 hybrids was unbranched inflorescence. While intermediate morphological characters displayed by F_1 hybrids were leaf serration, leaf colour, stem thickness. The most distinct morphological feature of both F_1 hybrids was self-sterility (i.e. fruit without seed content) as compared to self-fertility of their parents. Thus, interspecific sterility was observed in both F_1 plants (Table 10). These results are in agreement with findings of Thomas and Pratt (1981), Wu (1984), Imanishi *et al.* (1985) and Kalloo (1991).

The self-sterility observed in both interspecific F_1 hybrids can be correlated with their drastically reduced pollen viability (>50%). Similar observations were also recorded by Wu (1984).

The sterility of interspecfic F_1 hybrid may be chromosomal or genic, but the complete sterility observed because of genetical factors (Kalloo, 1991). Generally, normal pairing of chromosomes has been observed in interspecific F_1 hybrids indicates a high level of coherence between chromosome

morphology and pairing, and the regularity of chromosome behaviour. Generally, F_1 hybrids of all combinations of *Lycopersicon* species exhibit normal, or near normal chromosomal behaviour (Kalloo, 1991). Masum-Zade (1973) noticed that, in spite of perfect pairing of chromosome, some F_1 hybrids showed sterility due to the involvement of genetic factors.

5.5.2 Biochemical characterization with total protein analysis

With "total protein" analysis, two interspecific F_1 hybrid plants were easily identified. In both F_1 hybrid plants, more bands were observed than their parents and most of them were both parent specific and intermediate. More importantly, hybrid specific bands were observed in both F_1 hybrid plants. Thus, the additional bands of total protein could quantify the protein content in hybrids which exhibited the blood of both parents in hybrid. It proved that the plants recovered through study were true hybrids.

5.6 Screening tomato interspecific F_1 hybrids for biotic and abiotic stresses

5.6.1 Biotic stresses

Incidence of major pests and diseases were recorded for both hybrid plants along with their parents during *Kharif* 2002. The maternal parent tomato cv. Dhanashree showed maximum incidence for almost all pests in comparison with pollen parent, *L. peruvianum* and their hybrids. In both hybrid plants, pest incidence for all pests (e.g. leaf miner, mites, white fly and thrips) was intermediate which inclined towards their pollen parent *L. peruvianum* (Table 13).

These results revealed that neither wild tomato species, *L.* peruvianum nor their F_1 hybrids were totally free from pest infestation. But with compared to cultivated tomato, certainly lower pest incidence was observed in wild

parents and interspecific F_1 hybrids. Moreover, as white flies and thrips are vector for viral diseases like tomato leaf curl virus (TLCV) and tomato spotted wilt virus (TSWV) respectively their lower pest incidence in wild parents and F_1 hybrids may confer resistance to these viral diseases. Tomato cultivation in India is under major threat of viral diseases like TSWV and TLCV, which reduced the tomato yield potential upto 20 to 100 per cent. In this context, the main aim of tomato breeding in the world is to incorporate virus resistance in commercial tomato cultivars through interspecific breeding programme.

However, while screening of tomato interspecific F_1 hybrids along with their parents against major diseases, total different trend was observed than the pest incidence. The both F_1 hybrids and their wild parents were free from any disease symptom for important fungal and viral diseases while maternal parent, tomato cv. Dhanashree showed considerable disease reaction for all diseases screened under field conditions (Table 14). It showed that interspecific F_1 hybrids like their wild parent, *L. peruvianum* species should be used as source of resistance for important fungal and viral diseases in future tomato breeding programme. These observations are particularly important for tomato breeding for virus resistance, as susceptibility to viral diseases (i.e. TSWV, TLCV) is the major threat for tropical and field grown tomato cultivars. Viral diseases neither under chemical control nor any genetic resistance, particularly horizontal resistance for viral diseases has been transgressed effectively and permanently within commercial tropial tomato cultivars. Considering seriousness of viral diseases in tomato, two interspecific F_1 hybrid plants developed under present study may play an important role in development of tomato cultivars for virus resistance. Cho *et al.* (1989), Kalloo (1991) and Muniyappa (1991) reported that resistant sources for TSWV and TLCV

diseases are available in distantly related *Lycopersicon* species in *L. peruvianum* and *L. chilense.* But still no commercial tomato variety is developed for horizontal resistance against these viral diseases due to various physiological races or strains of virus and resistant is under polygenic control (Cho *et al.*, 1989).

