IDENTIFICATION OF POTENTIAL DONORS FOR SUPERIOR FRUIT QUALITY TRAITS AND GENES FOR RESISTANCE TO TOMATO LEAF CURL VIRUS (ToLCV) IN TOMATO AND ALLIED SPECIES

by

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(2014 - 21 - 122)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

KERALA, INDIA

2017

DECLARATION

I, hereby declare that this thesis entitled **"IDENTIFICATION OF POTENTIAL DONORS FOR SUPERIOR FRUIT QUALITY TRAITS AND GENES FOR RESISTANCE TO TOMATO LEAF CURL VIRUS (ToLCV) IN TOMATO AND ALLIED SPECIES"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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ACKNOWLEDGEMENT

With regards to sweet memories.....

Any tedious task is made light and smooth by God's grace. At this moment, I would remember 'the almightily god', who has made each impossible work a reality in my life. The fruit of which, I am expressing here.

The work presented in this thesis would not have been possible without my close association with many people. I take this opportunity to extend my sincere gratitude and appreciation to all those who made this Ph.D thesis possible.

First and foremost, I would like to extend my sincere gratitude to my research guide Dr. Jayalekshmy V. G. Professor, Department of Plant Breeding and Genetics, College of Agriculture, Vellayani for introducing me to this exciting field of Plant science and for her dedicated help, advice, inspiration, encouragement and continuous support, throughout my Ph.D. Her enthusiasm, integral view on research and her mission for providing high-quality work, has made a deep impression on me. She has taught me another aspect of life, that, "goodness can never be defied and good human beings can never be denied". Her constant guidance, cooperation, motivation and support have always kept me going ahead.

I feel highly obliged and have immense pleasure in expressing my sincere thanks to the members of my Advisory Committee Dr. Arya K, Professor and Head, (Plant Breeding and Genetics), Dr. Umamaheshwaran. K, Associate Director of Research, NARP (SR), College of Agriculture, Vellayani, Dr. Thomas George, Professor, Department of Soil Science & Agril. Chemistry,

College of Agriculture, Vellayani, Dr. Geetha. D. Professor, Department of Plant Pathology, College of Agriculture, Vellayani, for their intellectual enlightment and persistent suggestions throughout the pursuit of this study.

I cordially offer my profound gratitude to my teachers in Department of Plant Breeding and Genetics Dr. P. Manju, Dr. D.S. Radha Devi, Dr. Maya Devi, P., Dr. Sunny K, Oommen, Dr. Wilson D, Lekha Rani, Dr. Mareen Abraham and Dr. Beena Thomas for their excellent teaching and guidance which enabled me complete my course work.

On my personal note, it is an immense pleasure to express my sincere gratitude and heartfelt respect to the blessings of my parents Shri Raghvendra M. Nadkarni and Smt. Rupa R. Nadkarni for their boundless love, needy inspiring, unshakable confidence with me, would it be all envisaging to offer the humblest solution at the lotus of my parents, who imbued me with energy and enthusiasm through the ramifying path of thick and thin of my effort and become what I am today. I express my deep sense of gratitude to Reema, Riddhesh and Rishan all the family members for their moral support without whose affection I would not have come up to this level.

I am extremely grateful to skilled labours and all workers of College of Agriculture, Vellayani for their help and co-operation during my research.

A special mention of thanks to my friends Harikrishna, Vinay, Darshan, Bibishan and Brunda for their constant support and cooperation during my stay. Their timely help and friendship shall always be remembered.

I find myself in short of words to express my warmest thanks to my beloved senior friends Sreenivas ,Gangadhar, Ramling, Rajib, Kranti and beloved junior friends Sathishkumar, Shivamurty, Asha, Jeena, Athul, Nikitha,

Jaslam, Manu, Dilip, shahiba and srilakshmi whose cheerful company I have never felt my work as burden. Finally I am thankful to my batch mates, junior friends and senior friends of UG and PG degree programme for their kind support.

One last word; since it is practically impossible to list all the names who contributed contributions to my work, it seems proper to issue a blanket of thanks for those who helped me directly or indirectly during the course of study.

Any omission in the short of manuscript does not mean lack of gratitude.

Nadkarni Siddhesh Raghvendra

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LISTOF ABBREVIATIONS

%	-	per cent
μ	-	Mean
μl	-	Micro litre
μM	-	Micro molar
^{0} C	-	Degree Celsius
SCAR		Sequence Characterized Amplified Regions
ANOVA	-	Analysis of Variance
bp	-	base pairs
CAPS	-	Cleaved Amplified Polymorphic Sequences
CD	-	Critical difference
cm	-	centimeter
d.f	-	degrees of freedom
dNTP	-	Deoxyribonucleoside triphosphate
EDTA	-	Ethylene diamine tetra acetic acid
et al.	-	and co-workers/co-authors
F_1	-	First filial generation
Fig.	-	Figure
g	-	gram
i.e.	-	that is
ISSR	-	Inter-simple sequence repeats
kg	-	kilogram
mm	-	millimeters
M	-	Molar
mg	-	milligram
min	-	minutes
mM	-	Milli molar

Nacl	-	Sodium chloride
ng	-	Nanogram
PCR	-	Polymerase Chain Reaction
RFLP	-	Restriction Fragment Length Polymorphism
RAPD	-	Random amplified polymorphic marker
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease
rpm	-	revolutions per minute
S.E(d)	-	Standard Error deviation
SE	-	Standard Error
spp.	-	Species
SSR	-	simple sequence repeat
TAE	-	Tris –Acetate- EDTA
Taq	-	*Thermus aquaticus*
Tm	-	Annealing Temperature
Viz.,	-	namely
ToLCV	-	Tomato leaf curl virus
kg	-	Kilo grams
ml	-	Millilitre

Introduction

1. INTRODUCTION

Vegetables play an important role in nutritional security, economic viability and fit well into the predominant intensive cropping systems prevailing in different parts of our country. More than 60 kinds of vegetables are grown in India in tropical, subtropical and temperate agro-climates. Tomato [*Solanum lycopersicum* L. 2n=2x=24] belongs to the large and diverse family Solanaceae, which includes more than 3000 species, occupying a wide variety of habitats (Knapp, 2002). It is one of the most important vegetables crops grown throughout the world. In fact, it is the fifth important cultivated crop after rice, wheat, maize and potato. The fruits are consumed either as raw or cooked or processed into various products like juice, ketchup, sauce, paste, puree etc. The popularity of tomato is on the rise among consumers, not only because of its good taste, but also because it contains high levels of vitamin A, vitamin C, potassium, phosphorus, magnesium and calcium. It also contains lycopene and carotene, which are anti-oxidants that promote good health. The high demand for tomato makes it a high value crop that can generate much income to farmers.

The major tomato growing countries in the world are China, U.S.A, India, Turkey, Italy, Iran, Egypt, Brazil, Spain and Mexico. Tomatoes are one of the most widely consumed vegetables in the world. The annual worldwide production of tomatoes has been estimated as 163 million tonnes cultivated in an area of about 4.8 million ha with a productivity of 33.9 tonnes/ha (Anonymous, 2015). India is the second largest producer of tomato after China with an annual production of 18.73 million tonnes from an area of 0.88 million ha with productivity of 21.2 tonnes/ha (Anonymous, 2015).

In India, tomato has become a popular vegetable during last five decades because of its suitability for growing in all seasons. Hence, cultivation of tomato remains in the focus of the agricultural industry. Quality has gained importance in India after signing and notification of the GATT recommendations under WTO. The straight implications of this development are the gross reductions in import duties leading to cheaper imports, which include fresh as well as processed vegetables. Thus, it is high time to redefine our breeding and production objectives to include fruit quality traits in general and nutritional quality in particular as integrated objective with disease resistance along with high yield.

Among the various diseases infecting tomato, Tomato leaf curl virus (ToLCV) is one of the most serious diseases of tomato in Indian sub-continent and many other tropical and subtropical Asian countries. This disease is caused by gemini virus transmitted by the whitefly *Bemisia tabaci* (Gennadius) (Anbinder *et al.,* 2009). The affected tomato plants exhibit curling, puckering, reduction in leaflet size, severe stunting and reduction in fruit set. However, severely infected young plants almost fail to produce any fruits. This disease can cause yield losses up to 99-100% (Singh *et al.,* 2008). Chemical control measures as well as integrated pest management (IPM) strategies employed for controlling the vector have not been successful in controlling the disease. Under these circumstances breeding for resistant varieties appears to be a promising and eco-friendly approach for controlling the disease.

It is believed that primary and secondary gene centre of cultivated plants are the best place to find genuine resistance to common diseases (Leppik, 1970). In primary centre of origin of tomato, this virus is completely absent. Therefore the resistance sources to ToLCV are expected to be in secondary centre of origin. Wild tomato species have been screened for their response to the virus and a number of ToLCV resistant genotypes have been identified in wild species such as *Solanum chilense, S. habrochaites, S. peruvianum, S. pimpinellifolium* and *S. cheesmaniae.* Thus, breeding programs have been based on the transfer of resistance genes from accessions of wild origin to the cultivated tomato (Pico *et al.,* 1999).

Use of molecular markers linked to genes for resistance is a tool, which can be used efficiently in plant breeding for the indirect selection of quantitative resistance and for accelerated transfer of resistance from different sources into a single genotype.

Resistance breeding taken up in Kerala Agricultural University has resulted in the development of bacterial wilt resistant variety ''Anagha''. This variety is reported to be susceptible to serious disease caused by Tomato leaf curl virus (ToLCV) necessitating transfer of resistance to ToLCV disease as well.

Development of a variety resistant to both ToLCV and bacterial wilt disease through traditional breeding methods and molecular markers with superior fruit quality traits and high yield will be a boon to tomato cultivators in Kerala and elsewhere. Keeping this as the ultimate aim, the present study was undertaken with the following objectives.

- Evaluating varieties and allied species of tomato for fruit quality traits biochemical through analysis and genes for resistance to ToLCV through molecular markers.

- To study compatibility for hybridization and seed set to transfer ToLCV genes to bacterial wilt resistant variety ''Anagha'' from donors of related species.

Review of Literature

2. REVIEW OF LITERATURE

Tomato leaf curl virus (ToLCV) is one of the major devastating disease affecting tomato production in most tropical and sub-tropical countries. This disease is also called as tomato yellow leaf curl in other parts of the world because of the occurence of leaf yellowing, in addition to leaf curling. Incidence of this virus was reported in Middle East and India by many researchers (Cohen and Nitzany, 1966; Nour El- Din *et al.*, 1969; Yassin, 1985 and Barky, 1972). It is now established that ToLCV, which is spread by white fly *Bemisia tabaci* (Cohen and Nitzany, 1996) is an important threat to tomato production in India. Laterrot (1995) reported that genetic control of ToLCV is possible by breeding procedure using resistant Wild species. Molecular markers linked to disease resistance have immense use for rapid screening and gene pyramiding for production of resistant varieties and hybrids.

Vegetable breeder is primarily concerned with improvement of both quantitative and qualitative plant characters along with resistance. Hence, adequate knowledge of genetics of various traits is very important in vegetable breeding program for obtaining desirable results. Variability for crop improvement is present in different genotypes, lines, strains, varieties, wild relatives and their relatives, which constitute the germplasm of a specific crop. However the success of breeding depends on the magnitude and extent of variability existing in the germplasm. At the same time improvement is possible on the basis of heritable variations only. Hence, for the improvement of tomato, heritable variations in quantitative and quality traits are necessary. Therefore detailed information about genetic architecture of yield and quality attributes should be the main concern.

A brief review of available literature in consonance with the objectives of the present investigation in respect of tomato is reviewed and presented under the following headings.

2.1 TOMATO LEAF CURL VIRUS (ToLCV)

2.1.1 Distribution

Incidence of ToLCV was noticed in Asia particularly Israel during 1939. From Asia, the virus spread to Africa by 1966, Europe by 1974 and finally reached America by 1986 (Pico *et al.*, 1996).

First report on natural occurrence of *tobacco leaf curl virus* (TobLCV) in India was observed in tomato by Pruthi and Samuel (1939). Serious nature of leaf curl disease of tomato was reported in North India by Vasudeva and Samraj (1948) and later from Pune (Varma, 1959), Coimbatore (Ramakrishnan *et al.*, 1964), Delhi (Vasudeva, 1959; Nariani and Vasudeva, 1968), Karnataka (Govindu, 1964; Sastry and Singh, 1973), Kerala (Nair and Wilson, 1969), Punjab (Butler and Rataul, 1973), Lucknow (Srivastava *et al.*, 1975), Pantnagar, U.P. (Saklani and Mathai, 1978), Maharashtra (Datar, 1981) and Hissar (Varma and Poonam, 1977 and Varma *et al.*, 1980). This virus confronts the entire Peninsular and North Indian plains during summer season (Mayee *et al.*, 1974). It therefore becomes virtually impossible to successfully cultivate tomatoes in the states of Rajastan, Punjab, Haryana, U.P., M.P., West Bengal, Orissa, during April-August and in Southern states during March-June.

2.1.2 Symptomatology

The symptoms of the disease described by different researchers in the literature have been found to be mostly similar with little differences. The differences in symptoms have been to the type of cultivars infected, age of the cultivar at the time of infection, climate, strain of the virus and inoculum load etc. The common symptoms of the disease described in the literature on leaves and fruits were as follows

First ToLCV symptoms on tomato plants appear 2-4 weeks after inoculation and become fully developed after a period of upto two months (Credi *et al.*, 1989). Leaflets become hook like due to downward / upward and inward cupping and later developing leaves are mis-shapen and small (Jordan, 1993). Petioles of older leaves twist and plants become severely stunted, grow erect with many small branches and shortened internodes (Credi *et al.*, 1989). Early infected plants become unfruitful due to severe flower shedding (Ioannou, 1985). So, nationally the ToLCV affected plants have been described by several workers, Sastry and Singh (1973), Saklani and Mathai (1977), Raychaudhuri and Nariani (1977), Reddy (1978), Muniyappa (1980), Saikia (1985) and Saikia and Muniyappa (1989).

The leaf curl infected tomato plants exhibit vein clearing, greening and thickening of veins of leaflets, reduction in leaf size and stunted growth. The reduction in leaf size is more pronounced in the successive leaves accompanied by shortening of the internodes resulting in curling and crowding of leaves. The leaflets are deformed and

their margins curl inward or outward. The leaflets show a tendency to become stiff and crinkled with their tips coiled or twisted in the form of corkscrew. The younger leaves are pale yellow in color with intermingling of light green and dark green areas. Puckering of the leaflets is a characteristic symptom and plants have a greater tendency to produce stunted lateral branches imparting a bushy appearance. The plants infected in young age seldom attain height of more than 25 to 37 cm. The disease induces non-fruitfulness due to deformed floral structure. The infected plants usually develop purple patches especially on the older leaves Muniyappa (1980).

The ToLCV infected plants were assigned with a disease score according to the following scale of symptoms. 0 = no visible symptoms, 1 = very mild curling of up to 25% leaves of the total plant, 2 = curling, puckering of 26-50% leaves of the total plant, 3 = severe curling, puckering of 51-75% leaves of the total plant, 4 = very severe curling, puckering of 76-100% leaves of the total plant. In all the genotypes two classes, resistant (score 0) and susceptible (score l-4) were made for the inoculation studies (Banerjee and Kalloo, 1987a).

2.1.3 Morphology of ToLCV

The virus has a characteristic geminate particle (20 x 30 nm) made of two incomplete icosahedra and circular single stranded (ss) DNA encapsulated by viral coat protein. The genome of isolates from Israel, Sardinia, Egypt and Spain are monopartite (Crespi *et al.*, 1995) while those from Thailand are bipartite (Rochester *et al.*, 1994). Also, the isolates from Sudan and Australia (TLCV) are different from TYLCV (*Tomato Yellow Leaf Curl Virus*) isolates prevailing in other places (Dry *et al.*, 1993).

Gemini (Gemini=twins) viruses are characterized in having a genome of circular single stranded DNA contained in geminate particles that typically measure about 30 nm x 20 nm (Muniyappa *et al.*, 1991). The genome of the south Indian strain is monopartite (Muniyappa *et al.*, 1991) while that from north India is bipartite (Papidam *et al.*, 1995) also the Indian isolate (ToLCV) is different from the TYLCV isolates prevailing in other places (Muniyappa *et al.*, 1991).

2.1.4 Host range of ToLCV

Whitefly transmitted gemini viruses generally have a narrow host range among different cotyledonous plants and ToLCV is not an exception (Francki *et al.*, 1991).

In this respect, plants from six botanical families have been found to be host of ToLCV *viz.*, Asclepiadaceae, Compositae, Legumonosae, Malvaceae, Solanaceae and Umbelliferae (Cohen and Antignus, 1994).

2.1.5 Transmission of virus

The virus is naturally transmitted only through the tobacco whitefly (*Bemisia tabaci* Genn.). *Bemisia tabaci* occurs as a series of biotypes that have different geographical distributions, which differ in their ability to transmit the virus (Bedford *et al.*, 1994). The mobility of the Gemini viruses from one host to another depends on the mobility of their vector, determined by vector-host compatibility (Brown, 1994). Recently appearance of B biotype of greater fecundity, strong pesticide resistance and a broad host range has increased the importance of geminivirus infections, broadening the range of infected crops (Bedford *et al.*, 1994). Frequency of transmission depends on the whitefly culture and the virus isolates (Mc Grath and Harrison, 1995).

Whitefly *(Bemisia tabaci* Genn.) is proved to be the sole vector responsible for ToLCV transmission (Vasudeva and Samraj, 1948; Channarayappa *et al.*, 1992 and Nagaraja, 1995). White fly is tiny insect with piercing and sucking type of mouthparts belonging to the family Aleurodidae and order Homoptera.

2.1.6 Biology of the vector

The activity of the adult whitefly is influenced by temperature, light and rainfall (Leuschener, 1978). The life cycle of whitefly lasted for 17-32 days from August-March. The longest life cycle noticed was 39 days during December and shortest being the 11 days in April. The longevity of the adult whitefly is prolonged during winter and reduced during summer season. There is rapid multiplication of whitefly during April to October when the average maximum temperature ranges from 12° to 35°C (Butler *et al.*, 1983). The optimum RH for insect development is between 30-60 per cent. Rain, extreme temperatures and low humidity can impair oviposition.

Saikia (1985) reported a positive correlation between whitefly population and temperature and negative association with relative humidity. The reduction in population of whitefly during the cooler part of the year may be attributed to the influence of temperature rather than humidity.

2.1.7 Loss due to ToLCV and its incidence :

ToLCV infection results in severe yield losses often reaching upto 100 per cent particularly when the infection occurs before flowering (Polston *et al.*, 1994). Size reduction of fruits due to early infection affects fruit quality. The incidence, severity and spread of the disease have seasonal variations significantly correlated with fluctuations in the vector population (Cohen *et al.*, 1988). Under adequate conditions of disease spread, it reaches epidemic proportions leading to abandonment of cultivated fields in many regions (Abou *et al.*, 1995). In Mediterranean regions ToLCV incidence is very severe in late summer and autumn crops.

The studies of Saikia and Muniyappa (1989), which were carried out in the same region, revealed that, 90-100 per cent of plants were infected in plots sown between February and end of May. They also noted that during July to November, the low incidence of ToLCV was due to fall in whitefly population brought about by low temperatures. Several other workers (Banerjee and Kalloo, 1987a) also reported 100 per cent ToLCV incidence during summer season.

2.1.8 Screening and Inheritance of resistance to ToLCV

Resistance to tomato leaf curl virus is reported in the following *Solanum* species.

Solanum lycopersicon

Friedmann *et al.*, (1998) have worked on the inheritance of resistance in the resistant (R) line TY172. It was crossed with a susceptible (S) female parent L 27. F_1s exhibited mild symptoms with low viral content compared to L 27 and the F_2 population segregated in 7 symptom less (SL): 64 susceptible (S) ratio and the back cross progenies (F_1 x TY172) were symptom less or exhibited mild symptoms indicating resistance to *ToLCV* in TY172 to be partially dominant. When infected scions were grafted on to healthy susceptible and resistant root stocks, the viral DNA concentration in L 27 after 10 days of inoculation was around 50 per cent while in TY172 it was around 10 per cent even after three months of inoculation. Thus TY172 is a symptom less carrier and not a resistant line. This confirmed the findings of Lapidot *et al.*, (1997).

Solanum peruvianum

Pilowsky and Cohen (1990) from an interspecific cross between *L. peruvianum* line M-60 (resistant) and *L. esculentum* line 10 (susceptible) identified that resistance to

ToLCV in the Line M-60 is controlled by recessive gene action as all F_1s and BC1s were susceptible and F_2 and BC_2 segregated in 1:1000 and 1:31 (Tolerant: Susceptible) ratio respectively.

Solanum pimpinellifolium

Kasrawi (1989) has given the inheritance of resistance to *ToLCV* in resistant lines Hirsute-INRA and LA 1478. These were crossed with a susceptible line Special Back. F_2 and back cross populations of both the resistant parents segregated in the typical 3:1 and 1:1 ratios clearly indicating single dominant gene inheritance operating in ToLCV resistance.

Resistance in the line LA 1582 appears to be governed by a single dominant gene since all its F_1's with the susceptible female parent VF 134-1-2 were symptom less and the F_2 generation segregated in 3 (Resistant): 1 (Susceptible) ratio (Yassin, 1985).

Solanum hirsutum

Crosses between LA 386 (resistant) and VF 145-B-787 (susceptible) were made by Hassan *et al.*, (1984), all the F_1s were resistant suggesting that resistance is dominant but, the segregating populations (F_2 and BCs) exhibited varied responses indicating the effect of more than one gene (modifiers).

Solanum cheesmanii

Hassan *et al.*, (1984) have also given the genetics of resistance to ToLCV in the line LA 1401. It was crossed with a susceptible line UC 82 and out of the 59 F_2 plants screened, 6 produced no symptoms, 16 exhibited slight symptoms, 14 had moderate symptoms and 24 produced severe symptoms suggesting resistance to be controlled by recessive genes.

Solanum chilense

Pico *et al.*, (1999) have worked out the inheritance of resistance to ToLCV in four lines (LA 1932, LA 1938, LA 1960 and LA 1971) by crossing them with susceptible *L. esculentum* line NE 1. All the F_1s were tolerant with or without exhibiting symptoms and with no to very low virus accumulation indicating resistance to be dominant in these lines.

According to Banerjee and Kalloo (1987b) the inheritance of resistance to *tomato leaf curl virus* (ToLCV) was studied in the progenies derived from interspecific crosses between ToLCV resistant *Lycopersicon hirsutum f. glubratum* line B 6013 and five susceptible cultivars (HS 101, HS 102, HS 110, Pusa Ruby and Punjab Chhuhara) of *L. esculentum*. P1, P2, F_1, F_2, B_1 and B_2 progenies of the five crosses were artificially inoculated with local strains of ToLCV by means of the vector whitefly, *Bemisia tabaci* and the disease reaction was studied in all the crosses. Reaction of parents, F_1, F_2 and backcrosses suggests that resistance derived from *L. hirsutum f. glubratum* B 6013 is based on two epistatic genes, one from the wild parent and one from the cultivated one, resulting in a 13: 3 segregation in the F_2.

Zakay *et al.*, (1991) screened twenty three tomato accessions to tomato leaf curl virus under field conditions and examined that accessions of wild species *Lycopersicon pimpinellifolium*, *Lycopersicon hirsutum and Lycopersicon peruvianum* showed variance in their response to infection, however *Lycopersicon chilense* showed highest degree of resistance against the disease.

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

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

The resistant line 902 developed from accessions LA1777 and LA386 of the wild tomato species *L. hirsutum* was used to develop the new resistant lines referred to as "Favi". Line 902 was a stable line that is resistant to TYLCV from Israel, had *L. esculentum* plant morphologies, and was self-compatible. Subsequent crosses between Line 902 and a very prolific and large size tomato line but susceptible to TYLCV resulted in the hybrid Favi-9. Favi-9 was resistant to TYLCV-Is. Six resistant tomato lines, Favi -21, Favi -22, Favi -23, Favi -24, Favi -25 and Favi -27 were derived from the hybrid Favi -9. All the Favi lines and were found to be resistant to TYLCV in Israel (Vidavsky and Czosnek, 1998).

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

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

Rai *et al.,* (2001) screened twenty genotypes for resistance against tomato leaf curl virus (ToLCV) in Madhya Pradesh, India and reported that the cultivar Hisar Anmol and Hisar Gaurav were resistant to tomato leaf curl disease.

Sajeed *et al.,* (2002) screened ten tomato cultivars against ToLCV at 45 days after planting and observed that among all the cultivars Punjab Chhuhara showed higher degree of resistance against tomato leaf curl virus.

Maruthi *et al.,* (2003) screened a total of thirty-four tomato genotypes for resistance to ToLCV under glasshouse and field conditions and found that Lycopersicon hirsutum LA 1777 and PI 390659 were best sources of resistance to the virus.

Four tomato lines introgressed from *Lycopersicon chilense* were compared with the commercial F1 hybrids 'ARO 8479' and 'HA 3108', which are tolerant to Tomato yellow leaf curl virus, and the cv. 'Campbell 28' as a susceptible control. Resistance was evaluated by the use of grafted diseased scions as well as in a field trial where plants infected by viruliferous whiteflies and disease-free plants were transplanted in paired rows. The new lines LD 3, LD 4, LD 5 and LD 6 showed no disease symptoms after grafting or in the field trial (Gomez *et al.,* 2004).

Thirty-two hybrids were produced and evaluated along with ToLCV tolerant commercial hybrids (Mruithyunjaya-2, Sasya 9449 and Laxmi) during summer 2005. Of the 32 hybrids tested, 16 hybrids showed resistant reaction to ToLCV. Four hybrids *viz.*, Sankranthi x PKM-1, Sankranthi x Arka Meghali, LCR-9 x Vaibhav and Vaibhav x PKM-1 were found more promising with respective to resistance, yield and other horticultural characters (Shankarappa *et al.,* 2006).

Yadav and Awasthi (2009) screened twenty-two cultivars of tomato against ToLCV in Faizabad and out of twenty-two cultivars screened, none of the cultivar was found resistant against the disease. However Hisar anmol was found moderately resistant to the virus, while three cultivars were categorized as moderately susceptible and eighteen were found susceptible to tomato leaf curl virus.

Anjali (2007) found that Hawaii 7998, H-24, H-86, LE-474, LE-640 and LE-658 were completely free from ToLCV incidence.

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

Sadashiva *et al.,* (2007) screened the reported sources of resistance to ToLCV against Tomato Leaf Curl Bangalore Virus (ToLCBV) which is most prevalent in South India. Eight tomato lines *viz;* IIHR-2101 (*Lycopersicon hirsutum* LA-1777), IIHR-2195, IIHR-2205, IIHR-2406, IIHR-2413, IIHR-2611 and two *L. peruvianum* accessions (IIHR-1943 & IIHR-1970) were found to be resistant to ToLCBV.

Shankarappa *et al.,* (2008) developed hybrids by crossing three varieties Sankranthi, Nandi and Vaibhav (which are resistant to ToLCV) with 12 tomato genotypes with superior agronomic characteristics. From those selected 20 hybrids (named BLRH-1 to BLRH-20, Bangalore leaf curl virus-resistant hybrid) which are and evaluated for their resistance to ToLCV. Of the 20 hybrids evaluated, 11 were found resistant to ToLCV in the field, but only three (BLRH-3, BLRH-9 and BLRH-16) remained resistant when challenged with high virus inoculum pressure in the glasshouse through whitefly-mediated inoculations.

The screening of tomato germplasm against ToLCV was carried out in Ghana by Osei *et al.,* (2012) evaluated thirty accessions against the disease under field conditions at 30, 45 and 60 days after transplanting and found that no accessions provided complete resistance to tomato leaf curl virus.

Singh (2014) screened thirty-two genotypes for resistance against tomato leaf curl disease during rabi season at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. It was observed that one wild accession, H-88-78-1 showed immune reaction against ToLCV, three genotypes *viz.*, Hissar lalima, TLBRH-6 and NS-515 showed resistant reaction and eight genotypes *viz.,* Hissar Anmol, Kishi Vishesh, Kashi Amrit, Kashi Sharad, KS-17, KS-118, Avinash-2 and US-1008 were found moderately resistant against ToLCV.

Zeshan *et al.,* (2016) screened twenty-seven tomato varieties/lines for the source of resistance against tomato leaf curl virus disease (ToLCV) under field conditions and

found that three varieties were highly susceptible, six were susceptible, four were moderately susceptible. No variety/line was highly resistant or immune against tomato leaf curl virus disease.

2.1. 9 Confirmation of ToLCV resistance by Grafting:

Som and Choudhary (1976) examined for resistance source by growing plants in summer using *Bemisia tabaci* for inoculation and graft transmission.

Hassan *et al.,* (1984) employed vector inoculation graft transmission and progeny tests for screening ToLCV resistant lines.

Sankari *et al.,* (2002) screened 36 F_1 hybrids and 13 parents of tomato for their resistance to tomato leaf curl virus (ToLCV) by graft inoculation under glasshouse conditions. The hybrids FLCR5 x MLCR4 and FLCR5 x MLCR1 and the parents FLCR1, FLCR3, FLCR5, MLCR4, MLCR5 and MLCR6 recorded the lowest disease incidence.

Ahmed (2014) investigated possible positive effects of grafting and use of different TYLCV resistant rootstocks on the tolerance/resistance level and tomato fruit yield and quality. Tomato cvs used as scions were TYLCV-susceptible cv. Castlerock and TYLCV-tolerant hybrid cv. TH99806 (Nirouz). The rootstocks were TYLCV resistant accessions *Solanum chilense* LA2779, *S. habrochaites* LA1777 and *S. pennellii* LA716 and TYLCV-susceptible *S. lycopersicum* CGN14330 cv. and confirmed that grafting increased TYLCV tolerance in susceptible plants, expressed as delay in the appearance of TYLCV symptoms and an increase of yield components compared to non-grafted plants.

2.2 MOLECULAR MARKERS LINKED TO TOMATO LEAF CURL VIRUS RESISTANCE GENE

Zamir *et al.*, (1994) reported that the wild tomato species *Lycopersicon chilense*, which was resistant to the virus, was crossed to the cultivated tomato, *L. esculentum*. The backcross-1 selfed (BC_1S_1) generation was inoculated and a symptomless plant was selected. That plant was analyzed using 61 molecular markers, which span the tomato genome, to determine which *L. chilense* chromosome segments were introgressed. A TYLCV-tolerance gene with partial dominance, *Ty-1*, was mapped to chromosome 6; two modifier genes were mapped to chromosomes 3 and 7. Field and whitefly-mediated

cage inoculations of nearly isogenic lines in BC_3S_3 supported the conclusion that *Ty-1* is the major TYLCV-tolerance locus.

