SCREENING OF MAPPING POPULATION THROUGH MARKER ASSISTED SELECTION FOR IMPARTING DISEASE RESISTANCE IN TOMATO (*Solanum lycopersicum* L.)

By

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(2012-11-107)

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VELLANIKKARA, THRISSUR - 680 656

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THESIS

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KERALA, INDIA

2014

DECLARATION

I hereby declare that this thesis entitled **"Screening of mapping population through marker assisted selection for imparting disease resistance in tomato (*Solanum lycopersicum* L.)"** is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Screening of mapping population through marker assisted selection for imparting disease resistance in tomato (*Solanum lycopersicum* L.)"** is a bonafide record of research work done independently by Mr. Dheemanth T L (2012-11-107) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the advisory committee of **Mr. Dheemanth T L** a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Biotechnology**, agree that the thesis entitled **"Screening of mapping population through marker assisted selection for imparting disease resistance in tomato (*Solanum lycopersicum* L.)"** may be submitted by Mr. Dheemanth T L, in partial fulfillment of the requirement for the degree.

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Dheemanth T L


To my loving
parents and
guide

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ABBREVIATIONS

%	Percentage
>	Greater than
µg	Microgram
µl	Microlitre
A	Ampere
AFLP	Amplified Fragment Length Polymorphism
BGMV	Bean golden mosaic virus
Bp	Base pair
CI `	Coefficient of infection
cm	Centimetre
CPBMB	Centre for Plant Biotechnology and Molecular Biology
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
dNTPs	Deoxyribo Nucleoside Triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EST	Expressed sequence tags
g	Gram
GD	Genetic Distance
ha	Hectare
ISSR	Inter Simple Sequence Repeat
kb	Kilo basepairs
L	Litre
M	Molar
MAS	Marker Assisted Selection
mg	Milligram
ml	Millilitre
mM	Millimole
NBPGR	National Bureau of Plant Genetic Resources

ng	Nanogram
ºC	Degree Celsius
OD	Optical Density
PCR	Polymerase Chain Reaction
PDI	Per cent disease incidence
PDS	Per cent disease severity
pH	Hydrogen ion concentration
pM	Pico molar
PVP	Poly vinyl pyrolidone
PWI	Per cent wilt incidence
QTL	Quantitative trait loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RILs	Recombinant Inbred Lines
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Revolutions per minute
SCAR	Sequence Characterized Amplified Region
SSR	Simple Sequence Repeats
STMS	Sequence Tagged Microsatellite Sites
STR	Short Tandem Repeat
STS	Sequence Tagged Sites
TAE	Tris Acetate EDTA
TE	Tris EDTA
TZC	Tetrazolium chloride
U	Unit
UV	Ultra violet
V	Volts
β	Beta

Introduction

1. INTRODUCTION

The cultivated tomato originated in the wild form from Peru-Ecuador-Bolivia area of the Andes (South America). The first known record of tomato is in the year 1554 in South America. Since 1800, tomatoes are being used as food all over the world. Tomato was originally named *Solanum lycopersicum* by Linnaeus. In 1754, Miller separated tomatoes and designated the genus *Lycopersicon* and the species *esculentum* for the cultivated tomato and *Lycopersicon pimpinellifolium* to the wild form of tomato. Based on molecular and morphological information, a new taxonomic classification of tomato and readoption of *Solanum lycopersicum* for the cultivated tomato had been suggested a while ago (Foolad, 2007). Tomato has now been renamed as *Solanum lycopersicum* (Gupta *et al.,* 2006; Riccardia *et al.,* 2007).

Bacterial wilt caused by *Ralstonia solanacearum* (Yabuuchi *et al.,* 1995) has remained a major destructive plant disease in the warm humid tropics of the world. The pathogen is known to attack a wide range of host plants. It attacks more than 200 plant species belonging to 33 families. Of these, family *solanaceae* has the largest number of hosts (Kelman, 1953). The disease was first reported by E. F. Smith from Florida in 1897 (Rolfs, 1898). The first report on bacterial wilt of tomato in India was by Hedayathullah and Saha (1941).

Ralstonia solanacearum, earlier referred as *Pseudomonas solanacearum* is a very complex pathogen, differing in pathogenicity and host range. Geographical variation is seen in the organism. Buddenhagen *et al*. (1962) classified *Pseudomonas solanacearum* isolates from Central and Southern America based on host range, pathogenicity and colony appearance on TZC medium. It was classified into 3 races i.e., race 1, race 2 and race 3.

Erwin. F. Smith published the first description of *Pseudomonas solanacearum* E. F. Smith, which causes a wilt disease of solanaceous plants (Smith, 1896).

Leaf curl caused by the Tomato Leaf Curl virus (ToLCV), a heterogenous complex of whitefly transmitted geminivirus is a serious production constraint of tomato worldwide, particularly in the Indian subcontinent and many other Asian countries. The disease is spread through the whitefly. The effect of the disease is near total loss of crops. Each year ToLCV causes millions of dollars damage to tomato crops all over the world.

Geminiviruses form the second largest family of plant viruses, the Geminiviridae, represented by four genera namely Mastrevirus, Curtovirus, Topocuvirus and Begomovirus. During the last two decades these viruses have emerged as devastating pathogens, particularly in the tropics and sub tropics causing astronomic economic losses and threatening crop production. Compared to other virus groups of the Geminiviridae family, begomoviruses have emerged as more serious problems in a variety of crop especially tomato. Major contributory factors for the emergence and spread of these viruses are evolution of variants of the viruses and increase in vector population.

Adoption of disease resistant varieties/hybrids is the most practical way to combat the menace of ToLCV and bacterial wilt. Several ToLCV and bacterial wilt resistant tomato varieties and F_1 hybrids are being economically cultivated in India. However, none of them is resistant to all the prevalent ToLCV strains and *Ralstonia solanacearum* in Kerala. Identification of resistance source for the diseases and isolation of resistance genes by the help of molecular markers linked to resistance followed by pyramiding of these genes could be the most feasible way to overcome the problem of ToLCV and bacterial wilt.

Despite the efforts taken up all over the world so far, tomato leaf curl virus disease and bacterial wilt (BW) still continues to be the major limiting factors in tomato cultivation. The leaf curl virus infects the crop in all locations while bacterial wilt is more severe in warm humid tropics. Acidic soils, humid climate and high temperature favour bacterial wilt incidence in Kerala and it affects the crop at all stages of growth resulting in total crop loss. Leaf curl virus incidence is

also gaining importance in the state recently and hence it is the need of the hour to develop varieties with combined resistance.

Conventional breeding has helped to develop location specific varieties and molecular breeding have identified several Resistant Gene Analogues and QTLs mapped on different chromosomes. Considering the importance of bacterial wilt in Kerala, KAU has developed varieties with relatively good tolerance (eg- Mukthi), but are susceptible to ToLCV and fruit qualities are not superior. Genotypes resistant to different strains of ToLCV have been developed at Indian Institute of Horticultural Research (eg- IIHR-2195) and this project is an attempt to incorporate combined resistance to BW and ToLCV through molecular breeding. The markers that will be validated will be of great use in marker assisted selection. An ideal genotype with ToLCV resistance in bacterial wilt resistance background and having desirable horticultural traits is targeted in the programme.

Review of Literature

2. REVIEW OF LITERATURE

A brief review of literature collected with reference to the importance of the crop, diseases, pathogen, symptomology, resistant sources and molecular aspects are dealt in this chapter.

2.1 The crop

Tomato is the second most consumed vegetable, next to potato and it occupies largest number of cultivated varieties than any other vegetable crop. India is the sixth largest producer of tomato in the world with an area of 0.50 million hectares under cultivated and with a productivity of 17.4 MT/ha (Chamber *et al.*, 2006). Miller in 1978 gave the name *Lycopersicon esculentum* to cultivated tomato. More recently based on much molecular and morphological information, a new taxonomic classification of tomato and readoption of *Solanum lycopersicum* for cultivated tomatoes have been suggested and is being adopted (Majid, 2007). Tomato is used as a fresh vegetable and also can be processed and canned. Nutritionally tomato is a significant dietary source of vitamin A and C.

The cultivated tomato originated in a wild form in the Peru-Ecuador-Bolivia area of Andes *i.e.*, South America. Presently, the genus *Lycopersicon* has been recognized as having nine species, one cultivated and eight wild forms; all having identical genomes 2n = 2x = 24. Cultivated form is *Lycopersicon esculentum* and wild forms are *L. pimpinellifolium, L. cheesmanii, L. chilense, L. hirsutum, L. peruvianum, L. parvifolium, L. chinense, L penelli* etc. Almost all disease resistance genes in cultivated tomato originated from wild tomato species (Thamburaj and Singh, 2001).

As a crop plant, tomato is one of the best characterized plant systems. It has a relatively small genome of 950 Mb per haploid nucleus (Arumuganathan and Earle, 1991) and features such as diploidy, self-pollination and a relatively short generation time make it amenable to genetic analysis.

The wild species bear a wealth of genetic variability. Less than 10 per cent of the total genetic diversity in the *Lycopersicon* gene pool is found in *L. esculentum* (Miller and Tanksley, 1990). The cultivated *Lycopersicon esculentum* is genetically deploited compared to its wild relatives due to domestication and modern plant breeding (Rick and Chetelat, 1997).

Recent researches have revealed that resistance against nearly all serious diseases is available in wild *Lycopersicon* species (Tiwari *et al.,* 2002). Now the resistant genes from the wild species have been combined in commercial cultivars which can more easily utilized as donor parent (Kallo, 1986).

2.2 Evaluation of tomato genotypes for incidence of bacterial wilt

2.2.1 The pathogen

Bacterial wilt caused by the soil born bacterium *Ralstonia solanacearum* (Yabuuchi *et al.,* 1995) earlier referred as *Pseudomonas solanacearum* is one of the most destructive plant disease in the warm humid regions of the world. The pathogen is found to infect a wide range of host plants.

Ralstonia solanacearum is a very complex pathogen, differing in pathogenicity and host range. Geographical variation is seen in the organism. Buddenhagen *et al*. (1962) classified *Pseudomonas solanacearum* isolates from Central and Southern America based on host range, pathogenicity and colony appearance on TZC medium. It was classified into 3 races i.e., race 1, race 2 and race 3.

1. Race 1 (Solanaceous strain) – It is characterized by wide host range and is seen distributed throughout the low lands of tropics and subtropics. They infect tomato, tobacco and many solanaceous crops and other weeds.
2. Race 2 (Musaceous strain)- Host range is restricted to *Musa spp.* and a few perennial hosts. Initially limited to American tropics and spreading to Asia.

3. Race 3 (Potato strain) – Host range is restricted to potato and few alternate host in tropics and subtropics.

Two new races were proposed later, in addition to the first 3 races, one from ornamental ginger as race 4 (Aragaki and Quinon, 1965) and one from mulberry as race 5 (He *et al.*, 1983).

Hayward (1964) classified *Pseudomonas solanacearum* into biotypes or biochemical types namely biotype I, biotype II, biotype III and biotype IV based on their ability to oxidize various carbon sources and on other bacteriological reactions.

1. Biotype I – does not oxidise disaccharides and sugar alcohols.
2. Biotype II – oxidise only disaccharides.
3. Biotype III – oxidises both disaccharides and sugar alcohols.
4. Biotype IV– oxidises only hexahydric alcohols.

Cook and Sequeira (1988) used RFLP technique to study the relationship between biovars I to IV of Hayward and race1, 2 and 3. They divided *P. solanacearum* into two groups viz. Group 1 (includes strains of race 1, biovars III and IV) and Group II (include strains of race 1 bivar 1 and race 2 and 3). The gel pattern suggested that race 3 is a homogeneous group and fell into three distinct groups representing strains from different geographical origin. In contrast, race 1 strains exhibited highly variable RFLP gel patterns suggesting that race 1 is highly heterogeneous. Another recent classification of *P. solanacearum*, based on RFLP and other genetic fingerprinting studies (Hayward, 2000), is classified into Division I (biovars 3, 4 and 5 originating in Asia) and Division II (bivars 1, 2A and 2T, originating in South America).

Kumar *et al.* (1993) differentiated twelve isolates of *P. solanacearum* from solanaceous hosts into biovars following Hayward's classification. All the isolates from tomato, potato, aubergine and bell pepper were identified as biovar III or as sub type in biovar III. Yabuuchi *et al.* (1992) transferred several species of the

rRNA homology group II *Pseudomonas*, including *Pseudomonas solanacearum* to the genus *Burkholderia.* Sequencing information of 19S rRNA genes and polyphasic taxonomy led to the proposal of genus *Ralstonia* and the pathogen was renamed as *Ralstonia solanacearum* (Yabuuchi *et al.,* 1995).

Paul (1998) identified bacterial wilt infecting tomato and chilli isolates as race 1 biovar III. Studies conducted on the isolates of *R. solanacearum* from tomato, brinjal and chilli suggested that the pathogen belongs to race 1, biovar III and biovar V (Mathew *et al.,* 2000).

Yabuuchi *et al.* (1992) transferred several species of the rRNA homology group II *Pseudomonas*, including *Pseudomonas solanacearum* to the genus *Burkholderia*. Sequencing information of 16S rRNA genes and polyphasic taxonomy led to the proposal of genus *Ralstonia* and the pathogen was renamed as *Ralstonia solanacearum* (Yabuuchi *et al.,* 1995)

Variability studies conducted on the isolates of *R. solanacearum* of tomato, brinjal and chilli from different locations of Kerala showed the existence of pathogen belonging to race 1, race 3 and biovar III, III A and V (James, 2001 and Mathew, 2001).

Thwaites *et al.* (1997) studied the genetic variation among strains of *R. solanacearum* using polymerase chain reaction with random primers. A transposon induced mutant *R. solanacearum* has lost pathogenicity on its natural host, banana; but is still retaining the ability to wilt tomato.

2.2.2 Ecology of the pathogen

The ecology of the pathogen in infested soils is poorly understood. It is inferred that the primary inoculum came from the soil but there was no conclusive evidence that the pathogen is a ubiquitous inhabitant in the soil (Buddenhagen and Kelman, 1964). Under natural conditions, the pathogen was able to survive saprophytically in the soil for as long six years (Chester, 1950).

Ralstonia solanacearum does not survive in the soil for prolonged periods because it is not a strong competitor. It does not survive in the soil for prolonged periods because it is not a strong competitor but survives on or in plant roots. The bacterium appears to survive by continually infecting the root of susceptible or carrier plants or by colonizing the rhizospheres of non-host plant (Sequeira, 1993). Survival of *Pseudomonas solanacearum* in the rhizosphere has been documented by Granada and Sequeira (1983) who reported that the bacterium invades the roots of presumed non-hosts such as bean and maize. Long term survival was associated with localized or systemic infection of plants that did not express symptoms of bacterial wilt.

2.2.3 Symptomatology

First expression of the disease is wilting of lower leaves of the plants and it leads to entire wilting of the plants (Walker, 1952). Dwarfing or stunting may also occur. The entry of the pathogen through the root system and it was believed that a wound is necessary for the entry (Walker, 1952; Kelman, 1953; Chupp and Sherf, 1960). The entry of the pathogen through natural opening of the plant has been reported by Hildebrandt (1950).

In advanced stages, dark brown to black areas develop due to decay of root system and the whole plant dies off. A very distinct characteristic indication of bacterial wilt is the appearance of bacterial ooze from the injured vascular regions (Ashrafuzzaman and Islam, 1975). It is reported that break down of plant tissues by the pathogen, continued tissue decay and plugging finally result in death of the entry of the pathogen into the host plant (Kelman, 1953; Chupp and Sherf, 1960).

According to Kelman (1954), the virulence might be explained, at least in part by the quantitative difference in EPS (extra cellular polysaccharides), the bacterium can produce IAA, which helps in initiation of tylose formation and increases cell wall plasticity. Ethylene production is also associated with the disease development. Seqeira (1993) reported that there is no cytological evidence

for how the bacterium reaches the vascular systems. It is assumed that the pathogen has to digest its way through the primary wall of the weakened cortical cells as well as of the tracheary elements, where it is exposed between the spiral thickenings. Allen (1997) reported that *R. solanacearum* passes much of its life cycle living in harmony or in an uneasy truce with its plant hosts.

Allen *et al.* (1993) reported that there is no cytological evidence for how the bacterium reaches the vascular system. It is assumed that the bacterium has to digest its way through the primary wall of the weakened cortical cells as well as of the treachery elements, where it is exposed between the spiral thickenings.

2.2.4 Disease cycle and epidemiology

Ralstonia solanacearum is a soil borne and water borne pathogen; which can survive and disperse for various periods of time in infested soil or water, which can form a reservoir source of inoculum. The bacterium usually infects tomato plants through the roots (through wounds or at the points of emergence of lateral roots). Soil borne organisms, such as the root-knot nematode, can cause injury to plant roots and favour penetration of the bacterium.

Plant infection can also occur through stem injuries caused by cultural practices or insect damage. In some cases, plant to plant spread can occur when bacteria moves from roots of infected plants to roots of near by healthy plants, often via irrigation practices. Spread of bacteria by aerial means and subsequent plant contamination through foliage is not known to occur, thus making *R. solanacearum* a non-air born pathogen. High temperatures (29-35^{0}C) play a major role in pathogen growth and disease development. Several other factors that may affect pathogen survival in soil and water may also favour disease development, including soil type and structure, soil moisture content, organic matter in soil, water pH and salt content, and the presence of antagonist microorganisms.

The bacterium also has an “exterior” phase (epiphyte) in which it can reside on the outside of the plant. It is of minor importance in epidemiology of the

pathogen since bacteria do not survive epiphytically for long periods of time when exposed to hot conditions or when relative humidity is below 95%.

Under favourable conditions, tomato plants infected with *R. solanacearum* may not show any disease symptoms. In this case, latently infected plants can play a major role in spread of the bacterium. Transplants are either field grown (not common anymore) or container – grown in green houses. Cultural practices at either field production (over head irrigation or plant handling) may favour plant infection and spread of the pathogen from infected tomato transplants production sites to healthy growing sites.

2.2.5 Diagnosis and identification

Symptom identification is the first step for early diagnosis of bacterial wilt of tomato. Accurate identification of *R. solanacearum* from either symptomatic or asymptomatic plants and from water or soil samples demands multiple microbiological and molecular methods. A battery of complementary tests that differ in their sensitivity and specificity should be used for field or laboratory analyses for unambiguous identification of bacteria to species and biovar.

Screening tests can facilitate early detection and identification of bacteria in potentially infected plants or contaminated soil and water samples by *R. solanacearum.* They can not be used to identify the race or biovar of the organism. These screening tests include stem streaming, plating on semi-selective medium (modified SMSA), immunodiagnostic assay using *R. solanacearum.*

A biochemical growth tests is used for biovar determination of *R. solanacearum.* This test is based on the differential ability of strains of the pathogen to differentially produce acid from several carbohydrate sources, including disaccharides and sugar alcohols.

At the sub-species level, identification of strains of *R. solanacearum* can be assessed with several nucleic acid based methods such as DNA probe

hybridization and especially polymerase chain reaction (PCR) amplification with specific probe and primers.

Race determination is not generally possible because *R. solanacearum* strains usually have numerous hosts and do not have race-cultivar specificity on plant hosts.

2.3 Sources of bacterial wilt resistance

Tikoo *et al.* (1983) reported the presence of two independent genes for wilt resistance. The resistance was reported to be governed by multiple recessive genes in CRA 66 Sel A from Hawaii and another by single dominant gene in 663-12-3 from Taiwan. Sreelathakumari (1983) reported a complimentary and hypostatic type of digenic recessive gene system for wilt resistance.

Ramachandran *et al.* (1980) evaluated 36 tomato lines for their resistance to bacterial wilt in Kerala. They observed resistance in La-Bonita and CL-32 d-0-1 – 19GS. Celine (1981) collected 78 lines to isolate resistance ones and the field screening indicated the tolerance of LE-79, a line from AVRDC, Taiwan.

In an experiment conducted by Henderson and Jenkins (1972) to evaluate resistance in several genotypes, they found the genotypes such as Venus, Saturn and Beltsville-3814 to be resistant to bacterial wilt.

Ferrer (1974) identified CRA-66 from the Caribbean area, Hawaii 7997, Hawaii 7981, PI 126408 were sources of resistance to *Pseudomonas solanacearum*.

Rao *et al.* (1975) tested 23 wilt resistant cultivars and lines from USA and Philippines for their reaction to an Indian isolates of *R. solanacearum* and only one line CRA 66 selection A from Hawaii was founded to be resistant. Jenkins and Nesmith (1976) evaluated the resistance of cultivars Venus and Saturn against two isolates of *R. solanacearum* from America and India. They found that both

the cultivars were highly resistant to American isolate and also reported that the Indian isolate were more pathogenic than American isolate.

Sonoda (1977) evaluated 121 lines for resistance to bacterial wilt in the parent, VC 9-1, but it was unstable at soil temperatures above 32^0 C.

Augustine (1978) found that the lines PI 365950, PI 212441 and PI 263722 resistant to bacterial wilt.

Villareal and Lai (1979) reported that lines VC-11-7 and Kewalo derived their resistance from *Lycopersicon pimpinellifolium* PI- I27085A.

Ramachandran *et al.* (1980) evaluated 36 tomato lines for their resistance to bacterial wilt in Kerala. They observed resistance in La-Bonita and CL 32 d-0-19 GS cultivars. Similarly Celine (1981) reported field tolerance in the line CL 32 D-0-1-19GS.

Wilt resistance in cultivar Venus and the line CL 32d-0-1-19 GS from Taiwan and was broken down when *Meloidogyne incognita* larvae were added at the rate of 100/10 cm pot at the time of inoculation with bacteria (Goth *et al.*, 1983). He also suggested that nematode should be considered as a factor in the development of bacterial resistant lines.

Scorpio is a derivative from a cross between VC 9-1 and Floradel and found to be resistant to bacterial wilt with good fruit quality attributes (Peterson *et al.,* 1983).

Moffett (1986) reported bacterial wilt resistance in cultivars Scorpio, Redlander and Redlands Summer taste.

BWR-1 a pure line selection with a dominant gene for bacterial wilt resistance was developed from AVRDC accession L33 (VC 8-1-2-1) (Tikko *et al.,* 1986).

Nirmaladevi (1987) reported that resistance to bacterial wilt in CRA 66 Sel A was under polygenic control.

Ho (1988) screened resistance in genotypes for bacterial wilt and observed that cultivar MT-1 was highly resistant and MT-2, MT-3, MT-5, MT-7, MT-8, MT-10, MMT-11 were moderately resistant and Banting, MT-9 were susceptible and MT-6 was highly susceptible.

The most widely used bacterial wilt stocks in the breeding programmed are Venus (USA), Saturn (USA), L366 (Unknown origin), VC 11-3-1-8 (Philippines), VC 48-1 (Philippines), PI 406994 (Panama) and a few more, mostly coming from the tropical Southeast Asian countries (Opena *et al.*, 1989).

A bacterial wilt tolerant multiline called NTR has become popular in the highlands of West Java. It has also been tested in the swampy lowland areas of South Sumatra where it performed better than Intan, Ratna, Berlian and C1-1094 (Permadi, 1989).

Kapoor *et al.* (1991) screened exotic and Indian tomato lines/varieties for resistance to *R. solanacearum* during 1987-89. Of the 62 varieties screened, nine were immune, 26 resistant, five moderately resistant, four moderately susceptible and 18 were susceptible.

In field trails at Bangladesh Agriculture University, Mymersingh, the tomato cultivars Manik and Asa-4 were highly resistant to natural infection by *R. solanacearum*, Tustic and Bikash were resistant, while Oxheart, TM 008, Ratan and TM-0003 were moderately resistant (Islam and Rahman, 1991).

The lines LE-214, LE-217, LE-79, LFG, LE-79 DG and LE-79 SPF were found to be resistant (Peter *et al.*, 1992).

The most resistant germplasm in the AVRDC collection is L 285, a primitive type (*L. esculentum var.cerasiforme*) and CLN 65-349. (Opena *et al.*, 1992).

Varieties identified to have resistance to bacterial wilt included CLN-475-BC1 F2-265-9-0, CL-6046 (AVRDC); LV-2100 and LV 2099 (Indonesia); BL-7802, FMTT-13 and BC3F2-51-0-20-5-1 5-14-1(Philippines) (AVRDC, 1993).

In Thailand, results of IBWDN (International bacterial wilt disease nurseries) trial showed three out of 16 tomato accessions with resistant reaction to bacterial wilt. These were BL342, CL143-9-10-3-0-1-10 and CL 1 131 0-0-43-4-1 2 (AVRDC, 1993).

Prior *et al.* (1994) reported that the bacterial wilt resistance in cultivated tomato originated from *L. esculentum var. cerasiforme or L. pimpinellifolium*

A new source of resistance was identified from *Lycopersicon esculentum var. cerasiforme* LA 1421 (Mohamed, 1994); the genetic nature of this new resistance has not been reported.

The bacterial wilt resistant tomato varieties (KWR, T245, T146) are released by the Department of Agriculture, Srilanka were popular among the formers in the past; however, some of these varieties are now susceptible to bacterial wilt in some areas (Gunathilake *et al.*, 1994).

A monogenic dominant resistance was reported in Hawaii 7996 (Grimault *et al.*, 1995)

Sadhankumar (1995) screened 68 tomato genotype for resistance to bacterial wilt and found the Sakthi, LE-79-5, LE-415, LE-214, CAV-5 and LE-382-1 were resistant and he also found that the genes responsible for resistance in these lines were recessive.

William and William (1995) compared *R. solanacearum* resistant tomato cultivars as hybrid parents and it was found that hybrids with Hawaii 7998 as one of their resistant parents transmitted greater resistance than the other resistant parents used.

In a preliminary field screening in Pakistan five tomato lines BL 350, BL 342, BL 341, BL 333, L 285 showed tolerance to bacterial wilt (AVRDC, 1996).

In a work carried out by In-Mooseong *et al.* (1996) to identify resistance among 31 tomato cultivars, they found that the cultivars Naebyongchangsu, Kwangmying and Seojin were mildly resistant to *R. solanacearum* and the remaining cultivars were susceptible.

Protein bands PPO-1, PPO-4, PPO-7, POO-10, PPO-11 and PPO-12 were observed in the root and leaf samples of resistant genotypes namely Sakthi, Mukthi, LE 474 which could be considered as a marker for resistance to bacterial wilt in tomato (Bose, 1999). He also noticed high total phenol and OD phenol content in the resistant lines.

Yui *et al.* (1999) obtained four RAPD markers, which are useful for preliminary selection of bacterial wilt resistance, introduced from a bacterial wilt resistant parent Hawaii 7998.

Patil (2001) reported bacterial wilt infection ranging from 6-79 per cent in TLB 182 and Arka Vikas.

Kurian and Peter (2001) evaluated F_1 hybrids of bacterial wilt resistant/tolerant genotypes Sakthi, LE-214 and LE-206 with HW-208F, St-64, Ohio 8129, TH 318 and Fresh market and they found that these hybrids were completely susceptible to bacterial wilt.

Sadashiva and Madhavi (2001) confirmed and demonstrated that two bacterial wilt resistant tomato varieties viz; Ratan (Bangladesh) and T-89 (Sri

Lanka) were promising with to yield bacterial wilt resistance. But both the succumbed to wilt during summer indicating their cultivation would be restricted to cooler climate.

Two heat tolerant tomato lines TML 114 and TML 216 were developed, that are resistant to three biovars of bacterial wilt (Deanon *et al.,* 2002).

High humidity and acidic soil conditions favours bacterial wilt and this made cultivation of tomato very difficult in Tripura. Bacterial wilt resistant varieties like Arka Abha, Arka Alok, Arka Abhijit, Arka Shreshta, RCMT-6, Udaipur local, Sikkim local, Tura local, Sakthi, CS-714 and F_1 hybrids like All Rounder, Gotya S-41 and Samrudh are recommended for cultivation in Tripura to overcome bacterial wilt problems (Singh, 2006).

Over seventy genotypes of tomato from all over the world have been screened at KAU for resistance to ToLCV and BW (Yadav, 2011).

2.4 Evaluation of tomato genotypes for the incidence of tomato leaf curl virus (ToLCV)

2.4.1 Pathogen

Tomato leaf curl virus diseases (ToLCV) are caused by geminivirus belonging to family geminiviridae and genus begmovirus (Anbinder *et al.,* 2009). Tomato leaf curl virus (ToLCV) is a whitefly-transmitted (*Bemisia tabaci*) virus belonging to the family *Geminiviridae* and genus Begomovirus. Hussain (1932) was the first to report the leaf curl disease in tomato. Thung (1932) reported that the Tobacco leaf curl virus causes leaf curl disease in tomato. In India, occurrence of leaf curl disease was first observed in the Northern plains by Pal and Tandon (1937) and later reported by Pruthi and Samuel (1939).

Tomato is affected by 30 different viruses belonging to 16 different taxonomic groups. Among them, the gemini virus group, which causes leaf curl

disease, is more frequently found in sub- tropical and tropical environments. In tomato, leaf curl virus disease is an exhaustive one causing astronomic losses to the crop. Gemini viruses form the second largest family of plant viruses. This family is represented by four genera: Mastrevirus, Curtovirus, Topocuvirus and Begomovirus.

