EVALUATION OF TOMATO GENOTYPES FOR TOMATO LEAF CURL VIRUS (ToLCV) RESISTANCE

By

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(2019-11-181)

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VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled "**Evaluation of tomato genotypes for tomato leaf curl virus (ToLCV) resistance**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
10-12-2021

Anjitha A. R
2019-11-181

CERTIFICATE

Certified that this thesis, entitled **"Evaluation of tomato genotypes for tomato leaf curl virus (ToLCV) resistance"** is a bonafide record of research work done independently by **Ms. Anjitha A. R. (2019-11-181)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship, or associateship to her.

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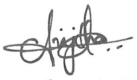

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INTRODUCTION

1. INTRODUCTION

Tomato (*Solanum lycopersicum,* 2n=24) is the most cultivated vegetable crop after potato worldwide. India ranks second in area as well as production of tomato. The major tomato producing states in India are Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Chhattisgarh, Bihar, Telangana, Tamil Nadu, Uttar Pradesh, Maharashtra, Haryana and Himachal Pradesh. These states account for 90 per cent of the total production in India.

Tomato belongs to the nightshade family (Solanaceae) and is believed to have originated from the Peru-Ecuador region. It is a widely produced and popular vegetable in India, having wide variability for plant traits (Tripathy and Mallikarjunarao, 2020). Tomato has very few competitors in the value addition chain of processing. Lycopene is the principal carotenoid in tomato, having antioxidant activity, which in turn reduces the risk of several diseases. It is a rich source of folate, vitamin C, potassium, fiber and different carotenoids. Health benefits associated with tomato include improved eyesight, reduced risk of cardiovascular diseases, improved digestion, good skin health etc.

It is a herbaceous sprawling plant, growing with weak woody stem and yellow colored bisexual flowers. Tomato is predominantly self-pollinated warm season crop. Based on the growth habit, it may be determinate, indeterminate or semi-determinate type. Botanically, tomato fruit is berry and can be flat round, slightly flattened, round, oval, heart shaped, pyriform etc. in shape.

Tomato can be cultivated on varied soil types, ranging from sandy to heavy clay. Sandy loam or light textured soils are best for tomato cultivation and it grows well in a pH range from 5.5-6.8.

Worldwide, tomatoes are susceptible to many diseases like damping off, bacterial wilt, early blight, leaf curl virus disease, leaf spot etc. Among them, tomato leaf curl virus disease (ToLCD), caused by whitefly-transmitted begomoviruses is more severe (Lapidot,

2007). Begomoviruses are the most devastating genera affecting tomatoes, especially in tropical and subtropical regions. Severe infection can cause yield loss up to 100 per cent (Ray *et al*., 2017). Resistance to ToLCV is influenced by morphological characters (colour, shape, pubescence and architecture), olfactory cues and environmental conditions (Vaishampayan *et al*., 1975). ToLCV is now being controlled utilising sticky traps as a vector management strategy. Tomato production is hampered by lack of ToLCV resistant genotypes. Identification of resistant sources will aid in virus resistance breeding. Since pesticides are not effective in controlling ToLCV and can be harmful to human health, utilising plant natural defenses found in wild tomato relatives may provide a solution for ToLCV management. Although ToLCV resistance is not found in *S. lycopersicum*, different levels of resistance were observed in many wild species like *S. pimpinellifolium, S. peruvianum, S. chilense, S. habrochaites and S. cheesmaniae* (Ji *et al*., 2009). Traditional breeding methods had previously been used to transfer ToLCV resistance genes to cultivated tomato species. The ToLCV resistance gene Ty-1 was the first to be introduced into tomato from *S. chilense* (Zamir *et al*., 1994). Till date, six ToLCV resistance genes (*Ty-1, Ty-3, Ty-2, Ty-4, Ty-5,* and *Ty-6*) have been identified (Hutton and Scott, 2014). Grafting, gene pyramiding, genome editing etc. have also been used to combat ToLCV.

Molecular markers are useful for genetic mapping as well as identification and characterization of genes and QTLs for many important traits in tomato, including disease and insect resistance (Foolad and Panthee, 2012). Marker Assisted Selection (MAS) has been used effectively for quick germplasm screening for disease resistance. MAS is not only faster than phenotypic selection but also cheaper and more effective.

The development of a variety or hybrid resistant to ToLCV is of utmost importance for attaining sustainable yield. Hence, the present study "evaluation of tomato genotypes for tomato leaf curl virus (ToLCV) resistance" was taken up with the objective to evaluate the ToLCV resistance in tomato genotypes by field screening and using molecular markers of Ty linked genes.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

A brief review of the available literature on the importance of the crop, viral diseases affecting tomato, resistance sources, variability in morphological traits, genes for ToLCV resistance and marker assisted selection is dealt with in this chapter.

2.1 Importance of the crop

Tomato is a warm season vegetable crop grown extensively for its fruits which is a rich source of vitamin C and lycopene. Lycopene is an antioxidant that scavenges free radicals and improves immunity. Tomato helps in lowering blood pressure and bad cholesterol, and is rich in potassium, vitamin B, E and other nutrients. The health benefits associated with the crop shows its importance in our diet. Although tomato is mainly served as cooked vegetable or eaten raw in salads, the processed products such as ketchup, puree, paste, juice etc. also have high demand throughout the world.

Tomato (*Solanum lycopersicum*) belongs to the family Solanaceae and is believed to have originated from Peru-Ecuador region. It is a model species for genomic studies with chromosome number 2n=2x=24. The major tomato producing states in India are Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Chhattisgarh, Bihar, Telangana, Tamil Nadu, Uttar Pradesh, Maharashtra, Haryana and Himachal Pradesh. The major production constraints in tomato cultivation include poor fruit set and yield loss due to the pest and disease incidence. Gatahi (2020) reported 100 per cent crop loss in tomato due to pest and disease attack. Management practices using chemicals is not much effective and are hazardous to human health and environment. Lack of resistant varieties/hybrids to various diseases and pests limits tomato production worldwide.

2.2 Major diseases in tomato

Tomato, a versatile vegetable crop cultivated in India, is often seriously affected by the incidence of diseases leading to severe crop loss. Tomato is susceptible to bacterial,

fungal and viral diseases, including bacterial wilt, early blight, damping off, leaf curl virus disease etc. In a study conducted by Tipu *et al*. (2021) in tomato, viral disease accounted for 100% crop loss and 41.67 per cent severity, whereas disease severity of wilt and phomopsis blight is 20 and 13.33 per cent respectively. Viral diseases of tomato are a major threat for its cultivation and among the viral diseases, Tomato Leaf Curl is a major and the most dangerous disease, causing severe yield loss in tomato.

Table 1. Viruses infecting tomato

Virus	**Genus**	**Family**
Tomato torrado virus (ToTV)	Torradovirus	Secoviridae
Tomato infectious chlorosis virus (TICV)	Crinivirus	Closteroviridae
Tomato chlorosis virus (ToCV)	Crinivirus	Closteroviridae
Tomato necrotic spot virus (TNSV)	Ilarvirus	Bromoviridae
Pelargonium zonate spot virus (PZSV)	Anulavirus	Bromoviridae
Alfalfa mosaic virus (AMV)	Alfamovirus	Bromoviridae
Tobacco streak virus (TSV)	Ilarvirus	Bromoviridae
Tomato yellow ring virus (TYRV)	Tospovirus	Bunyaviridae
Capsicum chlorosis virus (CaCV)	Tospovirus	Bunyaviridae
Tomato zonate spot virus (TZSV)	Tospovirus	Bunyaviridae
Pepino mosaic virus (PepMV)	Potexvirus	Flexiviridae
Tomato yellow leaf curl virus (TYCLV)	Begomovirus	Geminiviridae
Beet curly top virus (BCTV)	Curtovirus	Geminiviridae
Ageratum yellow vein virus (AYVV)	Begomovirus	Geminiviridae
Tomato leaf curl virus (ToLCV)	Begomovirus	Geminiviridae
Tomato mottle mosaic virus (ToMMV)	Tobamovirus	Virgaviridae
Tomato brown rugose fruit virus (ToBRFV)	Tobamovirus	Virgaviridae
Tobacco etch virus (TEV)	Potyvirus	Reoviridae
Tomato bushy stunt virus (TBSV)	Tombusvirus	Tombusviridae

(Hanssen *et al*., 2010)

2.3 Geminivirus

In most tropical and subtropical regions of the world, geminiviruses cause economically significant diseases. Geminiviruses are non-enveloped, small viruses having circular, single-stranded DNA genomes ranging from 2500 to 5200 bases (Zerbini *et al*., 2017). Geminiviruses are spread by whiteflies, leafhoppers, treehoppers and aphids. Whiteflies transmit members of the genus Begomovirus, specific leafhoppers transmit members of the genera Becurtovirus, Curtovirus, Grablovirus, Mastrevirus, and Turncurtovirus, a single member of the genus Topocuvirus is transmitted by a treehopper, and aphid transmits one member of the genus Capulavirus. Geminivirus can infect both monocot and dicot plants. They have twinned (geminate) incomplete icosahedral virions. They lack DNA polymerase and rely on host factors to complete replication. Their transcription is bi-directional and for gene expression, geminiviruses use overlapping transcripts (Prasad *et al*., 2020).

2.3.1 Begomovirus genome organization and variability

Begomoviruses are found in different parts of the world with high genetic diversity. They have unique twinned quasi isometric particles encasing a single-stranded, circular, covalently closed DNA genome. Tomato Leaf Curl Virus (ToLCV) is a distinct virus species of the genus Begomovirus in the family Geminiviridae.

It may have a monopartite or bipartite genome (designated as DNA-A and DNA-B). Bipartite genome is composed of two components, DNA-A and DNA-B each comprising ~2.6 kb whereas, monopartite genome corresponds to DNA-A component (~2.8 kb) (Souza *et al*., 2020).

However, ToLCV infected plants have been found to have satellite molecules that are around half the size of begomovirus genomes. Beta satellites, alpha satellites, and delta satellites are three types of circular DNA satellites associated with begomoviruses. These

alpha, beta, and delta satellites are not autonomous and must be replicated and maintained by a helper virus. These satellites are not always specific to a virus, although they can be linked to a variety of viruses that serve as helpers. In many cases, beta satellites influence virulence by suppressing host gene silencing. Delta satellites do not encode any proteins and alpha satellites are yet to be ascribed any specific activity. Virus symptoms that are enhanced vary depending on the virus/satellite complex and the host plant (Bragard and Schmutz, 2020).

In begomoviruses, the species demarcation threshold is 90 percent nucleotide sequence identity in the DNA A (full-length component). Strains of begomoviruses are defined as isolates with a 94 percent identity. Due to significant mutation and recombination both within and among species, begomovirus evolves quickly and produces novel variants. As a result, new isolates produce unique disease characteristics, host shifts, and host range expansions (Bragard and Schmutz, 2020).

2.4 Tomato leaf curl disease (ToLCD)

About 146 plant viruses belonging to 33 genera are known to infect tomato around the world. Among the viral diseases, ToLCV infection is devastating and causes significant yield loss when incidence is severe (Kumar and Kumar, 2018).

The first report of ToLCV disease was in Australia, by a monopartite begomovirus (Subhasmita *et a*l., 2021). In India, Tomato leaf curl disease was first reported by Vasudeva and Samraj in 1948. The emergence and spread of the disease throughout the world is rapid. In 1960s the disease was reported from Middle East; 1970s from Jordan (Makkouk, 1978); 1980s from Egypt and Turkey (Czosnek *et al*., 1990); 1990s from Italy, Spain and Portugal (Czosnek *et al*., 1990); 2000s from Greece (Avgelis *et al*., 2001). Monopartite Tomato leaf curl Bangalore virus (ToLCBV), is found in Southern India and bipartite tomato leaf curl New Delhi virus (ToLCNDV) found in Northern India. In addition, tomato leaf curl Karnataka virus (ToLCKV), tomato leaf curl Kerala virus (ToLCKeV), tomato leaf curl

Gujarat virus (ToLCGV), tomato leaf curl Palampur virus (ToLCPalV), tomato leaf curl Joydebpur virus (ToLCJoV) etc. are found in different parts of India (Kaushal *et al*., 2020). The disease is still spreading to new areas with more severity.

Table 2. Classification of tomato leaf curl viruses based on genome organization

Virus	**Acronym**	**Genome**
Tomato leaf curl Bangalore virus	ToLCBV	Monopartite
Tomato leaf curl Karnataka virus	ToLCKV	”
Tomato leaf curl Patna virus	ToLCPaV	”
Tomato leaf curl Rajasthan virus	ToLCRV	”
Tomato leaf curl Pune virus	ToLCPuV	”
Tomato leaf curl Kerala virus	ToLCKeV	”
Tomato leaf curl Ranchi virus	ToLCRV	”
Tomato leaf curl Joydebpur virus	ToLCJoV	”
Tomato leaf curl New Delhi virus	ToLCNDV	Bipartite
Tomato leaf curl Gujarat virus	ToLCGV	”
Tomato leaf curl Palampur virus	ToLCPalV	”

2.4.1 Host range of ToLCV

ToLCV causes severe yield losses in tomato and other economically important crops such as potato, cucurbits and cotton. In potato, the disease is characterized by leaf crinkling, apical leaf curling, stunting and mosaic or chlorotic blotching (Usharani *et al*., 2004). In cotton, ToLCV has been associated with monopartite begomovirus causing cotton leaf curl disease. In brinjal, the virus is associated with the yellow mosaic disease. The virus is also a major cause of yellow mosaic disease in sponge gourd causing yield loss in infected plants. In chrysanthemum, symptoms of mosaic, and a leaf curl disease were associated with the presence of ToLCV (Ashwathappa *et al*., 2020). The virus causes severe leaf curl and mosaic symptoms in cucurbits also.

In addition to tomato, the following are the host for tomato leaf curl virus,

Table 3. Different hosts for ToLCV

Family	**Host**
Solanaceae	*Capsicum annuum, C. frutescens, Datura stramonium, Solanum. hirsutum, S. peruvianum, S. pimpinellifolium, S. nigrum, Nicotiana sylvestris, N. benthamiana*
Fabaceae	*Arachis hypogea, Phaseolus vulgaris*
Malvaceae	*Gossypium hirsutum, Hibiscus syriacus, Corchorus tinctorius, Malva arvensis*
Pedaliaceae	*Sesamum indicum*
Euphorbiaceae	*Euphorbia heterophylla*
Acanthaceae	*Achyranthes aspera*

2.4.2 Symptoms

Tomato is susceptible to ToLCV at all the growth stages. Most plants show symptoms of begomovirus infection on their leaves throughout the plant. The disease symptoms include stunting of plant growth, reduction in leaf size, upward cupping of leaves, yellowing of the leaf margins, flower drop, reduced fruit set and fruit yield (Cohen and Antignus, 1994).

The plants infected at an early stage of growth remain severely stunted, their terminal and axillary shoots tend to stay erect and leaflets are reduced in size and abnormally shaped. The leaves that develop quickly after infection have downward cupping, whereas leaves that develop at later stages are chlorotic and distorted, with leaf margins rolled upwards and curled between the veins. (Cohen and Antignus, 1994). Yellowing of the edges of the leaves, inward curling with significant wrinkling and dwarfism are some of the other disease symptoms (El-Dougdoug, 2006).

Hassan *et al.* (2020) reported that ToLCV infected plants showed atrophy or weakness of the leaf and stem tissues. The number of xylem and phloem vessels, and capillary size were reduced in the infected plants compared to uninfected plants. If the infection starts before the reproductive stage of the plant, it will lead to flower abortion and affect the appearance of the fruit. Severe infection leads to stunting, coupled with significant yield loss. The disease can cause mild to very severe symptoms and ultimately lead to plant death.

2.4.3 Environmental conditions favouring the disease spread

The spread of ToLCV is dependent on the presence of the vector whitefly (*Bemisia tabaci)*. Climatic conditions, primarily determined by temperature and humidity, determine the population density and spatial distribution of this insect vector in the open fields. During summer in southern India and autumn in northern India, this disease represents a serious constraint for tomato production. In southern India, maximum temperature and rainfall are significant factors in disease spread, while in northern India, minimum temperature and relative humidity have an effect on the whitefly population. In southern India, the incidence of ToLCD during summer can result up to 100% loss in quantitative and qualitative yield (Prasanna *et al*., 2015).

2.4.4 Virus transmission

Whitefly (*Bemisia tabaci)* is the only known vector of ToLCV. Its wide distribution is a result of human activities and its ability to adapt to diverse host plants. It is a major pest of tomato, cucumber, pepper, brinjal etc. The efficient propagation of the virus is aided by large populations of adult whiteflies visiting many plants, and the virus is transmitted through circulative manner. The virus move from foregut to the mid and hind gut of the vector and finally to the salivary gland, from where they are released to the plants during feeing. The minimum acquisition and inoculation period is 15-30 minutes and the virus replicates shortly after the acquisition. Once the whitefly acquired the virus from an

infected plant, it will be viruliferous throughout its life span. The transmission rate increases with the increase in whitefly population density. Female whiteflies can transmit the virus more efficiently; six fold better than their male counterparts (Yadav, 2011). The virus can remain in the vector, but will not be transmitted to the progeny. ToLCV's wide host range makes it easier for the virus to disseminate and establish itself in locations where the insect vector thrives.

Polyphagous whitefly can transmit satellite molecules that can be coupled with ToLCV or other begomoviruses, as well as cause mixed virus infections of numerous begomoviruses. Population of whitefly fluctuate significantly throughout the year with the density attaining its peak during summer season when the temperature is high and rainfall is low. ToLCV spreads long distances through the transit of virulent *B. tabaci*, as well as the trading of infected plants for planting, pieces of infected plants (e.g. cut flowers), and probably seeds.

ToLCV can spread through plant sap and are mechanically transmitted through plant injury during crop management practises, such as pruning and training. ToLCV can reach high levels in infected plants, and infections can arise during germination as a result of virus contamination on the seed coat.