According to Chague *et al.*, (1997) in tomato, Bulked Segregant Analysis was used to identify Random Amplified Polymorphic DNA (RAPD) markers linked to a quantitative trait locus (QTL) involved in the resistance to the Tomato Yellow Leaf Curl Virus. F_4 lines were distributed into two pools, each consisting of the most resistant and of the most susceptible individuals, respectively. Both pools were screened using 600 random primers. Four RAPD markers were found to be linked to a QTL responsible for up to 27.7 per cent resistance. These markers, localized in the same linkage group within a distance of 17.3 cM, were mapped to chromosome 6 on the tomato RFLP map.

According to Brenda *et al.*, (2007), two sets of primers, T0302F/T0302R and T0302F/TY2R1, effectively detected the two genotypes, ty2/ty2 and Ty2/Ty2, and the T0302F/TY2R1 primer set also gave clearer bands with the heterozygous plants than the T0302F/T0302R primers. No false positives were detected, when 59 inbred lines and hybrids were evaluated. But it is possible that this marker might not detect all lines that have the *Ty2* gene, since it is not known how closely linked this marker is to the *Ty2* gene.

De Castro *et al.*, (2007) reported in the breeding programme that several resistance genes have been introgressed into tomato (*Solanum lycopersicum*) cultivars from different wild tomato relatives. A number of these resistance genes have been mapped to chromosome 6. Among them, *Ty-1* and *Mi*, which confer resistance to Tomato yellow leaf curl disease and to Meloidogyne *spp.*, respectively, are in most cases incorporated in commercial hybrids. The study was conducted in order to find an informative molecular marker linked to *Ty-1*. One allele of *JB-1* marker showed association with *Ty-1*. Furthermore this analysis enabled the location of CT21, the RFLP marker from which *JB-1* was designed.

Ji *et al.*, (2007) said that resistance to begomoviruses including bipartite Tomato mottle virus (*ToMoV*) and monopartite *Tomato yellow leaf curl virus* (TYLCV) has been introgressed to cultivated tomato (*Solanum lycopersicum*) from *S. chilense* accessions LA 1932, LA 2779, and LA 1938. A begomovirus resistance locus, *Ty-3*, was mapped to the marker interval between cLEG-31- P16 and T1079 on the long arm of chromosome 6. In addition to the *Ty-3* locus, the large introgression also spans the

Ty-1 region near the *Mi* gene, suggesting the possible coexistence and linkage of resistance alleles at both *Ty-1* and *Ty-3* loci in these lines. In contrast, LA 1932 derived advanced breeding lines possess a much shorter introgression from cLEG-31-P16 to C2_ At5g41480, which also carries a begomovirus resistance locus that is probably allelic at the *Ty-3* locus.

Barbieri *et al.*, (2008) reported that the study is focused on the development of traditional Italian varieties of tomato resistant to *TYLCD*. In order to investigate the effectiveness of two of such resistance loci, they screened lines LA3473 and H24, carrying respectively *Ty-1* and *Ty-2* genes, against TYLCD isolates collected in tomato production regions in the south of Italy. *Ty-1* gene has shown to provide tolerance to TYLCSV isolate whereas *Ty-2* has proven to be fully effective against TYLCV isolate. Two CAPS markers linked to each gene, TG178 and TG436 for *Ty-1*, TG105A and C2_At5g25760 for *Ty-2*, were screened for their utility in marker-assisted breeding programs. F_2 populations from crosses between resistant and susceptible lines were marker analyzed and selected F_3 progenies were phenotyped for their resistance.

According to Anbinder *et al.*, (2009) the breeding line TY172, originating from *Solanum peruvianum*, is highly resistant to TYLCV. To map quantitative trait loci (QTLs) controlling TYLCV resistance in TY172, appropriate segregating populations were analyzed using 69 polymorphic DNA markers spanning the entire tomato genome. Results showed that TYLCV resistance in TY172 is controlled by a previously unknown major QTL, originating from the resistant line, and four additional minor QTLs. The major QTL, which they termed *Ty-5*, maps to chromosome 4 and accounts for 39.7–46.6 per cent of the variation in symptom severity among segregating plants (LOD score 33–35). The minor QTLs, originated either from the resistant or susceptible parents, were mapped to chromosomes 1, 7, 9 and 11, and contributed 12 per cent to the variation in symptom severity in addition to *Ty-5*.

Hilal *et al.,* (2009) concluded in the study that, F_3 plants originated from 11 F_2 populations (individual numbers varied from 10 to 14 for each population, a total of 131 individuals) (*Lycopersicum esculentum*) were screened for resistance to *Tomato yellow leaf curl virus* (TYLCV) using Random Amplified Polymorphic DNA (RAPD) and Cleaved Amplified Polymorphic Sequence (CAPS) marker techniques. After DNA extraction from plants, CAPS primers were applied and screened for primer annealing

of gene locus. Out of 131 plants, 120 plants were detected containing gene locus. After that, the amplicons, obtained from PCR with CAPS primers (REX-F1 and REX-R3), were digested with *TaqI* restriction endonuclease enzyme to identify whether the lines carrying resistance gene is homozygous or heterozygous.

Ji *et al.*, (2009) reported that they have identified a 14-cM *S. chilense* introgression on the long arm of chromosome 3 in some resistant breeding lines derived from LA1932. A new begomovirus resistance locus, *Ty-4*, was mapped to the 2.3-cM marker interval between C2_At4g17300 and C2_At5g60160 in the introgression. Analysis of a population segregating for *Ty-3* and *Ty-4* demonstrated that *Ty-3* accounted for 59.6 per cent of the variance, while *Ty-4* only accounted for 15.7 per cent, suggesting that *Ty-4* confers a lesser effect on TYLCV resistance. Recombinant inbred lines (RILs) with *Ty-3* and *Ty-4* had the highest level of TYLCV resistance. The PCR based markers tightly linked to the *Ty-4* locus as well as the *Ty-3* locus have been recently used in breeding program for efficient selection of high-levels of begomovirus resistance and now allowed for efficient breeding by marker-assisted selection.

Shamprasad (2010) screened and validated three molecular markers *Ty1*, *Ty2* and *Ty3* linked to ToLCV resistance and confirmed genotypes two advanced breeding lines IIHR-2822 and IIHR-2823 showed the presence of the all three genes *Ty1, Ty2* and *Ty3* for ToLCV resistance, the wild accession *S. habrochaites* LA 1777 (IIHR-2101) showed the presence of two genes *Ty2* and *Ty3,* Abhinava showed the presence of *Ty1* gene and Hisar Anmol (H-24), Vaibhav, Arka Ananya, Lakshmi, NS-501 showed the presence of only *Ty2* gene.

2.3 MORPHOLOGICAL VARIABILITY AND FRUIT QUALITY PARAMETERS IN TOMATO

2.3.1 Yield parameters

Blay *et al.*, (1999) studied the morphological and agronomic characteristics of eight tomato accessions and a high variability was detected in plant height at flowering, fruit set, number of fruits plant $^{-1}$, fruit weight, number of locules fruit $^{-1}$ and yield.

Parthasarathy and Aswath (2002) evaluated twenty three genotypes of tomato during summer rainy season. There was considerable diversity among genotypes for morphological characters *viz.*, Plant height, number of fruits and fruit size contributing

to the divergence. *S. Pimpenellifolium* was the most divergent among genotypes. Crosses involving IIHR-1872, Pant Bahar, L-964 and L-154 with Arka Alok, Arka Abha, Floradude and LE-79 were recommended for improvement of yield and better size.

Hamid and Salih (2010) conducted a field experiment in two tomato cultivars like Peto 86 and Red Star. The results revealed that, the variety Red Star had plant height (53.70 cm), No. of Branches/ Plant highest (19.19) a Days to first fruit set (49.54), No. of fruits/ plant (71.46), Days to first fruit set (46.86), No. of fruits/ plant (45.75), Fruit size (5.01 cm), fruit weight (62.517) and highest yield. The highest plant density (71. 42 plant/ha) gave the highest and marketable yield. Also concluded sowing at October 1st increased the productivity of tomato as it positively influenced the plant height, days to 50 per cent flowering, fruit yield and marketable yield.

Shankar *et al.*, (2013) used twenty four hybrids along with eleven parents to study the genetic variability and recorded mean of following parameters Plant height (153.63 48.33 cm), No. of primary branches/ plant (10.60-5.33), No. of fruits/ cluster (3.60-1.17), Average fruit Wt. (105.53-40.20 g) and Yield/ plant (1-3.90 kg/ plant).

Reddy *et al.*, (2013) evaluated nineteen Tomato genotypes. The genotypes exhibited a wide range of variability for all the characters studied *viz.*, like Plant height (138.12 cm), Number of primary branches plant^{-1} (30.14), Days to 50 per cent flowering (51.667), Days to first fruit set (57.25), Days to first fruit harvest (98.16), Number of fruits plant^{-1} (74.18), Fruit weight (102.33 g), Fruit yield (2.72 Kg/plant). Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters. High heritability combined with high genetic advance was observed.

2.3.2 Fruit quality parameters

The various components of tomato fruit quality *viz.,* total soluble solids, lycopene, ascorbic acid, total titratable acidity and pH are essential in relation to specifications for grades and standards, selections in breeding programs and evaluation of fruit responses to various environmental factors (Kader *et al.*, 1986). Plant breeders in collaboration with physiologists should continue to select genotypes that have good

flavour (*i.e.*, high total soluble solids and acid contents and good potential for development of volatiles associated with desirable tomato flavour). Work on improving the nutritional quality of tomatoes via increased ascorbic acid content should be an integral part of tomato improvement programmes.

The accumulation of soluble sugars in ripe tomato fruit is perhaps the primary determinant of fruit quality and taste, together with the additional taste components that include acids and volatiles among primary and secondary metabolites (Davies and Hobson, 1981 and Grierson and Kadar, 1986).

Berry *et al.*, (1988) investigated stability and variation of fruit yield, soluble solids and citric acid content of eight tomato cultivars over six years. Cultivars Ohio-7814 exhibited above average yield and yield stability. The cultivars showed a wide range of variation for percent total soluble solids and citric acid.

Blay *et al.*, (1999) found variations in tomato with respect to percent total soluble solids (3.9-5.0 0 Brix) and pH (3.9-4.4).

Lycopene is a carotenoid that is present in tomatoes, processed tomato products and other fruits. It is one of the most potent antioxidants among dietary carotenoids. Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with a decreased risk of chronic diseases, such as cancer and cardiovascular disease (Agarwal and Rao, 2000).So for fresh market as well as for processing purpose, the lycopene content of tomatoes should be high.

Lycopene content was found to be in the range of 80.27 mg/100 g to 120.67 mg/100 g of fresh tomato harvested in northern California (Takeoka *et al.*, 2001).

Lycopene extracted from tomato varied from of 150 to 250 mg/kg (Rath and Math., 2001).

Tomato fruits with high total soluble solids, pH less than 4.5, high ascorbic acid, lycopene and total acid content were preferred for processing purpose (Bose *et al.*, 2002). While the fruits usually red but vary in colour, less total soluble solids and total titrable acidity are preferred for fresh market purpose. The tomato fruits with high lycopene content are used both for processing and table purpose.

Dewanto *et al.*, (2002) reported that, the lycopene concentration in the raw tomato slurry ranged between 31 mg/100 g to 67 mg/100 g of tomato.

The different quality parameters like total soluble solids, total titrable acidity, pH, ascorbic acid and lycopene content were studied by Prashanth (2003) for different tomato genotypes. He observed that the total soluble solids ranging from 3.19° Brix to 5.83° Brix, total titratable acidity from 0.21 per cent to 0.70 per cent and pH ranged from 4.07 to 5.33. While, ascorbic acid content of tomato fruits ranged from 9.37 to 22.85 mg/100 g and lycopene content ranged from 4.43 to 17.78 mg/100 g.

Different extraction methods also cause differences in the lycopene level even in the same sample of tomato. Periago *et al.*, (2004) showed best extraction yield was obtained by extraction using a mixture of hexane, acetone, and methanol solvent.

Ashwini (2005) evaluated parents and hybrids for total soluble solids and pH content of tomato. She observed that, the total soluble solids content of fruits from parents ranged from 2.91 to 4.96° Brix, while for hybrids it ranged from 3.15 to 5.34° Brix. Whereas, pH content for parents ranged from 3.21 to 4.34 and for hybrids from 3.04 to 4.89.

Toor *et al.*, (2004) studied the major antioxidants and antioxidant activity in different fractions (skin, seeds and pulp) of three tomato cultivars (Excell, Tradiro and Flavourine). It was found that the skin fraction of all cultivars had significantly ($p < 0.05$) higher levels of total phenolics, total flavonoids, lycopene, ascorbic acid and antioxidant activity compared to their pulp and seed fractions. The amount of antioxidants in each fraction was calculated on the basis of their actual fresh weights in whole tomato and it was found that the skin and seeds of the three cultivars on average contributed 53% to the total phenolics, 52% to the total flavonoids, 48% to the total lycopene, 43% to the total ascorbic acid and 52% to the total antioxidant activity present in tomatoes.

Choudhari and Ananthanarayan (2006) used a cellulase and pectinase enzymes to extract the lycopene from the tomato. Enzyme aided extraction of lycopene from whole tomatoes under optimised conditions resulted in an increase in the lycopene yield by 132 ng/g (198%) in cellulase treated sample and 108 ng/g (224%) in case of

pectinase treated sample. Extraction from tomato peel under optimised conditions showed a remarkable increase in the yield of lycopene by 429 ng/g (107%) and 1104 ng/g (206%), for cellulase and pectinase treated samples respectively.

Collins and Veazie (2006) reported that the lycopene is a pigment that imparts a red orange to some fruits and vegetables. This carotenoid studied over the last ten years because of its antioxidant activity and medical evidence that dietary intake can reduce the incidence of cardio-vascular disease and cancers.

Javanmardi and Kubota (2006) reported the TSS and lycopene content ranging from 5.0-5.1° Brix and 48-68 mg/kg respectively during storage.

The quality parameters like ascorbic acid, total titratable acidity, per cent juice recovery and total soluble solids were studied by Kulkarni (2006) for seven parents and twenty one hybrids. The ascorbic acid content ranged from 16.42 to 27.00 mg/100 g in parents and 3.92 to 17.08 mg/100 g in hybrids. While, the total titratable acidity ranged from 0.27 to 0.43 for parents and 0.27 to 0.44 mg/100 g for hybrids. Per cent juice recovery ranged from 27.92 to 39.5 per cent for parents and 19.37 to 40.67 per cent for hybrids. While, the total soluble solids ranged from 3.47 to 6.23° Brix and 4.00 to 6.37° Brix in parents and hybrids, respectively.

Lopez *et al.*, (2007) in a study, observed that the lycopene content of three different tomato cultivars *viz.*, Bodar, Cherry and Cocktail showed a lycopene content range in between 37- 51 mg/kg. Significant differences were found among cultivars.

Revanasiddappa (2008) evaluated F_1, F_2 and F_3 populations for fruit quality parameters during a study. Total soluble solids, ascorbic acid and total titratable acidity observed range was 4.40° Brix, 19.09 mg/100 g and 0.56 per cent, respectively.

Radzevicius *et al.,* (2009) used edible tomato cultivars to evaluate the best cultivar for fruit quality. The lycopene content was higher in cultivars Rani 310 (13.56 mg/100 g) and Elbrus (12.57 mg/ 100 g).

Laleye *et al.,* (2010) evaluated the lycopene content in fifteen varieties of tomatoes. In addition, three brands of tomato paste, three brands of ketchup and three brands of tomato hot sauce were evaluated for lycopene content. The lycopene content in different varieties of tomatoes analysed by spectrophotometry and HPLC methods ranged from < 0.05 to 5.82 mg/100 g, and from 0.01 to 4.90 mg/100 g respectively, whereas the lycopene content in the imported unknown tomato varieties ranged from 2.40 to 5.98 mg/100 g (spectrophotometry) and from 1.78 to 5.46 mg/100 g (HPLC). The analysis of variance using SPSS software indicated a highly significant ($P \leq 0.05$) variation in the total lycopene content between the fifteen varieties tested. Similar variation was observed in the pH and conductivity of the samples. The lycopene content in some processed tomato products ranged from 4.57 to 14.48 mg/ 100 g (spectrophotometry) and from 4.13 to 13.82 mg/100 g (HPLC).

Aghel (2011) reported that lycopene is a pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and products. Lycopene, as a natural source of antioxidants, has attracted attentions due to its biological and physicochemical properties.

Gupta *et al.*, (2011) reported two different hybrids like Hisar Arun and ARTH-3.Shown different amount of ascorbic acid, lycopene and beta-carotene (31.33 and 27.82, 3.12 and 4.03, 5.90 and 6.78 mg per 100 g in raw tomatoes respectively).

Naz *et al.*, (2011) found maximum TSS in cultivar 'Avinash' (5.50 Brix) followed by Yaqui (5.40 Brix) whereas it was found to be minimum in Roma (4.90 Brix) cultivar. 'Lyreka' had the most abundant ascorbic acid (16.03 mg/100 g) followed by 'Rio Grand' (15.86 mg/100 g). The highest titratable acidity was found in 'Yaqui' (0.38%) while 'Rio Grand' had the lowest (0.31%) in this respect.

Shankar *et al.*, (2013) found a range of TSS (5-3.170 Brix), Vit-C (40.67- 14.67 mg/100 g) and Lycopene content (11.73-2.07 mg/100 g) in twenty four hybrids along with their eleven parents.

Reddy *et al.*, (2013) evaluated nineteen tomato genotypes with varied quantity of Ascorbic acid (37.46 mg/100 g), Acidity (0.87%), TSS (10.35° Brix), Shelf life (34.50 days).

2.3.3 Genetic Variability

The magnitude of variability and its genetic components are the most important aspects of breeding material. Hence, basic understanding of the genetic variability is a pre requisite for the planning of breeding programme. The variability available in the population can be partitioned into heritable and non- heritable components using the genetic parameters *viz.,* phenotypic and genotypic coefficients of variation, heritability and genetic advance on which selection can be effectively carried out.

Hanson *et al.,* (1956) proposed heritability as the ratio of genotypic variance to the total variance in a non-segregating population. Heritability (h^2) measures the relevant amount of heritable portion of variability, while. Genetic advance is the measure of improvement that can be achieved by practicing selection in a population. Therefore, the components of variance, heritable components with genetic parameters such as GCv, PCV, heritability and genetic advance as percent mean are important tools of plant breeding. Literature pertaining to these aspects are summarized and presented in table 1.

2.3.3.1 Genotypic and phenotypic coefficient of variation

Variability in tomato germplasm has been investigated by number of research centers, usually as a first step in plant breeding. Variability studied is reviewed in Table 1.

Heritability

Heritability of the quantitative and quality characters studied is reviewed in Table 1.

Genetic advance

Genetic advance for the quantitative and qualitative characters studied is reviewed in Table 1.

Table 1. Summary of review of literature on variability, heritability and genetic advance in tomato by different authors

Character	Coefficient of variation		h^2 (%)	GA (%)	References
	GCV	PCV			
Plant height (cm)	H	H	H	H	Nandapuri *et al.*, (1977)
	L	M	M	L	Singh *et al.*, (1988)
	H	H	H	H	Bora *et al.*, (1993)
	H	H	H	H	Kumari and Subramanian (1994)
	L	M	M	L	Sahu and Mishra (1995)
	L	H	L	H	Narendra Kumar and Arya (1995)
	H	H	H	H	Anandgowda (1997)
	M	H	H	H	Mala and Vadivel (1999)
	H	H	H	H	Mohanty (2003)
	M	M	M	H	Arun *et al.*, (2003)
	L	M	L	L	Prashanth (2003)
	M	H	M	H	Aradhana and Singh (2003)
	M	H	M	H	Veershetty (2004)
	H	H	H	H	Akhilesh and Gulshanlal (2005)
	H	H	H	H	Singh (2005)
	H	H	M	M	Upadhayay *et al.*, (2005)
	M	M	H	H	Dhankar and Dhankar (2006)
	H	H	H	H	Samadia *et al.*, (2006)
	H	M	L	H	Kumar *et al.*, (2006)
	H	H	H	H	Kumari *et al.*,(2007)
	L	M	-	-	Mehta *et al.*, (2007)
	M	H	H	H	Kumar and Thakur (2007)
	H	H	H	H	Asati *et al.*, (2008)
	M	M	-	H	Ara *et al.*, (2009)
	H	H	H	H	Singh (2009)
	L	L	L	M	Prema *et al.*, (2011)

Table 1. Cont.

Character	Coefficient of variation		h^2 (%)	GA (%)	References
	GCV	PCV			
Number of primary branches	M	-	H	H	Paranjothi and Muthukrishnan (1979)
	L	M	M	-	Reddy and Gulshanlal (1987)
	H	H	H	H	Singh *et al,.* (1988)
	M	M	M	L	Kumari and Subriamaniam (1994)
	L	M	M	L	Sahu and Mishra (1995)
	L	H	L	H	Narendra Kumar and Arya (1995)
	H	H	H	H	Anandgowda (1997)
	M	H	H	H	Mala and Vadivel (1999)
	H	H	H	H	Mohanty (2003)
	L	M	L	L	Prashanth (2003)
	M	H	M	H	Aradhana and Singh (2003)
	H	H	H	H	Akhilesh and Gulshanlal (2005)
	H	H	H	H	Singh (2005)
	H	M	L	H	Kumar *et al.,* (2006)
	H	H	H	H	Kumari *et al.,* (2007)
	L	M	-	-	Mehta *et al.,* (2007)
	H	H	H	H	Asati *et al.,* (2008)
	M	M	-	H	Ara *et al.*, (2009)
	H	H	H	H	Singh (2009)
	L	L	L	M	Prema *et al.,* (2011)
Days to 50% flowering	H	H	H	M	Pujari *et al.*, (1995)
	L	L	H	M	Anupam *et al.*, (2002)
	L	L	L	L	Aradhana and Singh (2003)
	M	M	H	H	Prashanth (2003)
	L	M	M	M	Veershetty (2004)
	L	L	H	L	Singh (2005)
	H	H	H	M	Upadhayay *et al.*, (2005)
	M	M	M	L	Dhankar and Dhankar (2006)
	L	L	H	M	Samadia *et al.,* (2006)
	L	L	H	H	Prema *et al.*, (2011)
Days to first fruit harvest	L	M	H	M	Mohanty (2003)
	L	L	-	M	Ara *et al.*, (2009)

Table 1. Cont.

Character	Coefficient of variation		h^2 (%)	GA (%)	References
	GCV	PCV			
Number of fruits Plant^{-1}	M	H	L	H	Srivastava and Sachan (1973)
	H	H	H	M	Singh *et al.*, (1974)
	H	H	H	H	Prasad and Prasad (1976)
	H	H	H	H	Nandapuri *et al.*, (1977)
	H	H	H	H	Paranjothi and Muthukrishnan (1979)
	H	H	H	L	Bhutani *et al.*, (1983)
	M	H	L	M	Rattan *et al.*, (1983)
	H	H	H	M	Reddy and Gulshanlal (1987)
	L	H	L	L	Singh *et al.*, (1988)
	H	H	H	H	Bora *et al.*, (1993)
	H	H	H	H	Kumari and Subramanian (1994)
	H	H	H	H	Narendrakumar and Arya (1995)
	H	H	H	H	Pujari *et al.*, (1995)
	H	H	H	H	Sahu and Mishra (1995)
	H	H	H	H	Anandgowda (1997)
	H	H	H	H	Das *et al.*, (1998)
	H	H	H	H	Brar *et al.*, (1998)
	H	H	H	H	Mala and Vadivel (1999)
	H	H	H	H	Singh *et al.*, (2000)
	H	H	H	H	Anupam *et al.*, (2002)
	H	H	H	H	Prashanth (2003)
	H	H	H	H	Mohanty (2003)
	H	H	M	H	Aradhana and Singh (2003)
	H	H	H	H	Arun *et al.*, (2003)
	M	M	H	H	Singh (2005)
	H	H	H	H	Upadhyay *et al.*, (2005)
	H	H	H	H	Dhankar and Dhankar (2006)
	H	H	H	H	Samadia *et al.*, (2006)
	H	H	H	H	Kumari *et al.*, (2007)
	H	H	H	H	Asati *et al.*, (2008)
	H	H	-	H	Ara *et al.*, (2009)
	H	H	H	H	Singh (2009)

Table 1. Cont.

Character	Coefficient of variation		h^2 (%)	GA (%)	References
	GCV	PCV			
Fruit yield (kg/plant)	H	H	H	H	Srivastava and Sachan (1973)
	M	-	H	H	Singh *et al.*, (1974)
	H	H	H	H	Prasad and Prasad (1976)
	H	H	H	H	Paranjothi and Muthukrishnan (1979)
	H	H	H	H	Bhutani *et al.*, (1983)
	M	H	L	M	Rattan *et al.*, (1983)
	H	H	H	-	Reddy and Gulshanlal (1987)
	M	M	H	H	Singh *et al.*, (1988)
	H	H	H	H	Bora *et al.*, (1993)
	H	H	H	H	Kumari and Subramanian (1994)
	M	H	L	M	Narendra Kumar and Arya (1995)
	M	M	H	H	Pujari *et al.*, (1995)
	H	H	H	H	Sahu and Mishra (1995)
	H	H	H	L	Das *et al.*, (1998)
	H	H	H	H	Das *et al.*, (1998)
	H	H	H	H	Brar *et al.*, (1998)
	H	H	H	H	Mala and Vadivel (1999)
	M	M	H	H	Singh *et al.*, (2002)
	H	H	H	H	Prashanth (2003)
	H	H	H	H	Prashanth (2003)
	H	H	H	H	Akhilesh and Gulshanlal (2005)
	H	-	H	H	Arun Kumar and Veeraragavathatham (2005)
	H	H	H	H	Mayavel *et al.*, (2005)
	H	H	H	H	Samadia *et al.*, (2006)
	H	H	H	H	Kumari *et al.*, (2007)
	H	H	H	H	Kumar and Thakur (2007)
	H	H	H	H	Asati *et al.*, (2008)
	H	H	-	H	Ara *et al.*, (2009)
	H	H	H	H	Singh (2009)
	H	H	H	L	Prema *et al.*, (2011)

Table 1. Cont.

Character	Coefficient of variation		h^2 (%)	GA (%)	References
	GCV	PCV			
Vitamin C	M	M	H	H	Kumari *et al.*, (2007)
	H	H	H	H	Asati *et al.*, (2008)
	M	M	-	H	Ara *et al.*, (2009)
TSS (Brix)	L	L	H	M	Pradeep kumar and Tewari (1999)
	M	M	M	H	Prasad and Mathurarai (1999)
	L	M	L	L	Mala and Vadivel (1999)
	M	M	H	H	Aradhana and Singh (2003)
	L	M	M	M	Veershetty (2004)
	M	H	M	M	Akhilesh and Gulshanlal (2005)
	M	M	H	H	Arun kumar and Veeraragavathatham (2005)
	M	M	H	M	Kumari *et al.*, (2007)
	M	M	H	H	Kumar and Thakur (2007)
	M	H	-	H	Ara *et al.*, (2009)
	H	H	H	L	Prema *et al.*, (2011)
Shelf life	H	H	H	M	Prema *et al.*, (2011)

Where,

PCV and GCV: Phenotypic and genotypic coefficient of variation

h^2: Heritability

GA: Genetic Advance

Characterization of values		
PVC and GCV	**h^2**	**GA**
L: Low (< 10%)	L: Low (<30%)	L: Low (<10%)
M: Moderate (10-20%)	M: Moderate (30-60%)	M: Moderate (10-20%)
H: High (>20%)	H: High (>60%)	H: High (>20%)

2.3.3.2 Correlation coefficient analysis

Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for improvement in yield. Correlation studies provide information that the selection for one character will result in progress for all correlated characters. Simple correlations are of three types viz., phenotypic, genotypic and environmental. Phenotypic correlations is the observable correlation between variables, measures the environmental deviation together with non additive gene action. Genotypic correlation on the other hand is the inherent association between two variables.

The available literature on the association of various traits of tomato is presented in Table 2.

2.3.3.3 Path coefficient analysis

The study of simple correlation does not provide an exact picture of relative importance of direct and indirect influence of each of the component character towards the desired character. So, the plant breeder tries to partition the correlation coefficients into components of direct and indirect effects by employing the path coefficient analysis. Which involves measurement of influence of one trait upon the set of the other traits through standardized partial regression coefficient to increase the efficiency of selection.

Thorough review of literature pertaining to the direct and indirect effects of various components on Weight of fruits plant^{-1} (kg/Plant) is tabulated in Table 3 and 4.

Table 2. Summary of review if literature on association of component characters on yield and yield related components

Character	Nature of correlation	
	Positive	**Negative**
Plant height (cm)	Dudhi and Kalloo (1982), Rattan *et al.,* (1983), Patil (1998), Manivannan and Irulappan (1986), Sidhu and Singh (1989), Patil and Bojappa (1993), Raijadhav *et al.*, (1996), Aravindakumar and Mulge (2002), Tiwari (2002), Prashanth (2003), Joshi *et al.,* (2004), Lakshmikant and Mani (2004), Mayavel *et al.* (2005), Raut *et al.*, (2005), Ara *et al.*, (2009), Indurani *et al.,* (2010), Bernousi *et al.*, (2011)	Das *et al.,* (1998), Prasad and Mathura Rai (1999), Mohanty (2003), Dhankar and Dhankar (2006), Singh (2009)
Number of primary branches	Nardar *et al.,* (1980), Patil and Bojappa (1993), Manivannan and Iruppan (1986), Reddy and Gulshanlal (1987), Supe and Kale (1992), Raijadhav *et al.*, (1996), Anandgowda (1997), Rathod (1997), Patil (1998), Mohanty (2002), Aravindkumar and Mulge (2002), Prashanth (2003), Tiwari (2002), Dhankar *et al.*, (2001), Lakshmikant and Mani (2004), Raut *et al.,* (2005), Mayavel *et al.,* (2005), Dhankar and Dhankar (2006), Ara *et al.,* (2009)	Reddy and Gulshanlal (1987), Mohanty (2003)
Days to 50% flowering	Patil and Bojappa (1993), Shushila *et al.,* (1990), Dhankar and Dhankar (2006), Samadia *et al.,* (2006)	Singh (2005)
Number of fruits per Plant	Singh *et al.*, (1974), Nandapuri *et al.,* (1977), Ponnuswamy and Muthukrishnan (1977), Prasad and Prasad (1977), Bangaru *et al.,* (1983), Reddy and Gulshanlal (1987), Sushila *et al.,* (1990), Supe and Kale (1992), Indunair and Thamburaj (1996), Raijadhav *et al.,* (1996), Singh *et al.,* (1997) Rathod (1997), Patil (1998), Dhankar *et al.,* (2001), Tiwari (2002), Mohanty (2003), Prashanth (2003), Lakshmikanth and Mani (2004), Prasanna *et al.,* (2005), Raut *et al.,* (2005), Dhankar and Dhankar (2006), Kumar *et al.*, (2006).	Susie *et al.,* (2002), Prashanth (2003), Joshi *et al.,* (2004)

Table 2. Cont.