The disease incidence is correlated with the size of the *B. tabaci* population and attributed to the failure of the crop. Several weed species occurring in nature are known to be hosts for both virus and the vector and reported to be major contributors of ToLCV inoculation for the disease outbreak (Gameel, 1977).

During the last two decades these viruses have emerged as devastating pathogens, particularly in the tropics and sub-tropics, causing huge economic losses and threatening crop production (Varma and Malathi, 2003).

The yield loss accounted in tomato due to ToLCV infection ranges from 50 to 70 per cent (Gururaj *et al.,* 2002). The loss may be sometimes as high as 90 per cent and this varies with season and stage of crop growth at which the infection occurs (Saikia and Muniyappa, 1989).

This destructive disease of tomato has been reported in many regions of India. East Asia and Australia (Thamburaj and singh, 2001). Species of the genus Begomovirus are transmitted by the whitefly, *Bemisia tabaci,* in a persistent, circulative manner and infect dicotyledonous plants (Lapidot and Friedmann, 2002).

The typical symptoms of the disease are leaf curling, yellowing, upward leaf rolling, bunched and stunted growth with distorted leaves in initial stages, which becomes more adverse in advanced stages (Kumar *et al.,* 2002).

The vector of ToLCV is a polyphagous insect with more than 300 hosts comprising of a lot of cultivated plants and weed (Reddy *et al.,* 1986). It was

observed that the influence of ToLCV in tomato from 17 to 53 per cent during July to November and up to 100 per cent during February to May (Saikia and Muniyappa, 1989).

Besides India, tomato leaf curl virus has also been reported from Sudan (Cowland, 1932), Israel (Cohen and Harpaz, 1964), Srilanka (Shivanathan, 1983), Egypt (Nour-Eldir *et al.,* 1969), Philippines (Retuerma *et al.,* 1971), Somalia (Castellani *et al.,* 1981), Thailand (Thanapase *et al.,* 1983) and from Taiwan (Green *et al.,* 1987).

2.4.2 Symptomatology

In tomato symptoms vary depending on the growth stage at the time of initial infection, environmental conditions and the variety of tomato plant and include severe stunting, marked reduction in leaf margins, mottling, flower abscission and partial or complete sterility if infection occurs at an early stage of plant development (Sastry and Singh, 1973; Saikia and Muniyappa, 1989).

Yassin and Nour (1965a) described tomato leaf curl symptoms viz., leaf curling, stunting of the plants, thickening, greening of the veins of the leaves as similar to those described by Vasudeva and Sam Raj (1948).

Gevorkyan *et al.* (1976) reported that the growth and development of tomato plant infected by leaf curl virus were considerably delayed. The disease accompanied by decreased content of green and yellow pigments and increased total nitrogen and accumulations of hexose and sucrose.

2.4.3 Historical back ground and spread of yellow leaf curl virus disease

A Tomato Yellow Leaf Curl Virus (TYLCV) like disease was first reported in Israel in 1939-1940 associated with outbreaks of *Bemisia tabaci*. Twenty years later, in 1959, the entire tomato crop was destroyed by a disease with TYLCV- like symptoms in the Jordan Valley (Cohen and Antignus, 1994).

Cohen and Harpaz (1964) published the first description of this new disease transmitted by the whitefly *Bemisia tabaci.* It has since become an economically important disease in many countries of the Middle East, Southern Asia, Eastern and Western Africa and the Mediterranean Basin.

The first report of tomato yellow leaf curl disease in America came from the region of Sonora in Mexico, where a new TYLCV-like tomato disease, transmitted by *Bemisia tabaci*, were observed in 1986. The lack of accurate diagnostic method hampered the correct virus identification. ToLCV isolates have been reported from North America and its presence has been confirmed in Mexico and India (McGlashan *et al.,* 1994).

Before virus isolation, the detection and diagnosis of TYLCV relied on symptom expression, transmission mode and host range. This situation led to some confusion, since the variety of symptoms associated with TYLCV disease makes it difficult to identify. In this sense, tomato leaf curl disease caused by the Tomato leaf curl virus (ToLCV), reported from Sudan, India or Australia has been considered caused by the same viral agent, TYLCV. However, more studies consider both diseases caused by different viral agent (Muniyappa *et al.,* 1991; Dry *et al.,* 1993).

Electron microscopic observation of geminate viral particles and ultra-structural modification in the cell nucleus of infected plants provided evidence of the viral nature of the disease (Russo *et al.,* 1980). In a study, the causal agent of the tomato yellow leaf curl disease was isolated from diseased tomato and *Datura stramonium* plants. Reproduction of the disease using the isolated virus proved the association of viral particles with TYLCV symptoms. Data of particle morphology, mode of transmission and properties of TYLCV genome confirm this whitfly-transmitted geminivirus as the causal agent of this tomato disease (Czosnek *et al.,* 1988).

2.4.4 Importance of tomato leaf curl virus (ToLCV) disease

ToLCV was reported to be serious disease on tomato throughout India. Each year this disease causes millions of dollar damage to tomato crops all over the world.

Sastry and Singh (1973) reported that ToLCV infected plant produced very few fruits when infested within 20 days after transplanting and resulted upto 92.30 per cent yield loss, whereas plants infected at 35 and 50 days after transplanting resulted in 74 and 22.9 per cent yield loss respectively.

Banerjee and Kalloo (1987b) reported that the major constraint in the cultivation of tomato was the outbreak of ToLCV during summer in south India and autumn in north India.

Sadashiva *et al.* (2006) reported that incidence of the disease results in yield loss between 70 and 100 per cent.

Saikia and Muniyappa (1989) reported that tomato plants were susceptible to infection by ToLCV at all stages of their growth. The incidence of ToLCV in some tomato growing areas of Karnataka, India, ranged from 17-53 per cent in July-November to 100 per cent in crops grown in February-May (summer). In sequential sowings, 90-100 per cent of plants were infection in plots sown between the end of January and end of May. Infection in plots sown later was progressively less. 50 to 70 per cent yield loss was observed in tomato cv. Pusa Ruby in February – May. A strong correlation was obtained between the percentage incidence of ToLCV and *B. tabaci* number (r= +0.970, P=0.01)

2.4.5 Host range of ToLCV

In nature, the virus mainly infects tomato. The experimental host range of ToLCV is narrow, mainly infecting some species of the Solanaceae, Compositae, and Caprifoliaceae. Vasudeva and Sam Raj (1948) reported that ToLCV exhibits

leaf curl symptoms on *Nicotiana tabaccum L.* cvs. White Burley, Samsum and Harrison special, *Solanum tuberosum L.* Cv. Craig defiance, *Datura stramonium L, N. Sylvestris spegaz* and *N.glutinosa L,* when inoculated by grafting. Varma (1959) transmitted ToLCV by *B. tabaci* to *N. rustica L., Zinnia elegans* Jacq., *Datura stramonium L, Salvia splendens* Selle, *Althea rosea* Cav, *Petunia hybridia* Vilm, *Euphorbia geniculate* Orteg and *Cassia tora L*.

Sastry *et al.,* (1978) listed three different categories viz., weeds, ornamental and cultivated plants as host plants which had been harbouring ToLCV as well as vector, *B. tabaci*. Out of the 32 different plant species listed, some of them were perennials (*Gossypium arborium L.* and *Hibiscus rosa sinensis L.*) which acted as a reservoir not only for virus but also for the whitefly throughout year.

Natural infection of ToLCV up to 30 per cent amongst weed population at the Gezira Agriculture Research Station locality was observed. Weed hosts *Solanum dubium Frasn* and *Withania somnifera* Dun., occurring in continuous cycle were found perpetually infective throughout the year, whereas, short duration annuls such as *Acalypha indica L.* and *Helitropium sundanicum* Andr. Seem to occur during the limited growing season of tomato in the locality (Yassin and Dafalla, 1980).

Saikia and Muniyappa (1989) transmitted ToLCV by *B. tabaci to Acanthosperum hispidum, Ageratum conyzoides, Bidens biternata (*Lour.*)* Sheriff, *Centratherum anthelminticum* (L) Kuntze, *Conyza stricta, Galinsoga paraviflora, Sonchus brachyotis, Syndrella nodiflora* Gaertn., *Zinnia elegans, Euphorbia geniculata, Althea rosea, Oxalis acetosella* L., *Capsicum annum, Datura stramonium, Lycopersicon esculentum L. gladulosum* Mull., *L. hirsutum* Humb, and Bonpl., *L.peruvianum* (L.) Mill., *N. benthamiana Domin., N. glutinosa, N. tabaccum, Physalis minima, Solanumnigrum*, and *Galinsoga parviflora*.

Sastry (1984) reported that, weed hosts such as *Acanthospermum hispidum, Ageratum conyzoides, Parthenium hysterophorus, Datura stramonium, Euphorbia geniculate* and *Gynandropsis pentaphylla* were source of inoculum for tomato. ToLCV was transmitted to *Acanthosperum hispidum, Ageratum conyzoides, Conyza stricta, Datura stramonium, Euphorbia geniculate, G. parviflora, Oxalis corniculata, Parthenium hysterophorus, S. Nigrum, Sonchus brachyotis, Stachyterpicta indica, Syndrella nodiflora, Nicotiana benthamiana* through *B. tabaci* (Ramappa, 1993).

2.4.6 Strains of ToLCV

Whitefly transmitted tomato geminiviruses from southeast and East Asia constitute a cluster of distinct from those of the Middle East, southeastern Europe and America (Zeidan *et al.,* 1998).

Engel *et al.* (1998) reported that tomato infecting Gemini viruses from Panama named Tomato leaf curl virus (ToLCV-Pan) resembled other whitefly-transmitted geminiviruses, and produces the same kind of symptoms in tomatoes.

Malathi (2006) reported the presence of different strains such as a bipartite Tomato leaf curl New Delhi virus, a monopartite Tomato leaf curl Gujarat virus with a DNA B component, a Tomato leaf curl Bangalore virus with an additional satellite DNA β component tomato in the India subcontinent.

The presence of two subgroups of viruses causing tomato leaf curl disease in India on sequence analysis has been reported. Isolates belonging to subgroup I had a bipartite genome and were conserved among themselves, showing 94 to 95 per cent nucleotide sequence homology, while isolates belonging to the second sub group had monopartite genome and showed 73 to 75 per cent homology with subgroup I (Sinha *et al.,* 2004).

2.5 Sources of tomato leaf curl virus resistance

Vasudev and Sam Raj (1948) screened more than sixty varieties of tomato and reported all of them to be susceptible to ToLCV.

Nariani and Vasudeva (1963) tested 98 varieties of tomato and *Lycopersicon spp.* including lines of *L. pimpinellifolium*, *L. hirsutum* and *L. peruvianum* but did not find resistant genotypes.

The lines of *L. pimpinellifolium*, XXXII-354-A Silestra and P13-2247 are reported to show mild reaction to ToLCV (Som and Choudhury, 1976).

Varma *et al.* (1980) reported the resistance in accession of *L. esculentum* EC 104395.

Joshi and Choudhury, (1981) reported the resistance of *L. chilense* viz., 414-2 x 414-1 SIB, LA 267, 55 L-Antogagster to ToLCV.

Hassan *et al.* (1985) tested 46 tomato cultivars and breeding lines against tomato yellow leaf curl virus and found none of them are resistant.

Banerjee and Kalloo (1987b) screened 122 varieties, lines and wild accessions of *Lycopersicon* and recorded that *L. hirsutum f. typicum* (A 1904), *L. peruvianum* possessed resistance to ToLCV and observed no disease symptoms in *L. pimpinellifolium* (A 1921) till 90 days of age.

Banerjee and Kalloo (1989) observed that two lines *viz.,* A-1921 (*L. pimpinellifolium*) and B-6031 (*L. hirsutum f. glabratum*) were resistance for ToLCV.

In 1986, the first commercial TYLCV-resistant tomato hybrid TY20 was released (Pilowsky and Cohen, 1990).

Moustafa and Nakhla (1990) reported that 6 lines of *L. cheesnmanii*, *L. peruvianum* and *L. pimpinellifolium* showed resistance to ToLCV.

Kandeel (1991) reported that the cross between Clivia X Aurgia was recorded ToLCV resistance.

Among *L. esculentum* accessions LE-812, LE-376 and AVRDC lines were grown in natural infested condition and the genotypes were examined for presence of viral DNA and symptom development at two weeks interval. An accession of *L. chilense* showed highest level of resistance.

A very high level of resistance was found in one accession of *L. hirsutum* (LA 1777) (Moustafa, 1990).

Davino *et al.* (1992) studied the reaction of tomato F_1 hybrids Turguesa, Samar, Arlette, Rita and Mereto and varieties M46, M47, M48 and RS9020 to ToLCV in green house. Cherry type tomato variety RS9020 had the lowest disease incidence.

Moustafa and Hassan (1993) screened 17 true breeding cultivars, four tolerant hybrids and the local control Castlerock for ToLCV resistance. The four hybrids Typhoon, TY 20, BB234 and BB235 and true breeding cultivar T22 showed better virus resistance.

Two ToLCV resistant varieties (Hisar Anmol and Hisar Gaurov) derived from a backcross pedigree of *L. hirsutumf. glabratum* x *L. esculentum* have been identified by the variety evaluation committee of Haryana Agricultural University, Hissar (Kalloo and Banerjee 1993).

The intermediate TYLCV resistance gene *Ty1*, introgressed from *S. chilense* LA 1969 (Michelson *et al.*, 1994).

Kasrawi and Mansour (1995) developed F_7 lines (T27, T37 and T62) from the cross between *L. peruvianum, L. pimpinellifolium, L. hirsutum* with susceptible cv. Special Black. These lines remained symptom less for two years in the field trails and are seen source of stable resistance.

Raghupathi *et al.* (1997) screened one hundred and sixty germplasm entries of tomato against ToLCV. Under natural conditions only two wild species namely *L. hirsutum* (LA 1353) and *L. hirsutum f. glabratum* (LA 1223) were free from ToLCV infection.

Barg *et al.,* (1997) reported that the TYLCV-tolerant breeding line MP-1, which has been is highly amenable to transformation compared with the commonly utilized tomato cultivars. The tomato line MP-1 excels the cultivars commonly used for transformation with regard to the speed of regeneration, percentage of transformation and frequency of phenotypically normal transgenic plants. These characteristics, together with its tolerance to Tomato Yellow Leaf curl Virus, make line MP-1 very suitable for large scale generation of transgenic tomatoes.

Mishra *et al.* (1998) reported resistance to tomato leaf curl virus in the tomato crosses of Anand T-1 X BT-12 and H-24 X BT-12.

Resistance to leaf curl virus was also reported in tomato genotypes viz. H-11, H-22, H-106 and H-107 (Banerjee and Kallo, 1998).

Thirteen tomato varieties of different geographic origin were screened for resistance to tomato yellow leaf curl virus (TYLCV). BL937, BL938, FLA582-17, and TY-King did not show any TYLCV symptoms, while Hirseptyle was severely infected. Avinash#2, FLA438-17 and CLN 2117dcl-26-19-15 had both TYLCV resistance and favorable horticultural characteristics (Li, 1999).

Kallo and Banerjee (2000) reported the performance of H-24 with respect to yield and reaction to ToLCV under field and artificial inoculation. They found

that mean PDI values of H-24, Sel-7 and Punjab Chhuhara were 18.83%, 50.23% and 67.57% respectively.

Six of the most promising tomato yellow leaf curl virus (TYLCV) resistant hybrids (HA3017A, HA3017B, HA3044, HA3048, Px150420 and Ps150535) were evaluated along with, two grower standard cultivars, 'Sanibel' (Petoseed) and 'FL47' (Asgrow) in trials. Of these HA3017A, HA3044 and Px150420 are resistant to TYLCV compare to standard cultivars (Gilreath *et al.*, 2000).

New and old accessions of *Lycopersicon chilense* (LA 1932, LA 1938 and LA 1963), *L. peruvianum* (PI-143679 and PI-126944) and *L. hirsutum* (UPV-16910) reported as resistant to TYLCV (Pico *et al.*, 2002).

Muniyappa *et al.* (2002) developed three open- pollinated (OP) tomato varieties (Sankranthi, Nandi and Vybhav, previously referred to as TLB-111, TLB-130 and TLB-182, respectively) that are resistant to South Indian ToLCVs. These varieties derived virus resistance from the variety H-24, which in turn derived its resistance originally from wild species *Solanum habrochaites* (previously *Lycopersicon hirsutum f. glabratum*).

A total of 90 genotypes of *Lycopersicon* species were tested for resistance to the Tomato leaf curl geminivirus (ToLCV) by agroinoculation and the vector whitefly (*Bemisia tabaci* Genn.).Of the 38 cultivars and 11 breeding lines of *L. esculentum* tested, none was highly resistant. On the other hand, among the 38 commercial cultivars screened, 16 (42.1%) were highly susceptible in vector inoculation and 31 (81.6%) in agroinoculation. Among the exotic collection (EC) accessions six were highly resistant, eleven resistant to whitefly inoculation and none was highly susceptible in either of the two tests. While only one accessions of *L. cheesmanii* was tested, it could not be infected by either of the two methods. *L. pimpinellifolium* genotype EC 251580 was similarly resistant. In *L peruvianum*, five EC accessions could not be infected by whitefly inoculation, with three of

these being resistant and two moderately resistant in agroinoculation (Savarni and Varma, 2002).

Evaluation of tomato resistance were done under field (natural infections) and screen house conditions (natural and controlled infections) using genotypes of diverse origins, comprising cultivars, hybrids, breeding lines, populations and wild tomato species. Out of these resistance found in the *L. peruvianum* access LA 444-1, in the IAC 14-2 series, in the F_4 line TySw5 and in the hybrids 'Franco' and BX 1653088 ('Densus'), with ratings close to absence of symptoms (Matos *et al.,* 2003).

Five commercial tomato cultivars (Amoretto, Birloque, Royesta, Tovigreen and Ulises) naturally infected by TYLC viruses. The analyses showed that Ulises, Birloque and Tovigreen exhibited a moderate resistance and Ulises was also highly tolerant (Rubio *et al.,* 2003).

In order to obtain breeding materials, four TYLCV resistant varieties were screened. Those four varieties showed resistance to TYLCV. Aichi line and were proved to grown normally in spite of infection TYLCV by field resistance. It is considered that 'Athyla' is an elite line for breeding material causing of field resistance test (Masashi *et al.,* 2005).

A total of 25 lines were screened for tolerance to high temperature and ToLCV. Of which, sixteen lines viz; IIHR-2195, IIHR-2196, IIHR-2197, IIHR-2199, IIHR-2000, IIHR-2201, IIHR-2202, IIHR-2223, IIHR-2230, IIHR-2231, IIHR-2234, IIHR-2239, IIHR-2243, IIHR-2248, IIHR-2249 and IIHR-2251 were found to be tolerant to high temperature and resistant to ToLCV and all the lines had high per cent fruit set under field conditions (Singh and Sadashiva, 2007).

Shankarappa *et al.* (2008) developed hybrids by crossing three varieties Sankranthi, Nandi and Vybhav (which are resistant to ToLCV) with 12 tomato genotypes with Superior agronomic characteristics. From those selected 20 hybrids (named BLRH-1 TO BLRH-20, *Bangalore leaf curl virus-resistant*

hybrid) which are and evaluated for their resistance to ToLCV. Of the 20 hybrids evaluated, 11 were found resistant to ToLCV in the field, but only three (BLRH-3, BLRH-9 and BLRH-16) remained resistant when challenged with high virus inoculum pressure in the glasshouse through whitefly-mediated inoculations.

2.6 Molecular characterization

Chunwongse *et al.* (1994) reported the tagging of powdery mildew resistance gene, *Lv*, in tomato using RAPD and RFLP markers. Screening was carried out with 300 random primers that were used to amplify DNA of resistant and susceptible cultivars.

Chague *et al.* (1996) identified RAPD markers linked to *Sw-5* gene, which confers resistant to spotted wilt virus disease in tomato. They have identified four RAPD markers for *Sw-5*. Markers R2 and S are tightly linked to gene. For RAPD analysis DNA was extracted from each plant of the segregating population and the parent cultivars as described by Dellaporta *et al*. (1983).

The AFLP analysis method is more reproducible and robust than RAPD analysis and it displays more fragments than other fingerprinting techniques (Savelkoul *et al.,* 1999). AFLP markers were also reported to be more efficient than RAPD markers to discriminate tomato lines though they did not reveal more polymorphism (Saliba-Columbani *et al.,* 2000).

The use of randomly amplified polymorphic DNA to be detected molecular markers linked to the tomato mosaic virus (ToMV) resistance gene '*Tm2nv*' in tomato has been reported by Tian *et al.* (2000).

Gang *et al.* (2002) reported the use of RAPD markers analysis for identification of polymorphic markers for bacterial wilt resistance between resistant and susceptible bulk DNA of *Solanum phureja* using 300 random primers. The primers OPG09 gave a 960bp reproducible band in resistant clones in the population.

Tosti and Negri (2002) reported that though RAPD could efficiently discriminate among genetically distant accessions of cowpea, AFLP was more useful to analyze the diversity of cowpea population that probably shared a more similar genetic pool.

Sharma (2003) reported that recent development in molecular biology techniques particularly the advent of various DNA markers have greatly influenced plant protection methods. Various PCR-based and hybridization–based DNA markers techniques can be used for the characterization of genetic variability in pathogens and molecular tagging of disease resistance genes. DNA markers linked to specific resistance gene can be used in marker assisted selection for resistance breeding, gene pyramiding and map based cloning of the resistance genes.

Menezes *et al.* (2003) from Brazil reported the presence of 21 AFLP primers revealing DNA bands unique to genotype resistant to tomato spotted wilt virus and five primers revealing DNA bands association with susceptible from a total of 170 AFLP primer combinations surveyed for screening of tomato genotypes.

Langella *et al.* (2004) reported the use of molecular marker assisted breeding schemes using a CAPS marker derived from an RFLP marker CT220 for efficiently transferring TSWV resistance to two tomato elite lines AD-17 and Poly 39 from cultivar ‘Stevens’ carrying the *Sw-5-5* resistance gene in homozygous condition. They also highlight all the advantages of using molecular markers for selection.

The lack of polymorphism in RAPD analysis between eight *Solanum torvum* accessions and difficulties for molecular characterization of cultivars in other solanceae species has been reported by Clain *et al.* (2004). They also reported that due to strong homologies between genomes of the solanaceae species, AFLP’s may not be more polymorphic than RAPD’s.

Abraham *et al.* (2006) identified PCR based markers TG105 to the *Ty2* gene conferring resistance to TYLCV in tomato, which could be used as a molecular marker. They also reported the presence of an SSR marker to determine a tomato's susceptible or resistance to ToLCV and aid in the creation of a commercial acceptable resistant hybrid.

Many complications of a phenotype based assay can be mitigated through direct identification of genotype with a DNA based diagnostic assay. For this reason, DNA-based genetic markers are being integrated into several plant systems and are expected to play an important role in the future of plant breeding. The RAPD assay can be used for a number of applications, which include development of genetic maps, targeting molecular markers, pooling of genomic DNA from individual that are known to be fixed at a particular locus and study of individual identity and taxonomic relationship in both eukaryotic and prokaryotic organisms (Tingey and del Tufo, 1993).

In tomato, the development of a saturated RFLP map has facilitated mapping of several disease resistant genes through the use of molecular markers. These genes have been located through out the tomato genome in different chromosomes (Young *et al.,* 1988).

It has been reported that RAPD markers differ according to variations in experimental conditions. Wolf *et al.* (1993) reported that RAPD markers vary according to changes in concentration of $MgCl_2$ and the type of thermal cycler, while Schiewater *et al.* (1993) reported that the variation occurs with change in quantity and quality of *Taq* polymerase enzyme.

Williams *et al.* (1990) developed a method (RAPD) that uses random primers in a polymerase chain reaction to rapidly generate polymorphic markers that can be used to create genetic linkage maps. They reported that RAPD is a dominant marker inherited in Mendelian fashion.

Martin *et al.* (1993) reported the success of the map based cloning strategy in the cloning of a disease resistance gene in tomato. The *Pto* gene that confers resistance to bacterial speck disease in tomato was cloned through a map based strategy.

Three markers generated by random primers polymorphic in Near Isogenic Lines (NILs) of tomato have been identified. These markers were reportedly linked to the *Pto* gene, which is supposed to confer resistance to the bacterial disease caused by *Pseudomonas* (Martin *et al*., 1991).

Vos *et al.* (1995) described a novel technique for DNA fingerprinting, namely AFLP (Amplified Fragment Length Polymorphism). They illustrated how this technique can be used in fingerprinting of genomic DNA of λ-phage, Acinetobacter, yeast, *Arabidopsis*, cucumber, tomato, human, etc. In tomato, the reaction was carried out using the combination of *Eco*RI and *Mse*I enzymes. They reported that AFLP procedure is insensitive to template DNA concentration. According to their finding, AFLP is an effective tool to reveal polymorphism.

Haanstra *et al.* (1999) reported the development of an integrated high density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum x Lycopersicon pennellii* F_2 populations. This map spanned 1482rcM and contained 67 RFLP markers, 1078 AFLP markers obtained with 22 *Eco*RI + *Mse*I primer combinations and 97 AFLP markers obtained with five *Pst*I + *MSe*I primer combinations, 231 AFLP markers being common to both populations.

Smeich *et al.* (2000) reported the use of RAPD analysis using 271 primers to identify five primers, which enable distinction of resistant and susceptible forms of tomato.

Archak *et al.* (2002) reported low levels of polymorphism using RAPDs in tomato and difficulties for molecular characterization of cultivar in other diploid autogamous solanaceae species.

Balatero *et al.* (2002) reported high resolution detection using non-radioactive silver staining detection method for the construction of a molecular linkage map of F_6 recombinant inbred lines of tomato 'Hawaii 7996 x Wva 700' using polymerase chain reaction based markers such as amplified fragment length polymorphism (AFLP), resistance gene analogues (RGA) and simple sequence repeats (SSR) for development of molecular markers for wilt resistance in tomato.

Chandrashekhara *et al.* (2003) reported high levels of genetic similarity between four species of tomato by the use of RAPD analysis using Operon primers belonging to the OPC, OPD, OPF, OPH and OPK series. The genetic dissimilarity coefficient between these species ranged from 0.16 to 0.40.

Chandrashekhara *et al.* (2003) reported the use of RAPD markers analysis to determine the extent of inter specific genetic diversity in tomato. RAPD assay was carried out using 12 random primers and four accessions representing four species of tomato namely *L. esculentum, L. pimpinellifolium, L. glandulosum* and *L. hirsutum.*

Mejia *et al.* (2004) reported the absence of molecular markers for Begomovirus resistance gene hotspots (Rgh) on chromosomes six and eleven of the tomato genome. They also identified tomato breeding lines Gh13, Gc9 and Gc173 that are resistant to bipartite begomoviruses in Guatemala. Gh13 is the F_7 generations and is a homogenous breeding lines with resistance derived from *Lycopersicon hirsutum*. Gc173 and Gc9 are F_8 breeding lines with resistance genes introgressed from *Lycopersicon chilense*.

Molecular markers linked to ToLCV and BW resistance have been identified and mapping populations developed for integrating combined resistance (Nazeem *et al.,* 2010).

Materials and Methods

3. MATERIALS AND METHODS

The Introgression of ToLCV resistance in bacterial wilt resistant tomato varieties was carried out at CPBMB and Department of Olericulture, College of Horticulture, Kerala Agriculture University, Vellanikkara, Thrissur. Methodology adopted and materials used in this experiment have been described in this chapter.

3.1 Materials

3.1.1 Plant materials

3.1.1.1 Raising of parental population

Bacterial wilt resistant variety Mukthi, released from Kerala Agricultural University and ToLCV resistant genotype IIHR-2195 (Plate 1) identified at Indian Institute of Horticulture Research, Bangalore, were raised in pots during March-June, 2013, for screening the primers (SSR and SCAR). Thirty days old seedlings were transplanted in pots filled with sterilised potting mixture. Soil sterilisation was done with 4 per cent formaldehyde solution. The cultural and agronomic practices were followed as per the package of practices recommendation (KAU, 2011).

3.1.1.2 Raising of F_3 population

Seeds were collected from F_2 population (F_2-54, F_2-47, F_2-34, F_2-41 and F_2-38) of the cross between Mukthi X IIHR-2195 at Department of Olericulture, College of Horticulture, Kerala Agricultural University, Thrissur and F_3 population was raised in the wilt sick field (Plate 2) of the Department, for screening the plants resistant to both bacterial wilt and ToLCV. Selfed seeds were collected from the resistant plants of F_3.

a. Mukthi

b. IIHR-2195

Plate 1. Parental plants Mukthi and IIHR-2195 in pot

Plate 2. F_3 Population in field

3.1.1.3 Raising of F_4 population

F_4 population was raised by sowing the F_3 resistant plant seeds for further screening the segregating population (Plate 3). Selfed seeds were collected from the resistant plants of F_4.

3.1.2 Laboratory chemicals, glass wares and equipment items

The chemicals used in the study were of good quality (AR grade) procured from Merck India Ltd., HIMEDIA and SISCO Research Laboratories. The *Taq* DNA polymerase, dNTPs, Taq buffer and molecular markers used were supplied by Bangalore Genei Ltd. All the plastic ware used were obtained from Axygen and Tarson India Ltd. SSR and SCAR primers were obtained from Sigma Aldrich Chemicals Pvt. Ltd. Centrifugation was done in High speed refrigerated centrifuge (KUBOTA 6500). NanoDrop^R ND-1000 spectrophotometer was used for the estimation of quality and quantity of DNA. The DNA amplification was carried out in Veriti Thermal Cycler (Applied Biosystem, USA) and Agelent. Agarose gel electrophoresis was performed in horizontal gel electrophoresis unit (BIO-RAD, USA).