2.5 Screening for ToLCV resistance under natural field conditions

Identification of hotspots and favourable seasons are important for disease screening under natural field conditions. ToLCV incidence is higher in North-west plains and East India during early autumn (Banerjee and Kalloo, 1987). In semi-tropical regions of Egypt, the incidence is higher towards the end of summer (Dhaliwal *et al*., 2019).

Paul (2014) reported 64 to 88.8% disease severity for ToLCV in Vellanikkara, Kerala during summer season. They reported four types of symptoms such as upward

curling and cupping (Type I), curling and rolling (Type II), curling with purple tint (Type III), and yellowing and curling (Type IV). Banerjee and Kalloo (1987) developed a scale for classifying disease reaction of tomato leaf curl virus.

Dheemanth (2014) reported 48.48 to 88.46% disease severity in F_3 segregants and 53.84 to 95.65% disease severity in F_4 segregants in Vellanikkara, Kerala. Yadav (2011) reported 0 to 100% disease severity under natural filed conditions in Vellanikkara, Kerala. Divakaran (2008) reported 0 to 82.5% disease severity under pot culture conditions and 0 to 83.8% severity under natural field conditions in Vellanikkara, Kerala.

Banerjee and Kalloo (1987) have identified resistance sources such as *S. habrochaites, S. peruvianum* and *S. pimpinellifolium* under high disease pressure generated by natural infection in the field. There are chances of plants escaping from the infection due to low levels of inoculum pressure. Occurrence and dynamics of whitefly population, affinity of the vector to particular genotypes, weather conditions and plant stage at which the infection occurs are the factors influencing ToLCV incidence under natural field conditions.

Vidavsky and Czosnek (1998) found that, under the natural disease screening, 50 per cent of the susceptible tomato plants showed disease symptoms 30 days after transplanting and 10 per cent of the susceptible plants escaped from the infection at 90 days after transplanting. Such a high level of escapes will reduce the efficiency of screening and lead to erroneous selection of resistant genotypes. Sometimes ToLCV resistant plants may be infected by other virus or pathogen and be erroneously scored as susceptible, thus rejecting a resistant desired genotype (Lapidot, 2007). Even though the efficiency of selection under the natural disease condition may be variable and non-reproducible, it is useful while handling germplasm or breeding populations and will minimize the bulk of the material for artificial screening.

2.6 Whitefly mediated artificial inoculation technique

Mass inoculation or individual plant inoculation technique using whitefly is commonly adopted for ToLCD screening. The problem of non-preference is encountered during mass inoculation technique as in the case of natural screening. This problem can be overcome by inoculating individual plants in cages. The seedlings at the first true leaf stage are preferred for inoculation. According to Lapidot *et al*. (1997) an optimized controlled inoculation consists of allowing whiteflies to feed on ToLCV infected plants for 48 h acquisition access period, followed by the inoculation feeding on the young seedlings by 30–50 viruliferous whiteflies per plant, which upon feeding, transmit the virus with 100 per cent success rate. Vijeth (2015) found that, under natural field conditions symptom severity was milder than the artificial inoculation. Rajasri and Vijayalakshmi (2013) confirmed ToLCV resistance of the wild accession EC 251672 after artificial inoculation. Paul (2014) followed whitefly mediated artificial inoculation technique after the field screening, in order to confirm ToLCV resistance.

2.7 Sources of ToLCV resistance

Genes controlling resistance to ToLCV have been identified in several wild tomato species like *S. pimpinellifolium, S. peruvianum, S. chilense* etc. Introgression of these genes will be helpful for the development of ToLCV resistant varieties.

2.7.1 *Solanum pimpinellifolium*

S. pimpinellifolium is a wild species commonly used for breeding programs in tomato, since it is not having any hybridization barriers and allows recovery of fruit size. *S. pimpinellifolium* accession A1921 (Pilowsky and Cohen, 1974), Hirsute-INRA, LA 1478 (Kasrawi, 1989), PI 407543 and PI 407544 (Hassan and Abdel-Ati, 1999) etc. are ToLCV resistance sources. Pico *et al.* (2000) identified ToLCV resistance in the accessions UPV-17049, UPV-16953, UPV-16991 and UPV-16990. Even though some resistance

sources have been reported in *S. pimpinellifolium* accessions, the resistance level is not consistent and not effective in some environments. Agnihotri *et al*. (2013) reported *Ty-1* in *S. pimpinellifolium.*

2.7.2 *Solanum peruvianum*

The first ToLCV resistant hybrid TY 20 was derived from PI 126935. TY 172, TY 198, TY 536 and TY 197 are other ToLCV resistant hybrids derived from the *S. peruvianum* accessions PI 26926, PI 26930, PI 390681 and LA 441 (Dhaliwal *et al*., 2019). Hutton *et al*. (2014) reported *ty-5* in *S. peruvianum.*

2.7.3 *Solanum chilense*

S. chilense accessions, LA 1969, LA 1932, LA 1938, LA 2779, LA 1960 and LA 1971 are resistant to ToLCV. LA 1969 is a source for Ty-1 gene imparting ToLCV resistance (Zamir *et al*., 1994). Ji *et al*. (2007) identified ToLCV resistant gene *(Ty-3)* from LA 2779 and LA 1932. The cross incompatibility limitations with the *S. chilense* made it difficult to utilize them in breeding programs. These hindrances can be overcome by pollen mixture, bridging species and embryo culture thus facilitating breeding programs. Hutton and Scott (2012) reported *Ty-6* in *S. chilense.*

2.7.4 *Solanum habrochaites*

S. habrochaites accessions LA0386, LA 1252, LA 1295, LA 1352, LA 1393, LA 1624 and LA 1691 are highly resistant to ToLCV. Hassan *et al*., (1984) reported that the resistance in LA0386 was controlled by more than one dominant gene. Accessions *viz.,* UPV 16910 and UPV 16911 are also highly resistant to ToLCV (Pico *et al*., 2000). Kalloo and Banerjee (1990) reported *Ty-2* in *S. habrochaites.*

2.8 Management of ToLCV disease

It is estimated that the genome of cultivated tomato contains <5% of the genetic variation of their wild relatives (Miller and Tanksley, 1990). Limited genetic variation makes the crop vulnerable to disease and insect epidemics (Tanksley and McCouch, 1997). The major approach for begomovirus control is to keep the whitefly population under control. To control whitefly populations, a variety of methods are used, including the use of insecticides and physical barriers such as insect-proof netting, UV-absorbing plastic

mulches, and reflecting plastic mulches (Kaushal *et al*., 2020). However, due to the high rate of vector reproduction and virus multiplication, current vector population management approaches have not given an acceptable and long-term solution. Breeding tomatoes that are genetically resistant or tolerant to begomoviruses is the best sustainable strategy for begomovirus management. Therefore, begomovirus resistance sources in the tomato gene pool, such as wild relatives and other cultivated tomatoes, must be identified and investigated.

2.9 Genetic variability in morphological characters

Genetic variability is the pre-requisite for any crop improvement programme. Variability provides scope for selection of desirable genotypes in the population. Tripathy and Mallikarjunarao (2020) reported that plant height, primary branches/ plant, fruit weight, fruits/ plant and fruit size are significantly and positively correlated with yield. Ara *et al.* (2009) recommended that characters such as minimum days to first fruit, average fruit weight, fruit size, plant height, prolonged harvest period, and number of primary branches/plant be prioritized over other factors for selecting genotypes with better yields. Reddy *et al.* (2013) reported that determinate tomatoes have positive and significant association with yield than indeterminate types. Determinate varieties are preferred in open field conditions and indeterminate varieties are preferred in greenhouses (Gatahi, 2020).

Generally, variability in a population is measured by genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV). According to Kumar *et al*. (2013), fruit yield /plant, fruit weight, number of fruits /plant and plant height in tomato recorded high GCV and PCV. Similarly, Khan and Samadia (2012) found that PCV and GCV were high in tomato for fruit weight, fruits /plant, fruit yield /plant and plant height.

Hasan *et al*. (2016) reported that PCV was higher than the GCV for plant height, primary branches/plant and days to flowering indicating the influence of environment on the expression of these characters.

2.9.1 Morphological traits for ToLCV resistance

ToLCV incidence is highly dependent on vector population and its preference (Firdaus, 2012). Whiteflies choose the host mainly based on morphological and olfactory cues and depends on its host preference and antibiosis.

Host preference is determined by morphological characters such as colour, pubescence and architecture. Among these, colour is one of the most important factors for host recognition by whiteflies. In tomato, whiteflies prefer light yellow-green leaves (Vaishampayan *et al*., 1975). A less open plant canopy is also preferred by whiteflies (Srinivasan and Uthamasamy, 2005). They use olfactory cues to make choice from afar. Whiteflies prefers thin leaves with thin cuticular layer, as a thick cuticular layer will inhibit the insertion of stylet into the epidermis and phloem vessels. They prefer the abaxial side of leaves than adaxial side, in order to avoid direct light and natural enemies. Chemical constitution, age, nutritional value, leaf pubescence and trichome density have a role in whitefly preference and host suitability.

Six types of trichomes have been identified in tomato and its wild relatives. Type III and V are non-glandular trichomes and type I, IV, VI and VII are glandular trichomes. Whiteflies prefer to lay eggs on tomato leaves with a high density of non-glandular trichomes (Heinz and Zalom 1995). Non-glandular trichomes provide suitable microclimate and shelter for them. Although whiteflies prefer hairy leaves, the presence and high density of glandular trichomes has a negative effect on the adult whitefly survival and oviposition rate (Channarayappa *et al*., 1992).

The presence of glandular trichomes is the most important characteristic that contributes to whitefly antibiosis in tomato. In *S. habrochaites f. glabratum* type VI trichomes were associated with whitefly resistance (Fridman *et al*., 2005). Channarayappa *et al.* (1992) found high positive correlation between the presence of type IV trichomes and whitefly resistance in tomato. Wild tomato relatives resistant to whiteflies had abundant

glandular trichomes. They will act as a physical barrier and interfere with whitefly landing, feeding, and oviposition. The exudate of glandular trichomes are sticky traps for whiteflies. The phytochemicals produced by the glandular trichomes include terpenoids, methyl ketones and acyl sugars. Compounds such as monoterpenes and sesquiterpenes produced by type IV and VI trichomes repel the whiteflies (Bleeker *et al*., 2009).

S. habrochaites and *S. pennellii* produce a lot of monoterpenes and sesquiterpenes (Fridman *et al*., 2005; Bleeker *et al*., 2009) and are associated with reduced whitefly preference and survival. Monoterpenes contributing whitefly resistance are p-cymene, γ-terpinene, α-terpinene and α-phellandrenes (Martin-Luengo *et al*., 2010). Other terpenoid compounds toxic to whiteflies are sesquiterpenes, such as zingiberene and curcumene (Azevedo *et al*., 2003). Acyl sugars produced by Type IV trichomes, which are sticky and toxic to whiteflies, operates as a glue trap. Approximately 90% of *S. pennellii* (LA716) type IV trichome exudates are acyl sugars (Burke *et al*., 1987).

Hence, the previous studies indicate that while the presence of non-glandular trichomes is preferred by whitefly, glandular trichomes negatively affect vector oviposition and feeding.

2.10 Marker Assisted Selection

Marker assisted selection is the process where a trait of interest is selected based on a marker, which can be morphological, biochemical or molecular, linked to the trait of interest. Molecular markers have been used extensively for genetic mapping as well as identification and characterization of genes and QTLs for many agriculturally important traits in tomato (Foolad and Panthee, 2012). Marker assisted selection has been used effectively for quick germplasm screening for disease resistance. It is not only faster than phenotypic selection, but also cheaper and more effective (Chen *et al*., 2015).

2.11.1 ToLCV resistance genes

Many ToLCV resistance genes (*Ty-1, Ty-3, Ty-2, Ty-4, Ty-5* and *Ty-6*) have been identified till date, most of which are identified from wild tomato species. *Ty-1, Ty-2, Ty-3, Ty-4, Ty-5*, and *Ty-6*, have been discovered and mapped on chromosomes 6, 11, 6, 3, 4, and 10 respectively, using DNA markers (Hutton and Scott, 2014).

Two partially dominant genes *Ty-1* and *Ty-3* are mapped on chromosome 6 (Zamir et al., 1994). *Ty-1* and *Ty-3* were originally mapped as independent loci, but they were later discovered to be allelic, and the locus is now known as *Ty-1/3*, which codes for an RNA-dependent RNA polymerase (Verlaan *et al*., 2013). *Ty-1/3* is known to promote viral genome cytosine methylation, and plants carrying it develop resistance to ToLCV.

A dominant gene *Ty-2* was mapped on chromosome 11 (Hanson *et al*., 2000). *Ty-2* gene, also known as TYNBS1, produces a leucine-rich repeat-containing (NB-LRR) protein and a nucleotide-binding domain. *Ty-2* gene alone does not confer effective resistance to monopartite and bipartite begomoviruses (Prasanna *et al*., 2015).

Ty-4 is a minor QTL on chromosome 3 that accounted for only 16% variation in symptom severity (Ji *et al*., 2009). Besides, a recessive gene *ty-5* was mapped on chromosome 4 (Hutton et al., 2012). Recently, Hutton and Scott (2014) have identified the *Ty-6* gene on chromosome 10 that confers resistance against the bipartite ToLCV. *Ty-6* is effective in complementing the resistance conferred by *Ty-3* and *Ty-5* (Gill *et al*., 2019). It also confers resistance to tomato mottle virus (ToMoV), implying that *Ty-6* is capable of controlling both mono and bipartite begomoviruses in tomatoes.

2.11.2 Markers associated with ToLCV resistance genes

The major ToLCD resistance associated markers identified so far include TG178 (*Ty-1*), P6-25 (*Ty-3, Ty-3a, Ty-3b*), FLUW25 (*Ty-3, Ty-3a, Ty-3b*), SCAR2 (*Ty-2*) and

P116 (*Ty-2*). ACY-1 marker with a broad-spectrum application with the ability to simultaneously detect the presence of *Ty-3, Ty-3a*, and *Ty-1* resistance alleles was also identified (Nevame *et al*., 2018). ISSR HB 12 and SCAR Ualty 16 Markers were identified for ToLCV resistance screening (Ashru, 2014). Marker SSR-306 was associated with the (ToLCV) disease resistance genes in tomato cultivar EC-538408 (derived from *S. chilense*), and SSR-218 was associated with a ToLCV disease resistance gene of EC-520061(*S. hirsutum*) (Kumar *et al*., 2018).

Table 4. Reported molecular markers for Ty genes

Gene	Marker	References
Ty-1	TG178, JB1CAPS	Chen *et al*., 2015; Mahfouze and Mahfouze, 2019
Ty-2	TG105A, 20IY10, T0302, TES0344, SCAR2	Prabhandakavi *et al*., 2021; Lee *et al*., 2021; Chen *et al*., 2015
Ty-3	P6-25, CAPSFER-G8F	Prabhandakavi et al., 2021; Mahfouze and Mahfouze, 2019
Ty-1/3	Ty-1/3_K, 14IY218	Prabhandakavi *et al*., 2021; Lee *et al*., 2021
Ty-4	18IY23, 18IY13	Lee *et al*., 2021
ty-5	SINAC1, 14IY5, TM719, SLM4-34, TM81, TM80, TM70	Prabhandakavi *et al*., 2021; Lee *et al*., 2021; Chen *et al*., 2015
Ty-6	SLM 10–46	Prabhandakavi *et al*., 2021

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled 'Evaluation of tomato genotypes for Tomato Leaf Curl Virus (ToLCV) resistance' was carried out during 2019-2021. Experiments were carried out at Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara. The objective of the study was the evaluation of ToLCV resistance in tomato genotypes, by disease screening and using molecular markers for ToLCV resistance linked Ty genes. The study focused on the identification of resistant genotypes for the development of ToLCV resistant varieties/hybrids. The materials used and methodologies adopted are presented in this chapter.

3.1 Materials

3.1.1 Experimental site

The field experiments were conducted at the experimental field plot of Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, Thrissur, Kerala. The area is situated 10º32′47″ and 76º16′43″ at an altitude of 97m above MSL. The pH of the soil was 5.67 and EC was 97µs/m.

3.1.2 Experimental material

The material consisted of 27 tomato genotypes including eight NBPGR (National Bureau of Plant Genetic Resources) accessions, five breeding lines from World Vegetable Center, Taiwan, nine commercial hybrids, four KAU varieties and one local collection. The details of the genotypes used are presented in Table 5. These 27 genotypes were screened for ToLCV resistance under natural conditions during January- May 2021. The weather parameters such as temperature, rainfall and relative humidity during the period of natural field screening (January-May) at Vellanikkara is given in Table 6.

Table 5. Genotypes screened for resistance to ToLCV

Sl. No.	Genotypes	Source
1	AVTO 1726	World Vegetable Centre,Taiwan
2	AVTO 1727	"
3	AVTO 1707	"
4	AVTO 1706	"
5	AVTO 0922	"
6	Ansal	Private company hybrid (Bayer)
7	Virang	"
8	Kaustubh	"
9	Aryaman	"
10	Durg	"
11	Raymond	"
12	Abhiraj	"
13	Pranay	"
14	EC 519806 (*S. pimpinellifolium*)	NBPGR
15	EC 528360	"
16	EC 620428	"
17	EC 521067 B	"
18	EC 538153	"
19	EC 620486	"
20	EC 315489	"
21	EC 567305	"
22	Manuprabha	KAU, Vellanikkara
23	Manulekshmi	"
24	Anagha	"
25	Akshaya	
26	Arka Rakshak	IIHR, Bengaluru
27	Local Collection	Idukki

Plate 1. Field screening of genotypes

Whitefly rearing cage

Whiteflies on brinjal leaves

Acquisition feeding of whiteflies on infected tomato plant

Inoculation feeding of whiteflies on healthy tomato plant

Plate 2. Artificial inoculation

Table 6. Weather parameters at Vellanikkara during January-May 2021

Month	Mean temperature (ºC)	Mean relative humidity (%)	Rainfall (mm)
January	32.3	64	45.7
February	34.6	54	0.0
March	36.8	59	31.8
April	34.9	74	72.4
May	32.7	84	550.5

3.2 Methods

3.2.1 Seedling production

The seeds of the 27 selected genotypes were treated with *Pseudomonas fluorescence* (8g/ Kg seeds for 8h) and were sown separately in protrays. The potting mixture comprised coir pith, vermiculite and perlite in 3:1:1 ratio. Portrays helped in proper germination as well as reduced mortality rate, and 30 day old seedlings were transplanted to the main field.