Character	Nature of correlation	
	Positive	**Negative**
Fruit weight (g)	Dudhi and Kalloo (1982), Rattan *et al.,* (1983), Patil and Bojappa (1993), Reddy and Gulshanlal (1987), Sidhu and Singh (1989), Fageria and Kohli (1996), Das *et al.,* (1998), Brar *et al.,* (1998). Prasad and Mathura Rai (1999), Mohanty (2002), Prashanth (2003), Joshi *et al.,* (2004), Raut *et al.,* (2005), Singh and Cheema (2006). Prasanna *et al.*, (2005), Samadia *et al.*, (2006), Kumar *et al.,* (2006), Singh *et al.*, (2007), Singh (2009), Ara *et al.*, (2009), Indurani *et al.*, (2008).	Srivastava and Sachan (1973), Dhankar and Dhankar (2006), Fageria and Kohli (1996), Mohanty (2002), Mohanty (2003), Singh *et al.,* (2007), Bernousi *et al.*, (2011)
Vitamin C	Anitha *et al.*, (2007), Indurani *et al.*, (2008)	Ara *et al.*, (2009)

Table 3. Summary of review of literature on direct effects of component characters on fruit yield (kg/plant) in tomato

Characters	Direction and magnitude of direct effects (High)	
	Positive	**Negative**
Plant height (cm)	Bhutani and kalloo (1989), Sharma and Verma (2000), Joshi *et al.*, (2004), Kumar and Thakur (2007), Asati *et al.*, (2008)	Mehta *et al.*, (2007). Indu Rani *et al.*, (2008)
Number of primary branches Plant^{-1}	Sonone *et al.*, (1987), Supe and Kale (1992), Mohanty (2002), Mayavel *et al.*, (2005)	Asati *et al.*, (2008)
Days to 50 per cent flowering	Singh (2004)	Asati *et al.*, (2008)
Days to first fruit harvest	Singh (2004), Kumar and Thakur (2007), Asati *et al.*, (2008)	-
Number of fruits plant^{-1}	Padda *et al.*, (1971), Srivastava and Sachan (1973), Nandapuri *et al.*, (1977), Dudhi and Kalloo (1982), Bhutani and Kalloo (1989), Patil (1998), Vikram and Kohli (1998), Sharma and Verma (2000), Kumar *et al.*, (2003), Mohanty (2003), Lakshmikant and Mani (2004), Singh (2004), Kumar and Thakur (2007), Indu Rani *et al.*, (2008)	Asati *et al.*, (2008)
Fruit weight (g)	Singh *et al.*, (1973), Dudhi and kalloo (1982), Singh *et al.*, (1989), Vikram and Kohli (1998), Sharma and Verma (2000), Dhankar *et al.*, (2001), Mohanty (2002), Kumar *et al.*, (2003), Prashanth (2003), Singh (2005), Kumar and Thakur (2007), Asati *et al.*, (2008), Indu Rani *et al.*, (2008)	Srivastava and Sachan (1973), Prashanth (2003), Asati *et al.*, (2008)
Vitamin C	Asati *et al.*, (2008)	-
TSS	Kumar and Thakur (2007)	Indu Rani *et al.*, (2008), Asati *et al.*, (2008)

Table 4. Review of literature on indirect effects of component characters on fruit yield (kg/plant) in tomato

Character showing indirect effect on yield	**Characters through which effect is expressed**	**Direction magnitude of indirect effect (High)**	
		Positive	**Negative**
Plant height	Number of branches plant $^{-1}$	Singh (2005)	-
	Number of fruits plant^{-1}	Singh and Cheema (2006)	-
Number of branches plant^{-1}	Plant height	Singh and Cheema (2006)	-
	Number of fruits plant $^{-1}$	Patil (1998)	-
	Average fruit weight	Singh and Cheema (2006)	-
Number of fruits plant^{-1}	Number of branches plant $^{-1}$	Singh (2005)	-
	Days to 50 per cent flowering	-	Singh (2005)
Average fruit weight	Number of fruits plant $^{-1}$	-	Singh and Cheema (2006)
TSS	Number of locules	-	Bhutani and Kalloo (1989)

Material and Methods

3. MATERIALS AND METHODS

The present investigation on "Identification of potential donors for superior fruit quality traits and genes for resistance to tomato leaf curl virus (ToLCV) in tomato and allied species" was carried out in the Department of plant breeding and Genetics, College of Agriculture, Vellayani, during 2014-2017. The objectives of the experiment were to evaluate the varieties and allied species of tomato for quality traits and genes for resistance to ToLCV through biochemical analysis and molecular markers and to study compatibility for hybridization and seed set to transfer ToLCV genes to bacterial wilt resistant variety ''Anagha'' from donors of related species.

The experiment site is located at 8.5° North latitude and 76.9^{0} East longitude, at an altitude of 29.00 m above mean sea level. Predominant soil type of the experimental site is red loam to Vellayani series, texturally classified as sandy clay loam. The area enjoys a warm humid tropical climate. The study was conducted in four different experiments.

1. Screening of genotypes under natural field condition for Tomato leaf curl virus (ToLCV) resistance.

2. Evaluation of genotypes for yield and biochemical quality analysis of fruit quality parameters.

3. Presence or absence of the marker linked to the genes of resistance to ToLCV.

4. Evaluation of successful F_1 hybrids and parents for yield, quality, resistance and fertility status.

3.1 SCREENING OF GENOTYPES UNDER NATURAL FIELD CONDITION FOR TOMATO LEAF CURL VIRUS (ToLCV) RESISTANCE.

3.1.1 Materials

The experimental material comprised of thirty-four tomato genotypes collected from different sources (Table 5). The seedlings were raised in greenhouse and 30 days old seedlings of thirty-four tomato genotypes were transplanted in field during summer

2015 for screening against tomato leaf curl virus (ToLCV) under natural field conditions.

Design	: RBD (Randomized Block Design)
Replication	: 3
Treatment	: 34 genotypes
Spacing	: 60 cm x 60 cm
Plot size	: 7.2 m^2

3.1.2 Raising Seedlings

Tomato seedlings were raised in protrays. Seeds of each genotype were sown separately in protrays and kept in a polyhouse provided with insect proof netting on all sides. Thirty days old healthy seedlings were used for transplanting in the main field.

3.1.3 Cultural Operations

The field was prepared to fine tilth by ploughing, harrowing, clod crushing and leveling. Plants were transplanted in main field at a spacing of 60 cm x 60 cm. The crop was not sprayed with any pesticides or insecticides in any stage of the crop and other management steps were as per the package of practices recommendation of Kerala Agricultural University (KAU, 2011).

3.1.4 Reaction of tomato genotypes against ToLCV under field condition

The scale given by Banarjee and Kalloo (1987a) was employed for scoring the disease reaction (Table 6) and (Plate 1).

3.1.5 Recording of Observations

Ten plants were randomly selected in each genotype in each replication and symptom severity grade was assessed based on natural infection of tomato leaf curl virus (ToLCV).

Table 5. List of thirty-four tomato genotypes used for screening against leaf curl virus (ToLCV) under natural field screening

Sl. No.	Genotypes	Source
1	Palam pride	CSK HPKV, Palampur
2	Surya	CSK HPKV, Palampur
3	BWR-5	CSK HPKV, Palampur
4	S 7	CSK HPKV, Palampur
5	Arka Vikas	IIHR, Bengaluru
6	Hawaii	CSK HPKV, Palampur
7	Manulekshmi	KAU, Kerala
8	Arka Meghali	IIHR, Bengaluru
9	Anagha	KAU, Kerala
10	Akshay	KAU, Kerala
11	Vellayani vijai	KAU, Kerala
12	Vaibhav	UAS, Bengaluru
13	Arka Abha	IIHR, Bengaluru
14	PKM 1	Ashok Farm Aids
15	Nandi	UAS, Bengaluru
16	Arka Alok	IIHR, Bengaluru
17	S 22	Soccar Seeds
18	EC620419	NBPGR
19	EC362944	NBPGR
20	EC168283 (*Solanum pimpinellifolium* L.)	NBPGR
21	EC620545	NBPGR
22	IC549835	NBPGR
23	EC165751	NBPGR
24	EC322634	NBPGR
25	EC326142	NBPGR
26	IIHR2372 (*Solanum lycopersicum* L.)	IIHR, Bengaluru
27	EC16786	NBPGR
28	IIHR1970 (*Solanum peruvianum* L.)	IIHR, Bengaluru
29	EC541109 (*Solanum pimpinellifolium* L.)	NBPGR
30	EC16465	NBPGR
31	IIHR2200 (*Solanum lycopersicum* L.)	IIHR, Bengaluru
32	EC320574	NBPGR
33	IC247508	NBPGR
34	LA2805 (*Solanum lycopersicum var. cerasiforme* L.)	UAS, Bengaluru

Table 6. Scale used for classifying reaction of *Solanum sp.* to Tomato leaf curl virus (Banarjee and Kalloo, 1987a)

Symptom	Symptom severity grade	Response value	Coefficient of infection	Reaction
No visible symptoms	0	0	0-4	Highly resistant (HR)
Very mild curling upto 25% leaves	1	0.25	5-9	Resistant (R)
Curling & puckering upto 26-50% leaves	2	0.50	10-19	Moderately Resistant (MR)
Severe curling & puckering upto 51-75% leaves	3	0.75	20-39	Moderately Susceptible (MS)
Very severe curling & puckering upto 76-100% leaves	4	1.00	40-69	Susceptible (S)
			70-100	Highly susceptible (HS)

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

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

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

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

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

$$\text{CI} = \frac{\text{PDS x PDI}}{100}$$

0

1

2

3

4

Plate 1. Scoring scale of tomato leaf curl virus

3.1.6 Confirmation of identified resistant genotypes under artificial inoculation by grafting

Infected plants with Tomato leaf curl virus (ToLCV) symptoms were collected from field and planted in pots in green house for the confirmation of ToLCV by graft inoculation. Scion of resistant genotypes were grafted on these susceptible plants by wedge grafting as suggested by (Bausher, 2013). The grafted portion was wrapped tightly with parafilm and covered with polythene bags. Non grafted resistant plants were kept as control.

3.2 EVALUATION OF GENOTYPES FOR YIELD AND BIOCHEMICAL QUALITY ANALYSIS OF FRUIT QUALITY PARAMETERS

The experimental material comprised of thirty-four tomato genotypes collected from different sources (Table 5). The seedlings were grown in greenhouse and 30 days old seedlings of thirty-five tomato genotypes were transplanted during *Rabi* season 2015-16 for Yield estimation and biochemical quality analysis of fruits.

Design : RBD (Randomized Block Design)

Replication : 3

Treatment : 34 genotypes

Spacing : 60 cm x 60 cm

Plot size : 7.2 m^2

3.2.1 Cultural Operations

The field was prepared to fine tilth by ploughing, harrowing, clod crushing and leveling. Plants were transplanted in main field at a spacing of 60 cm x 60 cm. The crop was raised as per the package of practices recommendation of Kerala Agricultural University (KAU, 2011).

3.2.2 Recording of Observations

Five plants were randomly selected in each treatment in each replication tagged and observations with respect to morphological and fruit quality traits were recorded. Details of the characters studied is given below.

3.2.2.1 Yield Characters

1) Plant height (cm)

The height of plant was measured in centimeters from the base of main shoot to the top most leaf bud at the time of final harvest stage using a meter scale expressed in centimeters.

2) Number of primary branches plant $^{-1}$

The total number of primary branches of each observational plants at harvest was recorded.

3) Spread of the plant (cm)

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

4) Number of days to 50% flowering

Number of days from transplanting to first flower appearance in 50 per cent of the randomly selected plants in each row was recorded.

5) Number of days to first fruit harvest

Number of days taken from transplanting to the first fruit harvest.

6) Number of fruits plant $^{-1}$

The number of fruits harvested from each observational plant in a plot was recorded.

7) Weight of fruits plant $^{-1}$ (Kg)

Weight of all fruits harvested from observational plants per harvest was recorded and the total worked out and expressed in Kilograms.

8) Weight of fruit (g)

Weight of the fruits was found out using an electronic balance and average of ten fruits in each observational plant was recorded.

9) Number of locules fruit $^{-1}$

From each observational plant randomly fruits were selected and number of locules was counted in ten fruits and mean number of locules per fruit was estimated.

10) Volume of the fruit (ml of water displaced)

From each observational plant fruits were selected randomly and volume estimated in milliliters by water displacement method. Average of ten fruits plant^{-1} was worked out.

3.2.2.2 Fruit quality parameters

1) Pericarp thickness (mm)

The pericarp thickness was measured using vernier calipers in millimeters from randomly selected ten fruits from observational plant in a plot after cutting the fruits transversely.

2) Lycopene (mg/100g)

Lycopene is responsible for red color of tomato, its content varies depending on the potential of the accession to accumulate the same, and hence the lycopene content was estimated using the protocol proposed by Ranganna (1976). The carotenoids in the sample were extracted in acetone and then separated by using petroleum ether. Lycopene has absorption maxima at 473 nm and 503 nm. One mole of lycopene when dissolved in one liter petroleum (40-60 °C) and measured in a spectrophotometer at 503 nm in one cm light path gives an absorbance of 17.2 X 10^4. Therefore, a concentration of 3.1206 µg lycopene/ml gives unit absorbance.

Materials required:

Acetone (AR grade)

Petroleum ether 40-60 (AR)

Anhydrous Sodium sulphate

5% Sodium sulphate

Procedure:

1. Three to four tomato fruits were taken in a warming blender and pulped it well to a smooth consistency.
2. Five to ten grams of this pulp was weighed.
3. Extracted the pulp repeatedly with acetone using pestle and mortar or a waring blender until the residue was colorless.
4. Pooled the acetone extracts and transferred to a separating funnel containing about20 ml petroleum ether and mixed gently.
5. Added 20 ml of 55 Sodium sulphate solution and shaked the separating funnel gently. (Volume of petroleum ether might be reduced during these processes because of its evaporation. So added 20 ml of petroleum ether to the separating funnel for clear separation of two layers). Most of the color was noticed in the upper petroleum ether layer.
6. Separated the two phases and re-extracted the lower aqueous phase with additional 20 ml petroleum ether until the aqueous phase was colorless.
7. Pooled the petroleum ether extracts and washed once with a little distilled water.
8. Poured the washed petroleum ether extract containing carotenoids into a brown Bottle containing about 10 g anhydrous sodium sulphate. Kept it aside for 30 min or longer.
9. Decanted the petroleum ether extract into a 100 ml volumetric flask through a funnel containing cotton wool. Washed sodium sulphate slurry with petroleum ether until it was colorless and transferred the washings to the volumetric flask.
10. Made up the volume and measured the absorbance in a spectrophotometer at 503 nm using petroleum ether as blank.

Calculation:

Absorbance (1 unit) = 3.1206 µg lycopene/ml.

$$\textbf{mg lycopene in 100 g sample} = \frac{31.206 \times \text{Absorbance}}{\text{Wt. of sample (g)}}$$

3) Vitamin C (mg/100g)

Vitamin C content of tomato fruits was estimated using 2, 6- dichlorophenol indophenole dye method (Sadasivam and Manickam, 1996).

Reagents :

1. Oxalic acid (four per cent)

2. Ascorbic acid (standard)

Stock solution was prepared by dissolving 100 mg of ascorbic acid in 100 ml of 4% oxalic acid. Ten ml of this stock solution was diluted to 100 ml with 4% oxalic acid to get working standard solution.

3. 2, 6-dichlorophenol indophenole dye

Sodium bicarbonate (42 mg) was dissolved in a small volume of distilled water. 52 mg of 2, 6 dichlorophenol indophenole was added into this and made upto 200 ml with distilled water.

4. Working standard

Ten ml of stock solution was diluted to 100 ml with 45 oxalic acid. The concentration of working standard is 100 mg ml^{-1}.

Procedure

Five ml of the working standard solution was pippeted out into a 100 ml conical flask and 10 ml of 4% oxalic acid was added. This was titrated against the dye (V_1). End point is the appearance of pink colour which persisted for at least 5 seconds.

Five gram of fresh fruit was extracted in four per cent oxalic acid medium, the extract was filtered and volume was made upto 100 ml using oxalic acid. From this five ml aliquot was taken, 10 ml of 45 oxalic acid was added and titrated as above against the dye and the end point (V_2) was determined.

Vitamin C content of the sample was calculated using formula

$$\textbf{Amount of Vitamin C in mg/ 100 g sample} = \frac{0.5 \times V_2 \times 100}{V_1 \times 5 \times \text{Weight of sample}} \times 100$$

4) Carotene (mg /100g)

Carotene is a red orange pigment abundant in fruits, vegetables and cereals. It is precursor of vitamin A and was estimated by the protocol given by Joy *et al.,* (2015).

Reagent

Water saturated n-butanol: (Mix n-butanol and water in ratio of 6:2 (v/v) and shake vigorously. Then allow to stand till it separates into two phases, the upper clear layer is water saturated n-butanol).

Procedure

1) Dispersed 10 g of sample in 50 ml water saturated n-butanol to make a homogenous suspension.

2) Shaken gently and allow to stand overnight (16 hours) at room temperature in dark.

3) Filtered through Whatman filter paper no. 14 and made the volume of filtrate to 100 ml.

4) Measure the absorbance (A) of the clear filtrate at 440 nm in spectrophotometer using saturated n-butanol as a blank.

Formula for calculating carotene (ppm) = 0.0105 + 23.5366 x A

5) pH of juice

It was determined by using pH meter. A probe dipped in a homogenate fruit solution from each accession and expressed value were determined as fruit juice pH.

6) Total soluble solids (^{0}Brix)

Total soluble solids of tomato fruits were recorded using a hand refractrometer (0-32 ^{0} Brix). A drop of tomato juice was used to determine the TSS content with the help of refractrometer and the value was expressed in per cent at room temperature.

7) Shelf life (days)

Fruits at breaker stage (80% maturity) were harvested and kept at the ambient temperature. The shelf life was decided when more than 50 per cent of fruits started shriveling which was judged by visual scoring.

3.3 PRESENCE OR ABSENCE OF THE MARKER LINKED TO THE GENES OF RESISTANCE TO TOLCV

3.3.1 Plant material

As a part of the identification of a specific resistant genes to tomato leaf curl virus in thirty-four genotypes were screened with SCAR molecular markers.

3.3.2 Isolation of Genomic DNA

Genomic DNA from these accessions were isolated using QIAGEN DNeasy plant mini kit. Samples were disrupted (≤100mg wet weight or ≤ ≥20 mg lyophilized tissue) using the mortar and pestle with liquid nitrogen. 400µl of buffer AP_1 and 4 µl of RNAse A were added, vortexed and incubated for 10min at 65°C. The tube was inverted 2-3 times during incubation. 130 µl buffer P3 was added and mixed and incubated for 5 min on ice. The lysate was centrifuged for 5 min at 20,000 x g (14000 rpm), the lysate was pipetted into a QIA shredder spin column placed in a 2 ml collection tube and centrifuged for 2 min at 20,000 x g. The flow–through was transferred into a new tube without disturbing the pellet if present. 1.5 volumes of buffer AW1 was added by pipette and mixed well. Then 650 µl of the mixture was transferred into a DNeasy mini spin column placed in a 2 ml collection tube and centrifuged for 1 min at ≥ 6000 x g (≥8000 rpm).The flow –through was discarded and this step with the remaining sample was repeated. The spin column was placed into a new 2 ml collection tube, 500 µl Buffer AW2 were added and centrifuged for 1 min at ≥6000 x g. The flow through was discarded, another 500 µl Buffer AW_2 were added and centrifuged for 2 min at ≥20000 x g. The spin column was transferred to a new 1.5 ml or 2 ml micro centrifuge tube and 100 µl Buffer AE was added for elution. Then incubated for 5 min at room temperature (15-25° C) and centrifuged for 1 min at ≥6000 x g. These DNA samples were stored at -20°C.

Agarose Gel Electrophoresis:

Stock solutions

50X TAE Buffer

Tris base	240 g
Acetic acid	57.1 ml
0.5M EDTA (pH-8.0)	186.12 g
Final volume (Distilled H_2O)	1000 ml

6X loading dye

Sucrose	4.0 g
Bromophenol blue	0.025 g
Volume (Distilled H_2O)	10 ml

(Loading dye solution was stored at 4°C)

Agarose gel electrophoresis was carried out in a BIO-SYS, horizontal gel electrophoresis Unit. Agarose (0.8 g) was weighed and melted in 1 x TAE buffer. After cooling the solution to 42-45°C, ethidium bromide was added at the rate of 3 µl for 100 ml. The solution was then poured on to a preset, sealed gel casting tray with a comb fixed in position, to a height of 3 mm-5 mm. The gel was allowed to solidify for 15-20 min. The comb and sealing tapes were then removed and tray was submerged in electrophoresis tank filled with 1x TAE buffer ensuring that the buffer covered the gel to height of 1mm. Required volume of DNA sample and loading dye [glycerol 30% + bromophenol blue] were mixed in the ratio 5:1 and loaded into the slots of gel using a micropipette near the negative terminal. The cathode and anode of the electrophoresis unit were attached to the power supply and a constant voltage of 60 V was used for the run. The power was turned off when the loading dye moved about $3/4^{th}$ of the gel. The gel was documented using SYNGENE gel documentation system.

3.3.3 Quantification of DNA

DNA quantification was done using spectrophotometric (Systronics) measurement of UV absorption at wavelengths 260 and 280 nm. The TE buffer in which the DNA was already dissolved was taken in cuvette to calibrate the spectrophotometer at 260 and 280 nm wavelengths. The optical density of the DNA samples dissolved in TE buffer was recorded both at 260 and 280 nm wavelengths. The quality of DNA could be judged from the ratio of the O.D. values recorded at 260 and 280 nm. A ratio between 1.8 and 2 indicates good quality DNA. The quantity of DNA in sample was estimated by using the following formula:

Concentration DNA (ng/µl) $= A_{260} \times 50 \times$ dilution factor

3.3.4 PCR analysis of genomic DNA using SCAR markers specific for resistance to tomato leaf curl virus (ToLCV) disease.

SCAR Molecular Markers specific to three ToLCV Resistance genes were selected for this study *viz.,* Ty2 gene reported by Brenda *et al.,* (2007), Ty3 gene reported by Melinda *et al.,* (2007) and Ty3a gene by Jensen *et al.,* 2007. The details of molecular markers linked to ToLCV resistance genes are given in Table 7.

3.3.4.1 PCR Amplification for Ty2 ToLCV resistant Marker

PCR Reaction mixture

The PCR reaction was carried out in a total volume of 25 µl containing:

10x Incomplete buffer		: - 2.5 µl
2.5 mM MgCl2		: - 2.5 µl
5 µM of each primer	F	: - 2.5 µl
	R	: - 2.5 µl
1 mM dNTPs		: - 5 µl
3U/ µl of Taq polymerase		: - 0.5 µl
20 ng of template DNA		: - 2.5 µl
Water		: - 7.0 µl
		Total: 25 µl

Table 7. Details of Molecular Markers Linked to ToLCV Resistance genes

Name of the marker	Primer name	Forward sequence	Reverse sequence	Expected Amplicon Size		Annealing temperature	Reference
				Resistant	Susceptible		
Ty2	TG0302F/ TY2R1	5'TGGCTCATCCTGAAG CTGATAGCGC 3'	5'TGAT(T/G)TGATGTTCTC (T/A)TCTCT(C/A)GCCTG 3'	600 bp	450 bp	55 °C	Brenda *et al.*, (2007)
Ty3	FLUW 25	5'CAAGTGTGCATATAC TTCATA(T/G)TCACC 3'	5'CCATATATAACCTCTGTT TCTATTTCGAC 3'	640 bp or 600 bp or 450 bp	480 bp	53 °C	Melinda *et al.*, (2007)
Ty3a	P6-25	5'GGTAGTGGAAATGA TGCTGCTC 3'	5'GCTCTGCCTATTGTCCCA TATATAACC 3'	630 bp	320 bp	53 °C	Jensen *et al.*,(2007)

3.3.4.2 PCR Conditions for Ty2 ToLCV resistant Marker

The amplification was carried out in an eppendorf mastercycler with the following conditions

The amplification profile was as follows:

a) Initial Denaturation	94°C	5 min	
b) Denaturation	94 °C	30 sec	} 35 cycles
c) Primer annealing (primer specific)	55 °C	1 min	
d) Primer extension	72 °C	2 min	
e) Complete primer extension	72 °C	8 min	
f) Hold	4 °C	till remove	

3.3.4.3 PCR Amplification for Ty3 and Ty3a ToLCV resistant markers

The amplification was carried out in an Eppendorf Mastercycler with the following conditions

The amplification profile was as follows:

a) Initial Denaturation	94°C	5 min,	
b) Denaturation	94 °C	30 sec	} 35 cycles
c) Primer annealing (primer specific)	53 °C	1 min	
d) Primer extension	72 °C	2 min	
e) Complete primer extension	72 °C	8 min	
f) Hold	4 °C	till remove	

3.3.5 Electrophoresis and Visualization of Amplified Products:

The amplified products were usually smaller than 1.5kb size. Hence they were separated on 1.55 agarose gels, visualized by staining with ethidium bromide and viewed under UV light.

Protocol:

1. The gel tray was set by taping the open ends, and placed on a level surface.
2. Agarose gel (1.5%) was prepared in 1X TAE buffer boiling, and cooled to 40 °C added ethidium bromide solution of 16.6 µl (0.5 g/ml). Agarose solution was poured into the gel tray with the comb in place, avoiding air bubbles and allowed to set for 20 min.
3. After removing comb, the gel was placed in the electrophoresis tank containing 0.5 X TAE buffer till the gel was fully submerged.
4. 25 µl sample of PCR was transferred into the wells and suitable DNA marker was used to assess the size of the PCR product. The leads were connected to the power source and the gel was run at constant voltage of 75 V/cm^2.
5. The run was stopped as the bromophenol blue dye reached almost 2/3 the length of the gel.
6. The gel was viewed in a gel documentation system and photographed.

3.4 EVALUATION OF SUCCESSFUL F_1 HYBRIDS AND PARENTS FOR YIELD, QUALITY, RESISTANCE AND FERTILITY STATUS

3.4.1 Selfing and crossing techniques

In tomato, anthesis occurs between 7 and 8 a.m. The well developed flower buds which are expected to open next day morning were emasculated by the removal of anthers using forceps during evening hours and bagged using butter paper covers. On the next day morning (between 7 and 8 a.m.) emasculated flower buds were pollinated by the male parents (testers). The pollinated buds were again bagged with paper bags and labeled. The mature crossed fruits were harvested and the seeds were collected separately from each cross. For maintenance of parental genotypes, flower buds of parental genotypes were selfed by bagging the individual buds and properly tagged and later seeds were collected from the mature fruits. (Table 8, 9, and 10)

Percentage of fruit set was calculated by total number of flowers pollinated and number of fruit set for all crosses and depicted in percentage.

$$\textbf{Percentage of fruit set} = \frac{\text{Number of fruit set}}{\text{Number of flowers pollinated}} \times 100$$

Table 8. Details of parental lines (ToLCV resistant genotypes) used for hybridization

Sl. No.	Code Number	Genotypes
1	T_1	Vaibhav
2	T_2	Nandi
3	T_3	EC168283 (*Solanum pimpinellifolium* L.)
4	T_4	IIHR2372
5	T_5	EC541109 (*Solanum pimpinellifolium* L.)
6	T_6	IIHR2200
7	T_7	LA2805 (*Solanum lycopersicum var. cerasiforme* L.)

Table 9. Details of tester (Bacterial wilt resistant genotype) used for hybridization

Sl. No.	Code Number	Genotypes
1	L_1	Anagha

Table 10. Details of successful hybrid combinations

Sl. No.	Parents	Cross combinations
1	$L_1 \times T_1$	Anagha x Vaibhav
2	$L_1 \times T_2$	Anagha x Nandi
3	$L_1 \times T_3$	Anagha x EC168283 (*Solanum pimpinellifolium* L.)
4	$L_1 \times T_4$	Anagha x IIHR2372
5	$L_1 \times T_5$	Anagha x EC541109 (*Solanum pimpinellifolium* L.)
6	$L_1 \times T_6$	Anagha x IIHR2200
7	$L_1 \times T_7$	Anagha x LA2805 (*Solanum lycopersicum var. cerasiforme* L.)

3.4.2 Fertility status of F_1 hybrids

At flowering stage pollen fertility recorded as microscopic pollen grain count. Pollen grains from each hybrids were collected from its flower and were squashed in a drop of 1% Iodine potassium iodide (I_2KI) solution on a glass slide separately and observed under a light microscope. The stained pollen grains were counted as fertile and unstained pollens were counted as unfertile. The total counts of fertile pollen grains were observed in relation to the pollen grain in thye five microscopic fields. The mean of five microscopic fields was than calculated. These mean values for fertile pollens and total pollens were used for calculating the pollen fertility percentage.

$$\textbf{Hybrid fertility} = \frac{\text{No. of fertile pollen grains}}{\text{Total no. of pollen grains}} \times 100$$

Observations for yield parameters and fruit quality parameters were carried out same as experiment 3.2

3.5 STATISTICAL ANALYSIS

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

3.5.1 Analysis of variance

The mean values of genotypes in each replication were used for analysis of variance. The analysis of variance and covariance for individual character and for the character pairs respectively, were carried out using the mean values of each plot following the method given by Panse and Sukhatme (1985) (Table 11).

ANOVA

Table 11. Analysis of variance (ANOVA) for the quantitative characters of thirty-four genotypes and seven hybrids

Source of variation	DF	MSS	Cal F
Replications	(r-1)	RMSS	
Genotypes	(g-1)	GMSS	GMSS/EMSS
Error	(r-1) (g-1)	EMSS	
Total	(rg-1)		

Where,

r = Number of replications

g = Number of treatments (genotypes)

The standard error was calculated

$$S.Em = \frac{\sqrt{EMSS}}{r}$$

After testing for significance of the differences among the means of different genotypes for each character, further computations were done as detailed below.

3.5.2 Variability studies

Phenotypic and genotypic variance

Phenotypic variance and genotypic variance were estimated as per the formulae suggested by Lush (1949); Choudhary and Prasad (1998).

$$\text{Genotypic variance } (\sigma g^2) = \frac{GMSS - EMSS}{r}$$

Error of variance σg^2 = EMSS

Phenotypic variance $\sigma p^2 = \sigma g^2 + \sigma e^2$

Where,

GMSS = Mean sum of square due to genotypes

EMSS = Mean sum of square due to error

σg^2 = Genotypic variance

σe^2 = Error variance

R = Number of replications

Phenotypic and genotypic coefficient of variation

The method suggested by Burton and De Vane (1953) was followed for computation of the parameters. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) are as follows.

$$\text{Phenotypic coefficient of variability (PCV\%)} = \frac{\sqrt{Phenotypic\ var\ iance}}{Grandmean} x100$$

$$\text{Genotypic coefficient of variability (GCV\%)} = \frac{\sqrt{Genotypic\ var\ aiance}}{Grandmean} x100$$

Categorization of the range of variation was effected as proposed by Sivasubramanian and Madhavamenon (1973).