3.2 METHODS

3.2.1 Morphological analysis

The F_3 and F_4 populations were raised in the bacterial wilt sick plot maintained in the Department of Olericulture and the following observations were recorded.

1. Bacterial wilt incidence
2. ToLCV incidence and severity
3. Days to flower
4. Days to fruit
5. Plant height (cm)

Plate 3. F_4 Population in field

6. Fruit size (cm)
7. Average fruit weight (g)
8. Number of fruits per plant
9. Yield per plant
10. Growth habit

1. Bacterial wilt incidence

Incidence of bacterial wilt was recorded as and when wilt was observed and final count was computed.

Per cent wilt incidence was calculated by the following formula:

$$\text{Per cent wilt incidence} = \frac{\text{Number of plant infected}}{\text{Total number of plant observed}} \times 100$$

Based on the per cent wilt incidence the F_3 and F_4 segregating populations were categorised into four groups as suggested by Mew and Ho (1976).

PWI	**Disease reaction**
0-20	Resistant
20-40	Moderately resistant
40-60	Moderately susceptible
60-100	Susceptible

2. ToLCV incidence and severity

Based on the per cent of curling and puckering of leaves, the plants were scored using 0-4 scale as suggested by Banerjee and Kalloo (1987).

0 : Symptoms absent

1 : Very mild curling (Upto 25 % leaves)

2 : Curling and puckering of 26-50% leaves

3 : Curling and puckering of 51-75% leaves

4 : Sever curling and puckering of >75% leaves

Based on the disease score, per cent disease severity (PDS) was calculated using the following formula:

$$\text{PDS} = \frac{\text{Sum of numerical rating}}{\text{Total number of plants observed} \times \text{Maximum disease grade}} \times 100$$

Per cent disease incidence (PDI) was calculated using the following formula.

$$\text{PDI} = \frac{\text{Number of plant infected}}{\text{Total number of plants observed}} \times 100$$

Based on the per cent disease severity (PDS) and per cent disease incidence (PDI) the coefficient of the infection (CI) was calculated using following formula.

$$\text{Coefficient of infection} = \frac{\text{PDS} \times \text{PDI}}{100}$$

Based on the coefficient of infection the genotypes were categorised into six groups (PDVR, 1997).

0-4 : Highly resistant (HR)

4.1-9 : Resistant (R)

9.1-19 : Moderately resistant (MR)

19.1-39 : Moderately susceptible (MS)

39.1-69 : Susceptible (S)

69.1-100 : Highly susceptible (HS)

3. Days to flower

The number of days from sowing to the appearance of first flower was recorded.

4. Days of fruit

The number of days from sowing to the appearance of first fruit was recorded.

5. Plant height (cm)

The plant height was measured from ground level to tip of the plant at the time of crop senescence and plant height was expressed in centimetres.

6. Fruit size (cm)

Equatorial and polar diameter of the fruits were recorded.

7. Average fruit weight (g)

The best five fruits were weighted and the average fruit weight was worked out and expressed in grams (g).

8. Number of fruits per plant

Fruits harvested periodically from each plant were added to obtain the total number of fruits per plant.

9. Fruit yield per plant (g)

The weight of fruits from each picking was recorded from the each plant. Total yield per plant was worked out by adding yield of all harvests and was expressed in gram (g) per plant.

10. Growth habit

Determinate, semi-determinate and indeterminate growth habit of the plants was recorded.

3.2.2 Molecular analysis

Molecular analysis of the varieties (Mukthi and IIHR-2195) and F_3 and F_4 populations were carried out with two different marker systems- Simple sequence repeats (SSR) and Sequenced characterised amplified region (SCAR).

3.2.2.1 Genomic DNA extraction

Young tender, pale green leaves (first to third from the tip) were collected on ice from individual plants. The surface was cleaned by washing with sterile water and wiping with 70 per cent alcohol. The fresh leaves were ground into a fine powder in liquid nitrogen along with β -mercaptoethanol and PVP using ice-cold mortar and pestle in order to prevent browning due to phenol oxidase activity.

Among the most commonly used protocols, CTAB method developed by Rogers and Bendich (1994) was used for the extraction of genomic DNA. The reagents required for DNA isolation are given in Appendix-I.

Reagents used are,

I. CTAB buffer (2X):

- 2 per cent CTAB (w/v)

- 100mM Tris (pH 8)

- 20mM EDTA (pH 8)

- 1.4M NaCl

- 1 per cent PVP

II. 10 per cent CTAB solution:

- 10 per cent CTAB (w/v)

- 0.7M NaCl.

III. TE buffer:

-10mM Tris (pH 8)

-1mM EDTA (reagent 1 and 3 autoclaved and stored at room temperature)

IV. Chloroform: Isoamyl alcohol (24:1 v/v)

V. Isopropanol

VI. Ethanol 70 per cent and 100 per cent

VII. Sterile distilled water

Procedure:

- 0.2 gram of clean leaf tissues was ground in pre-chilled mortar and pestle in the presence of liquid nitrogen.

- 1ml of extraction buffer (2X), 10µl of β-Mercaptoethanol and a pinch of Poly Vinyl Pyrolidone (PVP) were added to the mortar.
- The homogenized sample was transferred into an autoclaved 2ml centrifuge tube and 1ml of pre-warmed extraction buffer was added (total 1ml).
- The contents were mixed well and incubated at 65ºC for 20 to 30 minutes with occasional mixing by gentle inversion.
- Equal volume (1ml) of chloroform: isoamyl alcohol (24:1) was added and mixed by inversion to emulsify. Spun at 12,000 rpm for 15 minutes at 4ºC
- After centrifugation the contents got separated into three distinct phases.

Aqueous topmost layer	-	DNA and RNA
Interphase	-	fine particles and proteins
Lower layer	-	Chloroform, pigments and cell debris

- Transferred the top aqueous layer to a clean centrifuge tube and added $1/10^{th}$ volume of 10 per cent CTAB solution and equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inversion.
- Centrifuged at 12,000 rpm for 15 minutes at 4ºC.
- Transferred the aqueous phase into a clean centrifuge tube and added 0.6 volume of chilled isopropanol and mixed by quick gentle inversion till the DNA precipitated. Kept at -20ºC for half an hour for complete precipitation.
- Centrifuged at 10,000 rpm for 15 minutes at 4ºC. Gently poured off the supernatant.
- Washed the DNA pellet with 70 per cent ethanol followed by 100 per cent ethanol.
- Spun for 5 min at 10,000 rpm and decanted the ethanol.
- Air dried the pellet, dissolved in 50µl of sterilized water and stored at -20ºC.
- DNA samples were loaded on 0.8 per cent agarose gel to observe the quantity and quality of DNA.

3.2.2.2 Purification of DNA

The DNA which had RNA as contaminant was purified by RNase treatment and further precipitation.

Reagents used are,

- Phenol: chloroform mixture (24:1, v/v)
- Chilled isopropanol
- 70 per cent ethanol
- TE buffer
- Chloroform: Isoamyl alcohol (24:1, v/v)
- 1 per cent RNase

One per cent solution was prepared by dissolving RNase (Sigma, USA) in TE buffer at 100^0C for 15 minutes. The solution was cooled to room temperature, dispensed into aliquots and stored at -20^0C.

Procedure

- To 100 µl DNA sample, RNase solution (2 µl) was added and incubated at 37^0Cin dry bath for 1 hour.
- The volume was made up to 250 µl with distilled water.
- Added equal volume of chloroform: isoamyl alcohol (24: 1) mixture and mixed gently.
- Centrifuged at 12,000 rpm for 15 minutes at 4^0C.
- Transferred the aqueous phase into a fresh micro centrifuge tube and added equal volume of chloroform: isoamyl alcohol (24: 1)
- Centrifuged at 12,000 rpm for 15 minutes at 4^0C.
- Transferred the aqueous phase into a clean centrifuge tube and added 0.6 volume of chilled isopropanol and mixed by quick gentle inversion till the DNA precipitated.

- Incubated the mixture at -20^{0}C for 30 minutes and centrifuged at 10,000 rpm for 15 minutes at 4^{0}C.
- Washed the DNA pellet with 70 per cent ethanol
- Air dried the pellet, dissolved in 50 to 100 µl sterilized water.
- Loaded the samples on 0.8 per cent agarose gel at constant voltage of 100 V to test the quality and to find whether there was any shearing during RNase treatment.

3.2.2.3 Electrophoresis of DNA

The quality of isolated DNA was evaluated through agarose gel electrophoresis (Sambrook *et al*., 1989).

Reagents

1. Agarose - 0.8 per cent (for genomic DNA)

   - 2 per cent (for PCR SCAR and SSR samples)

2. 50X TAE buffer (pH8.0)

   - Tris buffer

   - Glacialacetic acid

   - EDTA

3. Tracking/loading dye (6X)

   -Bromophenol blue

   -Xylene cyanol

   -Glycerol

4. Ethidium bromide (stock 10 mg/ml; working concentration 0.5 µg/ml)

Composition of reagents is provided in Appendix-II.

Procedure

- The gel tray was prepared by sealing the ends with tape. Comb was placed in gel tray about 1 inch from one end of the tray and positioned the comb vertically such that the teeth are about 1 to 2 mm above the surface of the tray.
- Prepared 0.8 per cent agarose (0.8 g in 100ml) in a glass beaker or conical flask with 100 ml 1X TAE buffer. Micro waved for 45 to 60 seconds until agarose was dissolved and solution was clear.
- Solution was allowed to cool to about 42 to 45 ^{0}C before pouring. (4µl Ethidium bromide was added at this point to a concentration of 10 µl/ml) and mixed well.
- Poured this warm gel solution into the tray to a depth of about 5 mm. allowed the gel to solidify for about 30 to 45 minutes at room temperature.
- To run, gently removed the comb and the tape used for sealing, placed the tray in electrophoresis chamber, and covered (just until wells are submerged) with electrophoresis buffer (1X TAE).
- To prepare samples for electrophoresis, added 1 µl of 6x gel loading dye for every 5µl of DNA solution. Mixed well and loaded 6µl DNA sample per well. Loaded suitable molecular weight marker (λDNA*Eco*RI/ *Hind*III double digest) in one lane.
- Electrophoresed at 80 volts until dye has migrated two third the length of the gel.
- Intact DNA appears as orange fluorescent bands. If degraded, it appears as a smear because of the presence of a large number of bands, which differ in base length. The gel profile was examined for intactness, clarity of DNA band, presence of RNA and protein.

3.2.2.4 Gel Documentation

Gel documentation was done with BioRad Gel Documentation System using PDQuest™ software. PDQuest is a software package for imaging, analysing and databasing 2-D electrophoresis gels. PDQuest can acquire images of gels using any of several Bio-Rad imaging systems. An image of a gel is captured using the controls in the imaging device window and displayed on computer screen.

3.2.2.5 Assessing the quality and quantity of DNA by spectrophotometer

The purity of DNA was further checked by using NanoDrop ND-1000 spectrophotometer. Nucleic acid shows absorption maxima at 260nm where as proteins show peak absorbance at 280nm. Absorbance was recorded at both wavelength and purity was indicated by the ratio OD_{260}/OD_{280}. The values between 1.8 and 2.0 indicated that the DNA was pure and free from proteins. The quantity of DNA in the pure sample was calculated using the relation 1 OD_{260} equivalent to 50µg double stranded DNA/ml sample.

1 OD at 260 nm = 50 µg DNA/ml

Therefore $OD_{260} \times 50$ gives the quantity of DNA in µg/ml.

Procedure for quantity detection using Nanodrop

- Connected the Nanodrop spectrophotometer to the System and opened the operating software ND-100.
- Selected the option Nucleic acid.
- With the sampling arm open, pipetted 1µl distilled water onto the lower measurement pedestal.
- Closed the sampling arm and initiated a spectral measurement using the operating software on the PC. The sample column is automatically drawn

between the upper and lower measurement pedestals and the spectral measurement is made.

- Set the reading to zero with sample blank.
- 1µl sample was pipetted onto measurement pedestal and select measure.
- When the measurement was complete, opened the sampling arm and wiped the sample from both the upper and lower pedestals using a soft laboratory wipe. Simple wiping prevents sample carryover in successive measurements for samples varying by more than 1000 fold in concentration.

3.2.2.6 Molecular Markers used for the study

Two types of markers were used for the study which includes SSR (Simple Sequence Repeats) and Sequenced characterised amplified region (SCAR). Under each marker analysis system, DNA from parents (Mukthi and IIHR-2195) were screened separately with all the selected primers. After screening all the selected primers with parent (Mukthi and IIHR-2195), got some primers which showed polymorphic bands for bacterial wilt and ToLCV resistance. Those primers which showed the polymorphic bands were selected for screening the F_3 and F_4 population.

3.2.2.7 DNA amplification conditions

The PCR conditions required for effective amplification in SSR and SCAR analysis include appropriate proportions of the component of the reaction mixture. The reaction mixture included template DNA, assay buffer A or B, $MgCl_2$, Taq DNA polymerase, dNTPs and primers. The aliquot of this master mix were dispensed into 0.2ml PCR tubes. The PCR was carried out in Veriti Thermal Cycler (Applied Biosystem, USA) or Agilent.

Another important factor, which affect amplification rate is the temperature profile of thermal cycle. The thermocycler was programmed for desired times and temperature for denaturation, annealing and polymerization based on the nature of primer used.

3.2.2.8 SSR (Simple Sequence Repeat) analysis

Good quality genomic DNA (20 to 25ng/µl) isolated from tomato leaf samples (F_3 and F_4 population). SSR primers supplied by sigma, USA were used for amplification of DNA. SSR primers for the assay were selected after an initial screening of primers with parents (Mukthi and IIHR-2195).

The amplification was carried out in Veriti Thermal Cycler (Applied Biosystem, USA) or Agilent. PCR amplification was performed in a 20 µl reaction mixture which consisted of,

a) Genomic DNA (30ng)	-	2.0µl
b) 10X Taq assay buffer A	-	2.0µl
c) dNTP mix (10mm each)	-	1.5µl
d) Taq DNA Polymerase (1U)	-	0.3µl
e) Forward Primer (10pM)	-	0.75µl
f) Reverse Primer (10pM)	-	0.75µl
g) Autoclaved Distilled Water	-	12.7µl
Total volume	-	20.0µl

The thermocycler was carried out with the following programme

94^0C for 3 minutes	-	Initial denaturation	
94^0C for 1 minute	-	Denaturation	35 cycles
56.5^0C to 58.9^0C for 1 minute	-	Primer annealing	
72^0C for 1 minute	-	Primer extension	
72^0C for 5 minutes	-	Final extension	
4^0C for infinity to hold the sample			

3.2.2.9 Screening of SSR primers and analysis

Five primers were screened with parents (Mukthi and IIHR-2195) and those primers which gave polymorphism were selected for screening F_3 and F_4 population by PCR for SSR analysis and are listed in Table 1. The amplified products were run on two per cent agarose gel using 1X TAE buffer stained with ethidium bromide along with marker (100bp ladder). The profile was visualized under UV (312 nm) transilluminator and documented. The documented SSR profiles were carefully examined for amplification of DNA as bands.

Table 1: List of SSR primers screened with tomato samples

Sl No	Name of Primers	Sequence	
1	LEaat 007	F	5’ CAA CAG CAT AGT GGA GGA GG 3’
		R	5’ TAC ATT TCT CTC TCT CCC ATG AG 3’
2	SSR 20	F	5’ GAG GAC GAC AAC AAC AAC GA 3’
		R	5’ GAC ATG CCA CTT AGA TCC ACC A 3’
3	LEaat 002	F	5’ GCG AAG AAG ATG AGT CTA GAG CAT AG 3’
		R	5’ CTC TCT CCC ATG AGT TCT CCT CTT C 3’
4	LEat 006	F	5’ CAT AAT CAC AAG CTT CTT TCG CCA 3’
		R	5’ CAT ATC CGC TCG TTT CGT TAT GTA AT 3’
5	SSR 306	F	5’ ACA TGA GCC CAA TGA ACC TC 3’
		R	5’ AAC CAT TCC GCA CGT ACA TA 3’

3.2.2.10 SCAR (Sequenced characterised amplified region) analysis

Good quality genomic DNA (30ng/µl) isolated from tomato leaf samples (F_3 and F_4 population). SCAR primers supplied by sigma, USA were used for amplification of DNA. SCAR primers for the assay were selected after an initial screening of primers with parents (Mukthi and IIHR-2195).

The amplification was carried out in Veriti Thermal Cycler (Applied Biosystem, USA) or Agilent. PCR amplification was performed in a 20 µl reaction mixture which consisted of,

a) Genomic DNA (30ng)	-	2.0µl
b) 10X Taq assay buffer A	-	2.0µl
c) dNTP mix (10mm each)	-	1.5µl
d) Taq DNA Polymerase (1U)	-	0.3µl
e) Forward Primer (10pM)	-	0.75µl
f) Reverse Primer (10pM)	-	0.75µl
g) Autoclaved Distilled Water	-	12.7µl
Total volume	-	20.0µl

The thermocycler was carried out with the following programme

94^0C for 3 minutes	-	Initial denaturation	
94^0C for 1 minute	-	Denaturation	35 cycles
50.7^0C to 54.2^0C for 1 minute	-	Primer annealing	
72^0C for 1 minute	-	Primer extension	
72^0C for 5 minutes	-	Final extension	

4^0C for infinity to hold the sample

3.2.2.11 Screening of SCAR primers and analysis

Six primers were screened with parents (Mukthi and IIHR-2195) and those primer give the polymorphism was selected for screening F_3 and F_4 population by PCR for SCAR analysis and are listed in Table 2. The amplified products were run on two per cent agarose gel using 1X TAE buffer stained with

ethidium bromide along with marker (100bp ladder). The profile was visualized under UV (312 nm) transilluminator and documented. The documented SCAR profiles were carefully examined for amplification of DNA as bands.

Table 2: List of SCAR primers screened with tomato samples

Sl No	Name of Primers	Sequence	
1	TSCAR AAT/CGA	F	5‘ TAG ATG GAA TCC AAT ATC AGG 3‘
		R	5’ AAC CAC AGT GAA GGA ATA TAC A 3’
2	Ualty3a	F	5’GAC CTT CAA AAT GAT CAG ATA 3’
		R	5’ TGG ACC CTT TTT ACC CTA AGC 3’
3	Ualty3b	F	5’ CTC CAC AGC TTC AAT GCA AA 3’
		R	5’ CGT GAA TAC CTT GAT TCT TGA 3’
4	Ualty 5	F	5’ TAG GAA ATG TTG AAC TAT TGT GTT 3‘
		R	5’ TCA TGC GAT GAA GAG GTC TAT G 3‘
5	Ualty 6	F	5’ TGT TGT GAT TGT TAT TGT CAA C 3‘
		R	5’ CTG GCA AGC GTG TAA CTC AC 3’
6	Ualty 11	F	5’ TTA ATT CTA GGG ATT TGG CAG T 3’
		R	5’ CCC AAG CCA TCA TGA GAT TC 3’

Results

4. RESULTS

Results obtained from the research on screening of mapping population through marker assisted selection for imparting disease resistance in tomato (*Solanum lycopersicum* L.) are described in this chapter.

4.1 Evaluation of F_3 population

F_3 population was raised from the seeds obtained from five F_2 plants which showed resistance. Total 337 plants were evaluated in the bacterial wilt sick field. The disease reaction is presented in Table 3. In total population, 71.8 per cent plants thrived over bacterial wilt, while only 6.53 per cent (22 plants) were found tolerant to both bacterial wilt and ToLCV. Maximum number of plants with combined resistance were observed among the progenies segregated from the plant F_2-38. None of the segregants from F_2-47 showed combined resistance (Table 3).

4.1.1 Disease reaction of F_3 segregants for bacterial wilt and tomato leaf curl virus

Per cent disease incidence was calculated in the segregating progenies of F_3 for bacterial wilt and ToLCV. The segregants were classified into four groups based on their reaction to bacterial wilt (Table 4 and Plate 4). Of these, 3 segregants were resistant and 2 segregants were moderately susceptible. The segregants were classified into six groups based on their reaction to tomato leaf curl disease (Table 5 and Plate 5). Of these, 3 segregants were moderately susceptible, 1 was moderately resistant and other one was susceptible.

4.1.2 Days to flower

Days to flower ranged from 31 days to 87 days in the F_3 progenies (Table 6a). Maximum days to flower was recorded in the F_3 progenies of F_2-54 (87 days) followed by F_2-34, 38, 41 and 47 (75 days), the minimum days to flower was

Table-3 Reaction of F_3 population to bacterial wilt and tomato leaf curl virus (ToLCV)

	F_2 Plants	F_3 Plants								
	F_2 Parents	Total number of plants	Number of plants resistant to both BW and ToLCV	Bacterial wilt		ToLCV infection				
				Resistant	Susceptible	Disease score				
						0	1	2	3	4
1.	F_2-34	78	2	64	14	2	11	31	25	2
2.	F_2-38	56	14	45	11	14	10	8	10	6
3.	F_2-41	65	2	37	28	2	3	13	15	8
4.	F_2-47	72	0	43	29	0	5	35	11	2
5.	F_2-54	66	4	53	13	4	10	6	10	6
Total		337	22	242	95	22	39	93	71	24

Table-4 Reaction of F_3 segregants to bacterial wilt

S.No	Segregants	PWI	Disease reaction
1.	F_2-34	17.94	Resistant
2.	F_2-38	19.64	Resistant
3.	F_2-41	43.07	Moderately Susceptible
4.	F_2-47	40.27	Moderately Susceptible
5.	F_2-54	19.69	Resistant

PWI- Per cent wilt incidence

Table-5 Reaction of F_3 segregants to tomato leaf curl virus (ToLCV)

S.No	Segregants	PDI	PDS	C.I	Category
1.	F_2-34	88.46	50	44.23	Susceptible
2.	F_2-38	60.71	35.71	21.67	Moderately Susceptible
3.	F_2-41	60	40.76	24.45	Moderately Susceptible
4.	F_2-47	73.61	40.27	29.64	Moderately Susceptible
5.	F_2-54	48.48	37.12	17.99	Moderately Resistant

PDI- Per cent disease incidence, PDS- Per cent disease severity, CI- Coefficient of infection

Plate 4. Bacterial wilt disease incidence in tomato

Plate 5. ToLCV disease incidence in tomato

Table-6 Mean performance of F_3 progenies

a. Days to flower

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_2-34	54.91	36	75	39	1.06
2.	F_2-38	52.21	32	75	43	1.05
3.	F_2-41	56.29	34	75	41	1.4
4.	F_2-47	56.95	33	75	42	1.14
5.	F_2-54	52.37	56	87	31	1.19

b. Days to fruit

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_2-34	59.47	49	80	43	1.2
2.	F_2-38	56.41	32	79	47	1.05
3.	F_2-41	60.82	36	82	46	1.4
4.	F_2-47	64.03	48	93	45	1.39
5.	F_2-54	58.42	49	93	44	1.37

observed in the F_3 progenies of F_3-54 (31 days) followed by F_2-34 (39 days). Highest mean value for days to flower was recorded in the F_2-47 (56.97 days) followed by F_2-41 (56.29 days). Lowest mean value was observed in F_2-38 (52.21 days) followed by F_2-54 (52.37 days).

4.1.3 Days to fruit

Days to fruit varied from 43 days to 93 days in F_3 population (Table 6b). Maximum days to fruit was recorded in the F_3 progenies of F_2-47 and F_2-54 (93 days) followed by F_2-41 (82 days), the minimum days to fruit was recorded in the F_3 progenies of F_2-34 (31 days) followed by F_2-54 (44 days). Highest mean value for days to fruit was recorded in the F_2-47 (64.03 days) followed by F_2-41 (60.82 days). Lowest mean value was observed in F_2-38 (56.41 days) followed by F_2-54 (58.42 days).

4.1.4 Biometric evaluation of F_3 plants with combined resistance to bacterial wilt and tomato leaf curl virus (ToLCV)

The performance of 22 F_3 plants (Plate 6) having combined resistance to bacterial wilt and ToLCV is presented in Table 7. The number of days to flower ranged between 38 and 61 days and days to fruit ranged between 44 and 67 days. The average fruit weight ranged between 31 g and 84 g and number of fruits varied between 4 and 40. The plant F_3-38-9 having combined resistance gave the maximum fruit yield (1060 g) followed by the plant F_3-38-14 (1048 g). The rest of the 20 plants recorded less than 900 g with the minimum 180 g recorded by F_3-38-49.

4.2 Evaluation of F_4 population

F_4 population was raised from the seeds obtained from 22 F_3 plants which showed resistance. Total 584 plants were evaluated in the bacterial wilt sick field. The disease reaction is presented in Table 8. In the total population, 84.76 per cent plants thrived over bacterial wilt, while only 5.99 per cent (35 plants) were found

Plate 6. Selected F_3 resistant (BW and ToLCV) plants

Table-7 Biometric characters of F_3 plants with combined resistance to bacterial wilt and tomato leaf curl virus (ToLCV)

S.No	Plant number	Days to flower	Days to fruiting	Average fruit weight (g)	Number of fruits per plant	Yield/Plant (g)
1.	F_3-38-09	50	54	44	34	1060
2.	F_3-38-14	58	61	58	34	1048
3.	F_3-38-12	58	61	44	29	810
4.	F_3-38-18	50	54	51	33	752
5.	F_3-38-28	55	58	61	23	673
6.	F_3-38-50	44	48	56	15	527
7.	F_3-38-45	44	48	69	12	459
8.	F_3-38-06	58	61	61	10	413
9.	F_3-38-29	55	58	50	20	400
10.	F_3-38-48	44	50	70	11	375
11.	F_3-38-55	44	48	34	19	262
12.	F_3-38-33	60	64	54	16	248
13.	F_3-38-27	55	58	45	12	211
14.	F_3-38-49	44	49	60	4	180
15.	F_3-54-31	55	58	31	31	683
16.	F_3-54-43	53	60	39	26	592
17.	F_3-54-57	38	44	65	19	505
18.	F_3-54-67	43	46	41	9	298
19.	F_3-41-33	54	58	84	8	410
20.	F_3-41-11	61	67	64	6	229
21.	F_3-34-52	59	62	37	40	820
22.	F_3-34-20	57	61	41	27	748
Mean		51.77	55.81	52.68	19.90	531.95

Table-8 Reaction of F_4 population to bacterial wilt and tomato leaf curl virus (ToLCV)

S.No	F_3 Plants	F_4 Plants								
	F_3 Parents	Total number of plants	Number of plants resistant to both BW and ToLCV	Bacterial wilt		ToLCV infection				
				Resistant	Susceptible	Disease score				
						0	1	2	3	4
1.	F_3-38-06	30	2	26	4	2	5	11	4	4
2.	F_3-38-09	30	1	28	2	1	3	20	4	0
3.	F_3-38-12	33	1	24	9	1	9	9	3	2
4.	F_3-38-14	23	0	22	1	0	8	9	3	2
5.	F_3-38-18	22	0	21	1	0	10	6	4	1
6.	F_3-38-27	6	0	5	1	0	1	3	1	0
7.	F_3-38-28	31	0	29	2	0	9	9	7	4
8.	F_3-38-29	32	0	23	9	0	5	12	2	4
9.	F_3-38-33	15	0	12	3	0	6	4	1	1

10.	F_3-38-45	15	2	13	2	2	5	4	2	0
11.	F_3 -38-48	25	0	19	6	0	10	6	2	1
12.	F_3-38-49	15	3	15	0	3	8	4	0	0
13.	F_3-38-50	34	5	31	3	5	11	13	2	0
14.	F_3-38-55	8	0	7	1	0	2	1	3	1
15.	F_3-41-11	21	0	18	3	0	3	13	2	0
16.	F_3-41-33	42	2	36	6	2	5	27	3	0
17.	F_3-34-20	47	1	41	6	1	8	24	8	0
18.	F_3-34-52	32	0	30	2	0	6	21	3	0
19.	F_3-54-31	38	6	26	12	6	7	11	1	1
20.	F_3-54-43	26	2	23	3	2	7	12	2	0
21.	F_3-54-57	26	4	18	8	4	5	9	0	0
22.	F_3-54-67	33	6	28	5	6	10	5	6	1
Total		584	35	495	89	35	143	233	63	22

tolerant to both bacterial wilt and ToLCV. Maximum number of plants with combined resistance were observed among the progenies segregated from the plants F_3-54-67 and F_3-54-31. 10 segregants have not given combined resistance.

4.2.1 Disease reaction of F_4 segregants for bacterial wilt and tomato leaf curl virus

Per cent disease incidence was calculated in the segregating progenies of F_4 for bacterial wilt and ToLCV (Table 9 and 10). The segregants were classified into four groups based on their reaction to bacterial wilt (Table 9 and Plate 4). Of these, 17 segregants were resistant and 5 segregants were moderately resistant. The segregants were classified into six groups based on their reaction to tomato leaf curl disease (Table 10 and Plate 5). Of these, 2 segregants were moderately resistant, 14 were moderately susceptible and 6 were susceptible.