Eventhough, some attempts were made to incorporate virus resistance in tomato. Banerjee and Kalloo (1987) reported resistance against TLCV as a digenic character from *L. esculentum* x *L. hirsutum* f. *glabratum* interspecific hybridization. To incorporate resistance against TSWV, the pioneer effort was made by Kikuta and Frazier (1946) and first tomato cultivar, Pearl Harbour, was developed from *L. pimpinellifolium.* But unfortunately, this resistance was strain specific and could not confer resistance to other strains of TSWV. Furthermore, Finlay (1953) reported that the character resistance to TSWV is under polygenic control and 5 genes are responsible to confer resistance to five physiological strains of TSWV. Furthermore, Finlay (1953) observed that *L. peruvianum* is the most effective source of resistant to all five strains of TSWV. Thus, interspecific hybridization with *L. peruvianum* is the most vital breeding aspect in development of tomato cultivar for TSWV resistance. In this context, the two F_1 hybrids derived from *L. peruvianum* under present studies showed resistance to TSWV will be valuable breeding material for further studies for development of resistant lines.

However, wild genome of tomato served as a source of resistance for other viral, bacterial, fungal and nematodal diseases (Kalloo, 1991). For Tobacco Mosaic Virus (TMV) disease, the resistance was reported through tomato interspecific hybridization with *L. peruvianum* i.e. in F_1 hybrids (Bonito and Ancora, 1986), backcrossing of F_1 ybrids (Saccardo and Monii, 1987) and

backcrossing of tomato somatic hybrids (Patil *et al.*, 1993). In this regard a notable example for source of TMV horizontal resistance is obtained from *L. peruvianum* (Alexander, 1963) and subsequently transferred into commercial glasshouse, European tomato cultivars as a single dominant gene which termed as TM-2^2 (Alexander, 1971) but original plant was afterward correctly designated as *L. chilense* (Holle *et al.*, 1979).

5.6.2 Abiotic stresses

The major abiotic stresses in tomato is fruit setting at lower temperatures (<15°C) for temperate regions and at higher temperatures (>38°C) for tropical regions like India.

Eventhough genetical sources for cold temperatures are reported in wild tomato taxa i.e. *L. hirsutum* f. *glabratum* and *L. peruvianum* (Kalloo, 1991) and incorporated in commercial tomato cultivars (Kalloo and Banerjee, 1990), the fruit setting at higher temperatures under tropics is still unsolved problem. Therefore, in present studies, the interspecific F_1 hybrids and their wild parents (*L. peruvianum*) were screened for fruit setting at high temperatures (40 to 45°C) during summer, 2003. However, unfortunately no resistance source was observed for this trait among F_1 hybrids and their parents.

5.7 Plant regeneration studies in genus *Lycopersicon*

Plant regeneration is an important feature in tomato for *in vitro* multiplication; cell, callus and tissue culture techniques required in distant hybridization and for biotechnological studies like genetic transformation, protoplast culture, somatic hybrid development. Tomato (*L. esculentum*) historically, has played an important role in the development of tissue culture technology. White (1934) first time demonstrated with tomato roots, the

possibility of growing tissues *in vitro*. However, plant regeneration potential of *Lycopersicon* species are both explant and genotypic dependent (Morgan and Cocking, 1982). Hence, present investigation was undertaken to evaluate plant regeneration from two explants i.e. leaf and cotyledon of two tomato (*L. esculentum*) cultivars i.e. cv. Dhanashre and Bhagyashree and *L. peruvianum* accessions (EC106294) on 20 MS based culture media. The observations were recorded on number of days required for callus induction, per cent callus induction and per cent shoot generation.

For callus induction, the parameters viz., leaf segment, cv. Dhanashree and *L. peruvianum* accession and medium M15 were crucial. The maximum callus induction (90%) was recorded by leaf segment of cv. Dhanashree on M15 medium. In general, media containing 1 mg l^{-1} NAA induced callus more efficiently in this genus, *Lycopersicon* (Table 16). These results were in agreement with the findings of Patil (1994) who noticed that NAA was the best auxin source for callus induction in the genus, *Lycopersicon*.

The 0 to 100% range was observed for shoot regeneration studies in the genus *Lycopersicon*. The MS culture media with lower concentration of plant growth regulators (M_1 and M_2) could not show any shoot regeneration in both explants of three genotypes (Table 17). However, MS culture media supplemented with 1.0 mg l^{-1} NAA and 1.5-5.0 mg l^{-1} BAP showed 100% plant regeneration from leaf explants of three genotypes. Thus, it is observed that plant regeneration in genus *Lycopersicon* was dependent on explant, genotype and culture medium. These results confirmed the findings of Morgan and Cocking (1982), Kinsara *et al.* (1986) and Patil (1994). Hence, the efficient plant regeneration system observed in present investigation will provide an excellent basis for genetic manipulation of these genotypes.