<10%	:	low
10-20%	:	moderate
>20%	:	high

3.5.3 Heritability in broad sense (h^2):

The broad sense heritability (h^2_{bs}) was estimated for all characters as the ratio of genotypic variance to the total variance as suggested Lush (1949).

$$h^2 = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

According to Johnson *et al.,* (1955) heritability estimates in cultivated plants can be placed in following categories.

5-10% - Low; 10.1-30% - Moderate; 30.1-60% - High

3.5.4 Genetic advance (GA):

Genetic advance for each character was estimated by using the formula of Johnson *et al.,* (1955).

$$GA = h^2_{bs} \times \sigma_p \times K$$

Where,

h^2_{bs} = Heritability estimate in broad sense

σ_p = Phenotypic standard deviation of the trait

K = Standard selection differential which is 2.06 at 5 per cent selection intensity

Further, the genetic advance as per cent of mean was computed by using the following formula

$$\text{GA as per cent of mean} = \frac{\text{GA}}{\text{Grand mean}} \times 100$$

Genetic advance as per cent mean was categorized as given below as suggested by Johnson *et al.,* (1955).

0- 10% - Low; 10.1-20% - Moderate; >20.1% - High

3.5.5 Character correlation analysis

The correlation coefficient analysis among all possible character combination at phenotypic (rp) level were estimated employing the following formula

$$\text{Correlation} = \frac{\sqrt{\text{COV.XY (p)}}}{\sqrt{[\text{VX(p) x VY (p)}]}}$$

Where,

$COV._{XY}(p)$ = Phenotypic co-variance between X and Y characters

$V_X(p)$ = Phenotypic variance of X characters

$V_Y(p)$ = Phenotypic variance of Y characters

The test of significance for association between characters was done by comparing table 'r' values at (n-2) error degrees of freedom for phenotypic correlation with estimated values

Results

4. RESULTS

The experimental results obtained from the present investigation on "Identification of potential donors for superior fruit quality traits and genes for resistance to tomato leaf curl virus (ToLCV) in tomato and allied species" are presented under the following headings.

4.1 Screening of genotypes under natural field condition for tomato leaf curl virus resistance (ToLCV)

4.2 Evaluation of genotypes for yield and fruit quality parameters

4.3 Presence or absence of the marker linked to the genes of resistance to ToLCV

4.4 Evaluation of successful F_1 hybrids and parents for yield, quality, resistance and fertility status.

4.1 SCREENING OF GENOTYPES UNDER NATURAL FIELD CONDITION FOR TOMATO LEAF CURL VIRUS RESISTANCE (ToLCV)

Individual plant scores for tomato leaf curl virus with 0-4 scale score of thirty-four genotypes were scored with symptom severity grade depending upon visual symptoms on the plants, Eight genotypes out of thirty-four showed zero disease scale for all plants *viz.*, IIHR 2372, IIHR 2200, EC 168283, IIHR 1970, Vaibhav, EC 541109, Nandi and LA 2805 as descripted in Table 12 and Plate 2.

4.1.1 Per cent disease severity (PDS)

Per cent disease severity result as indicated in Table 14 revealed that tomato genotypes exhibited a wide range of resistance reaction to the tune of 0 to 100% against ToLCV under field condition during summer season. Among the thirty-four genotypes, eight genotypes IIHR 2372, IIHR 2200, EC 168283, IIHR 1970, Vaibhav, EC 541109, Nandi and LA 2805 recorded disease severity of 0.00% without any symptoms. Genotype (EC 165751) recorded disease severity of 14.17%. Genotype (EC 620545) recorded a disease severity of 18.33%. Genotypes S 22, Arka Meghali, EC 362944, IC 549835, Akshay, Arka Vikas, EC 320574, EC 322634, IC 247508, Manulekshmi, Anagha and EC 164656 recorded disease severity from 31-40%. Genotypes Vellayani

Table 12. Number of plants in five classes (score) of tomato leaf curl virus symptoms in natural field conditions

Disease score							
Sr. No.	Genotypes	No. of plants scored	Disease scale				
			0	1	2	3	4
1	Palam Pride	30	0	0	0	14	16
2	Surya	30	0	0	11	8	11
3	BWR 5	30	0	0	12	10	8
4	S 7	30	0	12	13	5	0
5	Arka Vikas	30	4	11	13	2	0
6	Hawaii	30	3	5	11	6	5
7	EC 320574	30	3	12	15	0	0
8	IC 247508	30	2	14	10	4	0
9	IIHR 2372	30	30	0	0	0	0
10	EC 164656	30	0	14	16	0	0
11	IIHR 2200	30	30	0	0	0	0
12	Manulekshmi	30	0	15	15	0	0
13	EC 620419	30	0	1	5	12	12
14	EC 362944	30	3	16	11	0	0
15	EC 168283	30	30	0	0	0	0
16	EC 620545	30	10	18	2	0	0
17	IC 549835	30	3	16	11	0	0
18	Arka Meghali	30	3	18	9	0	0
19	EC 165751	30	15	13	2	0	0
20	Anagha	30	0	15	15	0	0
21	EC 322634	30	1	16	13	0	0
22	Akshay	30	3	14	13	0	0
23	EC 326142	30	0	2	5	8	15
24	EC 16786	30	0	2	3	6	19
25	Vellayani Vijai	30	0	9	21	0	0
26	IIHR 1970	30	30	0	0	0	0
27	Vaibhav	30	30	0	0	0	0
28	Arka Abha	30	0	6	11	11	2
29	EC 541109	30	30	0	0	0	0
30	PKM-1	30	0	8	12	10	0
31	Nandi	30	30	0	0	0	0
32	Arka Alok	30	0	3	12	15	0
33	S 22	30	5	13	11	1	0
34	LA 2805	30	30	0	0	0	0

Symptom severity grade and symptoms
0 - No visible symptoms
1 - Very mild curling upto 25% leaves
2 - Curling & puckering upto 26-50% leaves
3 - Severe curling & puckering upto 51-75% leaves
4 - Very severe curling & puckering upto 76-100% leaves

Plate 2. General view of experimental plot (Experiment I)

Vijai, S 7, Hawaii, PKM 1, Arka Abha, Arka Alok, BWR 5, Surya recorded disease severity from 41-75%, whereas genotypes EC 620419, EC 326142, EC 16786 and Palam Pride showed disease severity from 76-100% (Table 13) and (Plate 3).

4.1.2 Per cent disease incidence (PDI)

The per cent disease incidence was calculated using formula the number of plants infected divided by the total number of plant observed multiplied by 100. The result of per cent disease incidence mentioned in Table 14. Out of thirty-four genotypes, eight genotypes IIHR 2372, IIHR 2200, EC 168283, IIHR 1970, Vaibhav, EC 541109, Nandi and LA 2805 were not infected by the tomato leaf curl virus, it means 0% per cent disease incidence. All other genotypes *viz.*, EC 165751, EC 620545, S 22, Arka Vikas, Arka Meghali, EC 362944, IC 549835, Akshay, Hawaii and EC 320574 recorded per cent disease incidence from 50-90%. Whereas Genotypes IC 247508, EC 322634, Manulekshmi, Anagha, EC 164656, Vellayani Vijai, S 7, PKM 1, Arka Abha, Arka Alok, BWR 5, Surya, EC 620419, EC 326142, EC 16786 and Palam Pride showed disease incidence from 91-100% (Table 13).

4.1.3 Coefficient of the infection (CI)

The coefficient of the infection of thirty-four tomato genotypes is mentioned in Table 14. Based on the coefficient of infection, the genotypes were categorized into six groups by Banerjee and Kalloo (1988). Highly resistant reaction was found in eight genotypes, among these eight highly resistant genotypes four genotypes EC 541109 (*Solanum pimpinellifolium* L.) EC 168283 (*Solanum pimpinellifolium* L.) IIHR 1970 (*Solanum peruvianum* L.) and LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) were wild species and IIHR 2372, IIHR 2200, Vaibhav and Nandi were four cultivated species of tomato all these eight genotypes recorded (0%) of Coefficient of the infection (CI) and were under highly resistant category for tomato leaf curl virus disease.

Genotype EC 165751 recorded a (7.08%) of Coefficient of the infection (CI) and was under resistant category for tomato leaf curl virus disease. Whereas Genotype EC 620545 recorded (12.22%) and was under moderately resistant category for tomato leaf curl virus disease. Under moderately susceptible category twelve genotypes were recorded with different percentage of Coefficient of the infection (CI) *viz.*, S 22 (26.39%), Arka Meghali (27%), EC 362944 (28.5%), IC 549835 (28.5%), Akshay

Table 13. Reaction of thirty-four genotypes to local strains of tomato leaf curl virus (ToLCV) in field conditions

Sr. No.	Genotypes	PDS (%)	PDI (%)	CI	Category
1	Palam Pride	88.33	100.00	88.33	HS
2	Surya	75.00	100.00	75.00	HS
3	BWR-5	71.67	100.00	71.67	HS
4	S 7	44.17	100.00	44.17	S
5	Arka Vikas	35.83	86.67	31.06	MS
6	Hawaii	54.17	90.00	48.75	S
7	EC 320574	35.00	90.00	31.50	MS
8	IC 247508	38.33	93.33	35.78	MS
9	IIHR 2372	0.00	0.00	0.00	HR
10	EC 164656	38.33	100.00	38.33	MS
11	IIHR-2200	0.00	0.00	0.00	HR
12	Manulekshmi	37.50	100.00	37.50	MS
13	EC 620419	79.17	100.00	79.17	HS
14	EC 362944	31.67	90.00	28.50	MS
15	EC 168283	0.00	0.00	0.00	HR
16	EC 620545	18.33	66.67	12.22	MR
17	IC 549835	31.67	90.00	28.50	MS
18	Arka Meghali	30.00	90.00	27.00	MS
19	EC 165751	14.17	50.00	7.08	R
20	Anagha	37.50	100.00	37.50	MS
21	EC 322634	35.00	96.67	33.83	MS
22	Akshay	33.33	90.00	30.00	MS
23	EC 326142	80.00	100.00	80.00	HS
24	EC 16786	85.00	100.00	85.00	HS
25	Vellayani Vijai	42.50	100.00	42.50	S
26	IIHR 1970	0.00	0.00	0.00	HR
27	Vaibhav	0.00	0.00	0.00	HR
28	Arka Abha	57.50	100.00	57.50	S
29	EC 541109	0.00	0.00	0.00	HR
30	PKM 1	51.67	100.00	51.67	S
31	Nandi	0.00	0.00	0.00	HR
32	Arka Alok	60.00	100.00	60.00	S
33	S 22	31.67	83.33	26.39	MS
34	LA 2805	0.00	0.00	0.000	HR

Anagha Palam Pride

EC 620419 **BWR 5**

Plate 3. Susceptible genotypes with ToLCV symptoms

Surya

Hawaii

EC 16786

EC 326142

Plate 3 a. Susceptible genotypes with ToLCV symptoms

(30%), Arka Vikas (31.06%), EC 320574 (31.5%), EC 322634 (33.83%), IC 247508 (35.78%), Manulekshmi (37.5%), Anagha (37.5%) and EC 164656 (38.33%). Under susceptible category six genotypes recorded between 40 to 60 percentage of Coefficient of the infection (CI) *viz.,* Vellayani Vijai (42.5%), S 7 (44.17%), Hawaii (48.75%), PKM 1 (51.67%), Arka Abha (57.5%) and Arka Alok (60%). Six genotypes under Highly susceptible category recorded range between 70 to 100 percentage of Coefficient of the infection (CI) *viz.,* BWR 5 (71.67%), Surya (75%), EC 620419 (79.17%), EC 326142 (80%), EC 16786 (85%) and Palam Pride (88.33%) (Table 13).

4.1.4 Screening of identified highly resistant genotypes under artificial inoculation by grafting

Eight genotypes which showed highly resistant reaction against ToLCV in natural field conditions were used for grafting for confirmation studies, scions of resistant genotypes were grafted on susceptible root stock with symptoms of ToLCV. These grafted plants were kept under green house for 30-45 days after grafting and plants were scored for ToLCV by the scale given by Banerjee and Kalloo (1987) (Table 14) and (Plate 4).

Individual plant scores for tomato leaf curl virus with 0-4 scale score of eight grafted genotypes were scored with symptom severity grade depending upon visual symptoms on the plants, Five grafted genotypes out of eight showed zero disease scale for all plants *viz.,* IIHR 2200, EC 168283, IIHR 1970, EC 541109, and LA 2805 as descripted in Table 15.

4.1.5 Per cent disease severity

Per cent disease severity result as indicated in Table 16, revealed that tomato grafted genotypes exhibited high range of resistance reaction to against ToLCV under green house condition kept after grafting. Among the eight genotypes, five genotypes IIHR 2200, EC 168283, EC 541109, IIHR 1970 and LA 2805 recorded disease severity of 0.00% without any symptoms. Genotype (IIHR 2372) recorded disease severity of 1.67%, whereas genotype Vaibhav and Nandi recorded a disease severity of 3.33%.

4.1.6 Per cent disease incidence

The per cent disease incidence was calculated using formula the number of plants infected divided by the total number of plant observed multiplied by 100. The

Table 14. List of highly resistant genotypes used as scions for grafting for confirmation of ToLCV resistance

Sr. No.	Genotypes
1	Vaibhav
2	Nandi
3	EC 168283 (*Solanum pimpinellifolium* L.)
4	IIHR 2372
5	IIHR 1970 (*Solanum peruvianum* L.)
6	EC 541109 (*Solanum pimpinellifolium* L.)
7	IIHR 2200
8	LA 2805 (*Solanum lycopersicum var. cerasiforme* L.)

Vaibhav **Nandi**

EC 541109 **IIHR 2200**

Plate 4. Resistant scions after successful graft transmission of ToLCV

Table 15. Number of plants in five classes (score) of tomato leaf curl virus symptoms after grafting on susceptible root stock

Disease score of grafted plants							
Sr. No.	**Genotypes**	**No. of grafts scored**	**0**	**1**	**2**	**3**	**4**
1	Vaibhav	15	13	2	0	0	0
2	Nandi	15	13	2	0	0	0
3	IIHR 2372	15	14	1	0	0	0
4	IIHR 2200	15	15	0	0	0	0
5	EC 168283	15	15	0	0	0	0
6	EC 541109	15	15	0	0	0	0
7	IIHR 1970	15	15	0	0	0	0
8	LA 2805	15	15	0	0	0	0

Symptom severity grade and symptoms

0- No visible symptoms

1- Very mild curling upto 25% leaves

2- Curling & puckering upto 26-50% leaves

3- Severe curling & puckering upto 51-75% leaves

4- Very severe curling & puckering upto 76-100% leaves

Table 16. Reaction of eight resistant genotypes to local strains of tomato leaf curl virus by grafting on susceptible root stock

Sr. No.	Genotypes	PDS (%)	PDI (%)	CI	Category
1	Vaibhav	3.33	13.33	0.44	HR
2	Nandi	3.33	13.33	0.44	HR
3	IIHR 2372	1.67	6.67	0.11	HR
4	IIHR 2200	0.00	0.00	0.00	HR
5	EC 168283	0.00	0.00	0.00	HR
6	EC 541109	0.00	0.00	0.00	HR
7	IIHR 1970	0.00	0.00	0.00	HR
8	LA 2805	0.00	0.00	0.00	HR

result of per cent disease incidence mentioned in Table 16. Out of eight grafted genotypes, five genotypes IIHR 2200, EC 168283, EC 541109, IIHR 1970 and LA 2805 were not infected by the tomato leaf curl virus, it means 0% disease incidence. Genotype IIHR 2372 recorded per cent disease incidence of 6.67% and genotypes Vaibhav and Nandi showed disease incidence of 13.33%.

4.1.7 Coefficient of the infection (CI)

The coefficient of the infection of eight tomato grafted genotypes is mentioned in Table 16. Based on the coefficient of infection, the genotypes were categorized into six groups by Banerjee and Kalloo (1988). Highly resistant reaction was found in all eight genotypes, among these eight highly resistant genotypes four genotypes EC 541109 (*Solanum pimpinellifolium* L.) EC 168283 (*Solanum pimpinellifolium* L.) IIHR 1970 (*Solanum peruvianum* L.) and LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) were wild species and IIHR 2372, IIHR 2200, Vaibhav and Nandi were four cultivated species of tomato all these eight genotypes recorded (0 to 0.44%) of Coefficient of the infection (CI) and were under highly resistant category for tomato leaf curl virus disease.

4.2 EVALUATION OF GENOTYPES FOR YIELD AND FRUIT QUALITY PARAMETERS

4.2.1 Analysis of variance

The results of variance for 30 genotypes and 4 wild accessions of tomato for ten quantitative and seven qualitative traits are furnished separately in Table 17 to 17c and 18. Highly significant differences among the check and genotypes were observed for all seventeen characters, this is an indication of presence of good amount of genetic variability among the genotypes (Plate 5).

4.2.2 Mean performance

The observation for each genotype in three replications for fruit yield and its components characters were used for calculating the mean performance. The observations were recorded on five randomly selected tagged competitive plants from each replication and averaged. The mean performance of different genotype and its components characters are presented in Table 17 to 17 c and 18 are described below.

Plate 5. General view of experimental plot (Experiment II)

4.2.2.1 Yield parameters

Plant height (cm)

Plant height of the genotypes ranged from 58.51cm to 153.46 cm with a mean of 100.60 cm (Table 17). The plant height of 30 genotypes compared in Fig. 1. The maximum plant height was recorded in EC 320574 (153.46 cm) followed by EC 620545 (147.47), EC 165751 (142.47), EC 326142 (137.22) and EC 322634 (133.40), whereas minimum plant height was recorded in Arka Abha (62.83) followed by Anagha (62.60) and Surya (58.52).The check variety Vellayani Vijai recorded a plant height of 69.82 cm.

Number of primary branches plant $^{-1}$

Number of primary branches plant $^{-1}$ in genotypes ranged from 4.33 to 14.40 with a mean of 9.33 (Table 17). The highest number of primary branches plant^{-1} was recorded in EC 320574 (14.40) and EC 165751 (14.20). Further, EC 620545 with (13.47) followed by EC 362944 (12.93) and EC 322634 (12.87). Lowest number of primary branches was recorded in Anagha (4.33). The check variety Vellayani Vijai recorded an average number of primary branches 5.33 plant^{-1} (Fig. 2).

Spread of the plant (cm)

Spread of the plant ranged from 43.88 cm to 84.44 cm with a mean of 66.36 cm (Table 17). Highest spread of the plant was observed in EC 620545 (84.44 cm) followed by EC 320574 (82.00 cm), EC 326142 (81.89 cm), EC 165751 (81.33 cm) and EC 322634 (81.22 cm), all these four genotypes had no significant difference with respect to this character. Lowest spread of the plant was observed in PKM1 (28.00). Check variety Vellayani Vijai recorded a spread of plant 62.78 cm (Fig. 3).

Number of days to 50% flowering

Number of days to 50% flowering ranged from 29.66 days to 43.13 days with a mean of 34.55 days (Table 17). The check variety Vellayani vijay took minimum number of days to 50% flowering (29.67 days) followed by Arka Alok (30.07 days), Anagha (30.33 days), Arka Vikas (31.33 days) and BWR 5 (31.67 days). Maximum number of days to 50% flowering was observed in IC 247508 43.13 days (Fig. 4).

Table 17. Mean performance of 30 genotypes for seventeen characters in tomato

Sr. No	Genotype	Plant height (cm)	No. of primary branches plant^{-1}	Spread of plant (cm)	No. of days to 50% flowering	No. of days to first fruit harvest
1	Palam Pride	116.33	9.80	72.42	32.00	61.33
2	Surya	58.52	4.73	45.94	33.00	67.80
3	BWR 5	69.58	9.60	54.67	31.67	60.33
4	S 7	68.94	5.07	62.83	34.60	65.93
5	Arka Vikas	88.45	8.93	72.11	31.33	58.00
6	Hawaii	88.93	8.07	67.45	33.20	64.53
7	EC 320574	153.47	14.40	82.00	40.73	72.87
8	IC 247508	124.17	12.33	79.67	43.13	72.67
9	IIHR 2372	110.70	9.47	53.89	32.67	64.73
10	EC 164656	120.63	12.60	63.56	41.73	71.93
11	IIHR 2200	127.77	8.27	63.33	32.40	66.80
12	Manulekshmi	69.20	8.13	60.00	32.47	64.73
13	EC 620419	120.62	12.13	71.56	35.33	67.27
14	EC 362944	112.37	12.93	74.61	35.80	74.20
15	EC 620545	147.47	13.47	84.44	34.93	72.07
16	IC 549835	127.40	12.73	78.78	34.80	68.00
17	Arka Meghali	73.70	7.00	61.50	34.27	64.80
18	EC 165751	142.47	14.20	81.33	34.93	70.80
19	Anagha	62.60	4.33	63.84	30.33	61.60
20	EC 322634	133.40	12.87	81.22	40.87	74.40
21	Akshay	124.17	9.53	77.44	33.00	62.87
22	EC 326142	137.22	11.73	81.89	41.60	73.87
23	EC 16786	115.90	8.00	73.33	38.07	67.73
24	Vaibhav	90.75	8.53	68.44	32.73	67.33
25	Arka Abha	62.83	5.00	60.44	33.53	64.00
26	PKM 1	68.20	7.53	47.83	33.20	63.07
27	Nandi	73.31	8.67	49.05	31.73	64.47
28	Arka Alok	74.41	8.07	43.89	30.07	60.47
29	S 22	84.85	6.53	50.61	32.73	67.20
30	Vellayani vijay	69.82	5.33	62.78	29.67	59.27
	Mean	100.60	9.33	66.36	34.55	66.50
	C.D. (5%)	3.04	0.50	1.22	0.95	1.15
	S.E (m)	1.08	0.18	0.43	0.34	0.41
	C.V.	1.85	3.29	1.12	1.68	1.06