4.2.2 Plant height

Plant height ranged from 10 cm to 75 cm in the F_4 progenies (Table 11). Maximum height was recorded in the F_4 progenies of F_3-38-49 (75 cm) followed by F_3-38-50 and F_3-41-33, the minimum height was observed in the F_4 progenies of F_3-38-06 (10 cm) followed by F_3-38-28 (15 cm). Highest mean value for plant height was recorded in the F_3-38-27 (60.8 cm) followed by F_3-38-48 (60.42 cm). Lowest mean value was observed in F_3-38-06 (42.04 cm) followed by F_3-38-09 (51.14 cm).

4.2.3 Days to flower

Days to flower ranged from 30 days to 71 days in the F_4 progenies (Table 12). Maximum days to flower was recorded in the F_4 progenies of F_3-54-57 (71 days) followed by F_3-38-6 (70 days), the minimum days to flower was observed in the F_4 progenies of F_3-41-11 (30 days) followed by F_3-38-12 (32 days). Highest mean value for days to flower was recorded in the F_3-38-55 (59.25 days) followed

Table-9 Reaction of F_4 segregants to bacterial wilt

S.No	Segregants	PWI	Disease reaction
1.	F_3-38-06	13.33	Resistant
2.	F_3-38-09	6.66	Resistant
3.	F_3-38-12	27.27	Moderately Resistant
4.	F_3-38-14	4.34	Resistant
5.	F_3-38-18	4.54	Resistant
6.	F_3-38-27	16.66	Resistant
7.	F_3-38-28	6.45	Resistant
8.	F_3-38-29	28.12	Moderately Resistant
9.	F_3-38-33	20	Resistant
10.	F_3-38-45	13.33	Resistant
11.	F_3 -38-48	24	Moderately Resistant
12.	F_3-38-49	6.66	Resistant
13.	F_3-38-50	8.82	Resistant
14.	F_3-38-55	12.5	Resistant
15.	F_3-41-11	14.28	Resistant
16.	F_3-41-33	14.28	Resistant
17.	F_3-34-20	12.76	Resistant
18.	F_3-34-52	6.25	Resistant
19.	F_3-54-31	31.57	Moderately Resistant
20.	F_3-54-43	11.53	Resistant
21.	F_3-54-57	30.76	Moderately Resistant
22.	F_3-54-67	15.15	Resistant

PWI- Per cent wilt incidence

Table-10 Reaction of F_4 segregants to tomato leaf curl virus (ToLCV)

S.No	Segregants	PDI	PDS	C.I	Category
1.	F_3-38-06	80	45.83	36.66	Moderately Susceptible
2.	F_3-38-09	90	45.83	41.25	Susceptible
3.	F_3-38-12	69.69	33.33	23.22	Moderately Susceptible
4.	F_3-38-14	95.65	46.73	44.70	Susceptible
5.	F_3-38-18	95.45	43.18	41.21	Susceptible
6.	F_3-38-27	83.33	45.83	38.19	Moderately Susceptible
7.	F_3-38-28	93.54	51.61	48.27	Susceptible
8.	F_3-38-29	71.87	39.84	28.63	Moderately Susceptible
9.	F_3-38-33	80	35	28	Moderately Susceptible
10.	F_3-38-45	73.33	35	25.66	Moderately Susceptible
11.	F_3 -38-48	76	32	23.36	Moderately Susceptible
12.	F_3-38-49	80	26.66	21.33	Moderately Susceptible
13.	F_3-38-50	76.47	31.61	24.17	Moderately Susceptible
14.	F_3-38-55	87.5	53.12	46.48	Susceptible
15.	F_3-41-11	85.71	41.66	35.71	Moderately Susceptible
16.	F_3-41-33	83.33	40.47	33.72	Moderately Susceptible
17.	F_3-34-20	85.10	42.55	36.21	Moderately Susceptible
18.	F_3-34-52	93.75	44.53	41.74	Susceptible
19.	F_3-54-31	39.47	23.68	9.34	Moderately Resistant
20.	F_3-54-43	80.76	35.57	28.73	Moderately Susceptible
21.	F_3-54-57	53.84	22.11	11.90	Moderately Resistant
22.	F_3-54-67	66.66	31.81	21.21	Moderately Susceptible

PDI- Per cent disease incidence, PDS- Per cent disease severity, CI- Coefficient of infection

Table-11 Mean performance of F_4 progenies for plant height (cm)

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_3-38-06	42.04	51	61	10	2.65
2.	F_3-38-09	51.14	27	62	35	1.45
3.	F_3-38-12	57.04	38	69	31	1.63
4.	F_3-38-14	55.95	25	63	38	1.61
5.	F_3-38-18	55.35	33	62	29	1.95
6.	F_3-38-27	60.8	2	62	60	0.37
7.	F_3-38-28	55.83	48	63	15	2.19
8.	F_3-38-29	54	44	69	25	2.25
9.	F_3-38-33	57.58	17	67	50	1.26
10.	F_3-38-45	58.92	14	69	55	1.05
11.	F_3 -38-48	60.42	24	69	45	1.21
12.	F_3-38-49	59.53	32	75	43	2.29
13.	F_3-38-50	58.83	32	71	59	1.31
14.	F_3-38-55	58.42	9	63	54	1.26
15.	F_3-41-11	56.11	22	67	45	1.29
16.	F_3-41-33	58.25	48	71	23	1.42
17.	F_3-34-20	55.94	38	67	29	1.13
18.	F_3-34-52	55.9	24	69	45	1.04
19.	F_3-54-31	58.1	41	69	28	1.58
20.	F_3-54-43	57.04	25	68	43	1.17
21.	F_3-54-57	59.11	23	68	45	1.3
22.	F_3-54-67	57.85	20	69	49	1.17

Table-12 Mean performance of F_4 progenies for days to flower

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_3-38-06	51.46	31	70	39	1.56
2.	F_3-38-09	45.89	21	60	39	1.03
3.	F_3-38-12	48.10	25	57	32	1.11
4.	F_3-38-14	54.45	25	68	43	1.63
5.	F_3-38-18	54.04	14	61	47	0.75
6.	F_3-38-27	53.33	26	67	41	3.55
7.	F_3-38-28	52.51	12	61	49	0.45
8.	F_3-38-29	53.89	18	63	45	0.76
9.	F_3-38-33	56.15	16	67	51	1.24
10.	F_3-38-45	51.80	17	61	44	0.95
11.	F_3 -38-48	54.35	12	61	49	0.72
12.	F_3-38-49	54.73	10	61	51	0.90
13.	F_3-38-50	53.38	10	60	50	0.41
14.	F_3-38-55	59.25	08	64	56	0.92
15.	F_3-41-11	51.61	31	61	30	1.26
16.	F_3-41-33	54.56	11	62	51	0.54
17.	F_3-34-20	54.39	09	59	50	2.53
18.	F_3-34-52	53.93	18	67	49	0.70
19.	F_3-54-31	54.28	14	64	50	0.57
20.	F_3-54-43	51.84	10	59	49	0.43
21.	F_3-54-57	54.29	20	71	51	0.83
22.	F_3-54-67	53.37	11	61	50	0.45

by F_3-38-33 (56.15 days). Lowest mean value was observed in F_3-38-9 (45.89 days) followed by F_3-38-12 (48.10 days).

4.2.4 Days to fruit

Days to fruit varied from 40 days to 89 days in F_4 population (Table 13). Maximum days to fruit was recorded in the F_4 progenies of F_3-38-14 (89 days) followed by F_3-54-57 (77 days), the minimum days to fruit was recorded in the F_4 progenies of F_3-38-9 (40 days) followed by F_3-38-12 (42 days). Highest mean value for days to fruit was recorded in the F_3-38-55 (65.28 days) followed by F_3-38-49 (62.06 days). Lowest mean value was observed in F_3-38-9 (52.50 days) followed by F_3-38-12 (55.5 days).

4.2.5 Average fruit weight

Average fruit weight ranged from 15 g to 122 g in the F_4 progenies (Table 14). Maximum average fruit weight was recorded in the F_4 progenies of F_3-38-49 (140 g) followed by F_3-38-14 (125 g) and the minimum average fruit weight was observed in the F_4 progenies of F_3-38-18 (15 g) followed by F_3-41-33 (20 g). Highest mean value for average fruit weight was recorded in the F_3-38-49 (66.33 g) followed by F_3-38-50 (66.03 g). Lowest mean value was observed in F_3-54-31 (48.93 g) followed by F_3-38-27 (50 g).

4.2.6 Number of fruits per plant

Number of fruits ranged between 0 and 56 in F_4 population (Table 15). The highest number of fruits were produced by F_4 population of F_3-54-57 (56 fruits/plant) followed by F_3-38-6 (40 fruits/plant). Lowest number of fruits (0 fruits/plant) produced by 21 segregants among 22 F_4 segregants. Highest mean value was recorded by F_3-38-27 (21.33 fruits/plant) followed by F_3-38-18 (17.95 fruits/plant). Lowest mean value for number of fruits per plant was recorded by F_3-38-29 (8.63 fruits/plant) followed by F_3-38-55 (8.87 fruits/plant).

Table-13 Mean performance of F_4 progenies for days to fruit

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_3-38-06	56.67	30	74	44	1.48
2.	F_3-38-09	52.50	28	68	40	1.09
3.	F_3-38-12	55.5	22	64	42	0.98
4.	F_3-38-14	61.04	39	89	50	1.99
5.	F_3-38-18	60.29	12	67	55	0.72
6.	F_3-38-27	61.17	18	74	56	2.65
7.	F_3-38-28	58.57	19	70	51	0.78
8.	F_3-38-29	60.21	16	69	53	0.94
9.	F_3-38-33	61.77	14	71	57	1.09
10.	F_3-38-45	58.08	13	65	52	1
11.	F_3 -38-48	61.2	10	67	57	0.61
12.	F_3-38-49	62.06	10	68	58	0.9
13.	F_3-38-50	58.87	7	63	56	0.34
14.	F_3-38-55	65.28	6	69	63	0.77
15.	F_3-41-11	59.38	18	67	49	0.82
16.	F_3-41-33	60.57	15	68	53	0.57
17.	F_3-34-20	60.16	18	68	50	0.58
18.	F_3-34-52	60.56	16	71	55	0.72
19.	F_3-54-31	60.28	15	71	56	0.55
20.	F_3-54-43	57.65	10	64	54	0.45
21.	F_3-54-57	60.16	20	77	57	0.81
22.	F_3-54-67	59.5	12	67	55	0.48

Table-14 Mean performance of F_4 progenies for average fruit weight (g)

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_3-38-06	60.73	65	90	25	4.03
2.	F_3-38-09	62.37	70	100	30	2.97
3.	F_3-38-12	62.62	65	100	35	3.83
4.	F_3-38-14	55.75	90	121	35	4.73
5.	F_3-38-18	52.26	72	87	15	4.48
6.	F_3-38-27	50	17	57	40	3.35
7.	F_3-38-28	57.07	86	121	35	4.2
8.	F_3-38-29	56.75	45	80	35	2.5
9.	F_3-38-33	61.75	55	90	35	4.38
10.	F_3-38-45	54.31	40	75	35	3.57
11.	F_3 -38-48	58	76	107	31	3.75
12.	F_3-38-49	66.33	90	122	50	5.99
13.	F_3-38-50	66.03	80	120	40	3.32
14.	F_3-38-55	54.85	30	75	45	3.81
15.	F_3-41-11	65.77	69	120	51	4.17
16.	F_3-41-33	56.36	60	80	20	2.07
17.	F_3-34-20	54.09	55	90	35	1.58
18.	F_3-34-52	52.86	36	67	31	1.32
19.	F_3-54-31	48.93	37	67	30	1.63
20.	F_3-54-43	59.36	60	95	35	2.76
21.	F_3-54-57	54.16	20	65	45	1.3
22.	F_3-54-67	60.37	45	80	35	2.22

Table-15 Mean performance of F_4 progenies for number of fruits per plant

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_3-38-06	12.13	40	40	0	2.05
2.	F_3-38-09	17.30	39	39	0	2.22
3.	F_3-38-12	10.46	31	31	0	1.69
4.	F_3-38-14	15.61	33	33	0	2.02
5.	F_3-38-18	17.95	35	35	0	2.14
6.	F_3-38-27	21.33	34	34	0	4.92
7.	F_3-38-28	12.13	25	25	0	1.31
8.	F_3-38-29	8.63	25	25	0	1.46
9.	F_3-38-33	11.13	22	22	0	1.94
10.	F_3-38-45	15.4	30	30	0	2.29
11.	F_3 -38-48	11.84	29	29	0	1.73
12.	F_3-38-49	11.2	17	18	1	1.2
13.	F_3-38-50	13.5	26	26	0	1.06
14.	F_3-38-55	8.87	18	18	0	2.01
15.	F_3-41-11	12.42	30	30	0	2.03
16.	F_3-41-33	12.83	27	27	0	1.12
17.	F_3-34-20	13.97	35	35	0	1.13
18.	F_3-34-52	15.34	30	30	0	1.4
19.	F_3-54-31	10.26	30	30	0	1.32
20.	F_3-54-43	16.14	31	31	0	1.52
21.	F_3-54-57	14.26	56	56	0	2.46
22.	F_3-54-67	15.5	28	28	0	1.62

0 = values relate to plants which failed to give fruits due to sever disease infection.

4.2.7 Polar diameter

Polar diameter ranged from 1 cm to 5 cm in the F_4 progenies (Table 16). Maximum equatorial diameter was recorded in the F_4 progenies of F_3-41-11 (5 cm), F_3-41-33 (5 cm) and F_3-38-49 (5 cm) followed by F_3-38-12 (4.5 cm) and the minimum polar diameter was observed in the F_4 progenies of F_3-38-06 (1 cm) followed by F_3-34-20 (1.2 cm). Highest mean value for polar diameter was recorded in the F_3-38-27 (2.92 cm) followed by F_3-38-12 (2.84 cm). Lowest mean value was observed in F_3-38-29 (2.01 cm) followed by F_3-38-33 (2.11 cm).

4.2.8 Equatorial diameter

Equatorial diameter ranged from 2 cm to 9 cm in the F_4 progenies (Table 17). Maximum equatorial diameter was recorded in the F_4 progenies of F_3-41-11 (9 cm) followed by F_3-38-50 (8 cm) and the minimum equatorial diameter was observed in the F_4 progenies of F_3-38-06 (2 cm), F_3-38-49 (2 cm) and F_3-54-31 (2 cm) followed by F_3-54-57 (2.2cm). Highest mean value for equatorial diameter was recorded in the F_3-41-11 (4.63 cm) followed by F_3-38-50 (4.37 cm). Lowest mean value was observed in F_3-54-57 (3.24 cm) followed by F_3-54-31 (3.28 cm).

4.2.9 Yield per plant

Yield per plant varied from 0 g to 1751 g in F_4 population (Table 18). Maximum yield per plant was recorded in the F_4 progenies of F_3-38-9 (1751 g) followed by F_3-38-06 (1750 g) and the minimum of 0 g yield per plant was observed in the 21 F_4 progenies among 22 F_4 progenies. Highest mean value for yield per plant was recorded in the F_3-38-45 (723.6 g) followed by F_3-38-18 (710.45 g). Lowest mean value was observed in F_3-38-29 (389.53 g) followed by F_3-38-6 (400 g).

Table-16 Mean performance of F_4 progenies for polar diameter (cm)

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_3-38-06	2.57	3	4	1	0.14
2.	F_3-38-09	2.60	2.00	4	2	0.12
3.	F_3-38-12	2.84	3	4.5	1.5	0.16
4.	F_3-38-14	2.83	2.1	4.1	2	0.16
5.	F_3-38-18	2.62	2.3	4	1.7	0.16
6.	F_3-38-27	2.92	0.8	3.5	2.7	0.16
7.	F_3-38-28	2.63	2	4	2	0.11
8.	F_3-38-29	2.01	2	3.5	1.5	0.1
9.	F_3-38-33	2.11	1.4	2.7	1.3	0.13
10.	F_3-38-45	2.38	1.3	3	1.7	0.13
11.	F_3 -38-48	2.33	2	3.5	1.5	0.14
12.	F_3-38-49	2.4	3.8	5	1.2	0.26
13.	F_3-38-50	2.58	2	4	2	0.08
14.	F_3-38-55	2.28	1.3	3	1.7	0.17
15.	F_3-41-11	2.79	3.7	5	1.3	0.25
16.	F_3-41-33	2.4	3.5	5	1.5	0.11
17.	F_3-34-20	2.19	1.9	3.1	1.2	0.07
18.	F_3-34-52	2.14	2.1	3.3	1.2	0.09
19.	F_3-54-31	2.16	1.8	3	1.2	0.07
20.	F_3-54-43	2.18	1.8	3	1.2	0.1
21.	F_3-54-57	2.12	1.8	3	1.2	0.11
22.	F_3-54-67	2.46	2.5	4	1.5	0.13

Table-17 Mean performance of F_4 progenies for equatorial diameter (cm)

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_3-38-06	3.86	4	6	2	0.26
2.	F_3-38-09	4.19	4.5	7	2.5	0.21
3.	F_3-38-12	4.4	4.3	7.3	3	0.28
4.	F_3-38-14	4.12	3.2	6.2	3	0.24
5.	F_3-38-18	3.99	3	6	3	0.2
6.	F_3-38-27	4.32	2.6	5.5	2.9	0.46
7.	F_3-38-28	4.11	3	6	3	0.19
8.	F_3-38-29	3.53	3.5	6	2.5	0.18
9.	F_3-38-33	4.03	4	6.5	2.5	0.38
10.	F_3-38-45	3.98	2	5	3	0.25
11.	F_3 -38-48	3.75	3.3	6	2.7	0.2
12.	F_3-38-49	4.12	5	7	2	0.36
13.	F_3-38-50	4.37	5	8	3	0.25
14.	F_3-38-55	3.52	1	4	3	0.16
15.	F_3-41-11	4.63	6.5	9	2.5	0.42
16.	F_3-41-33	3.73	4.5	7	2.5	0.15
17.	F_3-34-20	3.58	1.7	4.7	3	0.07
18.	F_3-34-52	3.54	2	4.9	2.9	0.09
19.	F_3-54-31	3.28	2.2	4.2	2	0.11
20.	F_3-54-43	3.41	1.8	4.3	2.5	0.1
21.	F_3-54-57	3.24	1.8	4	2.2	0.11
22.	F_3-54-67	3.62	2.1	5	2.9	0.09

Table-18 Mean performance of F_4 progenies for yield per plant (g)

S.No	Parentage	Mean	Range	Maximum	Minimum	Standard error
1.	F_3-38-06	400	1750	1750	0	85.09
2.	F_3-38-09	542.60	1751	1751	0	80.28
3.	F_3-38-12	428.64	1254	1254	0	73.62
4.	F_3-38-14	665	1500	1500	0	86.29
5.	F_3-38-18	710.45	1300	1300	0	73.52
6.	F_3-38-27	876.5	1300	1300	0	199.85
7.	F_3-38-28	542.61	1200	1200	0	65.27
8.	F_3-38-29	389.53	1200	1200	0	68.18
9.	F_3-38-33	615.47	1100	1100	0	101.58
10.	F_3-38-45	723.6	1200	1200	0	97.92
11.	F_3 -38-48	563.8	1400	1400	0	83.05
12.	F_3-38-49	579.6	795	885	90	66.94
13.	F_3-38-50	677.14	1061	1061	0	49.39
14.	F_3-38-55	471.25	1100	1100	0	123.1
15.	F_3-41-11	575.61	1500	1500	0	87.66
16.	F_3-41-33	556.04	956	956	0	49.42
17.	F_3-34-20	586.59	1100	1100	0	43.49
18.	F_3-34-52	650.65	1500	1500	0	64.9
19.	F_3-54-31	417	995	995	0	54.75
20.	F_3-54-43	671	1200	1200	0	55.52
21.	F_3-54-57	558.57	1257	1257	0	79.98
22.	F_3-54-67	609.68	1100	1100	0	63.44

0 = values relate to plants which failed to give yield due to sever disease infection.

4.2.10 Biometric evaluation of F_4 plants with combined resistance to bacterial wilt and tomato leaf curl virus (ToLCV)

The performance of 35 (Plate 7) F_4 plants having combined resistance to bacterial wilt and ToLCV is presented in Table 19. The number of days to flower ranged between 42 to 71 days and days to fruit ranged between 50 and 77 days. With respect to growth habit, sixteen plants were semideterminate, ten were determinate and nine were indeterminate in growth habit. Plant height ranged between 41 cm to 71 cm. Polar diameters was ranged between 1.7 cm to 4 cm and equatorial diameter was ranged between 2 cm to 7cm. The average fruit weight ranged between 30 g to 86 g and number of fruits varied between 3 and 40. The plant F_3-38-6-19 having combined resistance gave the maximum fruit yield (1447 g) followed by the plant F_3-54-57-21 (1257 g), the fruit yield in rest of the 33 plants were quite low (less than 1100 g) and the lowest fruit yield was recorded in F_3-54-31-19 (107 g).

4.3 Molecular analysis

4.3.1 Isolation and Quantification of DNA

CTAB method developed by Rogers and Bendich (1994) was used for the isolation of genomic DNA from tender tomato leaves. The quality of DNA was tested using agarose gel electrophoresis. Better quality DNA indicated by discrete bands and lesser RNA contamination was obtained by the Rogers and Bendich method.

RNA contamination in the sample DNA was removed by treatment with RNase A (Sambrook *et al.*, 1989). The electrophoretic profile obtained after RNase treatment revealed clear bands without RNA contamination (Plate 8).

The quality and quantity of DNA isolated by Rogers and Bendich method was estimated byNanoDrop ND-1000 spectrophotometry. Absorbance at 260nm

Plate 7. Selected F_4 resistant (BW and ToLCV) plants

Table-19 Biometric characters of F_4 plants with combined resistance to bacterial wilt and tomato leaf curl virus (ToLCV)

S.No	Plant number	Plant height (cm)	Growth habit	Days to flower	Days to fruit	Average fruit weight (g)	Number of fruits per plant	Polar diameter (cm)	Equatorial diameter (cm)	Yield/Plant (g)
1.	F_3-54-67-5	67	ID	53	61	75	25	2	3	1100
2.	F_3-54-67-18	57	SD	55	58	70	21	3	4	953
3.	F_3-54-67-23	51	SD	55	64	60	22	4	5	842
4.	F_3-54-67-22	59	SD	51	62	70	14	4	3	601
5.	F_3-54-67-8	53	D	56	64	75	17	3	4	375
6.	F_3-54-67-28	67	ID	55	59	40	4	3	4	160
7.	F_3-54-31-7	60	D	51	56	65	23	2.5	3.5	995
8.	F_3-54-31-47	63	D	51	58	50	18	2	3	658
9.	F_3-54-31-25	53	D	55	63	30	11	2	2	337
10.	F_3-54-31-20	63	SD	55	59	39	5	2	3	185
11.	F_3-54-31-19	69	ID	63	71	30	5	2	2	107

S.No	Plant number	Plant height (cm)	Growth habit	Days to flower	Days to fruit	Average fruit weight (g)	Number of fruits per plant	Polar diameter (cm)	Equatorial diameter (cm)	Yield/Plant (g)
12.	F_3-54-31-33	65	D	51	57	50	3	2	2.5	110
13.	F_3-38-50-26	71	ID	55	59	72	22	3	5	955
14.	F_3-38-50-18	67	SD	50	57	50	16	2	3	772
15.	F_3-38-50-35	70	ID	53	58	40	26	2	3	771
16.	F_3-38-50-39	58	D	55	60	40	11	2	3	580
17.	F_3-38-50-31	71	ID	56	62	70	10	3	4	552
18.	F_3-54-57-21	59	SD	54	59	45	25	3	4	1257
19.	F_3-54-57-5	57	SD	53	59	45	23	2.1	3.3	896
20.	F_3-54-57-1	65	SD	71	77	51	18	2.5	3.1	825
21.	F_3-54-57-2	61	SD	59	64	50	16	2	3	735
22.	F_3-38-49-2	70	SD	60	65	50	11	2	3	545
23.	F_3-38-49-13	59	SD	54	58	70	10	2	4	439

S.No	Plant number	Plant height (cm)	Growth habit	Days to flower	Days to fruit	Average fruit weight (g)	Number of fruits per plant	Polar diameter (cm)	Equatorial diameter (cm)	Yield/Plant (g)
24.	F_3-38-49-16	71	ID	51	58	70	6	2	5	260
25.	F_3-54-43-30	55	D	53	59	50	14	2	3	672
26.	F_3-54-43-29	65	D	51	57	50	10	2	3	521
27.	F_3-41-33-42	68	D	55	63	70	19	2	3	853
28.	F_3-41-33-41	67	SD	53	58	80	13	3.5	7	627
29.	F_3-38-45-5	60	ID	51	59	60	30	3	5	1089
30.	F_3-38-45-13	69	ID	52	62	75	23	1.7	5	1077
31.	F_3-38-6-19	61	SD	45	51	85	40	3	5	1447
32.	F_3-38-6-3	53	SD	42	50	86	23	4	6	929
33.	F_3-38-9-28	41	D	45	50	73	21	2	4	970
34.	F_3-38-12-23	62	SD	51	60	65	19	2	3.5	110
35.	F_3-34-20-30	50	SD	53	58	71	11	3.1	4.7	339
Mean		61.62		53.51	59.85	59.2	16.71	2.49	3.76	675.54

D- Determinate, SD- Semi determinate, ID- Indeterminate

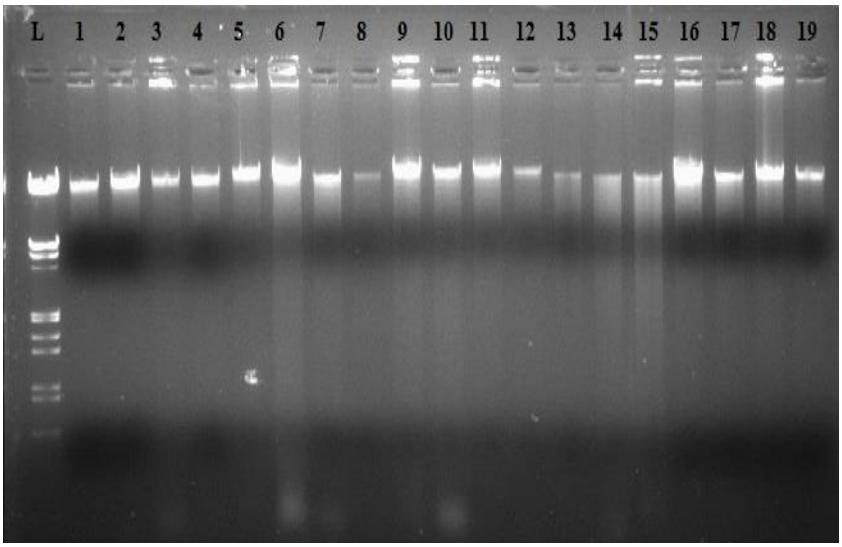


Plate 8. DNA isolated from tender tomato leaves by Rogers and Bendich method

and 280nm were estimated using the NanoDrop ND-1000 spectrophotometry. The ratio of absorbance at 260nm to 280nm ranged from 1.78 to 1.88 indicating relatively pure DNA in the samples.

4.3.2 Primer Screening

Eleven primers (5 SSR and 6 SCAR) were screened using the DNA isolated from Mukthi and IIHR-2195 to select the primers showing good amplification and polymorphism among the parents so that it can be further used for screening F_3 and F_4 plants.

4.3.2.1 SSR primer screening

The number of bands obtained using the SSR primers ranged from 1 to 2. The amplification pattern obtained for SSR primers is shown in Plate 9 and the number of amplicon produced by each SSR primers is given in Table 20. Among the five SSR primers only one (SSR 20) gave polymorphism among the parents Mukthi and IIHR-2195. The primer SSR 20 gave two distinct bands for IIHR-2195 out of which shared one with the variety Mukthi, thus the band of size 180 bp was found polymorphic among the two parents. The other primers gave monomorphism among the parents so they were not selected for validation in F_3 and F_4 plants.

