LOCATING DONORS FOR GENES FOR BIOTIC STRESS RESISTANCE IN TOMATO THROUGH MOLECULAR MARKER ASSISTED SELECTION

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By

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THESIS

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KERALA, INDIA

2021

DECLARATION

I hereby declare that the thesis entitled "Locating donors for genes for biotic stress resistance in tomato through molecular marker assisted selection" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Locating donors for genes for biotic stress resistance in tomato through molecular marker assisted selection"** is a record of research work done independently by **KRISHNENDU M. R.** (2016-09- 025) under my guidance and supervision and this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Krishnendu M. R.

DEDICATED TO MY DEAR PARENTS, TEACHERS AND FRIENDS

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LIST OF ABBREVATIONS

%	Percentage
(L.)	Linnaeus
µg	Microgram
µl	Microlitre
µM	Micromolar
A	Adenine
A260	Absorbance at 260 nm wavelength
A280	Absorbance at 280 nm wavelength
AFLP	Amplified Fragment Length Polymorphism
BLAST	Basic Local Alignment Search Tool
bp	Base pair
C	Cytosine
CAPS	Cleaved Amplified Polymorphic Sequences
CCSHAU	Choudary Charan Singh Haryana Agricultural University
DNA	Deoxyribonucleic acid
dNTPs	Deoxy nucleotide tri phosphates
dsDNA	double stranded Deoxyribonucleic acid
E.coli	*Escherichia coli*
EDTA	Ethylene diamine tetra acetic acid
et al.	et alia
EtBr	Ethidium bromide
FAO	Food and Agriculture Organization
g	gram
G	Guanine
IARI	Indian Agricultural Research Institute
IIHR	Indian Institute of Horticultural Research
IIVR	Indian Institute of Vegetable Research
InDels	insertion-deletion
KAU	Kerala Agricultural University
Kb	Kilo bases
kg	Kilogram

L	Litre
LB	Late blight
LRR	leucin-rich-repeat
m	Meter
M	Molar
MAS	Marker assisted selection
Mb	megabases
mg	milligram
$MgCl_2$	Magnesium chloride
min	Minute
ml	Millilitre
mm	Millimeter
mM	Millimolar
NaCl	Sodium chloride
NBPGR	National Bureau of Plant Genetic Resources
NCBI	National Centre for Biotechnological Information
ng	Nanogram
Nm	Nanometer
O.D.	Optical density
^oC	Degree Celsius
PCR	Polymerase chain reaction
pH	Potential of hydrogen
PS	Phenotypic selection
PVP	Polyvinylpyrrolidine
QTL	Quantitative trait locus
R	Resistant
RAPD	Random amplified polymeric DNA
RFLP	Restriction fragment length polymorphism
RKN	Root knot nematodes
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute
s	second

SCAR	Sequence characterized amplified region
SNP	Single nucleotide polymorphism
sp.	Species
spp.	Species (plural)
SSR	Simple sequence repeat
T	Thymine
Ta	Annealing Temperature
TAE buffer	Tris Acetate EDTA buffer
Taq	*Thermus aquaticus*
TE	Tris-EDTA buffer
Tris HCl	Tris (Hydroxy methyl) aminomethane hydrochloride
U	Enzyme units
UAS	University of Agriculture Science
UV-VIS	Ultra Violet-Visible Spectroscopy
V	Volt
v/v	volume/volume
Vit	Vitamin
w/v	weight/volume

INTRODUCTION

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop of Solanaceae family, grown as a perennial in its native habitat and as an annual when grown outdoors in temperate climates. Presently it is estimated that about 160 million tonnes of tomato are produced annually worldwide, from 4.7 million hectares (Konuma and Gennari, 2014). Tomatoes are botanically a berry fruit, but they are used as a vegetable for culinary purposes and are consumed in variety of forms, including raw. Native to South America, tomato is cultivated extensively in all the states of India from Jammu and Kashmir to Kerala and Arunachal Pradesh to Gujarat.

Tomato prefers warm sunny weather for growth and is sensitive to extreme conditions of high and low temperature. It fairly withstands drought condition, but prevalence of low moisture supply, obstruct setting of fruit. Warm weather is essential for high yield, quality, color, and proper ripening of tomato. A temperature range of 14°C to 32°C is optimum for fruit setting and ripening and 18°C to 26°C temperature range is favored for germination in tomato. It grows well in all soil types with a pH range of 6 to 7, which is properly drained with fairly good water holding capacity and rich in organic matter. The plant grows to a height of 1-3 meters and often sprawls over the ground due to weak stem.

Tomato has gained popularity as the richest source of powerful antioxidant lycopene, which has anti-cancer properties and imparts red color to the fruit (Kim *et al*., 2016). Various other compounds with antioxidant properties present in tomato include beta carotene, chlorogenic acid, plastoquinones, rutin, tocopherol and xanthophylls (Leonardi *et al*., 2000). It is a vital source of dietary vitamins (Vit A and Vit C), minerals and fiber and is thus recognized as a healthy food that helps in providing protection against various human diseases like heart and numerous cancer diseases (Dhaliwal *et al*., 2020).

The cultivated tomato and its wild relatives in the genus *Solanum* are diploid (2n=2x=24) in nature with similar chromosome number and karyotype. With a widely characterized small genome size of 950 Mb, the greatest wealth of genetic variability is contributed by wild relatives of tomato (Chetelat and Ji, 2007). Tomato

being a dynamic academic model system in plant genetics for abiotic and biotic stress tolerance and plant - microbe interaction, is highly threatened by different types of pathogen attack, resulting in substantial production and yield losses (Lee *et al*., 2015). The major vulnerable diseases constituting biotic stress in tomato are caused by viruses, fungi, bacteria and nematodes.

Genes that impart resistance to diseases caused by several pathogens has been identified in wild relatives of tomato including *Solanum peruvianum, Solanum chilence, Solanum pimpinellifolium, Solanum pennelli* and *Solanum habrochaites* (Sajid and Eminur, 2019). New strategies based on Marker assisted selection (MAS) has been successfully employed in tomato breeding program to select for several qualitative disease resistance traits and to improve upon quantitative resistance to disease. Application of molecular markers in tomato breeding program for disease resistance unveil the possibility of locating donors for genes for resistance to multiple diseases from the germplasm.

The accuracy of MAS in tomato crop improvement program has been hugely elevated with the advent of PCR based gene specific markers linked to the genes for resistance, which detects DNA polymorphism within target genes. The gene based markers employed for screening tomato genotypes for biotic stress resistance is majorly based on genetic variations present in different alleles like Single nucleotide polymorphism (SNP) or InDels (insertion/deletion) resulting in critical phenotypic changes (Arens *et al*., 2010). Being free from environmental conditions, the use of molecular markers in MAS for disease resistance screening has overcome many limitations in developing commercial tomato cultivars and is a highly reproducible and reliable tool for introducing simple and complex traits in tomato breeding programs.

This study aims to screen 30 genotypes for genes for resistance to major diseases, ie, *Tomato yellow leaf curl virus* (*TYLCV*), Verticillium wilt, Fusarium wilt, Late blight and Root-knot that challenge tomato production using gene specific SCAR and CAPS molecular markers.

The study is expected to contribute donors for genes for resistance to *Tomato yellow leaf curl virus* (*TYLCV*), Verticillium wilt, Fusarium wilt, Late blight and Root-knot diseases in tomato.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The study focuses on screening 30 tomato genotypes for genes for resistance to major diseases like *Tomato yellow leaf curl virus (TYLCV)*, Verticillium wilt, Fusarium wilt, Late blight and Root-knot through molecular markers. A review of literature on *Solanum lycopersicum* L., disastrous pathogens resulting biotic stress in *Solanum lycopersicon*, major diseases and associated genes for resistance as well as molecular markers used for screening in the study, has been carried out in this chapter.

2.1 TOMATO (*Solanum lycopersicum*)

Cultivated tomato (*Solanum lycopersicum*) is an important warm season grown fruit vegetable worldwide, with second most ranking in consumption and global food production, after potatoes. It belongs to *Solanaceae* family, which consist of about 100 genera and 2500 species of high agronomic significance (Sajid and Eminur, 2019). Being one of the popular grown crops in garden, tomato varieties are sold worldwide in high numbers than any other vegetable crops.

With the recent revision of the phylogenetic classification in the family *Solanaceae*, the genus *Lycopersicon* got reintegrated into the genus *Solanum* and thus tomatoes became more commonly accepted in the genus *Solanum* (Child, 1990).The genus *Solanum* consist of 13 species of tomato, which include cultivated tomato and the wild relatives, constituting the genetic diversity.

Origin of wild tomato is centered in western South America, distributed along the mountainous regions of the Andes from Ecuador to northern Chile (Darwin *et al.*, 2003; Peralta and Spooner, 2005). Diversity in wild tomato has been a contribution of diverse ecological habits like arid coastal lowlands to isolated valleys of Andean topography and different climate conditions like the scorching deserts in northern Chile.

India is the second leading producer of tomato following China, with tomato being cultivated in all the states of India from Jammu and Kashmir to Kerala. India is not a home to naturally growing wild species of tomato, but possesses germplasm

lines of potential wild species like *Solanum pimpinellifolium, Solanum lycopersicumvar cerasiforme, Solanum peruvianum, Solanum habrochites etc*. The potent factors used to differentiate the germplasm of tomato in India are fruit weight, number of fruits per plant and plant height (Reddy *et al.*, 2013).

The most recent ancestor of cultivated tomato is identified to be the cherry tomato *Solanum lycopersicum* var. *cerasiforme* (Arumuganathan and Earle, 1991). Wild tomato species are diploid with similar chromosome number and structure with not much major differences. Wild species of tomato are recognized to possess definite source of genes for resistance to biotic and abiotic stress tolerance. The development of new resistant cultivars with improved economic traits and capability to survive biotic and abiotic stress has been achieved through various methods of genetic introgression from their corresponding wild relatives. However, high selection and inbreeding practices to breed similar ideotypes to achieve improved productivity has resulted in depletion of genetic diversity within modern tomato cultivars. Estimates shows that only less than 5% of genetic diversity of wild relatives were found in the genome of cultivated tomatoes (Miller and Tanksley, 1990) and this limited genetic variation is the prime reason of crop vulnerability to biotic stress causing diseases and insect epidemics.

2.1.1 Taxonomic Tree of *Solanum lycopersicum L.*

Domain	: Eukaryota
Kingdom	: Plantae
Phylum	: Spermatophyta
Subphylum	: Angiospermae
Class	: Dicotyledonae
Subclass	: Asteridae
Order	: Solanales
Family	: Solanaceae
Genus	: *Solanum*
Species	: *lycopersicum*

Tomato is a popular academic model plant for biology and plant genetics research, particularly in the areas of abiotic and biotic stress tolerance, fruit biology, and plant-microbe interactions (Sajid and Eminur, 2019). It has been examined extensively and scientifically in response to pathogen attacks. Various devastating diseases have been reported in tomato plants around the world in recent years, resulting in significant losses in production and yield. Crop yields are currently declining; as a result, various novel tactics have been developed to maintain output, stability, or sustainability of plant features.

2.2 BIOTIC STRESS IN TOMATO AND ASSOCIATED MAJOR GENES FOR RESISTANCE

Tomatoes are susceptible to more than 200 diseases caused by a variety of pathogens, including fungus, viruses, bacteria, and nematodes, posing enormous damage to plant health. Despite decades of rigorous disease resistant tomato breeding initiatives, a substantial variety of pathogens continue to pose threats to tomato production. *Tomato spotted wilt virus (TSWV), Tomato yellow leaf curl virus (TYLCV), Tomato mosaic virus (ToMV),* verticillium wilt caused by *Verticillium* spp., fusarium wilt caused by *Fusarium oxysporum*, late blight caused *Phytophthora infestans*, leaf mold caused by caused by *Cladosporium fulvum*, root knot caused by *Meloidogyne* spp., bacterial spot caused by *Xanthomonas* spp., bacterial speck caused by *Pseudomonas syringae* and bacterial wilt caused by *Ralstonia solanacearum* are some of the major diseases challenging tomato production worldwide.

Cultivated tomatoes have a limited gene pool due to rigorous breeding efforts focused at boosting economically useful traits. As a result, wild relatives like *S. pimpinellifolium, S. pennelli, S. habrochaites, S. peruvianum,* and *S. chilense* have been studied for their potential as disease resistance sources (Peirce, 1971; Stevens *et al*., 1991; Zamir *et al*., 1994; Diwan *et al*., 1999). Today, resistance genes derived from moderately wild species such as *Solanum peruvianum, Solanum chilence, Solanum pimpinellifolium, Solanum pennellii,* and *Solanum habrochaites* can be used to genetically control nearly 20 distinct infections in tomato. Molecular markers have been developed and used in tomato breeding programs to utilize the resistance conferred by these wild relatives in producing new tomato varieties.

2.2.1 *Tomato yellow leaf curl virus* disease (*TYLCVD*)

Since the 1980s, tomato yellow leaf curl disease (TYLCD) has posed a global threat to tomato (*Solanum lycopersicum*) production (Moriones and Navas-Castillo, 2000). *Tomato yellow leaf curl virus* (*TYLCV*) is a member of Begomovirus genus of the Geminiviridae family is one of the virus species among a cluster of related species that can cause TYLCD. In nature, *TYLCV* is transmitted in a persistent-circulative way by the invasive pest whitefly *Bemisia tabaci* (Gronenborn, 2007). The disease infects agriculture all over the world, in both protected and open field cultivations (Lapidot and Polston, 2006).

Tomatoes can be infected with *TYLCV* at any stage of development. Several weeks after the virus infection, the disease symptoms appear. Stunted plant growth, reduced leaf size, upward cupping of leaves, yellowing of the leaf margins, flower drop, and reduced fruit set and yield are all indications of the disease (Antignus and Cohen, 1994). Mosaic, mottling, necrosis, and leaf deformities are the most visible viral symptoms in the field (Green, 1991). These characteristics, however, are not very useful for identifying symptom-causing viruses on their own as they are influenced by a variety of other factors such as plant-water relations, sucking insect pest infestation, and so on.

Dı´az-Pendo´n *et al*. (2010) found that TYLCD is caused by a virus complex containing at least six different virus species. TYLCD-causing begomoviruses are collectively known as *TYLCV*-like viruses, with *TYLCV* being the most widely disseminated. Two *TYLCV* species have been identified in the Mediterranean basin based on sequence similarities, *Tomato yellow leaf curl virus*-Israel (*TYLCV-Isr*) (Navot *et al*., 1991) and *Tomato yellow leaf curl Sardinia virus* (*TYLCSV*) (Kheyr-Pour *et al*.,1991).

2.2.1.1 Genes for resistance to Tomato yellow leaf curl virus disease

The development of inoculation techniques, screening and confirmation of resistance sources, gene discovery and genetic mapping, resistance gene transfer to cultivated varieties, and field evaluation of introgressed lines are all part of the *TYLCV* resistance breeding programme. Six independently inherited genes (*Ty-1*, *Ty-*

2, *Ty-3*, *Ty-4*, *Ty-5*, and *Ty-6*) with varied levels of resistance have been identified and transferred to commercial cultivars from wild germplasm resources to date (Anbinder *et al*., 2009).

Major resistance loci *Ty-1* and *Ty-3* originate from LA1969 and LA2779 accessions of *S. chilense*, (Ji *et al*., 2007). They are alleles of the same gene that encodes a DFDGD –class RNA-dependent RNA polymerase (RDR), which is found on the long arm of chromosome 6 of tomato (Verlaan *et al*., 2011; Verlaan *et al*., 2013). *Ty-2*, found on the long arm of chromosome 11 and originated from *S. habrochaites* accession B6013, encodes an NB-LRR gene (Yang *et al*., 2014; Yamaguchi *et al*., 2018). In *Ty3*-carrying *S. chilense* LA1932 and LA2779, *Ty4* was identified on chromosome 3 as an additional resistance locus, which contributes minorly to *TYLCV* resistance (Ji *et al*., 2009). Another recessive resistance locus *ty5*, was identified on chromosome 4 in *S. peruvianum* (Anbinder *et al*., 2009). A *Pelo* gene, which is the messenger RNA surveillance factor Pelota, implicated in the ribosome recycling phase of protein synthesis, has recently been discovered as a gene involved for *ty5* resistance (Lapidot *et al*., 2015). *Ty6*, a recessive resistance gene on chromosome 10, has also been identified in *Ty3*-carrying LA2779 accession of *S. chilense* (Scott *et al*., 2015).

2.2.2 Verticillium wilt

Verticillium wilt also called as vascular wilt disease is a serious tomato disease caused by the soil-borne fungus *Verticillium dahliae* and *V. alboatrum* in many tomato growing locations across the world (Fradin and Thomma, 2006). Over 200 dicotyledonous species, including many important crop species, are infected by these fungi, which cause vascular diseases in them.

Yellow blotches on the lower leaves are the first symptom of verticillium wilt, followed by brown veins, and finally chocolate brown dead spots, which may emerge under favourable moisture and nutrition conditions. The spots resemble alternaria early blight, but they aren't the same, and they don't form concentric bull's-eye rings. It's possible that the leaves will wilt, die, and fall off. The plant becomes stunted as the disease signs move up the stem. The upper leaves are the only ones that remain

green. Because of the absence of leaves, the fruits remain small, develop yellow shoulders, and are susceptible to sunburn (Karagiannidis *et al*., 2002).

For the control of *Verticillium* spp., there are only a few options, and none of them are completely effective. Because soil fumigation is both costly and harmful to the environment, the most promising solutions for controlling these diseases rely mostly on genetic resistance (Corsini and Pavek, 1996).

2.2.2.1 Genes for resistance to Verticillum wilt

The *Ve* locus contains two closely linked, inversely orientated genes: *Ve1* and *Ve2*, confers resistance against verticillium wilt disease in tomatoes. The genes share 84% amino acid similarity and have no introns. Resistance to *Verticillium dahliae* race 0 was found on chromosome 9 in *S. lycopersicum* line Peru Wild (Schaible *et al*., 1951). The resistance spectrums of the resistance genes of *Ve* locus (both *Ve1* and *Ve2*) have been determined, which confers resistance to race 1 strains of *V. dahliae* and *Verticillium alboatrum*. The extracellular LRR receptor-like protein class of disease resistance proteins confers *Ve1* resistance (Kawchuk *et al*., 2001), which appear to be important for evoking an effector-triggered immunity in the host plant. The wild tomato species *Solanum licopersicoides* (Chai *et al*., 2003) and the wild potato species *Solanum torvum* both are recognized have *Ve* gene homologues (Fei *et al*., 2004).

Selection can be carried out at an early seedling stage of development using molecular markers associated to the traits of interest, and the Verticillium-resistant genotypes can be easily differentiated. *Ve* resistance is present in the majority of contemporary tomato cultivars. This resistance gene has been linked to a number of PCR-based markers (Kawchuk *et al*., 2001; Acciarri *et al*., 2007; Park *et al*., 2008). Many tomato-growing locations across the world have reported race 2 of the pathogen, which encompasses all strains of the pathogen, virulent to cultivars carrying *Ve* gene (Scott and Gardener, 2007). The resistance to this race is not based on a gene-for-gene mechanism. However, sources of *S. lycopersicum* that give partial resistance to race 2 have been discovered (de Miranda *et al*., 2010). In addition, there

have also been reports of SCAR and SNP markers linked to this resistance (Kawchuk *et al*., 2001).

Jung et al. (2015) reported several allele-specific molecular markers for *Ve1* and *Ve2*. Between the *Ve1* resistance allele and the *ve1* susceptibility allele, the major DNA variation is a single-bp deletion, TCA/T-A at nucleotide position 1,220, which causes a premature stop codon in the *ve1* susceptibility allele, which is associated with loss of function and disease resistance. As a result, the single-bp deletion-derived gene-based CAPS marker can be deemed as a functional marker for the *Ve1* resistance locus.

2.2.3 Fusarium wilt

Fusarium oxysporum f. sp. *Lycopersici* (Fol) is a common and destructive pathogen that causes wilt in tomato and endangers the industry of this economically predominant crop worldwide. Many host specific special forms of *F. oxysporum*, known as formae speciales (f. sp.) (Di Pietro *et al*., 2003), shows a gene-for-gene relationship between the pathogen and its diverse host range.

Fol is a soil-borne pathogen that penetrates tomato plants through the roots and can infect them at any stage of growth. Fol infection begins with the germination of soil-borne spores near developing roots, followed by adhesion to the root surface, root cortical penetration, and hyphae development within the root vasculature. Fol eventually infiltrates and colonises the parenchyma of the dying tomato plant, sporulating on its surface (Michielse and Rep, 2009).

Yellowing, wilthing, and browning of the leaves, stunted development, and eventually death of the plant are all symptoms of this disease. These symptoms are generated by hyphae within the xylem vessels, as well as tyloses, callose, gums, and gels produced by the host plant, obstructing water and nutrient flow (Bacon and Yates, 2006; Moretti, 2009).

To control this fungus in tomato fields, a variety of techniques are used, including soil steaming, fumigation, solarization, or a combination of these (Essarioui *et al*., 2016). All of these management strategies, however, are expensive

and primarily restricted to greenhouses. The use of resistant cultivars in the field is the only effective and practical approach of control.

2.2.3.1 Genes for resistance to Fusarium wilt

Three races of the pathogen (1, 2, and 3) have been identified, as well as four resistance loci (*I, I-1, I-2, and I-3*) that confer vertical resistance to the disease so far. Fol resistance has been established in a number of wild tomato varieties. The first gene, *I,* identified in *S. pimpinellifolium* accession PI 126915 (Bohn and Tucker, 1940) is found to confer resistance to race 1 of the pathogen, and was consequently mapped to the short arm of tomato chromosome 11. The *I-2* gene, which confers resistance to race 2 of the pathogen, was identified in *S. pimpinellifolium* accession PI 126915 and mapped to the long arm of chromosome 11, where it encodes a coiled coil, nucleotide-binding (CC-NB)-LRR resistance protein (Simons *et al*., 1998). *I-2* is one of six members of the *I-2C* gene family, which spans 90 kb within which the complex resistant locus includes *I-2, I-2C1, I-2C2, I-2C3*, *I-2C4,* and *I-2C5*. While *I* and *I-2* have been the most commonly used resistance genes for breeding (Scott, 2008), *I-3* is now the preferred resistance gene since race 3 is becoming more common than before, and there are also few tomato cultivars with the *I-3* resistance gene. *S. pennelli* LA716 (Scott and Jones, 1989) was found to have *I-3* resistance to Fol race 3 and this gene encodes an S-receptor-like kinase (SRLK) protein (Catanzariti *et al.,* 2015). *I-7* is part of a small gene family that consists of a homologue on chromosome 6 and a homologue on chromosome 8 next to it. In *S. pennellii* LA716, the *I-7* resistance gene was identified, which confers resistance to *Fol* races 1, 2, and 3. Also it encodes a typical extracellular LRR receptor-like protein (LRR-RLP) (Gonzalez-Cendales *et al*., 2016). Different SCAR and CAPS marker have been developed for all these disease resistance genes.

2.2.4 Late blight

Late blight (LB), caused by the oomycete *Phytophthora infestans*, is one of the most damaging tomato diseases in areas of high humidity and cool temperatures, and can result in crop loss of up to 100 percent in unprotected tomato fields or greenhouses. Reduced yield, lower fruit quality (such as low specific gravity),

reduced storability, and increased costs associated with fungicide applications are all possible losses.

Phytophthora infestans can decimate tomato crop at any stage of their growth. It can infect the plant's entire above-ground structure, causing necrosis of the leaves and stems, fruit rot, and eventual plant death. Tomato seed may also be infected by *Phytophthora infestans* (Rubin *et al*., 2001). Lesions of late blight (LB) first emerge near the leaflet margins. Water-soaked lesions that are purple, dark-brown, or black often develop a pale yellowish-green border that blends in with healthy tissue. Lesions can appear elsewhere on the leaves as the disease progresses. Leaflets shrivel and die as LB progresses, and the disease spreads to the rest of the vegetation, resulting in widespread defoliation. The dark brown LB lesions of the stem usually start at the top of the stem or at a node and spread down the stem (Rubin and Cohen, 2004).

2.2.4.1 Genes for resistance to Late blight

Late blight (LB) resistance in potatoes has been extensively studied, with more than 60 resistance genes identified and recognized on the genetic map (Rodewald and Trognitz, 2013) and only a few race-specific LB resistance loci in the tomato have been identified. In both traditional and organic agriculture, host plant resistance has proven to be an effective way to fight LB and other tomato diseases.