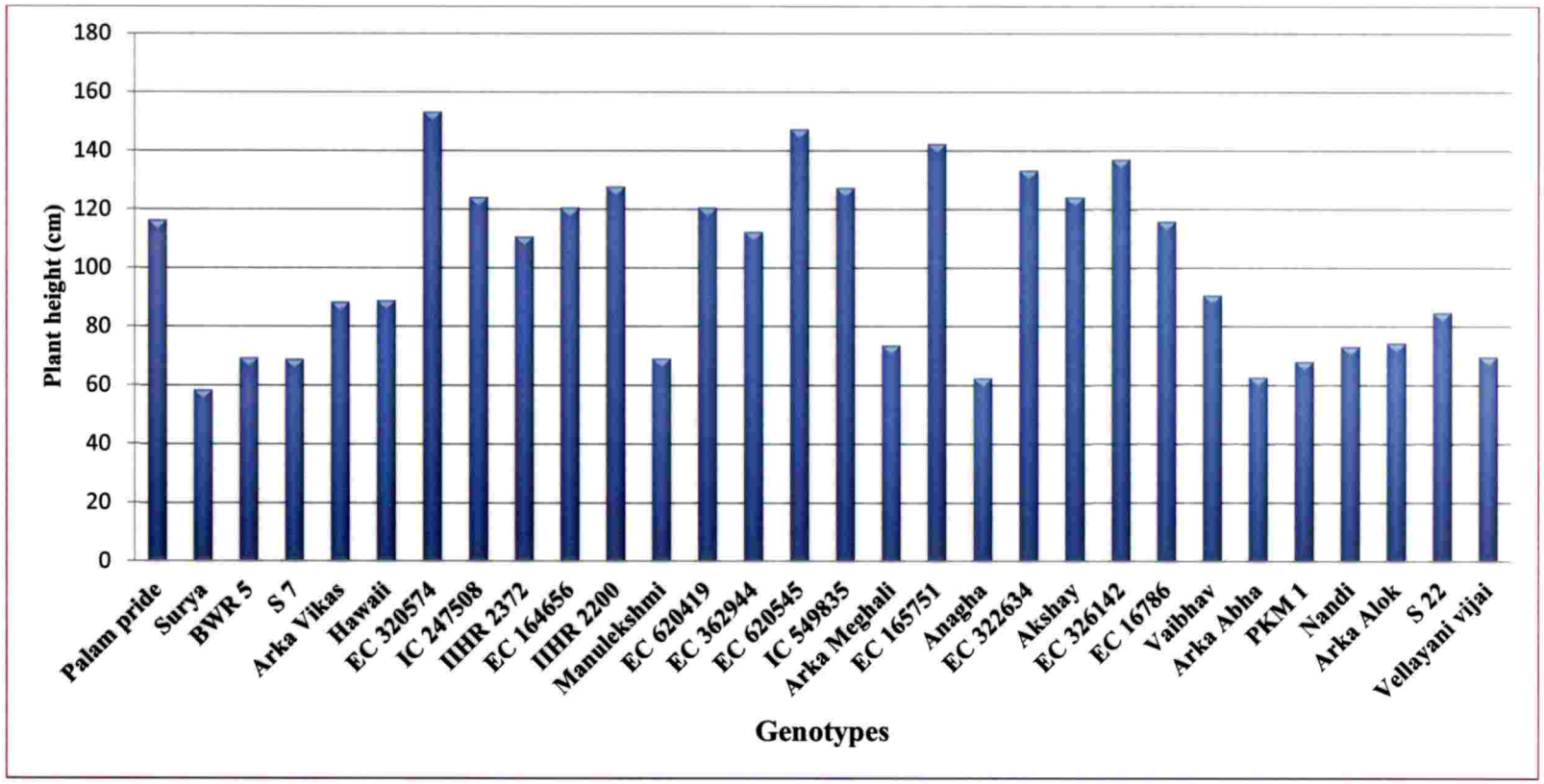

Fig 1. Plant height (cm) in various tomato genotypes

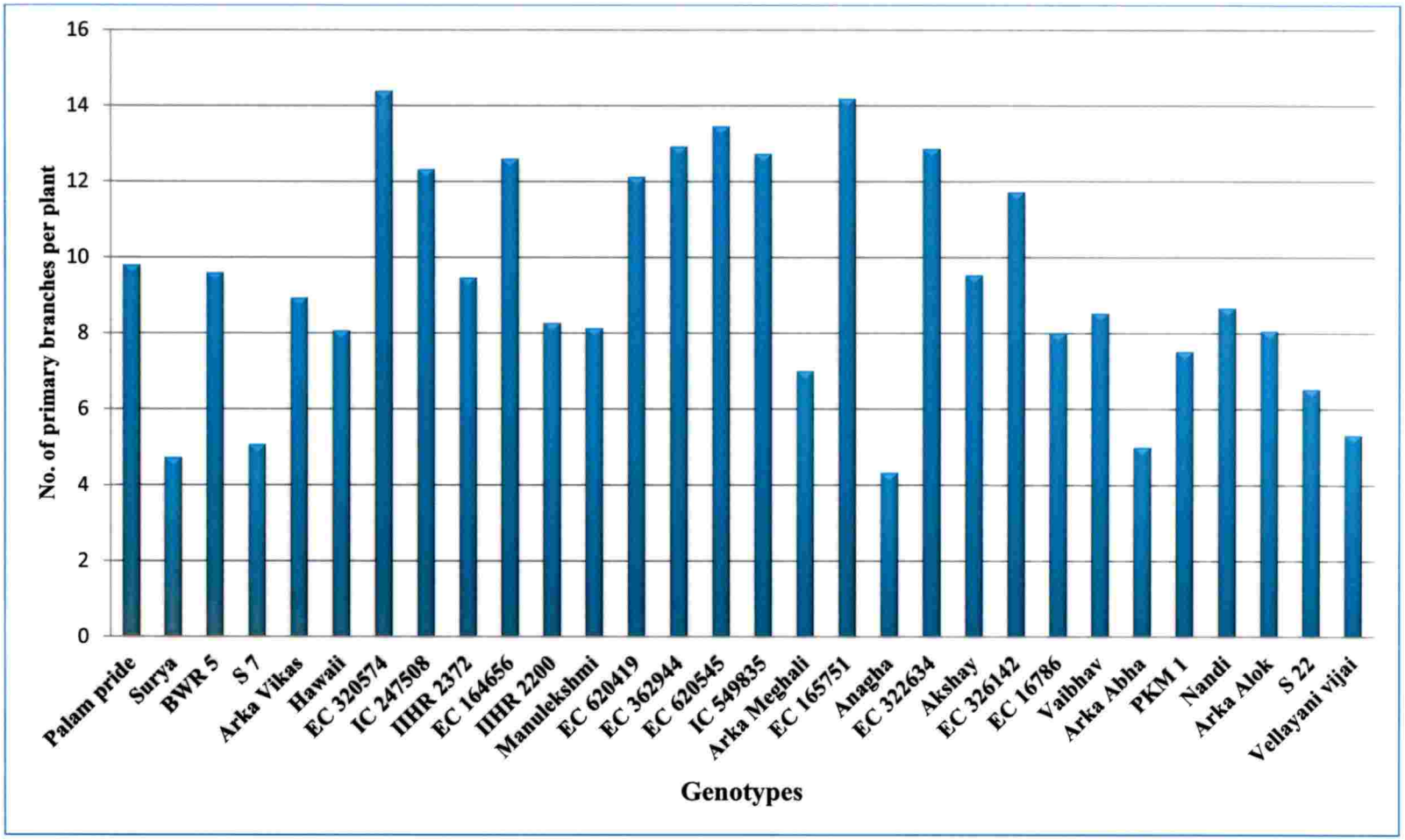

Fig 2. Number of primary branches per plant in various tomato genotypes

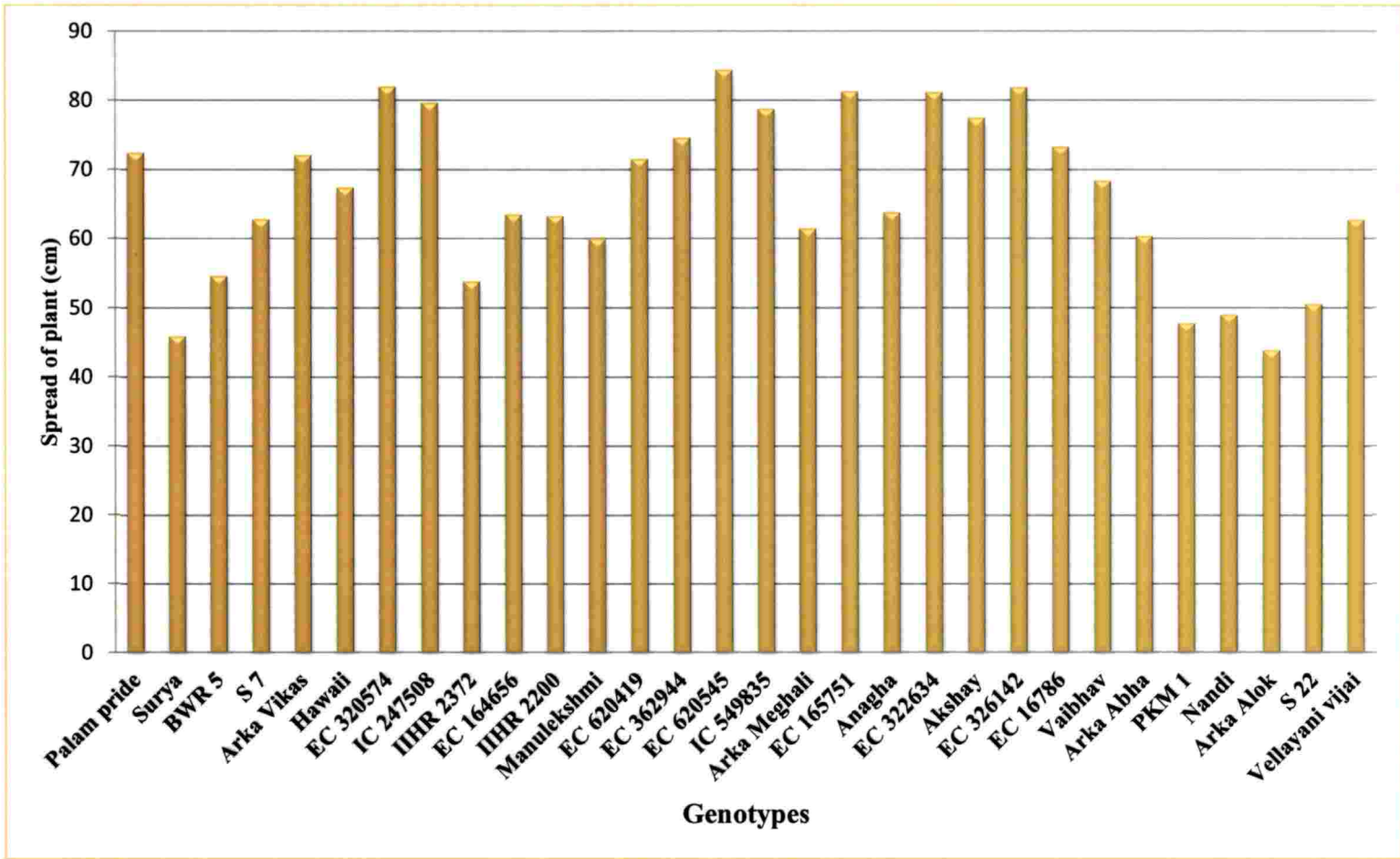

Fig 3. Spread of plants (cm) in various tomato genotypes

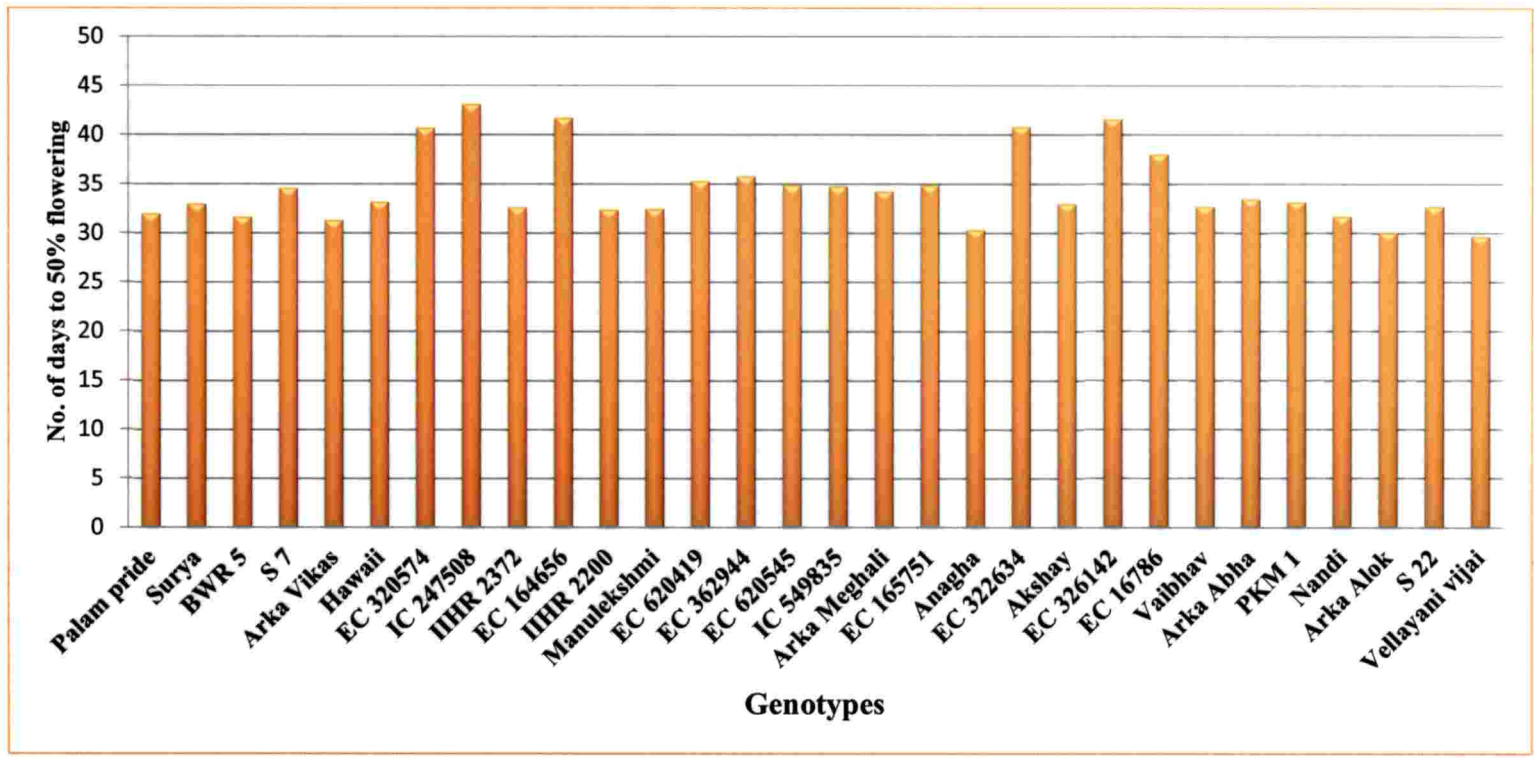

Fig 4. Number of days to 50% flowering in various tomato genotypes

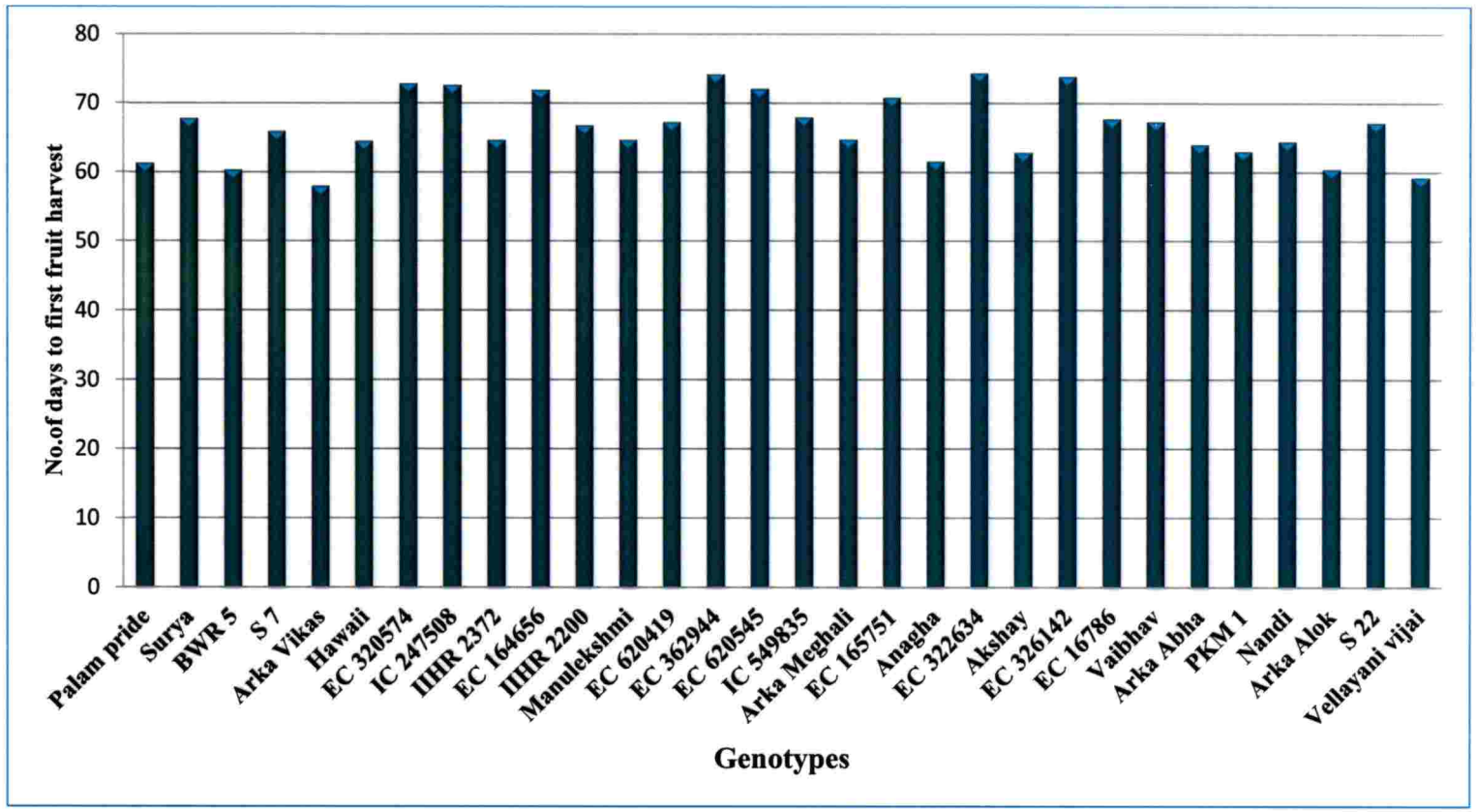

Fig 5. Number of days to first fruit harvest in various tomato genotypes

Number of days to first fruit harvest

Number of days to first fruit harvest ranged from 58.0 days to 74.40 days with a mean of 66.50 days (Table 17). Minimum number of days to first fruit harvest was observed in Arka Vikas (58.00 days) followed by check variety Vellayani Vijai (59.27 days), BWR 5 (60.33 days), Arka Alok (60.47 days) and Palam Pride (61.33 days). Check variety Vellayani Vijai and BWR 5 had no significant difference with respect to number of days to first fruit harvest. Maximum number of days to first fruit harvest was observed in EC 326142 (73.78 days) followed by EC 362944 (74.20 days) and EC 322634 (74.40) these three genotypes had no significant difference with respect to this character (Fig. 5).

Number of fruits plant $^{-1}$

Number of fruits plant^{-1} ranged from 14.33 to 47.36 with a mean of 30.05 fruits (Table 17a). Highest number of fruits plant^{-1} were recorded in EC 165751 (47.37) followed by IC 549835 (46.67), EC 362944 (46.13) these three genotypes had no significant difference with respect to number of fruits plant^{-1}, lowest number of fruits plant^{-1} was recorded in Surya (14.33), The check variety Vellayani Vijai recorded 19.60 number of fruits plant^{-1} (Fig. 6).

Weight of fruits plant^{-1} (Kg)

Weight of fruits plant^{-1} ranged from 0.49 kg to 2.41 kg with a mean of 1.19 kg (Table 17 a). Maximum weight of fruits plant^{-1} was observed in Vaibhav (2.41 kg) followed by EC 165751 (2.02 kg), EC 164656 (2.00 kg), IC 247508 (1.94 kg) and EC 16786 (1.92 kg). Genotype EC 165751 and EC 164656 were on par for this trait, similarly genotype IC 247508 and EC 16786 were also on par for this trait. Minimum weight of fruits plant^{-1} was observed in Manulekshmi (0.60 kg), Arka Alok (0.56 kg) and Surya (0.49 kg). The check variety Vellayani Vijai recorded (0.65 kg) weight of fruits plant^{-1} (Fig. 7).

Weight of fruit (g)

Weight of fruit ranged from 19.31 g to 61.46 g with a mean of 41.56 g. (Table 17 a). Maximum weight of fruit was recorded in Vaibhav (61.47 g) followed by S 7 (57.83 g), S22 (54.08 g), EC 620545 (51.08 g) and EC 620419 (50.55 g), While minimum weight of fruit was recorded in Anagha (27.95 g), EC 326142 (23.78 g) and

IC 549835 (19.31 g). The check variety Vellayani Vijai recorded 34.93 g weight of fruit (Fig. 8).

Number of locules fruit^{-1}

Number of locules fruit^{-1} ranged from 2.0 to 4.99 with a mean of 3.58 (Table 17 a). Maximum number of locules fruit^{-1} were observed in EC 164646 (4.99) followed by EC 322634 (4.89), S7 (4.89), EC 320574 (4.78) and Arka Meghali (4.78) all the five genotypes had no significant difference between them for number of locules. While minimum number of locules fruit^{-1} were observed in IIHR 2372 (2.00), IIHR 2200 (2.00), EC 620419 (2.00) and EC 326142 (2.00). Average number of locules fruit^{-1} in check variety Vellayani Vijai was 2.11 (Fig. 9).

Volume of fruit (ml of water displaced)

Volume of fruit ranged from 17.38 ml to 64.64 ml with a mean of 40.06 ml (Table 17 a). Maximum volume of fruit was recorded in Vaibhav (64.64 ml) followed by S 22 (58.77 ml), S 7 (55.82 ml), EC 620545 (46.87 ml) and EC 164656 (45.97 ml), Genotype EC 620545 and EC 164656 had no significant difference between them and were on par for this trait. Minimum volume of fruit was recorded in Anagha (27.42 ml) followed by EC 356142 (23.65 ml) and IC 549835 (17.38 ml), The check variety Vellayani Vijai recorded (34.10 ml) volume of fruit (Fig. 10).

4.2.2.2 Fruit quality parameters

Pericarp thickness

Pericarp thickness ranged from 3.25 mm to 9.34 mm with a mean of 5.36 mm (Table 17 b). Maximum pericarp thickness was observed in IIHR 2372 (9.35 mm) followed by EC 620419 (7.84 mm), Vaibhav (7.49 mm), EC 362944 (6.57 mm) and IC 247508 (6.56 mm), EC 362944 and IC 247508 had no significant difference for this trait. While minimum pericarp thickness was observed in EC 322634 (3.94 mm) followed by EC 326142 (3.72 mm) and IC 549835 (3.25 mm), Average of pericarp thickness in check variety Vellayani Vijai was 4.29 mm (Fig.11).

Lycopene (mg/100g)

Lycopene content ranged from 4.67 mg to 12.41 mg with a mean of 7.22 mg (Table 17 b). Highest content of lycopene was observed in check variety Vellayani Vijai

Table 17 a. Mean performance of 30 genotypes for seventeen characters in tomato

Sr. No	Genotype	No. of fruits plant^{-1}	Weight of fruit plant^{-1} (kg)	Weight of fruit (g)	No. of locules fruit^{-1}	Vol. of the fruit (ml)
1	Palam Pride	19.93	0.79	42.80	3.89	43.27
2	Surya	14.33	0.49	35.94	3.22	34.53
3	BWR 5	31.40	1.24	40.82	2.11	40.17
4	S 7	15.83	0.96	57.83	4.89	55.82
5	Arka Vikas	19.07	0.60	32.59	4.11	30.57
6	Hawaii	37.62	1.28	34.61	3.22	35.90
7	EC 320574	40.00	1.81	45.00	4.78	41.33
8	IC 247508	40.93	1.94	47.10	3.11	44.30
9	IIHR 2372	18.40	0.77	45.18	2.00	44.33
10	EC 164656	39.03	2.00	48.74	5.00	45.97
11	IIHR 2200	21.33	0.83	42.46	2.00	41.67
12	Manulekshmi	19.20	0.60	34.29	4.11	33.92
13	EC 620419	33.47	1.65	50.55	2.00	45.90
14	EC 362944	46.13	1.52	31.63	4.11	29.50
15	EC 620545	34.13	1.62	51.08	3.11	46.87
16	IC 549835	46.67	0.95	19.31	4.33	17.38
17	Arka Meghali	27.60	1.08	40.34	4.78	37.27
18	EC 165751	47.37	2.02	43.22	3.33	41.43
19	Anagha	25.80	0.73	27.95	2.11	27.42
20	EC 322634	42.08	1.71	42.51	4.89	37.93
21	Akshay	21.60	0.91	43.58	4.33	42.30
22	EC 326142	40.67	0.99	23.78	2.00	23.65
23	EC 16786	40.60	1.92	46.87	2.11	45.13
24	Vaibhav	42.53	2.41	61.47	4.11	64.65
25	Arka Abha	15.73	0.63	43.69	4.11	42.43
26	PKM 1	33.73	0.75	44.53	4.66	42.33
27	Nandi	33.82	1.47	44.59	4.00	39.82
28	Arka Alok	17.60	0.56	35.35	4.66	33.22
29	S 22	15.33	0.81	54.08	4.33	58.77
30	Vellayani vijay	19.60	0.65	34.93	2.11	34.10
	Mean	30.05	1.19	41.56	3.58	40.06
	C.D. (5%)	1.74	0.04	2.06	0.41	2.01
	S.E (m)	0.61	15.08	0.73	0.15	0.71
	C.V.	3.54	2.20	3.04	7.09	3.07

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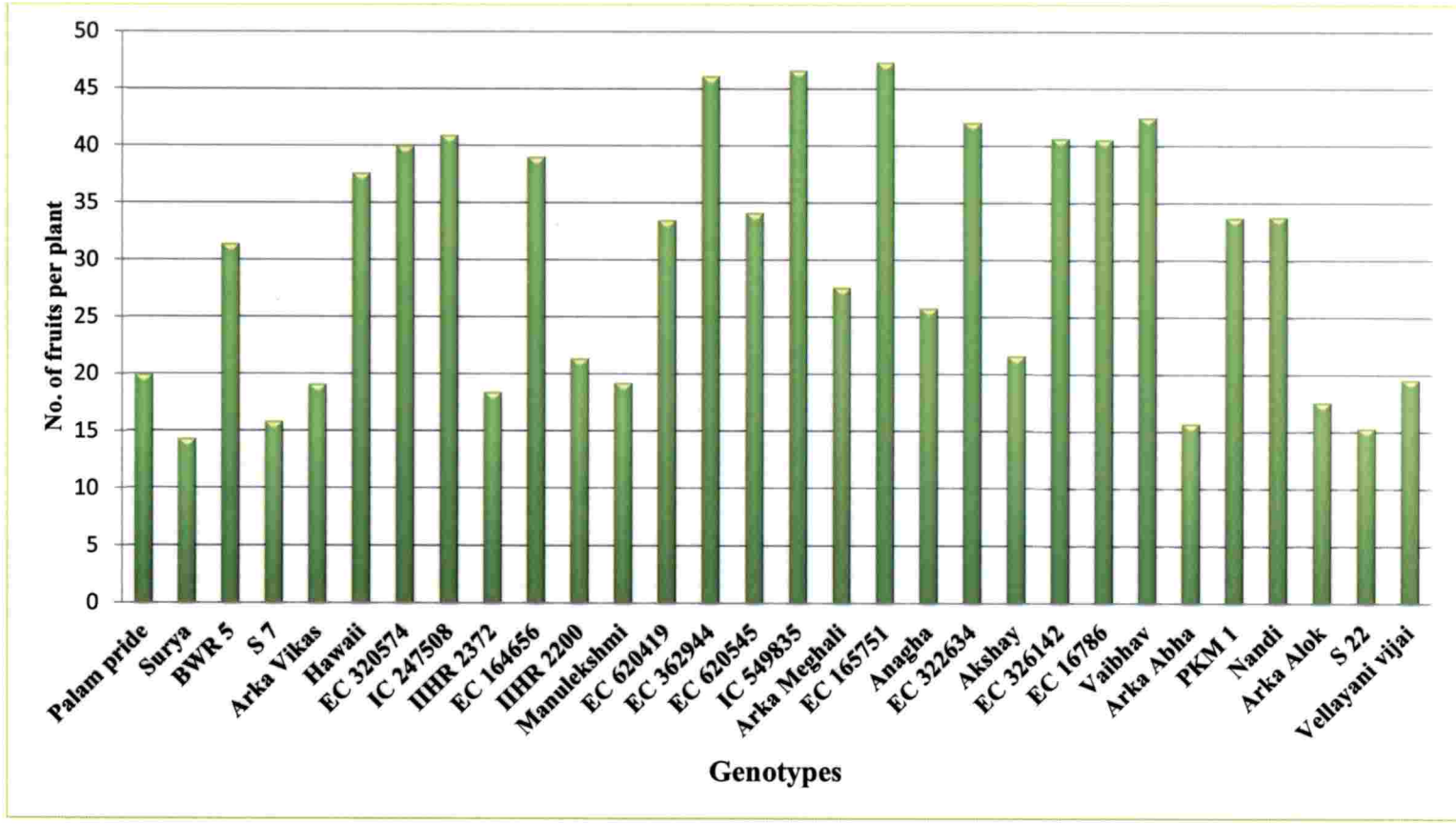