3.2.2 Design and layout

Season: Summer

Design: RBD

Replications: 2

Treatments: 27

Spacing: 0.6 X 0.6 m

No. of plants/ treatment/ replication: 20

Plot size/ replication: 16 X 13 m

3.2.3 Field preparation

The experimental field was ploughed thoroughly using tractor and brought to a fine tilth. Lime was incorporated at the rate of 500 Kg/ha. Ridges and furrows were prepared

and the seedlings were transplanted in furrows. Net was fixed around the filed in order to provide protection from birds and animals. The cultural and agronomic practices were followed as per the Package of Practices Recommendations: Crops (KAU, 2016).

3.2.4 Field screening under natural conditions

The disease was assessed by adopting the score chart suggested by Banerjee and Kalloo (1987).

0- Symptom absent
1- Very mild curling (up to 25% leaves)
2- Curling, puckering of 26-50% leaves
3- Curling, puckering of 51-75% leaves
4- Severe curling, puckering of >75% leaves

Based on the score, Disease Severity Index (DSI) was calculated using the formula,

$$\text{DSI} = \frac{\text{Sum of numerical rating}}{\text{Total no. of plants observed} \times \text{Max. disease grade}} \text{ x } 100$$

Per cent Disease Incidence (PDI) was calculated using the formula,

$$\text{PDI} = \frac{\text{No. of plants infected}}{\text{Total no. of plants observed}} \text{ x } 100$$

Based on the Disease Severity Index (DSI) and Per cent Disease Incidence (PDI) the Coefficient of Infection (CI) was calculated using the formula,

$$\text{CI} = \frac{\text{Disease Severity Index} \times \text{Per cent disease incidence}}{100}$$

Based on the coefficient of infection, the genotypes were categorized into six groups,

0-4 : Highly resistant (HR)
4.1-9 : Resistant (R)
9.1- 19 : Moderately resistant (MR)
19.1- 39 : Moderately susceptible (MS)
39.1- 69 : Susceptible(S)
69.1- 100 : Highly susceptible (HS)

3.3 Morphological growth characters

The morphological features were recorded at 60 days after transplanting on all the twenty plants per replication and the main observations on growth characters of the genotypes evaluated includes growth habit, plant height, number of primary branches per plant, spread of the plant and days to flowering.

3.3.1 Growth habit

Growth habit of the plants *viz.,* determinate, semi determinate or indeterminate was recorded.

3.3.2 Plant height (cm)

Height of the plant from ground level to the top most leaf bud was recorded.

3.3.3 Number of primary branches per plant

The number of branches arising from the main stem above the ground was recorded.

3.3.4 Spread of the plant (cm)

Spread of the plant was measured between the farthest two opposite leaf buds in the side branches.

3.3.5 Days to flowering

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

3.3.6 Hairiness

Trichome density (Number of trichomes/cm^2) of both glandular and non-glandular trichomes on abaxial as well as adaxial surface of leaf was recorded using stereo microscope Leica EZ4E with image analyser. The area of the leaf was measured using Leica Application Suite (LAZ). The total number of hairs per cm^2 was counted manually by making the image in grids.

3.4 Statistical analysis

The data recorded on different traits were subjected to statistical analysis.

The data on morphological characters related to growth was subjected to one-way analysis of variance (ANOVA) using KAU GRAPES software (Gopinath *et al*., 2021). Analysis of variance was done to test the significance of differences among genotypes using the mean value of each genotype in each replication.

Table 7. ANOVA for each character

Source of variation	Degrees of freedom	Mean squares	F values
Replication	(r-1)	RMS	RMS/EMS
Treatment	(t-1)	TMS	TMS/EMS
Error	(r-1)(t-1)	EMS	
Total	(rt-1)		

r = Number of replications

t = Number of treatments

RMS = Replication mean square

TMS = Treatment mean square

EMS = Error mean square

Standard Error of Mean (SEm) = $\sqrt{EMS/r}$

Critical difference (CD=0.05) = SEm$\sqrt{2}$ X t $_{(0.05,\ \text{error d.f})}$

where, $t_{(0.05, \text{error d.f})}$ is the student's t table value at error degrees of freedom and at five per cent level of significance.

3.4.1 Correlation between trichomes and ToLCV resistance

The correlation coefficients were calculated using SPSS (Statistical Package for the Social Sciences) software to determine the degree of association of glandular and non-glandular trichomes with Tomato Leaf Curl Virus disease resistance. Correlation between trichome density and coefficient of infection was determined by the method suggested by Pearson (1905).

3.5 Molecular characterization

Laboratory chemicals, glassware and equipment

The AR (analytical reagents) grade chemicals (extra pure) from Sisco Research Laboratories (SRL) were used in this study. The primers synthesized by Sigma Aldrich Chemical Pvt. Ltd., Bangalore were used.

High speed refrigerated centrifuge (Eppendorf 5804 R) was used for centrifugation. The quality and quantity of DNA were estimated using Nanodrop spectrophotometer (Jenway- Genova Nano). Eppendorf Mastercycler nexus gradient PCR machine was used for amplification. Horizontal gel electrophoresis unit by Bio-Rad, USA was used for agarose gel electrophoresis.

3.5.1 Isolation of DNA

DNA isolation was done adopting the method suggested by Rogers and Benedich (1994) with slight modification.

Reagents used:

a. CTAB buffer (2X)
   2 per cent CTAB (w/v)
   100 mM Tris base (pH 8.0)
   20 mM EDTA (pH 8.0)

1.4 M NaCl

1 per cent Polyvinyl pyrrolidone (PVP)

b. 0.2 per cent 2-β mercaptoethanol
c. Chloroform: Isoamyl alcohol (24:1 v/v)
d. Chilled isopropanol (100%)
e. Ethanol 100% and 70%
f. Autoclaved distilled water

Procedure:

- Tender leaf tissue (300 mg) was wiped with 70% ethanol and ground in pre-chilled mortar and pestle using 1 ml of pre-warmed 2X extraction buffer (CTAB). 10 µl of β-mercaptoethanol and a pinch of PVP were added to the mortar during grinding.
- The homogenized sample was transferred into a sterile 2.0 ml microcentrifuge tube.
- The contents were mixed well and incubated at 65ºC for 30 min. with occasional mixing by gentle inversion.
- Equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inversion to emulsify.
- Centrifuged at 12,000 rpm for 15 min. at 4ºC.
- Transferred the top aqueous layer to a microcentrifuge tube, added equal volume of chloroform: isoamyl alcohol (24:1) and mixed by inversion.
- Centrifuged at 12,000 rpm for 15 min. at 4ºC.
- The supernatant was transferred into a microcentrifuge tube, added 0.6 volume of chilled isopropanol and mixed gently.
- Centrifuged at 8,000 rpm for 5 min. at 4ºC. Gently removed the supernatant without disturbing the pellet.
- Added 200 µl of absolute alcohol and centrifuged at 8,000 rpm for 5 min. at 4ºC
- The alcohol was removed and the pellet was air dried for 30 min.
- Pellet was dissolved in 50 µl distilled water and stored at -20ºC.

3.5.2 Estimation of quality of DNA by agarose gel electrophoresis

Agarose gel electrophoresis was carried out to determine the quality of the isolated DNA.

Reagents used:

a. Agarose (0.8%)
b. TAE buffer 50X (pH 8.0)
   2 M Tris buffer
   Glacial acetic acid
   0.5 M EDTA
c. Ethidium bromide (stock 10mg/ml: working concentration 0.5µg/ml)
d. Loading dye (6X)

Procedure:

- The gel tray and comb were wiped with 70% ethanol and the ends were sealed. Comb was placed in gel tray about 1 inch from one end of the tray and positioned the comb vertically such that the teeth are about 1 to 2 mm above the surface of the tray.
- Prepared 0.8 per cent agarose (0.8 g in 100 ml) in a conical flask with 100 ml 1X TAE buffer. Agarose was dissolved in a microwave for 45 to 60 s. till the solution was clear.
- Solution was allowed to cool to about 42ºC to 45ºC and 4 µl Ethidium bromide from the working solution was added and mixed well.
- Poured the gel solution into the tray and allowed to solidify for about 30 to 45 min. at room temperature.
- Gently removed the comb and placed the tray in the electrophoresis chamber, and poured 1X TAE buffer until the wells were submerged.
- To prepare samples for electrophoresis, added 1µl of 6X gel loading dye for every 5 µl of DNA solution, mixed well and loaded 5µl DNA sample per well.

- Electrophoresed at 80 volts until dye migrated for two third the length of the gel.

Gel documentation

Gel documentation was done using BioRad molecular imager Gel Documentation system using imagelab software. The gel was captured using the controls in the imaging device window.

3.5.3 Assessing the quality and quantity of DNA

The purity of DNA was further checked using 2 µl of DNA sample in the JENWAY Nanodrop spectrophotometer as per the manufacturer's protocol. Proteins show maximum absorption at 280nm, whereas nucleic acid shows peak absorption at 260nm. Absorbance was recorded at both wavelength and purity was indicated by OD_{260}/OD_{280}. The values between 1.8 to 2.0 is indicative of pure DNA free from proteins. The quantity of DNA was calculated using the relation, 1 OD_{260} equivalent to 50µg double stranded DNA/ml sample.

1 OD at 260nm = 50µg DNA/ml

Quantity of DNA in µg/ml = OD_{260} X 50

3.5.4 Molecular markers used for the study

Screening with reported molecular markers for ToLCV resistance genes were carried out. Three types of markers were used for the study which includes SSR (Simple Sequence Repeats), Indel and SCAR (Sequence Characterised Amplified Region). Twenty seven genotypes were screened with seven primers in this study. The list of markers used in this study are given in the table 8.

Table 8. Molecular markers for ToLCV resistance

Gene	Marker	Sequence (5' to 3')	Reference
Ty-1	TG178(SCAR)	F: GAGTCCCTAACGAATGGTCCTACT R: GCAGACAAATGCTCAAAGGTCACACC	Barbieri *et al*., 2010
Ty-2	SCAR2	F: TGGCTCATCCTGAAGCTGATAGCGC R: AGTGTACATCCTTGCCATTGACT	Mahfouze and Mahfouze, 2019
Ty-3	P6-25 (SCAR)	F: GGTAGTGGAAATGATGCTGCTC R: GCTCTGCCTATTGTCCCATATATAACC	Ji *et al*., 2007
Ty-1/3	TY-1/3_K (SCAR)	F: ACAGGAAAAATGGGTGATCC R: CCTGCTCCTTGCAGATTCTA	Chen *et al*., 2015
Ty-4	18IY13 (Indel)	F: CTTCTGTTCTATGCAGGTGTG R: GGATACAACTGTCAACGCAC	Lee *et al*., 2021
Ty-5	SLM4-34(SSR)	F: GACCATTAACCTCGATCA R: GAAAGTCATGTGAATAGCAG	Kadirvel *et al*., 2013
Ty-6	SLM 10–46 (SSR)	F:TCGAGCTGGTACATAGCTTCAT R:CATCTGACACTTGGTCCAGAA	Kadirvel *et al*., 2013

3.5.5 DNA amplification by PCR

The PCR conditions required for effective amplification in SSR, Indel and SCAR analysis include, appropriate proportion of the components in the reaction mixture and temperature profile of the thermal cycle. The reaction mixture included 10X Taq assay buffer (with KCl and 15mm $MgCl_2$), dNTPs, Taq DNA polymerase, primers and autoclaved distilled water. The aliquots of this master mix were dispensed into 0.2ml PCR microcentrifuge tubes along with genomic DNA. PCR was carried out in Eppendorf Mastercycler nexus gradient. The thermocycler was programmed for appropriate temperature and duration for denaturation, annealing and extension based on the nature of the primer used and product size.

The reaction mixture consisted of,

Genomic DNA (50 ng) - 1.0 µl
10X Taq assay buffer - 2.5 µl
dNTP mix (200 µM) - 0.5 µl
Taq DNA polymerase (1U) - 0.3 µl
Forward primer (5 picomole) - 2.0 µl

Reverse primer (5 picomole) - 2.0 µl
Autoclaved distilled water - 16.7 µl
Total reaction volume - 25.0 µl

PCR thermal regimes for TG178 (SCAR) primer

Initial denaturation : 94ºC 4 min.
Denaturation : 94ºC 30 s.
Primer annealing : 60ºC 1 min.
Primer extension : 72 ºC 3 min.
Final extension : 72ºC 7 min.
Cold storage : 4ºC ∞

35 cycles

PCR thermal regimes for SCAR2, P6-25 (SCAR), TY-1/3_K (SCAR) and SLM 10–46 (SSR) primers

Initial denaturation : 94ºC 4 min.
Denaturation : 94ºC 30 s.
Primer annealing : 60ºC 1min.
Primer extension : 72 ºC 2 min.
Final extension : 72ºC 7 min.
Cold storage : 4ºC ∞

35 cycles

PCR thermal regimes for 18IY13 (Indel)

Initial denaturation : 94ºC 4 min.
Denaturation : 94ºC 30 s.
Primer annealing : 55ºC 1 min.
Primer extension : 72 ºC 2 min.
Final extension : 72ºC 7 min.
Cold storage : 4ºC ∞

35 cycles

PCR thermal regimes for SLM4-34(SSR)

Initial denaturation : 94ºC 4 min.

Denaturation : 94ºC 30 s.

Primer annealing : 50ºC 1 min.

Primer extension : 72 ºC 2 min.

} 35 cycles

Final extension : 72ºC 7 min.

Cold storage : 4ºC ∞

3.5.6 Screening of primers and analysis

All 27 genotypes were screened with seven selected primers. The amplified PCR products were separated on a 2 per cent (TG178 (SCAR), SCAR2, P6-25 (SCAR), TY-1/3_K (SCAR), SLM4-34(SSR)) or 4 per cent (18IY13 (Indel), SLM 10–46 (SSR)) agarose gel along with suitable DNA ladder (100bp ladder or 1kb plus ladder). The amplification profile was visualized and documented using BioRad Gel Documentation system and scored for amplification of relevant products.

3.6 Artificial inoculation

Genotypes resistant under the natural conditions were subjected to whitefly mediated artificial inoculation.

3.6.1 Rearing of whitefly

Thirty day old brinjal seedlings were planted in growbags and covered with insect proof net cage. Whiteflies were released to the fifty day old plants and allowed to multiply on them.

3.6.2 Cages for inoculation

Plastic bottles were used for making the cages. The base and neck portions of bottles were removed using knife. Top portion of the bottles were covered with muslin cloth and other end kept free for covering the plant.

3.6.3 Test plants

The genotypes identified as resistant to ToLCV were raised in pots (15 seedlings/genotype). Twenty day old seedlings were kept in separate cages for inoculation.

3.6.4 Source of inoculum

ToLCV infected plants were collected from the experimental field of Department of Plant Breeding and Genetics. These plants were used as source of inoculum.

3.6.5 Acquisition and inoculation access periods

Whiteflies were collected from the rearing cage using test tubes plugged with cotton. They were allowed to feed on the infected plants for 24 h (acquisition access period). The viruliferous whiteflies were collected and released onto the healthy seedlings (30-50 whiteflies/ seedling) and kept in separate cages for 48 h (inoculation access period). The inoculated plants were sprayed with insecticide, dimethoate (30 EC) to kill the whiteflies. Cages were removed and kept for symptom development. Symptoms were recorded at regular interval after artificial inoculation.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

Tomato leaf curl virus disease is a serious constraint in tomato production worldwide. Attempts on management of the disease have not resulted in an efficient control strategy. A viable method to combat this disease is the use of resistant varieties. Identification of resistance sources for utilization in resistance breeding programmes will be beneficial for the development of varieties/hybrids that are resistant to ToLCV.

4.1 Meteorological data

The meteorological data presented in the Table 5 for the period from January 2021 to May 2021showed that the mean temperature varied from 32.3ºC to 36.8ºC with the maximum in the month of March. The mean relative humidity ranged from 54 per cent to 84 per cent during summer season with maximum in the month of May. The mean monthly rainfall was low and it ranged from 31.8 mm to 550.5 mm. Paul (2014) reported that high temperature, less relative humidity and rainfall favored the high incidence of ToLCV in Vellanikkara, Kerala during summer season than rabi season. The meteorological data during the present study reveals that the experiment was conducted when temperature was high with less relative humidity and rainfall which favoured the high incidence of ToLCV.

4.2 Field screening of tomato genotypes for ToLCV resistance

Twenty seven tomato genotypes were screened for ToLCV resistance under natural conditions during January to May 2021. The genotypes were categorized into highly resistant, resistant, moderately susceptible, susceptible and highly susceptible (Table 9).