For several years, tomato genetic resistance to LB has been of interest, and three main resistance genes, *Ph-1, Ph-2*, and *Ph-3*, have been identified in the tomato wild species *S. pimpinellifolium*, and have been mapped to tomato chromosomes 7, 10, and 9, respectively. *Ph-1* is a single dominant gene on chromosome 7 that confers resistance to race T-0, but it was quickly surpassed by new pathogen races (Peirce, 1971). Currently, race T-1 of *P. infestans* predominates, causing *Ph-1* resistance ineffective. To many isolates of race T-1, the resistance conditioned by *Ph-2*, a single incomplete-dominant gene mapped to the lower end of tomato chromosome 10's long arm (Moreau *et al*., 1998), provides partial resistance (Peirce, 1971). *Ph-2* delays but does not hinder the progression of the disease (Moreau *et al*., 1998) and often fail in the presence of aggressive isolates. In *S. pimpinellifolium* accession L3708, a much

stronger resistance gene, *Ph-3*, was identified (Black *et al*., 1996). This gene is currently much more prominent than *Ph-1* and *Ph-2* (Chunwongse *et al*., 2002) as it confers incomplete dominant resistance to a wide variety of *P. infestans* isolates of tomato, including those that surpassed *Ph-1* and *Ph-2*. The *Ph-3* gene is found in a 26-kb region on chromosome 9's long arm, encodes a coiled-coil nucleotide-binding (NBS)-LRR (Zhang *et al*., 2014). However, it has been proposed that the maximum resistance conferred by L3708 (the original source of *Ph- 3*) is conferred by more than just the *Ph-3* locus, and the homozygous or heterozygous conditions of *Ph-3* alone, would not be commercially attractive because it does not impart substantial amount of resistance against aggressive isolates like US-7 and US-17 (Kim and Mutschler, 2005; Lee *et al*., 2006). As a result, it appears that combining *Ph-3* and *Ph-2* confers high resistance to the majority of these isolates. In *S. habrochaites* LA 1033, *Ph 4* on chromosome 2 was identified (Kole *et al*., 2006).

The breakdown or reduced effectiveness of established major LB resistance genes in tomato, as well as the introduction of new as well as more aggressive *P. infestans* isolates, necessitated the discovery and use of new resistance sources. A few highly resistant accessions within the tomato wild species *S. pimpinellifolium* were recently identified in an intense effort to find new sources of LB resistance in tomato (Foolad *et al*., 2008). The identified accessions were resistant to 7 isolates of *P. infestans* belonging to the US8, US13, US14, or US15 lineages. One of these accession PSLP153, was chosen for genetic analysis as well as for discovery and mapping of a possible new LB resistance genes. Two genomic regions on tomato chromosomes 1 (*Ph-5–1*) and 10 (*Ph-5–2*) have been identified as conferring resistance to tomato LB as a result of a targeted genotyping approach (Merk *et al*., 2012). The position of *Ph-5–1* and *Ph-5–2*, as well as the possibility of PCR-based markers associated with these new resistance genes, are currently being investigated.

2.2.5 Root knot nematode disease

Root knot nematodes (RKN) are endoparasites of plants belonging to the genus *Meloidogyne*, that inflict major damage to tomato crops all over the world, particularly in home gardens. These nematodes enter the plant through the roots and

move to the vascular cylinder, causing root knots that disrupt nutrient partitioning and water uptake (El-Sappah *et al*., 2019).

There are more than 90 species of root-knot nematode (RKN), some of which have many races. *M. incognita*, *M. javanica*, *M. hapla*, and *M. arenaria* are the four most economically damaging species globally (Kiewnick *et al*., 2009). *M. incognita*, on the other hand, is the most common species that causes crop damage. They are biotrophic parasites that can infect over 2000 plant species.

Root-knot nematode infection manifests itself in the roots below ground. Female nematodes and their egg masses can be detected in the galls or swellings that appear on the roots. This interferes with the intake of nutrients and water, causing the plants to wilt during the warmer part of the day. Stunting, chlorosis (yellowing), and wilting of plants are some of the above-ground symptoms that are typically associated with large nematode populations (Dahal *et al*., 2009).

2.2.5.1 Genes for resistance to Root knot nematode disease

In the 1940s, sources of RKN resistance in tomato were discovered in *S. peruvinaum*, and a dominant resistance gene, *Mi*, was later discovered in a hybrid of *S. lycopersicum* and *S. peruvianum* (Medina- Filho and Stevens, 1980). *Meloidogyne incognita-1 (Mi-1)* is a single dominant gene found on chromosome 6 that has important antimicrobial properties against *Meloidogyne incognita*, *Meloidogyne arenaria*, and *Meloidogyne javanica*, but not against *Meloidogyne hapla*. This gene also gives resistance to potato aphid and whitefly (Milligan *et al*., 1998). *Mi-1* is the most important commercially available source of resistance currently and this gene confers resistance in tomato plants as early as two weeks after germination. It is a member of the NBS-LRR class of R-genes, which codes for nucleotide-binding sites and leucine-rich repeats, that has a putative coiled-coil (CC) domain before the NBS (Williamson *et al*., 2000). *Mi-1* and its homologs are separated by 300 kb into two clusters, each with three and four copies (Seah *et al*., 2007). The *Mi* locus has three genes, *Mi-1.1*, *Mi-1.2*, and *Mi-1.3*, but resistance to RKNs is conferred only by the *Mi-1.2* gene (Milligan *et al*., 1998). Other *Mi-1* homologs exist now, ranging from *Mi-1.4* to *Mi-1.7* (Rossi *et al*., 1998). These seven homologs located within two

clusters, P1 and P2, are separated by 300 kb and reside inside a 650 kb area introgressed from *S. peruvianum*. According to other research, *Mi-1.1*, *Mi-1.2*, and *Mi-1.3* are the most effective for providing resistance to *Meloidogynes sp.* (Seah *et al*., 2007). *Mi-1.2*, found on the P1 cluster, among all *Mi* genes, is the highly specialized gene providing resistance to RKNs (Goggin *et al*., 2001).

Other resistance genes have been discovered, which include *Mi-2*, *Mi-3*, *Mi-4*, *Mi-5*, *Mi-6*, *Mi-7*, *Mi-8*, *Mi-HT*, and *Mi-9* (Veremis and Roberts, 1996). It's been proposed that wild tomato possesses a genetic system capable of generating variation at the nematode resistance locus, resulting in the production of new resistance specifications. Seven of the ten RKN resistance genes discovered in tomatoes which include *Mi-2*, *Mi-3*, *Mi-4*, *Mi-5*, *Mi-6*, *Mi-9*, and *MI-HT* are heat-stable (Wu *et al*., 2009). *Mi-3* is found on chromosome 12's short arm. Mi-3 and Mi-5 have the same chromosome 12 location. *Mi-9* is a *Mi-1* homolog that has been localised to short arm of chromosome 6. *Mi-HT* is found on short arm of chromosome 6. The remaining three heat-stable resistance genes have yet to be discovered.

2.3 MOLECULAR MARKER ASSISTED SELECTION IN TOMATO FOR BIOTIC STRESS RESISTANCE

The genetics and molecular mechanism of disease resistance to a range of diseases have been thoroughly explored due to the commercial and academic relevance of tomato, and the discoveries have contributed to MAS in tomato breeding efforts. Marker-assisted selection is defined as the selection of a trait based on the genotype of a related marker rather than the trait itself (Brautigam and Gowik, 2010). The related marker functions as an indirect selection criterion (Foolad, 2007).

Tomatoes were one of the first crop species for which genetic markers were proposed as an indirect selection criteria for breeding and for which molecular markers and maps were constructed. Furthermore, the widespread use of wild tomato relatives as resistance sources rather than cultivated tomatoes necessitated the use of molecular markers to track the resistance supplied by these wild relatives and transfer it to producing new tomato varieties (Arens *et al*., 2010).

According to Pillen *et al*. (1996) and Fulton *et al*. (2002), several molecular maps of tomato have been created during the last 30 years, including a high-density linkage map based on a *S. lycopersicum S. pennellii* cross. Furthermore, molecular markers linked to genes or QTLs underlying numerous agriculturally important traits in tomato have been found and mapped, including disease and insect resistance, abiotic stress tolerance, flower and fruit-related characteristics (Foolad and Panthee, 2013). As a result of these developments, it is now possible to pick economically relevant qualities based on the genotypes of related markers rather than the trait itself, through a method known as marker-assisted selection (MAS) (Panthee and Foolad, 2012). For at least some simply-inherited disease resistance traits in tomatoes, MAS is becoming routine practice in many breeding programmes, especially in the private sector.

2.3.1 Development of genetic markers in tomato

In the 1930s, systematic breeding efforts in tomato began with the goal of improving the overall horticultural characteristics of the plant. Breeding aims became more specialised as market demand for more specific features required by the fresh-market or processing tomato industries grew, and by the 1950s superior varieties were developed for either processing or fresh-market purposes (Gardner, 1982; Gardner, 2006). However, practically all breeding programes relied entirely on phenotypic selection until the early 1980s. While phenotypic selection is still an important part of most tomato breeding programs, it comes with a number of potential drawbacks.

Some of the constraints associated with phenotypic selection could be resolved by using molecular markers and MAS, such as (1) selection of traits in the absence of a selection environment, (2) early selection of traits that are developmentally controlled or phenotypically not visible until late in the season, (3) multiple-trait selection in a single season/environment, (4) selection for recessive traits without the need for progeny testing, (5) elimination of undesirable genetic backgrounds or plant material in early breeding generations and identification of the most desirable gene combinations or individuals in segregating populations, (6) breaking the undesirable links between favourable and unfavourable alleles (reducing linkage drag), (7)

selection of low heritability traits, (8) conducting multiple rounds of selection in a single year (Collard *et al*., 2005).

2.3.2 Molecular markers for marker assisted selection

A molecular marker is a particular segment of DNA that represents differences at the genome level. They are recognisable DNA sequences located at specific locations in the genome that are passed down through the generations according to the laws of inheritance (Chetelat and Ji, 2006). Characteristics of an ideal marker include: high degree of polymorphism; even distribution across the whole genome; provide adequate resolution of genetic differences; co-dominance in expression; have linkage to distinct phenotypes and genome specific in nature (Arens *et al*., 2010).

DNA markers have the advantage of being neutral in phenotypic reactions, thus they have no pleiotropic effect on the phenotype and are not affected in their segregation and inheritance during the growth conditions of the plant. Furthermore, unlike the season-bound nature of phenotypic selection, molecular markers can be identified at any stage of plant growth, allowing breeders to select plants based on their convenience (Reddy *et al*., 2013). Also, MAS reduces linkage drag in a backcross-breeding program by selecting against the undesirable donor genome and for desirable recurrent parent genome (background selection) as well as selecting for desirable donor alleles (foreground selection) (Foolad and Panthee, 2013). Markers have been widely used in both public and private-sector breeding systems for simple traits because of these advantages. At least for some disease resistance traits, it is estimated that MAS is not only faster than PS, but also cheaper and more effective. Many other factors influence the effectiveness of MAS, including the underlying genetic control of the traits of interest. Since the early twentieth century, MAS has been feasible, if not always realistic, for a wide range of qualitative/simple traits. MAS for improving polygenic traits, on the other hand, is more difficult, even though its utility has been recognised (Bernardo and Yu, 2007; Rutkoski *et al*., 2011).

While a number of molecular markers have been identified and linked to various agriculturally significant traits in tomatoes, not all of them are effective or immediately useful in breeding programes. Most markers haven't been validated

across tomato genotypes and aren't polymorphic within tomato breeding populations, limiting their usefulness in tomato improvement programmes. If markers detect associations with traits of interest across breeding lines and populations, they are considered useful. As a result, before markers are used as indirect selection criteria on a regular basis, their utility must be evaluated across genotypes (Nevame *et al*., 2018).

2.3.2.1 PCR based molecular markers

PCR based molecular markers are effective tools for genetic analysis. Because of the technical simplicity, ease of screening a large number of samples within short period of time, and ability to be performed in a moderately equipped laboratory, PCR-based markers have been commonly used. To target DNA motifs/genes of interest, different combinations of specific, semi-specific, and arbitrary primers can be used (Stevens and Robbins, 2007).

Presently, most markers used for tomato genetic mapping and breeding purposes are PCR-based, which include randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequence (CAPS), single nucleotide polymorphism (SNP) and insertion-deletion (InDel) markers (Lee *et al*., 2015).

2.3.2.1.1 Sequence characterized amplified region (SCAR)

A SCAR is a PCR-based monolocus codominant marker that uses two specific primers derived from the nucleotide sequence of a cloned RAPD fragment linked to a trait of interest. Specific primers of SCAR sequence for amplification may be placed within or flanking the specific RAPD amplicon to recognise the polymorphism in a population. SCAR markers may be both dominant as well as codominant in nature (Lim and Ha, 2013).

It's fast, dependable, less sensitive to reaction conditions, and simple to carry out in any laboratory. It can be done with unidentified genomic DNA from any stage of development and body part (Kethidi *et al*., 2003). As a result, once established,

SCAR markers provide a realistic method for accurately screening a large number of samples at once, increasing the experiment's cost efficiency (Kasai *et al*., 2000).

RAPD-PCR amplifications generate fragment polymorphisms that may not be reproducible always (Saiki *et al*., 1988). By translating RAPDs into SCARs (sequence-characterized amplified region), this limitation can be overcome. For genotype authentication and identification, the SCAR marker has proved to be more reliable (Tartarini *et al*., 1999).

RAPD-SCAR marker is developed by purifying the PCR fragments followed by designing of SCAR primers. In brief, from the agarose gel, a polymorphic band of interest is identified, selected and eluted (Tartarini *et al*., 1999). Nucleotide sequence of the eluted DNA fragment is then determined. The polymorphic DNA band's nucleotide sequence obtained is used to compare with the known DNA sequences in the NCBI database using BLAST, to determine sequence uniqueness (Paran and Michelmore, 1993). Finally, specific SCAR primers are synthesized using the nucleotide sequence of the polymorphic DNA band.

Indirect sequencing of RAPD amplicons is another choice. In short, polymorphic DNA fragments of interest are cloned in a suitable vector and transformed into competent cells of the *E. coli* DH-5α strain. Clones are further chosen for plasmid DNA isolation. From restriction enzyme digestion, the same size fragments that correspond to the RAPD markers can be retrieved, and the ends of the cloned fragments can be sequenced. The series of the initial 10-mer primer is combined with additional 11 - 15 bases to design the specific SCAR markers. For authentication of a specific trait/genotyping, the newly developed SCAR primers are validated using PCR (Kiran *et al*., 2010).

2.3.2.1.2 Cleaved amplified polymorphic sequences (CAPS)

CAPS, or Cleaved Amplified Polymorphic Sequences, are a group of well-known markers that have been successfully used, especially in plant biology. CAPS markers are based on polymorphisms in the restriction fragment lengths caused by SNPs or Indels that either create or abolish restriction endonuclease recognition sites in PCR amplicons, generated by locus-specific oligonucleotide primers. The

principle CAPS is based on three simple successive steps: (1) PCR with specific primers; (2) restriction enzyme digestion of amplification products (amplicons) and (3) separation of digested products in an agarose gel (Heubl, 2010; Lu *et al*., 2010; Hu *et al*., 2014). CAPS markers, in particular, combine commonly used PCR techniques with traditional Restriction Fragment Length Polymorphism (RFLP) methods, with the exception that they are based on the amplification of small DNA fragments rather than the entire genome.

Since exons are more highly conserved, primers to study genes of interest are often based on sequences within them. However, introns, where polymorphisms are more likely to be discovered, should be included in the amplification fragments discovered (Lee *et al*., 2012; Lim and Ha, 2013). CAPS markers, on the other hand, can be established successfully in other parts of the genome. CAPS primers may be generated using nucleotide sequences from published articles, databases, or additional experiments to develop sequences. As a result, the first step of CAPS, amplification of a particular gene of interest fragment, is actually a routine PCR.

The second step in CAPS is the application of endonucleases, and the possibility of genetic variation among DNA samples is critical. Single Nucleotide Polymorphisms (SNPs) and Insertion-Deletions (InDels) are the most common types of genetic variations in DNA (Wu *et al*., 2014; Jiang *et al*., 2015). Plant species, population, specific gene of interest, and even the amplicon location (introns, exons or non-coding regions) in the genome, all influence the frequency of genetic changes in DNA. Higher frequencies of genetic polymorphism in samples are clearly linked to easier and more successful CAPS marker development. Thus the development and application of CAPS markers are dependent on genetic differences in restriction enzyme recognition sites (Neff *et al*., 1998; Li *et al*., 2012). CAPS markers depend much on the detection of modified or unmodified restriction enzyme recognition sites in amplicons.

Traditionally, restriction products were isolated on agarose or polyacrylamide gels, but modern technology now permits for capillary separation of digested and fluorescence-labeled PCR products (Perovic *et al*., 2013). For studies with a large

number of samples, this approach will substantially speed up the identification of restriction fragments in CAPS markers.

CAPS markers are codominant in nature, allowing homozygotes and heterozygotes to be easily distinguished during genotyping (Shavrukov, 2016). CAPS are thus an ideal fit as an extra tool for more detailed and precise analyses, and this can provide a significant benefit in genetic investigations.

2.3.3 Marker assisted selection in tomato breeding program

Marker assisted selection has recently become a standard practice in many tomato-breeding programmes, due to the development of new molecular markers and maps, for a variety of reasons. First, to screen seed lots with a panel of molecular markers, MAS are frequently used to determine hybrid purity from overseas production. Second, MAS can be used to quickly screen germplasm for disease resistance or fruit quality when accurate markers closely linked to resistance genes are identified. Third, after definitive linkages between markers and simple traits of interest are established, MAS is used for marker-assisted backcrossing (MAB) (Brouwer and St. Clair, 2004; Collard *et al*., 2005).

For the usage of molecular markers in breeding populations, there must be phenotypic and genetic polymorphism and segregation in the breeding population for the trait of interest. A genetic linkage relationship must be established between genes or QTLs of interest and genetic markers. Polymorphism in the population for the associated genetic markers is needed. Because of the low prevalence of marker polymorphism within breeding populations, this has been a major problem in the use of molecular markers for tomato breeding (Stevens and Robbins, 2007). The majority of tomato genetic maps are focused on interspecific crosses between cultivated and related wild tomato species, where marker polymorphism is abundant. This is particularly problematic when the wild species is only distantly related to the cultivated tomato, such as *S. pennellii*, which was used to create the tomato high-density molecular linkage map (Pillen *et al*., 1996). Most tomato breeding populations, on the other hand, are focused on intraspecific crosses within the cultigen or crosses between closely related wild species like *S. pimpinellifolium.*

When compared to wide crosses, those populations have far less marker polymorphism. As a result, attempts must be made to identify markers in breeding populations with a higher rate of polymorphism and to enable their use in large populations, markers must be high throughput and economically affordable. Finally, the linkage between the gene or QTL of interest and the genetic marker must be strong enough to prevent unintended crossing-over, which could lead to false positive selection. The best genetic markers in this regard are those found within the gene of interest (Lecomte *et al*., 2004; Arens *et al*., 2010).

For many simple disease resistance traits in tomatoes, it appears that MAS is not only quicker, but also less expensive and more efficient. Over 35 pathogen resistance genes have been identified and mapped in tomato (van Ooijen *et al*., 2007). MAS is commonly used in the tomato seed industry to select for qualitative disease resistance traits such as fusarium wilt, late blight, verticillium wilt, bacterial wilt, *tomato yellow leaf curl virus*, and root-knot nematodes.

Table 1. Molecular markers associated with genes for resistance to *Tomato yellow leaf curl virus disease (TYLCVD)*

Marker	Type of marker	Gene	Chr. No.	Marker information	References
TG178	CAPS	*Ty1*	6	F:GGTACTCCTGGAAGGGTTAAGG R: CACGCTGGTTCTGTTGTATCTC (*TaqI*)	Barbieri, *et al*. (2010)
JB1	CAPS	*Ty1*	6	F: AACCATTATCCGGTTCACTC R: TTTCCATTCCTTGTTTCTCTG (*TaqI*)	Pérez, *et al*. (2007)
TG105A	CAPS	*Ty2*	11	F:CTTCAGAATTCCTGTTTTAGTCAGTTGAACC R: ATGTCACATTTGTTGCTTGGACCATCC (*TaqI*)	Maxwell, *et al*. (2006)
T0302	SCAR	*Ty2*	11	F: TGGCTCATCCTGAAGCTGATAGCGC R: AGTGTACATCCTTGCCATTGACT	Yang, *et al*. (2014)
FER-G8	CAPS	*Ty3*	6	F: CATCCCGTGCATCATCCAAAGTGAC R: CTAAGGGTGTACCCCAAGGGAAC (*TaqI*)	Jensen, *et al*. (2007)
P6- 25	SCAR	*Ty3*	6	F: GGTAGTGGAAATGATGCTGCTC R: GCTCTGCCTATTGTCCCATATATAACC	Ji, *et al*. (2008)
C2_At4g17300	CAPS	*Ty4*	3	F:ATTTAACCGTGTCTGGGCAACTCAATGG R: GCTCACTTTGCAAATCACATCCCCATTTCACC (*AflI*)	Ji, *et al*. (2009)
TG0302/TY2R1	SCAR	*Ty2*	11	F: TGGCTCATCCTGAAGCTGATAGCGC R:TGAT(T/G)TGATGTTCTC(T/A)TCTCT(C/A)GCCTG	Brenda, *et al*. (2007)
FLUW25	SCAR	*Ty3*	6	F:CAAGTGTGCATATACTTCATA(T/G)TCACC R:CCATATATAACCTCTGTTTCTATTTCGAC	Melinda, *et al*. (2007)

Table 2. Molecular markers associated with genes for resistance to Verticillum wilt

Marker	Type of marker	Gene	Chr. No.	Marker information	References
V1LeO2 new	CAPS	*Ve1*	9	F: GTTACATGCAATCTCTTTGG R: AGAGTAGTCCACATAGATGG (*Xba*I)	Acciarri, *et al.* (2007)
V2LeO3	CAPS	*Ve2*	9	F: CAAACATAGCTGGAAGAATC R:TAGGAGGAAAAGAATTGG (*Hinc*II)	Acciarri, *et al.* (2007)
Ve1-XbaI	CAPS	*Ve1*	9	F: CGAACTTGACTACATTGACCCTG R:CAGTCTTGAAAGGTTGCTCAGCC (*Xba*I)	Jung, *et al*. (2015)

Table 3. Molecular markers associated with genes for resistance to Fusarium wilt

Marker	Type of marker	Gene	Chr. No.	Marker information	References
At2	SCAR	*I1*	11	F: CGAATCTGTATATTACATCCGTCGT R: GGTGAATACCGATCATAGTCGAG	Arens, *et al*. (2010)
Z1063	SCAR	*I2*	11	F: ATTTGAAAGCGTGGTATTGC R: CTTAAACTCACCATTAAATC	Arens, *et al*. (2010)
P7-43DF1/ R1	SCAR	*I3*	7	F: GGTAAAGAGATGCGATGATTATGTGGAG R: GTCTTTACCACAGGAACTTTATCACC	Barillas, *et al*. (2008)
CAPS7774	CAPS	*I7*	8	F: AAGAAGTTCCCTTCTTCCCTTA R: GGAATAACCAAGGGGGTGTT (*AgeI*)	Gonzalez-Cendales, *et al.* (2016)

Table 4. Molecular markers associated with genes for resistance to Late blight (LB)

Marker	Type of marker	Gene	Chr. No.	Marker information	References
dTG63	CAPS	*Ph2*	10	F: CTACTCTTTCTATGCAATTTGAATTG R: AATAATTTTCAACCATAGAATGATT (*Hinf*I)	Panthee, *et al*. (2012)
Ph3-MspI	CAPS	*Ph3*	9	F: TCGATCGTATGTAGACGATG R: AGGCAAATCTTGAAGAAGCA (*Msp*I)	Jung, *et al*. (2015)
Ph3-SCAR	SCAR	*Ph3*	9	F: CTACTCGTGCAAGAAGGTAC R: TCCACATCACCTGCCAGTTG	Jung, *et al*. (2015)
TG328	CAPS	*Ph2*	10	F: GGTGATCTGCTTATAGACTTGGG R: AAGGTCTAAAGAAGGCTGGTGC (*BstN*1)	Robbins, *et al*. (2010)
TG591	CAPS	*Ph2*	10	F: AAGGCAAAGGAAGTTGGAGGTCA R: AGAGGTTGCAACTCGTGGATTGAG (*Acl*I)	Robbins, *et al*. (2010)

Table 5. Molecular markers associated with genes for resistance to Root knot nematode

Marker	Type of marker	Gene	Chr. No.	Marker information	References
Mi-23	SCAR	*Mi1.2*	6	F: TGGAAAAATGTTGAATTTCTTTTG R: GCATACTATATGGCTTGTTTACCC	Seah, *et al*. (2007)
Mi-J CAPS	CAPS	*Mi-J*	6	F: CTACGGAGGATGCAAATAGAA R: AATCATTATTGTCACACTTCCCC (*Nt*III)	Hoogstraten and Braun, (2005)
Mi1 CAPS	CAPS	*Mi 1*	6	F: AACCGTGGACTTTGCTTTGACT R: TAAGAACAGGGACTCAGAGGATGA (*Taq*I)	Hoogstraten and Braun, (2005)

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled “Locating donors for genes for biotic stress resistance in tomato through molecular marker assisted selection” was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellayani. This chapter details the experimental methods and materials adopted for the conduct of the study.