Fig 6. Number of fruits per plant in various tomato genotypes

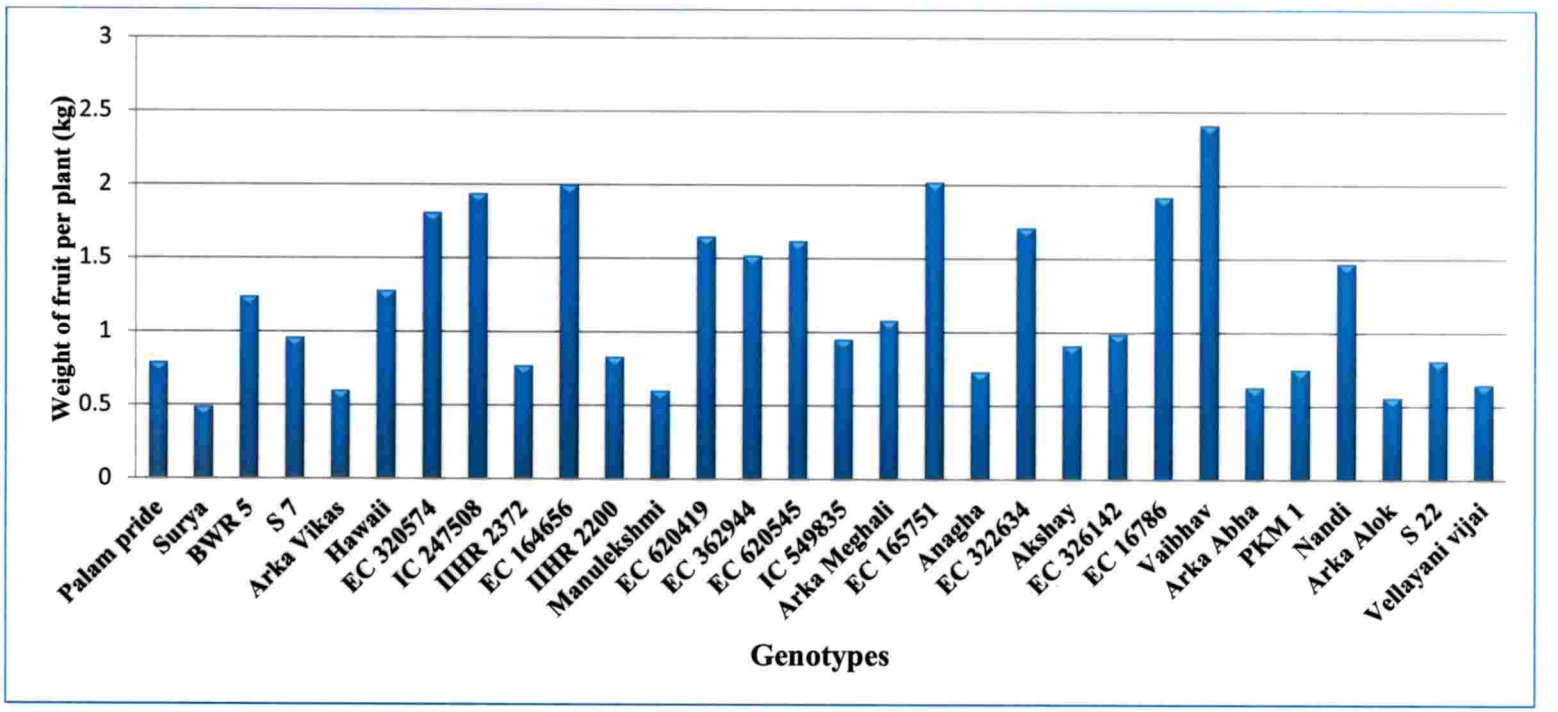


Fig 7. Weight of fruits per plant (kg) in various tomato genotypes

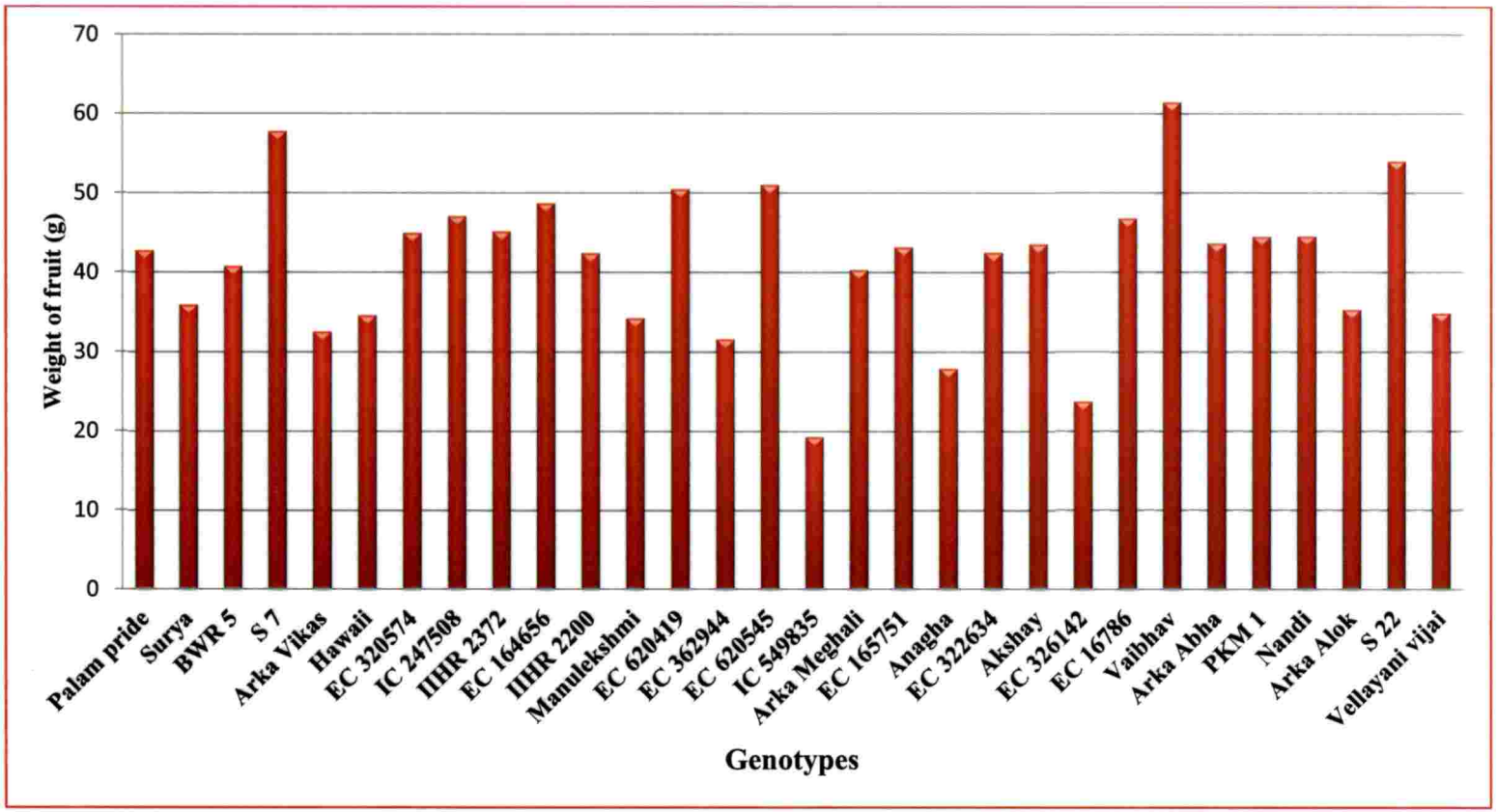


Fig 8. Weight of fruit (g) in various tomato genotypes

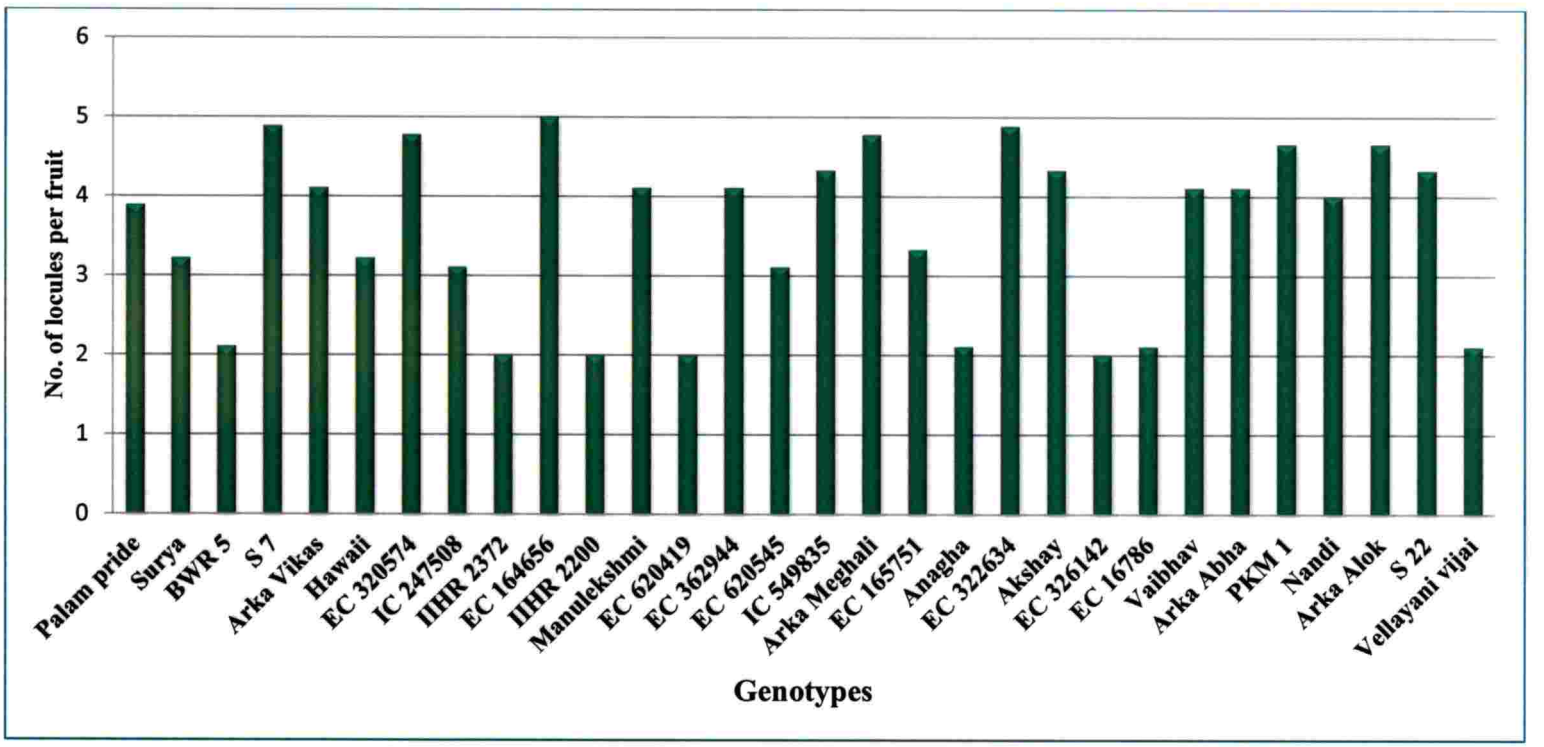


Fig 9. Number of locules in various tomato genotypes

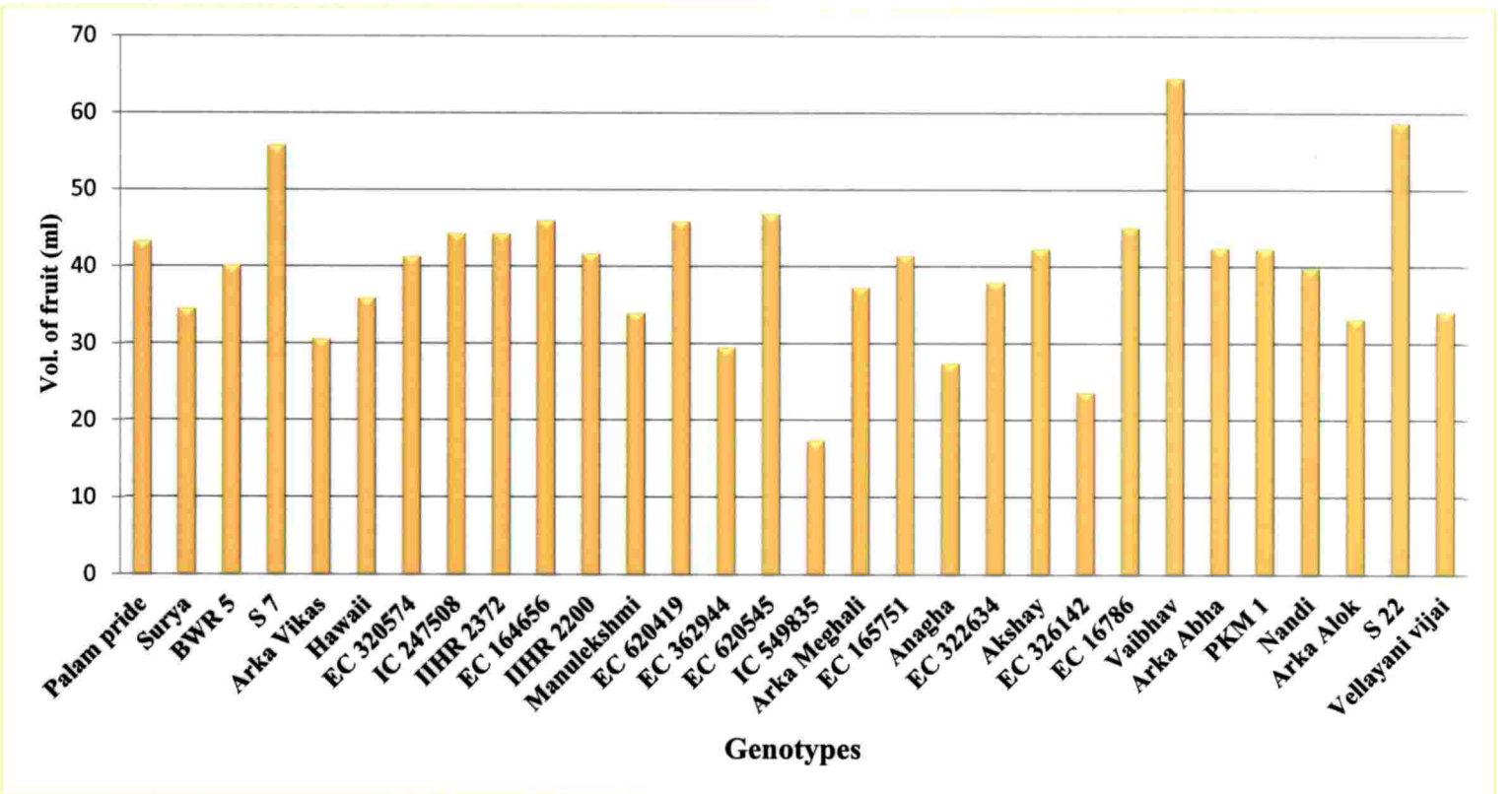


Fig 10. Volume of fruit (ml of water displaced) in various tomato genotype

(12.41 mg) followed by Akshay (11.60 mg), Anagha (10.45 mg), Manulekshmi (9.28 mg) and IIHR 2200 (8.50 mg), whereas the lowest content of lycopene was observed in EC 164656 (5.00 mg) followed by EC 322634 (4.93 mg) and EC 326142 (4.67 mg) respectively (Fig. 12).

Vitamin C (mg/ 100 g)

Vitamin C content ranged from 10.87 mg to 36.32 mg with a mean of 23.36 mg (Table 17 b) Highest content of vitamin C was observed in IIHR 2200 (36.23 mg) followed by Akshay (36.26 mg), Vaibhav (36.26), BWR 5 (36.23 mg) and EC 320574 (34.42 mg) all the five genotypes had no significant difference between them and were on par for this trait, Whereas lowest content of vitamin C was observed in EC 165751 (12.68 mg) followed by IC 549835 (12.68 mg) and EC 322634 (10.87 mg). The check variety Vellayani Vijai recorded (25.36 mg) content of vitamin C (Fig. 13).

Carotene (mg/100 g)

Carotene content ranged from 2.49 mg to 7.16 mg with a mean of 4.62 mg (Table 17 b). Highest content of carotene was observed in EC 326142 (7.16 mg) followed by IC 549835 (6.58 mg), EC 164656 (5.94 mg), EC 320574 (5.68 mg) and BWR 5 (5.66 mg), Genotypes EC 326142 and IC 549835 had no significant for carotene content, Genotypes EC 164656, EC 320574 and BWR 5 were on par for this trait, While lowest content of carotene was observed in IIHR 2200 (2.58 mg) followed by EC 620545 (2.53 mg) and Anagha (2.49 mg). Average of carotene content in check variety Vellayani Vijai was 3.04 mg (Fig. 14).

pH of juice

pH of juice ranged from 4.13 to 4.58 with a mean of 4.38 (Table 17 b). Highest pH was observed in EC 620545 (4.58) followed by Arka Alok (4.55), S22 (4.53), EC 362944 (4.52) and IC 549835 (4.51) whereas lowest pH was observed in S7 (4.28) followed by EC 320574 (4.25) and IIHR 2200 (4.13). Average of pH in check variety Vellayani Vijai was 4.38 (Fig. 15).

Total soluble solids (%)

Total soluble solids (%) ranged from 4.2% to 8.35% with a mean of 6.03% (Table 17 c). Highest total soluble solids (%) was observed in IIHR 2372 (8.35%)

followed by Arka Vikas (7.80%), Arka Alok (7.54%), EC 165751 (7.27%) and EC 326142 (7.23%), while lowest content of total soluble solids (%) was observed in IIHR 2200 (4.43%) followed by EC 164656 (4.23%) and EC 322634 (4.20%). The check variety Vellayani Vijai recorded (6.04%) of total soluble solids (Fig. 16).

Shelf life (days)

Shelf life ranged from 9.44 days to 17.44 days with a mean of 11.72 days (Table 17 c). Maximum shelf life was recorded in EC 16786 (17.44 days) followed by Vaibhav (16.33 days), PKM 1 (15.33 days), IIHR 2372 (14.66 days) and Akshay (14.55 days), Genotypes IIHR 2372 and Akshay had no significant difference between them and were on par. Whereas lowest shelf life was recorded in Nandi (9.66 days) followed by Palam Pride (9.55 days) and Arka Alok (9.44 days), Genotypes Nandi, Palam Pride and Arka Alok had no significant difference between them with respect to shelf life. Average of shelf life in check variety Vellayani Vijai was 10.89 days (Fig. 17).

4.2.2.3 Mean performance of wild genotypes for yield and fruit quality characters in tomato

Plant height (cm)

Plant height of the wild genotypes ranged from 115.12 cm to 160.50 cm with a mean of 145.69 cm (Table 18). The maximum plant height was recorded in EC 541109 and minimum plant height was recorded in IIHR 1970.

No. of primary branches plant^{-1}

Number of primary branches plant^{-1} in the wild genotypes ranged from 13.46 to 14.40 with a mean of 13.94 (Table 18). The maximum number of primary branches plant^{-1} was recorded in EC 541109 and minimum number of primary branches plant^{-1} was recorded in IIHR 1970.

Spread of plant (cm)

Spread of plant ranged from 76.11 cm to 89.56 cm with a mean of 82.14 cm (Table 18). The maximum spread of plant was recorded in EC 168283 and minimum spread of plant was recorded in LA 2805.

Table 17 b. Mean performance of 30 genotypes for seventeen characters in tomato

Sr. No.	Genotype	Pericarp thickness (mm)	Lycopene (mg/100g)	Vitamin C (mg/100 g)	Carotene (mg/100 g)	pH of juice
1	Palam Pride	6.34	7.50	23.55	4.89	4.36
2	Surya	4.32	6.38	19.93	5.35	4.28
3	BWR 5	4.38	7.18	36.23	5.66	4.45
4	S 7	5.21	6.28	21.74	3.96	4.28
5	Arka Vikas	4.95	7.44	14.49	4.04	4.45
6	Hawaii	4.29	6.75	16.30	4.95	4.33
7	EC 320574	4.35	5.77	34.42	5.68	4.25
8	IC 247508	6.56	7.84	30.80	4.58	4.34
9	IIHR 2372	9.35	8.33	34.42	5.40	4.31
10	EC 164656	4.64	5.00	14.49	5.94	4.30
11	IIHR 2200	6.31	8.50	36.23	2.58	4.13
12	Manulekshmi	4.33	9.28	21.74	4.65	4.37
13	EC 620419	7.84	6.58	16.30	4.96	4.39
14	EC 362944	6.57	6.95	25.36	3.98	4.52
15	EC 620545	6.29	5.36	27.17	2.53	4.58
16	IC 549835	3.25	5.67	12.68	6.58	4.51
17	Arka Meghali	4.75	5.95	21.74	5.48	4.43
18	EC 165751	4.79	5.26	12.68	4.39	4.46
19	Anagha	6.04	10.45	16.30	2.49	4.37
20	EC 322634	3.94	4.93	10.87	4.96	4.38
21	Akshay	5.29	11.60	36.23	4.96	4.28
22	EC 326142	3.72	4.67	25.36	7.16	4.48
23	EC 16786	5.51	7.51	16.30	5.14	4.35
24	Vaibhav	7.49	7.63	36.23	3.31	4.41
25	Arka Abha	5.91	6.28	23.55	4.59	4.46
26	PKM 1	4.49	6.75	18.11	4.51	4.29
27	Nandi	4.82	7.42	19.93	4.37	4.47
28	Arka Alok	4.77	6.79	30.80	5.31	4.55
29	S 22	6.17	8.15	21.74	3.31	4.53
30	Vellayani vijay	4.29	12.41	25.36	3.04	4.38
	Mean	5.36	7.22	23.36	4.62	4.38
	C.D. (5%)	0.12	0.16	4.56	0.59	0.02
	S.E (m)	0.04	0.06	1.61	0.21	0.01
	C.V.	1.37	1.33	11.96	7.80	0.21

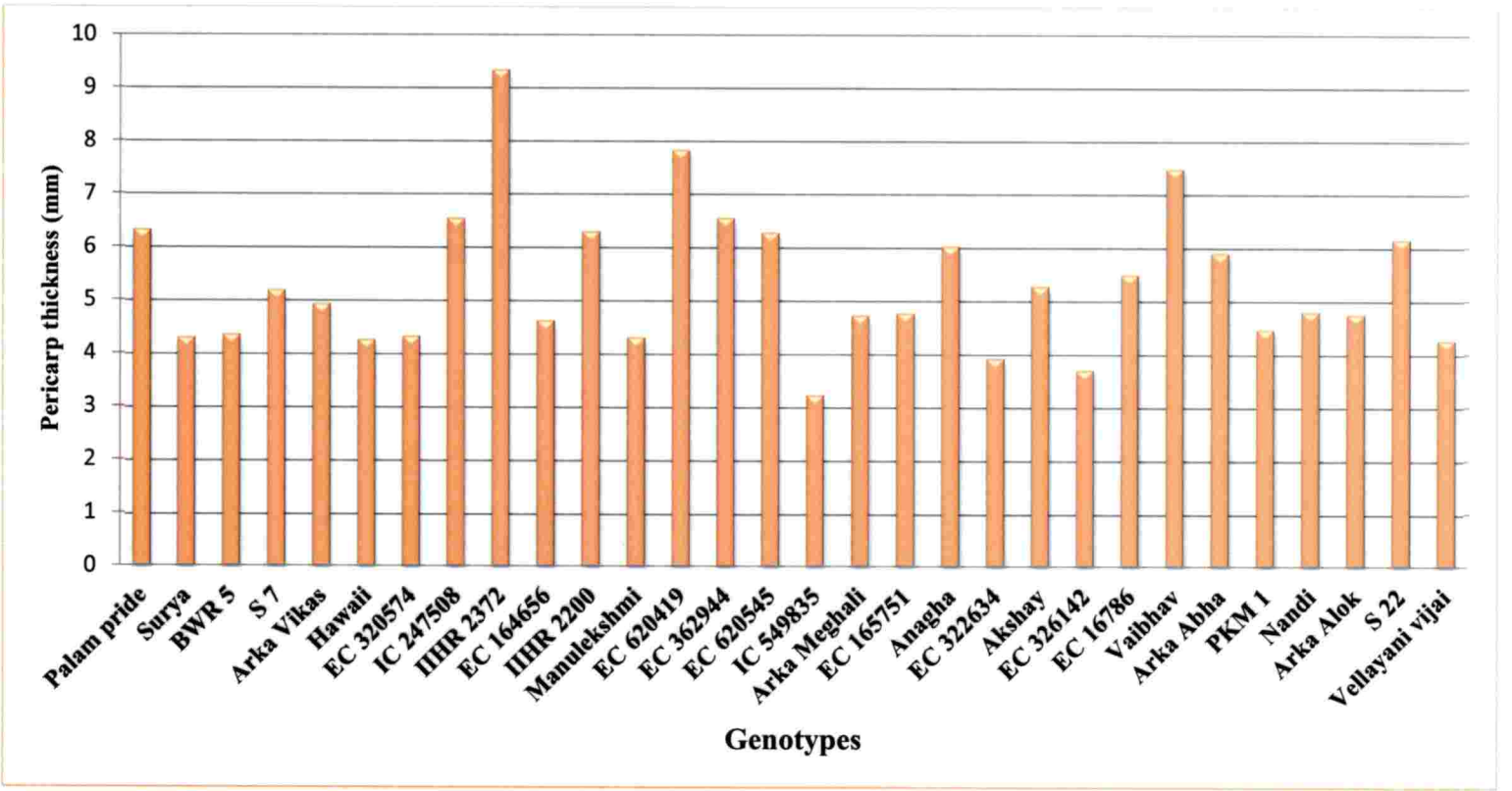


Fig 11. Pericarp thickness (mm) in various tomato genotypes

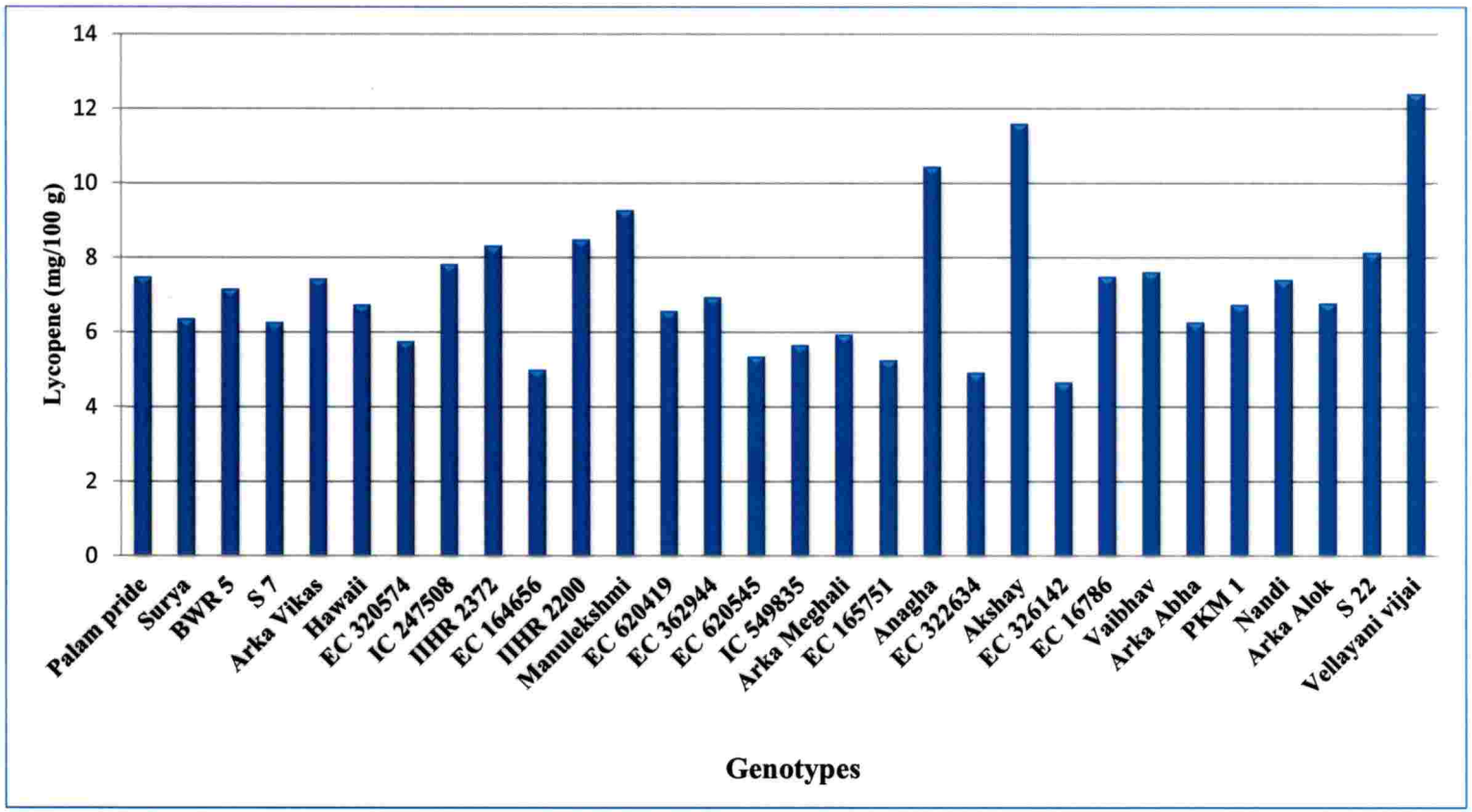


Fig 12. Lycopene (mg/100 g) in various tomato genotypes

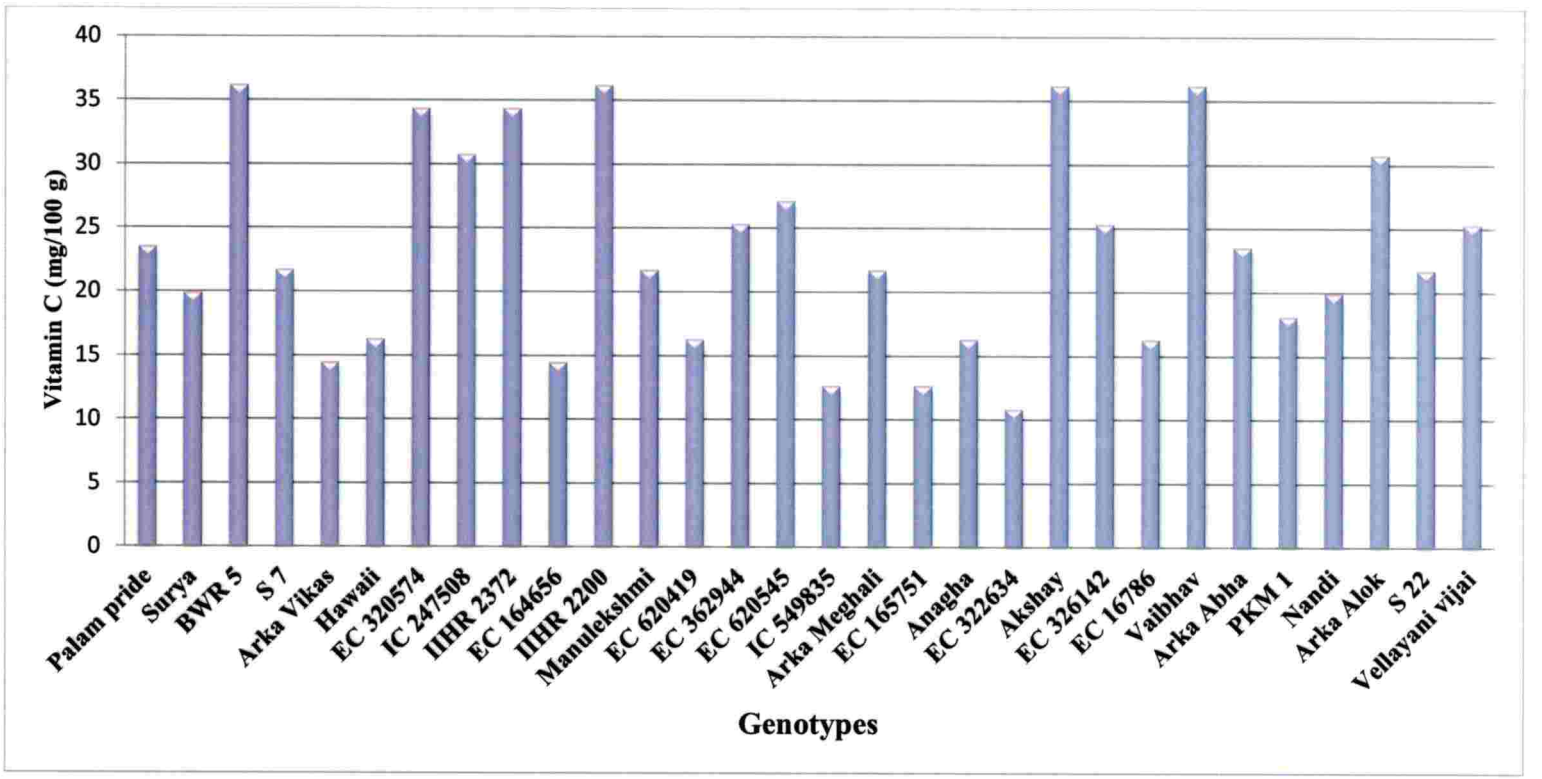


Fig 13. Vitamin C (mg/100 g) in various tomato genotypes

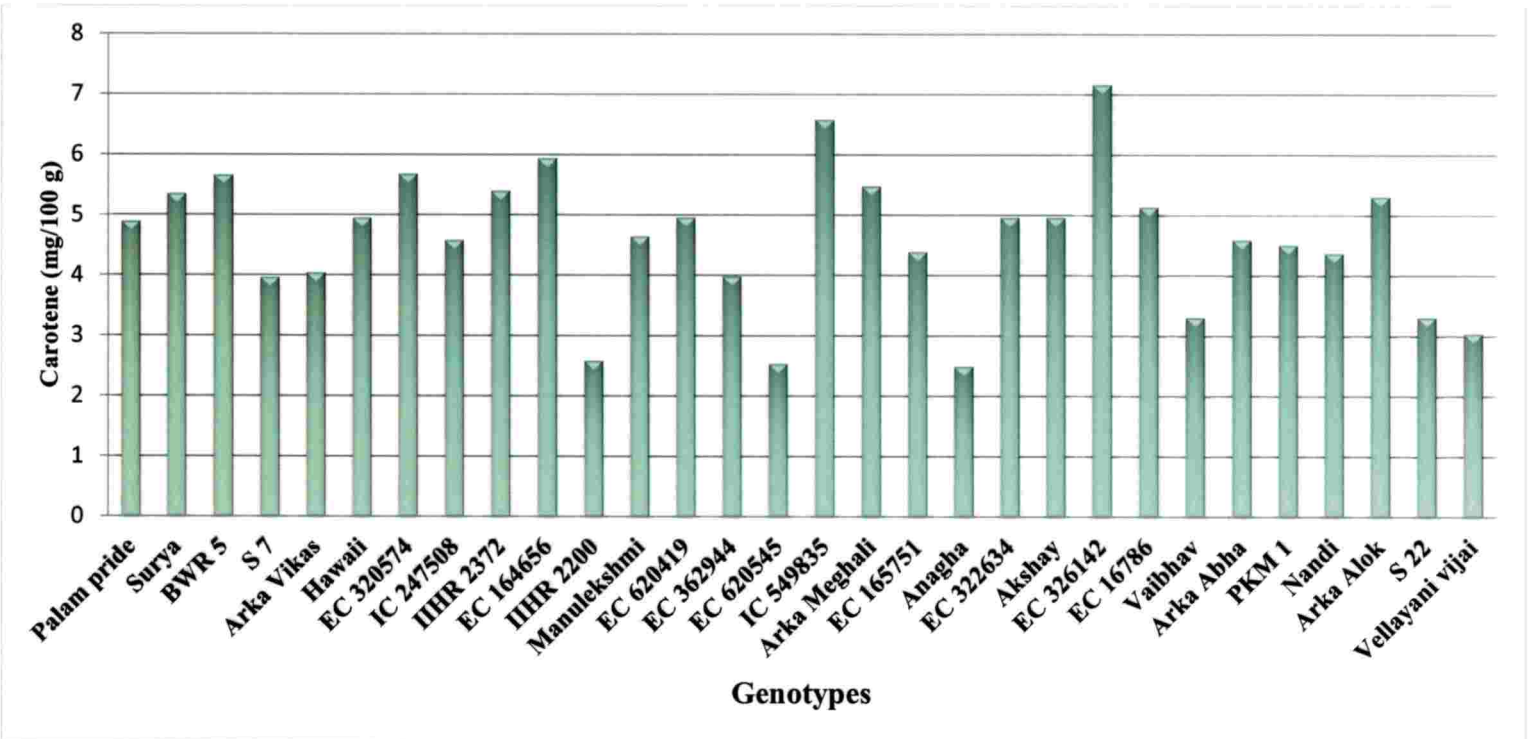


Fig 14. Carotene (mg/100 g) in various tomato genotypes

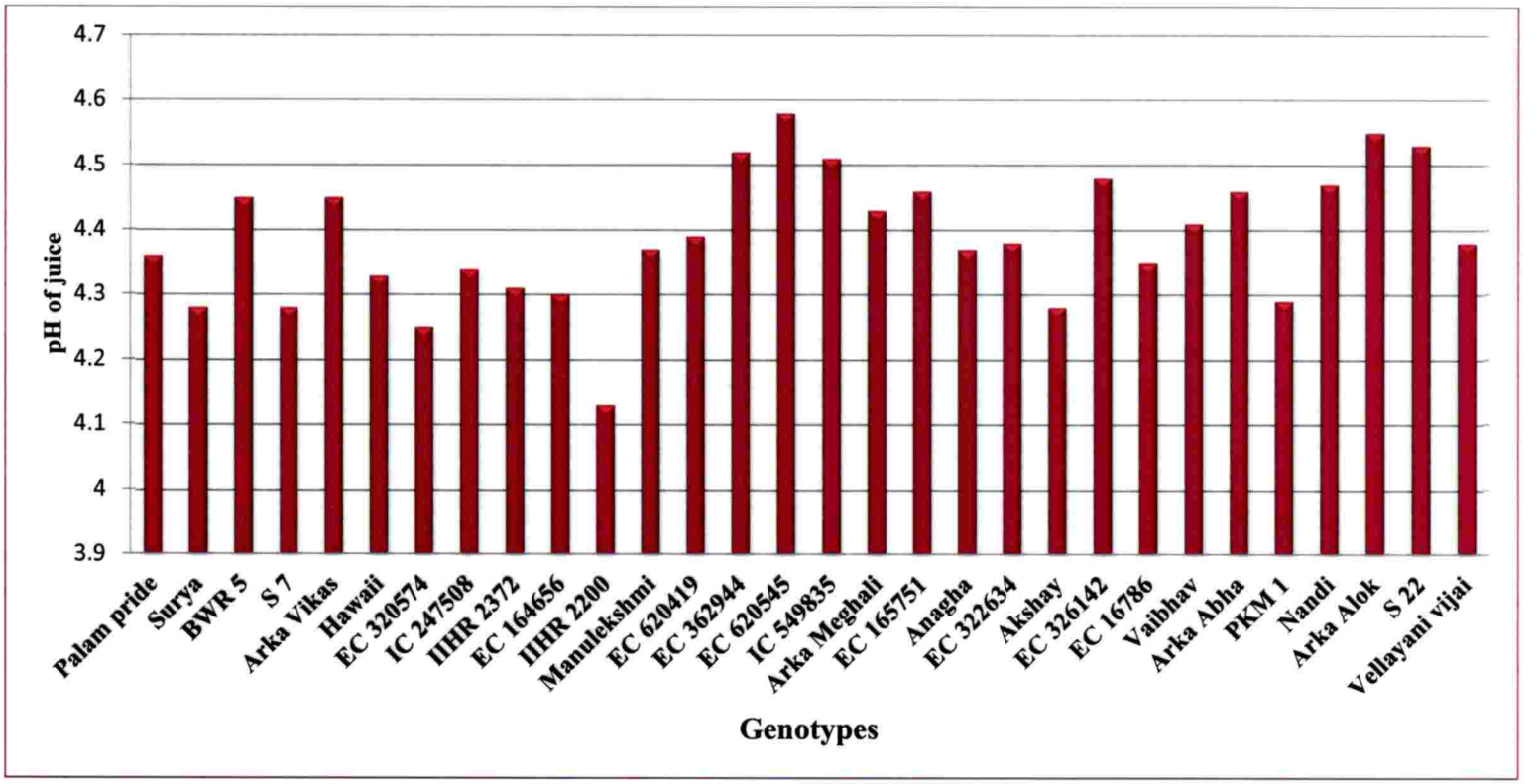


Fig 15. pH of juice in various tomato genotypes

Table 17 c. Mean performance of 30 genotypes for seventeen characters in tomato

Sr. No	Genotype	TSS (%)	Shelf life (days)
1	Palam Pride	6.45	9.55
2	Surya	6.01	10.66
3	BWR 5	4.45	10.44
4	S 7	6.79	11.66
5	Arka Vikas	7.80	10.66
6	Hawaii	5.44	12.78
7	EC 320574	5.57	10.89
8	IC 247508	6.21	9.78
9	IIHR 2372	8.35	14.66
10	EC 164656	4.23	10.66
11	IIHR 2200	4.43	11.89
12	Manulekshmi	6.48	10.78
13	EC 620419	5.23	11.33
14	EC 362944	5.77	10.89
15	EC 620545	5.79	12.33
16	IC 549835	6.24	10.55
17	Arka Meghali	6.05	10.78
18	EC 165751	7.27	9.77
19	Anagha	6.41	11.44
20	EC 322634	4.20	10.67
21	Akshay	6.23	14.55
22	EC 326142	7.23	12.89
23	EC 16786	5.22	17.44
24	Vaibhav	6.20	16.33
25	Arka Abha	5.77	10.44
26	PKM 1	5.83	15.33
27	Nandi	6.31	9.66
28	Arka Alok	7.54	9.44
29	S 22	5.59	12.55
30	Vellayani vijay	6.04	10.89
	Mean	6.03	11.72
	C.D. (5%)	0.06	0.75
	S.E (m)	0.02	0.27
	C.V.	0.61	3.91