4.3.2.2 SCAR primer screening

The number of bands obtained using the SCAR primers ranged from 1 to 2. The amplification pattern obtained for SCAR primers is shown in Plate 10a and Plate 10b. The number of amplicon produced by each SCAR primers is given in Table 21. Among six SCAR primers two primers (Ualty 3a and Ualty 3b) gave polymorphism among the parents Mukthi and IIHR-2195. The primer Ualty 3a gave two distinct bands for IIHR-2195 out of which shared one with the variety Mukthi, thus the band of size 700 bp found polymorphic among the two parents. The Ualty 3b gave only one band in IIHR-2195 and zero band in Mukthi, thus the

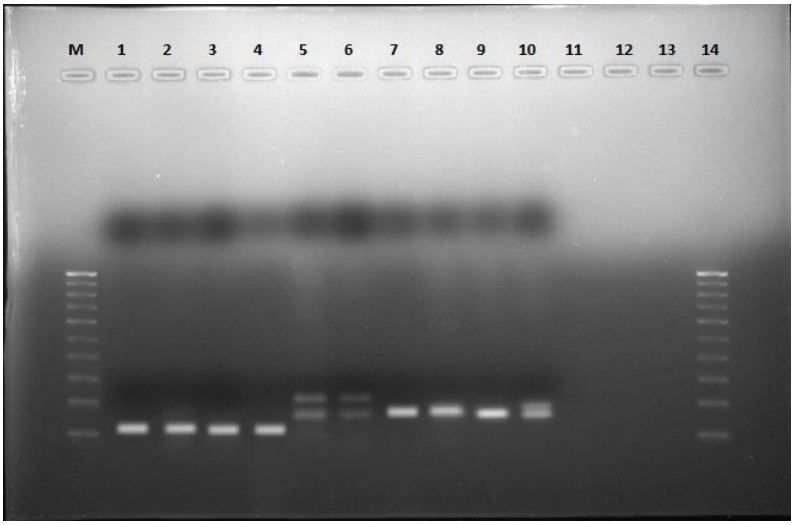


Plate 9. Screening of SSR primers with Mukthi and IIHR-2195

M and 14- Marker (100bp), 1- LEaat 007 (BW and ToLCV) with Mukthi, 2-LEaat 007 (BW and ToLCV) with IIHR-2195, 3- LEaat 002 (BW and ToLCV) with Mukthi, 4- LEaat 002 (BW and ToLCV) with IIHR-2195, 5- SSR 306 with Mukthi, 6- SSR 306 with IIHR-2195, 7 - LEaat 006 (BW and ToLCV) with Mukthi, 8- LEaat 006 (BW and ToLCV) with IIHR-2195, 9- SSR 20 (BW) with Mukthi, 10- SSR 20 (BW) with IIHR-2195.

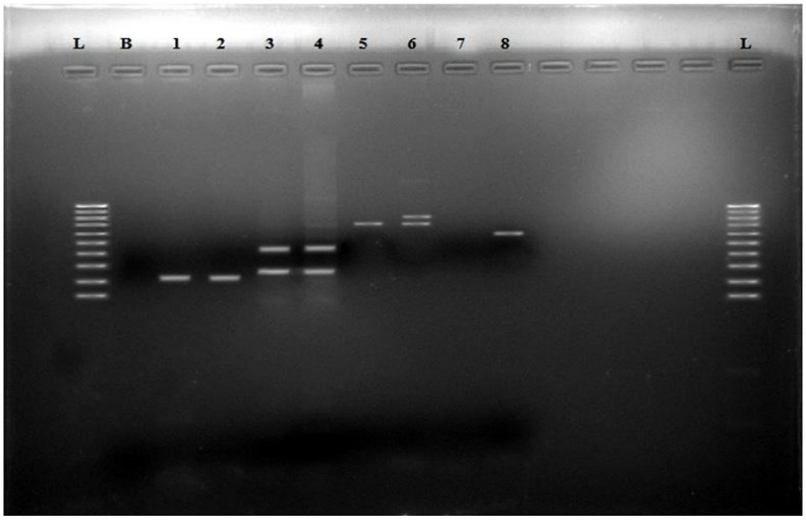


Plate 10a. Screening of SCAR primers with Mukthi and IIHR-2195

L– 100bp Ladder, B- Blank, 1- TSCAR $_{AAT/CGA}$ (Bacterial wilt) with Mukthi, 2- TSCAR $_{AAT/CGA}$ (Bacterial wilt) with IIHR-2195, 3- Ualty 5 (ToLCV) with Mukthi, 4- Ualty 5 (ToLCV) with IIHR-2195, 5- Ualty 3a (ToLCV) with Mukthi, 6- Ualty 3a (ToLCV) with IIHR-2195, 7- Ualty 3b (ToLCV) with Mukthi, 8- Ualty 3b (ToLCV) with IIHR-2195.

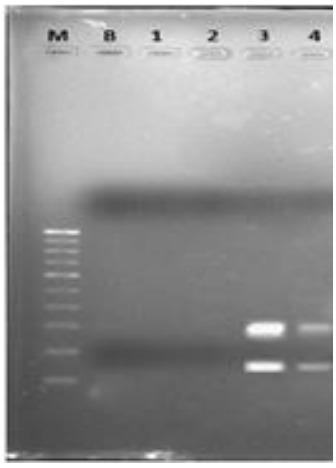


Plate 10b. Screening of SCAR primers with Mukthi and IIHR-2195

M- Marker (100bp ladder), B- Blank, 1- Ualty 6 (ToLCV) with Mukthi, 2- Ualty 6 (ToLCV) with IIHR-2195, 3- Ualty 11 (ToLCV) with Mukthi, 4- Ualty 11 (ToLCV) with IIHR-2195.

Table 20 Number of bands and amplification patterns of SSR primers

Sl.No	SSR Primers	No of bands		Amplification pattern
		Mukthi	IIHR-2195	
1.	LEaat 007	1	1	Monomorphic
2.	LEaat 002	1	1	Monomorphic
3.	SSR 306	2	2	Monomorphic
4.	LEaat 006	1	1	Monomorphic
5.	SSR 20	1	2	Polymorphic

Table 21 Number of bands and amplification patterns of SCAR primers

Sl.No	SCAR Primers	No of bands		Amplification pattern
		Mukthi	IIHR-2195	
1.	TSCAR $_{AAT/CGA}$	1	1	Monomorphic
2.	Ualty 3a	1	2	Polymorphic
3.	Ualty 3b	0	1	Polymorphic
4.	Ualty 5	0	0	Monomorphic
5.	Ualty 6	0	0	Monomorphic
6.	Ualty 11	2	2	Monomorphic

band of size 600 bp found polymorphic among the two parents. The other primers showed monomorphism and absence of bands among the parents so they were not selected for validation in F_3 and F_4 plants

4.3.3 Validation of Markers on F_3 and F_4 population

The selected SSR and SCAR markers were further tested on F_3 and F_4 population for confirming their segregation pattern.

4.3.3.1 SSR 20

The bacterial wilt specific primer SSR 20 showed polymorphism among the parents Mukthi and IIHR-2195. This particular primer was evaluated on F_4 progenies with combined resistance for bacterial wilt and ToLCV along with its F_3 parent and susceptible F_3 lines.

4.3.3.1.1 Validation in segregating lines of 54-31

Out of 38 F_4 progenies of 54-31, 6 showed combined resistance. The F_4 progenies of 54-31 having combined resistance and utilized for primer validation were 54-31-19, 54-31-25 and 54-31-33. The primer SSR20 was specific to bacterial wilt resistance and it gave a unique band of 180 bp in the bacterial wilt resistant variety Mukthi. These F_4 lines, their F_3 parent 54-31; when analyzed indicated monomorphism to wilt resistant parent Mukthi representing 180bp band. The bacterial wilt specific band was however also found present (Plate 11a) and absent in some of the susceptible F_3 progenies evaluated (Plate 11b).

4.3.3.1.2 Validation in segregating lines of 38-50

Thirty four F_4 progenies of the line 38-50 were evaluated for combined resistance and 5 plants were selected include 38-50-18, 38-50-26, 38-50-31, 38-50-35 and 38-50-39. All the five were utilized for the validation of the primer SSR20. These F_4 lines, their F_3 parent 38-50; when analyzed indicated

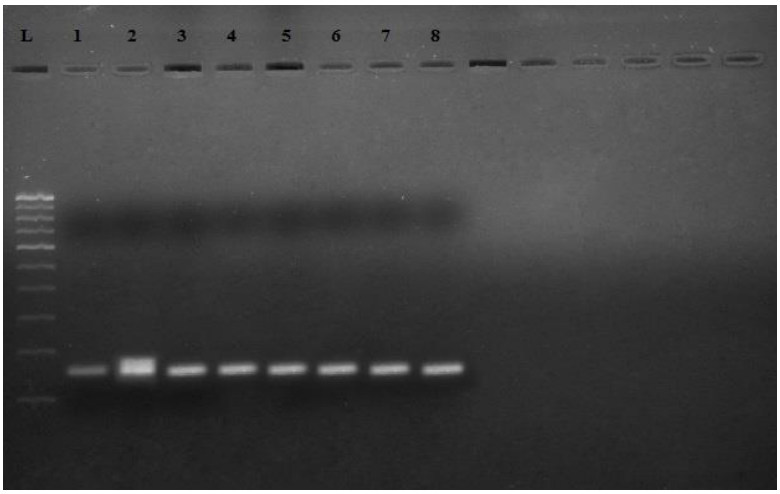


Plate 11a. Validation of SSR 20 in F_3 and F_4 (54-31 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-31), 4- F_4 resistant (F_3-54-31-19), 5- F_4 resistant (F_3-54-31-25), 6- F_4 resistant (F_3-54-31-33), 7- Susceptible (F_2-47-6), 8- Susceptible (F_2-47-14).

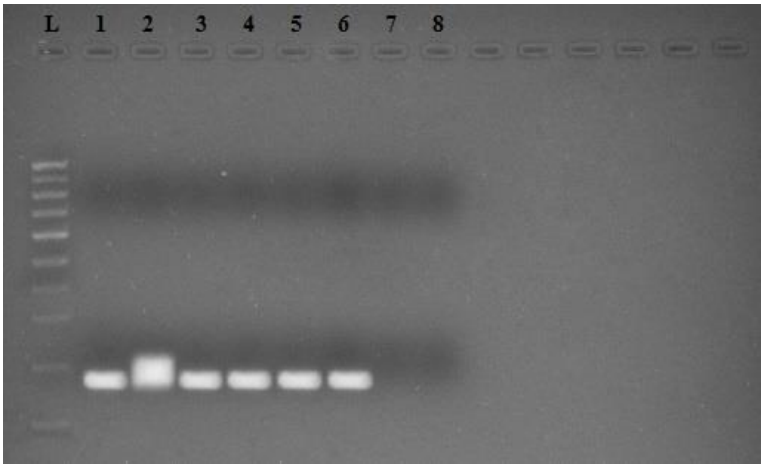


Plate 11b. Validation of SSR 20 in F_3 and F_4 (54-31 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-31), 4- F_4 resistant (F_3-54-31-19), 5- F_4 resistant (F_3-54-31-25), 6- F_4 resistant (F_3-54-31-33), 7- Susceptible (F_2-38-1), 8- Susceptible (F_2-38-3).

monomorphism to wilt resistant parent Mukthi. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. The bacterial wilt specific band was found absent in the susceptible F_3 progenies evaluated (Plate 12a). Bacterial wilt specific band (180 bp) was amplified in all 5 resistant segregants and their F_3 parent. The primer did not amplify any region in the susceptible F_3 lines (Plate 12a) but amplified ToLCV and bacterial wilt specific bands in other susceptible F_3 plants (Plate 12b).

4.3.3.1.3 Validation in segregating lines of 54-67

Among the 33 F_4 progenies evaluated in the line 54-67, 6 plants gave combined resistance. The F_4 progenies of 54-67 line having combined resistance and utilized for primer validation were 54-67-18, 54-67-22, 54-67-23 and 54-67-28. SSR20 primer was specific to bacterial wilt resistance and it gave a unique band of 180 bp in the bacterial wilt resistant variety Mukthi. These F_4 lines, their F_3 parent 54-67; when analyzed indicated monomorphism to wilt resistant parent Mukthi. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. The bacterial wilt specific band was found absent in the susceptible F_3 progenies evaluated (Plate 13a) but ToLCV and bacterial wilt specific bands were present in other susceptible F_3 plants (Plate 13b).

4.3.3.1.4 Validation in segregating lines of 38-49

Fifteen F_4 progenies of the line 38-49 were evaluated for combined resistance and 3 plants were selected include 38-49-2, 38-49-13 and 38-49-16. All the three were utilized for the validation of the primer SSR20. These F_4 lines, their F_3 parent 38-49; when analyzed indicated monomorphism to wilt resistant parent Mukthi. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. The bacterial wilt specific band was found absent in the susceptible F_3 progenies evaluated (Plate 14). Bacterial wilt specific band (180 bp) was amplified in all 3 resistant segregants and their F_3 parent. The primer did not amplify any region in the susceptible F_3 lines.

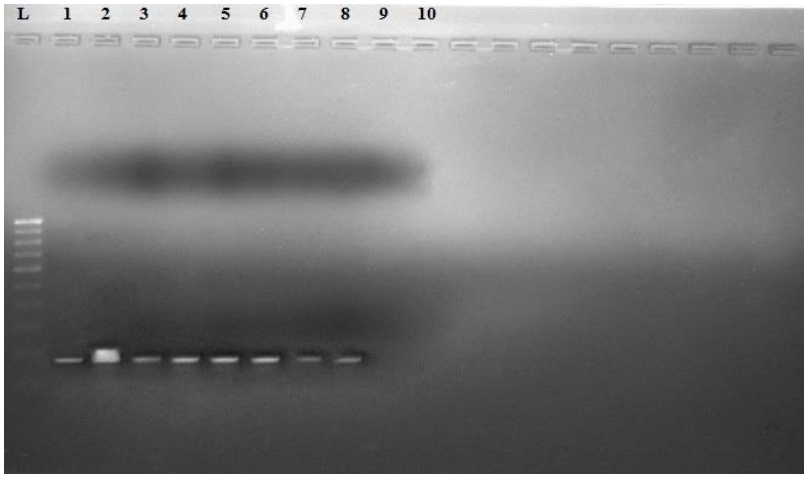


Plate 12a. Validation of SSR 20 in F_3 and F_4 (38-50 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-38-50), 4- F_4 resistant (F_3-38-50-18), 5- F_4 resistant (F_3-38-50-26), 6- F_4 resistant (F_3-38-50-31), 7- F_4 resistant (F_3-38-50-35), 8- F_4 resistant (F_3-38-50-39), 9- Susceptible (F_2-38-1), 10- Susceptible (F_2-38-3).

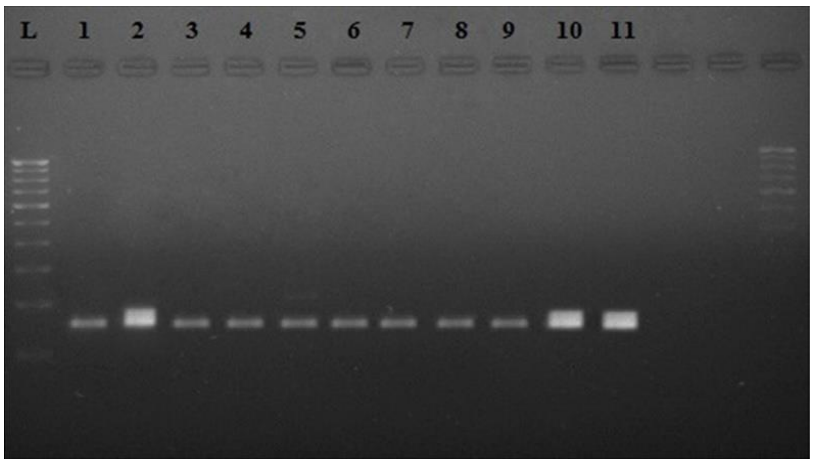


Plate 12b. Validation of SSR 20 in F_3 and F_4 (38-50 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-38-50), 4- F_4 resistant (F_3-38-50-18), 5- F_4 resistant (F_3-38-50-26), 6- F_4 resistant (F_3-38-50-31), 7- F_4 resistant (F_3-38-50-35), 8- F_4 resistant (F_3-38-50-39), 9- Susceptible (F_2-38-66), 10- Susceptible (F_2-41-5), 11- Susceptible (F_2-41-74).

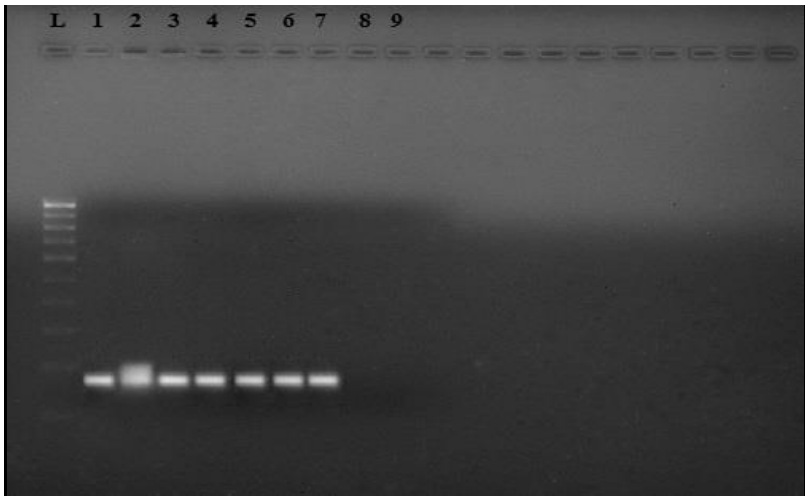


Plate 13a. Validation of SSR 20 in F_3 and F_4 (54-67 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-67), 4- F_4 resistant (F_3-54-67-18), 5- F_4 resistant (F_3-54-67-22), 6- F_4 resistant (F_3-54-67-23),7- F_4 resistant (F_3-54-67-28), 8- Susceptible (F_2-38-1), 9- Susceptible (F_2-38-3).

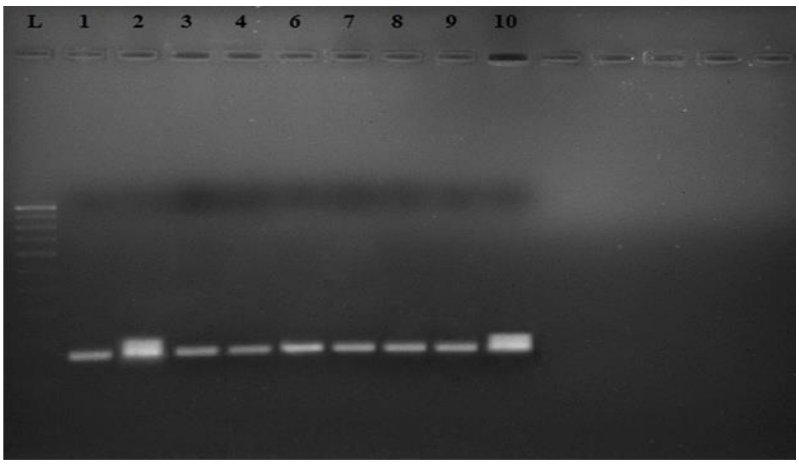


Plate 13b. Validation of SSR 20 in F_3 and F_4 (54-67 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-67), 4- F_4 resistant (F_3-54-67-18), 6- F_4 resistant (F_3-54-67-22), 7- F_4 resistant (F_3-54-67-23), 8- F_4 resistant (F_3-54-67-28), 9- Susceptible (F_2-38-66), 10- Susceptible (F_2-41-5).

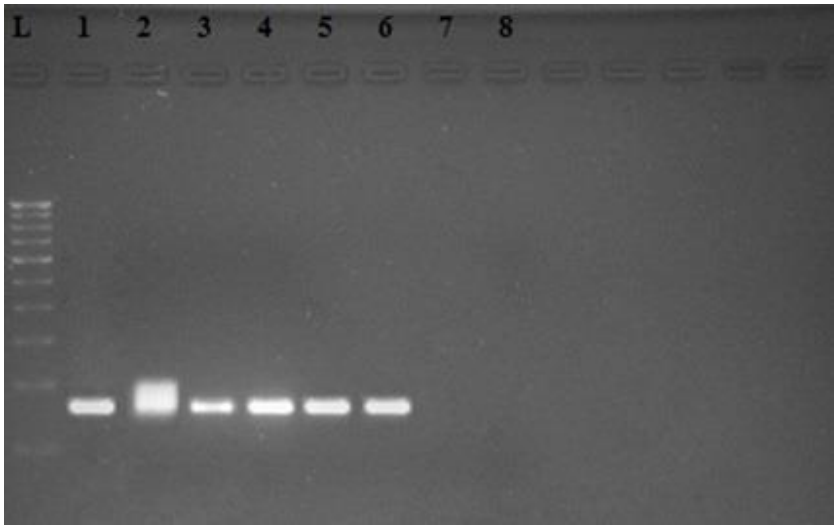


Plate 14. Validation of SSR 20 in F_3 and F_4 (38-49 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-38-49), 4- F_4 resistant (F_3-38-49-2), 5- F_4 resistant (F_3-38-49-13), 6- F_4 resistant (F_3-38-49-16), 7- Susceptible (F_2-41-3), 8- Susceptible (F_2-41-4).

4.3.3.1.5 Validation in segregating lines of 54-57

Out of 26 F_4 progenies of 54-57, 4 showed combined resistance. The F_4 progenies of 54-57 having combined resistance and utilized for primer validation were 54-57-1, 54-57-5 and 54-57-21. The primer SSR20 was specific to bacterial wilt resistance and it gave a unique band of 180 bp in the bacterial wilt resistant variety Mukthi. These F_4 lines, their F_3 parent 54-57; when analyzed indicated monomorphism to wilt resistant parent Mukthi representing 180 bp band. The bacterial wilt specific band was however found absent (Plate 15a) in the susceptible F_3 progenies evaluated but amplified ToLCV and bacterial wilt specific bands in other susceptible F_3 plants (Plate 15b).

4.3.3.2 SCAR Ualty 3a

The ToLCV specific primer SCAR Ualty 3a showed polymorphism among the parents Mukthi and IIHR-2195. This particular primer was evaluated on F_4 progenies with combined resistance for bacterial wilt and ToLCV along with its F_3 parent and susceptible F_3 lines.

4.3.3.2.1 Validation in segregating lines of 54-31

Out of 38 F_4 progenies of 54-31, 6 showed combined resistance. The F_4 progenies of 54-31 having combined resistance and utilized for primer validation were 54-31-19, 54-31-25 and 54-31-33. The primer SCAR Ualty 3a was specific to ToLCV resistance and it gave a unique 2 bands of 800 bp and 700 bp in the ToLCV resistant variety IIHR-2195. These F_4 lines, their F_3 parent 54-31; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195 representing 2 bands of 800 bp and 700 bp. Among 2 ToLCV specific bands, 800 bp band was found absent in the susceptible F_3 progenies evaluated (Plate 16).

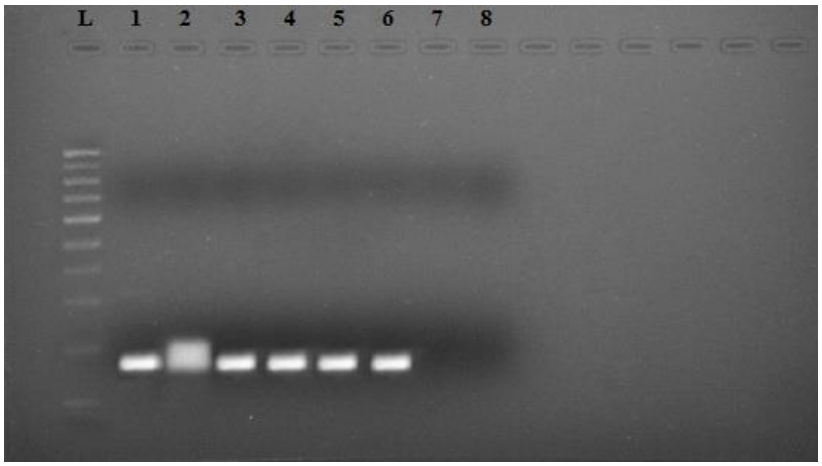


Plate 15a. Validation of SSR 20 in F_3 and F_4 (54-57 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-57), 4- F_4 resistant (F_3-54-57-1), 5- F_4 resistant (F_3-54-57-5), 6- F_4 resistant (F_3-54-57-21), 7- Susceptible (F_2-41-3), 8- Susceptible (F_2-41-4).

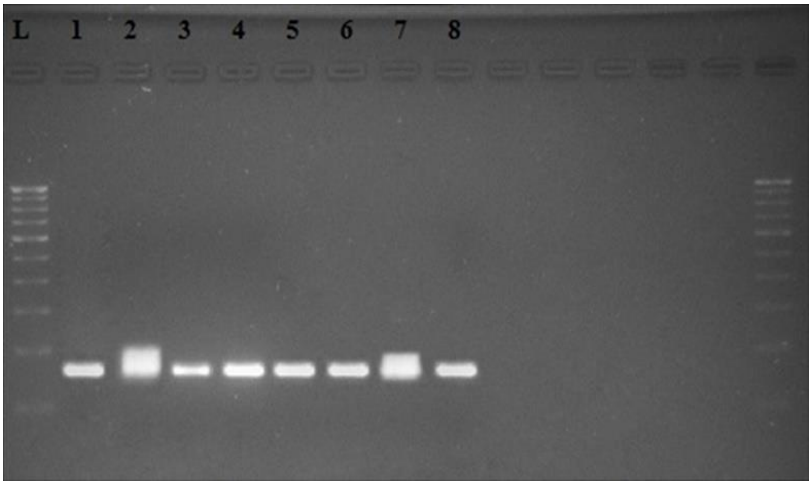


Plate 15b. Validation of SSR 20 in F_3 and F_4 (54-57 line) for bacterial wilt resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-57), 4- F_4 resistant (F_3-54-57-1), 5- F_4 resistant (F_3-54-57-5), 6- F_4 resistant (F_3-54-57-21), 7- Susceptible (F_2-41-5), 8- Susceptible (F_2-41-4).

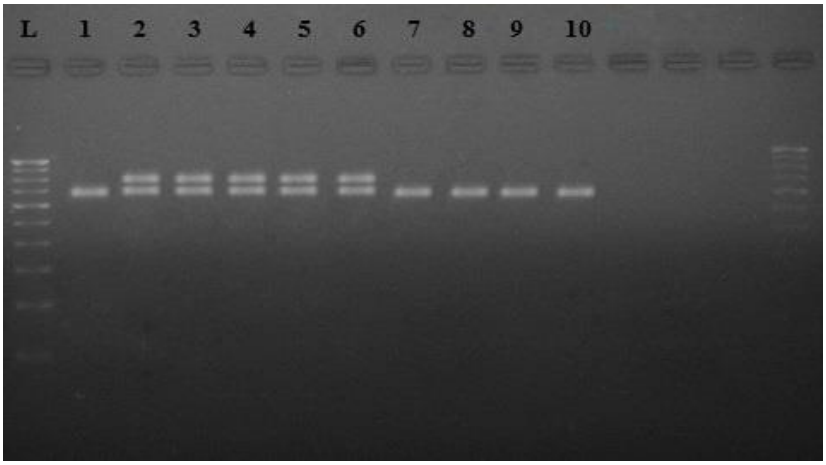


Plate 16. Validation of SCAR Ualty 3a in F_3 and F_4 (54-31 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-31), 4- F_4 resistant (F_3-54-31-19), 5- F_4 resistant (F_3-54-31-25), 6- F_4 resistant (F_3-54-31-33), 7- Susceptible (F_2-41-5), 8- Susceptible (F_2-41-74), 9- Susceptible (F_2-41-77), 10- Susceptible (F_2-34-2).

4.3.3.2.2 Validation in segregating lines of 38-50

Thirty four F_4 progenies of the line 38-50 were evaluated for combined resistance and 5 plants were selected include 38-50-18, 38-50-26, 38-50-31, 38-50-35 and 38-50-39. All the five were utilized for the validation of the primer SCAR Ualty 3a. These F_4 lines, their F_3 parent 38-50; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. ToLCV specific bands 800 bp and 700 bp were amplified in all 5 resistant segregants and their F_3 parent (Plate 17). Among 2 ToLCV specific bands, 800 bp band was found absent in the susceptible F_3 progenies evaluated.

4.3.3.2.3 Validation in segregating lines of 54-67

Among the 33 F_4 progenies evaluated in the line 54-67, 6 plants gave combined resistance. The F_4 progenies of 54-67 line having combined resistance and utilized for primer validation were 54-67-18, 54-67-22, 54-67-23 and 54-67-28. SCAR Ualty 3a primer was specific to ToLCV resistance and it gave a unique 2 bands of 800 bp and 700 bp in the ToLCV resistant variety IIHR-2195. These F_4 lines, their F_3 parent 54-67; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. Among 2 ToLCV specific bands, 800 bp band was found absent in susceptible F_2-41-74 and present in susceptible (F_2-38-58) along with 700 bp band in F_3 susceptible progenies evaluated (Plate 18).

4.3.3.2.4 Validation in segregating lines of 38-49

Fifteen F_4 progenies of the line 38-49 were evaluated for combined resistance and 3 plants were selected include 38-49-2, 38-49-13 and 38-49-16. All the three were utilized for the validation of the primer SCAR Ualty 3a. These F_4 segregants, their F_3 parent 38-49; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. ToLCV specific bands (700 bp and 800 bp) were

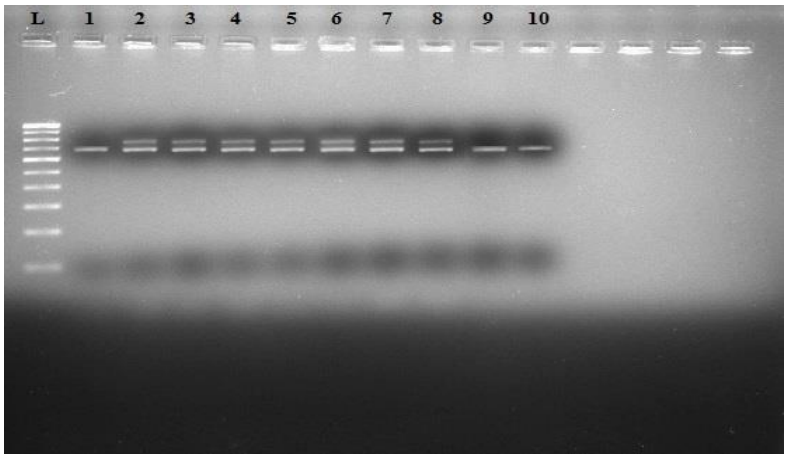


Plate 17. Validation of SCAR Ualty 3a in F_3 and F_4 (38-50 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-38-50), 4- F_4 resistant (F_3-38-50-18), 5- F_4 resistant (F_3-38-50-26), 6- F_4 resistant (F_3-38-50-31), 7- F_4 resistant (F_3-38-50-35), 8- F_4 resistant (F_3-38-50-39), 9- Susceptible (F_2-41-5), 10- Susceptible (F_2-41-74).