3.1 MAINTENANCE OF PLANT SAMPLES

3.1.1 Experimental material

Seeds of 30 tomato genotypes including cultivated varieties and wild species were collected for the study (Table 6). 5-7 seeds of each genotype were soaked overnight and planted in nursery bags. The potting mixture consisted of 1:1 ratio of coir pith and vermi-compost mixed in soil. The nursery bags were maintained in polyhouse and watered twice on a daily basis. The seeds started to germinate and sprout within 8 days. All the planted genotypes at the stage of two week were transplanted to medium sized pots.

3.1.2 Sample collection

Young leaves of three week old tomato genotypes were harvested and wrapped in aluminium foil. The leaf samples kept inside an ice chamber was immediately taken to the laboratory, where they were maintained at -20°C to retain the freshness. Prior to DNA isolation, the harvested leaves were thoroughly rinsed with distilled water to clean the leaf surface and sliced into smaller pieces using a sterilized scissor.

Table 6. List of tomato genotypes used in the study

Sl. no	Genotypes	Source of genotypes
1.	*Solanum pimpinellifolium*-EC 697275	NBPGR, New Delhi
2.	IIHR 2205	IIHR, Bangalore
3.	Akshaya	Kerala Agricultural University
4.	Arka Rakshak	IIHR, Bangalore
5.	EC 514124	NBPGR, New Delhi
6.	IIHR 2374	IIHR, Bangalore
7.	IIHR 2373	IIHR, Bangalore
8.	LA 1206	UAS Bangalore
9.	Vellayani Vijai	Kerala Agricultural University
10.	IC-45	NBPGR, New Delhi
11.	IIHR 2868	IIHR, Bangalore
12.	Shakthi	Kerala Agricultural University
13.	Pusa Ruby	IARI, New Delhi
14.	Nenmara local	Kerala
15.	CA 22053	Kerala
16	Manulekshmi	Kerala Agricultural University
17.	EC 620419	NBPGR, New Delhi
18.	IIHR 2204	IIHR, Bangalore
19.	Alathur local	Kerala
20.	Palakkad local	Kerala
21.	Hisar Lalit	CCSHAU, Hisar
22.	LA 1805	UAS, Bangalore
23.	Kuttichal local	Kerala
24.	Kashi Vishesh	IIVR, Varanasi
25.	IIHR 26	IIHR, Bangalore
26.	PKM 1	Tamil Nadu Agricultural University
27.	Arka Vikas	IIHR, Bangalore
28.	EC 677034	NBPGR, New Delhi
29.	PNR 7	Tamil Nadu Agricultural University
30.	*Solanum torvum*	Kerala

3.2 MOLECULAR SCREENING FOR GENES FOR RESISTANCE IN TOMATO GENOTYPES

3.2.1 DNA Isolation

The availability of good quality DNA is a prerequisite of any molecular biology experiments. The DNA extraction technique should be quick, affordable and simple, yielding a good quantity of intact DNA of reasonable purity from minimal amounts of tissue and extraction chemicals. Proper care and precautions must be taken in to account in order to obtain good quality DNA from plant samples.

Disruption of the cell wall, cell membrane and nuclear membrane to release the DNA into solution, followed by precipitation of the DNA while ensuring removal of contaminating biomolecules such as proteins, polysaccharides, lipids, phenols and other secondary metabolites, were the basic steps in all plant DNA extraction protocols. However, extracting good-quality DNA from plants has proven to be difficult. The fact that different plant species contain differing quantities of polysaccharides, polyphenols and other secondary metabolites was the reason. These components affect the quality and quantity of the extracted DNA. It's unlikely that a single DNA isolation protocol will work for all plant tissues. The majority of the DNA extraction techniques based on cetyltrimethyl ammonium bromide (CTAB) method targets internal components of each individual plant species.

DNA isolation of the 30 tomato genotypes used for the study was performed based on cetyltrimethylammonium bromide (CTAB) method with minor modifications (Murray and Thompson, 1998) and the procedure followed was briefly described below:

1 mL of extraction buffer (Appendix I) was preheated in a water bath maintained at 65°C for 30 minutes. Immediately before use, 1% Poly vinyl pyrrolidone (PVP) and 2% 2-β-mercaptoethanol was added freshly to the extraction buffer. 0.2 g leaf sample was ground with liquid nitrogen in a pre-chilled mortar and pestle and the powered samples were immediately added

with 1mL pre-heated extraction buffer to homogenize the contents, which was then transferred into a 2ml microfuge tube. After thorough mixing, the samples were incubated in water bath for 30 minutes at 65°C with intermittent mixing in-between, after every 10 minutes. The centrifuge 5804 R (eppendorf) was precooled to 4° C and the samples cooled to room temperature were spun at 13000 rpm for 15 minutes. The upper aqueous layer was carefully separated and transferred to a sterile tube. 5µl of RNase was added to the supernatant separated and incubated at 65°C for 15 minutes. After incubation, 25:24:1 ratio of Phenol: Chloroform: Isoamyl-alcohol was added in equal proportion to the supernatant, which was then gently inverted for 1-2 minutes to mix the contents and centrifuged at 10,000 rpm for 5 minutes. The supernatant was collected and equal volume of 24:1 ratio of Chloroform: Isoamyl-alcohol was added. The supernatant was slightly inverted to mix with Chloroform: Isoamyl-alcohol and centrifuged at 13,000 rpm for 10 minutes. Supernatent was obtained and added Sodium acetate, $1/10^{th}$ to the volume of the supernatant and isopropanol to equal volume of the supernatant. Tube was gently inverted until the formation of DNA threads was visible. The tubes were incubated at -20°C for 30 minutes and centrifuged at 13,000 rpm for 5 minutes. Supernatant was discarded and pellet was collected. The tubes containing DNA pellets were inverted on tissue paper to completely drain off the supernatant and allowed to air dry at room temperature for 25 minutes. The air dried pellets were washed twice using 500µl of 70% ethanol with centrifugation at 11,000 rpm for 5 minutes. The washed pellets were air dried to remove the traces of ethanol and dissolved in 80 µl 1X TE buffer (Appendix I). The DNA pellets dissolved in 1X TE buffer was aliquoted and stored at -80°C.

3.2.1.1 Agarose gel electrophoresis

The quality of the DNA isolated was checked using agarose gel electrophoresis. 5 µl DNA sample mixed with 2 µl of 6X loading dye was loaded on 0.8% agarose gel. Electrophoresis (Sub Cell GT, BIO-RAD) was carried out at 65V for 30 minutes using 1X TAE buffer (Appendix II). The gel

profile of the DNA samples was visualized with Syngene G: BOX Chemi XX6 gel documentation system using HyGene software.

3.2.1.2 Spectrophotometric analysis

UV absorbance measurement with a spectrophotometer is the standard technique for performing concentration and purity measurements. The absorption of UV light is used to determine nucleic acid concentrations. According to the Beer-Lambert law, the amount of light absorbed at 260 nm is proportional to the concentration of nucleic acid in solution.

The A260/A280 gives information about the type of nucleic acid present (dsDNA or RNA) as well as a general estimate of purity. Protein contamination is typically detected by a decrease in this ratio; RNA contamination is typically recognised by an increase in this ratio. Pure dsDNA has an A260/A280 ratio of 1.8 - 2.0 in buffered solutions. The concentration of dsDNA in sample is estimated by the formula:

$$\text{dsDNA concentration (ng/µl)} = OD_{260} \text{ x dilution factor x } 50$$

The concentration (ng/µl) and purity (A_{260}/A_{280}) of the isolated DNA was determined with UV-VIS Spectrophotometer (SL 218 ELICO). Cuvette containing 5 µl of DNA dissolved in 2995 µl of TE buffer with a dilution factor of 600 was used for obtaining the O. D. values at wavelength 260 nm and 280 nm.

3.2.2 PCR analysis

Molecular screening of genes for resistance to biotic stresses in 30 tomato genotypes was determined with gene specific molecular markers. Genes providing resistance to five vulnerable diseases namely, *Tomato yellow leaf curl virus* disease (TYLCVD) *caused by Tomato yellow leaf curl virus,* verticillium wilt caused by *Verticillium* spp., fusarium wilt caused by *Fusarium oxysporum*, late blight caused by *Phytophthora infestans* and root knot caused by *Meloidogyne* spp., were studied with respective SCAR and CAPS markers (Table 7).

Table 7. Details of molecular markers and primer sequences used in the study

Genes for resistance	Marker name and type	Primer sequence	Expected Amplicon size (bp)		Annealing temperature	References
			Resistant	Susceptible		
Ty 2	T0302F/TY2R1 - SCAR	F:5'TGGCTCATCCTGAAGCTGATAGCGC 3' R:5'TGAT(T/G)TGATGTTCTC(T/A)TCTCT(C/A)GCCT G 3'	600bp	450bp	55°C	Brenda, *et al*. (2007)
Ty 3	FLUW25-SCAR	F:5'CAAGTGTGCATATACTTCATA(T/G)TCACC3' R:5'CCATATATAACCTCTGTTTCTATTTCGAC3'	640bp	480 bp	53°C	Melinda, *et al*. (2007)
Ve 1	Ve1 XbaI-CAPS	F: 5' CGAACTTGACTACATTGACC 3' R: 5' CAGTCTTGAAAGGTTGCTCA 3' Restriction enzyme: *Xba I*	410bp, 332bp	410bp,310bp,22bp	55°C	Jung, *et al*. (2015)
Ve 2	V2LeO3F/V2LeO3R-CAPS	F: 5' CAAACATAGCTGGAAGAATC 3' R: 5' TAGGAGGAAAAGAATTGG 3' Restriction enzyme: *HincII*	428bp, 601bp	1029bp	47 °C	Acciarri, *et al*. (2007)
I3	P743DF1/R1-SCAR	F:5'GGTAAAGAGATGCGATGATTATGTGGAG3' R:5'GTCTTTACCACAGGAACTTTATCACC 3'	1270bp	1060bp	53°C	Barillas, *et al.* (2008)
I7	CAPS7774-CAPS	F: 5' AAGAAGTTCCCTTCTTCCCTTA 3' R: 5' GGAATAACCAAGGGGGTGTT 3' Restriction enzyme: *AgeI*	612bp, 196bp	808bp	55°C	Gonzalez-Cendales, *et al*. (2016)
Ph2	dtG63-CAPS	F:5'CTACTCTTTCTATGCAATTTGAATTG 3' R:5'AATAATTTTCAACCATAGAATGATT 3' Restriction enzyme: *HinfI*	245bp	221bp	55°C	Panthee and Foolad, (2012)
Ph3	Ph3-SCAR	F: 5' CTACTCGTGCAAGAAGGTAC 3' R: 5' TCCACATCACCTGCCAGTTG 3'	176bp	154bp	55°C	Jung, *et al*. (2015)
Mi-1.2	Mi23-SCAR	F: 5' TGGAAAAATGTTGAATTTCTTTTG 3' R: 5' GCATACTATATGGCTTGTTTACCC 3'	380bp	430bp	57°C	Seah, *et al*. (2007)

3.2.2.1 Annealing temperature optimization

Gradient PCR was carried out to primer pairs of molecular markers Ve1 XbaI, V2LeO3F/V2LeO3R, CAPS7774, dtG63, Ph3-SCAR and Mi23 with the annealing temperatures set at 47, 50, 52 and 55°C to standardize it. PCR reaction mixture of 25 µl (Table 8) was prepared and amplification reaction was performed using nexus gradient Mastercycler (Eppendorf) (Table 9). The amplified products were resolved on a 2.5% agarose gel and visualized with Syngene G: BOX Chemi XX6 gel documentation system using HyGene software.

3.2.2.2 PCR profile

PCR reaction mixture containing 20 ng/ µl of template DNA, 5 µM of each primer, 1mM of dNTPs, 10X PCR buffer, 2.5mM $MgCl_2$, and 3U/µl of Taq DNA polymerase was prepared with a reaction volume of 25 µl (Table 10). Amplification of the molecular markers corresponding to the genes for resistance studied was carried out using nexus gradient Mastercycler (Eppendorf). Amplification was performed with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min and extension at 72°C for 2 min. Final extension at 72°C of the amplification was extended to 8 min (Table 11). The PCR products after amplification were stored at -20°C and resolved on 2.5% agarose gel using 1X TAE buffer at 65V, until the loading dye moved about ¾th of the gel. The gel profile of the amplified PCR products were visualized with Syngene G: BOX Chemi XX6 gel documentation system using HyGene software.

Table 8. Reaction mix used for annealing temperature standardization

Reagent	Concentration	Volume (µl)
Buffer	10X	2.5µl
MgCl2	2.5mM	2.5µl
Forward primer	5µM	2.5µl
Reverse primer	5µM	2.5µl
dNTP	1mM	5µl
Taq Polymerase	3U/µl	0.5µl
Template DNA	20 ng/ µl	2.5µl
Nuclease free water	---	7 µl
Total volume	---	25 µl

Table 9. Thermal profile used for annealing temperature standardization

Steps	Stage	Repeat	Temperature (°C)	Time (s)
Initial denaturation	1	1	94	300
Denaturation	2	35	94	30
Annealing			47/50/52/55	60
Primer extension			72	120
Final extension	3	1	72	480

Table 10. Reaction mix used for PCR reaction

Reagent	Concentration	Volume (µl)
Buffer	10X	2.5µl
$MgCl_2$	2.5mM	2.5µl
Forward primer	5µM	2.5µl
Reverse primer	5µM	2.5µl
dNTP	1mM	5µl
Taq Polymerase	3U/µl	0.5µl
Template DNA	20 ng/µl	2.5µl
Nuclease free water	---	7 µl
Total volume	---	25 µl

Table 11. Thermal profile used for PCR reaction

Steps	Stage	Repeat	Temperature (°C)	Time (s)
Initial denaturation	1	1	94	300
Denaturation	2	35	94	30
Annealing			55	60
Primer extension			72	120
Final extension	3	1	72	480

Table 12. Reaction mix used for restriction digestion

Reagent	Concentration	Volume(µl)
PCR reaction mixture	0.1-0.5 µg of DNA	10µl
Buffer- Tango/O/R	10X	2ul
Restriction Enzyme-*HincII/XbaI/AgeI/HinfI*	10U/µl	1µl
Nuclease-free water	-	17µl
Total volume		30 µl

3.2.3 Restriction digestion

The amplified PCR products of the CAPS markers Ve1 XbaI, V2LeO3F/V2LeO3R, dtG63, and CAPS7774 of the genes *Ve1, Ve2, Ph2* and *I7* were restriction digested to distinguish the genotypes with alleles of genes for resistance from the genotypes without alleles of genes for resistance. Restriction digestion mixture of 30 µl (Table 12) was prepared, gently mixed and incubated in a water bath maintained at 37°C for 16 hours. The restriction digested products were resolved on a 2.5% agarose gel in 1X TAE buffer at 65V, until the loading dye moved about ¾th of the gel. The restriction digested products were visualized with Syngene G: BOX Chemi XX6 gel documentation system using HyGene software.

RESULTS

4. RESULTS

The results of the study entitled “Locating donors for genes for biotic stress resistance in tomato through molecular marker assisted selection” conducted in the Department of Seed Science and Technology, College of Agriculture, Vellayani during 2020-2021 is detailed in this chapter.

4.1 MOLECULAR MARKER BASED SCREENING OF TOMATO GENOTYPES FOR GENES FOR RESISTANCE TO BIOTIC STRESSES

4.1.1 DNA Isolation

DNA isolation of 30 tomato genotypes was carried out with cetyltrimethylammonium bromide (CTAB) method. The isolated DNA samples (Plate 1) were eluted to a final volume of 80 µl with TE buffer and stored at -80°C. The quality of the samples was analysed using agarose gel electrophoresis and gel profile was visualized with Syngene G: BOX Chemi XX6 gel documentation system using HyGene software.

4.1.2 Quantitative (ng/µl) and qualitative analysis (A260/A280) of the isolated DNA

The yield as well as purity of the isolated DNA of the 30 tomato genotypes were analysed using Spectrophotometer. The purity and concentration of the isolated DNA ranged from 1.684 to 2.248 and 354 ng/µl to 3222 ng/µl (Table 13).

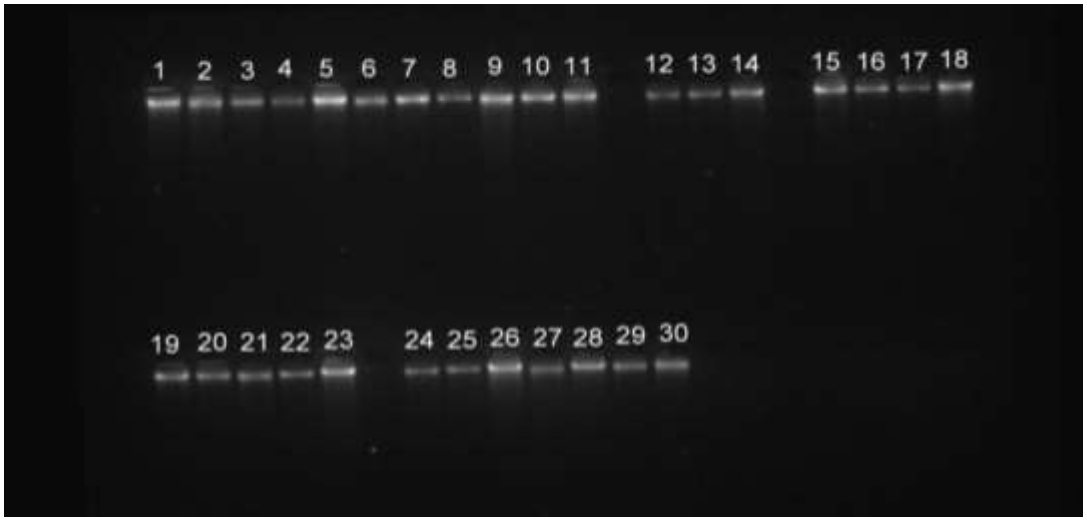

Plate 1: Gel profile of the DNA isolated from 30 genotypes resolved on 0.8% agarose gel Lane 1- *Solanum pimpinellifolium* -EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

Table 13. Quantitative and qualitative analysis of the isolated DNA

Sl. no	Genotypes	Absorbance at 260 nm	Absorbance at 280 nm	OD Ratio A260/A280	DNA Conc(ng/µl)
1.	*Solanum pimpinellifolium*- EC 697275	0.0365	0.020	1.825	1095
2.	IIHR 2205	0.029	0.016	1.812	870
3.	Akshaya	0.0221	0.011	2.009	663
4.	Arka Rakshak	0.0195	0.0097	2.010	585
5.	EC 514124	0.0306	0.0159	1.924	918
6.	IIHR 2374	0.0176	0.010	1.76	528
7.	IIHR 2373	0.032	0.019	1.684	960
8.	LA 1206	0.0153	0.0075	2.04	459
9.	Vellayani Vijai	0.1074	0.0588	1.826	3222
10.	IC-45	0.0598	0.032	1.868	1794
11.	IIHR 2868	0.0406	0.021	1.93	1218
12.	Shakthi	0.0236	0.0139	1.697	708
13.	Pusa Ruby	0.0254	0.0139	1.827	762
14.	Nenmara local	0.0369	0.019	1.942	1107
15.	CA 22053	0.0453	0.025	1.769	1359
16	Manulekshmi	0.022	0.013	1.692	660
17.	EC 620419	0.0201	0.011	1.827	603
18.	IIHR 2204	0.0521	0.028	1.86	1563
19.	Alathur local	0.0448	0.025	1.792	1344
20.	Palakkad local	0.0255	0.014	1.821	765
21.	Hisar Lalit	0.0278	0.015	1.853	834
22.	LA 1805	0.0118	0.0055	2.145	354
23.	Kuttichal local	0.0351	0.020	1.755	1053
24.	Kashi Vishesh	0.013	0.006	2.147	393
25.	IIHR 26	0.0174	0.0083	2.096	522
26.	PKM 1	0.0652	0.0290	2.248	1956
27.	Arka Vikas	0.0253	0.012	2.00	759
28.	EC 677034	0.0263	0.014	1.813	789
29.	PNR 7	0.0208	0.011	1.89	624
30.	*Solanum torvum*	0.0285	0.016	1.78	855

4.1.3 Standardization of annealing temperature

Annealing temperature standardization of the primer pairs of SCAR and CAPS molecular markers were determined using gradient PCR. Gradient temperatures ±5°C from the reported annealing temperatures were setup for the standardization. The Gradient PCR products at different temperatures were resolved on 2.5% agarose gel and the gradient temperature at which the most clear banding pattern generated on agarose gel was determined to be the standardized annealing temperature (Table 14).

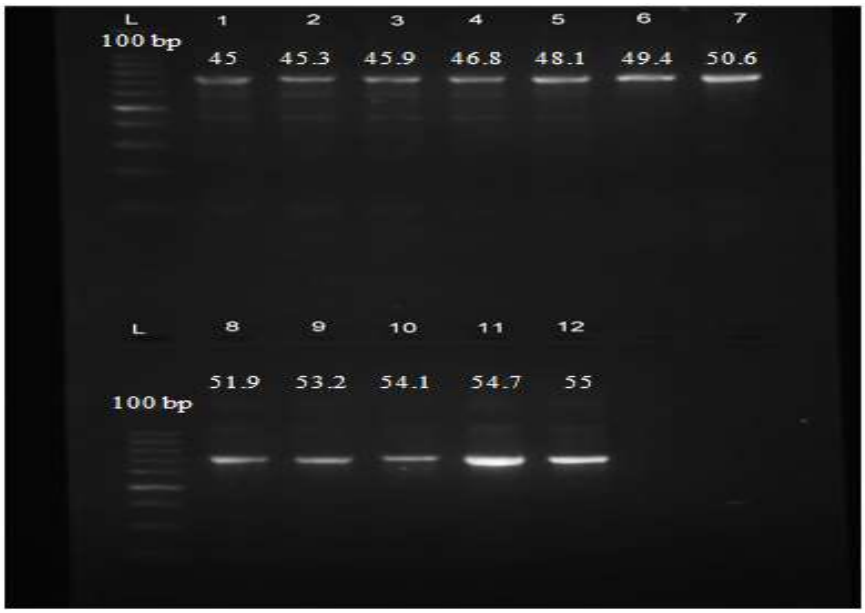

Plate 2: Gel profile of the gradient PCR of the marker Ve1 XbaI for the gene *Ve1* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- PCR product amplified at 45°C; Lane 2- PCR product amplified at 45.3°C; Lane 3- PCR product amplified at 45.9°C; Lane 4- PCR product amplified at 46.8°C; Lane 5- PCR product amplified at 48.1°C; Lane 6- PCR product amplified at 49.4°C; Lane 7- PCR product amplified at 50.6°C; Lane 8- PCR product amplified at 51.9°C; Lane 9- PCR product amplified at 53.2°C; Lane 10- PCR product amplified at 54.1°C; Lane 11- PCR product amplified at 54.7°C; Lane 12- PCR product amplified at 55°C.

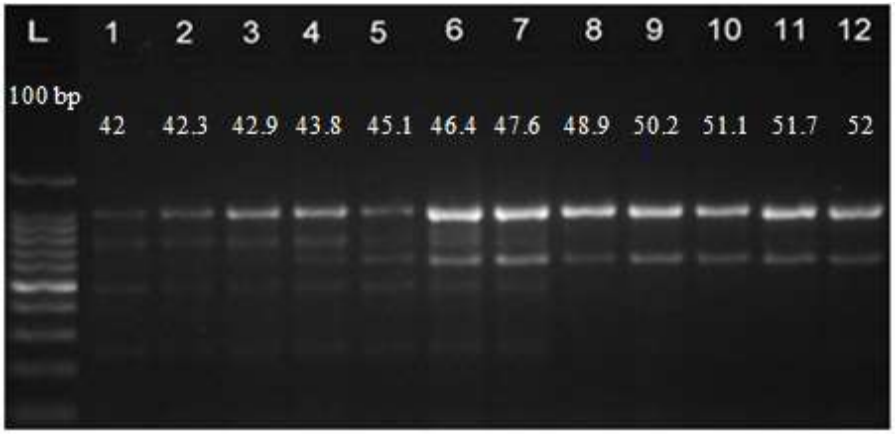

Plate 3: Gel profile of the gradient PCR of the marker V2LeO3F/V2LeO3R for the gene *Ve2* resolved on 2.5 % agarose gel Lane L- 100 bp DNA ladder; Lane 1- PCR product amplified at 42°C; Lane 2- PCR product amplified at 42.3°C; Lane 3- PCR product amplified at 42.9°C; Lane 4- PCR product amplified at 43.8°C; Lane 5- PCR product amplified at 45.1°C; Lane 6- PCR product amplified at 46.4°C; Lane 7- PCR product amplified at 47.6°C; Lane 8- PCR product amplified at 48.9°C; Lane 9- PCR product amplified at 50.2°C; Lane 10- PCR product amplified at 51.1°C; Lane 11- PCR product amplified at 51.7°C; Lane 12- PCR product amplified at 52°C.