Table 9. Reaction of tomato genotypes to ToLCV under natural condition

Sl. No.	Accession name	PDI (%)	DSI (%)	CI	Reaction
1	Ansal	40	10.00	4.00	Highly resistant
2	Kaustubh	45	16.25	7.31	Resistant
3	EC 519806	50	12.50	6.25	Resistant
4	Local collection (Idukki)	45	11.25	5.06	Resistant
5	Arka Rakshak	35	13.75	4.81	Resistant
6	AVTO 1726	90	35.00	31.50	Moderately susceptible
7	AVTO 1727	85	41.25	35.06	Moderately susceptible
8	Anagha	95	37.50	35.62	Moderately susceptible
9	Akshaya	85	33.75	28.68	Moderately susceptible
10	AVTO 1707	85	46.25	39.30	Susceptible
11	AVTO 1706	95	67.50	64.12	Susceptible
12	AVTO 0922	90	72.50	65.25	Susceptible
13	Virang	85	80.00	68.00	Susceptible
14	Durg	85	75.00	63.75	Susceptible
15	Abhiraj	90	65.00	58.50	Susceptible
16	EC 528360	85	76.25	64.80	Susceptible
17	EC 620428	80	76.25	61.00	Susceptible
18	EC 521067 B	85	73.75	62.68	Susceptible
19	Manuprabha	85	78.75	66.90	Susceptible
20	Manulekshmi	90	75.00	67.50	Susceptible
21	EC 620486	90	76.25	68.62	Susceptible
22	Aryaman	90	78.75	70.87	Highly susceptible
23	Raymond	95	86.25	81.93	Highly susceptible
24	Pranay	95	91.25	86.68	Highly susceptible
25	EC 538153	90	87.50	78.75	Highly susceptible
26	EC 315489	90	82.50	74.25	Highly susceptible
27	EC 567305	90	85.00	76.50	Highly susceptible

Highly resistant genotype

Ansal

Resistant genotypes

EC 519806

Local collection (Idukki)

Kaustubh

Arka Rakshak

Plate 3. Response of highly resistant and resistant genotypes to ToLCV infection under field conditions

Moderately susceptible genotypes

AVTO 1726

AVTO 1727

Anagha

Akshaya

Plate 4. Response of moderately susceptible genotypes to ToLCV infection under field conditions

Susceptible genotypes

AVTO 1707

AVTO 1706

AVTO 0922

Virang

Durg

Abhiraj

EC 528360

EC 620428

EC 521067 B

Manuprabha

Manulekshmi

EC 620486

Plate 5. Response of susceptible genotypes to ToLCV infection under field conditions

Highly susceptible genotypes

Aryaman

Raymond

EC 538153

Pranay

EC 315489

EC 567305

Plate 6. Response of highly susceptible genotypes to ToLCV infection under field conditions

Ansal

Kaustubh

EC 519806

Local collection (Idukki)

Arka Rakshak

Plate 7. Response of resistant genotypes after artificial inoculation

Manuprabha

Anagha

Plate 8. Response of susceptible checks after artificial inoculation

The Disease Severity Index (DSI) ranged from 10 to 91.25. Ansal recorded the minimum DSI (10%) and Pranay recorded the highest (91.25%). The percent disease incidence (PDI) ranged from 35 to 95. Minimum disease incidence was recorded in Arka Rakshak and maximum in Anagha, AVTO 1706, Raymond and Pranay. Coefficient of Infection (CI) ranged from 4 to 86.68. Ansal recorded the lowest CI and Pranay recorded the highest.

Among the 27 genotypes, one genotype was found to be highly resistant (Ansal), four were resistant (Kaustubh, EC 519806, Local collection (Idukki) and Arka Rakshak), four were moderately susceptible (AVTO 1726, AVTO 1727, Anagha and Akshaya), twelve were susceptible (AVTO 1707, AVTO 1706. AVTO 0922, Virang, Durg, Abhiraj, EC 528360, EC 620428, EC 521067 B, Manuprabha, Manulekshmi and EC 620486) and six were highly susceptible (Aryaman, Raymond, Pranay, EC 538153, EC 315489 and EC 567305) (Plate 3-6).

Arka Rakshak was found to be resistant to ToLCV. Arka Rakshak is the first triple resistant (Bacterial wilt, Tomato leaf curl virus disease, Early blight) hybrid released from IIHR (Subhasmita *et al*., 2021).

EC 528360 and EC 521067 B were found to be susceptible in the present study. Ponselvakumari *et al*. (2020) reported moderate susceptibility of EC 528360 and moderate resistance in EC-521067-B under field conditions. Our study indicated that EC 538153 was highly susceptible under the prevailing field conditions. Susceptibility of EC 538153 in Madurai, Tamil Nadu was reported by Ponselvakumari *et al*. (2020).

Anagha was observed to be moderately susceptible to ToLCV in our study. The result was in agreement with observations by Yadav (2011), where, Anagha was reported to be highly susceptible with a disease severity of 66.7 per cent. On the contrary, Anagha

as a highly resistant genotype with 10% disease severity both under field condition as well as in pot culture at Vellanikkara, Kerala was reported by Divakaran (2008).

The varieties Manuprabha and Manulekshmi were found to be susceptible to ToLCV. The result was in accordance with the observations by Nadkarni *et al*. (2017) where Manuprabha was reported to be susceptible to ToLCV in Kerala with 41.67% disease severity. They also reported that the variety Manulekshmi is moderately susceptible to ToLCV with 37.50% disease severity.

The highly resistant genotypes identified by Divakaran (2008) in Vellanikkara, Kerala are Hawaii 7998, H-24, H-86, LE-658, LE-651, and Anagha. The highly resistant genotypes identified by Yadav (2011) in Vellanikkara, Kerala are LE-574, LE-635, LE-640, LE-641, and LE-658. The resistant varieties identified by Nadkarni *et al*. (2017) in Kerala include Vaibhav, EC168283 (*S pimpinellifolium),* EC165751 and LA2805 (*Solanum lycopersicum var. cerasiforme* L.). From the present study, the hybrid Ansal was identified as the highly resistant and Kaustubh, EC 519806, Local collection (Idukki) and Arka Rakshak as resistant genotypes under field conditions prevailing at Vellanikkara in summer.

4.3 Morphological growth characters of genotypes evaluated

Most of the genotypes had a determinate growth habit (Table 10). Monamodi *et al*. (2013) reported that genotypes with determinate growth habit have high positive correlation with yield.

Table 10. Growth habit of the genotypes evaluated

Sl. No.	Genotype	Growth habit
1	AVTO 1726	Determinate
2	AVTO 1727	Determinate
3	AVTO 1707	Determinate
4	AVTO 1706	Determinate
5	AVTO 0922	Determinate
6	Ansal	Determinate
7	Virang	Determinate
8	Kaustubh	Determinate
9	Aryaman	Determinate
10	Durg	Determinate
11	Raymond	Determinate
12	Abhiraj	Determinate
13	Pranay	Determinate
14	EC 519806	Determinate
15	EC 528360	Indeterminate
16	EC 620428	Determinate
17	EC 521067 B	Determinate
18	EC 538153	Indeterminate
19	Local collection (Idukki)	Determinate
20	Manuprabha	Determinate
21	Manulekshmi	Determinate
22	Anagha	Determinate
23	Akshaya	Indeterminate
24	EC 620486	Determinate
25	EC 315489	Determinate
26	EC 567305	Determinate
27	Arka Rakshak	Determinate

The indeterminate genotypes include EC 528360, EC 538153 and Akshaya. The genotypes with determinate growth habit are AVTO 1726, AVTO 1727, AVTO 1707, AVTO 1706, AVTO 0922, Ansal, Virang, Kaustubh, Aryaman, Durg, Raymond, Abhiraj, Pranay, EC 519806, EC 620428, EC 521067 B, Local collection (Idukki), Manuprabha, Manulekshmi, Anagha, EC 620486, EC 315489, EC 567305 and Arka Rakshak.

Table 11. Morphological growth characters of the genotypes evaluated

Sl. No.	Genotype	Plant height (cm)	Number of primary branches per plant	Spread of the plant (cm)	Days to flowering
1	AVTO 1726	53.60^{abcd}	1.91^{efghij}	36.97	37.67^{fgh}
2	AVTO 1727	33.31^{ef}	1.39^{ij}	22.91	51.46^{a}
3	AVTO 1707	33.48^{ef}	1.50^{hij}	22.95	39.78^{ef}
4	AVTO 1706	39.77^{def}	1.84^{efghij}	32.85	34.36^{ijk}
5	AVTO 0922	60.02^{a}	2.59^{bcdefg}	36.40	33.27^{jkl}
6	Ansal	44.49^{abcdef}	1.18^{j}	22.43	42.53^{cd}
7	Virang	57.09^{abc}	1.49^{hij}	29.24	50.63^{a}
8	Kaustubh	50.69^{abcd}	1.19^{j}	26.63	47.36^{b}
9	Aryaman	53.33^{abcd}	1.30^{ij}	31.59	40.59^{de}
10	Durg	59.09^{abc}	$2.11^{defghij}$	36.67	32.43^{klm}
11	Raymond	56.93^{abc}	1.55^{ghij}	33.36	28.68^{op}
12	Abhiraj	48.59^{abcde}	1.62^{fghij}	30.08	43.86^{c}
13	Pranay	53.63^{abcd}	1.92^{efghij}	31.35	36.03^{ghi}
14	EC 519806	53.72^{abcd}	4.45^{a}	39.61	30.74^{mno}
15	EC 528360	59.46^{ab}	$2.31^{cdefghi}$	38.41	30.57^{mno}
16	EC 620428	45.13^{abcde}	1.41^{hij}	28.01	42.69^{cd}
17	EC 521067 B	50.19^{abcd}	3.07^{bcd}	36.72	29.76^{nop}

Sl. No.	Genotype	Plant height (cm)	Number of primary branches per plant	Spread of the plant (cm)	Days to flowering
18	EC 538153	58.80^{abc}	$2.03^{defghij}$	30.64	33.62^{jk}
19	Local collection (Idukki)	28.88^{f}	2.57^{bcdefg}	17.75	39.27^{ef}
20	Manuprabha	51.11^{abcd}	2.81^{bcde}	35.95	38.13^{fg}
21	Manulekshmi	59.12^{abc}	2.62^{bcdef}	38.14	30.34^{mno}
22	Anagha	46.34^{abcde}	$2.45^{bcdefgh}$	32.42	35.06^{ij}
23	Akshaya	58.22^{abc}	2.71^{bcde}	34.65	32.29^{klm}
24	EC 620486	53.00^{abcd}	$2.04^{defghij}$	27.29	35.40^{hij}
25	EC 315489	44.31^{bcdef}	3.21^{bc}	28.09	27.81^{p}
26	EC 567305	43.77^{cdef}	3.46^{ab}	31.52	36.16^{ghi}
27	Arka Rakshak	50.77^{abcd}	3.06^{bcd}	31.70	31.28^{lmn}
	CD ($p \leq 0.05$)	15.632	1.042	-	2.299
	CV (%)	15.25	22.92	25.11	3.05

The genotypes varied significantly with respect to plant height, number of primary branches/plant and days to flowering (Table 11). But there was no significant difference between the treatments in case of spread of the plant.

In the present study, plant height ranged from 28.88 cm to 60.02 cm. The plant height was highest in AVTO 0922 (60.02 cm) followed by EC 528360 (59.46 cm) and lowest in local collection (Idukki) (28.88 cm). All genotypes evaluated, except local collection (Idukki), were on par with Anagha for plant height, the determinate tomato variety released by Kerala Agricultural University for plant height. The plant height of local collection (Idukki) was found to be inferior to Anagha. Nadkarni *et al*. (2017), Dudhi and Kalloo (1982), Rattan *et al*. (1983) and Ara *et al*. (2009) also reported plant height as

a desirable characteristic for high fruit yield in tomato. On the contrary, Das *et al*. (1998) and Singh (2009) reported negative correlation between plant height and yield.

The number of primary branches/ plant was the highest in EC 519806 (4.45) followed by EC 567305 (3.46) and EC 315489 (3.21). The lowest number of primary branches/plant was recorded in Ansal (1.18). The genotypes AVTO 1727, Ansal, Kaustubh and Aryaman were inferior to Anagha in terms of number of primary branches/ plant. But the genotype EC 519806 was superior to Anagha. All other genotypes were on par with Anagha. Tripathy and Mallikarjunarao (2020), Ara *et al*. (2009) and Dhankar and Dhankar (2006) reported that number of primary branches/plant have positive and significant correlation with yield. Since the genotype EC 519806 is superior in terms of number of primary branches/plant and also ToLCV resistant, that can be further utilized in breeding programs. Rai *et al*. (2001) reported EC 519806 as a high yielder.

Days to flowering was the highest in AVTO 1727 (51.46) followed by Virang (50.63). The lowest days to flowering was recorded in EC 315489 (27.81). The genotypes AVTO 1727, AVTO 1726, AVTO 1707, Ansal, Kaustubh, Virang, Abhiraj, Aryaman, EC 620428, local collection (Idukki) and Manuprabha took more number of days to flower compared to Anagha. The genotypes Durg, Raymond, EC 519806, EC 528360, EC 521067 B, Manulekshmi, Akshaya, EC 315489 and Arka Rakshak had lower days for flowering than Anagha. Early flowering not only ensure early pickings and better returns but also widens fruiting period of a plant (Nadkarni, 2017). In the present study, symptoms of ToLCV infection were observed before the flowering stage in most of the genotypes. The genotypes EC 315489, EC 521067 B and Raymond, which took less than 30 days for flowering, were found to be susceptible (EC 521067 B) and highly susceptible (EC 315489 and Raymond) to ToLCV. Khan and Samadia (2012) reported that tomatoes are severely affected by the disease, especially when the infection starts before the flowering stage and observed reduced yield under such condition.

4.4 Variability in trichome character and correlation with ToLCV resistance

The genotypes varied significantly with respect to glandular as well as non-glandular trichome density on both abaxial and adaxial leaf surface (Table 12).

Table 12. Trichome density (Number/ cm^2) on the abaxial and adaxial leaf surfaces

Sl.No.	Genotype	Adaxial surface		Abaxial surface	
		Glandular trichome density	Non-glandular trichome density	Glandular trichome density	Non-glandular trichome density
1	AVTO 1726	6.33^{fghi} (40)	9.78^{h} (96)	10.92^{cde} (120)	22.28^{o} (496)
2	AVTO 1727	7.49^{efghi} (56)	9.82^{h} (96)	10.95^{cde} (128)	23.67^{n} (560)
3	AVTO 1707	4.88^{hi} (24)	7.49^{i} (56)	10.60^{cde} (136)	24.99^{m} (624)
4	AVTO 1706	4.06^{i} (16)	10.2^{h} (104)	7.33^{efg} (56)	32.01^{ghij} (1024)
5	AVTO 0922	4.06^{i} (16)	17.67^{a} (312)	4.88^{fg} (24)	32.25^{fghi} (1040)
6	Ansal	11.94^{bcde} (144)	7.49^{i} (56)	21.73^{a} (472)	28.28^{l} (800)
7	Virang	10.60^{cdef} (136)	9.78^{h} (96)	10.70^{cde} (128)	32.98^{ef} (1088)
8	Kaustubh	18.08^{a} (328)	7.52^{i} (56)	21.73^{a} (472)	21.17^{p} (448)
9	Aryaman	4.88 ^{hi} (24)	10.2^{h} (104)	8.92^{defg} (80)	33.47^{e} (1120)
10	Durg	6.94^{fghi} (56)	10.22^{h} (104)	8.51^{defg} (80)	31.75^{hij} (1008)
11	Raymond	5.51^{ghi} (32)	15.76^{bc} (248)	6.33^{efg} (40)	36.00^{b} (1296)
12	Abhiraj	4.06^{i} (16)	13.27^{ef} (176)	4.88^{fg} (24)	30.47^{k} (928)
13	Pranay	4.06^{i} (16)	14.71^{cd} (216)	4.88^{fg} (24)	37.10^{a} (1376)
14	EC 519806	16.24^{ab} (272)	12.98^{efg} (168)	21.00^{a} (448)	24.00^{n} (576)
15	EC 528360	9.49^{cdefgh}	16.70^{ab}	9.68^{cdef}	32.01^{ghij}

Sl.No.	Genotype	Adaxial surface		Abaxial surface	
		Glandular trichome density	Non-glandular trichome density	Glandular trichome density	Non-glandular trichome density
		(96)	(280)	(96)	(1024)
16	EC 620428	4.88^{hi} (24)	7.49^{i} (56)	4.88^{fg} (24)	31.24^{jk} (976)
17	EC 521067 B	15.42^{ab} (240)	15.49^{c} (240)	14.15^{bc} (200)	31.50^{ij} (992)
18	EC 538153	15.66^{ab} (248)	15.76^{bc} (248)	9.68^{cdef} (96)	35.56^{bc} (1264)
19	Local collection (Idukki)	4.06^{i} (16)	7.49^{i} (56)	16.95^{ab} (288)	23.67^{n} (560)
20	Manuprabha	9.31^{defgh} (88)	9.39^{h} (88)	4.06^{g} (16)	32.50^{fgh} (1056)
21	Manulekshmi	12.00^{bcde} (144)	12.02^{g} (144)	6.33^{efg} (40)	32.74^{efg} (1072)
22	Anagha	15.88^{ab} (256)	10.2^{h} (104)	10.21^{cde} (104)	24.00^{n} (576)
23	Akshaya	16.25^{ab} (264)	12.34^{fg} (152)	9.68^{cdef} (96)	21.17^{p} (448)
24	EC 620486	9.68^{cdefg} (96)	9.82^{h} (96)	8.92^{defg} (80)	32.99^{ef} (1088)
25	EC 315489	12.34^{bcd} (152)	12.35^{fg} (152)	12.97^{bcd} (168)	34.41^{d} (1184)
26	EC 567305	14.15^{abc} (200)	14.15^{de} (200)	6.86^{efg} (48)	34.87^{cd} (1216)
27	Arka Rakshak	18.54^{a} (344)	9.82^{h} (96)	17.42^{ab} (304)	32.01^{ghij} (1024)
	CD ($p \leq 0.05$)	4.74	1.18	5.12	0.801

* Original value is given in parenthesis

Glandular trichome density on the adaxial leaf surface was high in Arka Rakshak (344 no.s/ cm^2) followed by Kaustubh, EC 519806, Akshaya and Anagha. Among these genotypes, Arka Rakshak, Kaustubh and EC 519806 were resistant in field screening and artificial inoculation. The genotypes Virang, EC 620486, EC 528360, Manuprabha, AVTO 1727, AVTO 1726, Durg, AVTO 1707, Aryaman, Raymond, EC 620428, AVTO 1706, AVTO 0922, Abhiraj, Pranay and Local collection (Idukki) were inferior than Anagha

(moderately susceptible) in glandular trichome density on the adaxial leaf surface. Other genotypes were found to be on par with Anagha.