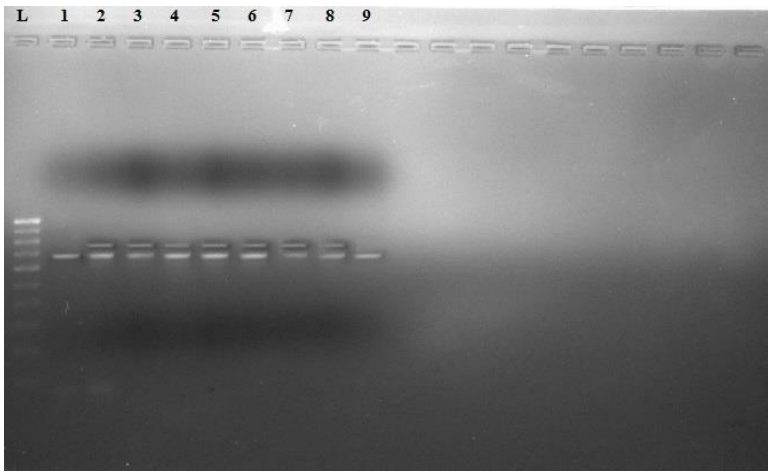


Plate 18. Validation of SCAR Ualty 3a in F_3 and F_4 (54-67 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-67), 4- F_4 resistant (F_3-54-67-18), 5- F_4 resistant (F_3-54-67-22), 6- F_4 resistant (F_3-54-67-23), 7- F_4 resistant (F_3-54-67-28), 8- Susceptible (F_2-38-58), 9- Susceptible (F_2-41-74).

amplified in all 3 resistant segregants and their F_3 parent. Among 2 ToLCV specific bands, 800 bp band was found absent in the susceptible F_3 progenies evaluated (Plate 19).

4.3.3.2.5 Validation in segregating lines of 54-57

Out of 26 F_4 progenies of 54-57, 4 showed combined resistance. The F_4 progenies of 54-57 having combined resistance and utilized for primer validation were 54-57-1, 54-57-5 and 54-57-21. The primer SCAR Ualty 3a was specific to ToLCV resistance and it gave a unique 2 bands of 800 bp and 700 bp in the ToLCV resistant variety IIHR-2195. These F_4 lines, their F_3 parent 54-57; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195 representing 700bp and 800bp band. Among 2 ToLCV specific bands, 800 bp band was found absent the susceptible F_3 progenies evaluated (Plate 20).

4.3.3.3 SCAR Ualty 3b

The ToLCV specific primer SCAR Ualty 3b showed polymorphism among the parents Mukthi and IIHR-2195. This particular primer was evaluated on F_4 progenies with combined resistance for bacterial wilt and ToLCV along with its F_3 parent and susceptible F_3 lines.

4.3.3.3.1 Validation in segregating lines of 54-31

Out of 38 F_4 progenies of 54-31, 6 showed combined resistance. The F_4 progenies of 54-31 having combined resistance and utilized for primer validation were 54-31-19, 54-31-25 and 54-31-33. The primer SCAR Ualty 3b was specific to ToLCV resistance and it gave a unique band of 600 bp in the ToLCV resistant variety IIHR-2195. These F_4 lines, their F_3 parent 54-31; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195 representing 600 bp band. The ToLCV specific band was found absent in the susceptible F_3 progenies evaluated (Plate 21).

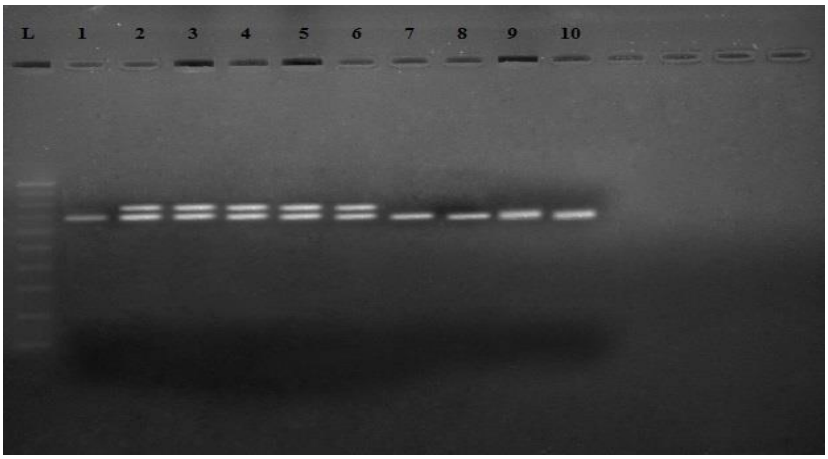


Plate 19. Validation of SCAR Ualty 3a in F_3 and F_4 (38-49 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-38-49), 4- F_4 resistant (F_3-38-49-2), 5- F_4 resistant (F_3-38-49-13), 6- F_4 resistant (F_3-38-49-16), 7- Susceptible (F_2-41-5), 8- Susceptible (F_2-41-74), 9- Susceptible (F_2-41-77), 10- Susceptible (F_2-34-2).

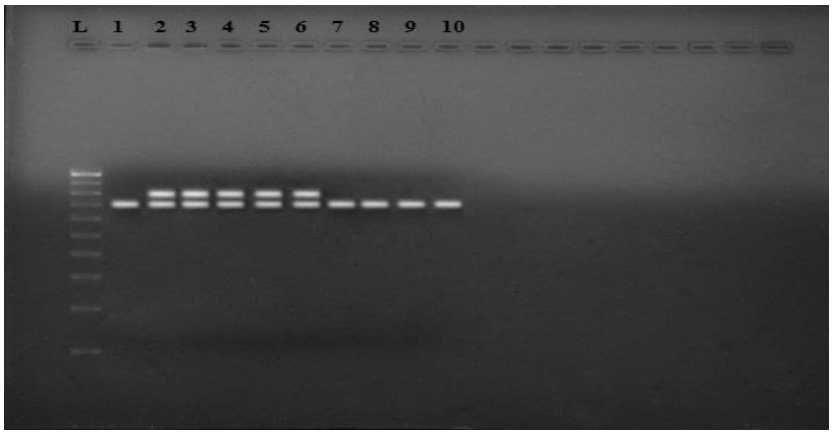


Plate 20. Validation of SCAR Ualty 3a in F_3 and F_4 (54-57 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-57), 4- F_4 resistant (F_3-54-57-1), 5- F_4 resistant (F_3-54-57-5), 6- F_4 resistant (F_3-54-57-21), 7- Susceptible (F_2-41-5), 8- Susceptible (F_2-41-74), 9- Susceptible (F_2-41-77), 10- Susceptible (F_2-34-2).

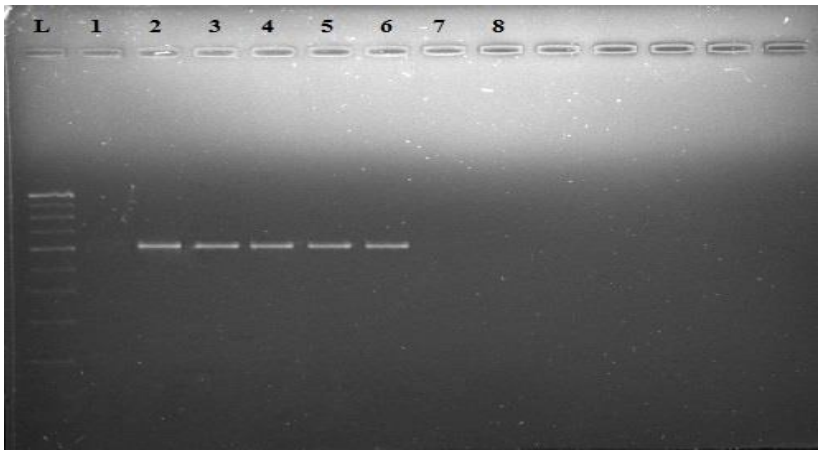


Plate 21. Validation of SCAR Ualty 3b in F_3 and F_4 (54-31 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-31), 4- F_4 resistant (F_3-54-31-19), 5- F_4 resistant (F_3-54-31-25), 6- F_4 resistant (F_3-54-31-33), 7- Susceptible (F_2-34-2), 8- Susceptible (F_2-34-84).

4.3.3.3.2 Validation in segregating lines of 38-50

Thirty four F_4 progenies of the line 38-50 were evaluated for combined resistance and 5 plants were selected include 38-50-18, 38-50-26, 38-50-31, 38-50-35 and 38-50-39. All the five were utilized for the validation of the primer SCAR Ualty 3b. These F_4 lines, their F_3 parent 38-50; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. The ToLCV specific band was found absent in the susceptible F_3 progenies evaluated (Plate 22). ToLCV specific band (600 bp) was amplified in all 5 resistant segregants and their F_3 parent. The primer did not amplify any region in the susceptible F_3 lines.

4.3.3.3.3 Validation in segregating lines of 54-67

Among the 33 F_4 progenies evaluated in the line 54-67, 6 plants gave combined resistance. The F_4 progenies of 54-67 line having combined resistance and utilized for primer validation were 54-67-18, 54-67-22, 54-67-23 and 54-67-28. SCAR Ualty 3b primer was specific to ToLCV resistance and it gave a unique band of 600 bp in the ToLCV resistant variety IIHR-2195. These F_4 lines, their F_3 parent 54-67; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. The ToLCV specific band was found absent in all the susceptible F_3 progenies evaluated but except in one F_3 susceptible progeny F_3-34-22 (Plate 23).

4.3.3.3.4 Validation in segregating lines of 38-49

Fifteen F_4 progenies of the line 38-49 were evaluated for combined resistance and 3 plants were selected include 38-49-2, 38-49-13 and 38-49-16. All the three were utilized for the validation of the primer SCAR Ualty 3b. These F_4 lines, their F_3 parent 38-49; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195. Both the F_3 parent and F_4 progenies gave monomorphic banding pattern. The ToLCV specific band was found absent in the susceptible F_3 progenies evaluated (Plate 24). ToLCV specific band (600 bp) was

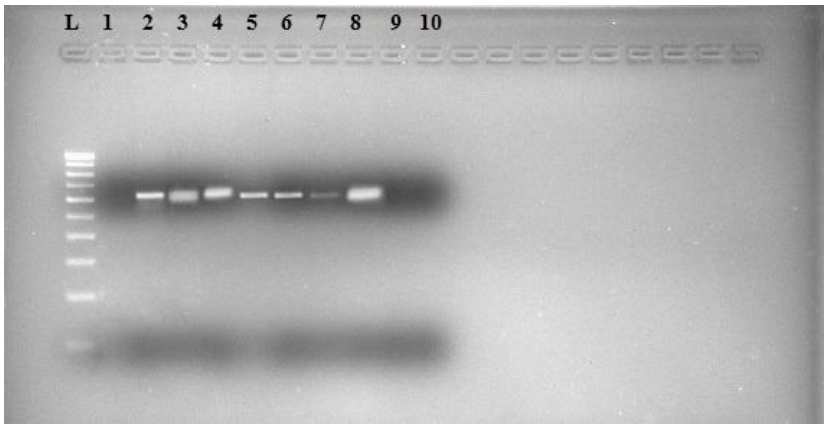


Plate 22. Validation of SCAR Ualty 3b in F_3 and F_4 (38-50 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-38-50), 4- F_4 resistant (F_3-38-50-18), 5- F_4 resistant (F_3-38-50-26), 6- F_4 resistant (F_3-38-50-31), 7- F_4 resistant (F_3-38-50-35), 8- F_4 resistant (F_3-38-50-39), 9- Susceptible (F_2-34-2), 10- Susceptible (F_2-34-84).

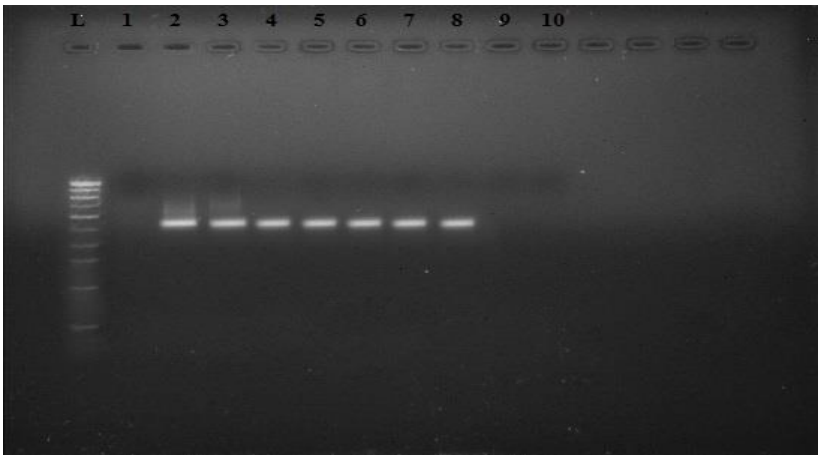


Plate 23. Validation of SCAR Ualty 3b in F_3 and F_4 (54-67 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-67), 4- F_4 resistant (F_3-54-67-18), 5- F_4 resistant (F_3-54-67-22), 6- F_4 resistant (F_3-54-67-23), 7- F_4 resistant (F_3-54-67-28), 8-Susceptible (F_2-34-22), 9- Susceptible (F_2-34-84), 10- Susceptible (F_2-34-56).

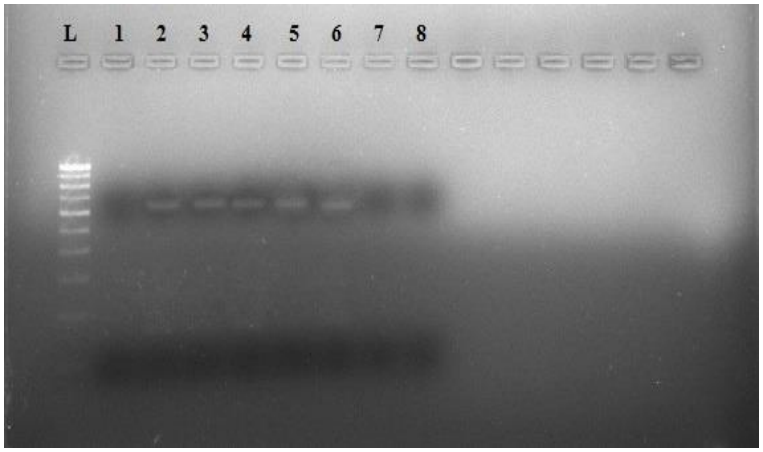


Plate 24. Validation of SCAR Ualty 3b in F_3 and F_4 (38-49 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-38-49), 4- F_4 resistant (F_3-38-49-2), 5- F_4 resistant (F_3-38-49-13), 6- F_4 resistant (F_3-38-49-16), 7- Susceptible (F_2-34-2), 8-Susceptible (F_2-34-84).

amplified in all 3 resistant segregants and their F_3 parent. The primer did not amplify any region in the susceptible F_3 lines.

4.3.3.3.5 Validation in segregating lines of 54-57

Out of 26 F_4 progenies of 54-57, 4 showed combined resistance. The F_4 progenies of 54-57 having combined resistance and utilized for primer validation were 54-57-1, 54-57-5 and 54-57-21. The primer SCAR Ualty 3b was specific to ToLCV resistance and it gave a unique band of 600 bp in the ToLCV resistant variety IIHR-2195. These F_4 lines, their F_3 parent 54-57; when analyzed indicated monomorphism to ToLCV resistant parent IIHR-2195 representing 600 bp band. The ToLCV specific band was however found absent in the susceptible F_3 progenies evaluated (Plate 25).

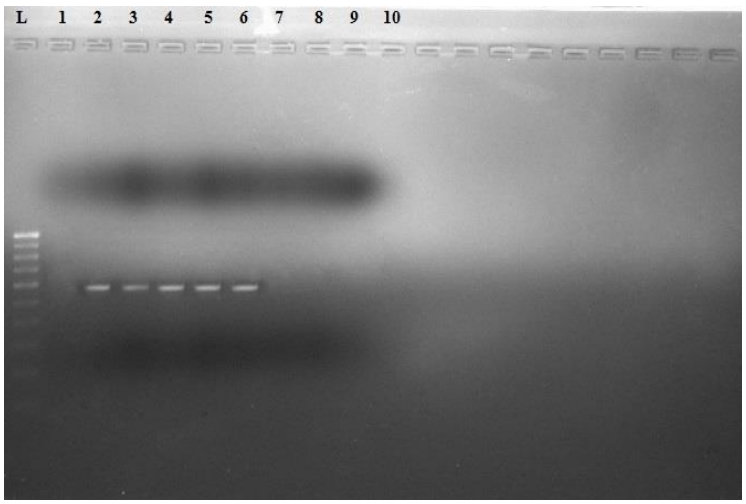


Plate 25. Validation of SCAR Ualty 3b in F_3 and F_4 (54-57 line) for ToLCV resistance in tomato

L- Ladder (100bp), 1- Mukthi, 2- IIHR-2195, 3- F_3 Parent (F_2-54-57), 4- F_4 resistant (F_3-54-57-1), 5- F_4 resistant (F_3-54-57-5), 6- F_4 resistant (F_3-54-57-21), 7- Susceptible (F_2-34-22), 8- Susceptible (F_2-34-84), 9- Susceptible (F_2-34-56).

Discussion

5. DISCUSSION

Tomato is the second most consumed vegetable, next to potato and it occupies largest number of cultivated varieties than any other vegetable crop. India is the sixth largest producer of tomato in the world with an area of 0.50 million hectares under cultivation and with a productivity of 17.4 MT per hectare.

Despite the efforts taken up all over the world so far, Tomato leaf curl virus (ToLCV) disease and bacterial wilt (BW) still continues to be the major limiting factors in tomato cultivation. The leaf curl virus infects the crop in all locations while bacterial wilt is more severe in warm humid tropics. Acidic soils, humid climate and high temperature favour bacterial wilt incidence in Kerala and it affects the crop at all stages of growth resulting in total crop loss. Leaf curl virus incidence is also gaining importance in the state recently and hence it is the need of the hour to develop varieties with combined resistance.

Conventional breeding has helped to develop location specific varieties and molecular breeding have identified several Resistant Gene Analogues and QTLs mapped on different chromosomes. Considering the importance of bacterial wilt in Kerala, KAU has developed varieties with relatively good resistance, but are susceptible to ToLCV and fruit qualities are not superior. Genotypes resistant to different strains of ToLCV have been developed at Indian Institute of Horticultural Research and this project is an attempt to incorporate combined resistance to BW and ToLCV through molecular breeding. Several molecular markers have been reported in tomato for those two diseases but each one of them have to be validated against the genotype used in molecular breeding. The markers that will be validated will be of great use in marker assisted selection. An ideal genotype with ToLCV resistance in bacterial wilt resistance background and having desirable horticultural traits is targeted in the programme.

In this context, the work entitled “Screening of mapping population through marker assisted selection for imparting disease resistance in tomato

(*Solanum lycopersicum* L.)" was under taken with the objective of validation of markers (SCAR and SSR) in parental population (Mukthi & IIHR-2195) and screening of mapping population (F_3 & F_4) for imparting combined resistance to bacterial wilt and ToLCV in tomato.

5.1 Comparison of F_3 and F_4 segregants based on biometric characters

The segregating population of 337 F_3 plants of 5 F_2 lines were evaluated in the wilt sick field for biometric characters and disease reaction. The progenies did not differ much for days to flower and days to fruit (Fig 1). With respect to disease reaction, 3 out of 5 lines were rated as resistant for bacterial wilt and the other 2 rated as moderately susceptible (Table 4). Since the female parent Mukthi is resistant to bacterial wilt and because selection was made for disease reaction in F_2, most of its progenies could be expected to have bacterial wilt resistance. With respect to ToLCV, progenies when evaluated as a whole the segregating population of F_2-54 was observed to be moderately resistant but others were susceptible to ToLCV (Table 5). The 22 F_3 plants were found to have combined resistance to bacterial wilt and ToLCV (Table 3). The biometric characters for the selected 22 F_3 plants indicated variation in important characters like days to flower, days to fruit, average fruit weight, number of fruits per plant and yield per plant (Fig 2 to 5). The yield varied between 180 to 1060 g/plant. Such variation in quantitative traits could be expected in the segregating population. Pradeep kumar et *al.* (2001) have reported highly significant differences among tomato cultivars in an evaluation of cultivars for yield, fruit quality and resistant to bacterial wilt screened under field conditions and pot culture conditions.

22 F_3 segregants were further forwarded to F_4 and the 584 progenies were evaluated under field condition for their performance and disease reaction (Table 8). Only 12 out of 22 F_3 lines gave F_4 with combined resistance. Only 3 lines 38-50, 54-31, 54-67 gave more than 5 resistant progenies while it was 1 to 4 in rest of the population. This may be due to polygenic control on disease reaction, recessive nature of the genes and insufficient population size for each of the

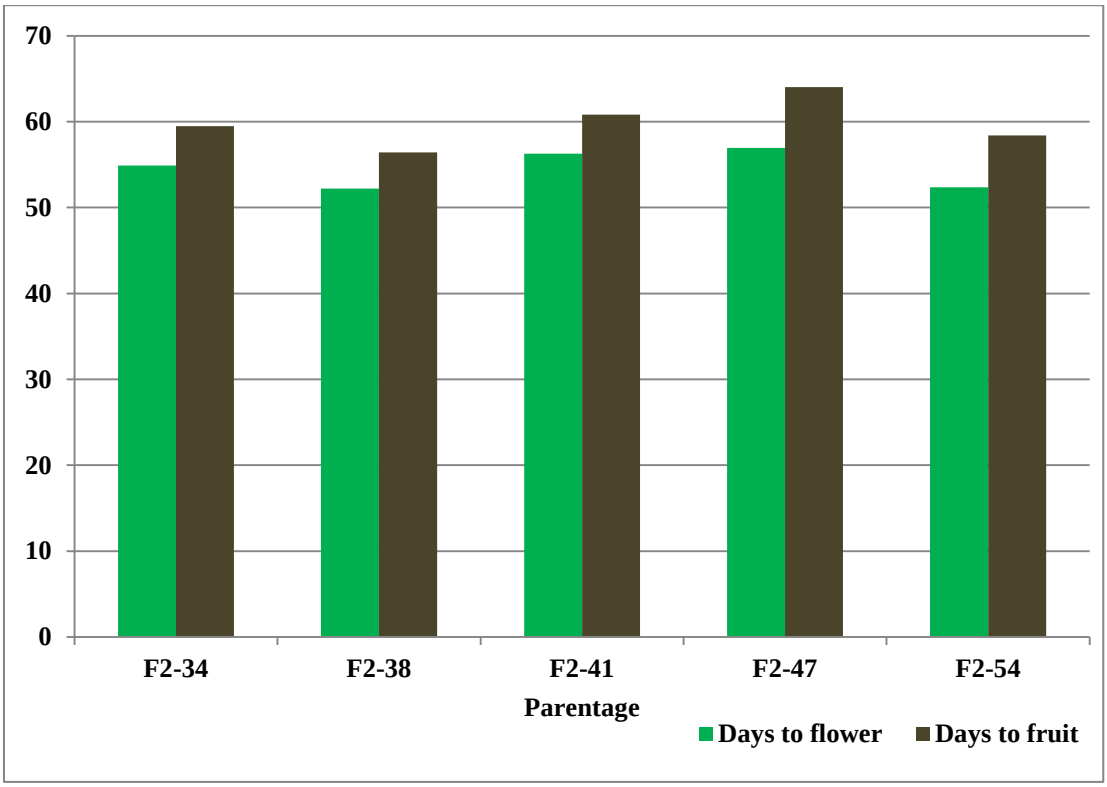


Fig 1. Mean performance of F_3 progenies for days to flower and days to fruit

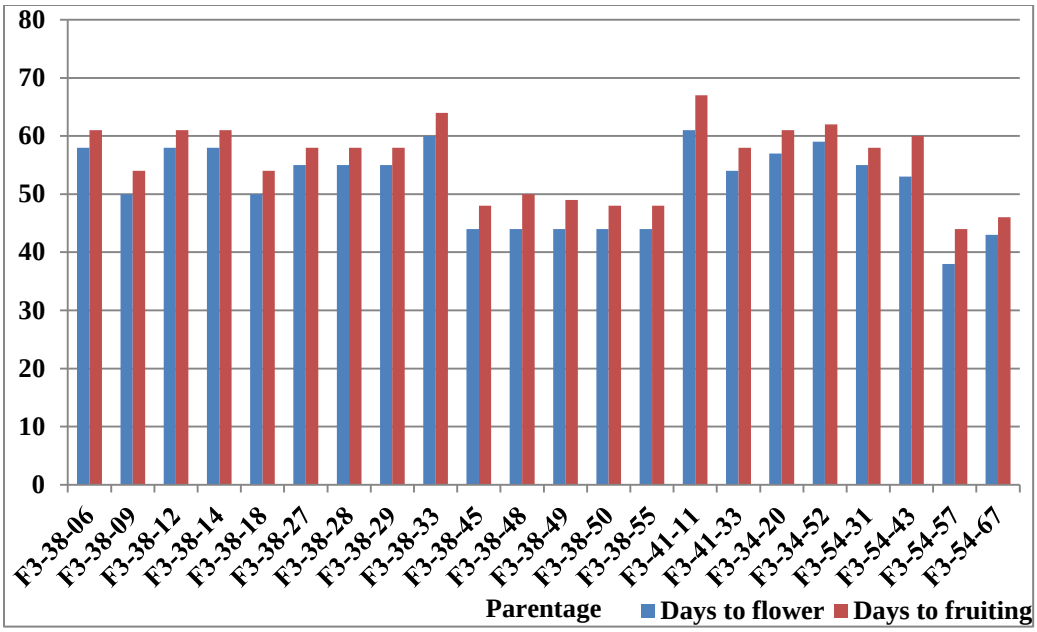


Fig 2. Variation in days to flower and days to fruit among the selected F_3 plants