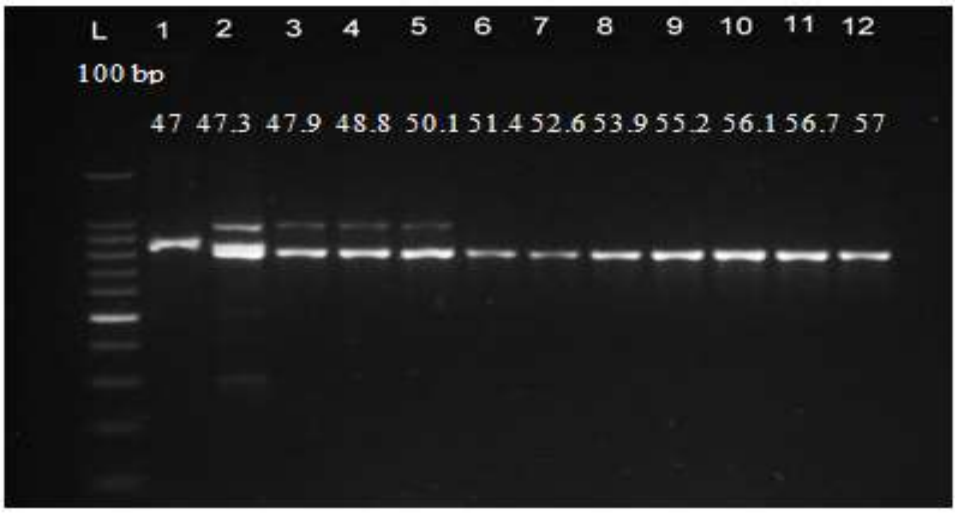

Plate 4: Gel profile of the gradient PCR of the marker CAPS7774 for the gene *I7* resolved on 2.5 % agarose gel Lane L- 100 bp DNA ladder; Lane 1- PCR product amplified at 47°C; Lane 2- PCR product amplified at 47.3°C; Lane 3- PCR product amplified at 47.9°C; Lane 4- PCR product amplified at 48.8°C; Lane 5- PCR product amplified at 50.1°C; Lane 6- PCR product amplified at 51.4°C; Lane 7- PCR product amplified at 52.6°C; Lane 8- PCR product amplified at 53.9°C; Lane 9- PCR product amplified at 55.2°C; Lane 10- PCR product amplified at 56.1°C; Lane 11- PCR product amplified at 56.7°C; Lane 12- PCR product amplified at 57°C.

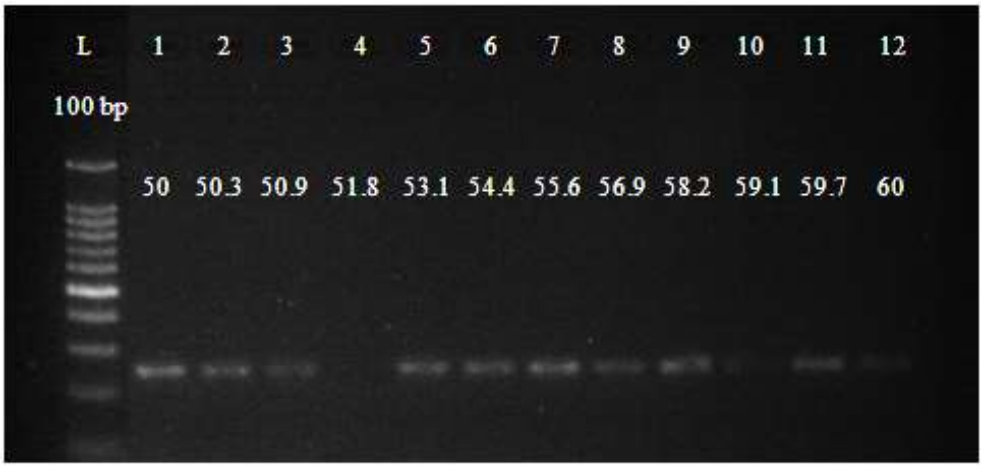

Plate 5: Gel profile of the gradient PCR of the marker dtG63 for the gene *Ph2* resolved on 2.5 % agarose gel Lane L- 100 bp DNA ladder; Lane 1- PCR product amplified at 50°C; Lane 2- PCR product amplified at 50.3°C; Lane 3- PCR product amplified at 50.9°C; Lane 4- PCR product amplified at 51.8°C; Lane 5- PCR product amplified at 53.1°C; Lane 6- PCR product amplified at 54.4°C; Lane 7- PCR product amplified at 55.6°C; Lane 8- PCR product amplified at 56.9°C; Lane 9- PCR product amplified at 58.2°C; Lane 10- PCR product amplified at 59.7°C; Lane 11- PCR product amplified at 56.7°C; Lane 12- PCR product amplified at 60°C.

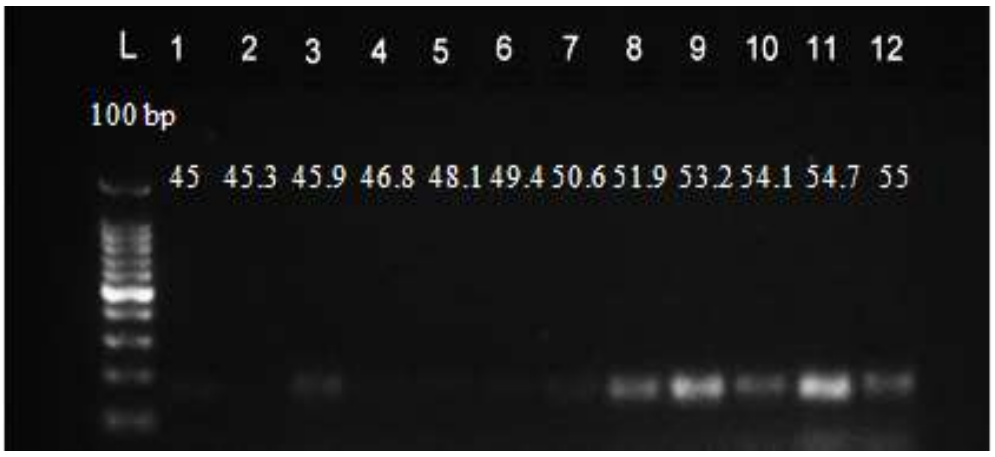

Plate 6: Gel profile of the gradient PCR of the marker Ph3-SCAR for the gene *Ph3* resolved on 2.5 % agarose gel Lane L- 100 bp DNA ladder; Lane 1- PCR product amplified at 45°C; Lane 2- PCR product amplified at 45.3°C; Lane 3- PCR product amplified at 45.9°C; Lane 4- PCR product amplified at 46.8°C; Lane 5- PCR product amplified at 48.1°C; Lane 6- PCR product amplified at 49.4°C; Lane 7- PCR product amplified at 50.6°C; Lane 8- PCR product amplified at 51.9°C; Lane 9- PCR product amplified at 53.2°C; Lane 10- PCR product amplified at 54.1°C; Lane 11- PCR product amplified at 54.7°C; Lane 12- PCR product amplified at 55°C.

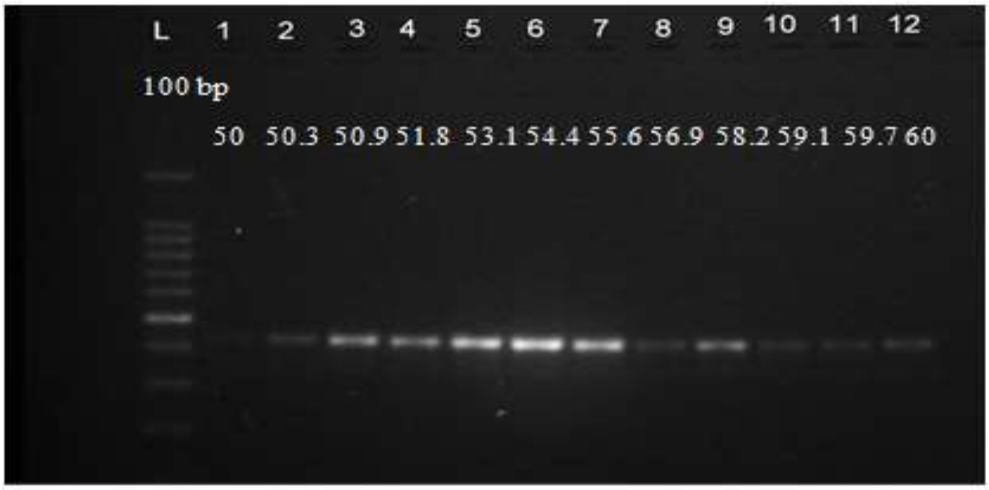

Plate 7: Gel profile of the gradient PCR of the marker Mi23 for the gene *Mi-1.2* resolved on 2.5 % agarose gel Lane L- 100 bp DNA ladder; Lane 1- PCR product amplified at 50°C; Lane 2- PCR product amplified at 50.3°C; Lane 3- PCR product amplified at 50.9°C; Lane 4- PCR product amplified at 51.8°C; Lane 5- PCR product amplified at 53.1°C; Lane 6- PCR product amplified at 54.4°C; Lane 7- PCR product amplified at 55.6°C; Lane 8- PCR product amplified at 56.9°C; Lane 9- PCR product amplified at 58.2°C; Lane 10- PCR product amplified at 59.7°C; Lane 11- PCR product amplified at 56.7°C; Lane 12- PCR product amplified at 60°

Table 14: Standardized annealing temperature of primer pairs of molecular markers

Genes for resistance	Marker name	Primer sequence	Standardized annealing temperature
Ty 2	T0302F/ TY2R1-SCAR	F: 5'TGGCTCATCCTGAAGCTGATAGCGC 3' R: 5'TGAT(T/G)TGATGTTCTC(T/A)TCTCT(C/A)GCCTG 3'	55°C
Ty 3	FLUW25 -SCAR	F: 5'CAAGTGTGCATATACTTCATA(T/G)TCACC3' R: 5'CCATATATAACCTCTGTTTCTATTTCGAC3'	53°C
Ve 1	Ve1 XbaI-CAPS	F: 5' CGAACTTGACTACATTGACC 3' R: 5' CAGTCTTGAAAGGTTGCTCA 3' Restriction enzyme: *Xba I*	55°C
Ve 2	V2LeO3 F/V2LeO 3R - CAPS	F: 5' CAAACATAGCTGGAAGAATC 3' R: 5' TAGGAGGAAAAGAATTGG 3' Restriction enzyme: *HincII*	47 °C
I3	P743DF1 /R1-SCAR	F: 5' GGTAAAGAGATGCGATGATTATGTGGAG3' R: 5' GTCTTTACCACAGGAACTTTATCACC 3'	53°C
I7	CAPS77 74-CAPS	F: 5' AAGAAGTTCCCTTCTTCCCTTA 3' R: 5' GGAATAACCAAGGGGGTGTT 3' Restriction enzyme: *AgeI*	55°C
Ph2	dtG63-CAPS	F: 5' CTACTCTTTCTATGCAATTTGAATTG 3' R: 5' AATAATTTTCAACCATAGAATGATT 3' Restriction enzyme: *HinfI*	55°C
Ph3	Ph3-SCAR	F: 5' CTACTCGTGCAAGAAGGTAC 3' R: 5' TCCACATCACCTGCCAGTTG 3'	55°C
Mi-1.2	Mi23-SCAR	F: 5' TGGAAAAATGTTGAATTTCTTTTG 3' R: 5' GCATACTATATGGCTTGTTTACCC 3'	55°C

4.1.4 Gel documentation of the amplification profile of the molecular markers

Genotypes with potential genes for resistance to biotic stresses, *Tomato yellow leaf curl virus* disease (TYLCVD) caused by *Tomato yellow leaf curl virus,* Verticillium wilt caused by *Verticillium* spp., Fusarium wilt caused by *Fusarium oxysporum*, Late blight caused by *Phytophthora infestans* and Root knot caused by *Meloidogyne* spp., were located with respective SCAR and CAPS molecular markers. The amplification profiles were resolved on 2.5% agarose gel and visualized with Syngene G: BOX Chemi XX6 gel documentation system using HyGene software.

4.1.4.1 Tomato Yellow Leaf Curl virus

30 tomato genotypes with genes for resistance to *Tomato Yellow Leaf Curl virus* were determined using the SCAR markers TG0302/TY2R1 specific to *Ty2* gene and FLUW25 specific to *Ty3* gene for resistance.

4.1.4.1.1 Amplification profile of the molecular marker T0302F/TY2R1 for the gene Ty2

The SCAR marker TG0302/TY2R1 for the gene *Ty2* amplified a band of size 600bp for genotypes with *Ty2/Ty2* alleles and 450 bp for genotypes with *ty2/ty2* alleles.

In the study, amplified band of size 450 bp was generated by the SCAR marker T0302F/TY2R1 for the gene *Ty2,* corresponding to genotypes of *ty2/ty2* alleles and the amplified products corresponding to *Ty2/Ty2* alleles was not obtained for all of the 30 genotypes screened (Plate 8). From the study, none of the 30 genotypes screened were identified for the presence of the gene *Ty2*, providing resistance to *Tomato Yellow Leaf Curl virus* (Table 15).

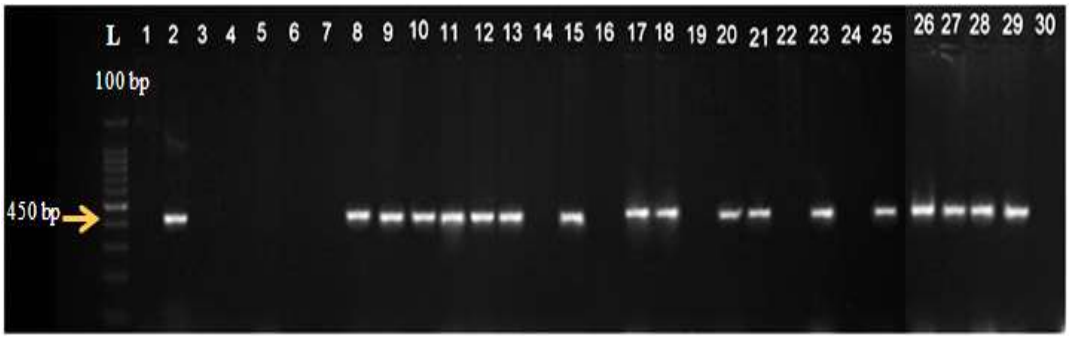

Plate 8: Amplification profile of the molecular marker T0302F/TY2R1 for the gene *Ty2* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* - EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum.*

Table 15. Screening of the 30 tomato genotypes for the presence or absence of the gene *Ty2* for resistance to *Tomato Yellow Leaf Curl virus*

Sl. no	Genotypes	Presence/Absence of gene for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	A
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	A
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	A
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	A
13.	Pusa Ruby	A
14.	Nenmara local	A
15.	CA 22053	A
16	Manulekshmi	A
17.	EC 620419	A
18.	IIHR 2204	A
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	A
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	A
25.	IIHR 26	A
26.	PKM 1	A
27.	Arka Vikas	A
28.	EC 677034	A
29.	PNR 7	A
30.	*Solanum torvum*	A

4.1.4.1.2 Amplification profile of the molecular marker FLUW25 for the gene Ty3

The SCAR marker FLUW25 specific to the gene *Ty3,* amplified a band of size 640 bp for the genotypes with *Ty3/Ty3* alleles and 480 bp for the genotypes with *ty3/ty3* alleles.

In the study, the SCAR marker FLUW25 generated amplified bands of size 480 bp corresponding to genotypes of *ty3/ty3* alleles and the amplified products corresponding to genotypes of *Ty3/Ty3* alleles was not obtained (Plate 9). Presence of the gene *Ty3,* for resistance to *Tomato Yellow Leaf Curl virus* was not identified in the 30 genotypes screened (Table 16).

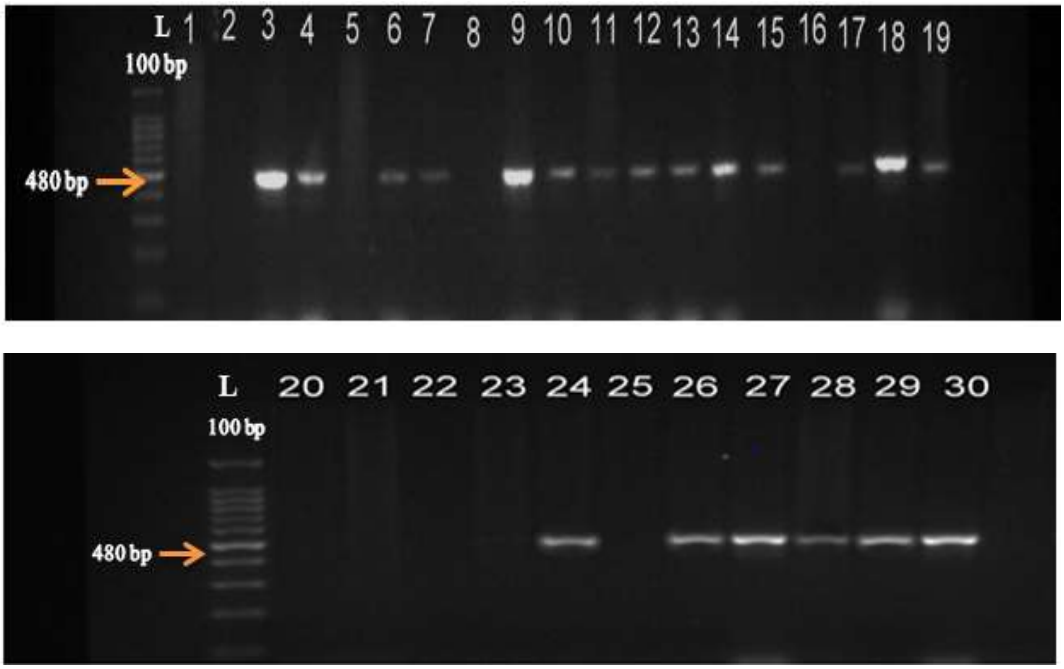

Plate 9: Amplification profile of the molecular marker FLUW25 for the gene *Ty3* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* -EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

Table 16. Screening of the 30 tomato genotypes for the presence or absence of the gene *Ty3* for resistance to *Tomato Yellow Leaf Curl virus*

Sl. no	Genotypes	Presence/Absence of gene for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	A
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	A
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	A
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	A
13.	Pusa Ruby	A
14.	Nenmara local	A
15.	CA 22053	A
16	Manulekshmi	A
17.	EC 620419	A
18.	IIHR 2204	A
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	A
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	A
25.	IIHR 26	A
26.	PKM 1	A
27.	Arka Vikas	A
28.	EC 677034	A
29.	PNR 7	A
30.	*Solanum torvum*	A

4.1.4.2 Verticillium wilt

Resistance to Verticillium wilt is provided by two closely linked, inversely oriented genes, *Ve1* and *Ve2* in which screening of genotypes for *Ve1* was done with CAPS marker Ve1 XbaI and *Ve2* with CAPS marker V2LeO3F/V2LeO3R.

4.1.4.2.1 Amplification profile of the molecular marker Ve1 XbaI for the gene Ve1

Ve1 gene screened using the CAPS marker Ve1 XbaI, generated 410 bp and 332bp fragments for genotypes with *Ve1/Ve1* alleles and fragment size of 410 bp, 310 bp and 22 bp for genotypes with *ve1/ve1* alleles.

In the study, the primer pairs, specific to the CAPS marker *Ve1 XbaI* for the gene *Ve1* generated the amplification profile (Plate 10). The restriction digestion profile was generated by the CAPS marker *Ve1 XbaI* for the gene *Ve1* (Plate 11) in which the restriction digested fragments of size 410 bp and 332 bp, corresponds to genotypes of *Ve1/Ve1* alleles and fragments of size 410 bp and 310 bp corresponds to genotypes of *ve1/ve1* alleles. Among the 30 genotypes screened, the genotypes, IIHR 2374, Kashi Vishesh and PNR 7 were identified for the presence of the gene *Ve1* (Table 17).

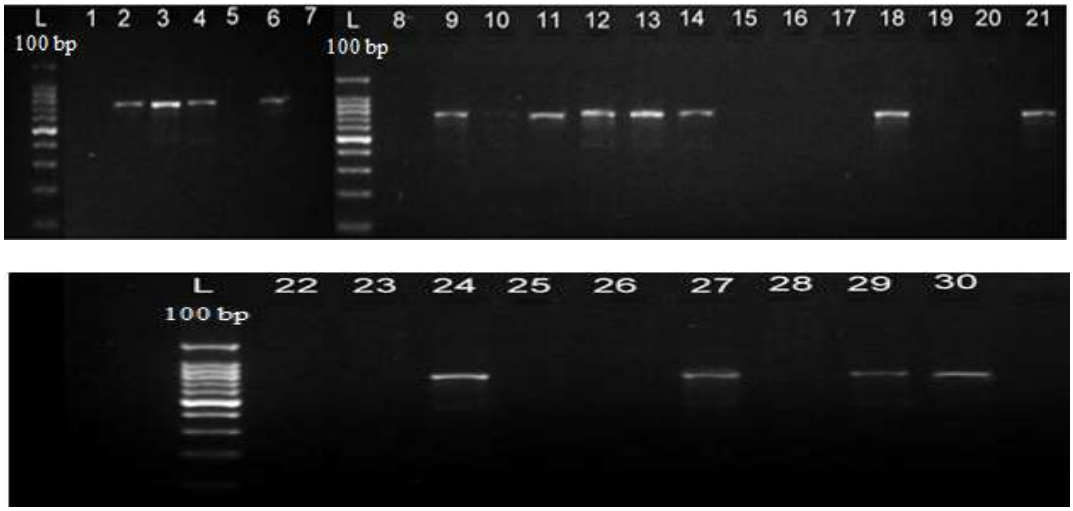

Plate 10: Amplification profile of the molecular marker *Ve1 XbaI* for the gene *Ve1* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* -EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum.*

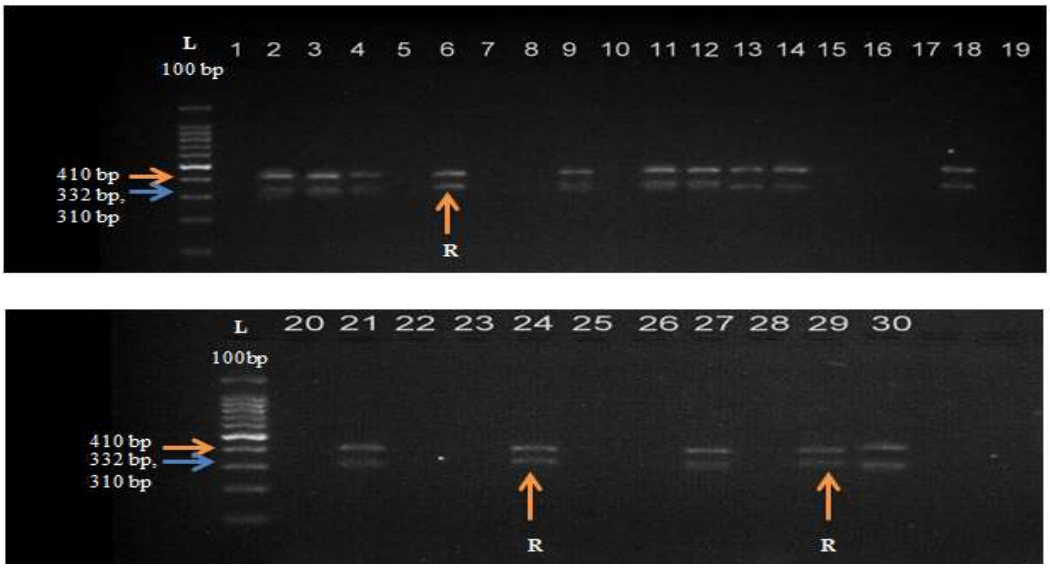

Plate 11: Restriction digestion profile of the molecular marker Ve1 XbaI for the gene *Ve1* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* - EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

Table 17. Screening of the 30 tomato genotypes for the presence or absence of the gene *Ve1* for resistance to Verticillium wilt

Sl. no	Genotypes	Presence/Absence of gene for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	A
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	**P**
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	A
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	A
13.	Pusa Ruby	A
14.	Nenmara local	A
15.	CA 22053	A
16	Manulekshmi	A
17.	EC 620419	A
18.	IIHR 2204	A
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	A
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	**P**
25.	IIHR 26	A
26.	PKM 1	A
27.	Arka Vikas	A
28.	EC 677034	A
29.	PNR 7	**P**
30.	*Solanum torvum*	A

4.1.4.2.2 Amplification profile of the molecular marker V2LeO3F/V2LeO3R for the gene Ve2

Ve2 gene was screened using the CAPS marker V2LeO3F/V2LeO3R, which generated fragment of size 601 bp and 428 bp for genotypes with *Ve2/Ve2* alleles and fragments of size 1029 bp for genotypes with *ve2/ve2* alleles.