Non-glandular trichome density was found to be higher than glandular trichome density in most of the genotypes in both abaxial and adaxial surface. Non-glandular trichome density on adaxial leaf surface was high in AVTO 0922 (312 no.s/ cm^2) followed by EC 528360 and Raymond respectively. These genotypes showed ToLCV susceptibility in the field screening. Kaustubh, AVTO 1707, EC 620428, Ansal and local collection (Idukki) were inferior to Anagha. Among these genotypes Kaustubh, Ansal and local collection (Idukki) were resistant in field screening and artificial inoculation. Non-glandular trichome density on the adaxial side of the leaf was significantly and positively correlated with per cent disease incidence and disease severity index. Hence, the low non-glandular trichome density of Kaustubh, Ansal and local collection (Idukki) might have contributed to the low ToLCV infection in these genotypes.

Glandular trichome density on the abaxial leaf surface was high in Ansal (472 no.s/ cm^2) and Kaustubh (472 no.s/ cm^2) followed by EC 519806, Arka Rakshak and local collection (Idukki). Non-glandular trichome density on abaxial leaf surface was high in Pranay followed by Raymond. The genotypes Ansal, Kaustubh, EC 519806, Arka Rakshak and local collection (Idukki) showed ToLCV resistance in field screening and artificial inoculation and were superior to Anagha in glandular trichome density on the abaxial leaf surface. Other genotypes were on par with Anagha, except Manuprabha which had significantly lower glandular trichome density. Firdaus (2012) reported that whitefly repellence in tomato is due to the monoterpenes and sesquiterpenes produced by the glandular trichomes.

The present study also demonstrates that the presence of glandular trichomes can confer resistance to ToLCD. Whitefly prefers abaxial surface than adaxial surface in order to avoid direct sunlight and enemies (Srinivasan and Uthamasamy, 2005). Non-glandular trichome density on abaxial leaf surface was high in Pranay (1376 no.s/ cm^2) followed by

Raymond, EC 538153 and EC 567305 respectively. These genotypes were found to be highly susceptible under field screening. The genotypes AVTO 1726, Kaustubh and Akshaya were inferior to Anagha. Among these genotypes Kaustubh was resistant in field screening. AVTO 1726 and Akshaya showed moderate susceptibility to ToLCV.

Table 13. Correlation of trichome density with PDI and DSI

Trichome density	PDI	DSI
Glandular abaxial trichome density	-0.884**	-0.795**
Non-glandular abaxial trichome density	0.420*	0.824**
Glandular adaxial trichome density	-0.444*	-0.393*
Non-glandular adaxial trichome density	0.404*	0.496**

*Correlation is significant at the 0.05 level (2 tailed)
**Correlation is significant at the 0.01 level (2 tailed)

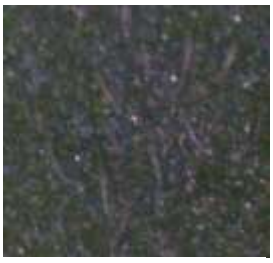
Channarayappa *et al*., (1992) found high positive correlation between presence of type IV glandular trichomes and resistance to whiteflies in tomato.

Correlation analysis showed that glandular trichome density on abaxial and adaxial surfaces of leaf was significantly and negatively correlated with per cent disease incidence and disease severity index. However, the negative correlation was stronger between the abaxial glandular trichome density and ToLCV resistance. This has been attributed to the production of terpenes (Fridman *et al*., 2005; Bleeker *et al*., 2009) and acyl sugars which operates as glue trap (Burke *et al.,* 1987), thus interfering with the landing and oviposition of white flies. Non-glandular trichome density on abaxial and adaxial sides of the leaf was significantly and positively correlated with per cent disease incidence and disease severity index. Whiteflies prefer to lay eggs on tomato leaves with a high density of non-glandular trichomes (Heinz and Zalom 1995). The non-glandular trichomes provide suitable microclimate and shelter and there by the plants are more susceptible to the disease.

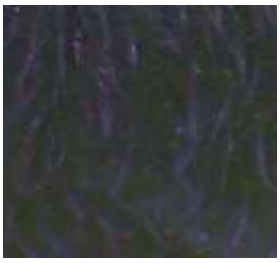
Abaxial trichomes

EC 538153

Raymond



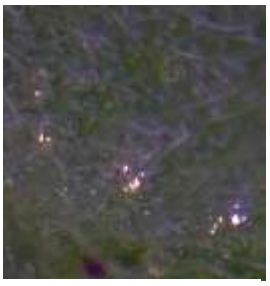
AVTO 1706



Manulekshmi

Aryaman

EC 528360

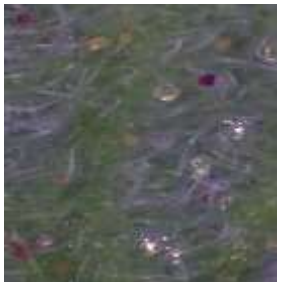


AVTO 1707

Akshaya

EC 519806

Virang

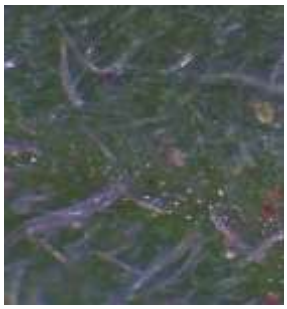


Local collection (Idukki)

EC 567305

Kaustubh

Durg



Pranay

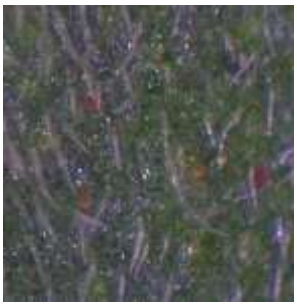
EC 519806

Ansal

Arka Rakshak

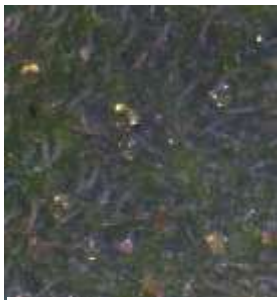
AVTO 1727

EC 521067 B



AVTO 0922

Anagha

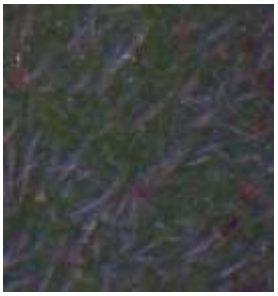


EC 315489

Abhiraj

EC 620428

AVTO 1726



Manuprabha

Plate 9. Trichomes on abaxial leaf surface

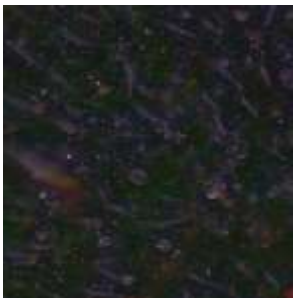
Adaxial trichomes

EC 528360

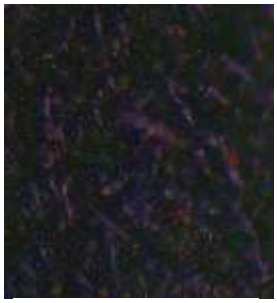
Durg

Raymond

EC 576305

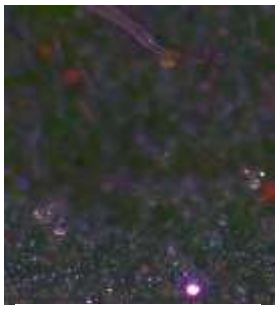


EC 521067 B



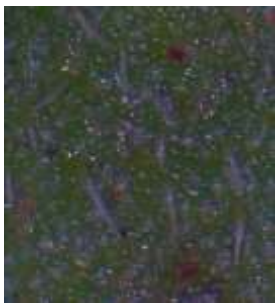
AVTO 1727

Manulekshmi



EC 315489

Anagha



Manuprabha

AVTO 1726

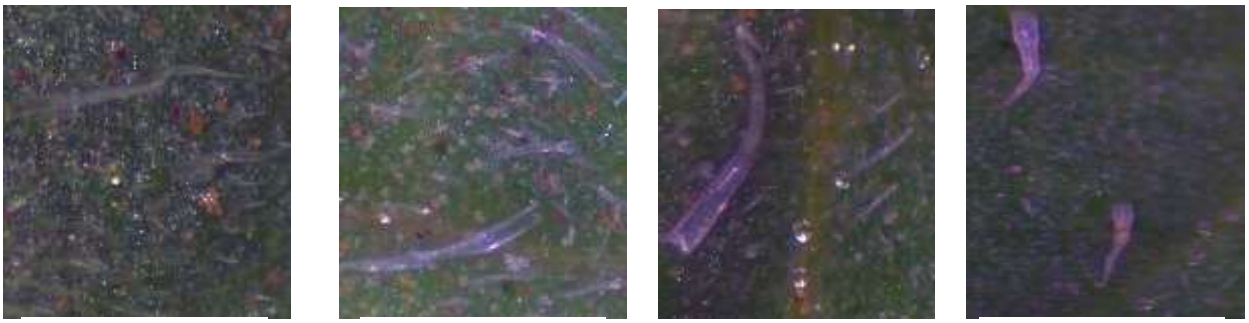
Local collection (Idukki)

AVTO 1706

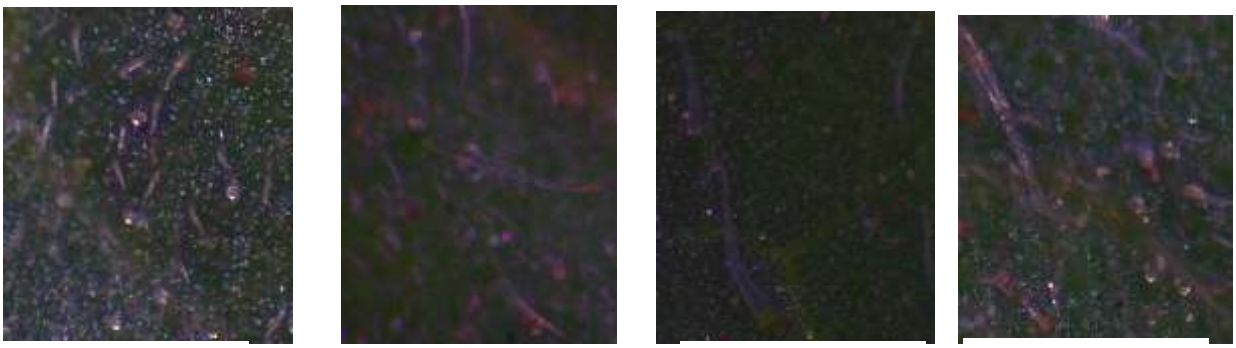
Arka Rakshak

EC 538153

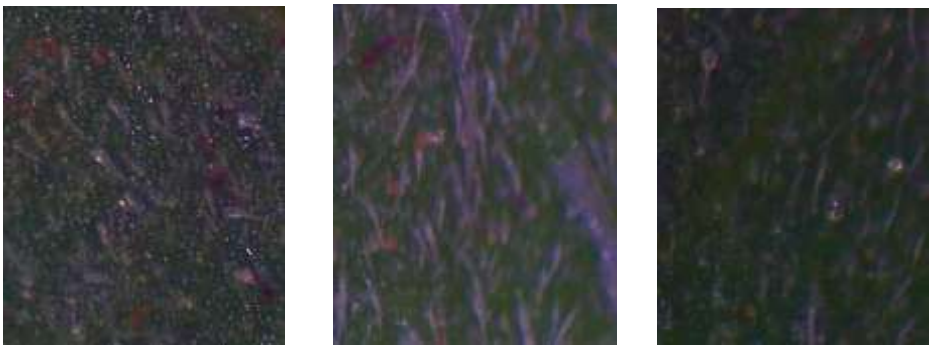
Abhiraj



EC 620486 Virang Akshaya AVTO 0922

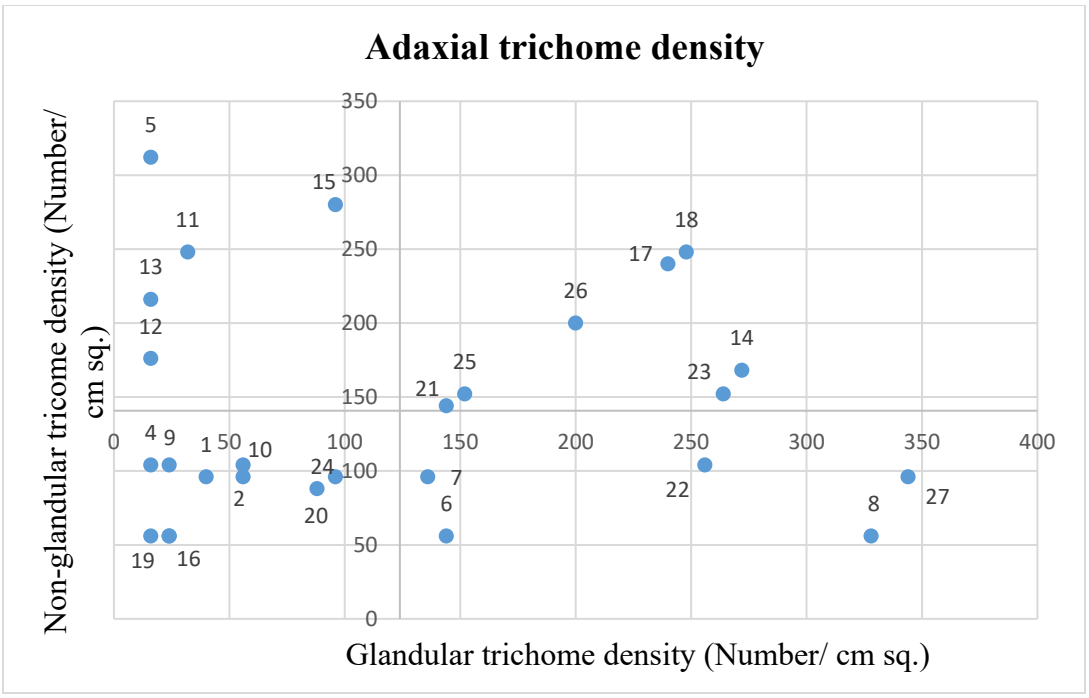


Kaustubh EC 620428 Pranay EC 519806



AVTO 1707 Aryaman Ansal

Plate 10. Trichomes on adaxial leaf surface



1-AVTO 1726
2- AVTO 1727
3- AVTO 1707
4- AVTO 1706
5- AVTO 0922
6- Ansal
7-Virang
8- Kaustubh
9- Aryaman
10- Durg
11- Raymond
12- Abhiraj
13- Pranay
14- EC 519806
15- EC 528360
16- EC 620428
17-EC 521067 B
18- EC 538153
19-Local collection (Idukki)
20- Manuprabha
21- Manulekshmi
22- Anagha
23- Akshaya
24- EC 620486
25- EC 315489
26- EC 567305
27- Arka Rakshak

Fig. 1 Scatter plot showing trichome density on adaxial leaf surface of genotypes

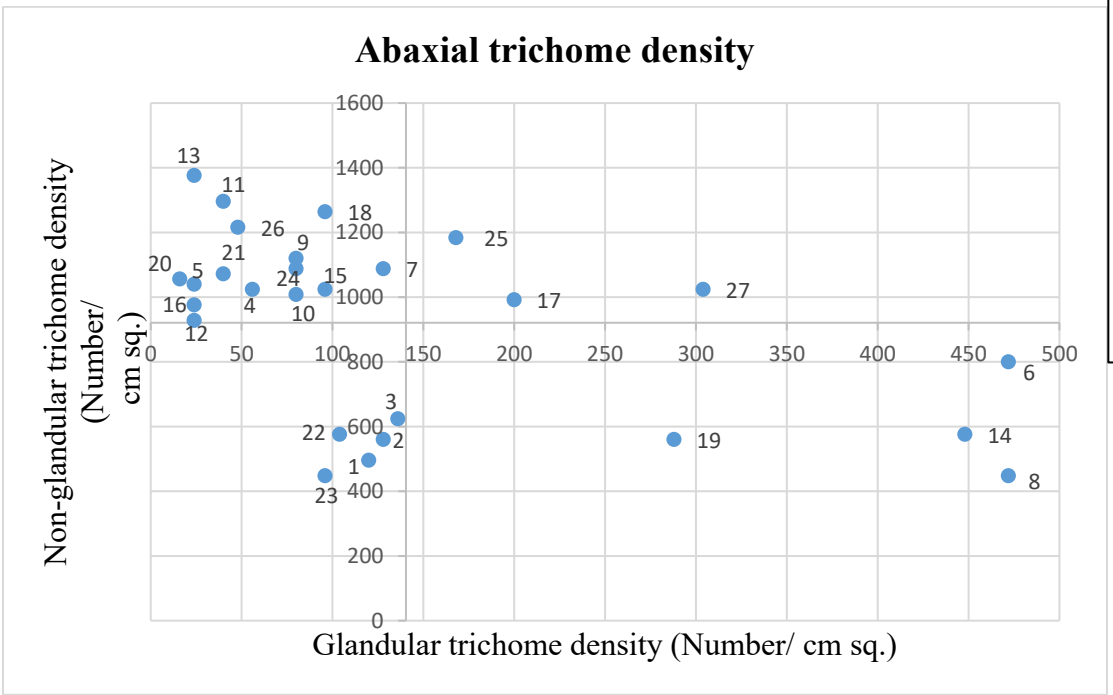


Fig. 2 Scatter plot showing trichome density on abaxial leaf surface of genotypes

Hence, it is evident that the genotypes having high density of glandular trichomes, especially on the abaxial leaf surface, and low density of non-glandular trichomes will have better ToLCD resistance. From the scatter plot we can see that Ansal, Kaustubh, EC 519806, local collection (Idukki) and Arka Rakshak, had lower non-glandular trichome density and higher glandular trichome density than the average value, especially on the abaxial surface, and is better equipped to have ToLCD resistance (Fig. 1 & 2). This was evident from the fact that Ansal, Kaustubh, EC 519806, local collection (Idukki) and Arka Rakshak genotypes exhibited resistance to ToLCV infection under field screening as well as artificial infection.

4.5 Molecular characterisation of tomato genotypes for Ty genes

Genomic DNA was isolated from all the 27 genotypes (Table 15) and were analysed for the presence of six reported Ty genes.