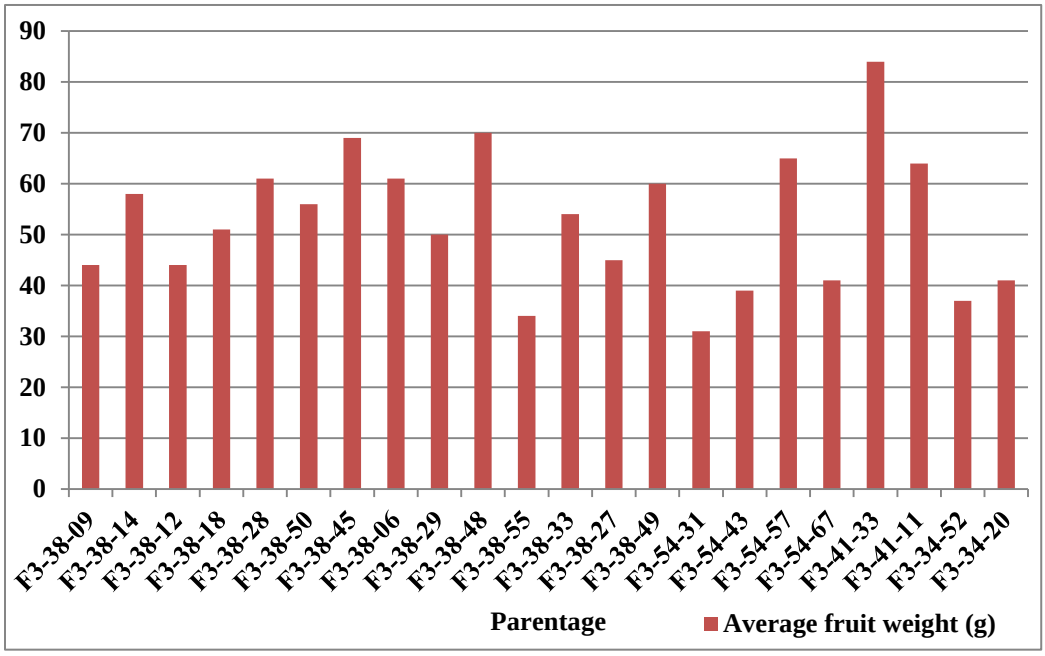


Fig 3. Variation in average fruit weight among the selected F_3 plants

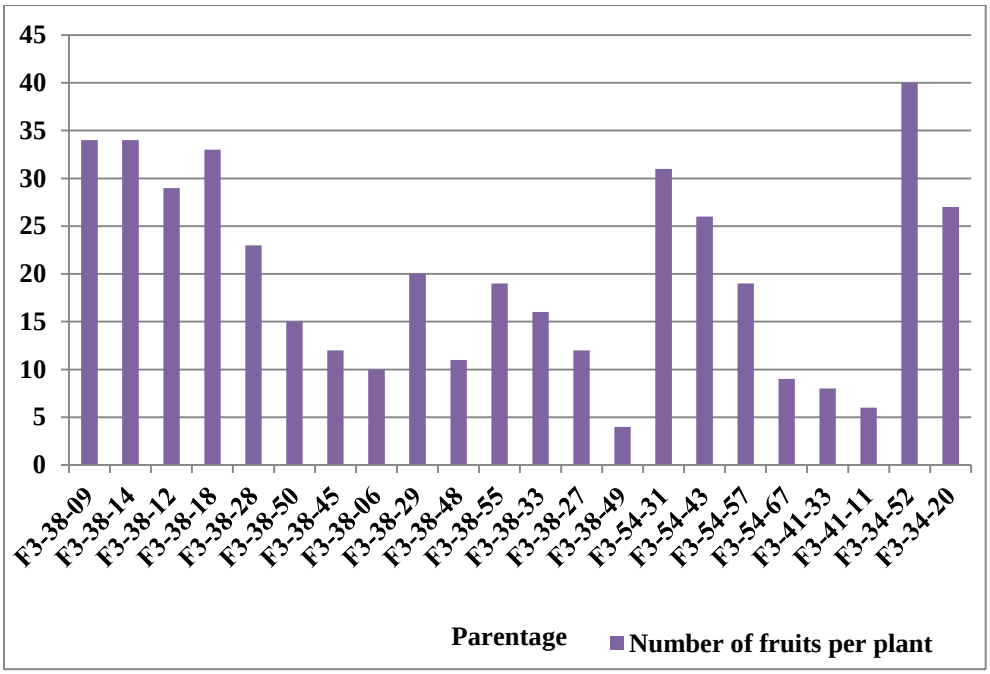


Fig 4. Variation in number of fruits/plant among the selected F_3 plants

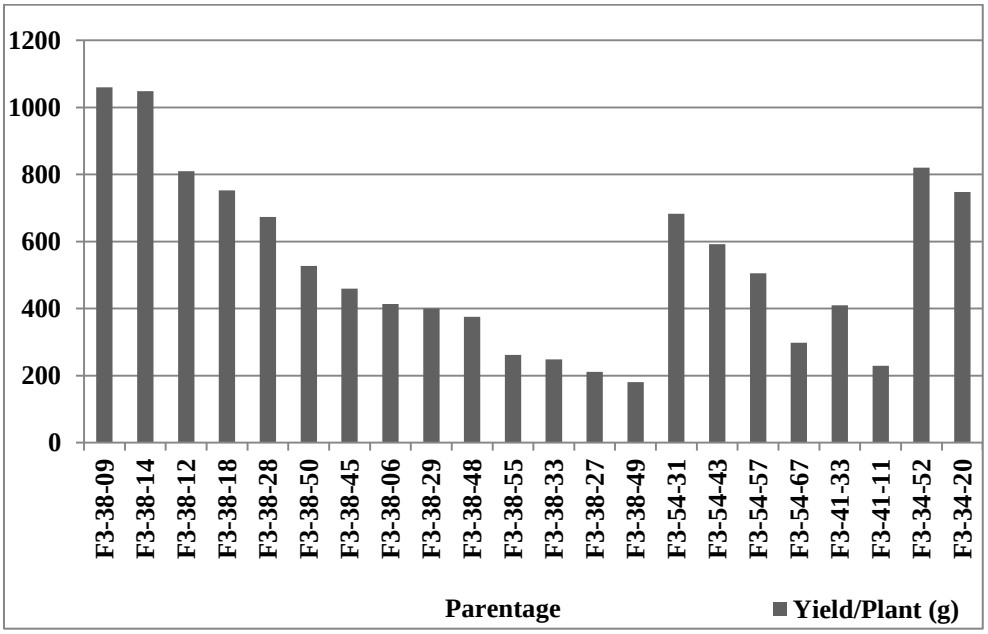
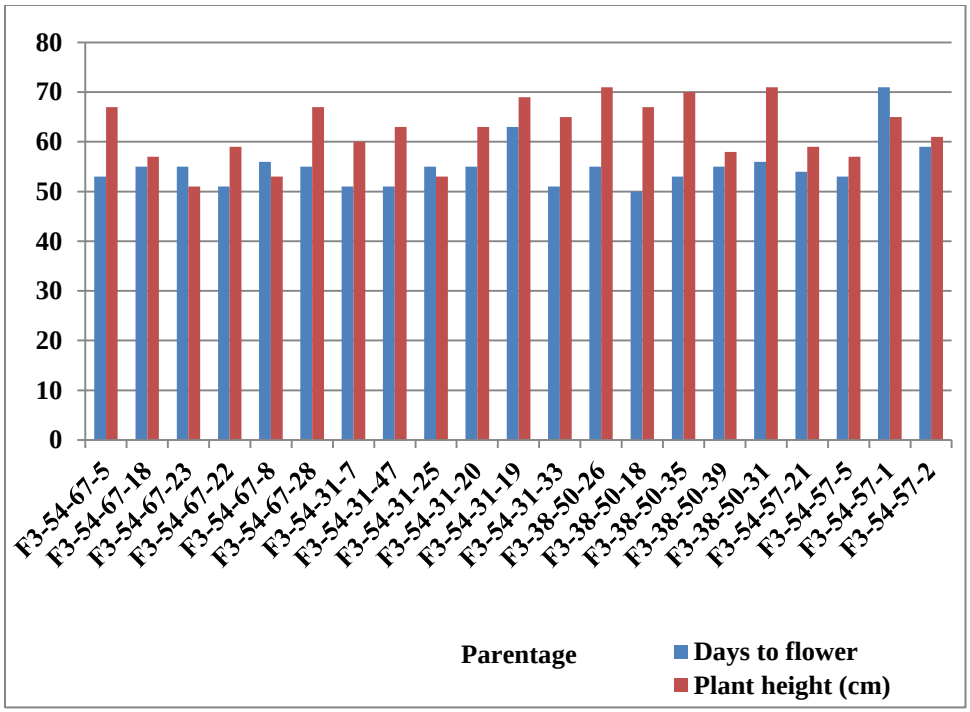


Fig 5. Variation in yield/plant among the selected F_3 plants

selected F_3 line. Tikoo *et al.* (1983) reported the presence of two independent genes for wilt resistance. The resistance was reported to be governed by multiple recessive genes in CRA 66 Sel A from Hawaii and another by single dominant gene in 663-12-3 from Taiwan. Sreelathakumari (1983) reported a complimentary and hypostatic type of digenic recessive gene system for wilt resistance. Rajan and Peter (1986) reported a monogenic incompletely dominant gene action in the resistant line LE-79. BWR-1 a pure line selection with a dominant gene for bacterial wilt resistance was developed from AVRDC accession L33 (VC 8-1-2-1) (Tikko *et al.,* 1986). Anand *et al.* (1992) reported dominant gene action in the F_1S of BWR-1, BWR-5, 1661, 15 SB and 1836 and incomplete dominance in the F_1S of 1881 and Sonali for resistance to bacterial wilt. Resistance in *L. Pimpinellifolium* (A1921) was found to be monogenic and incompletely dominant (Banerjee and Kalloo 1987a) and resistance in *L. hirsutum f. glabratum* (B 6013) was governed by two epistatic genes (Banerjee and Kalloo 1987b).

In the F_4 population 35 progenies were screened for having combined resistance to bacterial wilt and ToLCV. The biometric characters for the selected 35 F_4 lines indicated variation in important characters like plant height, days to flower, days to fruit, average fruit weight, number of fruits per plant, polar diameter, equatorial diameter and yield per plant (Fig 6 to 10).

The mean performance of F_4 segregating progenies of the 22 selected F_3 lines indicated variation with respect to plant height, days to flower, days to fruit, average fruit weight, number of fruits per plant, polar diameter, equatorial diameter and yield per plant (Fig 11 to 15). However the variation with respect to number of fruits/plant and yield per plant were observed to be more than the other characters studied. Among the F_3 lines, which gave good resistance; the progenies F_3-54-43 gave better number of fruits and yield/plant. However the line 54-67 gave more number plants with combined resistance (6 plants) and having good yield but next to 54-43. While developing varieties, yield attributes also will be given priority along with combined resistance. Thus the F_4 lines selected for yield attributes and combined resistance could be further forwarded for developing



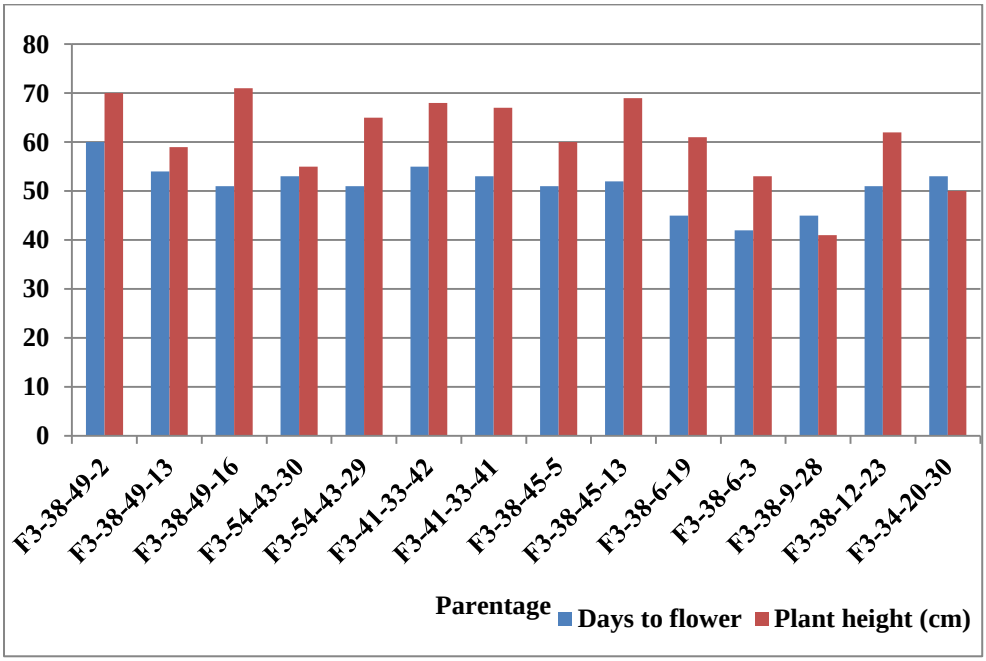
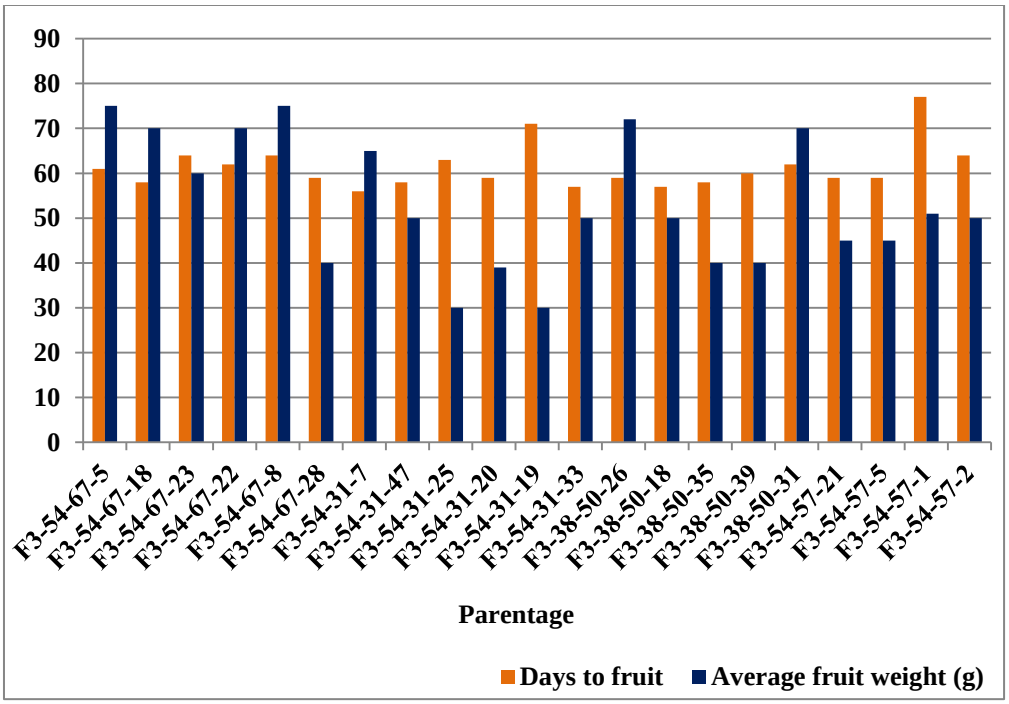


Fig 6. Variation in days to flower and plant height among the selected F_4 plants



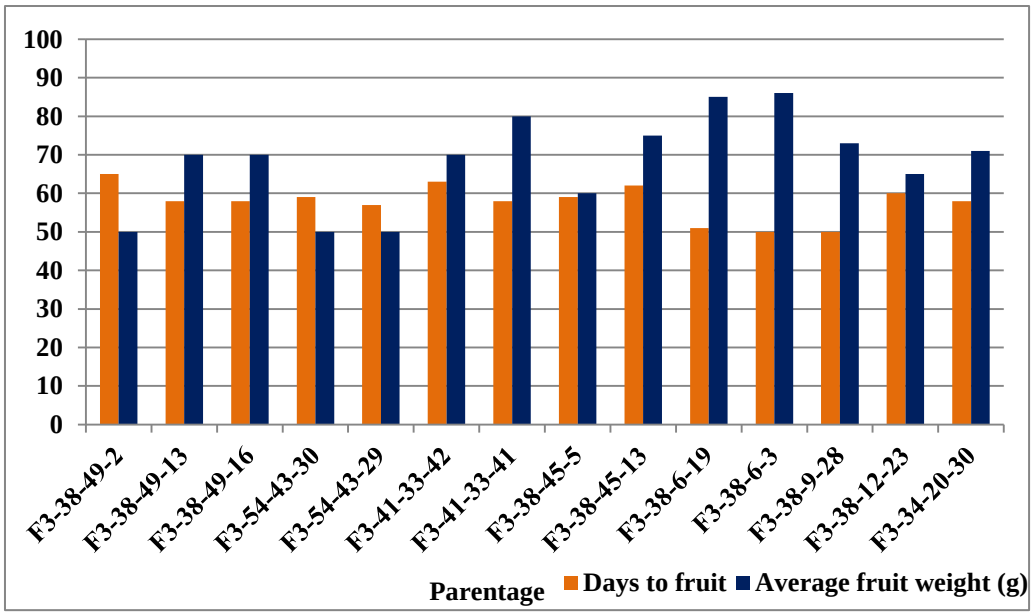
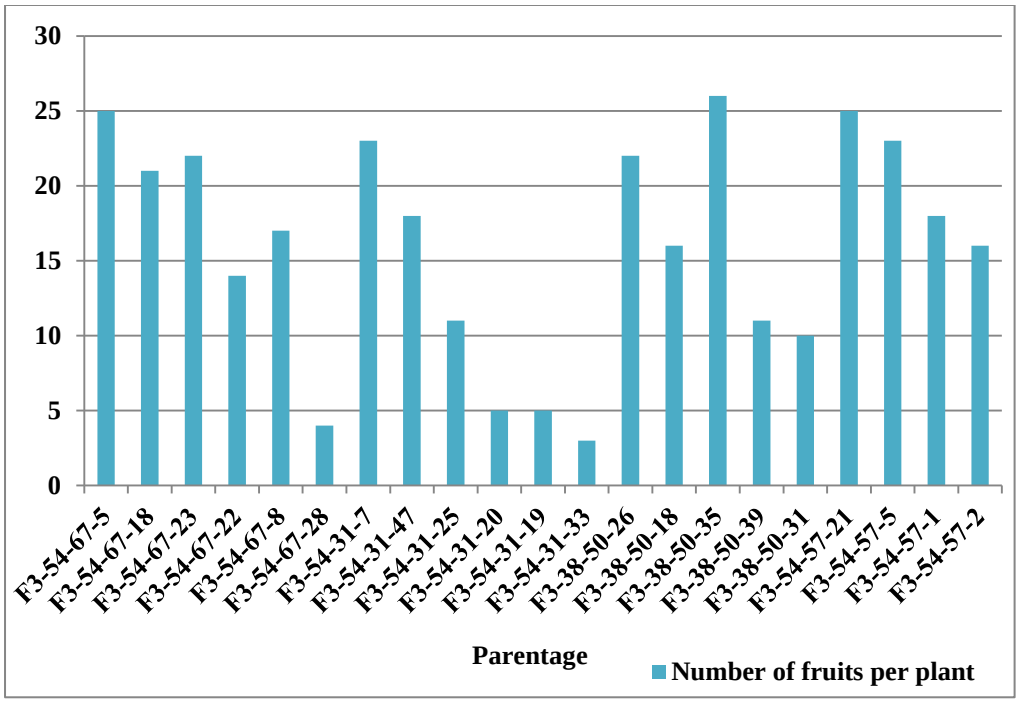


Fig 7. Variation in days to fruit and average fruit weight among the selected F_4 plants



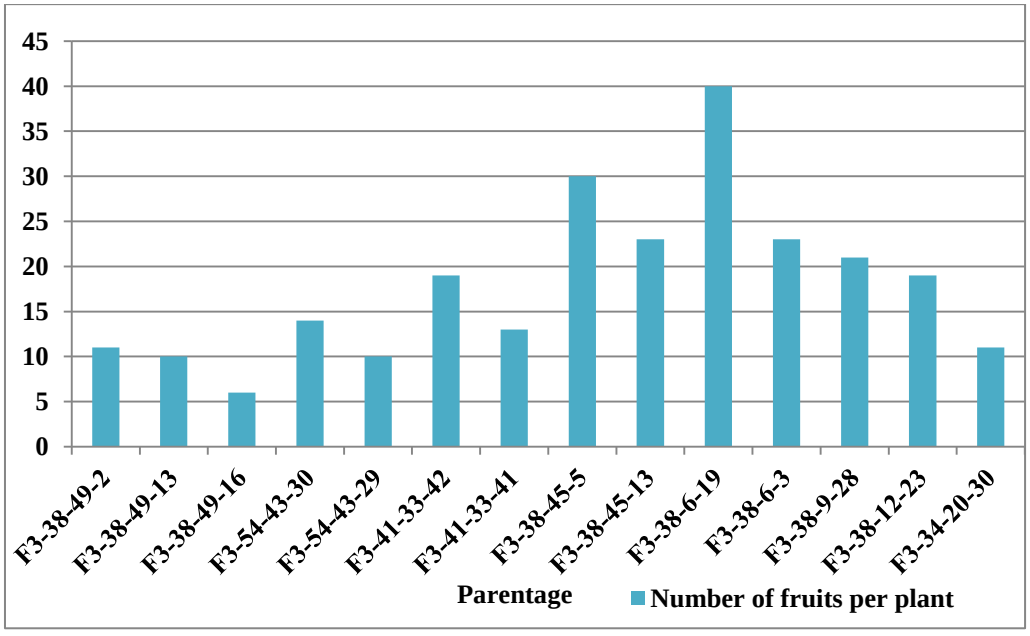
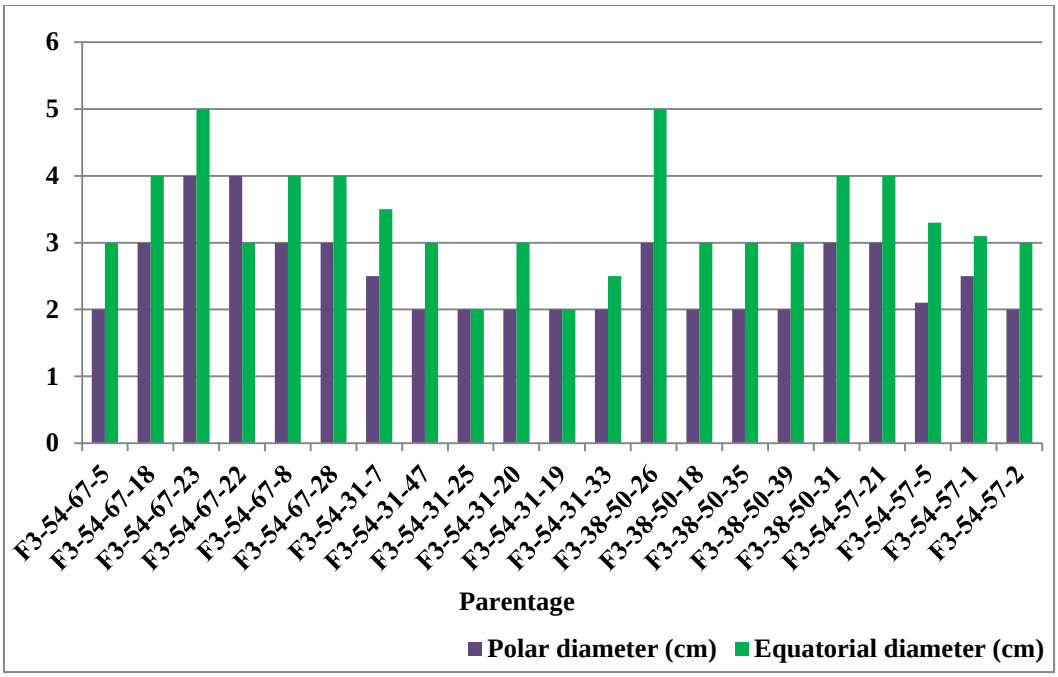


Fig 8. Variation in number of fruits/plant among the selected F_4 plants



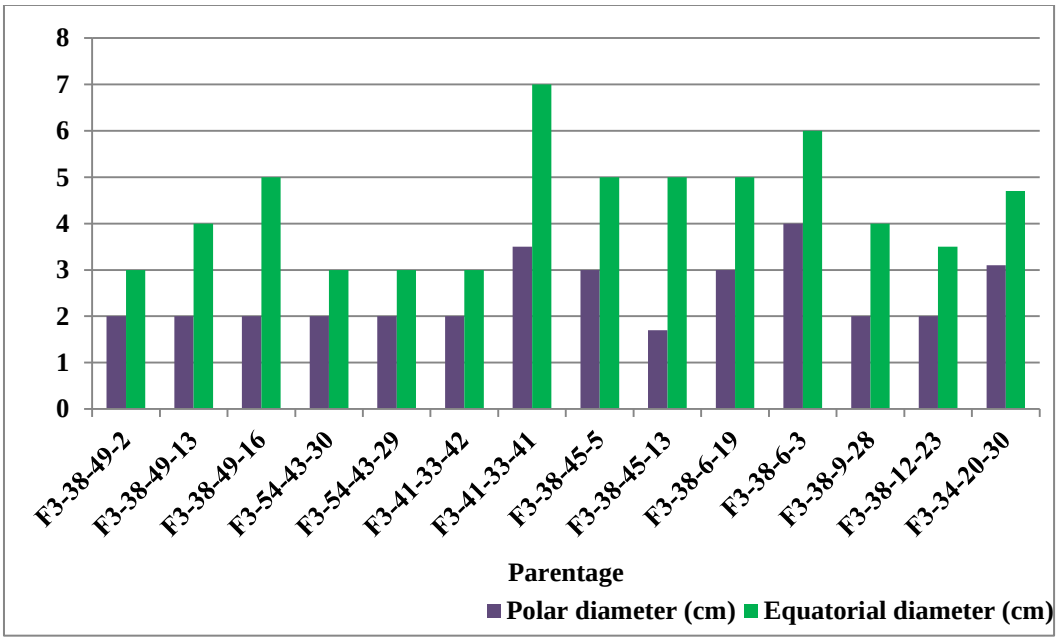
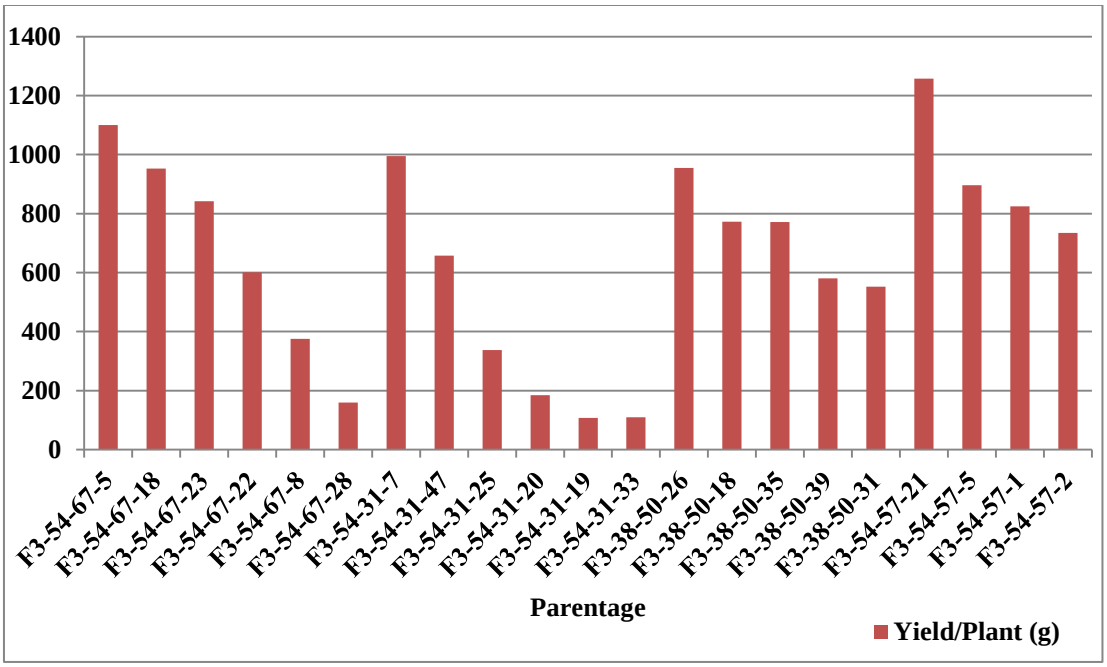


Fig 9. Variation in polar diameter and equatorial among the selected F_4 plants



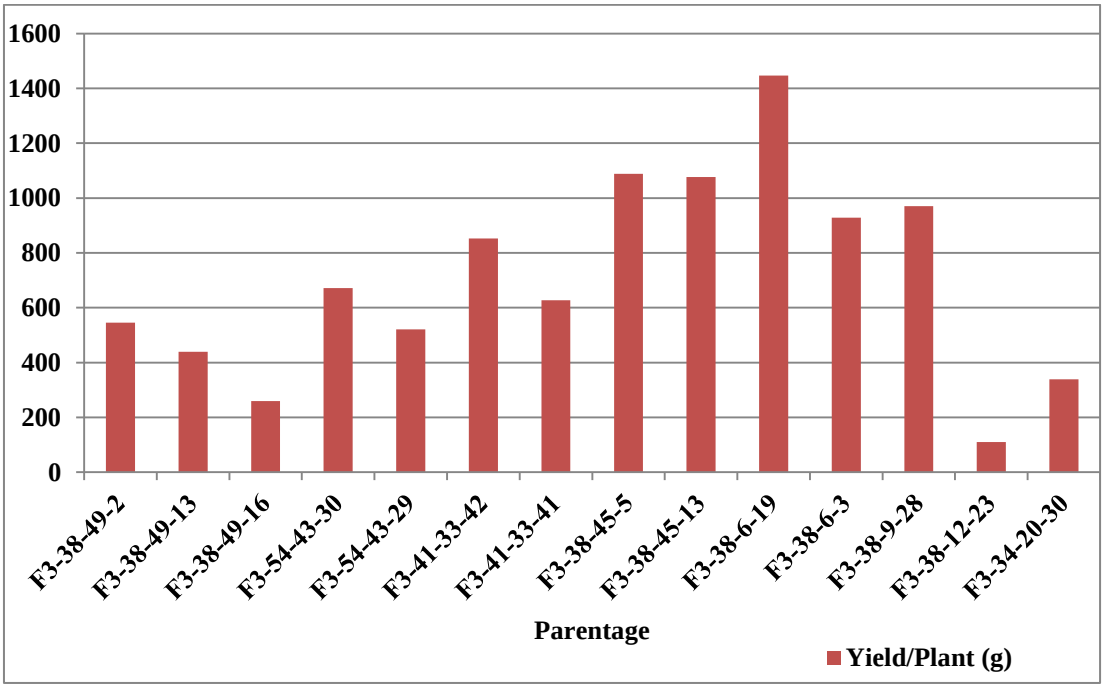


Fig 10. Variation in yield/plant among the selected F_4 plants

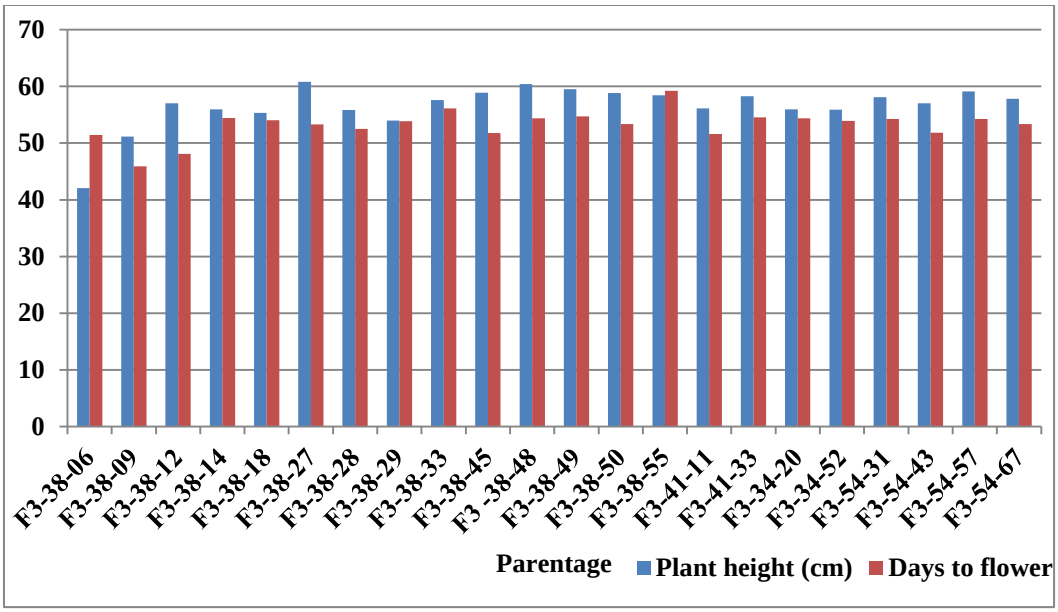


Fig 11. Mean performance of F_4 progenies for plant height (cm) and days to flower