The primer pairs, specific to the CAPS marker V2LeO3F/V2LeO3R for the gene *Ve2* generated the amplification profile (Plate 12). Restriction digestion profile was generated by the CAPS marker V2LeO3F/V2LeO3R for the gene *Ve2* (Plate 13) in which the genotypes IIHR 2374 and Kashi Vishesh were identified with restriction digestion fragments of 601 bp and 428 bp corresponding to *Ve2/Ve2* alleles. The presence of gene *Ve2* was identified in genotypes IIHR 2374 and Kashi Vishesh (Table 18).

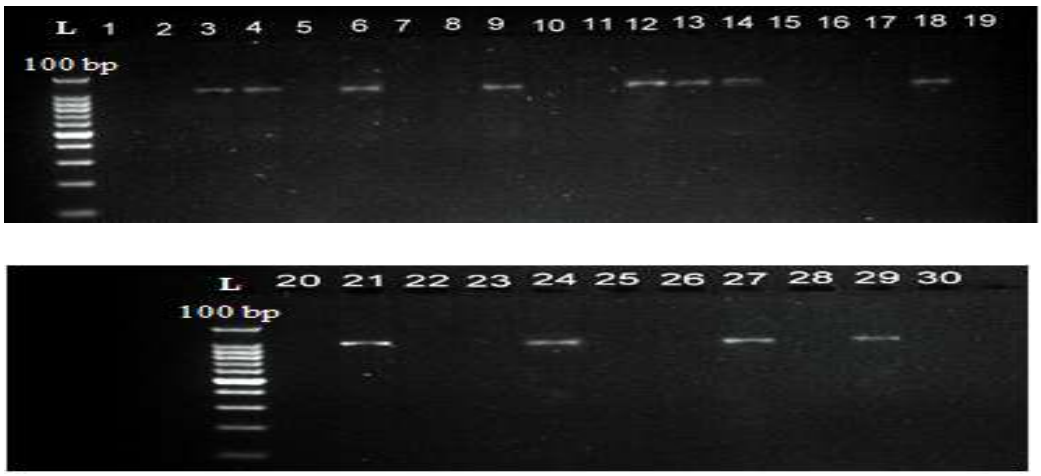

Plate 12: Amplification profile of the molecular marker V2LeO3F/V2LeO3R for the gene *Ve2* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* -EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

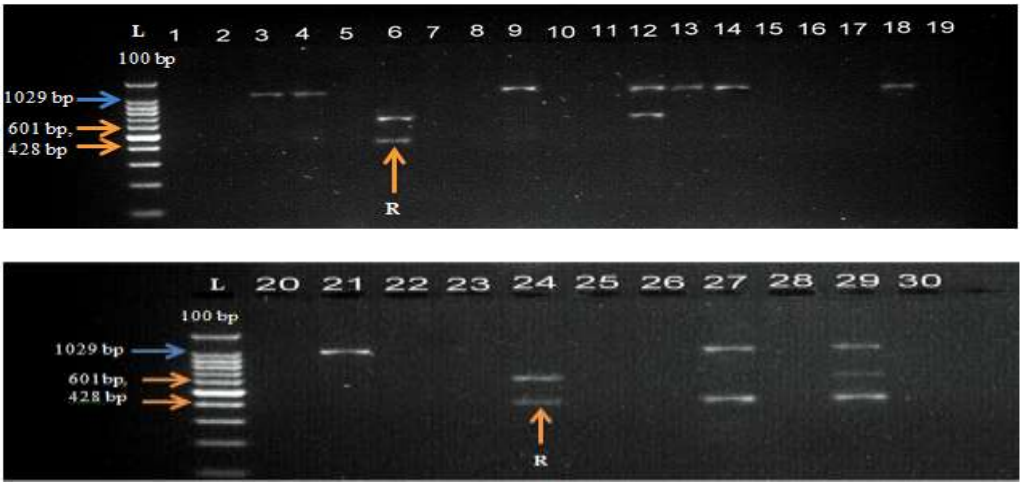

Plate 13: Restriction digestion profile of the molecular marker V2LeO3F/V2LeO3R for the gene *Ve2* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* -EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

Table 18. Screening of the 30 tomato genotypes for the presence or absence of the gene *Ve2* for resistance to Verticillium wilt

Sl. no	Genotypes	Presence/Absence of gene for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	A
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	**P**
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	A
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	A
13.	Pusa Ruby	A
14.	Nenmara local	A
15.	CA 22053	A
16	Manulekshmi	A
17.	EC 620419	A
18.	IIHR 2204	A
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	A
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	**P**
25.	IIHR 26	A
26.	PKM 1	A
27.	Arka Vikas	A
28.	EC 677034	A
29.	PNR 7	A
30.	*Solanum torvum*	A

4.1.4.3 Fusarium wilt

Genotypes resistant to Fusarium wilt were screened for the presence of genes *I3* and *I7* using SCAR marker P743DF1/R1 and CAPS marker CAPS7774.

4.1.4.3.1 Amplification profile of the molecular marker P743DF1/R1 for the gene I3

The SCAR marker P743DF1/R1 for the gene *I3* amplified a band size of 1270 bp for genotypes with *I3/I3* alleles and 1060 bp for genotypes with *i3/i3* alleles.

In the study, the amplified bands of size 1060 bp, generated by the SCAR marker P743DF1/R1 for the gene *I3* corresponds to the genotypes of *i3/i3* alleles and none of the 30 genotypes screened were identified for *I3/I3* alleles (Plate 14). Presence of the gene *I3* for resistance to fusarium wilt was not identified in the 30 genotypes (Table 19).

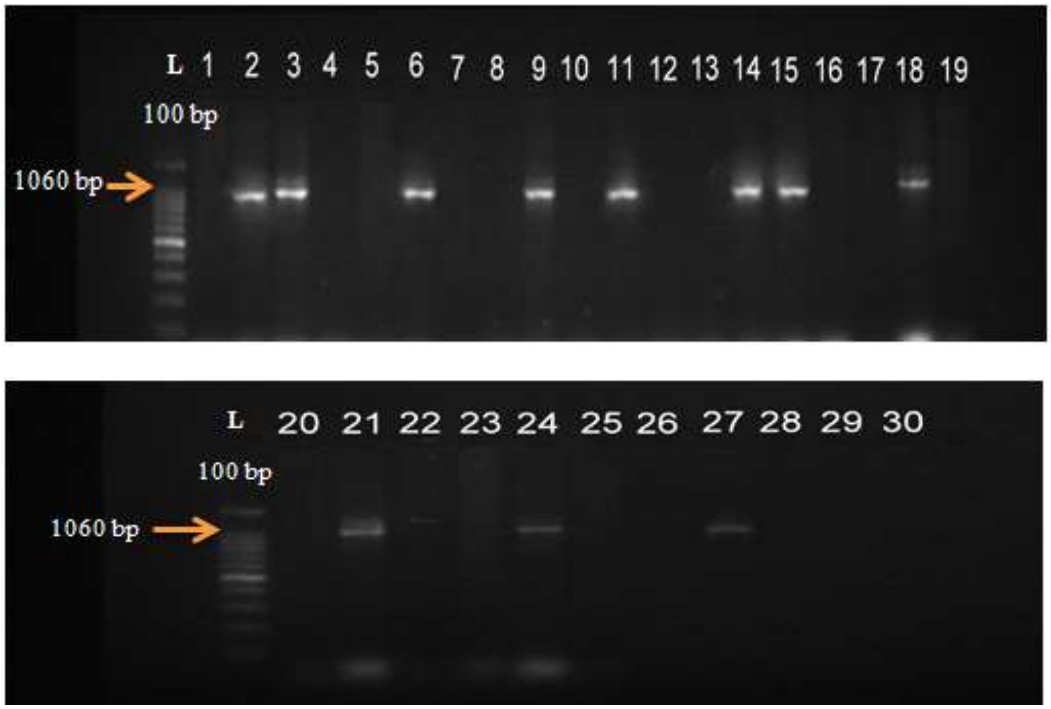

Plate 14: Amplification profile of the molecular marker P743DF1/R1 for the gene *I3* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* - EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

Table 19. Screening of the 30 tomato genotypes for the presence or absence of the gene *I3* for resistance to Fusarium wilt

Sl. no	Genotypes	Presence/Absence of genes for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	A
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	A
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	A
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	A
13.	Pusa Ruby	A
14.	Nenmara local	A
15.	CA 22053	A
16	Manulekshmi	A
17.	EC 620419	A
18.	IIHR 2204	A
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	A
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	A
25.	IIHR 26	A
26.	PKM 1	A
27.	Arka Vikas	A
28.	EC 677034	A
29.	PNR 7	A
30.	*Solanum torvum*	A

4.1.4.3.2 Amplification profile of the molecular marker CAPS7774 for the gene I7

The CAPS marker CAPS7774 for the gene *I7* generated fragments of size 612 bp and 196 bp for genotypes with *I7/I7* alleles and fragments of size 808 bp for genotypes with *i7/i7* alleles.

In the study, the primer pairs specific to the CAPS marker CAPS7774 for the gene *I7*, generated amplification profile (Plate 15). Restriction digestion profile was generated by the CAPS marker CAPS7774 for the gene *I7* (Plate 16) in which the restriction digested fragments of size 612 bp and 196 bp, obtained for the genotypes IIHR 2205, IIHR 2374 and Vellayani Vijai corresponds to *I7/I7* alleles. Presence of the gene *I7* for resistance to Fusarium wilt was identified in the genotypes IIHR 2205, IIHR 2374 and Vellayani Vijai (Table 20).

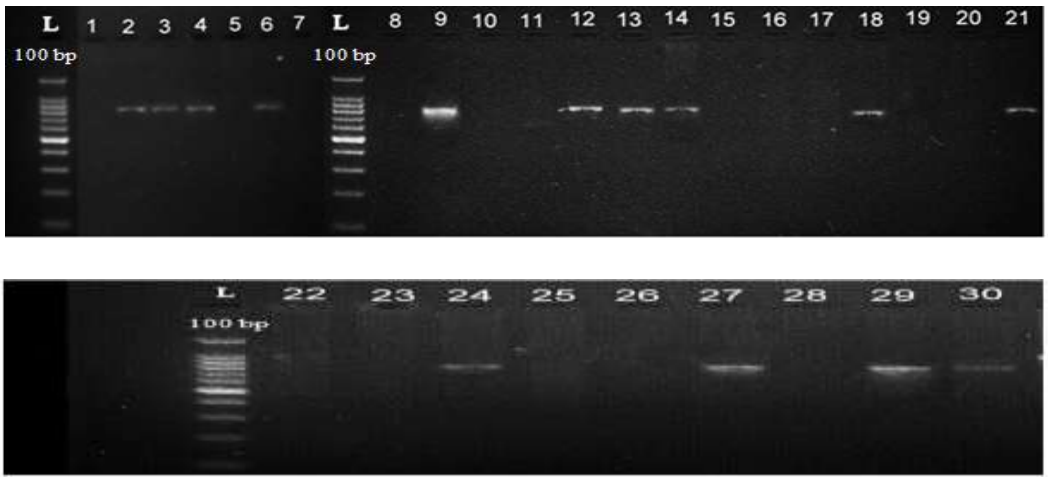

Plate 15: Amplification profile of the molecular marker CAPS7774 for the gene *I7* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* -EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

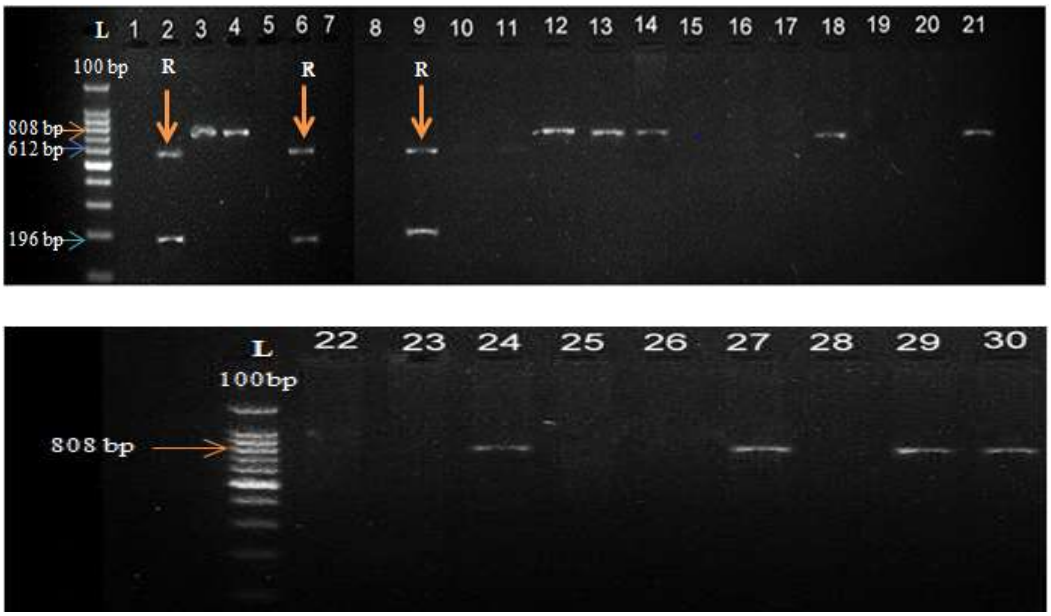

Plate 16: Restriction digestion profile of the molecular marker CAPS7774 for the gene *I7* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* - EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum.*

Table 20. Screening of the 30 tomato genotypes for the presence or absence of the gene *I7* for resistance to Fusarium wilt

Sl. no	Genotypes	Presence/Absence of genes for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	**P**
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	**P**
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	**P**
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	A
13.	Pusa Ruby	A
14.	Nenmara local	A
15.	CA 22053	A
16	Manulekshmi	A
17.	EC 620419	A
18.	IIHR 2204	A
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	A
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	A
25.	IIHR 26	A
26.	PKM 1	A
27.	Arka Vikas	A
28.	EC 677034	A
29.	PNR 7	A
30.	*Solanum torvum*	A

4.1.4.4 Late blight

Screening of genotypes for resistance to Late blight was done using the SCAR marker Ph3-SCAR specific to *Ph3* gene and the CAPS marker dtG63 specific to *Ph2* gene.

4.1.4.4.1 Amplification profile of molecular marker Ph3-SCAR for gene Ph3

Screening for the presence of *Ph3* gene was done using the SCAR marker Ph3-SCAR that amplified band of size 176 bp for genotypes with *Ph3/Ph3* alleles and 154 bp for *ph3/ph3* alleles.

In the study, an amplification profile was generated by the SCAR marker Ph3-SCAR for the gene *Ph3* (Plate 17) in which the amplified band of size 176 bp, corresponds to genotypes of *Ph3/Ph3* alleles and 154 bp for genotypes of *ph3/ph3* alleles. Among the 30 genotypes screened, the genotypes Pusa Ruby, CA 22053, Hisar Lalit, PKM-1 and Arka Vikas were identified for the presence of the gene *Ph3* (Table 21).

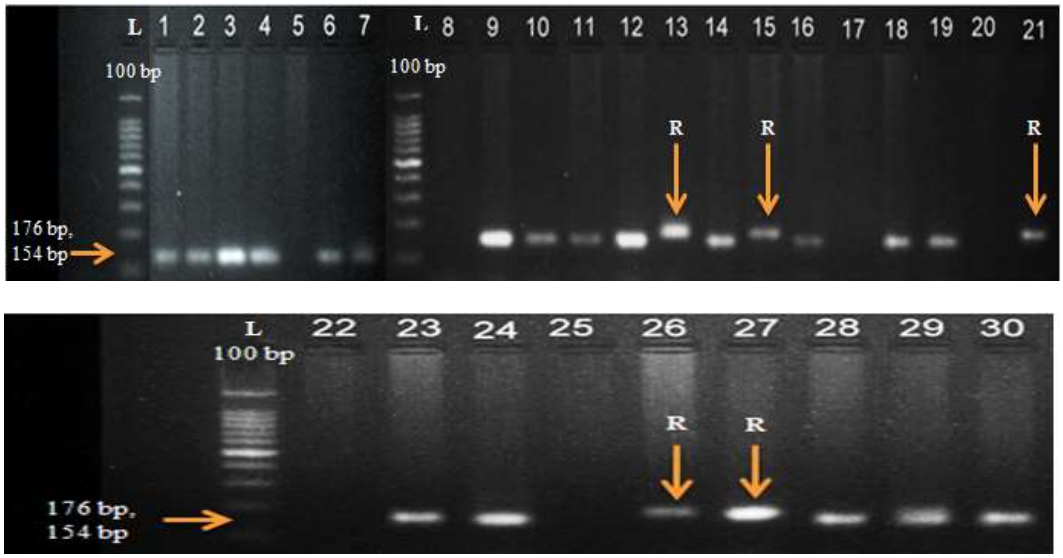

Plate 17: Amplification profile of the molecular marker Ph3-SCAR for the gene *Ph3* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* - EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

Table 21. Screening of the 30 tomato genotypes for the presence or absence of the gene *Ph3* for resistance to Late blight

Sl. no	Genotypes	Presence/Absence of genes for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	A
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	A
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	A
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	A
13.	Pusa Ruby	**P**
14.	Nenmara local	A
15.	CA 22053	**P**
16	Manulekshmi	A
17.	EC 620419	A
18.	IIHR 2204	A
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	**P**
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	A
25.	IIHR 26	A
26.	PKM 1	**P**
27.	Arka Vikas	**P**
28.	EC 677034	A
29.	PNR 7	A
30.	*Solanum torvum*	A

4.1.4.4.2 Amplification profile of the molecular marker dtG63 for the gene Ph2

Presence of *Ph2* gene was screened using the CAPS marker dtG63, which generated fragments of size 245 bp for genotypes with *Ph2/Ph2* alleles and fragments of size 221 bp for genotypes with *ph2/ph2* alleles.

In the study, the primer pairs specific to the CAPS marker dtG63 for the gene *Ph2,* generated the amplification profile (Plate 18). The restriction digestion profile was generated by the CAPS marker dtG63 for the gene *Ph2* in which the restriction digested bands of size 245 bp corresponds to genotypes of *Ph2/Ph2* alleles and 221 bp corresponds to genotypes of *ph2/ph2* alleles (Plate 19). The genotypes Shakthi, Pusa Ruby, Nenmara local, CA 22053, Manulakshmi, IIHR 2204, PNR 7 and *Solanum torvum* were identified for the presence of the gene *Ph2* (Table 22).

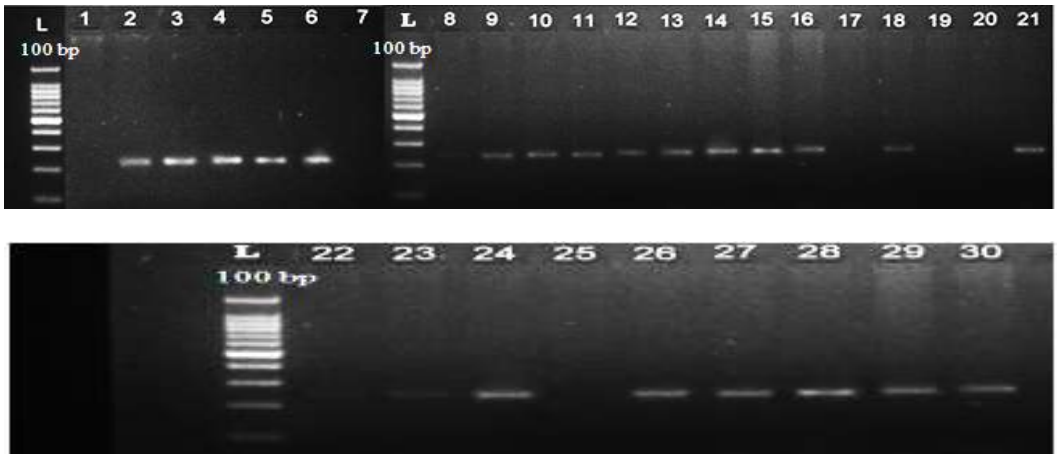

Plate 18: Amplification profile of the molecular marker dtG63 for the gene *Ph2* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* -EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

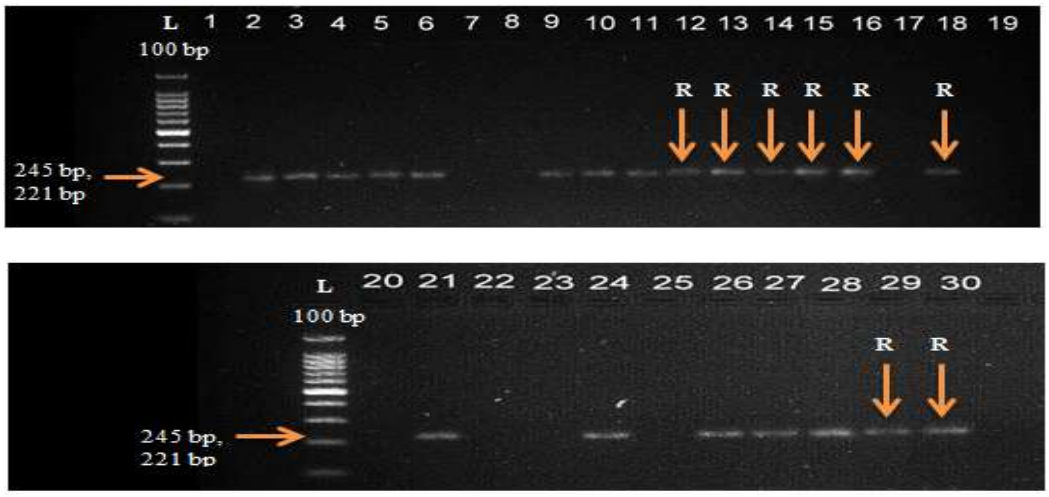

Plate 19: Restriction digestion profile of the molecular marker dtG63 for the gene *Ph2* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* - EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

Table 22. Screening of the 30 tomato genotypes for the presence or absence of the gene *Ph2* for resistance to Late blight

Sl. no	Genotypes	Presence/Absence of genes for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	A
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	A
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	A
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	**P**
13.	Pusa Ruby	**P**
14.	Nenmara local	**P**
15.	CA 22053	**P**
16	Manulekshmi	**P**
17.	EC 620419	A
18.	IIHR 2204	**P**
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	A
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	A
25.	IIHR 26	A
26.	PKM 1	A
27.	Arka Vikas	A
28.	EC 677034	A
29.	PNR 7	**P**
30.	*Solanum torvum*	**P**

4.1.4.5 Root knot nematode

Root knot resistance is contributed by the gene *Mi 1.2* which was screened by the SCAR marker Mi23. The SCAR marker Mi23 amplified a band of size 380 bp for genotypes with *Mi/Mi* alleles for resistance and 430 bp for genotypes which are susceptible in nature.

The SCAR marker Mi23 for the gene *Mi1.2* generated the amplification profile in which the amplified bands of 430 bp corresponds to genotypes of *mi/mi* alleles and none of the 30 genotypes were amplified for *Mi/Mi* alleles (Plate 20). None of the genotypes among the 30 genotypes screened were identified for the presence of the gene *Mi1.2* (Table 23).