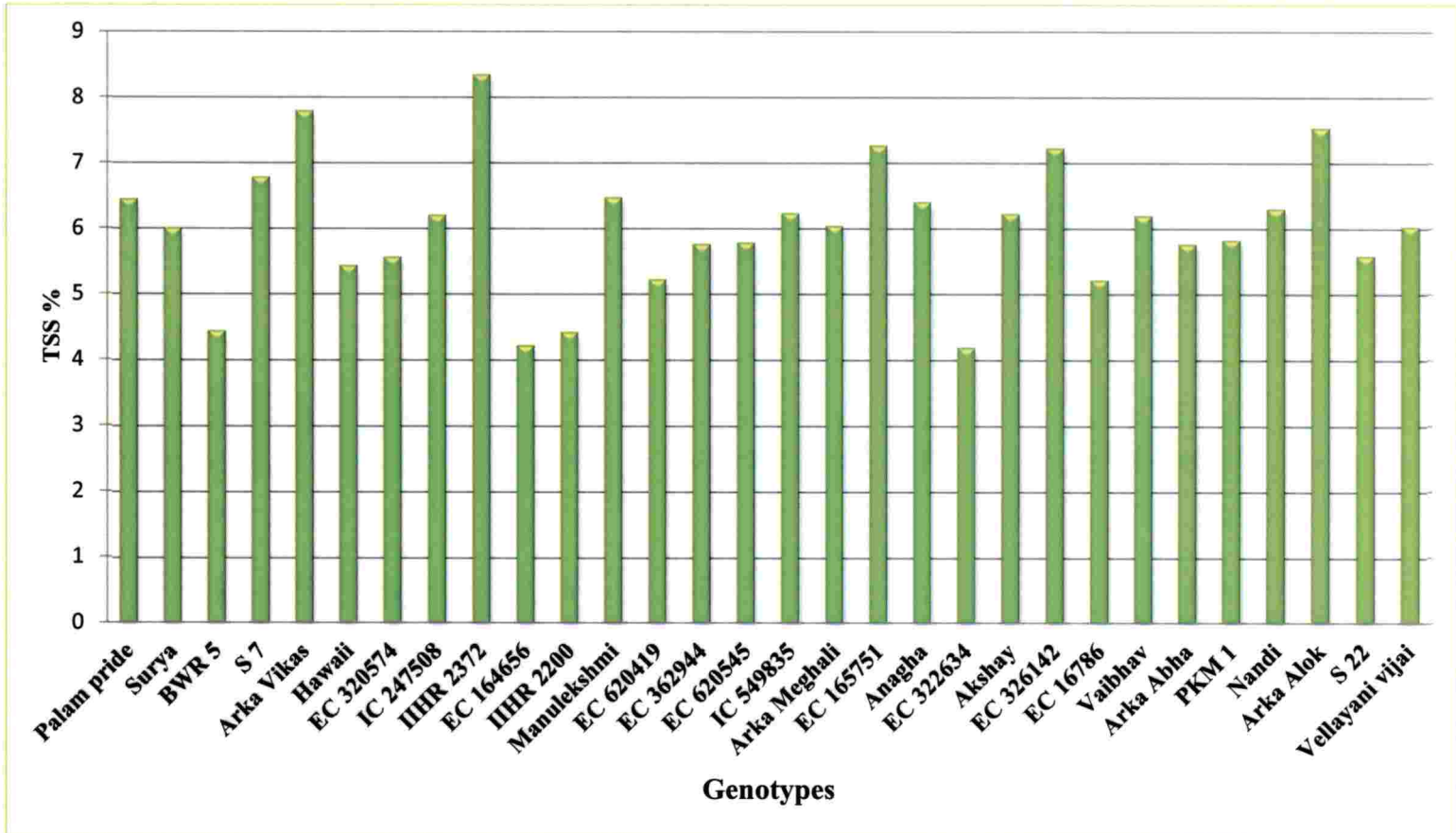


Fig 16. TSS% in various tomato genotypes

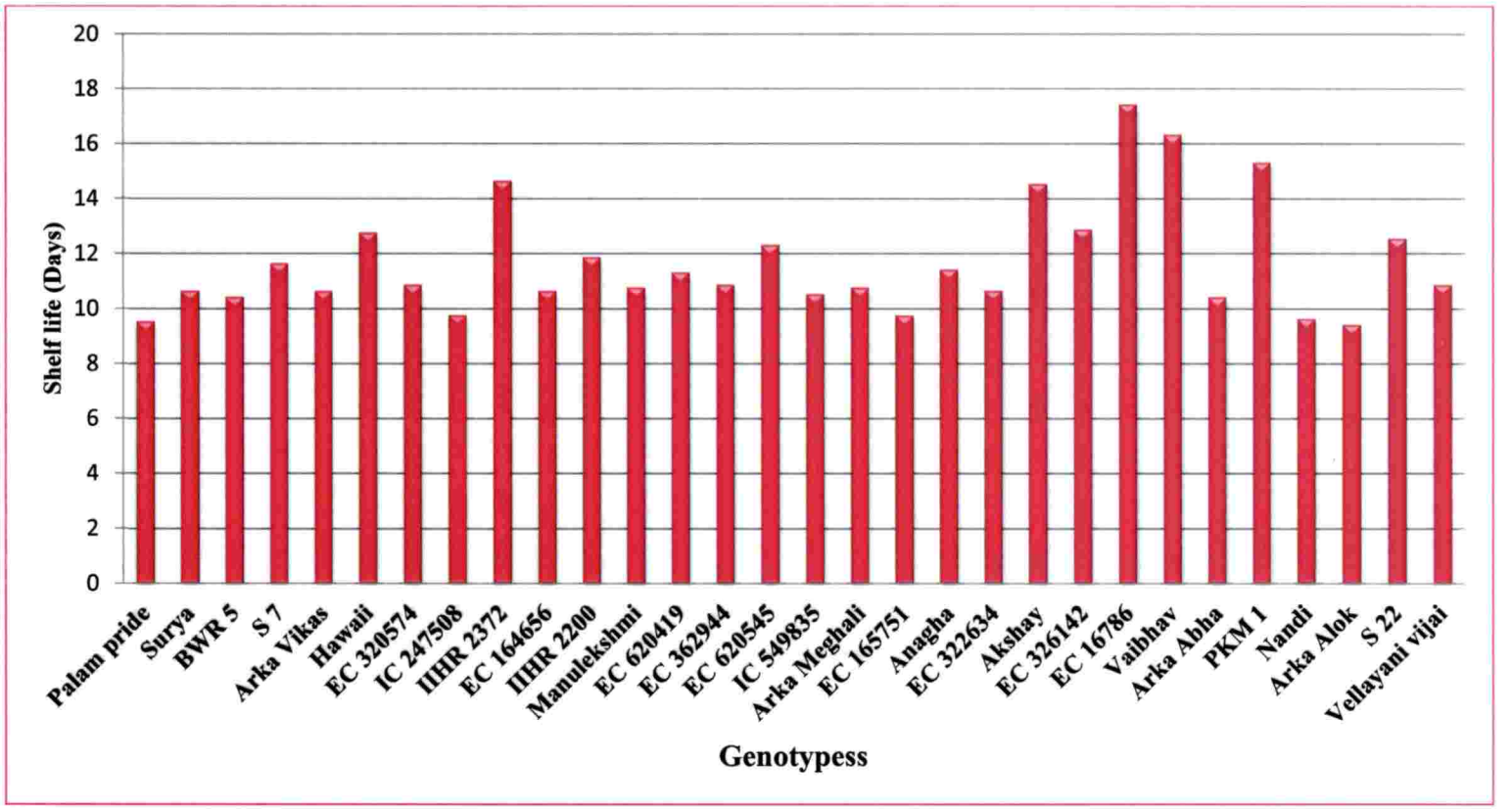


Fig 17. Shelf life (Days) in various tomato genotypes

No. of days to 50% flowering

Number of days to 50% flowering ranged from 42.27 days to 51.07 days with a mean of 46.68 days (Table 18). The minimum number of days to 50% flowering was observed in EC 168283 and maximum minimum number of days to 50% flowering was observed in IIHR 1970.

No. of days to first fruit harvest

Number of days to first fruit harvest ranged from 71.40 days to 75.93 days with a mean of 73.58 days (Table 18). The minimum number of days to first fruit harvest was observed in EC 168283 and maximum number of days to first fruit harvest was observed in IIHR 1970.

No. of fruits plant^{-1}

Number of fruits plant^{-1} ranged from 214.27 to 397.20 with a mean of 279.2 (Table 18). The maximum number of fruits plant^{-1} was observed in EC 541109 and minimum number of fruits plant^{-1} was recorded in IIHR 1970.

Weight of fruit plant^{-1} (kg)

Weight of fruit plant^{-1} ranged from 0.41 kg to 0.96 kg with a mean of 0.70 kg (Table 18). The maximum weight of fruit plant^{-1} was observed in EC 541109 and minimum weight of fruit plant^{-1} was recorded in IIHR 1970.

Weight of fruit (g)

Weight of fruit ranged from 2.11 g to 2.83 g with a mean of 2.49 g (Table 18). The maximum weight of fruit was recorded in EC 168283 and minimum weight of fruit was recorded in IIHR 1970.

Number of locules fruit^{-1}

Number of locules fruit^{-1} was 2 and same for all wild genotypes (Table 18).

Volume of the fruit (ml)

Volume of the fruit ranged from 2.58 ml to 2.72 ml with a mean of 2.63 ml (Table 18). The maximum volume of the fruit was recorded in LA 2805 and and minimum volume of the fruit was recorded in IIHR 1970.

Pericarp thickness (mm)

Pericarp thickness ranged from 2.15 mm to 2.72 mm with a mean of 2.32 mm (Table. 18 a). The maximum pericarp thickness was recorded in LA 2805 and minimum pericarp thickness was recorded in EC 168283.

Lycopene (mg/100 g)

Lycopene content ranged from 4.55 mg to 13.58 mg with a mean of 9.86 mg (Table 18 a). The maximum lycopene content was recorded in EC 541109 and minimum lycopene content was recorded in IIHR 1970.

Vitamin C (mg/100g)

Vitamin C content ranged from 21.74 mg to 30.80 mg with a mean of 26.25 mg (Table 18 a). The maximum vitamin C content was recorded in EC 168283 and minimum vitamin C content was recorded in LA 2805.

Carotene (mg/100 g)

Carotene content ranged from 3.47 mg to 9.35 mg with a mean of 6.81 mg (Table 18 a). The maximum carotene content was recorded in EC 541109 and minimum carotene content was recorded in IIHR 1970.

pH of juice

pH of juice ranged from 4.37 to 4.57 with a mean of 4.49 (Table 18 a). The maximum pH of juice was recorded in EC 541109, LA 2805 and minimum pH of juice was recorded in EC 168283.

TSS (%)

TSS content ranged from 4.41% to 10.34% with a mean of 7.52% (Table 18 a). The maximum TSS content was recorded in EC 541109 and minimum TSS content was recorded in EC 168283.

Shelf life (days)

Shelf life ranged from 10.89 days to 12.55 days with a mean of 11.85 days (Table 18 a). The maximum shelf life was recorded in EC 541109 and minimum shelf life was recorded in IIHR 1970.

Table 18. Mean performance of wild genotypes for yield and fruit quality characters in tomato

Sr. No	Genotype	Plant height (cm)	No. of primary branches plant^{-1}	Spread of plant (cm)	No. of days to 50% flowering	No. of days to first fruit harvest	No. of fruits plant^{-1}	Weight of fruit plant^{-1} (kg)	Weight of fruit (g)	No. of locules fruit^{-1}	Vol. of the fruit (ml)
1	EC 168283	149.27	13.60	89.56	42.27	71.40	269.53	0.81	2.83	2.00	2.65
2	IIHR 1970	115.12	13.46	76.33	51.07	75.93	214.27	0.41	2.11	2.00	2.58
3	EC 541109	160.50	14.40	86.56	47.73	75.53	397.20	0.96	2.57	2.00	2.60
4	LA 2805	157.90	14.31	76.11	45.67	71.47	235.80	0.62	2.48	2.00	2.72
	Mean	145.69	13.94	82.14	46.68	73.58	279.2	0.70	2.49	2.0	2.63
	C.D. (5%)	2.325	0.679	1.600	2.458	1.023	15.321	35.27	0.155	-	0.057
	S.E (m)	0.659	0.192	0.454	0.697	0.290	4.343	10.00	0.044	-	0.016
	C.V.	0.783	2.390	0.957	2.585	0.683	2.694	2.452	3.047	-	1.069

Table 18 a. Mean performance of wild genotypes for yield and fruit quality characters in tomato

Sr. No	Genotype	Pericarp thickness (mm)	Lycopene (mg/100 g)	Vitamin C (mg/100 g)	Carotene (mg/100 g)	pH of juice	TSS (%)	Shelf life (days)
1	EC 168283	2.15	9.24	30.80	5.36	4.37	4.41	12.44
2	IIHR 1970	2.16	4.55	27.12	3.47	4.46	5.14	10.89
3	EC 541109	2.27	13.58	25.36	9.35	4.57	10.34	12.55
4	LA 2805	2.72	12.10	21.74	9.09	4.57	10.19	11.55
	Mean	2.32	9.86	26.25	6.81	4.49	7.52	11.85
	C.D. (5%)	0.114	0.311	3.690	0.506	0.014	0.082	0.443
	S.E (m)	0.032	0.088	1.046	0.143	0.004	0.023	0.126
	C.V.	2.399	1.547	6.900	3.644	0.154	0.536	1.833

4.2.3 Variability, Heritability (h^2) and Genetic advance

The results pertaining to grand mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in sense (h^2) and expected genetic advance as per cent of mean (GA) for all the seventeen characters are furnished in Table 19 to 19 a. (Fig. 18 and Fig. 19). The character wise details of these variability parameters are presented below (Plate 6 to 6c and 7).

Plant height (cm)

Plant height shown high PCV and GCV of 29.58 and 29.52 per cent, High estimates of heritability (99.61%), genetic advance (61.07) and GA as per cent of mean (60.70) for this character (Table 19).

Number of primary branches plant^{-1}

The genotypes recorded high estimates of PCV (32.23%), GCV (32.12%) and High heritability (98.96%), low genetic advance (6.14) and High GA as per cent of mean (65.82) for number of primary branches plant^{-1} (Table 19).

Spread of the plant (cm)

The genotypes recorded moderate PCV (18.12) GCV (18.08) and High heritability (99.62%), Moderate genetic advance (24.67) and High GA as per cent of mean (37.18) for spread of the plant (Table 19).

Number of days to 50% flowering

The genotypes recorded Moderate PCV (10.72) GCV (10.58), High heritability (97.54%), low genetic advance (7.44) and high GA as per cent of mean (21.54) for number of days to 50% flowering (Table 19).

Number of days to first fruit harvest

Low PCV (7.11), Low GCV (7.03), High heritability (97.79%), low genetic advance and Moderate GA as per cent of mean (14.32) for number of days to first fruit harvest (Table 19).

Number of fruits plant^{-1}

Number of fruits plant^{-1} had high estimates of PCV (36.95), GCV (36.78), High heritability (99.08%), High genetic advance and High GA as per cent of mean 75.43 (Table 19).

Weight of fruits plant $^{-1}$ (Kg)

The genotypes recorded high PCV (45.91), GCV (45.86), High heritability (99.78%), Low genetic advance 91.12) and high GA as per cent of mean (94.37) for weight of fruits plant $^{-1}$ (Table 19).

Weight of fruit (g)

The genotypes recorded high PCV (22.69), GCV (22.49), High heritability (98.21%), Moderate genetic advance (19.08) and high GA as per cent of mean (45.91) for weight of fruit (Table 19).

Number of locules fruit $^{-1}$

The genotypes recorded high PCV (30.49), GCV (29.66), High heritability (94.60%), Low genetic advance and high GA as per cent of mean (59.42) for number of locules fruit $^{-1}$ (Table 19 a) and (Plate 8 to 8c).

Volume of fruit (ml of water displaced)

The genotypes recorded high PCV (24.38), GCV (24.18), High heritability (98.41%), Moderate genetic advance (19.80) and high GA as per cent of mean (49.42) for volume of fruit (Table 19 a).

Pericarp thickness

The genotypes recorded high PCV (24.99), GCV (24.96), High heritability (99.70%), Low genetic advance (2.75) and high GA as per cent of mean (51.34) recorded for pericarp thickness (Table 19 a)

Lycopene (mg/100g)

High PCV (25.61), GCV (25.57), High heritability (99.73%), Low genetic advance (3.79) and high GA as per cent of mean (52.61) recorded for lycopene content (Table 19 a).

Vitamin C (mg/ 100 g)

The genotypes recorded high PCV (35.51), GCV (33.44), High heritability (88.67%), Moderate genetic advance (15.16) and high GA as per cent of mean (64.87) for vitamin C content (Table 19 a).

Carotene (mg/100 g)

High PCV (25.46), GCV (24.24), High heritability (90.63%), Low genetic advance (2.19) and high GA as per cent of mean (47.54) for carotene content (Table 19 a).

Total soluble solids (%)

The genotypes recorded moderate PCV (16.58) GCV (16.57) and High heritability (99.87%), Low genetic advance (2.06) and High GA as per cent of mean (34.12) for total soluble solids (Table 19 a).

pH of juice

The genotypes did not differ significantly for this character and recorded Low PCV (2.32), GCV (2.31), High heritability (99.13%), Low genetic advance (0.20) and High GA as per cent of mean (47.46) for pH of juice (Table 19 a).

Shelf life (days)

The genotypes recorded moderate PCV (17.79) GCV (17.36) and High heritability (95.17%), Low genetic advance (4.09) and High GA as per cent of mean (34.89) for shelf life (Table 19 a).

4.2.4 Phenotypic and genotypic correlation coefficient analysis

The genotypic and phenotypic correlation coefficient for fruit yield and its component characters in tomato are presented in Table 20 & 21 and only significant correlations are discussed here

4.2.4.1 Genotypic Correlation among fruit yield and its associated traits

Plant height (cm)

Plant height showed highly positive significant genotypic correlation with number of primary branches plant^{-1} (0.867), spread of the plant (0.803), number of days to 50% flowering (0.642), number of days to first fruit harvest (0.680), number of fruits

Table. 19. Estimates of variability, heritability and genetic advance as per cent of mean for seventeen characters in 30 genotypes of tomato

Character	Range		Mean ± S.Em.	Variance		Coefficient of variation (%)		h^2 (%)	Genetic Advance	GA(%) of mean
	Minimum	Maximum		Phenotypic	Genotypic	Phenotypic	Genotypic			
Plant height (cm)	58.51	153.46	100.60 ± 1.07	885.92	882.45	29.58	29.52	99.61	61.07	60.70
No. of primary branches plant^{-1}	4.33	14.40	9.33 ± 0.17	9.08	8.98	32.28	32.12	98.96	6.14	65.82
Spread of plant (cm)	43.88	84.44	66.36 ± 0.42	144.63	144.08	18.12	18.08	99.62	24.67	37.18
No. of days to 50% flowering	29.66	43.13	34.55 ± 0.33	13.72	13.38	10.72	10.58	97.54	7.44	21.54
No. of days to first fruit harvest	58	74.40	66.50 ± 0.40	21.87	22.37	7.11	7.03	97.79	9.52	14.32
No. of fruits plant $^{-1}$	14.33	47.36	30.05 ± 0.61	123.35	122.22	36.95	36.78	99.08	22.66	75.43
Weight of fruit plant $^{-1}$ (kg)	0.49	2.41	1.19 ± 15.08	0.29	0.29	45.91	45.86	99.78	1.12	94.37
Weight of fruit (g)	19.31	61.46	41.56 ± 0.72	88.98	87.39	22.69	22.49	98.21	19.08	45.91

Table. 19 a. Estimates of variability, heritability and genetic advance as per cent of mean for seventeen characters in 30 genotypes of tomato

Character	Range		Mean ±S.Em	Variance		Coefficient of variation (%)		h^2	Genetic Advance	GA (%) of mean
	Minimum	Maximum		Phenotypic	Genotypic	Phenotypic	Genotypic			
No. of locules fruit $^{-1}$	2	4.99	3.58 ± 0.14	1.19	1.12	30.49	29.66	94.60	2.12	59.42
Vol. of the fruit	17.38	64.64	40.06 ± 0.71	95.41	93.89	24.38	24.18	98.41	19.80	49.42
Pericarp Thickness (mm)	3.25	9.34	5.36 ± 0.04	1.79	1.79	24.99	24.96	99.70	2.75	51.34
Lycopene (mg/100g)	7.49	12.40	7.22 ± 0.05	3.41	3.41	25.61	25.57	99.73	3.79	52.61
Vitamin C (mg/100 g)	10.87	36.23	23.36 ± 1.61	68.88	61.08	35.51	33.44	88.67	15.16	64.87
Carotene (mg/100 g)	2.49	7.16	4.62 ± 0.20	1.38	1.25	25.46	24.24	90.63	2.19	47.54
pH of juice	4.13	4.58	4.38 ± 0.005	0.01	0.01	2.32	2.31	99.13	0.20	47.46
TSS (%)	4.2	8.35	6.037 ± 0.02	1.00	1.00	16.58	16.57	99.87	2.06	34.12
Shelf life (Days)	9.44	17.44	11.72 ± 0.26	4.35	4.14	17.79	17.36	95.17	4.09	34.89

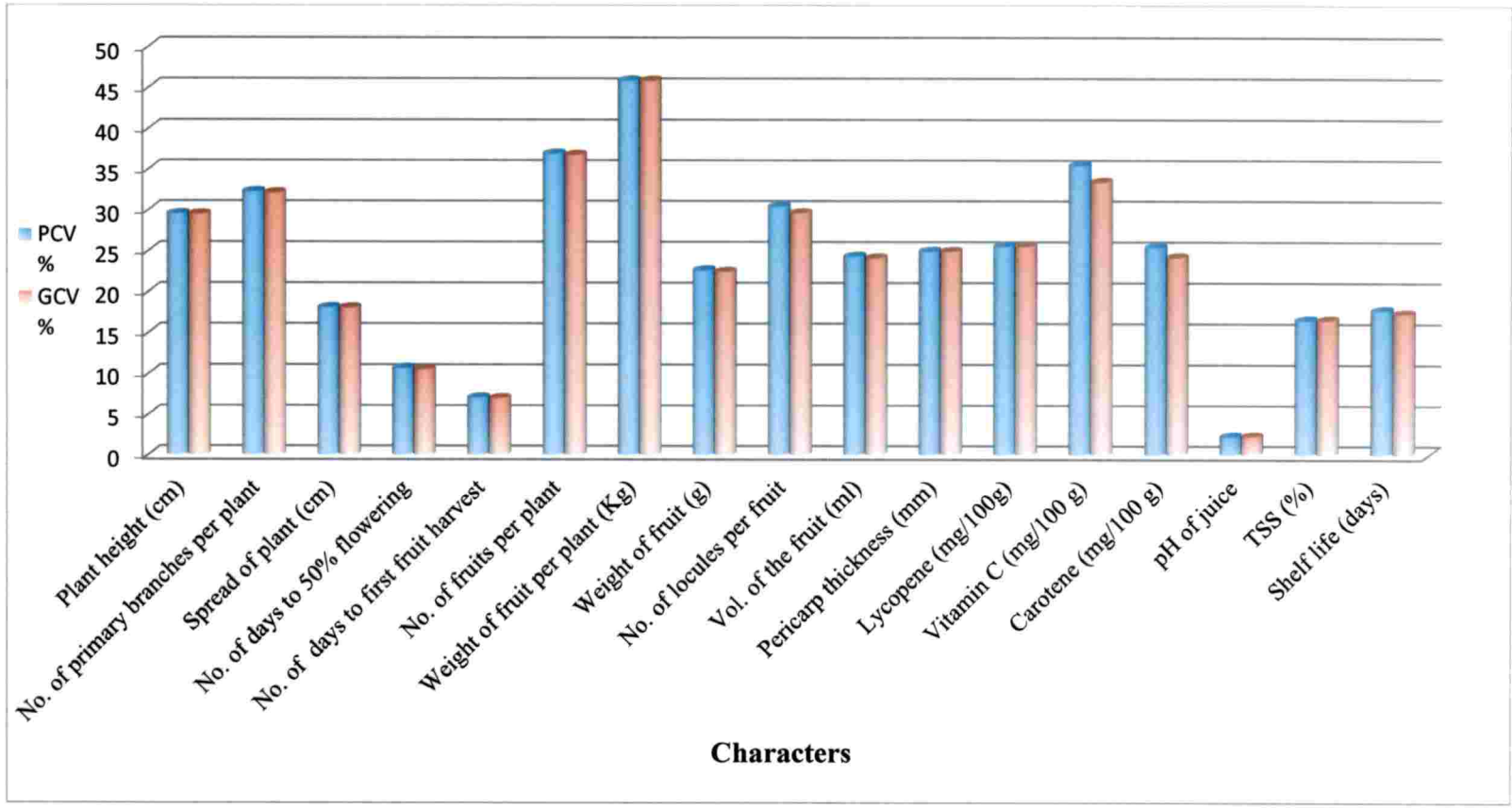


Fig 18. PCV and GCV for seventeen characters in tomato

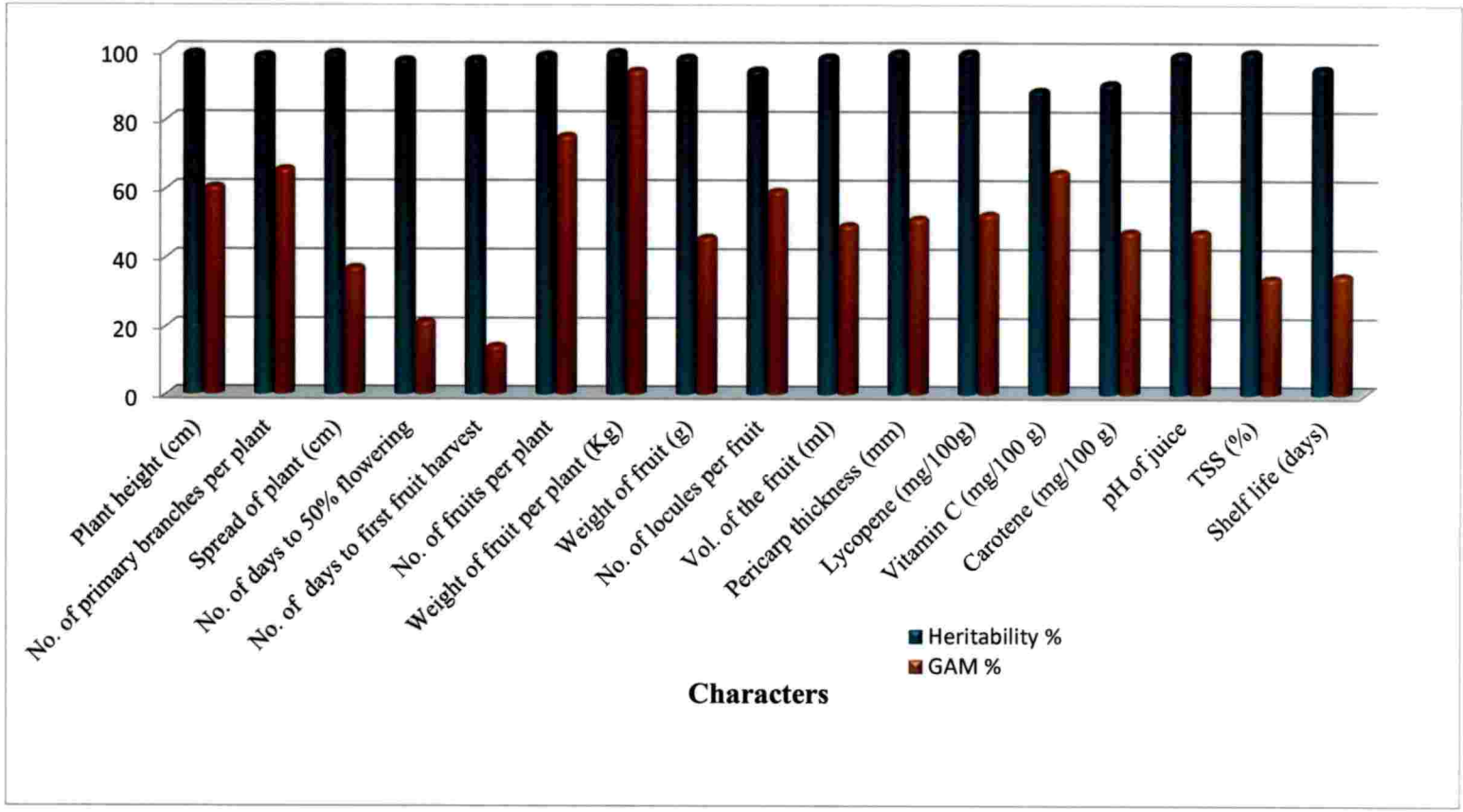
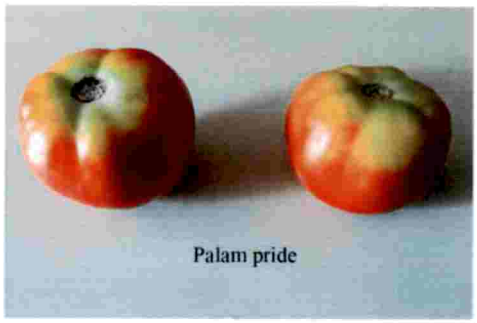
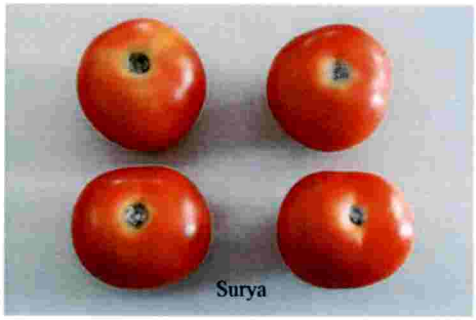
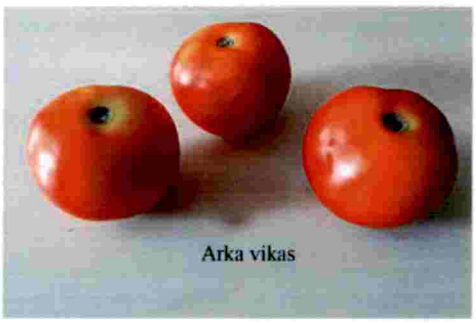