Table 14. Quantity of DNA isolated from tomato genotypes

Sl.No.	Genotype	A_{260}/A_{280}	Quantity (µg/ml)
1	AVTO 1726	2.12	2783.8
2	AVTO 1727	2.14	2841.5
3	AVTO 1707	1.93	345.13
4	AVTO 1706	2.02	208.96
5	AVTO 0922	2.10	696.64
6	Ansal	2.07	517.86
7	Virang	2.10	2007.30
8	Kaustubh	2.13	407.19
9	Aryaman	2.17	388.16
10	Durg	2.09	951.18
11	Raymond	2.16	666.63
12	Abhiraj	2.16	982.09
13	Pranay	1.84	686.74
14	EC 519806	2.14	1915.4
15	EC 528360	2.04	549.62
16	EC 620428	2.17	182.29
17	EC 521067 B	2.15	230.35
18	EC 538153	2.20	725.23
19	Local collection (Idukki)	2.09	446.83
20	Manuprabha	2.17	1048.8
21	Manulekshmi	2.13	928.84
22	Anagha	2.14	717.92
23	Akshaya	2.10	755.59
24	EC 620486	2.13	957.73
25	EC 315489	2.09	1261.0
26	EC 567305	2.17	394.97
27	Arka Rakshak	2.08	1601.8

Table 15. Amplification of PCR products from tomato genotypes using primers for selected markers of Ty genes

Sl. No	Genotype	Genes and markers						
		Ty-1 TG178 (SCAR)	*Ty-2* SCAR2	*Ty-3* P6-25 (SCAR)	*Ty-4* 18IY13 (Indel)	*Ty-5* SLM 4-34 (SSR)	*Ty-6* SLM 10–46 (SSR)	*Ty-1/3* TY-1/3_K (SCAR)
	Amplicon size of resistant genotype	Multiple bands	900	450	228	Multiple bands	255	114
	Amplicon size of susceptible genotype		800	320	200		230	102
1	AVTO 1726	NA	+	+	-	✓	NA	+
2	AVTO 1727	NA	±	±	NA	NA	NA	+
3	AVTO 1707	NA	+	+	NA	NA	NA	+
4	AVTO 1706	NA	+	NA	NA	NA	NA	+
5	AVTO 0922	*	+	-	-	NA	-	-
6	Ansal	*	±	-	-	✓	-	±
7	Virang	*		-	-	NA	NA	±
8	Kaustubh	NA	NA	-	-	✓	-	NA
9	Aryaman	*	±	-	-	NA	-	±
10	Durg	*	-	-	-	NA	-	±
11	Raymond	*	-	-	NA	NA	NA	±
12	Abhiraj	NA	-	-	-	NA	NA	±
13	Pranay	NA	-	NA	NA	NA	NA	±
14	EC 519806	*	-	-	NA	NA	NA	NA
15	EC 528360	NA	NA	NA	-	NA	NA	-
16	EC 620428	*	-	-	-	✓	-	+
17	EC 521067 B	NA	-	-	-	NA	NA	-
18	EC 538153	*	-	-	-	✓	-	+
19	Local collection (Idukki)	NA	-	-	-	NA	NA	-
20	Manuprabha	*	-	-	-	✓	-	-
21	Manulekshmi	*	-	-	-	✓	-	-
22	Anagha	*	-	-	NA	✓	-	-
23	Akshaya	*	-	-	-	✓	NA	-
24	EC 620486	*	-	-	-	✓	NA	+
25	EC 315489	*	-	-	NA	NA	-	-
26	EC 567305	*	-	-	-	NA	-	-
27	Arka Rakshak	*	±	-	-	NA	-	-

NA: No Amplification
* 2kb fragment amplified
✓Multiple bands
- Amplicon corresponding to susceptible genotype
+ Amplicon corresponding to resistant genotype
± Heterozygous condition

Seventeen out of 27 genotypes screened were found to carry the gene Ty-1 (Plate 12). However, there was no clear difference between the resistant and susceptible genotypes with respect to the amplification of the marker TG178 linked to *Ty-1*. Prasanna *et al.* (2015) also reported the inability of the TG178 marker to discriminate between the *Ty-1* positive and negative genotypes. This warrants further evaluation of these genotypes with alternative markers for the detection of the Ty-1 gene.

Ty-3, a partially dominant gene, is reported to be allelic with Ty-1 and code for RNA dependent RNA polymerase (RDR). Resistant allele for *Ty-3* was present in the genotype AVTO 1726 and AVTO 1707 in homozygous state. Whereas, it was in heterozygous condition in AVTO 1727. Amplicon corresponding to the susceptible allele of *Ty-3* was detected in 21 genotypes screened in the present study (Plate 14). The presence of a four amino acid deletion in the amino-terminal part of the RDR protein leads to the allele conditioning susceptibility at the *Ty-1/3* locus compared to the *Ty-1* and *Ty-3* resistance alleles (Chen *et al*., 2015). Seven genotypes recorded the presence of *Ty-1/3* in homozygous condition (AVTO 1727, AVTO 1726, AVTO 1706, AVTO 1707, EC 538153, EC 620428 and EC 620486) and seven genotypes in heterozygous condition (Ansal, Virang, Aryaman, Durg, Raymond, Abhiraj, Pranay) (Plate 15). All the genotypes harbouring *Ty-1/3* locus in heterozygous state were commercial hybrid varieties.

Four genotypes each carried the resistant allele for *Ty- 2* in the homozygous state (AVTO 1726, AVTO 1706, AVTO 1707 and AVTO 0922) and in the heterozygous state (AVTO 1727, Ansal, Arka Rakshak and Aryaman) (Plate 13). Sadasiva *et al*. (2017) had reported the presence of only Ty-2 gene in Arka Rakshak. All the AVTO lines (received from World Vegetable Centre, Taiwan) are known to harbour Ty-2 gene and amplification of the 900 bp amplicon corresponding to the resistant allele of Ty-2 gene was detected in the present study as expected.

Ten genotypes produced amplicons using SLM4-34 marker associated with Ty-5 gene (AVTO1726, Ansal, Kaustubh, EC 620428, EC 538153, Manuprabha, Manulekshmi,

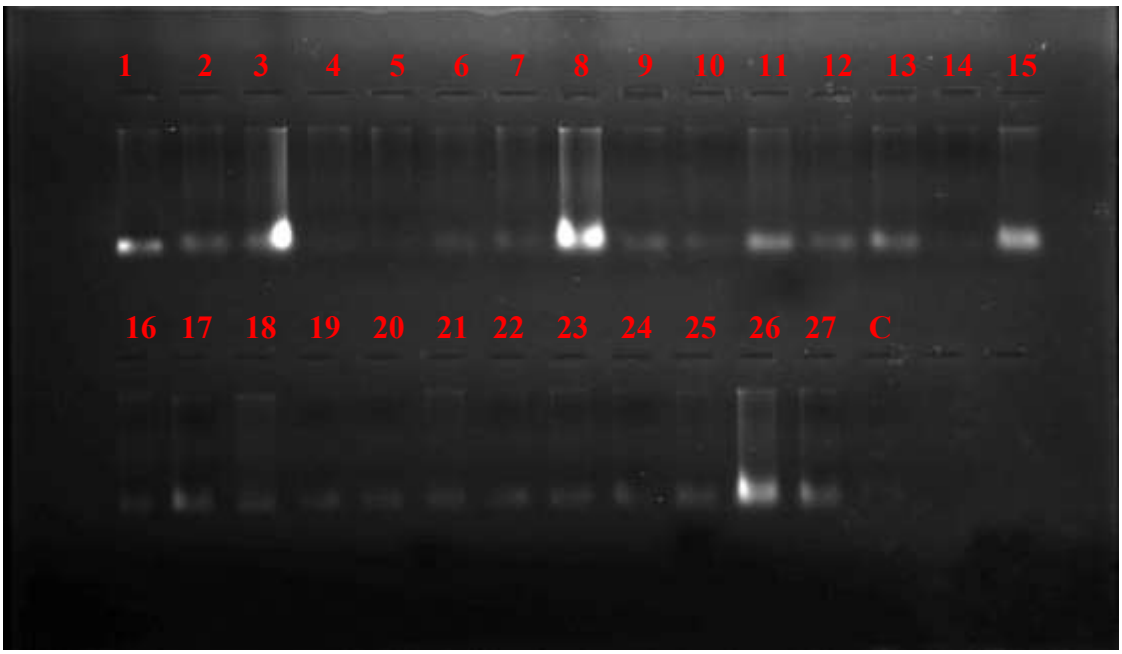
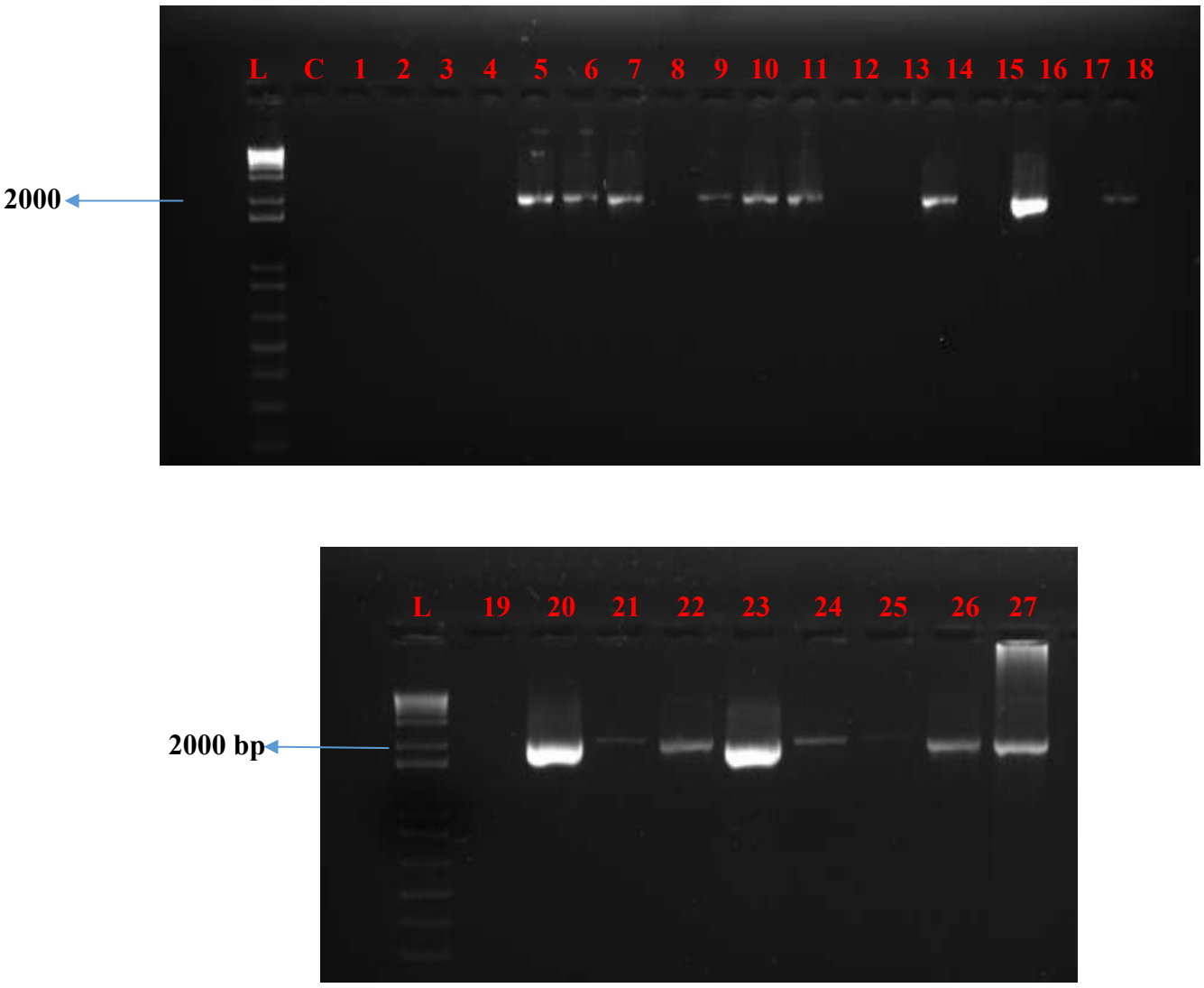


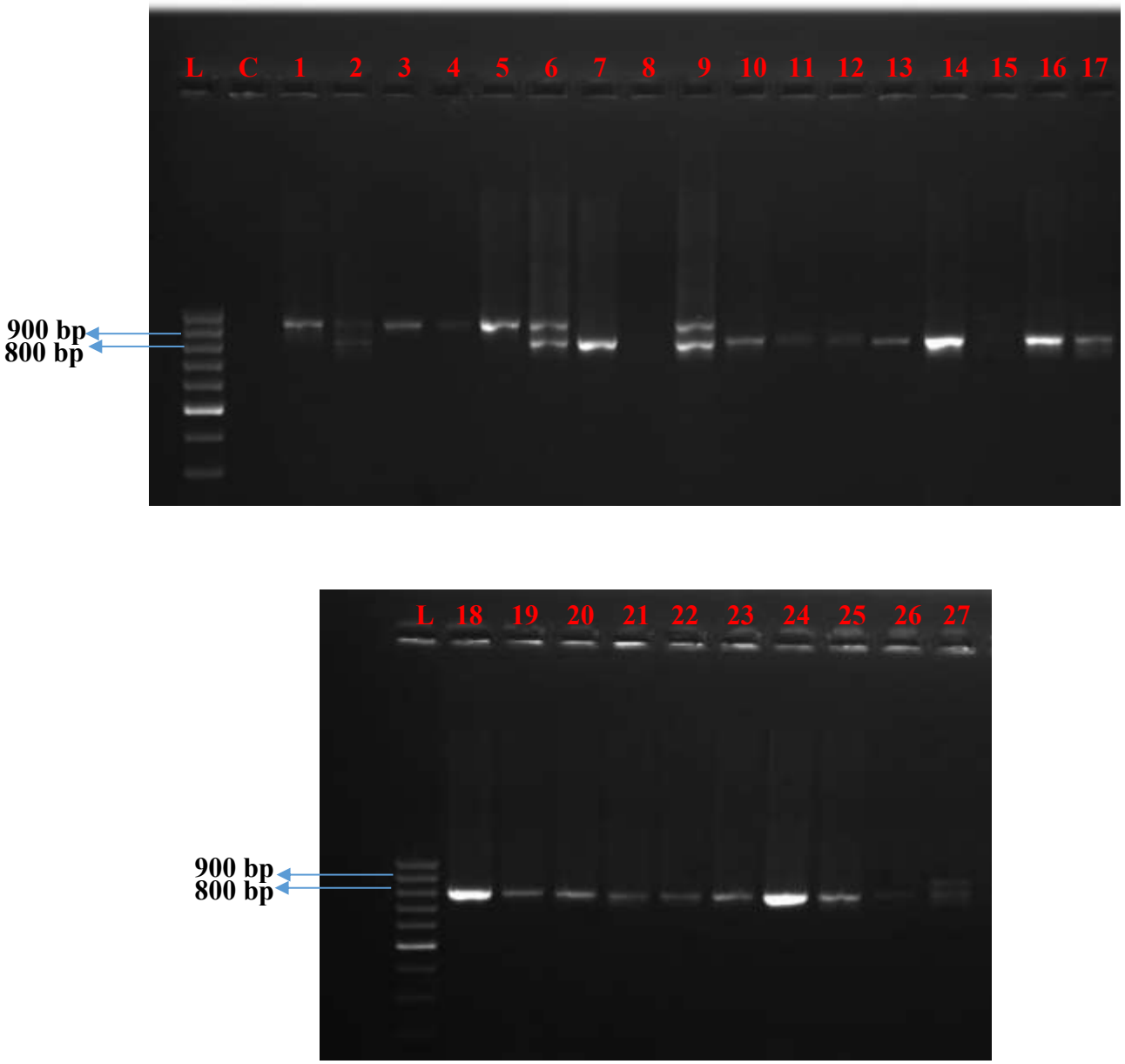
Plate 11. DNA isolation

1-AVTO 1726 2- AVTO 1727 3- AVTO 1707 4- AVTO 1706 5- AVTO 0922 6- Ansal 7- Virang 8- Kaustubh 9- Aryaman 10- Durg 11- Raymond 12- Abhiraj 13- Pranay 14- EC 519806 15- EC 528360 16- EC 620428 17- EC 521067 B 18- EC 538153 19- Local collection (Idukki) 20- Manuprabha 21- Manulekshmi 22- Anagha 23- Akshaya 24- EC 620486 25- EC 315489 26- EC 567305 27- Arka Rakshak