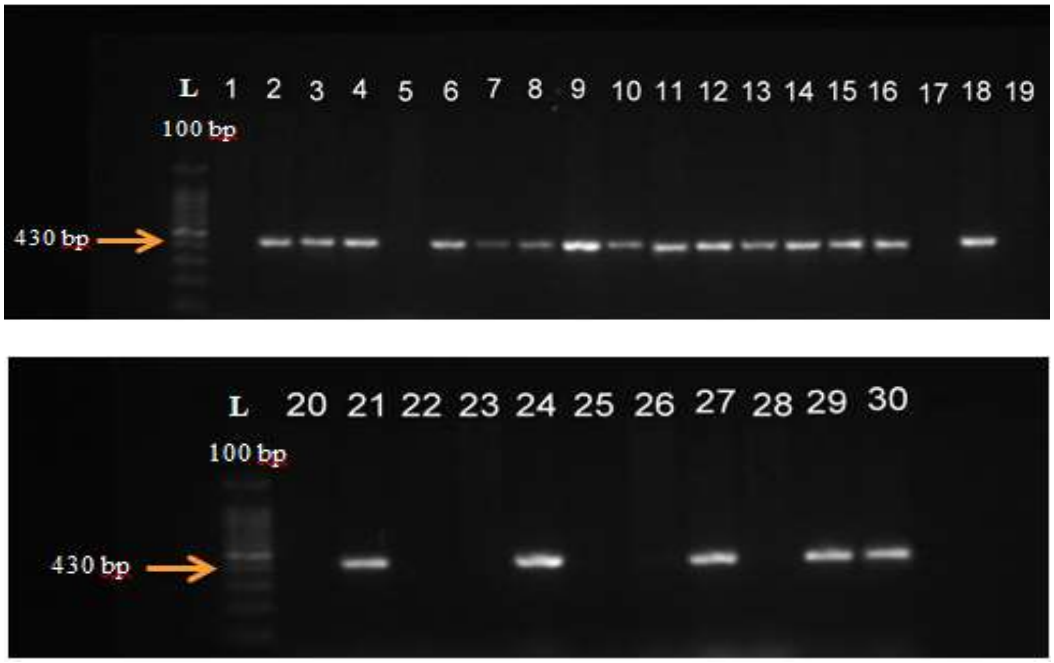

Plate 20: Amplification profile of the molecular marker Mi23 for the gene *Mi1.2* resolved on 2.5% agarose gel Lane L- 100 bp DNA ladder; Lane 1- *Solanum pimpinellifolium* -EC 697275; Lane 2- IIHR 2205; Lane 3-Akshaya; Lane 4- Arka Rakshak; Lane 5-EC 514124; Lane 6- IIHR 2374; Lane 7- IIHR 2373; Lane 8- LA 1206; Lane 9- Vellayani Vijai; Lane 10- IC-45; Lane 11- IIHR 2868; Lane 12- Shakthi; Lane 13- Pusa Ruby; Lane 14- Nenmara local; Lane 15- CA 22053; Lane 16- Manulakshmi; Lane 17- EC 620419; Lane 18- IIHR 2204; Lane 19- Alathur local; Lane 20- Palakkad local; Lane 21- Hisar Lalit; Lane 22- LA 1805; Lane 23- Kuttichal local; Lane 24- Kashi Vishesh; Lane 25- IIHR 26; Lane 26- PKM-1; Lane 27- Arka Vikas; Lane 28- EC 677034; Lane 29- PNR 7; Lane 30- *Solanum torvum*.

Table 23. Screening of the 30 tomato genotypes for the presence or absence of the gene *Mi1.2* for resistance to Root knot nematode

Sl. no	Genotypes	Presence/Absence of genes for resistance
1.	*Solanum pimpinellifolium*- EC 697275	A
2.	IIHR 2205	A
3.	Akshaya	A
4.	Arka Rakshak	A
5.	EC 514124	A
6.	IIHR 2374	A
7.	IIHR 2373	A
8.	LA 1206	A
9.	Vellayani Vijai	A
10.	IC-45	A
11.	IIHR 2868	A
12.	Shakthi	A
13.	Pusa Ruby	A
14.	Nenmara local	A
15.	CA 22053	A
16	Manulekshmi	A
17.	EC 620419	A
18.	IIHR 2204	A
19.	Alathur local	A
20.	Palakkad local	A
21.	Hisar Lalit	A
22.	LA 1805	A
23.	Kuttichal local	A
24.	Kashi Vishesh	A
25.	IIHR 26	A
26.	PKM 1	A
27.	Arka Vikas	A
28.	EC 677034	A
29.	PNR 7	A
30.	*Solanum torvum*	A

Table 24. Genotypes identified as donors for genes for resistance located from the study

Sl. no	Genes for resistance	Donors for genes for resistance located from the study
1.	***Ty2***	-
2.	***Ty3***	-
3.	***Ve1***	IIHR 2374, Kashi Vishesh, PNR 7
4.	***Ve2***	IIHR 2374, Kashi Vishesh
5.	***I3***	-
6.	***I7***	IIHR 2205, IIHR 2374, Vellayani Vijay
7.	***Ph3***	Pusa Ruby, CA 22053, Hisar Lalit, PKM 1, Arka Vikas
8.	***Ph2***	Shakthi, Pusa Ruby, Nenmara local, CA 22053, Manulakshmi, IIHR 2204, PNR 7, *Solanum torum*
9.	***Mi 1.2***	-

All the genotypes identified as donors for genes for resistance to the diseases studied, located from the study were enlisted in Table 24. From the 30 genotypes of tomato screened for the presence of genes for resistance to *Tomato yellow leaf curl virus* (*TYLCV),* Verticillium wilt, Fusarium wilt, Late blight and Root knot nematodes, none were identified to carry the genes screened for resistance to *Tomato yellow curl virus* and Root knot nematodes. The genotypes IIHR 2374, Kashi Vishesh and PNR 7 were identified for the presence of gene *Ve1* and the genotypes IIHR 2374 and Kashi Vishesh were identified for the presence of gene *Ve2* for resistance to Verticillium wilt. The genotypes IIHR 2205, IIHR 2374 and Vellayani Vijai were identified to contain the gene *I7* and none for the gene *I3* for resistance to Fusarium wilt. Presence of the gene *Ph3* was identified in the genotypes Pusa Ruby, CA 22053, Hisar Lalit, PKM-1 and Arka Vikas and presence of the gene *Ph2* was identified in the genotypes Shakthi, Pusa Ruby, Nenmara local, CA 22053, Manulakshmi, IIHR 2204, PNR 7 and *Solanum torvum* for resistance to late blight.

DISCUSSION

5. DISCUSSION

The cultivated tomato (*Solanum lycopersicum* L.) is the world's most popular garden crop, coming in second only to potatoes and undeniably the most widely consumed vegetable crop. According to the data from 2001 to 2016, the world harvested area and the total tomato production have registered a striking advance of approximately 22.8% and 63.5%, respectively (Gardner, 2006). With the expansion in the growing areas of tomato, the yield per hectare has been highly compromised by several factors, among which, biotic stresses resulted from various pathogenic attacks were considered to be the most significant cause. Generally, more than 60 pathogenic agents including bacteria, fungi, oomycetes, viruses, and nematodes, were known to pose a threat to tomato production worldwide (Sajid and Eminur, 2019). To effectively control those diseases causing biotic stress, it is advocated to have a high-efficient combination of corresponding genes for resistance, as well as pyramiding them into commercial tomato varieties as a pragmatic breeding strategy (Miller and Tanksley, 1990).

In recent years, significant progress has been achieved in the development of resistant tomato cultivars against the most disastrous diseases. To provide tomato breeding programme with genetically diversified sources of resistance, knowledge of distinct genetic backgrounds such as breeding lines or germplasms carrying different types of genes for resistance is required (Collard *et al*., 2005). The major sources of genes for resistance that have been introgressed into cultivated tomatoes were from wild relatives of tomato species like *S. pimpinellifolium, S. pennellii, S. habrochaites, S. peruvianum*, and *S. chilense* (Reddy *et al.,* 2013). Marker assisted selection is a frequently employed technique for identifying the genes for resistance (Foolad, 2007). Various molecular markers were extensively and successfully employed in breeding programmes with a major focus on promoting and improving disease resistance in tomatoes.

In the present study, 30 genotypes of tomato including wild related species and cultivated varieties, were screened to locate donors for genes for resistance to biotic stresses namely, *Tomato Yellow Leaf Curl Virus* (*TYLCV*), Verticillium wilt, Fusarium wilt, Late blight and Root knot through molecular marker assisted selection.

DNA isolation from 3-4 week old genotypes was carried out using CTAB method with minor modifications, as described by Murray and Thompson (1988). The isolated DNA resolved on 0.8 % agarose gel was displayed to be intact bands with a slight degradation in few of the samples, which might have occurred due to the handling errors during the conduct of the isolation procedure. The concentration and purity analysis of the DNA samples determined by spectrophotometer indicated values, which were satisfactory for carrying out PCR reaction for the majority of the samples and the presence of slight protein and RNA contamination in some of the samples, which might not be an issue for PCR analysis due to the lower levels of contamination. The DNA samples with a concentration above 1000 ng/µl were diluted to a 1:10 ratio to a final concentration of 20ng/µl for PCR amplification (Aboul-Maaty and Oraby, 2019).

Standardization of annealing temperatures for the primer pairs of molecular markers was carried out with the gradient temperature ranges, set with ±5°C from the reported temperature (Yang *et al*., 2005). The standardized annealing temperatures obtained for all the markers were identical or showed a maximum deviation of only 2°C from the reported temperatures, hence PCR amplifications were carried out with reaction conditions as reported specific to each of the molecular markers.

Screening of tomato genotypes for genes for resistance was carried out using gene based functional molecular markers, that detects DNA polymorphism like single nucleotide polymorphisms(SNPs) or insertions/ deletions (InDels), which are physically located adjacent to the gene of interest, critically responsible for providing resistance to various diseases (Jung *et al*., 2015).

The screening of genotypes for *Tomato yellow leaf curl virus* resistance was done using the SCAR marker T0302F/TY2R1 for the gene *Ty2* located on chromosome 11. Homozygous resistant genotypes to *Tomato yellow leaf curl virus* carry *Ty2/Ty2* alleles and susceptible genotypes carry *ty2/ty2* alleles. According to Brenda *et al*. (2007), the T0302/TY2R1 SCAR marker developed generated a band of size 600 bp, for genotypes with *Ty2/Ty2* alleles of gene for resistance as observed from the breeding line H-24, which consists of *Ty 2* gene present in the introgressed region derived from the *S. habrochaites* and 450 bp for the genotypes which are susceptible, indicating the presence of *ty2/ty2* alleles, similar as to the banding pattern obtained for the susceptible inbred line HUJ-VF. In this study, among the 30 genotypes screened, a band of size 600 bp corresponding to gene for resistance was not amplified for any of the genotypes. The genotypes IIHR 2205, LA 1206, Vellayani Vijai, IC-45, IIHR 2868, Shakthi, Pusa Ruby, CA 22053, EC 620419, IIHR 2204, Hisar Lalit, Kuttichal local, IIHR 26, PKM-1, Arka Vikas, EC 677034, PNR 7 and *Solanum torvum* displayed amplified bands corresponding to 450 bp as similar as obtained by Brenda *et al*. (2007) and the remaining genotypes showed no amplification due to the absence of alleles for the gene *Ty2*. In a similar study, by Maxwell *et al.* (2006), the CAPS marker TG105AF/TG105AR was used to screen the presence of gene *Ty2* in the tomato genotypes. The restriction enzyme, *Taq I*, generated restriction digested products of 330 bp for *Ty2/Ty2* alleles and 220 bp for *ty2/ty2* alleles. In another study, Yang *et al*. (2014) screened tomato genotypes for the presence of gene *Ty2* using the SCAR marker T0302F/T0302R, which scored band of size 900 bp for *Ty2/Ty2* alleles and 800 bp for *ty2/ty2* alleles.

The genotypes with the gene *Ty3* located on chromosome 6 for resistance to *Tomato yellow leaf curl virus* was screened with the SCAR marker FLUW25 reported by Melinda *et al*. (2007). Ji *et al*. (2007) mapped *Ty3* gene from *S chilense* accession LA 2779 to an introgression near the FER locus on chromosome 6 and cloned in BAC 56B23. The primers developed to amplify the sequences in the 5'end of the BAC clone by Ji *et al*. (2007) was used to amplify PCR fragments from susceptible heritage tomato, *S lycopersicum*

'Purple Russian' and resistant breeding line, Gc43 by Mejia *et al*. (2005). The sequences from the PCR amplifications generated by Mejia *et al*. (2005) were used to design the primers for the marker FLUW25. The SCAR marker FLUW25 amplified a band of size 640 bp in genotypes with *Ty3/Ty3* alleles and 480 bp in susceptible genotypes with *ty3/ty3* alleles. In this study, none of the 30 genotypes showed amplification for alleles for resistance. The genotypes Akshaya, Arka Rakshak, IIHR 2374, IIHR 2373, Vellayani Vijai, IC-45, IIHR 2868, Shakthi, Pusa Ruby, Nenmara local, CA 22053, EC 620419, IIHR 2204, Alathur local, Kashi Vishesh, PKM-1, Arka Vikas, EC 677034, PNR 7 and *Solanum torvum* showed the presence of susceptible alleles and remaining genotypes showed no amplification due to the absence of alleles for the gene *Ty3*. In a similar study conducted by Jensen *et al*. (2007), the CAPS marker FER-G8F/FER-G8R using the restriction enzyme *Taq I*, distinguished the tomato genotypes of *Ty3/Ty3* alleles with a restriction digested products of size 200 bp and 300 bp from genotypes of *ty3/ty3* alleles with an undigested product of size 500 bp. According to Ji *et al*. 2008, the SCAR marker P6-25F/ P6-25R amplified a band of size 450 bp for genotypes of *Ty3/Ty3* alleles and 320 bp for genotypes of *ty3/ty3* alleles.

Ve1 and *Ve2* are two closely linked genes of the *Ve* locus located on chromosome 9, which independently confers resistance to the same pathogen (Arens *et al*., 2010). Verticillium wilt resistance conferred by the gene *Ve1* in genotypes was screened using the CAPS marker Ve1 XbaI reported by Jung *et al*. (2015). *Ve* locus is associated with several SNPs (Kawchuk *et al*., 2001). In the study conducted by Jung *et al*. (2015) the Ve1 XbaI marker is developed based on a single bp deletion of TCA/T-A at nucleotide position 1220 on the exon sequence, resulting in a premature stop codon. This SNP based generation of truncated protein in susceptible genotypes is responsible for the loss of resistance and is utilized for marker development, in which the site of SNP forms the recognition site for the restriction enzyme *Xba I.* The PCR amplified products generated by the marker were subjected to restriction digestion by *Xba I.* The restriction enzyme digested the susceptible alleles with bands of size 410 bp, 310bp, and 22 bp and resistant alleles with bands

of size 410 bp and 332 bp. In the present study, resistant alleles were amplified for the genotypes IIHR 2374, Kashi Vishesh and PNR 7. Susceptible alleles were amplified for the genotypes IIHR 2205, Akshaya, Arka Rakshak, Vellayani Vijai, IIHR 2868, Shakthi, Pusa Ruby, Nenmara local, IIHR 2204, Hisar Lalit, Arka Vikas and *Solanum torvum* and remaining genotypes showed no amplification due to the absence of alleles for the gene *Ve1*. In a study, Arens *et al*. (2010) developed SNP marker Ve1-2072F/Ve1-2651R based on the study of Kawchuk *et al.* (2001) to screen for the presence of *Ve2* in tomato genotypes, which generated amplification products of size 476 bp for *Ve1/Ve1* alleles and 158 bp for *ve1/ve1* alleles.

Genotypes with the gene *Ve2* for resistance to verticillium wilt were screened using the CAPS marker V2LeO3F/V2LeO3R, developed by Acciarri *et al*. (2007). According to the study conducted by Acciarri *et al*. (2007), the CAPS marker V2LeO3F/V2LeO3R was developed based on the SNP, C to G at nucleotide position 2827 in the *Ve2* locus of the susceptible cultivar L98A, which forms the recognition site for the restriction enzyme *HincII*. The susceptible genotypes were undigested by the restriction enzyme with a fragment of size 1029 bp and the genotypes with *Ve2/Ve2* alleles showed two distinct bands of 601 bp and 428 bp respectively. In this study conducted to screen 30 genotypes, two genotypes, IIHR 2374 and Kashi Vishesh showed amplified bands corresponding to *Ve2/Ve2* alleles. Akshaya, Arka Rakshak, Hisar Lalit, Pusa Ruby, Nenmara local, IIHR 2204, Vellayani Vijai were the genotypes amplified with a band of size 1029 bp, corresponding to *ve2/ve2* alleles. Shakthi, Arka Vikas and PNR 7 showed banding pattern similar to heterozygous genotypes, which might be due to certain possible cross pollination events occurred during their selfing to propagate from homozygous parents and the remaining genotypes showed no amplification due to the absence of alleles for the gene *Ve2*. In a relatable study, Arens *et al*. (2010) retrieved sequences of primer pairs from the study conducted by Kawchuk *et al*. (2001), to develop a SNP marker Ve2-2720F/Ve2-3040R to screen *Ve2* gene in tomato genotypes, which generated band of size 242 bp for *Ve2/Ve2* alleles and 131 bp for *ve2/ve2* alleles.

Tomato genotypes with resistance to fusarium wilt were screened for the presence of the gene *I3* located on chromosome 7, using the SCAR marker P743DF1/R1 reported by Barillas *et al*. (2008). The SCAR marker P743DF1/R1 developed by Barillas *et al*. (2008), amplified band of size 1270 bp for genotypes with *I3/I3* alleles and 1060 bp for genotypes with *i3/i3* alleles. In this study, among the 30 genotypes screened for the resistance, none showed amplified band size of 1270 bp corresponding to *I3/I3* alleles. The genotypes IIHR 2205, Akshaya, IIHR 2374, Vellayani Vijai, Arka Vikas, Nenmara local, CA- 22053, IIHR 2204, Hisar Lalit, Kashi Vishesh and IIHR 2868 were amplified with 1060 bp corresponding to *i3/i3* alleles while the remaining genotypes showed no amplification due to the absence of alleles for the gene *I3*. In a similar study, Barillas *et al*. (2008) developed a CAPS marker P7-43B with the restriction enzyme *NsiI* to screen for the presence of *I3* gene in tomato genotypes. The marker generated restriction digested products of size 320 bp for *I3/I3* alleles and 800 bp for *i3/i3* alleles. In the same study, Barillas *et al*. (2008) developed another CAPS marker PTG-190 in which the restriction enzyme *Alu I* generated restriction digested products of size 250 bp and 380 bp for *I3/I3* alleles and 380 bp and 180 bp for *i3/i3* alleles.

Genotypes resistant to fusarium wilt were screened for the presence of the gene *I7* located on chromosome 8 using the CAPS marker CAPS7774 reported by Gonzalez-Cendales *et al.* (2015). In the study carried out by Gonzalez-Cendales *et al*. (2015), the CAPS marker CAPS7774 was developed to identify the gene *I7*, from the *S. pennellii* introgressed region in the tomato cultivar Tristar. The marker generated restriction digested fragments of the amplified PCR products using the restriction enzyme *AgeI*, of size 612 bp and 196 bp corresponding to *I7/I7* alleles and undigested fragments of the PCR products of size 808 bp for *i7/i7* alleles. Among the 30 genotypes screened for the presence of *I7* genes in this study, the genotypes IIHR 2205, IIHR 2374 and Vellayani Vijai showed band sizes of 612 bp and 196 bp, corresponding to *I7/I7* alleles. The genotypes Akshaya, Arka Rakshak, Shakthi, Pusa Ruby, Nenmara local, IIHR 2204, Hisar Lalit, Kashi Vishesh, Arka Vikas, PNR-7,

Solanum torvum generated undigested fragments of the PCR products of size 808 bp corresponding to *i7/i7* alleles, while the remaining genotype showed no amplification due to the absence of alleles for the gene *I7*. In a study carried out by Arens *et al*. (2010) using the SCAR marker At2 to screen the tomato genotypes for the presence of *I* gene for resistance to fusarium wilt, amplified fragments of size 130 bp were generated for genotypes corresponding to *I/I* alleles. Based on the primer sequences developed by Simons *et al*. (1998), Arens *et al*. (2010) developed another SCAR marker Z1063 to screen the tomato genotypes for the presence of the gene *I2*, which generated a band of size 940 bp for *I2/I2* alleles.

Resistance to *Phytophthora infestans* in tomato is conferred by a coiled-coil nucleotide-binding leucine-rich repeat (NBS–LRR) gene *Ph3* located on chromosome 9. The presence of the gene *Ph3* in tomato genotypes were screened using the SCAR marker Ph3-SCAR reported by Jung *et al*. (2015). In the study conducted by Jung *et al*. (2015), the marker Ph3-SCAR was developed based on a two 11 bp deletions with a separation of 56 bps in between, in the exonic sequence of chromosome 9, resulting in the generation of a premature stop codon, which renders the loss of resistance due to the formation of a truncated proteins in genotypes carrying susceptible alleles of the gene *Ph3*. The marker amplified a band of size 174 bp in genotypes with *Ph3/Ph3* alleles and 154 bp in genotype with *ph3/ph3* alleles. In this study, the genotypes Pusa Ruby, CA 22053, Hisar Lalit, PKM-1 and Arka Vikas were amplified with a band of size 174 bp, corresponding to *Ph3/Ph3* alleles. The genotypes EC 514124, LA 1206, EC 620419, Palakkad local, LA 1805 and IIHR 26 showed no amplification due to the absence of alleles for the gene *Ph3* and the remaining genotypes were amplified with a band size of 154 bp corresponding to *ph3/ph3* alleles. A similar study was carried out by Robbins *et al*. (2010) to screen tomato genotypes for the presence of *Ph3* gene using the CAPS marker TG328 with the restriction enzyme *BstN1*, which generated restriction digested products of size 275 bp for genotypes of *Ph3/Ph3* alleles and 560 bp for genotypes of *ph3/ph3* alleles. In another study, Arens *et al*. (2010) screened tomato genotypes for the presence of the gene

Ph3 using the CAPS marker dtG328 with the restriction enzyme *BstN1*, which generated restriction digested products of size 270 bp for genotypes of *Ph3/Ph3* alleles and 380 bp for genotypes of *ph3/ph3* alleles.

Tomato genotypes with the gene *Ph2* located on chromosome 10 for resistance to *Phytophthora infestans* were located using the CAPS marker dtG63 reported by Panthee *et al*. (2012). In the study conducted by Panthee *et al*. (2012), the homozygous genotype NC1 CELBR for the alleles *Ph2/Ph2* was amplified with a band of size 245 bp by the marker dtG63 and the homozygous susceptible genotype NC 123S showed an amplified band size of 221 bp corresponding to *ph2/ph2* alleles. In this study conducted to screen genotypes for the gene *Ph2*, Shakthi, Pusa Ruby, Nenmara local, CA 22053, Manulakshmi, IIHR 2204, PNR 7 and *Solanum torvum* were the genotypes amplified of band size 245 bp corresponding to *Ph2/Ph2* genotypes. The genotypes *Solanum pimpinellifolium,* IIHR 2373, LA 1206, EC 620419, Alathur local, IIHR 26, LA 1805, Kuttichal local and Palakkad local showed no amplification due to the absence of alleles for the gene *Ph2* and the remaining genotypes were amplified of band size 221 bp corresponding to susceptible alleles. In a similar study, Arens *et al*. (2010) screened tomato genotypes for the presence of gene *Ph2* using the CAPS marker dTG422 with the restriction enzyme *HinfI*, which generated restriction digested products of band size 310 bp for *Ph2/Ph2* alleles and 290 bp for *ph2/ph2* alleles.

The genotypes with the gene *Mi-1.2* located on chromosome 6, for resistance to root knot nematode was screened using the SCAR marker Mi23 reported by Seah *et al*. (2007). The marker Mi23 developed by Seah *et al*. (2007), generated an amplified fragment of size 380 bp for the genotypes Motelle and Gh2 with the *Mi/Mi* alleles and an amplified fragment of size 430 bp for the genotypes M82-1-8 and Gh13 with the *mi/mi* alleles. On comparing the sequences of the amplified fragments between the genotypes carrying the gene for resistance and genotypes which were susceptible, indels of 1nucleotide and 56 nucleotides, accounting for the differences in the length between the band sizes of two sequences were observed, along with the

identification of 13 SNPs between these two sequences. In this study carried out to identify the genotypes with the gene *Mi1.2*, none showed bands of 380 bp corresponding to *Mi/Mi* alleles. The genotypes *Solanum pimpinellifolium* - EC 697275, EC 514124, EC 620419, Alathur local, Palakkad local, LA 1805, EC 677034, IIHR 26, PKM-1and Kuttichal local showed no amplification due to the absence of alleles for the gene *Mi-1.2* and the remaining genotypes were amplified for *mi/mi* alleles. In a similar study, El Mehrach *et al*. (2005) carried out screening of tomato genotypes for the presence of *Mi1.2* gene using the SCAR marker PMiF3, which generated amplified products of band size 550 bp for genotypes of *Mi/Mi* alleles and 350 bp for genotypes of *mi/mi* alleles. In another study, Michelson *et al*. (1994) screened tomato genotypes for the presence of *Mi1.2* gene using the CAPS marker MI-REX with the restriction enzyme *Taq I*, which generated restriction digested products of band sizes 180 bp and 570 bp for *Mi/Mi* alleles and 750 bp for *mi/mi* alleles.