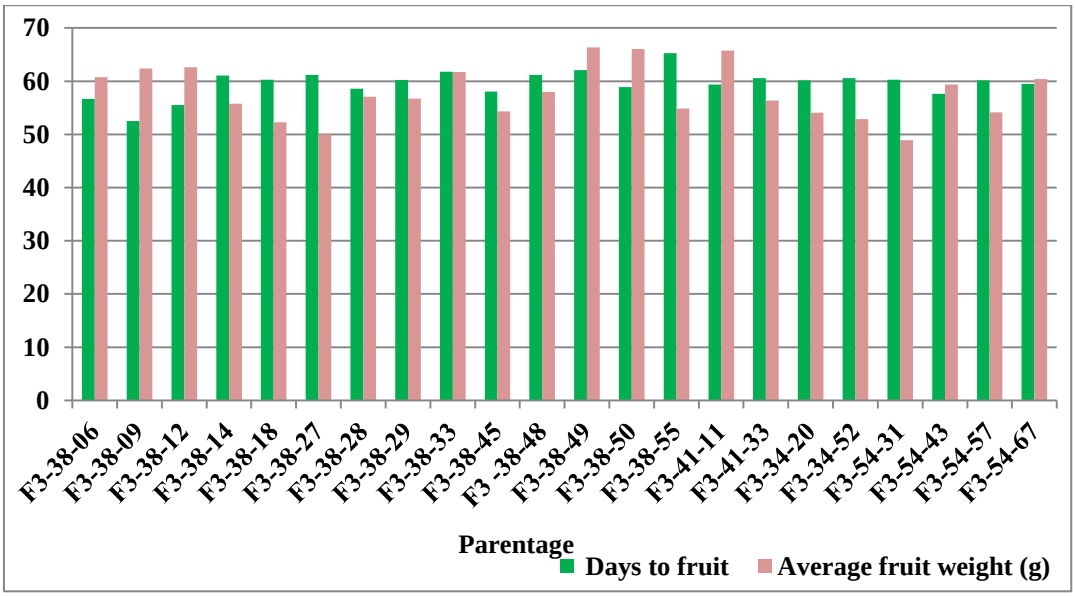


Fig 12. Mean performance of F_4 progenies for days to fruit and average fruit weight

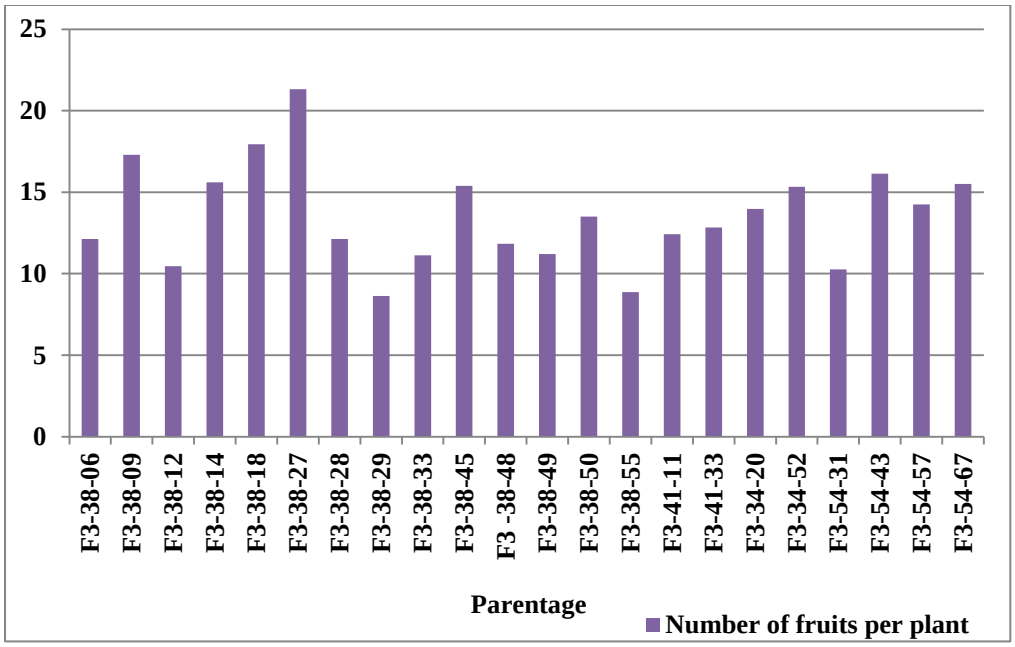


Fig 13. Mean performance of F_4 progenies for number of fruits per plant

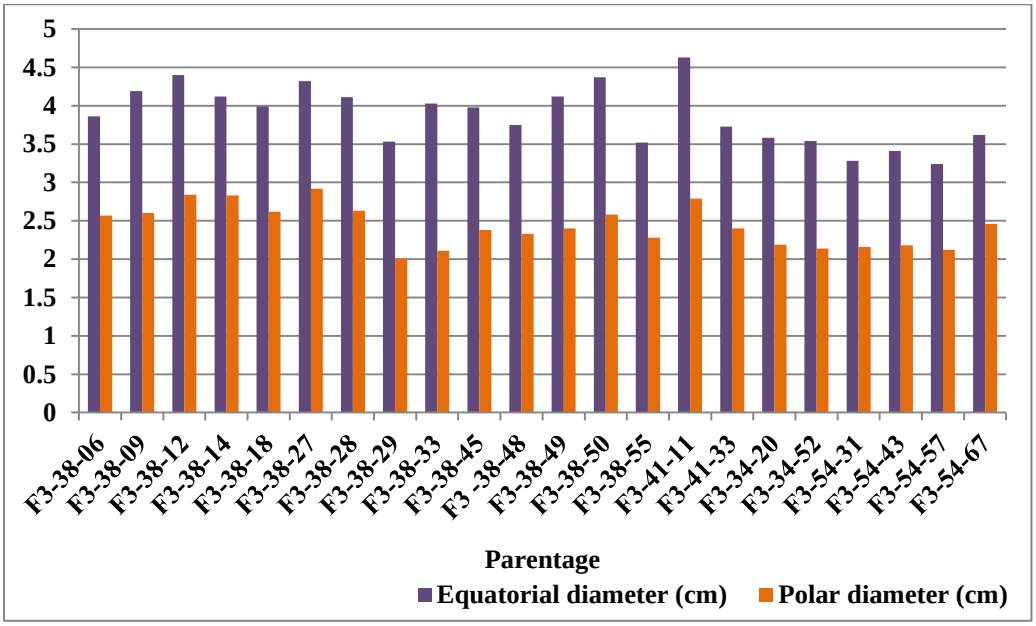


Fig 14. Mean performance of F_4 progenies for equatorial diameter and polar diameter (cm) of tomato fruit

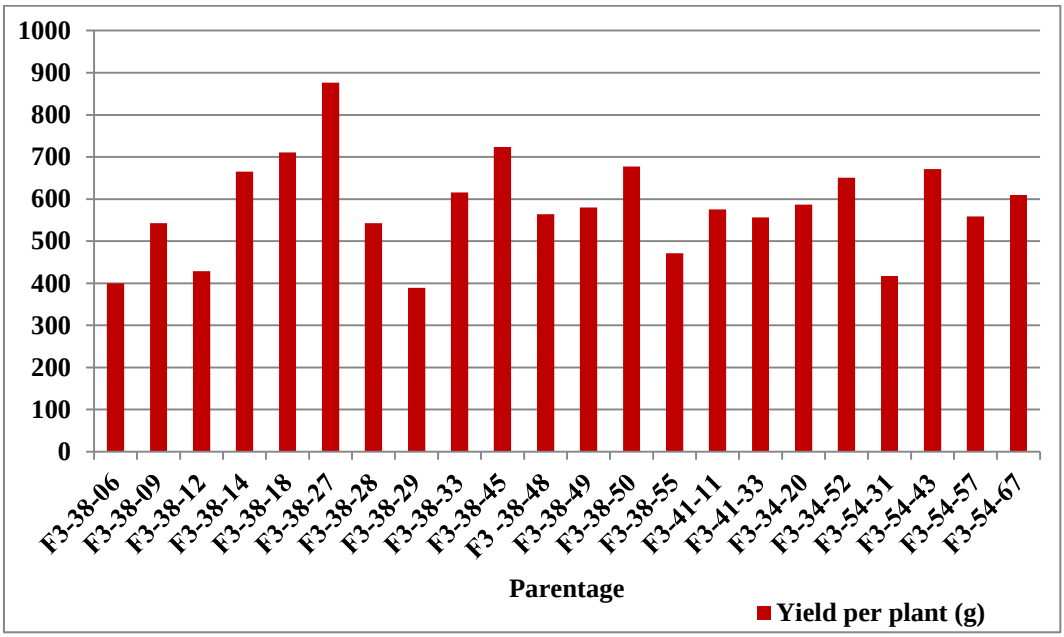


Fig 15. Mean performance of F_4 progenies for yield per plant (g)

varieties with favourable horticultural traits. Gururaj *et al.* (2002) have also reported a lack of positive correlation between yield and growth parameters in tomato. A further source of resistance was reported in *Lycopersicon pimpinellifolium* (PI 127805A) which had partial dominance at seedling stage and the resistance was controlled by recessive genes (Abeygunawardena and Siriwardena, 1963). The expression of the resistance in a variety is a function of the age of the plant and changes in temperature (Acosta *et al.*, 1964).

5.1.1 Comparison of yield of selected F_3 and F_4 segregants based on Frequency distribution

Frequency distribution of the selected F_3 and F_4 segregants was worked out based on the range value for yield. There was significant difference in the yield of F_3 and F_4 segregants having combined disease resistance. Among the 22 F_3 segregants, F_3-38-09 gave the maximum range of 1060-1148 g followed by F_3-38-14 (972 -1060 g). Five segregants recorded minimum were grouped within the range of 180 to 268 g as the lowest yielders (Table 22). Among the 35 F_4 segregants, F_3-38-6-19 gave the maximum yield with in the range of 1447-1583 g followed by F_3-54-57-21 (1179-1313 g). Five segregants were identified as minimum yielders within the range 107-241 g. However all the segregants with combined resistance in F_4 have to be evaluated further in F_5, F_6 etc for obtaining pure line with high yield and combined resistance (Table 23).

5.2 Marker Assisted Selection

Screening of mapping population through marker assisted selection for imparting disease resistance in tomato was carried out with the objective of validation of markers (SCAR and SSR) in parental population (Mukthi & IIHR-2195) and screening of mapping population (F_3 & F_4) for imparting combined resistance to bacterial wilt and ToLCV in tomato.

Table 22 Categorization of selected F_3 lines based on Frequency distribution for yield

Category	Yield (g/plant) Range	Number of Segregants	Name of Segregants
1	1060-1148	1	F_3-38-09
2	972-1060	1	F_3-38-14
3	884-972	0	
4	796-884	2	F_3-38-12, F_3-34-52.
5	708-796	2	F_3-38-18, F_3-34-20.
6	620-708	2	F_3-38-28, F_3-54-31.
7	532-620	1	F_3-54-43
8	444-532	3	F_3-38-50, F_3-38-45, F_3-54-57.
9	356-444	4	F_3-38-06, F_3-38-29, F_3-38-48, F_3-41-33.
10	268-356	1	F_3-54-67
11	180-268	5	F_3-38-55, F_3-38-33, F_3-38-27, F_3-38-49, F_3-41-11.

Table 23 Categorization of selected F_4 lines based on Frequency distribution for yield

Category	Yield (g/plant) Range	Number of Segregants	Name of Segregants
1	1447-1583	1	F_3-38-6-19
2	1313-1447	0	
3	1179-1313	1	F_3-54-57-21
4	1045-1179	3	F_3-54-67-5, F_3-38-45-5, F_3-38-45-13.
5	911-1045	5	F_3-54-67-18, F_3-54-31-7, F_3-38-50-26, F_3-38-6-3, F_3-38-9-28.
6	777-911	4	F_3-54-67-23, F_3-54-57-5, F_3-54-57-1, F_3-41-33-42.
7	643-777	5	F_3-54-31-47, F_3-38-50-18, F_3-38-50-35, F_3-54-57-2, F_3-54-43-30.
8	509-643	6	F_3-54-67-22, F_3-38-50-39, F_3-38-50-31, F_3-38-49-2, F_3-54-43-29, F_3-41-33-41.
9	375-509	2	F_3-54-67-8, F_3-38-49-13.
10	241-375	3	F_3-54-31-25, F_3-38-49-16, F_3-34-20-30.
11	107-241	5	F_3-54-67-28, F_3-54-31-20, F_3-54-31-19, F_3-54-31-33, F_3-38-12-23.

5.2.1 Isolation of Genomic DNA

Isolation of good quality DNA without any contamination is a pre-requisite for molecular study. Required quantity of DNA should be present to carry out SSR and SCAR assay. DNA was isolated in the morning from tender leaves so as to minimize the interference of polyphenols. The quality and quantity of DNA isolated was best when tender leaves were used as compared to mature and half matured leaf samples (Babu, 2000).

Due to the lower content of polyphenols, polysaccharides and other secondary metabolites, which co-precipitate with DNA in the extraction procedure, DNA extraction from plants is preferentially performed from young tissues (Zhang and Mc Stewart, 2000). High amount of these impurities which form co-precipitate with DNA, inhibit DNA digestion and molecular assay, presumably by irreversible interactions with DNA. The use of tender leaves for DNA isolation in tomato has been reported by Martin *et al.* (1991), Archak *et al.* (2002) and Langella *et al.* (2004).

DNA isolated by Rogers and Bendich (1994) protocol was free from chlorophyll and other pigments. This could be due to the fact that the protocol by Rogers and Bendich involves two treatment with chloro: isoamyl alcohol (24:1). These treatments ensure the removal of chlorophyll and other coloring substances such as pigments, dyes, etc.

Leaf tissue was ground into past form by using liquid nitrogen. Liquid nitrogen freezes the tissue and help to prevent the nucleic acid degradation and this could be one of the reasons for obtaining intact DNA. Similar observation have been made by Lodhi *et al.* (1994) in grape vine, Sharma *et al.* (2002) in sorghum, chick pea, wheat and soybean and Padmalatha and Prasad (2006) in medicinal and aromatic plants.

Tomato leaf DNA isolation can be hampered by high level of tannins and polyohenoic compounds. During tissue homogenization, phenolics become

oxidized and covalently bind to DNA giving it a brown color. The irreversible binding produces a gelatinous material, which is hard to separate from organelles and the DNA become unsuitable for amplification and digestion analysis.

During the isolation, β-mercapto ethanol and extraction buffer containing Poly Vinyl Pyrolidone (PVP) were added to overcome the problems due to high level of tannins and polyphenolic compounds. β-mercapto ethanol is a reducing agent, which protect DNA from peroxidase and polyphenol oxidase. β-mercapto ethanol disrupts the protein disulfide bonds and is thereby capable of initiating protein degradation. PVP complexes with secondary plant products especially polyphenols and tannins by binding them with hydrogen bond and can be separated from DNA by centrifugation. The use of β-mercapto ethanol and PVP for overcoming phenolics have already been reported by Nesbit *et al.* (1995) and Padmalatha and Prasad (2006).

The cationic detergent, CTAB (Cetyl trimethylammonium bromide), helps in recovery of relatively pure DNA by helping in lysis of cell membrane and release of nucleic acid and CTAB forms a complex with polysaccharides and prevents co-precipitation of polysaccharides with nucleic acid. On the other hand, it acts as a selective precipitant of nucleic acids in the solution. The DNA is soluble in presence of CTAB at high salt concentration (1.4 M Nacl). In addition, Nacl present in extraction buffer would also have helped in removal of polysaccharides. The nucleic acid form tight complexes with polysaccharides creating a gelatinous pellet that contains embedded DNA and polysaccharides (Sharma *et al.*, 2002).

Extraction buffer also contain EDTA which is a chelating agent and chelates the Mg^{2+} ion which is an essential co-factor for the enzyme to act. It prevents the indigenous endonucleases which act on nucleic acids TE buffer (Tris EDTA) also contain EDTA in which the DNA is dissolved finally.

In order to overcome the problem of RNA contamination, extracted DNA samples were treated with RNase A. Large amount of RNA in the samples can chelate Mg^{2+} ions and reduce the yield of polymerase chain reaction (PCR). The contaminating RNA that precipitates along with DNA causes interference with DNA amplification involving SSR and SCAR primers and improper priming of DNA templates during thermal cycle amplification. The importance of RNase treatment in order to yield RNA free pure DNA was also reported by Lodhi *et al.* (1994), Archak *et al.* (2002), Archak *et al.* (2003) and Padmalatha and Prasad (2006).

5.2.2 Primer screening

Out of the 11 primers reported by different authors, only three were found good to distinguish the parents Mukthi and IIHR-2195. Others did not amplify with one or both parents; or did not give polymorphic bands among the two parents. Since two diseases are considered at a time and no marker is so far reported to be linked with resistance to bacterial wilt and ToLCV; the reported markers were validated individually for 2 diseases. The genetics of bacterial wilt resistance and ToLCV resistance are reported in different ways. Som and Choudhary (1977) reported incompletely dominant polygenes to govern the ToLCV resistant trait. Two independent genes for resistance seem to be involved in these two wild species with that of *L. hirsutum f. glabratum* dominant over the other (Banerjee and Kalloo 1990).

Prior *et al.* (1994) reported that the bacterial wilt resistance in cultivated tomato originated from *L. esculentum* var. *cerasiforme* or *L. pimpinellifolium.* Banerjee and Kalloo (1989) observed that two lines *viz.*, A-1921 (*L. pimpinellifolium*) and B-6031 (*L. hirsutum f. glabratum*) were resistance for ToLCV. Sreelathakumari (1983) reported that no F_1 hybrids involving 10 lines from *Lycopersicon esculentum* as female and *L. Pimpinellifolium* as male showed resistance. She also reported a complementary and hypostatic type of digenic recessive gene system for wilt resistance.

Different sources of resistance and linkage of the markers with the QTL may be the reason for not obtaining polymorphism to characterize Mukthi and IIHR-2195 with all the reported markers in the present study. The marker selected for further screening and validation include (SSR 20, Ualty 3a and Ualty 3b). Abraham *et al.* (2006) identified PCR based markers TG105 to the *Ty2* gene conferring resistance to TYLCV in tomato, which could be used as a molecular marker. They also reported the presence of an SSR marker to determine a tomato's susceptible or resistance to ToLCV and aid in the creation of a commercial acceptable resistant hybrid.

5.2.3 Validation of the primer SSR 20

The selected F_4 plants derived from 5 F_2 lines were validated against the primer SSR 20 along with their corresponding F_3 parents and few susceptible lines. In all the 5 sets of resistant plants the specific band for wilt resistance (180 bp) was amplified. However the susceptible once gave different amplification patterns. Some of them gave heterozygous banding pattern as expected (Plate 15b, 13b, 12b). Few other susceptible once gave banding pattern similar to resistant once (Plate 11a, 12b, 13b, 15b) and others did not amplify at all (Plate 11b, 12a, 13a, 14, 15a).

5.2.4 Validation of the primer Ualty 3a (SCAR)

F_4 resistant plants and its F_3 parent with few susceptible plants were validated by Ualty 3a. ToLCV specific bands were amplified (700 and 800 bp) in all the 5 sets of resistant plants (Plate 16 to 20) and all susceptible plants showed bacterial wilt specific band which was present in Mukthi but in only one susceptible plant ToLCV specific bands were found (Plate 18) this may be because of segregating nature of population and lack full purity.

5.2.5 Validation of the primer Ualty 3b (SCAR)

Primer Ualty 3b was validated against the selected F_4 plants derived from 5 F_2 lines along with their corresponding F_3 parent and few susceptible lines. In all the 5 sets of resistant plants the specific ToLCV resistance band (600 bp) was amplified. However ToLCV specific band found absent in all the susceptible plants (Plate 21 to 25) but in one susceptible plant ToLCV specific band was amplified (Plate 23).

Summary

6. SUMMARY

The investigations on "Screening of mapping population through marker assisted selection for imparting disease resistance in tomato (*Solanum lycopersicum* L.)" were carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB) and Department of Olericulture, College of Horticulture, Vellanikkara during 2012 to 2014. The objective of the study was to validate markers (SCAR and SSR) in parental population (Mukthi & IIHR-2195) and screening of mapping population (F_3 & F_4) for imparting combined resistance to bacterial wilt and ToLCV in tomato. Two tomato genotypes Mukthi (bacterial wilt resistant) and IIHR-2195 (ToLCV resistant) were used to develop the mapping population.

The salient findings of the study are summarized as follows:

1. Bacterial wilt resistant variety Mukthi and ToLCV resistant genotype IIHR-2195 were raised in net house for extraction of genomic DNA. The protocol suggested by Roger and Bendich (1994) with RNase treatment yielded good quality DNA.

2. Parental DNA (Mukthi and IIHR-2195) was used for screening the reported primers which were specific for bacterial wilt and ToLCV resistance.

3. Six SCAR primers specific to BW and ToLCV were screened using the DNA isolated from Mukthi and IIHR-2195. Among the six, two primers (Ualty 3a and Ualty 3b) which gave polymorphism in the parents were selected for screening F_3 and F_4 population.

4. Five SSR primers were validated in parents (Mukthi and IIHR-2195). Only one primer (SSR 20) gave polymorphic banding pattern in the parents and it was selected for screening F_3 and F_4 population.

5. F_3 population was raised from the seeds obtained from five F_2 plants which showed combined resistance. Morphological characters and disease reactions were observed.

6. In F_3 population, 337 plants were evaluated in disease sick field. In the total population, 71.8 per cent plants thrived over bacterial wilt, while only 6.53 per cent (22 plants) were found tolerant to both bacterial wilt and ToLCV.

7. F_4 population was raised from the seeds obtained from 22 F_3 plants which showed combined resistance. Morphological characters and disease reactions were observed.

8. In F_4 population, 584 plants were evaluated in the disease sick field. In the total population 84.76 per cent plants thrived over bacterial wilt, while only 5.99 per cent (35 plants) were found tolerant to both the diseases and they were derived from 12 F_3 lines.

9. The three selected primers (SSR 20, Ualty 3a and Ualty 3b) were validated on resistant F_4, their corresponding F_3 parental lines, along with susceptible checks. The selected markers segregated with the trait in the F_3 and F_4 resistant plants.

10. SSR 20 was validated in resistant F_4 plants, their F_3 parents and few susceptible lines. In all the 5 sets of resistant plants derived from different F_3 lines, the specific band for wilt resistance (180 bp) was amplified. However the susceptible ones gave different amplification patterns. Some of them gave heterozygous banding pattern as expected. Few other susceptible ones gave banding pattern similar to resistant ones and others did not amplify at all. This can be expected in a segregating population for a trait controlled by recessive genes and multiple alleles.

11. The primer Ualty 3a gave ToLCV specific bands (700 and 800 bp) in all the 5 sets of resistant F_4 plants and their respective F_3 parents. None of the susceptible checks except one (F_2-38-58) gave ToLCV resistance specific amplicon.

12. The primer Ualty 3b was also specific to ToLCV. It gave specific amplicon (600 bp) in all the 5 sets of resistant F_4 plants and their corresponding F_3 parent. The ToLCV specific amplicon was not observed in most of the susceptible checks.

13. The study could identify 35 plants among the F_4 segregants as having combined resistance to bacterial wilt and ToLCV.

14. Great variations in yield parameters were observed among these resistant lines. Fourteen promising lines with better yield (>800 g) and combined disease resistance were screened out in the study; suggesting the potential for obtaining high yielders with good resistance in the coming generations.

15. The 3 markers identified in the study could be utilized for marker assisted selection with respect to ToLCV and BW in tomato.

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Appendices

APPENDIX-I

Reagents used for DNA isolation

Reagents:

1. 2x CTAB extraction buffer (100 ml)

CTAB	:	2g
(Cetyltrimethyl ammonium bromide)		
TrisHCl	:	1.21 g
EDTA	:	0.745 g
NaCl	:	8.18 g
PVP	:	1.0 g

Adjusted the pH to 8 and made up final volume up to 100 ml.

2. CTAB (10%, 100 ml)

CTAB	:	10 g
NaCl	:	4.09 g

3. Chloroform- Isoamyl alcohol (24:1 v/v)

To chloroform (24 parts), isoamyl alcohol (1 part) was added and mixed properly.

4. Chilled isopropanol

Isopropanol was stored in refrigerator at 0ºC and was used for the study.

5. Ethanol (70%)

To the 70 parts of absolute ethanol (100%), 30 parts of sterile distilled water was added to make 70 per cent ethanol.

6. TE buffer (pH 8, 100 ml)

TrisHCl (10 mM)	:	0.1576 g
EDTA (1 mM)	:	0.0372 g

The solution was prepared, autoclaved and stored at room temperature.

APPENDIX-II

Composition of buffers and dyes used for gel electrophoresis

1. TAE Buffer 50X

Tris base	:	242 g
Glacial acetic acid	:	57.1 ml
0.5M EDTA (pH 8.0)	:	100 ml

2. Loading Dye (6X)

0.25% bromophenol blue

0.25% xylene cyanol

30% glycerol in water

3. Ethidium bromide

The dye was prepared as a stock solution of 10 mg/ml in water and was stored at room temperature in a dark bottle.

SCREENING OF MAPPING POPULATION THROUGH MARKER ASSISTED SELECTION FOR IMPARTING DISEASE RESISTANCE IN TOMATO (*Solanum lycopersicum* L.)

By

DHEEMANTH T L

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ABSTRACT OF THE THESIS

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ABSTRACT

Tomato is the second most consumed vegetable, next to potato and it occupies largest number of cultivated varieties than any other vegetable crop. India is the sixth largest producer of tomato in the world with an area of 0.50 million hectares and with a productivity of 17.4 MT per hectare.

Despite the efforts taken up all over the world so far, tomato leaf curl virus (ToLCV) disease and bacterial wilt (BW) still continues to be the major limiting factors in tomato cultivation. The leaf curl virus infects the crop in all locations while bacterial wilt is more severe in the humid tropics. Acidic soils, humid climate and high temperature favour bacterial wilt incidence in Kerala and it affects the crop at all stages of growth resulting in total crop loss. Leaf curl virus incidence is also gaining importance in the state recently and hence it is the need of the hour to develop varieties with combined resistance.

Conventional breeding has helped to develop location specific varieties and molecular breeding have identified several Resistant Gene Analogues and QTLs mapped on different chromosomes. Considering the importance of bacterial wilt in Kerala, KAU has developed varieties with relatively good tolerance (eg- Mukthi), but are susceptible to ToLCV and fruit qualities are not superior. Genotypes resistant to different strains of ToLCV have been developed at Indian Institute of Horticultural Research (eg- IIHR-2195) and this project is an attempt to incorporate combined resistance to BW and ToLCV through molecular breeding. The markers that will be validated will be of great use in marker assisted selection. An ideal genotype with ToLCV resistance in bacterial wilt resistance background and having desirable horticultural traits is targeted in the programme.

The investigation was carried out during 2012 - 2014 for Screening of F_3 and F_4 population of a cross between Mukthi and IIHR-2195 for imparting combined disease resistance along with better horticultural traits.

Total 921 plants in F_3 and F_4 population were evaluated in a disease prone field of Department of Olericulture, College of Horticulture. Important biometric parameters were recorded, disease reaction scored and selected molecular markers were validated on resistant and susceptible lines.

DNA isolated from the parent Mukthi and IIHR-2195 was used to validate eleven primers already reported for BW and ToLCV resistance. Three primers which showed good polymorphism and reproducibility among parents were selected for further validation in F_3 and F_4 population.

Twenty two F_3 and 35 F_4 plants with combined resistance to BW and ToLCV were screened out based on disease reaction. The three selected primers (Ualty 3a, Ualty 3b and SSR 20) were validated on resistant F_4, their corresponding F_3 parental lines, along with susceptible checks. The selected markers segregated with the trait in the F_3 and F_4 resistant plants and were also found expressed in few susceptible checks.

The markers found to segregate along with the trait could be recommended for marker assisted selection in tomato. Fourteen promising lines with combined resistance and better yield (>800g) were identified for further evaluation and variety development.