5.8 Future line of work

The two tomato interspecific hybrid plants developed under present investigation are containing genomes of tomato (*L. esculentum*) cv. Dhanashree and two accessions of *L. peruvianum* i.e. EC 106294 and EC 252. Tomato cv. Dhanashree is an improved variety developed for high yield potential (60 to 80 t/ha) and also has field tolerance against viral diseases (Anon., 2002) and is suitable for genetic manipulation like protoplast culture and somatic hybridization (Patil *et al.*, 1994). While two accessions of *L. peruvianum* were of polymorphic nature and showed resistance against important diseases. Thus, these two interspecific F_1 plants will be effectively utilized in future crop improvement programme, especially in breeding for disease resistance, which is the most vital breeding objective in tomato. Therefore, these two interspecific F_1 hybrids will be further backcrossed with commercial tomato cultivars and effort should be made to locate source for multiple disease resistance. However, in future backcrossing of these interspecific F_1 hybrids it may required aid of tissue culture upto BC2 and BC3 generation since severe interspecific breeding barriers were noticed in these F_1 hybrids as both were self-sterile. In this regard, it is important to note that Kosova and Kiku (1979) in tomato interspecific hybridization with *L. peruvianum* reported hybrid sterility and abnormality in early segregating populations. Furthermore, Patil *et al.* (1993) employed immature seed culture upto BC3 generation while backcrossing tomato-*peruvianum* somatic hybrids. Therefore, backcrossing programme of these interspecific F_1 hybrids may be carried out with due care.

SUMMARY AND CONCLUSIONS

6. SUMMARY AND CONCLUSIONS

6.1 Summary

The present investigation entitled, "Interspecific hybridization in *Lycopersicon* species" was carried out at MPKV, Rahuri during 2002-2003. For tomato interspecific hybridization programme, two tomato (*L. esculentum*) cultivars were crossed reciprocally with six accession of *L. peruvianum* and observations were recorded for crossing index and unilateral incompatibility. While development of *in vitro* interspecific F_1 hybrids through immature seed culture, the various parameters such as performance of parents in individual cross combination, culture media and fruit growth stages were assessed. The morphological characterization and biochemical analysis was carried out to confirm hybridity of *ex-vitro* F_1 plants. The interspecific F_1 hybrids were screened for biotic and abiotic stresses under field conditions. Furthermore, plant regeneration studies was undertaken with two tomato cultivars and *L. peruvianum* accession. Effect of two explants and twenty MS based culture media was studied on callus induction and shoot regeneration.

The important findings of present investigation are summarized below.

1. Unilateral compatibility was observed while crossing *L. esculentum* with *L. peruvianum*.
2. Crossing index for interspecific hybridization was noticed within range of 0 to 51.86%.
3. Pre- and post-zygotic sterility was observed during tomato interspecific hybridization. In post-zygotic sterility, the immature seed development was observed.

4. For development of *in vitro* interspecific F_1 hybrids, the immature seed culture was employed and effect of twenty-six MS-based culture media and eight fruit growth stages were assessed on seed germination of 12 cross combinations. Total 24,960 immature seeds were cultured and seed germination was observed in 44 immature seeds. Thus, 0.18 per cent overall recovery of *in vitro* interspecific F_1 hybrids was noticed.

5. While evaluating twenty-six MS based culture media for immature seed culture, the MS culture medium without supplement of plant growth substances (i.e. M_1) was the most effective medium with recovery of 0.91 per cent *in vitro* F_1 hybrids. It was followed with M_2 and M_3 culture media containing MS salt supplemented with lower concentration of plant growth substances (i.e. 0.2 mg l^{-1} NAA and 2 and 3 mg l^{-1} BAP, respectively) with recovery of 0.62 and 0.52% *in vitro* hybrids, respectively. The normal plant growth and rooting of *in vitro* F_1 plants was observed in MS culture medium without supplement of plant growth hormones.

6. The distinct effect of fruit growth stages was noticed on immature seed culture. Total of eight fruit growth stages were evaluated at 5 days interval. Early (10 to 14 DAP) and late (40 to 49 DAP) fruit stages were found recalcitrant for seed germination. In general, the fruit stage at 30 to 34 DAP was found the most effective fruit stage with 0.48% seed germination. However, slightly different trend was observed with crosses involving different tomato cultivars as a maternal parent.

7. While assessing performance of parents for tomato interspecific hybridization, among tomato cultivars as a maternal parent, cv. Dhanashree showed superiority with 0.23% seed germination over cv.

85-1 (0.12%). Among 6 accessions of *L. peruvianum* as a pollen parent, EC 106294 was emerged as best pollen parent with 0.36% seed germination followed by EC 252 (0.29%). While two accessions EC 492 and EC 34479 showed severe crossing barriers.

8. During hardening of *ex-vitro* F_1 plants, severe mortality was observed under polyhouse conditions. Out of 44 plants transplanted in polyhouse only two F_1 hybrids were eventually survived and successfully grown under field conditions.