Tomato genotypes	Genes for resistance located from study				
	Ve1	*Ve2*	*Ph2*	*Ph3*	*I7*
1. *S. pimpinellifolium*- EC 697275					
2. IIHR 2205					–
3. Akshaya					
4. Arka Rakshak					
5. EC 514124					
6. IIHR 2374	–	–			–
7. IIHR 2373					
8. LA 1206					
9. Vellayani Vijai					–
10.IC-45					
11. IIHR 2868					
12. Shakthi			–		
13. Pusa Ruby			–	–	
14. Nenmara local			–		
15. CA 22053			–	–	
16. Manulekshmi			–		
17. EC 620419					
18. IIHR 2204			–		
19. Alathur local					
20. Palakkad local					
21. Hisar Lalit				–	
22. LA 1805					
23. Kuttichal local					
24. Kashi Vishesh	–	–			
25. IIHR 26					
26. PKM 1				–	
27. Arka Vikas				–	
28. EC 677034					
29. PNR 7	–		–		
30. *Solanum torvum*			–		

Figure 1. Genotypes and their located genes for resistance

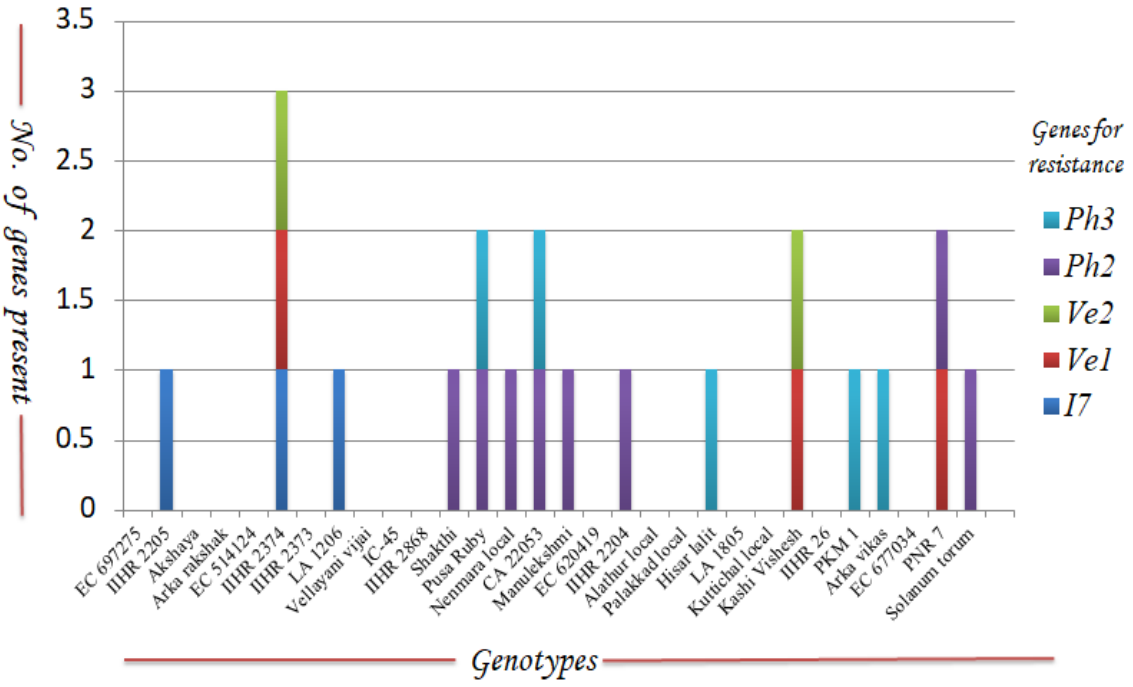

Figure 2. Presence or absence of the genes for resistance located in the 30 tomato genotypes

The presence of single or multiple genes for resistance were located in the 30 genotypes, (Figure 2) with the identified genes distinguishably represented with separate colored bars. From the study, a total of 15 genotypes among the 30 genotypes screened were identified for the presence of various genes for resistance studied. Among the 30 genotypes screened, presence of multiple genes for resistance was identified in five of the genotypes. Genotype IIHR 2374 was identified for the presence of the genes *Ve1* and *Ve2* for resistance to Verticillium wilt and *I7* for resistance to Fusarium wilt. Genotype Kashi vishesh was identified for the presence of genes *Ve1* and *Ve2* for resistance to Verticillium wilt. Genotypes Pusa Ruby and CA 22053 were identified with the presence of genes *Ph2* and *Ph3* for resistance to Late blight. Genotype PNR 7 was identified with the presence of genes *Ve1* for resistance to Verticillium wilt and *Ph2* for resistance to Late blight.

An evident limitation of the study carried out for the screening of genes for resistance in the tomato genotypes for the diseases, *Tomato Yellow Leaf Curl Virus (TYLCV)*; Verticillium wilt; Fusarium wilt; Late blight and Root knot would be the usage of a single molecular marker for each of the genes studied. According to Nevame *et al*. (2018), it may not be preferable to rely entirely on a single type of molecular marker for MAS, as it may not always provide accurate banding patterns under varying experimental conditions. Thus it is advisable to employ different markers for screening the presence of a gene, in order to circumvent the issues of reproducibility under different conditions.

Based on the results obtained from the study, the tomato genotypes in which the genes for resistance were located to the diseases studied, could be considered as potential donors (Figure 1) for the tomato resistance breeding program. As a future line of this study, phenotypic analysis of the genotypes identified for resistance to the concerned genes, can be carried out, under the field conditions to ensure the credibility of the screening conducted using the selected molecular markers.

SUMMARY

6. SUMMARY

The production of tomato (*Solanum lycopersicum* L.), one of the world's most significant vegetable crops, is frequently imperilled by disease infestation. Virus, fungus, bacteria, nematode, and oomycete are the major agents responsible for many of the diseases that threaten tomato production. With a strong focus on promoting and improving disease resistance in tomatoes, various functional molecular markers based on DNA polymorphisms within the genes, responsible for resistance have been employed, extensively and successfully in breeding programs.

The study entitled "Locating donors for genes for biotic stress resistance in tomato through molecular marker assisted selection" was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellayani with an objective to identify donors for genes for resistance to biotic stress inducing diseases like *Tomato Yellow Leaf Curl Virus (TYLCV),* verticillium wilt, fusarium wilt, late blight and root knot nematodes from tomato varieties and elite species through marker assisted selection using gene specific markers.

In the study, 30 genotypes including wild species as well as cultivated varieties of tomatoes, collected from inside and outside of Kerala were used. The genomic DNA isolated from the selected genotypes revealed a purity range of 1.684 and 2.248 and concentration ranged between 354ng/µl to 3222ng/µl. The standardization of the annealing temperatures of the primer pairs for the SCAR and CAPS molecular markers were done using gradient PCR and the annealing temperature standardized were 47°C, 53°C and 55°C.

Molecular screening of genotypes for genes for resistance was carried out using gene specific SCAR and CAPS markers. *Tomato Yellow Leaf Curl Virus (TYLCV)* resistance in genotypes were screened for the presence of the genes *Ty2* and *Ty3* using the SCAR markers T0302F/TY2R1 and FLUW25 respectively. T0302F/TY2R1 amplified a band of size 600 bp for genotypes of *Ty2/Ty2* alleles for resistance and 450 bp for susceptible genotypes of *ty2/ty2* alleles. All the genotypes screened were not identified for the presence of the gene *Ty2*. FLUW25 generates

amplified products of band size 640 bp for *Ty3/Ty3* alleles for resistance and 480 bp for *ty3/ty3* alleles. None of the genotypes screened were identified with the presence of *Ty3* gene for resistance.

Genotypes resistant to verticillium wilt were screened for the presence of two closely linked genes *Ve1* and *Ve2* of *Ve* locus using the CAPS markers Ve1 XbaI and V2LeO3F/V2LeO3R respectively. Ve1 XbaI marker specific to *Ve1* gene generated restriction digested products of band size 410 bp and 332 bp for resistant genotypes with *Ve1/Ve1* alleles and 410 bp, 310bp and 22 bp for susceptible genotypes with *ve1/ve1* alleles. The genotypes IIHR 2374, Kashi Vishesh and PNR 7 were identified for the presence of the gene *Ve1*. *Ve2* gene specific marker V2LeO3F/V2LeO3R distinguishes the genotypes of *Ve2/Ve2* alleles with a restriction digested products of band size 601 bp and 428 bp from an undigested product of 1029bp for *ve2/ve2* alleles. Presence of *Ve2* gene was identified in the genotypes IIHR 2374 and Kashi Vishesh.

The SCAR marker P743DF1/R1 and the CAPS marker CAPS7774 were used for the screening of the genes *I3* and *I7* for resistance to fusarium wilt in genotypes. The SCAR marker P743DF1/R1 generates amplified bands of size 1270 bp for *I3/I3* alleles and 1060 bp for *i3/i3* alleles. The CAPS marker generates restriction digested products of band size, 612 bp and 196 bp for *I7/I7* alleles and undigested products of 808 bp for *i7/i7* alleles. The genotypes IIHR 2205, IIHR 2374 and Vellayani Vijai were identified for the presence of the gene *I7* and none for the gene *I3* for resistance to fusarium wilt.

Genotypes resistant to late blight were screened for the presence of genes *Ph3* and *Ph2* using the SCAR marker Ph3-SCAR and CAPS marker dtG63 respectively. The SCAR marker Ph3-SCAR amplifies band size of 176 bp for genotypes of *Ph3/Ph3* alleles and 154 bp for *ph3/ph3* alleles. Presence of the gene *Ph3* was identified in the genotypes Pusa Ruby, CA 22053, Hisar Lalit, PKM-1 and Arka Vikas. The CAPS marker dtG63 generates restriction digested products of band size 245 bp for *Ph2/Ph2* alleles and 221 bp for *ph2/ph2* alleles. The genotypes Shakthi,

Pusa Ruby, Nenmara local, CA 22053, Manulakshmi, IIHR 2204, PNR 7 and *Solanum torvum* were identified for the presence of *Ph2* gene.

Root knot disease resistance in tomato genotypes were screened for the presence of the gene *Mi1.2* using the SCAR marker Mi23 which generates amplified bands of size 380 bp for *Mi/Mi* alleles and 430 bp for *mi/mi* alleles. Among the 30 genotypes screened, none of the genotypes were identified for the presence of *Mi1.2* gene for resistance.

From the study, 15 genotypes among the 30 genotypes screened were identified for the presence of one or more than one gene for resistance studied. All the genotypes identified with genes for resistance to the diseases studied can be expected to contribute to the tomato resistant breeding programs as potential donors.

REFERENCES

7. REFERENCES

Aboul-Maaty, N. A. F. and Oraby, H. A. S. 2019. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bull. Natl. Res. Cent*. 43(1): 1-10.

Acciarri, N., Rotino, G. L., Tamietti, G., Valentino, D., Voltattorni, S., and Sabatini, E. 2007. Molecular markers for Ve1 and Ve2 Verticillium resistance genes from Italian tomato germplasm. *Plant Breed. 126*(6): 617 - 621.

Anbinder, I., Reuveni, M., Azari, R., Paran, I., Nahon, S., Shlomo, H., Chen, L., Lapidot, M., and Levin, I. 2009. Molecular dissection of Tomato leaf curl virus resistance in tomato line TY172 derived from *Solanum peruvianum*. *Theor. Appl. Genet*. 119(3): 519-530.

Antignus, Y. and Cohen, S. 1994. Complete nucleotide sequence of an infectious clone of a mild isolate of *tomato yellow leaf curl virus* (*TYLCV*). *Phytopathol*. 84(7): 707 - 712.

Arens, P., Mansilla, C., Deinum, D., Cavellini, L., Moretti, A., Rolland, S., van der Schoot, H., Calvache, D., Ponz, F., Collonnier, C., and Mathis, R. 2010. Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. *Theor. Appl. Genet.* 120(3): 655-664.

Arumuganathan, K. and Earle, E. D. 1991. Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.* 9: 208-218.

Bacon, C. W. and Yates, I. E. 2006. Endophytic Root Colonization by *Fusarium* Species: Histology, Plant Interactions, and Toxicity. *Soil Biol.* 9: 133 - 152.

Barbieri, M., Acciarri, N., Sabatini, E., Sardo, L., Accotto, G. P., and Pecchioni, N. 2010. Introgression of resistance to two Mediterranean virus species causing *tomato yellow leaf curl* into a valuable traditional tomato variety. *J. Plant Pathol*. 92: 485–493.

Barillas, A. C., Mejia, L., Sanchez-Perez, A., and Maxwell, D. P. 2008. CAPS and SCAR markers for detection of *I3* gene introgression for resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 3. *Rep. Tomato Genet. Coop*. pp.11-17.

Bernardo, R. and Yu, J. 2007. Prospects for genomewide selection for quantitative traits in maize. *Crop Sci.* 47: 1082–1090.

Black, L. L., Wang, T. C., Hanson, P. M., and Chen, J. T. 1996. Late blight resistance in four wild tomato accessions: effectiveness in diverse locations and inheritance of resistance. *Phytopathol.* 86: S24.

Bohn, G. W. and Tucker, C. M. 1940. Studies on Fusarium wilt of the tomato. I. Immunity in *Lycopersicon pimpinellifolium* Mill and its inheritance in hybrids. *Research Bulletin, Missouri Agricultural Experiment Station*, 311: 1 - 82.

Brautigam, A. and Gowik, U. 2010. What can next generation sequencing do for you? Next generation sequencing as a valuable tool in plant research. *Plant Biol.* 12: 831–841.

Brenda, E. G., Elaine, G., Katie, S. J., Peter, H., Luis, M., and Maxwell, D. P. 2007. Co-dominant SCAR marker for detection of the *begomo virus* resistance *Ty2* locus derived from *Solanum habrochaites* in tomato germplasm. *Rep. Tomato Genet. Coop.* 57: 21 – 24.

Brouwer, D. J. and St. Clair, D. A. 2004. Fine mapping of three quantitative trait loci for late blight resistance in tomato using near isogenic lines (NILs) and sub-NILs. *Theor. Appl. Genet.* 108: 628 - 638.

Catanzariti, A. M., Lim, G. T., and Jones, D. A. 2015. The tomato *I-3* gene: a novel gene for resistance to Fusarium wilt disease. *New Phytologist*, 207(1): 106-118.

Chai, Y., Zhao, L., Liao, Z., Sun, X., Zuo, K., Zhang, L., Wang, S., and Tang, K. 2003. Molecular cloning of a potential *Verticillium dahliae* resistance gene SlVe 1 with multi-site polyadenylation from *Solanum licopersicoides*. *DNA Sequence*, 14(5): 375 - 384.

Chetelat, R.T. and Ji, Y. 2007. Cytogenetics and evolution. *Genetic improvement of solanaceous crops*, 2: 77 - 112.

Child, A. 1990. A synopsis of *Solanum* subgenus *Potatoe* (G. Don) (D'Arcy) [*Tuberarium* (Dun.)) Bitter (s.l.)]. *Feddes Repert*. 101: 209–235.

Chunwongse, J., Chunwongse, C., Black, L., and Hanson, P. 2002. Molecular mapping of the Ph-3 gene for late blight resistance in tomato. *J. Hortic. Sci. Biotechnol.* 77: 281- 286.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., and Pang, E. C. K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, 142: 169–196.

Corsini, D. and Pavek, J. J. 1996. Agronomic performance of potato germplasm selected for high resistance to Verticillium wilt. *Am. Potato J.* 73(6): 249-260.

Dahal, D., Heintz, D., Van Dorsselaer, A., Braun, H. P., and Wydra, K. 2009. Pathogenesis and stress related, as well as metabolic proteins are regulated in tomato stems infected with *Ralstonia solanacearum*. *Plant Physiol. Biochem*. 47: 838 – 846.

Darwin, S. C., Knapp, S., and Peralta, I. E. 2003. Tomatoes in the Galápagos Islands: morphology of native and introduced species of *Solanum* section *Lycopersicon* (Solanaceae). *Syst. Biodivers*. 1: 29-54.

de Miranda, B. E. C., Boiteux, L. S., Cruz, E. M., and Reis, A. 2010. Sources of resistance to *Verticillium dahliae* races 1 and 2 in accessions of *Solanum* (section *Lycopersicon*). *Hortic. Bras.* 28: 458 - 465.

Dhaliwal, M. S., Jindal, S. K., Sharma, A., and Prasanna, H. C. 2020. Tomato yellow leaf curl virus disease of tomato and its management through resistance breeding: a review. *J. Hortic. Sci. Biotechnol. 95*(4): 425 - 444.

Di Pietro, A., Madrid, M. P., Caracuel, Z., Delgado-Jarana, J., and Roncero, M. I. G. 2003. *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. *Mol. Plant Pathol.* 4: 315–325.

Dı´az-Pendo´n, J. A., Canizares, M. C., Moriones, E., Bejarano, E. R., Czosnek, H., and Navas-Castillo, J. 2010. *Tomato yellow leaf curl viruses*: Me´nage a ` trois between the virus complex, the plant and the whitefly vector. *Mol. Plant Pathol.* 11: 441 - 450.

Diwan, N., Fluhr, R., Eshed, Y., Zamir, D., and Tanksley, S. D. 1999. Mapping of *Ve* in tomato: a gene conferring resistance to the broad spectrum pathogen, *Verticillium dahliae* race 1. *Theor. Appl. Genet.* 98: 315-319.

El Mehrach, K., Mejía, L., Gharsallah-Couchane, S., Salus, M. S., Martin, C. T., Hatimi, A., Vidavski, F., Williamson, V., and Maxwell, D. P. 2005. PCR-based methods for tagging the *Mi-1* locus for resistance to root-knot

nematode in *begomovirus*-resistant tomato germplasm. *Acta. Hortic.* 695: 263–270.

El-Sappah, A. H., Islam. M. M, El-awady, H., Yan, H., Qi, S., Liu, J., Cheng, G.T., and Liang, Y. 2019. Tomato natural resistance genes in controlling the root-knot nematode. *Genes*, 10(11): 925.

Essarioui, A., Mokrini, F., and Afechtal, M. 2016. Molecular interactions between tomato and its wilt pathogen *Fusarium oxysporum* f. sp. *Lycopersici*-a review. *Revue Marocaine des Sciences Agronomiques et Vétérinaires*, *4*(1): 66-74.

Fei, J., Chai, Y., Wang, J., Lin, J., Sun, X., Sun, C., Zuo, K., and Tang, K. 2004. cDNA cloning and characterization of the *Ve* homologue gene *StVe* from *Solanum torvum* Swartz. *DNA Sequence*, 15(2): 88 - 95.

Foolad, M. 2007. Genome mapping and molecular breeding of tomato. *Int. J. Plant Genomics*, 2007: 64358.

Foolad, M. R., and Panthee, D. R. 2013. Marker-assisted selection in tomato breeding. *Crit. Rev. Plant Sci*. 31(2): 93-123.

Foolad, M. R., Merk, H. L., and Ashrafi, H. 2008. Genetics, genomics and breeding of late blight and early blight resistance in tomato. *Crit. Rev. Plant Sci*. 27(2): 75-107.

Fradin, E. F. and Thomma, B. P. H. J. 2006. Physiology and molecular aspects of Verticillium wilt diseases caused by *V. dahliae* and *V. alboatrum*. *Mol. Plant Pathol.* 7: 71-86.

Fulton, T. M., Van der Hoeven, R., Eannetta, N. T., and Tanksley, S. D. 2002. Identification, analysis, and utilizatioin of conserved ortholog set markers for comparative genomics in higher plants. *Plant Cell,* 14: 1457–1467.

Gardner, R. G. 1982. NC50–7 breeding line, ‘Cherokee’ and ‘Mountain Pride’ tomato. *Hortic. Sci.* 23: 92–93.

Gardner, R. G. 2006. ‘Plum Crimson’ fresh-market plum tomato hybrid and its parents, NC EBR-7 and NC EBR-8. *Hortic. Sci.* 41: 259–260.

Goggin, F. L., Williamson, V. M. and Ullman, D. E. 2001. Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the tomato resistance gene *Mi*. *Environ. Entomol*. 30: 101–106.

Gonzalez-Cendales, Y., Catanzariti, A. M., Baker, B., Mcgrath, D. J., and Jones, D. A. 2016. Identification of *I-7* expands the repertoire of genes for resistance to Fusarium wilt in tomato to three resistance gene classes. *Mol. Plant Pathol*. *17*(3): 448-463.

Green, S. K. 1991. *Guidelines for diagnostic work in plant virology*. Technical bulletin No. 15, Asian Vegetable Research and Development Center, Taiwan, 63p.

Gronenborn, B. 2007. The tomato yellow leaf curl virus genome and function of its proteins. In: Czosnek H. (ed.). *Tomato yellow leaf curl virus disease*. Springer, Dordrecht, pp. 67 - 84.

Heubl, G. 2010. New aspects of DNA-based authentication of Chinese medicinal plants by molecular biological techniques. *Planta Med.* 76(17): 1963 - 1974.

Hoogstraten, J. G. and Braun, C. J. 2005. Methods for coupling resistance alleles in tomato. US Patent No. 20050278804. Available: http://www.wipo.int/pctdb/en/wo.jsp?wo=2005079342[10 March 2009].

Hu, C. Y., Tsai, Y. Z., and Lin, S. F. 2014. Development of STS and CAPS markers for variety identification and genetic diversity analysis of tea germplasm in Taiwan. *Bot. Stud.* 55(1): 12.

Jensen, K. S., Martin, C. T., and Maxwell, D. P. 2007. A CAPS marker FER-G8 for detection of *Ty3* and *Ty3a* alleles associated with *S. chilense* introgression for *begomovirus* resistance in tomato breeding lines. Available:http://www.plantpath.wisc.edu/.

Ji, Y, Scott, J. W., and Schuster, D. J. 2009. Molecular mapping of *Ty- 4*, a new *Tomato yellow leaf curl virus* resistance locus on chromosome 3 of tomato. *J. Am. Soc. Hortic. Sci*. 134(2): 281-288.

Ji, Y., Schuster, D. J., and Scott, J. W. 2007. *Ty-3*, a begomovirus resistance locus near the *Tomato yellow leaf curl virus* resistance locus *Ty-1* on chromosome 6 of tomato. *Mol.Breed.* 20: 271-284.

Ji. Y, Betteray. B, Smeets, J., Jensen, K. S., and Mejia, L. 2008. Co- dominant SCAR marker, P6-25, for detection of *Ty-3*, *Ty-3a* and *Ty-3b* introgressions from three *Solanum chilense* accessions at 25 cM of chromosome 6 of Begomovirus-resistanttomatoes. Available: http://www.plantpath.wisc.edu/ Geminivirus Resistant Tomatoes/Markers/MAS-Protocols/P6-25-locus.pdf.

Jiang, Y., Jiang, Q., Hao, C., Hou, J., Wang, L., Zhang, H., Zhang, S., Chen, X., and Xhang, X. 2015. A yield-associated gene TaCWI, in wheat: Its function, selection and evolution in global breeding revealed by haplotype analysis. *Theor. Appl. Genet.* 128(1): 131–143.

Jung, J., Kim, H. J., Lee, J. M., Oh, C. S., Lee, H. J., and Yeam, I., 2015. Gene-based molecular marker system for multiple disease resistances in tomato against *Tomato yellow leaf curl virus*, late blight, and verticillium wilt. *Euphytica*, 205(2): 599 - 613.

Karagiannidis, N., Bletsos, F., and Stavropoulos, N. 2002. Effect of Verticillium wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. *Sci. Hortic.* 94: 145 - 156.

Kasai, K., Morikawa, Y., Sorri, V. A., Valkonen, J. P. T., Gebhardt, C., and Watanabe, K. N. 2000. Development of SCAR markers to the PVY resistance gene *Ryadg* based on a common feature of plant disease resistance genes. *Genome,* 43:1–8.

Kawchuk, L. M., Hachey, J., Lynch, D. R., Kulcsar, F., Van Rooijen, G., Waterer, D. R., Robertson, A., Kokko, E., Byers, R., Howard, R. J., and Fischer, R. 2001. Tomato *Ve* disease resistance genes encode cell surface-like receptors. *Proc. Natl. Acad. Sci.* 98(11): 6511-6515.

Kethidi, D. R., Roden, D. B., Ladd, T. R., Krell, P. J., Retnakaran, A., and Feng, Q. 2003. Development of SCAR markers for the DNA-based detection of the Asian long-horned beetle *Anoplophora ghbripennis* (Motschulsky). *Arch. Insect Biochem. Physiol*. 52: 193- 4.