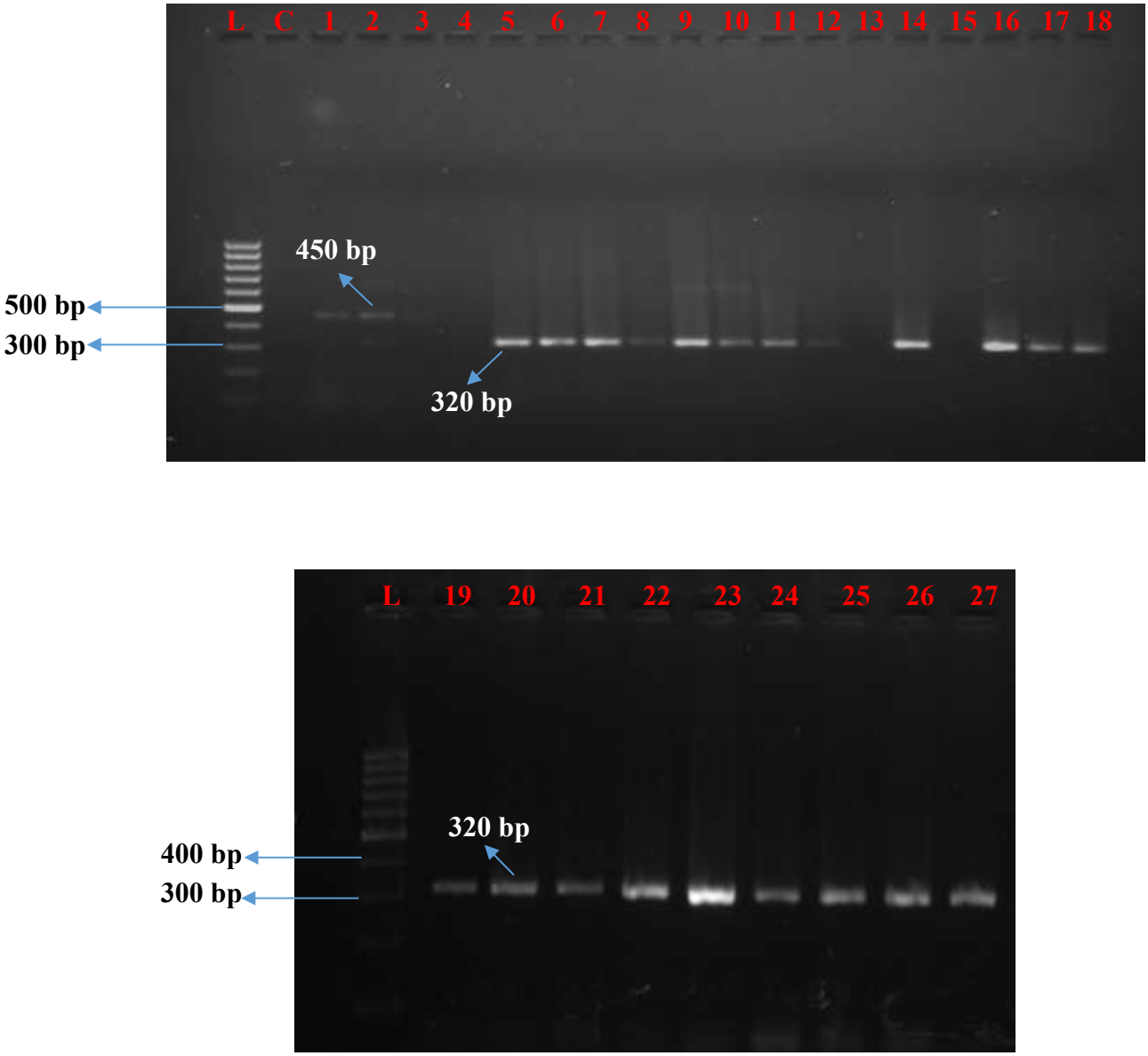
1-AVTO 1726 2- AVTO 1727 3- AVTO 1707 4- AVTO 1706 5- AVTO 0922 6- Ansal 7- Virang 8- Kaustubh 9- Aryaman 10- Durg 11- Raymond 12- Abhiraj 13- Pranay 14- EC 519806 15- EC 528360 16- EC 620428 17- EC 521067 B 18- EC 538153 19- Local collection (Idukki) 20- Manuprabha 21- Manulekshmi 22- Anagha 23- Akshaya 24- EC 620486 25- EC 315489 26- EC 567305 27- Arka Rakshak L- ladder C- control

Plate 12. Amplification pattern in genotypes using TG178 (SCAR) primer for Ty-1 gene



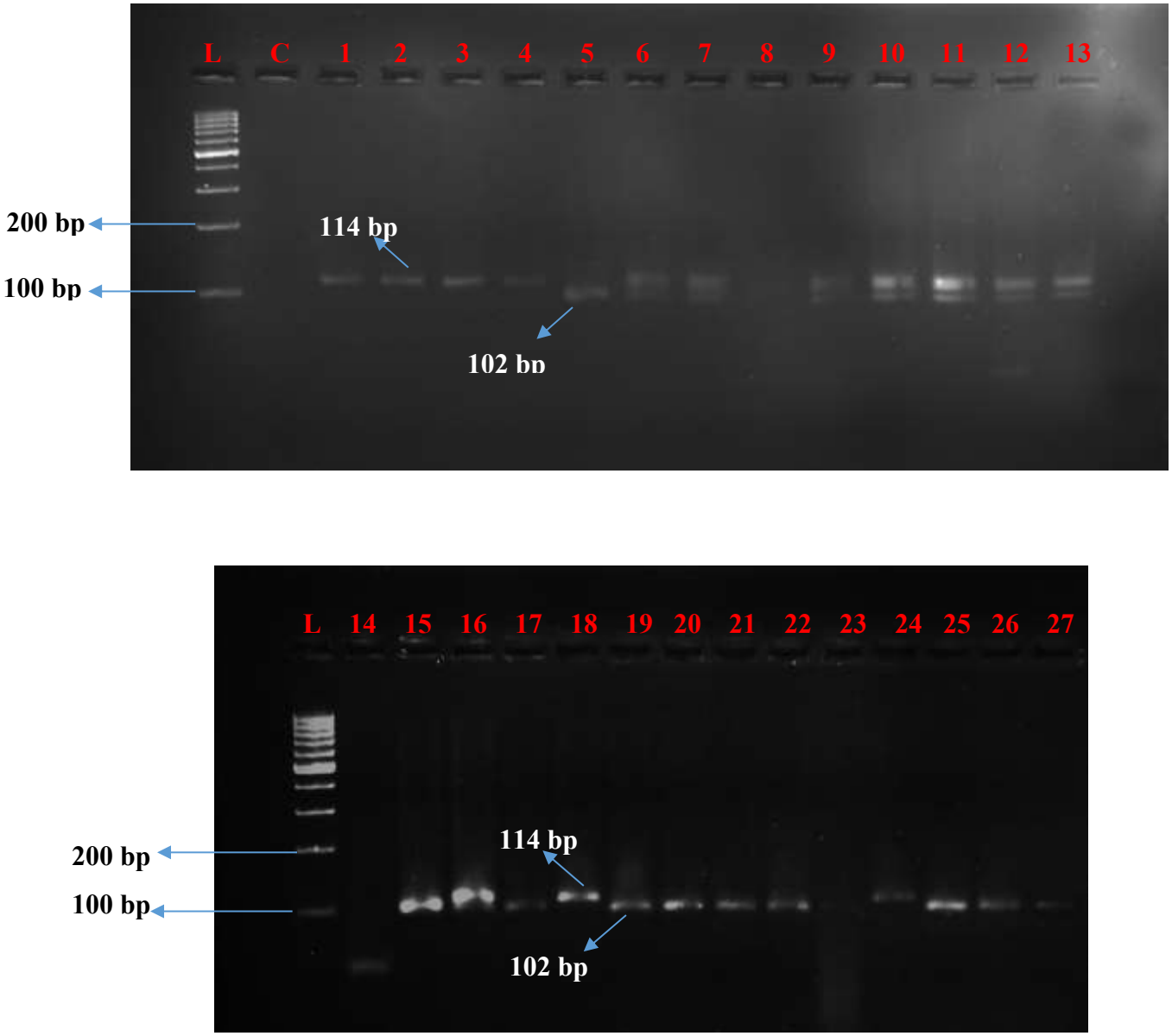
1-AVTO 1726 2- AVTO 1727 3- AVTO 1707 4- AVTO 1706 5- AVTO 0922 6- Ansal 7- Virang 8- Kaustubh 9- Aryaman 10- Durg 11- Raymond 12- Abhiraj 13- Pranay 14- EC 519806 15- EC 528360 16- EC 620428 17- EC 521067 B 18- EC 538153 19- Local collection (Idukki) 20- Manuprabha 21- Manulekshmi 22- Anagha 23- Akshaya 24- EC 620486 25- EC 315489 26- EC 567305 27- Arka Rakshak L- ladder C- control

Plate 13. Amplification pattern in genotypes using SCAR2 primer for Ty-2 gene



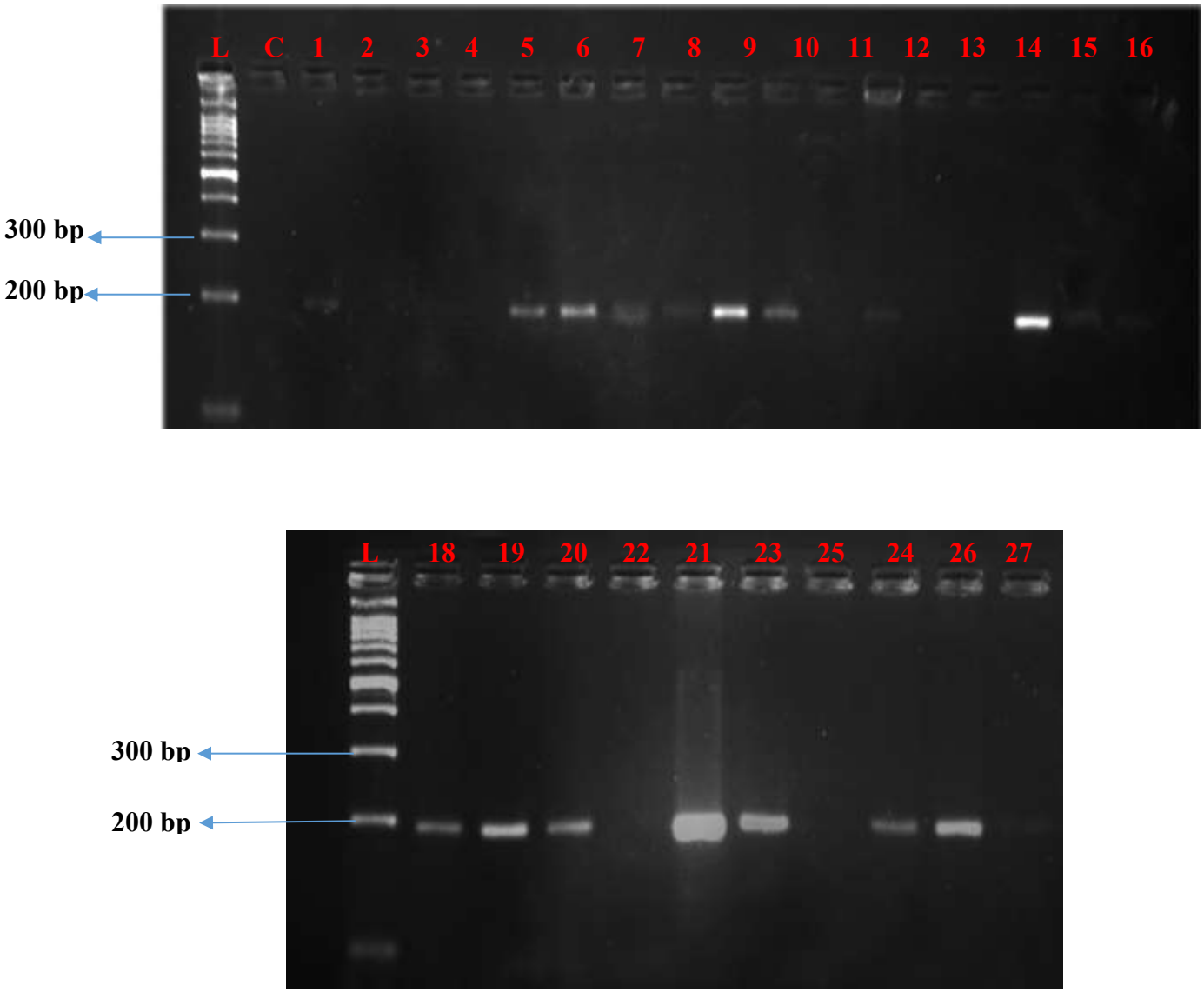
1-AVTO 1726 2- AVTO 1727 3- AVTO 1707 4- AVTO 1706 5- AVTO 0922 6- Ansal 7- Virang 8- Kaustubh 9- Aryaman 10- Durg 11- Raymond 12- Abhiraj 13- Pranay 14- EC 519806 15- EC 528360 16- EC 620428 17- EC 521067 B 18- EC 538153 19- Local collection (Idukki) 20- Manuprabha 21- Manulekshmi 22- Anagha 23- Akshaya 24- EC 620486 25- EC 315489 26- EC 567305 27- Arka Rakshak L- ladder C- control

Plate 14. Amplification pattern in genotypes using P6-25 primer for Ty-3 gene



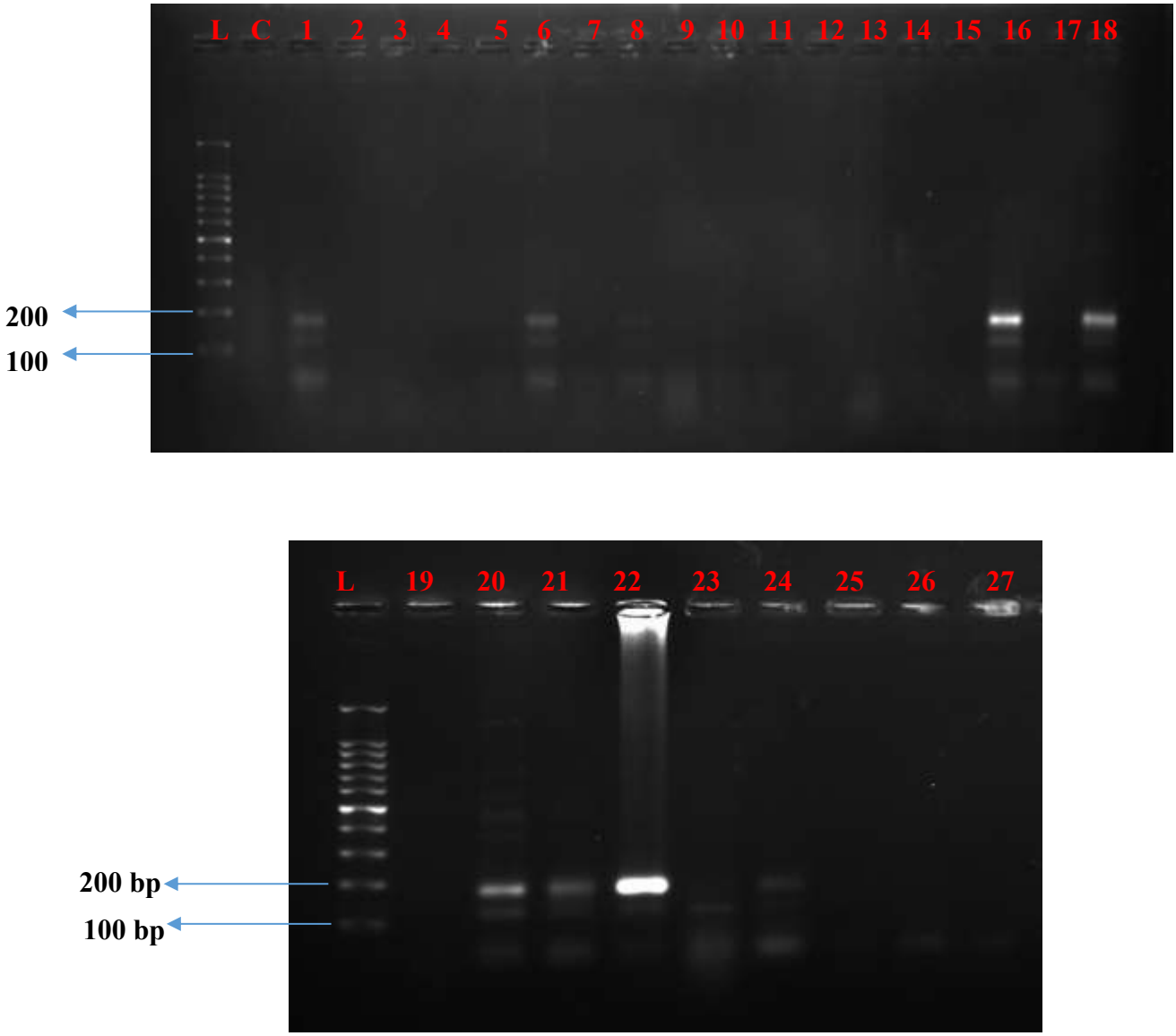
1-AVTO 1726 2- AVTO 1727 3- AVTO 1707 4- AVTO 1706 5- AVTO 0922 6- Ansal 7- Virang 8- Kaustubh 9- Aryaman 10- Durg 11- Raymond 12- Abhiraj 13- Pranay 14- EC 519806 15- EC 528360 16- EC 620428 17- EC 521067 B 18- EC 538153 19- Local collection (Idukki) 20- Manuprabha 21- Manulekshmi 22- Anagha 23- Akshaya 24- EC 620486 25- EC 315489 26- EC 567305 27- Arka Rakshak L- ladder C- control