9. To confirm hybridity of interspecific hybrids, morphological characterization was carried out for both F_1 plants were confirmed by expression of both parent-specific morphological features expressed in F_1 hybrids. Furthermore, distinct intermediate morphology was also recorded in F_1 hybrids. The most of the morphological features of F_1 hybrids were dominated by wild pollen parent, *L. peruvianum* especially indeterminate plant growth, cherry fruit size with green fruit ripening. The most distinguish feature observed in both F_1 hybrid was self-sterility; which expressed as "interspecific sterility" due to severe crossing barrier between two parental *Lycopersicon* species (i.e. *L. esculentum* and *L. peruvianum*).Thus hybrid-sterility was itself confirmation for interspecific hybridity.

10. The hybridity of interspecific F_1 hybrids were also confirmed by "total protein" content analysis. There was presence of hybrid specific bands in both F_1 hybrids along with number of intermediate bands and both parents specific bands.

11. The *ex-vitro* F_1 hybrid plants and their parents were screened for biotic stresses under field conditions. For major pests, both F_1 hybrids recorded

similar reaction like their wild parent, *L. peruvianum*. As compared to tomato cultivar, the considerably lower pest incidence was recorded among plant population of F_1 hybrids and *L. peruvianum*.

While screening of F_1 hybrids and their parents against major diseases, the F_1 hybrids and their wild parent, *L. peruvianum* were observed free from any diseases symptoms, particularly for TSWV and TLCV.

12. However, while screening of F_1 hybrids and their parents against major abiotic stress i.e. fruit setting at higher temperatures (>40°C) during summer season, no genetic source of resistance was observed.

13. While evaluating plant regeneration potential in the genus, *Lycopersicon*, the maximum callus induction (90%) was recorded by leaf segments of cv. Dhanashree in M_{15} culture medium. Moreover, 100% shoot regeneration was recorded by leaf segments of all three genotypes in three culture media (M_{13}, M_{14} and M_{15}) containing MS salt supplemented with 1.0 mg l^{-1} NAA and 1.5-5.0 mg l^{-1} BAP.

6.2 Conclusions

On the basis of results obtained in present investigations following conclusions were drawn.

1. In tomato interspecific hybridization programme, due to involvement of unilateral incompatibility, the tomato (*L. esculentum*) cultivars should be used as a maternal parent and wild taxa such as *L. peruvianum* should be used as a pollen parent.
2. As post-zygotic sterility observed during tomato interspecific hybridization, aid of tissue culture techniques (e.g. culture of immature seeds) was pre-requisite in development of *in vitro* F_1 plants.

3. For culture of immature interspecific hybrid seed, an optimal fruit growth stage is dependent on involvement of tomato cultivar as a maternal parent. For crosses involved tomato cv. Dhanashree as a maternal parent, 30 to 34 DAP fruit growth stage was effective. While for crosses involved tomato cv. 85-1 as a maternal parent, 25 to 29 DAP fruit growth stage was effective.
4. The best culture medium for immature seed culture was pure MS medium without supplement of any plant growth substances.
5. The parents with low crossing barriers should be used in tomato interspecific hybridization programme e.g. Tomato cv. Dhanashree and *L. peruvianum* accessions, EC 106294 and EC 252.
6. Through culture of immature seed, about 0.18% recovery of *in vitro* tomato interspecific F_1 hybrid was obtained.
7. Protocol for plant hardening of *ex-vitro* tomato plants needs to standardized especially for tropical climates. Similarly, plant hardening of *ex-vitro* tomato plants should be avoided during summer season.
8. Distinct morphological and biochemical markers can be used in characterization of F_1 plants and for confirmation of hybridity.
9. Interspecific F_1 hybrids can be used as a source of resistance for biotic stresses e.g. pest and disease incidence. In particular, these F_1 hybrids will be exploited effectively to incorporate resistance against viral diseases like TSWV and TLCV from *L. peruvianum* to tomato (*L. esculentum*) germplasm.
10. The protocol developed for efficient plant regeneration in genus *Lycopersicon* can be utilized for genetic manipulation of these tomato genotypes.

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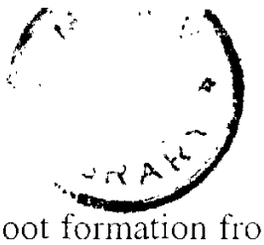

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* **Originals not seen.**

VITA

8. VITA

BALU JAGANNATH SHETE

A candidate for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

HORTICULTURE

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Major field : Horticulture

Biographical information

- **Personal** : Born at Sonai, Tal. Newasa, Dist. Ahmednagar on 1st December, 1961. Son of Sou. Chandrabhagabai and Shri. Jagannath Bhiku Shete of Rahuri Kd., Tal. Rahuri, Dist. Ahmednagar. Married with Nanda on 3rd April, 1986. One son Amit and one daughter Priyanka.

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