Kheyr-Pour, A., Bendahmane, M., Matzeit, V., Accotto, G. P., Crespi, S., and Gronenborn, B. 1991. Tomato yellow leaf curl virus from Sardinia is a whitefly-transmitted monopartite geminivirus. *Nucleic Acids Res.* 19(24): 6763–6769.

Kiewnick, S., Dessimoz, M., and Franck, L. 2009. Effects of the Mi-1 and the N root-knot nematode-resistance gene on infection and reproduction of *Meloidogyne enterolobii* on tomato and pepper cultivars. *J. Nematology,* 41(2): 134.

Kim, M. J. and Mutschler, M. A. 2005. Transfer to processing tomato and characterization of late blight resistance derived from *Solanum pimpinellifolium* L. L3708. *J. Am. Soc. Hortic. Sci.* 130: 877 - 884.

Kim, S. G., Hur, O. S., Ro, N. Y., Ko, H. C., Rhee, J. H., Sung, J. S., Ryu, K. Y., Lee, S.Y., and Baek, H. J., 2016. Evaluation of resistance to *Ralstonia solanacearum* in tomato genetic resources at seedling stage. *Plant Pathol. J*. 32(1): 58 - 64.

Kiran, U., Khan, S., Mirza, K. J., Ram, M., and Abdin, M. Z. 2010. Scar Markers: A Potential Tool for Authentication of Herbal drugs. *Fitoterapia*, 81: 969-976.

Kole, C., Ashrafi, H., Lin, G., and Foolad, M. 2006. Identification and molecular mapping of a new R gene, *Ph-4*, conferring resistance to late blight in tomato. In: Solanaceae Conference; University of Wisconsin, Madison, Abstract 449.

Konuma, H. and Gennari, P. 2014. FAO Statistical Yearbook. Asia and the Pacific Food and Agriculture. 80p.

Lapidot, M. and Polston, J. E. 2006. Resistance to Tomato yellow leaf curl virus in tomato. In: *Natural resistance mechanisms of plants to viruses*. Springer, Dordrecht, pp. 503-520.

Lapidot, M., Karniel, U., Gelbart, D., Fogel, D., Evenor, D., Kutsher, Y., Makhbash, Z., Nahon, S., Shlomo, H., Chen, L., Reuveni, M., and Levin, I. 2015. A novel route controlling Begomovirus resistance by the messenger RNA surveillance factor Pelota. *PLoS Genet.* 11: e1005538.

Lecomte, L., Duffe, P., Buret, M., Servin, B., Hospital, F., and Causse, M. 2004. Marker-assisted introgression of five QTLs controlling fruit quality traits

into three tomato lines revealed interactions between QTLs and genetic backgrounds. *Theor. Appl. Genet.* 109: 658 -668.

Lee, J. M., Oh, C. S., and Yeam, I. 2015. Molecular markers for selecting diverse disease resistances in tomato breeding programs. *Plant Breed. Biotechnol.* 3(4): 308 - 322.

Lee, J. W., Bang, K. H., Kim, Y. C., Seo, A. Y., Jo, I. H., Lee, J. H., Kim, O. T., Hyun, D. Y., Cha, S. W., and Cho, H. H. 2012. CAPS markers using mitochondrial consensus primers for molecular identification of *Panax* species and Korean ginseng cultivars (*Panax ginseng* C.A. Meyre). *Mol. Biol. Rep*. 39(1): 729 - 736.

Lee, S. J., Kelley, B. S., Damasceno, C. M. B., John, B. S., Kim, B. S., Kim, B. D., and Rose, J. K. C. 2006. A functional screen to characterize the secretomes of eukaryotic pathogens and their hosts in planta. *Mol. Plant-Microbe Interact.* 1: 1368 - 1377.

Leonardi, C., Ambrosino, P., Esposito, F., and Fogliano, V. 2000. Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. *J. Agric. Food Chem.* 48(10): 4723 - 4727.

Li, D., Lewis, R. S., Jack, A. M., Dewey, R. E., Bowen, S. W., and Miller, R. D. 2012. Development of CAPS and dCAPS markers for CYP82E4, CYP82E5V2 and CYP82E10 gene mutants reducing nicotine to nornicotine conversion in tobacco. *Mol. Breed.* 29(3):589–599.

Lim, S. H. and Ha, S. H. 2013. Marker development for the identification of rice seed color. *Plant Biotechnol. Rep.* 7(3): 391- 398.

Lu, K. T., Lee, H. C., Liu, F. S., Lo, C. F., and Lin, J. H. 2010. Identification of *Ginseng radix* in Chinese medicine preparations by

nested PCR-DNA sequencing method and nested PCR restriction fragment length polymorphism. *J. Food Drug Anal.* 18(1): 58 - 63.

Maxwell, D. P, Martin, C., Salus, M. S., Montes, L., and Mejía, L. 2006. Breeding tomatoes for resistance to tomato-infecting *begomoviruses*. International Plant Virology Laboratory, University of Wisconsin-Madison, Available: http://www.plantpath.wisc.edu/ Geminivirus Resistant Tomatoes/ index. htm.

Medina-Filho, H. and Stevens, M. 1980. Tomato breeding for nematode resistance: survey of resistant varieties for horticultural characterisitcs and genotype of acid phosphatase. *Acta. Hortic*. 100: 383–391.

Mejía, L., Teni, R. E., Vidavski, F., Czosnek, H., Lapidot, M., Nakhla, M. K., and Maxwell, D. P. 2005. Evaluation of tomato germplasm and selection of breeding lines for resistance to *begomoviruses* in Guatemala. *Acta. Hortic*. 695: 251-255.

Melinda, S., Martin, C. T. and Maxwell, D. P. 2007. PCR protocol for the co-dominant SCAR marker, FLUW -25, for detection of the introgression at 25 cM (*Ty-3* locus) of chromosome 6. University of Wisconsin-Madison, 1-4p.

Merk, H. L., Ashrafi, H., and Foolad, M. R. 2012. Selective genotyping to identify late blight resistance genes in an accession of the tomato wild species *Solanum pimpinellifolium*. *Euphytica*, 187: 63-75.

Michelson I., Zamir, D., and Czosnek, H. 1994. Accumulation and translocation of *Tomato yellow leaf curl virus* (*TYLCV*) in a *Lycopersicon esculentum* breeding line containing the *L. chilense TYLCV* tolerance gene *Ty-1*. *Phytopathol*. 84: 928-933.

Michielse, C. B. and Rep, M. 2009. Pathogen profile update: *Fusarium oxysporum*. *Mol. Plant Pathol.* 10:311 - 324.

Miller, J. C. and Tanksley, S. D. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theor. Appl. Genet.* 80: 437 - 448.

Milligan, S. B., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P., and Williamson, V. M. 1998. The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *The Plant Cell*, 10(8): 1307-1319.

Moreau, P., Thoquet, P., Olivier, J., Laterrot, H., and Grimsley, N. 1998. Genetic mapping of *Ph-2*, a single locus controlling partial resistance to *Phytophthora infestans* in tomato. *Mol. Plant-Microbe Interact.* 11: 259 - 269.

Moretti, A. N. 2009. Taxonomy of Fusarium genus, a continuous fight between lumpers and splitters. *Zbornik Matice srpske za prirodne nauke*, 117: 7-13.

Moriones, E. and Navas-Castillo, J. 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Res*. 71(1-2): 123-134.

Murray, H. G. and Thompson, W. F. 1998. Rapid isolation of high molecular weight DNA. *Nucleic Acids Res.* 8: 4321–4325.

Navot, N., Pichersky, E., Zeidan, M., Zamir, D., and Czosnek, H. 1991. *Tomato yellow leaf curl virus*: A whitefly- transmitted *geminivirus* with a single genomic component. *Virology*, 185: 151–161.

Neff, M. M., Neff, J. D., Chory, J., and Pepper, A. E. 1998. dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in *Arabidopsis thaliana* genetics. *Plant J.* 14(3): 387–392.

Nevame, A. Y. M., Xia, L., Nchongboh, C.G., Hasan, M. M., Alam, M. A., Yongbo, L., Wenting, Z., Yafei, H., Emon, R. M., Ismail, M. R., Efisue, A., Gang, S., Wenhu, L., and Longting, S. 2018. Development of a new molecular marker for the resistance to *tomato yellow leaf curl virus*. *Biomed Res. Int.* 46: 19–29.

Panthee, D. R. and Foolad, M. R., 2012. Retraction: A reexamination of molecular markers for use in marker-assisted breeding in tomato. *Euphytica*, 184: 165–179.

Paran, I. and Michelmore, R. W. 1993. Development of Reliable PCR-Based Markers Linked to Downy Mildew Resistance Genes in Lettuce. *Theor. Appl. Genet.* 85: 985-993.

Park, Y. H., Lee, Y. J., Kang, J. S., Choi, Y. W., and Son, B. G. 2008. Development of gene-based DNA marker for verticillium wilt resistance in tomato. *Korean J. Hortic. Sci. Technol.* 26: 313 - 319.

Peirce, L. C. 1971. Linkage tests with *Ph* conditioning resistance to race 0, *Phytophthora infestans*. *Tomato Genet. Coop.Rep*. 21: 30.

Peralta, I. E. and Spooner, D. M. 2005. Morphological characterization and relationships of wild tomatoes (*Solanum* L. Section *Lycopersicon*). *Monographs In Systematic Botany*, 104: 227-257.

Pérez de Castro, A., Díez, M. J., and Nuez, F. 2007. Inheritance of Tomato yellow leaf curl virus resistance derived from *Solanum pimpinellifolium* UPV16991. *Plant Dis*. 91: 879–885.

Perovic, J., Silvar, C., Koenig, J., Stein, N., Perovic, D., and Ordon, F. 2013. A versatile fluorescence-based multiplexing assay for CAPS genotyping electrophoresis systems. *Mol. Breed.* 32 (1): 61–69.

Pillen, K., Pineda, O., Lewis, C., and Tanksley, S. D. 1996. Status of genome mapping tools in the taxon Solanaceae. In: Paterson, A., (ed.), *Genome Mapping in Plants*. R. G. Landes, Austin, TX, USA, pp. 281–308.

Reddy, B. R., Reddy, M. P., Begum, H., and Sunil, N. 2013. Genetic diversity studies in tomato (*Solanum lycopersicum* L.). *J. Agric. Vet. Sci.* 4(4): 53-55.

Robbins, M. D., Masud, M. A. T. Panthee, D. R., Gardner, R. G., Francis, D. M., and Stevens, M. R. 2010. Marker-assisted Selection for Coupling Phase Resistance to Tomato spotted wilt virus and *Phytophthora infestans* (Late Blight) in Tomato. *Hortic. Sci.* 45(10): 1424 - 1428.

Rodewald, J. and Trognitz, B. 2013. Solanum resistance genes against *Phytophthora infestans* and their corresponding avirulence genes. *Mol. Plant Pathol.* 14(7): 740-757.

Rossi, M., Goggin, F. L., Milligan, S. B., Kaloshian, I., Ullman, D. E., and Williamson, V. M. 1998. The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proc. Natl. Acad. Sci.* USA. 95: 9750–9754.

Rubin, E. and Cohen, Y. 2004. Oospores associated with tomato seed may lead to seedborne transmission of *Phytophthora infestans. Phytoparasitica*, 32: 237–245.

Rubin, E., Baider, A., and Cohen, Y., 2001. *Phytophthora infestans* produces oospores in fruits and seeds of tomato. *Phytopathol*. 91(11): 1074-1080.

Rutkoski, J., Heffner, E., and Sorrells, M. 2011. Genomic selection for durable stem rust resistance in wheat. *Euphytica,* 179: 161–173.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. 1988. Primer Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase. *Sci.* 239: 487- 491.

Sajid, Q. and Eminur, E. 2019. Recent development in molecular markers system for disease resistance in Tomato. *Res. J. Biotechnol*. 14(12): 140 – 151.

Schaible, L., Cannon, O. S., and Waddoups, V. 1951. Inheritance of resistance to Verticillium wilt in a tomato cross. *Phytopathol.* 41: 986– 990.

Scott, J. W. 2008. Fresh market tomato breeding in the USA. *Acta. Hortic*. 789: 21 - 25.

Scott, J. W. and Gardner, R. G. 2007. Breeding for resistance to fungal pathogens. In: Razdan M. K. and Matto A. K. (eds), *Genetic improvement of solanaceous crops*. Science Publishers, 2: 421- 456.

Scott, J. W., Hutton, S. F., and Freeman, J. H. 2015. Fla. 8638B and Fla. 8624 Tomato Breeding Lines with *Begomovirus* Resistance Genes *ty-5* Plus *Ty-6* and *Ty-6*, respectively. *Hortic. Sci*. 50: 1405-1407.

Scott, J. W. and Jones, J. P. 1989. Monogenic resistance in tomato to *Fusarium oxysporum* f. sp. *lycopersici* race 3. *Euphytica*, 40: 49-53.

Seah, S. Williamson, V. M., Garcia, B. E., Mejia, L., Salus, M. S., Martin, C. T., and Maxwell, D. P. 2007. Evaluation of a co-dominant SCAR marker for detection of the *Mi-1* locus for resistance to root-knot nematode in tomato germplasm. *Rep. Tomato Genet. Coop*. 57: 37–40.

Shavrukov, Y. N. 2016. CAPS markers in plant biology. *Russ. J. Genet. Appl. Res.* 6(3): 279-287.

Simons, G., Groenendijk, J., Wijbrandi, J., Reijans, M., Groenen, J., Diergaarde, P., Van der Lee, T., Bleeker, M., Onstenk, J., de Both, M., Haring, M., Mes, J., Cornelissen, B., Zabeau, M., and Vos, P. 1998. Dissection of the Fusarium *I2* gene cluster in tomato reveals six homologs and one active gene copy. *Plant Cell*. 10: 1055-1068.

Stevens, M. R. and Robbins, M. D. 2007. Molecular markers in selection of tomato germplasm. In: Razdan, M. K. and Mattoo, A. K., (eds.), *Genetic Improvement of Solanaceous Crops.* Science Publishers, NH, USA, 2: 239–260.

Stevens, M., Scott, S., and Gergerich, R. 1991. Inheritance of a gene for resistance to *Tomato spotted wilt virus* (*TSWV*) from *Lycopersicon peruvianum* Mill. *Euphytica*, 59: 9-17.

Tartarini, S., Gianfranceschi, L., Sansavini, S., and Gessler, C. 1999. Development of reliable PCR markers for the selection of the *Vf* gene conferring scab resistance in apple. *Plant Breed.* 118: 183–186.

van Ooijen, G., van den Burg, H. A., Cornelissen, B. J., and Takken, F. L. 2007. Structure and function of resistance proteins in solanaceous plants. *Annu. Rev. Phytopathol. 45*: 43 - 72.

Veremis, J. C. and Roberts, P. A. 1996. Relationship between *Meloidogyne incognita* resistance gene in *Lycopersicon peruvianum* differentiated by heat sensitivity and nematode virulence. *Theor. Appl. Genet*. 93: 950 - 959.

Verlaan, M. G., Hutton, S. F., Ibrahem, R. M., Kormelink, R., Visser, R. G. F., Scott, J. W., Edwards, J. D., and Bai, Y. 2013. The *Tomato yellow leaf curl virus* resistance genes *Ty-1* and *Ty-3* are allelic and code for DFDGD-class RNA-dependent RNA polymerases. *PLoS Genet.* 9: e1003399.

Verlaan, M. G., Szinay, D., Hutton, S. F., de Jong, H., Kormelink, R., Visser, R. G., Scott, J.W., and Bai, Y. 2011. Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the *TYLCV* resistance gene *Ty-1*. *The Plant J*. *68*(6): 1093-1103.

Williamson, V. M., Hwang, C. F., Truesdell, G., Bhakta, A. V., and Fort, K. P. 2000. The nematode resistance gene, *Mi*. In: De Wit, P. J. G., Bisseling, T., and Stiekema, W. J. (eds.), *Biology of Plant Microbe Interactions*. International Society for Molecular Plant Microbe Interactions, St. Paul, MN, USA, 2: 88 – 92.

Wu, J., Cao, X., Guo, L., Qi, T., Wang, H., Tang, H., Zhang, J., and Xing, C. 2014. Development of a candidate gene marker for Rf_1 based on a *PPR* gene in cytoplasmic male sterile CMS-D2 Upland cotton. *Mol. Breed.* 34 (1): 231–240.

Wu, W. W., Shen, H., and Yang, W. 2009. Sources for Heat-Stable Resistance to Southern Root-Knot Nematode (*Meloidogyne incognita*) in *Solanum lycopersicum*. *Agric. Sci. China*, 8: 697–702.

Yamaguchi, H., Ohnishi, J., Saito, A., Ohyama, A., Nunome, T., Miyatake, K., and Fukuoka, H. 2018. An NB-LRR gene, TYNBS1, is responsible for resistance mediated by the *Ty-2 Begomovirus* resistance locus of tomato. *Theor. Appl. Genet.* 131(6): 1345-1362.

Yang, I., Kim, Y. H., Byun, J.Y., and Park, S. R. 2005. Use of multiplex polymerase chain reactions to indicate the accuracy of the annealing temperature of thermal cycling. *Anal. Biochem*. 338(2): 192-200.

Yang, X., Caro, M., Hutton, S. F., Scott, J. W., Guo, Y., Wang, X., Rashid, M. H., Szinay, D., de Jong, H., Visser, R. G., Bai, Y., and Du, Y. 2014. Fine mapping of the *Tomato yellow leaf curl virus* resistance gene *Ty-2* on chromosome 11 of tomato. *Mol. Breed.* 34: 749-760.

Zamir, D. 1., Ekstein-Michelson, I., Zakay, Y., Navot, N., Zeidan, M., Sarfatti, M., Eshed, Y., Harel, E., Pleban, T., van-Oss, H., Kedar, N., Rabinowitch, H. D., and Czosnek, H. 1994. Mapping and introgression of a *Tomato yellow leaf curl virus* tolerance gene, *Ty-1*. *Theor. Appl. Genet.* 88: 141-146.

Zhang, C., Liu, L., Wang, X., Vossen, J., Li, G., Li, T., Zheng, Z., Gao, J., Guo, Y., Visser, R. G., and Li, J., 2014. The *Ph-3* gene from *Solanum pimpinellifolium* encodes CC-NBS-LRR protein conferring resistance to *Phytophthora infestans*. *Theor. Appl. Genet.* 127(6): 1353 - 1364.

APPENDICES

8. APPENDICES

APPENDIX I

Reagents required for DNA Isolation

1. **DNA Extraction buffer (pH: 8)**

CTAB	2% (w/v)
NaCl	1.4 M
EDTA	20 mM
Tris HCl (pH: 8)	100 mM
PVP	2% (w/v)
β-Mercaptoethanol	0.2% (v/v)

Dissolve all the reagents except PVP and β-Mercaptoethanol and adjust the final pH to 8. Make up the final volume of the buffer to 1L using deionized water and store at room temperature. Add PVP and β-Mercaptoethanol to the buffer freshly before use.

2. **TE buffer (1X)**

Tris HCl (pH: 8)	10 mM
EDTA	1 mM

Dissolve the reagents and adjust the final pH to 8. Make up the final volume to 1L using deionized water. Store the buffer at -20℃.

3. **Chloroform: Isoamyl alcohol**

Chloroform	24 ml
Isoamyl alcohol	1 ml

Mix 24 parts of chloroform with 1 part of isoamyl alcohol, freshly before use.

4. 70% Ethanol

100% Ethanol	70 ml
Distilled water	30 ml

APPENDIX II

Reagents required for Agarose gel electrophoresis

1. **TAE buffer (10X) (Ph: 8)**

Tris base	48.4 g
Glacial acetic acid	11.42 ml
0.5 M EDTA (Ph: 8)	20 ml

Dissolve Tris base in about 800 ml of deionized water. Add glacial acetic acid and EDTA, and make up the final volume to 1 L using deionized water. Store the buffer at room temperature.

2. **Gel loading dye (6X)**

Bromophenol Blue	0.25 g
Sucrose	4 g
Milipore water	10 ml

ABSTRACT

LOCATING DONORS FOR GENES FOR BIOTIC STRESS RESISTANCE IN TOMATO THROUGH MOLECULAR MARKER ASSISTED SELECTION

by

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9. ABSTRACT

The study entitled “Locating donors for genes for biotic stress resistance in tomato through molecular marker assisted selection” was conducted at the Department of Seed Science and Technology, College of Agriculture, Vellayani, Thiruvananthapuram during 2020-2021. The primary objective of the study was to identify donors for genes for resistance to different biotic stresses viz., *Tomato Yellow Leaf Curl Virus (TYLCV),* verticillium wilt, fusarium wilt, late blight and root knot nematodes from tomato varieties and elite wild species through marker assisted selection using gene specific markers.

The study was conducted in 30 tomato genotypes, collected from inside and outside of Kerala which included wild species as well cultivated varieties. The genotypes were screened for the presence of genes for resistance to various biotic stress inducing diseases like *Tomato Yellow Leaf Curl Virus (TYLCV)*, verticillium wilt, fusarium wilt, late blight and root knot using tightly linked gene specific SCAR and CAPS markers.

The genotypes resistant to *Tomato Yellow Leaf Curl Virus (TYLCV)* were screened for the presence of gene *Ty2* located on chromosome 11 using the SCAR marker TG0302/TY2R1 which amplified a band of size 600 bp for genotypes with *Ty2/Ty2* alleles and 450 bp for susceptible genotypes. None of the 30 genotypes screened for *Ty2* gene were identified to consist the *Ty2/Ty2* alleles. Another gene for resistance to *TYLCV*, ie, *Ty3* located on chromosome 6, was screened using the SCAR marker FLUW25, which amplified the genotypes carrying *Ty3/Ty3* alleles with a band of size 640 bp and 480 bp for genotypes corresponding to *ty3/ty3* alleles. Presence of *Ty3* gene was not found in any of the genotypes screened in the study.

Verticillium wilt resistance in genotypes was screened for the presence of two closely linked genes, *Ve1* and *Ve2* of *Ve* locus. *Ve1* specific CAPS marker Ve1 XbaI, distinguished the genotypes of *Ve1/Ve1* alleles with restriction digested fragments of size 410bp and 332bp from the susceptible genotypes of restriction digested fragments 410bp, 310bp and 22bp. *Ve2* specific CAPS marker V2LeO3F/V2LeO3R generated restriction digested products, 601 bp and

428 bp for *Ve2/Ve2* alleles and undigested product of 1029bp for *ve2/ve2* alleles. Among the 30 genotypes screened in the study, IIHR 2374 and Kashi Vishesh were identified for the presence of *Ve1* and *Ve2* genes and PNR 7 for *Ve2* gene alone.

The genotypes resistant to Fusarium wilt were screened for the presence of the genes *I3* located on chromosome 7 and *I7* located on chromosome 8. The gene *I3* specific SCAR marker P743DF1/R1 amplified a band of size 1270bp for genotypes with *I3/I3* alleles and 1060 bp for genotypes with *i3/i3* alleles. None of the genotypes screened in the study were identified for the presence of *I3* gene. The CAPS marker CAPS7774 specific to *I7* gene generated restriction digested fragments of size 612bp and 196bp for genotypes of *I7/I7* alleles and undigested fragment of 808 bp for susceptible genotypes. The genotypes IIHR 2205, IIHR 2374 and Vellayani Vijai were identified for the presence of *I7/I7* alleles from the study.

The genotypes resistant to Late blight were screened for the presence of the genes *Ph3* located on chromosome 9 and *Ph2* located on chromosome 10. The SCAR marker Ph3-SCAR amplified the genotypes of *Ph3/Ph3* alleles with a band of size 176bp and 154 bp for the genotypes of *ph3/ph3* alleles. Presence of the gene *Ph3* was identified in the genotypes Pusa Ruby, CA 22053, Hisar Lalit, PKM-1 and Arka Vikas from the study. The CAPS marker dtG63 differentiated the genotypes of *Ph2/Ph2* alleles with a restriction digested fragment of band size 245 bp from 221 bp of susceptible genotypes. In the study, the genotypes Shakthi, Pusa Ruby, Nenmara local, CA 22053, Manulakshmi, IIHR 2204, PNR 7 and *Solanum torvum* were identified for the presence of *Ph2/Ph2* alleles.

Resistance to Root knot in genotypes were screened for the presence of gene *Mi1.2* using the SCAR marker Mi23, which amplified a band of size 380 bp for *Mi/Mi* alleles and 430 bp for *mi/mi* alleles. From the study it was confirmed that, none of the genotypes screened for the presence of *Mi 1.2* gene were resistant to root knot.

In accordance with the findings from the study, phenotypic analysis of the genotypes identified as donors for genes for resistance can be carried out, in

order to ensure the disease resistance in field conditions as well as the credibility of the molecular markers used in the study. The genes for resistance to the diseases studied, located in the 30 genotypes, screened from the study could indicate the donors for tomato resistance breeding programs.