Plate 15. Amplification pattern in genotypes using Ty1/3K primer for Ty-1/3 gene



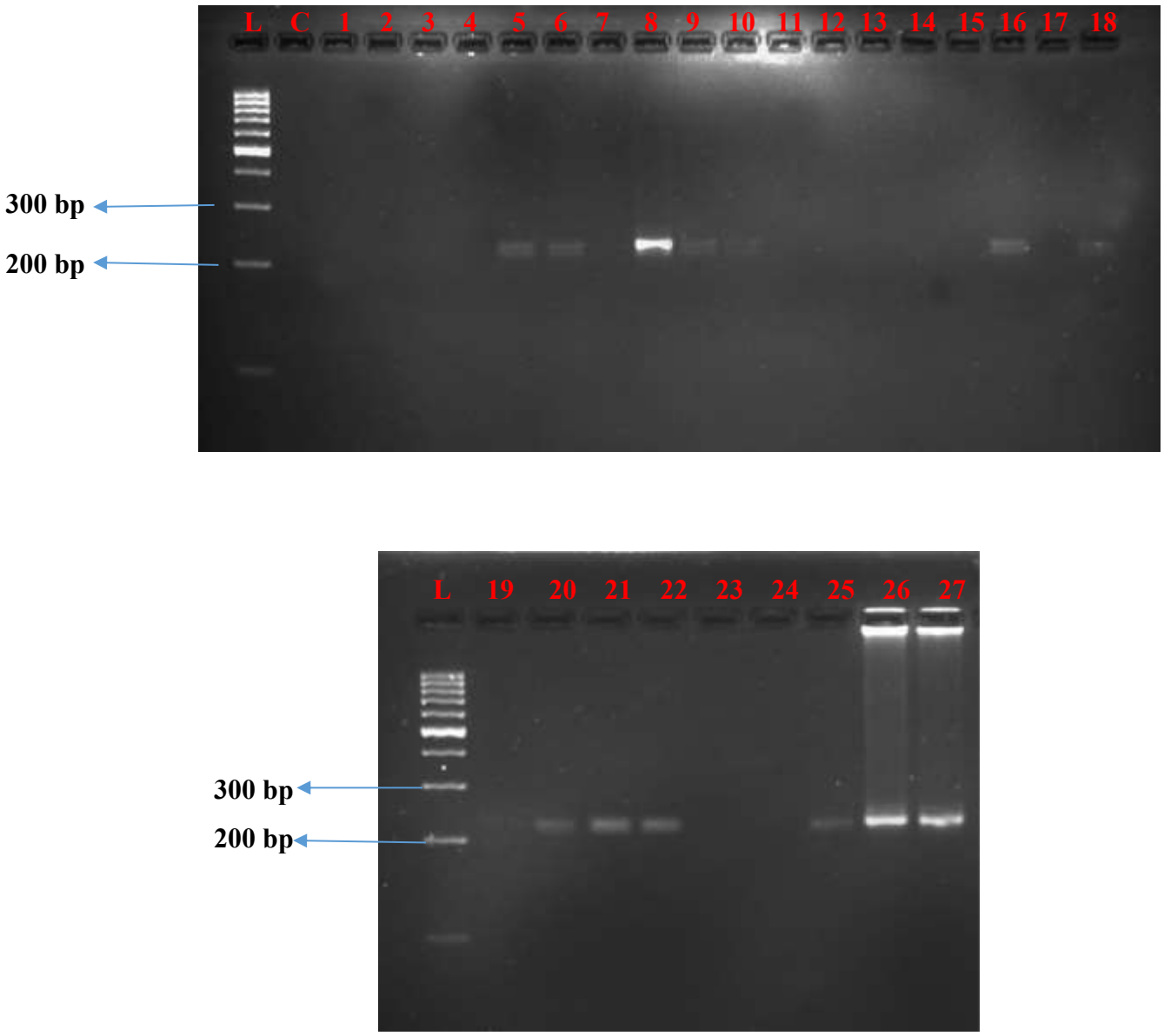
1-AVTO 1726 2- AVTO 1727 3- AVTO 1707 4- AVTO 1706 5- AVTO 0922 6- Ansal 7- Virang 8- Kaustubh 9- Aryaman 10- Durg 11- Raymond 12- Abhiraj 13- Pranay 14- EC 519806 15- EC 528360 16- EC 620428 17- EC 521067 B 18- EC 538153 19- Local collection (Idukki) 20- Manuprabha 21- Manulekshmi 22- Anagha 23- Akshaya 24- EC 620486 25- EC 315489 26- EC 567305 27- Arka Rakshak L- ladder C- control

Plate 16. Amplification pattern in genotypes using 181Y13 (Indel) primer for Ty-4 gene



1-AVTO 1726 2- AVTO 1727 3- AVTO 1707 4- AVTO 1706 5- AVTO 0922 6- Ansal 7- Virang 8- Kaustubh 9- Aryaman 10- Durg 11- Raymond 12- Abhiraj 13- Pranay 14- EC 519806 15- EC 528360 16- EC 620428 17- EC 521067 B 18- EC 538153 19- Local collection (Idukki) 20- Manuprabha 21- Manulekshmi 22- Anagha 23- Akshaya 24- EC 620486 25- EC 315489 26- EC 567305 27- Arka Rakshak L- ladder C- control

Plate 17. Amplification pattern in genotypes using SLM 4-34 primer for Ty-5 gene



1-AVTO 1726 2- AVTO 1727 3- AVTO 1707 4- AVTO 1706 5- AVTO 0922 6- Ansal 7- Virang 8- Kaustubh 9- Aryaman 10- Durg 11- Raymond 12- Abhiraj 13- Pranay 14- EC 519806 15- EC 528360 16- EC 620428 17- EC 521067 B 18- EC 538153 19- Local collection (Idukki) 20- Manuprabha 21- Manulekshmi 22- Anagha 23- Akshaya 24- EC 620486 25- EC 315489 26- EC 567305 27- Arka Rakshak L- ladder C- control

Plate 18. Amplification pattern in genotypes using SLM 10-46 primer for ty-6 gene

Anagha, Akshaya and EC 620486) (Plate 17). Chen *et al*. (2015) reported that although SLM4-34 was polymorphic between plants carrying the *Ty-5* resistance and susceptibility alleles it produced multiple bands. Multiple bands could also be observed in the present study during amplification with SLM4-34. However, no polymorphic band specific to the resistant genotypes could be detected in the present study.

None of the genotypes evaluated in the present study revealed the presence of amplicons corresponding to resistant allele of *Ty-4* and *Ty-6,* although corresponding susceptible amplicons were amplified for both in several genotypes.

The genotypes found to be resistant under natural field screening and artificial inoculation are Ansal, Kaustubh, EC 519806, Local collection (Idukki) and Arka Rakshak. Among the markers linked with Ty genes that were reliably amplified in the present study, *Ty-2* and *Ty-1/3* were present in Ansal. Ansal was found to be highly resistant in natural field screening as well as under artificial inoculation. Four lines (AVTO 1727, AVTO 1726, AVTO 1706 and AVTO 1707) in the present study have the combination of *Ty-2* +*Ty-1/3.* Among these, AVTO 1727 and AVTO 1726 were moderately susceptible to ToLCV in natural condition. Prasanna *et al.* (2015) reported that lines carrying *Ty2* + *Ty3* exhibited very low coefficient of infection compared with *Ty-2* alone. The lines carrying *Ty-2* alone have higher coefficient of infection than those of *Ty-3*, *Ty-2*+*Ty-3*, *Ty-2*+*ty-5* lines (Kaushal *et al*., 2020). The genotypes Akshaya and Anagha which were moderately susceptible under natural conditions, did not harbour *Ty-3, Ty-1/3* or *Ty-2*. Nadkarni (2017) reported the absence of Ty-2 and Ty-3 genes in Anagha and Akshaya using SCAR markers TG0302F/TY2R1 and P6-25 respectively. All the genotypes harbouring *Ty-1/3* locus in heterozygous state were commercial hybrid varieties, among which only Ansal showed resistance response to ToLCV.

Resistance of the genotypes are highly specific to the virus strain. Genotype found to be resistant under a particular environment may not be resistant under other

environment. Hassan (2020) reported that different ToLCV isolates have varying effects on the onset of symptoms as well as their type and severity.

Three resistant genotypes identified in the present study, *viz.*, Kaustubh, EC 519806 and local collection (Idukki) did not show amplification corresponding to resistant genotypes for any of the markers tested in this study. The susceptible alleles of *Ty-2* and *Ty-3* are carried by the genotype EC 519806; Kaustubh carry susceptible alleles of *Ty-3*, *Ty-4* and *Ty-6*; and local collection (Idukki) had amplicon corresponding to susceptible genotype for *Ty-3, Ty-1/3, Ty-4* and *Ty-2*. But these genotypes expressed resistance under the field conditions. Prasanna *et al*. (2015) reported that markers linked to a specific gene may not be useful for general use in other genetic backgrounds. Since the genotype EC 519806 (*S. pimpinellifolium*) belongs to another genetic background, and the species status of the local collection (Idukki), which is small fruited type is yet to be determined, we may have to attempt with some other markers associated with Ty genes for further validation in these genotypes. The possibility of these genotypes harbouring some important gene that can confer resistance to ToLCV which is yet to be deciphered exists, and the high glandular trichome density observed in EC 519806, Kaustubh and local collection (Idukki), may be functioning as a physical barrier for the whitefly from accessing and infecting these genotypes.

4.6 Reaction of genotypes to artificial inoculation

The susceptible checks Anagha and Manuprabha in the present study were found to be symptomatic after artificial inoculation. Symptoms were observed at weekly intervals and mild curling and upward cupping symptoms appeared in them at four week after artificial inoculation. The genotypes found to be resistant under the field screening (Ansal, Kaustubh, EC 519806, Local collection (Idukki) and Arka Rakshak) were also found to be resistant after artificial inoculation (Plates 7 & 8) whereas, mild curling was observed in Arka Rakshak, which was identified as resistant during the field screening.

Whitefly mediated inoculation technique have been able to ensure infection in susceptible genotypes, allowing reliable screening of tomato germplasm (Yadav, 2011). Gomez *et al*. (1994) and Paul (2014) also used whitefly transmission for screening against ToLCV in tomato. The genotypes F3524, F3522, Fiona and Tyking were identified as resistant after whitefly mediated artificial inoculation (Pico *et al*., 2000). Rajasri and Vijayalakshmi (2013), identified the genotypes EC 251672, Akash-918, NS 515, NS 539 and US 275 as resistant and Pusa Ruby, Arka Alok, Arka Vikas, Arka Sourabh and Marutham as susceptible to ToLCV after whitefly mediated artificial inoculation. Curling and upward cupping symptoms of ToLCV after artificial inoculation were also reported by them.

SUMMARY

5. SUMMARY

Tomato leaf curl disease (ToLCD), caused by tomato leaf curl virus (ToLCV), is one of the most devastating diseases of tomato and is a major constraint in the cultivation of tomato. Identification of sources for ToLCV resistance in wild or cultivated tomato, and their utilization in resistance breeding offers a sustainable solution for ToLCV management. Keeping the above facts in view, the present investigation entitled "Evaluation of tomato genotypes for tomato leaf curl virus (ToLCV) resistance" was taken up at the Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara from 2019-2021.

The salient findings of the study are summarised below,

- Among the 27 genotypes screened, one genotype was found to be highly resistant (Ansal), four were resistant (Kaustubh, EC 519806, Local collection (Idukki) and Arka Rakshak), four were moderately susceptible (AVTO 1726, AVTO 1727, Anagha and Akshaya), twelve were susceptible (AVTO 1707, AVTO 1706, AVTO 0922, Virang, Durg, Abhiraj, EC 528360, EC 620428, EC 521067 B, Manuprabha, Manulekshmi and EC 620486) and six were highly susceptible (Aryaman, Raymond, Pranay, EC 538153, EC 315489 and EC 567305).
- The genotypes found to be resistant under the field screening *viz.,* Ansal, Kaustubh, EC 519806, local collection (Idukki) and Arka Rakshak, were found to be resistant after artificial inoculation as well.
- The genotypes with determinate growth habit were AVTO 1726, AVTO 1727, AVTO 1707, AVTO 1706, AVTO 0922, Ansal, Virang, Kaustubh, Aryaman, Durg, Raymond, Abhiraj, Pranay, EC 519806, EC 620428, EC 521067 B, local collection (Idukki), Manuprabha, Manulekshmi, Anagha, EC 620486, EC 315489, EC 567305 and Arka Rakshak. The genotypes with indeterminate growth habit were EC 528360, EC 538153 and Akshaya.

- Morphological characters such as plant height, number of primary branches/plant, days to flowering and trichome density differed significantly between the genotypes evaluated.
- Plant height ranged from 28.88 cm to 60.02 cm. The plant height was highest in AVTO 0922 and the lowest in local collection (Idukki).
- The number of primary branches/plant ranged from 1.18 to 3.46. The number of primary branches/plant was highest in EC 519806 and lowest in Ansal.
- Days to flowering ranged from 27.81 days to 51.46 days. Shortest days to flowering was in EC 315489 and longest in AVTO 1727.
- Glandular trichome density on the adaxial leaf surface was high in Arka Rakshak, Kaustubh, EC 519806, Akshaya and Anagha. Glandular trichome density on the abaxial leaf surface was high in Ansal, Kaustubh, EC 519806, Arka Rakshak and local collection (Idukki).
- Glandular trichome density on abaxial leaf surface had strong and significant negative correlation with per cent disease incidence and disease severity index.
- Glandular trichome density on adaxial leaf surface was negatively correlated with per cent disease incidence and disease severity index.
- Non-glandular trichome density on adaxial and abaxial leaf surface were positively correlated with per cent disease incidence and disease severity index.
- Amplicon corresponding to the resistant allele for *Ty-3* was present in AVTO 1726 and AVTO 1707 in homozygous state and in heterozygous condition in AVTO 1727.
- Seven genotypes recorded the presence of *Ty-1/3* in homozygous condition (AVTO 1727, AVTO 1726, AVTO 1706, AVTO 1707, EC 538153, EC 620428 and EC 620486) and seven genotypes in heterozygous condition (Ansal, Virang, Aryaman, Durg, Raymond, Abhiraj, Pranay).
- Four genotypes each carried the resistant allele for *Ty- 2* in the homozygous state (AVTO 1726, AVTO 1706, AVTO 1707 and AVTO 0922) and in the heterozygous state (AVTO 1727, Ansal, Arka Rakshak and Aryaman).

- None of the genotypes evaluated in the present study revealed the presence of amplicons corresponding to resistant allele of *Ty*-1, *Ty-4, Ty-5* and *Ty-6.*
- Four lines (AVTO 1727, AVTO 1726, AVTO 1706 and AVTO 1707) in the present study have the combination of *Ty-2* and *Ty-1/3.*
- The highly resistant genotype identified in the present study, Ansal, carried *Ty-2* and *Ty-1/3,* while the resistant genotype Arka Rakshak carried *Ty-2.*
- The resistant genotypes Kaustubh, EC 519806 and local collection (Idukki) did not show amplification corresponding to resistant genotypes for any of the markers tested in this study.
- Donors of *Ty-2, Ty-3* and *Ty-1/3* identified in the present study can be used for future breeding programmes.
- Molecular markers Ty-1/3-K, SCAR2 and P6-25 for *Ty-1/3, Ty-2* and *Ty-3* respectively, validated in the present study can be used for marker assisted selection.
- Trichome density is a powerful morphological marker for screening for ToLCV resistance.

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EVALUATION OF TOMATO GENOTYPES FOR TOMATO LEAF CURL VIRUS (ToLCV) RESISTANCE

By

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ABSTRACT

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Abstract

Tomato (*Solanum lycopersicum,* 2n=24) is a widely grown vegetable in India, exhibiting wide variability for plant traits. Worldwide, tomatoes are susceptible to many diseases like damping off, bacterial wilt, early blight, leaf curl virus disease, leaf spot etc. Among them, tomato leaf curl virus disease (ToLCD), caused by whitefly-transmitted begomoviruses is more severe and it can cause up to 100 per cent yield loss. Although, the cultivated tomatoes are susceptible to Tomato leaf curl virus (ToLCV), different levels of resistance were identified in wild relatives of tomato. Utilization of these natural resources will aid ToLCV management through crop improvement.

The present study entitled 'Evaluation of tomato genotypes for Tomato Leaf Curl Virus (ToLCV) resistance' was carried out at Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara during 2019-21. The experimental material consisted of 27 tomato genotypes including eight NBPGR (National Bureau of Plant Genetic Resources) accessions, five breeding lines from the World Vegetable Centre, Taiwan, nine commercial hybrids, four KAU varieties and one local collection from Idukki.

All 27 genotypes were screened for ToLCV resistance under natural conditions in randomized block design with two replications during January- May 2021. The disease response was assessed after 30 days of transplanting by adopting the score chart suggested by Banerjee and Kalloo (1987). Among the genotypes tested, Ansal was found to be highly resistant, Kaustubh, EC 519806 (*S. pimpinellifolium*), Arka Rakshak and local collection (Idukki) were identified as resistant genotypes. The genotypes found to be highly resistant and resistant were subjected to whitefly mediated artificial inoculation. The resistant and highly resistant genotypes remained asymptomatic, whereas the susceptible genotypes (Anagha and Manuprabha) exhibited ToLCV symptoms after artificial inoculation.

The morphological growth characters such as plant height, number of primary branches per plant, days to flowering and trichome density differed significantly among the genotypes evaluated in the present study. Density of both glandular and non-glandular trichomes on both abaxial as well as adaxial leaf surfaces were recorded. Correlation analysis between trichome density, and disease severity index and per cent disease incidence showed that glandular trichome density on abaxial and adaxial surfaces of leaf

was significantly and negatively correlated with per cent disease incidence and disease severity index. However, the negative correlation was stronger between the abaxial glandular trichome density and ToLCV incidence. The glandular trichome density of the resistant genotypes identified in the field screening was found to be high. Non-glandular trichome density on abaxial and adaxial side of the leaf was significantly and positively correlated with per cent disease incidence and disease severity index.

Screening for the reported Ty genes using the primers TG178 (SCAR), SCAR-2, P6-25, TY-1/3_K (SCAR), 18IY13 (Indel), SLM 4-34 (SSR) and SLM 10-46(SSR) linked to the ToLCV resistance genes Ty-1, Ty-2, Ty-3, Ty-1/3, Ty-4, Ty-5 and Ty-6 respectively was done in the present study. Seventeen genotypes produced amplicon using TG178 and ten genotypes produced amplicons using SLM4-34. However, there was no pattern in the amplification of the markers TG178 and SLM 4-34. Seven genotypes recorded the presence of *Ty-1/3* in homozygous condition (AVTO 1727, AVTO 1726, AVTO 1706, AVTO 1707, EC 538153, EC 620428 and EC 620486) and seven genotypes in heterozygous condition (Ansal, Virang, Aryaman, Durg, Raymond, Abhiraj, Pranay). Four genotypes each carried the resistant allele for Ty-2 in the homozygous state (AVTO 1726, AVTO 1706, AVTO 1707 and AVTO 0922) and in the heterozygous state (AVTO 1727, Ansal, Arka Rakshak and Aryaman). Resistant allele for Ty-3 was present in the genotype AVTO 1726 and AVTO 1707 in homozygous state. Whereas, it was in heterozygous condition in AVTO 1727. None of the genotypes evaluated in the present study revealed the presence of amplicons corresponding to resistant allele of *Ty-4* and *Ty-6.*

The resistant genotypes identified in the present study *viz.,* Ansal, Kaustubh, Arka Rakshak, EC 519806 and local collection (Idukki) can be used for resistance breeding. The present study revealed that trichome density can be used as a reliable morphological marker for ToLCV resistance. The donors for *Ty-2*, *Ty-3* and *Ty-1/3* identified in this study can be used for gene pyramiding in future breeding programmes.