Fig 19. Heritability (h^2) and Genetic advance as percent mean for seventeen characters in tomato







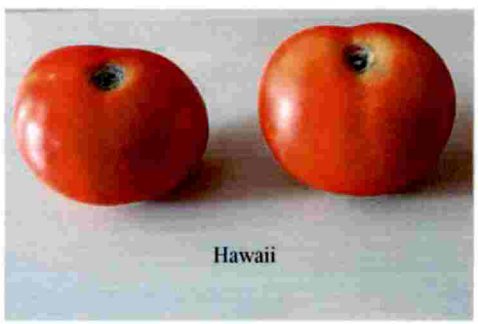
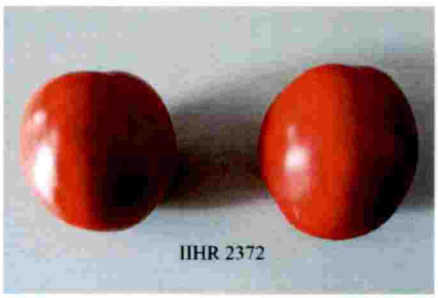


Plate 6. Variability of fruits in different tomato genotypes



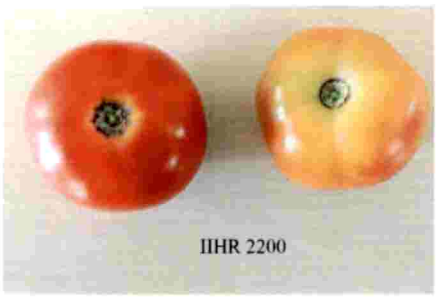
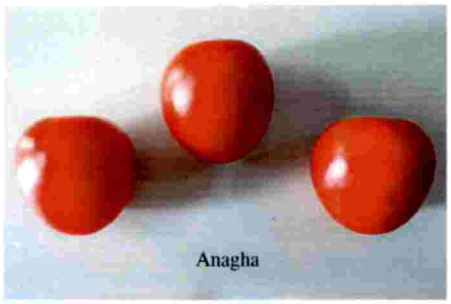
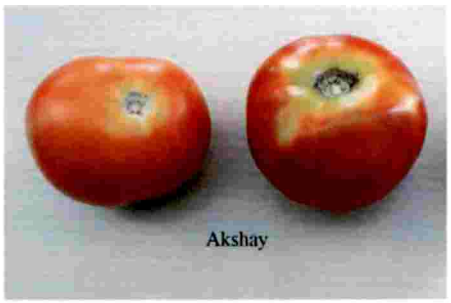


Plate 6 a. Variability of fruits in different tomato genotypes





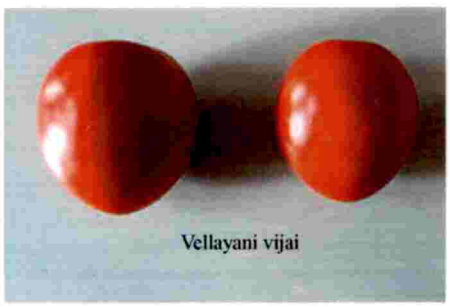


Plate 6 b. Variability of fruits in different tomato genotypes

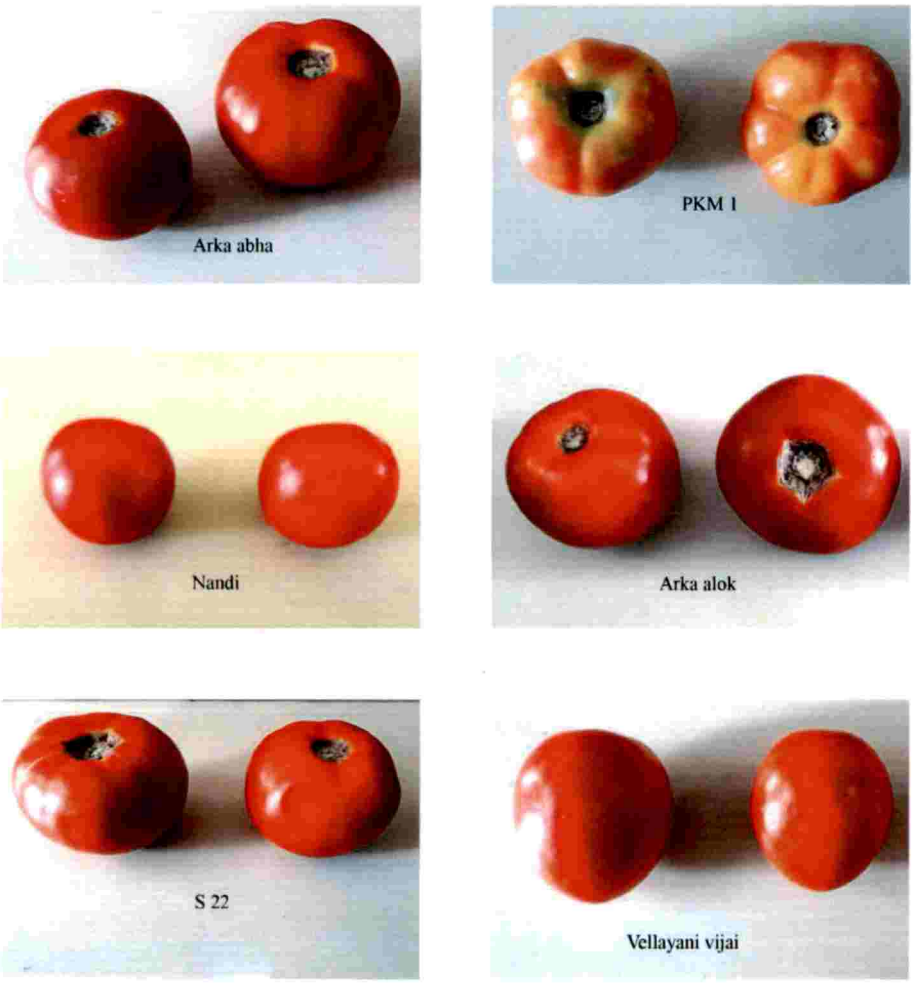


Plate 6 c. Variability in different tomato genotypes

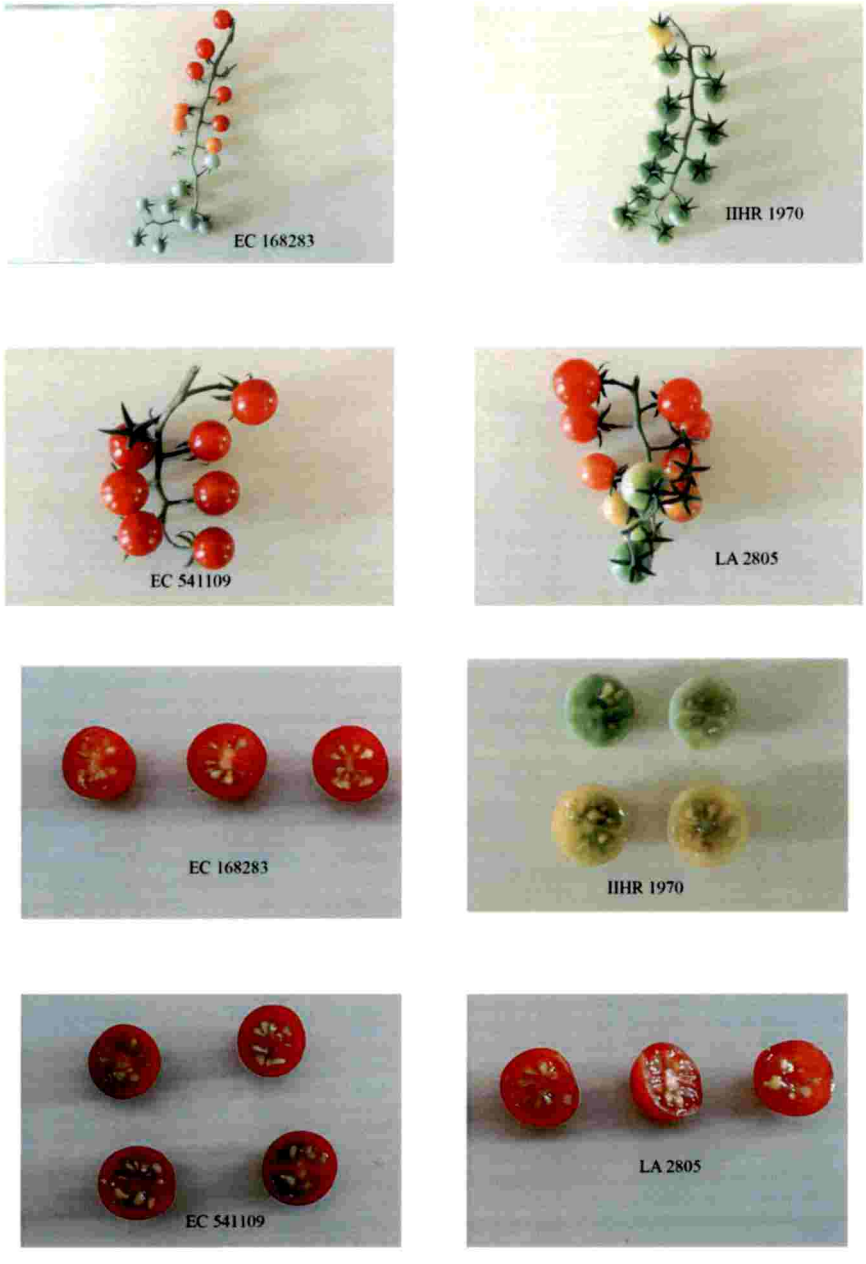


Plate 7. Variability in different fruits & locules of wild tomato genotypes

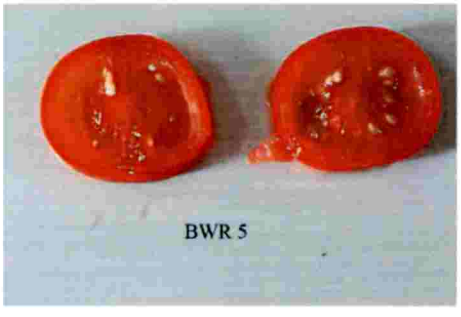
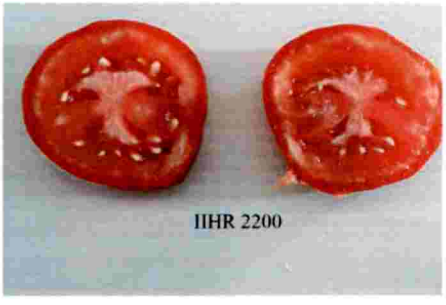


Plate 8. Variability in locules of different tomato genotypes



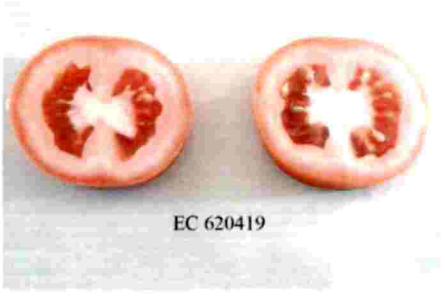


Plate 8 a. Variability in locules of different tomato genotypes

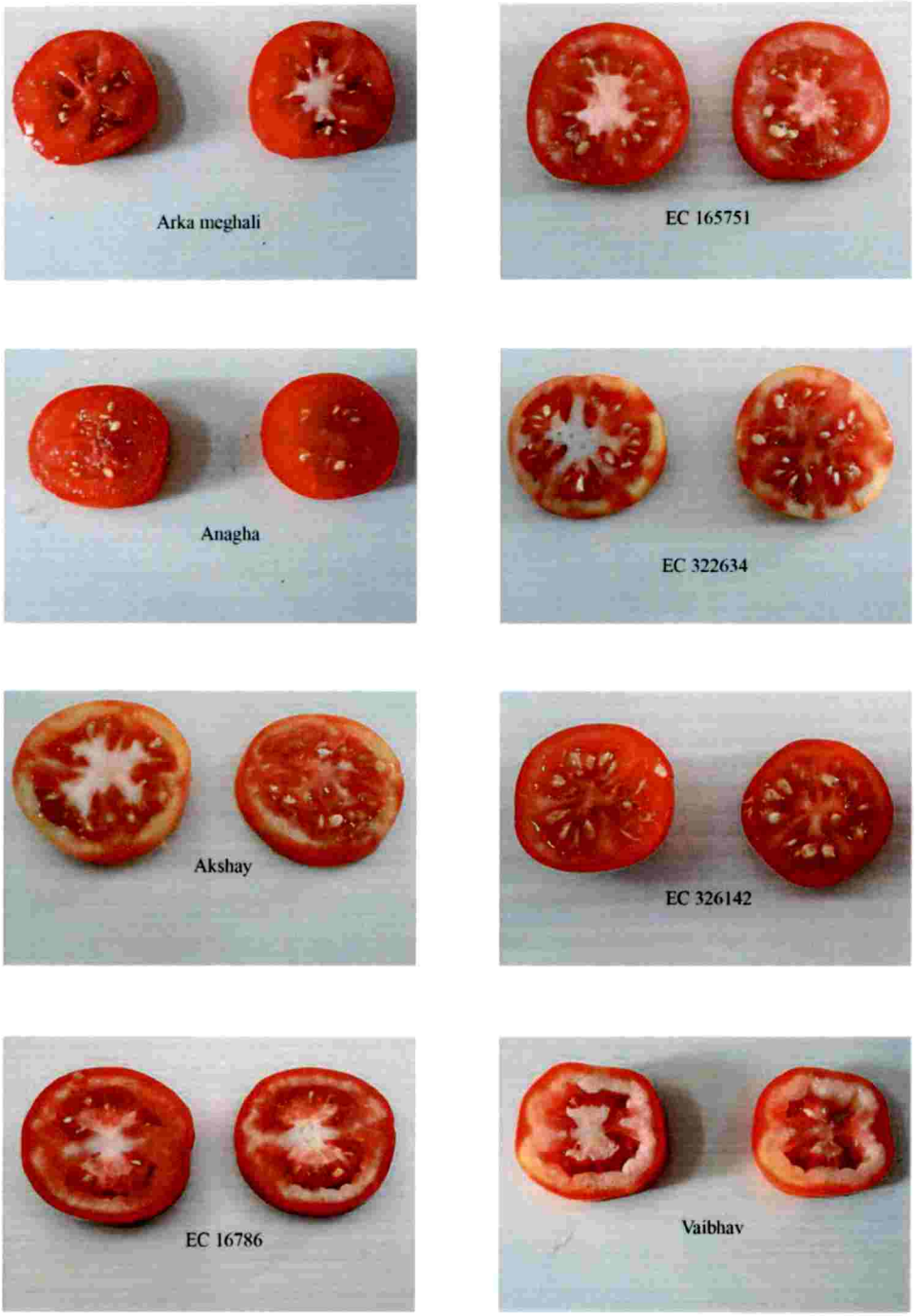


Plate 8 b. Variability in locules of different tomato genotypes

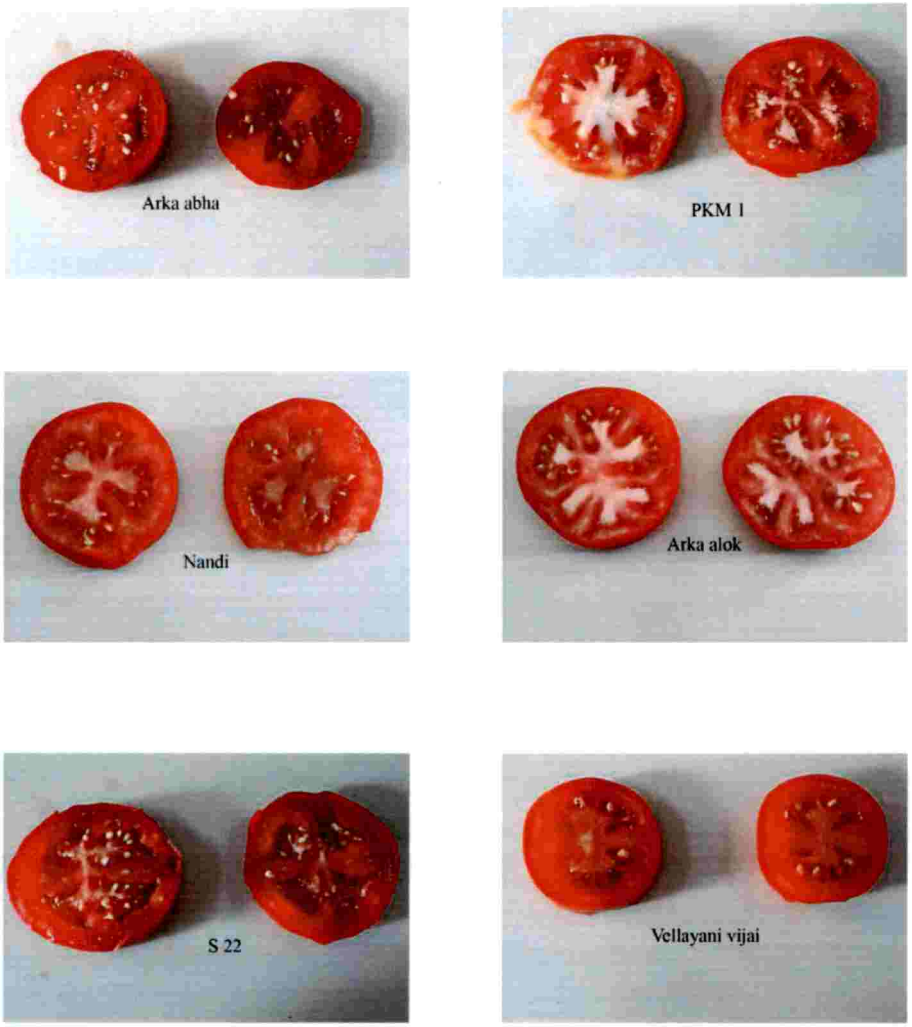


Plate 8 c. Variability in locules of different tomato genotypes

plant^{-1} (0.585) and weight of fruits plant^{-1} (0.549) at 1% and 5%, While it was significant and negatively correlated with lycopene (-0.346) at 1% and 5% (Table 20).

Number of primary branches plant $^{-1}$

Number of primary branches plant^{-1} had high positive significant genotypic correlation with spread of the plant (0.690), number of days to 50% flowering (0.630), number of days to first fruit harvest (0.666), number of fruits plant^{-1} (0.731), weight of fruits plant^{-1} (0.626) and carotene (0.340) at 1% and 5%, While it had significant negative genotypic correlation with lycopene (-0.489) at 1% and 5% (Table 20).

Spread of the plant

Spread of the plant had highly positive significant genotypic correlation with number of days to 50% flowering (0.581), number of days to first fruit harvest (0.553), number of fruits plant^{-1} (0.602) and weight of fruits plant^{-1} (0.517) at 1% and 5%, However it showed significance negatively genotypic correlation with lycopene (-0.219) at 1% (Table 20).

Number of days to 50% flowering

Number of days to 50% flowering showed highly positive significant genotypic correlation with number of days to first fruit harvest (0.839), number of fruits plant^{-1} (0.612), weight of fruits plant^{-1} (0.604), and carotene (0.456) at 1% and 5%, While it had significant negative genotypic correlation with lycopene (-0.547) and total soluble solids (-0.306) at 1% and 5% (Table 20).

Number of days to first fruit harvest

Number of days to first fruit harvest showed highly positive significant genotypic correlation with number of fruits plant^{-1} (0.655) and weight of fruits plant^{-1} (0.633) at 1% and 5%, While it had significant negative genotypic correlation with lycopene (-0.582) and total soluble solids (-0.294) at 1% and 5% (Table 20).

Number of fruits plant $^{-1}$

Number of fruits plant^{-1} showed highly positive significant genotypic correlation with weight of fruits plant^{-1} (0.794) and carotene (0.271) at 1% and 5%, While it was

significant and negatively correlated with lycopene (-0.464) and total soluble solids (-0.274) at 1% and 5%. It also showed significant negative genotypic correlation with vitamin C (-0.224) at 1% (Table 20).

Weight of fruits plant $^{-1}$ (kg)

Weight of fruits plant^{-1} showed high positive significant genotypic correlation with weight of fruit (0.472) and volume of fruit (0.408) at 1% and 5%, While it was significant and negatively correlated with lycopene (-0.376) and total soluble solids (-0.355) at 1% and 5% (Table 20).

Weight of fruit (g)

Weight of fruit showed high positive significant genotypic correlation with volume of fruit (0.981), pericarp thickness (0.503) and shelf life (0.321) at 1% and 5%, it also showed positive significant genotypic correlation with vitamin C (0.262) at 1%, While it was significant and negatively correlated with carotene (-0.345) at 5% and total soluble solids (-0.225) at 1% (Table 20).

Number of locules fruit $^{-1}$

Number of locules fruit^{-1} had no positive significant genotypic correlation with any of the characters. While it had significant negative genotypic correlation with pericarp thickness (-0.331) and lycopene (-0.312) at 1% and 5% (Table 20).

Volume of fruit

Volume of fruit showed high positive significant genotypic correlation with pericarp thickness (0.520), vitamin C (0.304) and shelf life (0.385) at 1% and 5%, While it was significant and negatively correlated with carotene (-0.369) at 1% and 5% (Table 20).

Pericarp thickness

Pericarp thickness showed positive significant genotypic correlation with vitamin C (0.383) and shelf life (0.309) at 1% and 5% and lycopene (0.224) at 1%, While it was significant and negatively correlated with Carotene (-0.397) at 1% and 5% (Table 20).

Lycopene

Lycopene showed positive significant genotypic correlation with vitamin C (0.341) at 1% and 5%, While it was significant and negatively genotypic correlated with carotene (-0.483) at 1% and 5% and pH of juice (-0.246) at 1% (Table 20).

pH of juice

pH of juice showed positive significant genotypic correlation with total soluble solids (0.260) at 1%. While it was significant and negatively genotypic correlated with shelf life -0.243 (Table 20).

4.2.4.2 Phenotypic Correlation among fruit yield and its associated traits

Plant height (cm)

Plant height showed high positive significant phenotypic correlation with number of primary branches plant^{-1} (0.860), spread of the plant (0.800), number of days to 50% flowering (0.632), number of days to first fruit harvest (0.670), number of fruits plant^{-1} (0.581) and weight of fruits plant^{-1} (0.546) at 1% and 5%, While it was significant and negatively correlated with lycopene (-0.345) at 1% and 5% (Table 21).

Number of primary branches plant $^{-1}$

Number of primary branches plant^{-1} had high positive significant phenotypic correlation with spread of the plant (0.686), number of days to 50% flowering (0.620), number of days to first fruit harvest (0.657), number of fruits plant^{-1} (0.724), weight of fruits plant^{-1} (0.623) and carotene (0.318) at 1% and 5%, While it was significant and negatively correlated with lycopene (-0.486) at 1% and 5% (Table 21).

Spread of the plant

Spread of the plant had highly positive significant phenotypic correlation with number of days to 50% flowering (0.572), number of days to first fruit harvest (0.549), number of fruits plant^{-1} (0.599) and weight of fruits plant^{-1} (0.515) at 1% and 5%, However it showed significant and negative correlation with lycopene (-0.219) at 1% (Table 21).

Number of days to 50% flowering

Number of days to 50% flowering showed highly positive significant phenotypic correlation with number of days to first fruit harvest (0.820), number of fruits plant^{-1}

Table 20. Genotypic correlation coefficient among seventeen characters in 30 genotypes of tomato

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1	0.867**	0.803**	0.642**	0.680**	0.585**	0.549**	0.056	-0.080	-0.005	0.104	-0.346**	0.072	0.202	-0.045	-0.127	0.066
2		1	0.690**	0.630**	0.666**	0.731**	0.626**	-0.015	0.087	-0.104	-0.011	-0.489**	-0.011	0.340**	0.178	-0.125	-0.159
3			1	0.581**	0.553**	0.602**	0.517**	-0.083	-0.060	-0.130	-0.037	-0.219*	-0.092	0.076^{NS}	0.067	-0.078	0.023
4				1	0.839**	0.612**	0.604**	0.108	0.126	0.029	-0.131	-0.547**	-0.130	0.456**	-0.137	-0.306**	0.015
5					1	0.655**	0.633**	0.130	0.105	0.069	-0.003	-0.582**	-0.094	0.203	0.030	-0.294**	0.016
6						1	0.794**	-0.076	0.012	-0.132	-0.163	-0.464**	-0.224*	0.271**	0.170	-0.274**	0.113
7							1	0.472**	0.049	0.408**	0.160	-0.376**	-0.022	0.032	0.034	-0.355**	0.179
8								1	0.183	0.981**	0.503**	-0.059	0.262*	-0.345**	-0.201	-0.225*	0.321**
9									1	0.149	-0.331**	-0.312**	-0.190	0.146	0.101	-0.024	-0.205
10										1	0.520**	0.015	0.304**	-0.369**	-0.190	-0.191	0.385**
11											1	0.224*	0.383**	-0.397**	-0.062	0.182	0.309**
12												1	0.341**	-0.483**	-0.246*	0.133	0.191
13													1	-0.107	-0.189	0.076	0.197
14														1	0.012	0.065	-0.078
15															1	0.260*	-0.243*
16																1	-0.008
17																	1

1 = Plant height (cm), 2 = Number of primary branches plant^{-1}, 3 = Spread of the plant (cm), 4 = Number of days to 50% flowering,
5 = Number of days to first fruit harvest, 6 = Number of fruits plant^{-1} 7 = Weight of fruits plant^{-1} (kg), 8 = Weight of fruit (g),
9 = Number of locules fruit^{-1}, 10 = Volume of fruit (ml of water displaced), 11= Pericarp thickness, 12= Lycopene (mg/100 g),
13= Vitamin C (mg/ 100 g), 14 = Carotene (mg/100g), 15 = pH of juice, 16 = Total soluble solids (%), 17= Shelf life (days)

**** (Significant at 5% & 1%) * (Significant at 1%)**

(0.603), weight of fruits plant^{-1} (0.597), and carotene (0.436) at 1% and 5%, While it had significant negative correlation with Lycopene (-0.539) and total soluble solids (-0.302) at 1% and 5% (Table 21).

Number of days to first fruit harvest

Number of days to first fruit harvest showed highly positive significant phenotypic correlation with number of fruits plant^{-1} (0.646) and weight of fruits plant^{-1} (0.626) at 1% and 5%, While it had significant and negative correlation with lycopene (-0.574) and total soluble solids (-0.291) at 1% and 5% (Table 21).

Number of fruits plant $^{-1}$

Number of fruits plant^{-1} showed highly positive significant phenotypic correlation with weight of fruits plant^{-1} (0.790) at 1% and 5%, and carotene (0.257) at 1%, While it had significant negative correlation with lycopene (-0.462) and total soluble solids (-0.273) at 1% and 5% (Table 21).

Weight of fruits plant^{-1} (kg)

Weight of fruits plant^{-1} showed highly positive significant phenotypic correlation with weight of fruit (0.468) and volume of fruit (0.404) at 1% and 5%, While it had significant and negative correlation with lycopene (-0.375) and total soluble solids (-0.355) at 1% and 5% (Table 21).

Weight of fruit (g)

Weight of fruit showed highly positive significant phenotypic correlation with volume of fruit (0.967), pericarp thickness (0.498) and shelf life (0.315) at 1% and 5%, it also showed positive significant correlation with vitamin C (0.243) at 1% , While it had significant and negative correlation with carotene (-0.319) at 5% and total soluble solids (-0.223) at 1% (Table 21).

Number of locules fruit $^{-1}$

Number of locules fruit^{-1} had no positive significant phenotypic correlation with any of the characters. While it had significant and negative correlation with pericarp thickness (-0.322) and lycopene (-0.305) at 1% and 5% (Table 21).

Volume of fruit

Volume of fruit showed highly positive significant phenotypic correlation with pericarp thickness (0.514), vitamin C (0.280) and shelf life (0.376) at 1% and 5%, While it had significant and negative correlation with carotene (-0.361) at 1% and 5% (Table 21).

Pericarp thickness

Pericarp thickness showed positive significant phenotypic correlation with vitamin C (0.363) and shelf life (0.302) at 1% and 5% and lycopene (0.223) at 1%, While it had significant and negative correlation with carotene (-0.375) at 1% and 5% (Table 21).

Lycopene

Lycopene showed positive significant phenotypic correlation with vitamin C (0.322) at 1% and 5%, While it had significant and negative correlation with carotene (-0.461) at 1% and 5% and pH of juice (-0.246) at 1% (Table 21).

pH of juice

pH of juice showed positive significant phenotypic correlation with Total soluble solids (0.258) at 1%. While it had significant and negative correlation with shelf life (-0.232) (Table 21).

4.2.5 Path coefficient analysis

Direct and indirect effect of deferent character on total fruit yield is presented in Table 22. The genotypic correlation coefficient of weight of fruits plant^{-1} and along with its components was partitioned into direct and indirect effect taking weight of fruits plant^{-1} as depended variable

Number of fruits plant^{-1} 0.7612 expressed highest positive direct effect on weight of fruits plant^{-1} followed by weight of fruit (0.7151), spread of the plant (0.1019), lycopene (0.0892), number of primary branches plant^{-1} (0.0766), number of days to 50% flowering (0.0548) and number of days to first fruit harvest (0.0234) whereas, negative direct effect on volume of fruit (-0.1775), plant height (-0.1062) and total soluble solids -0.0036 (Table 22).

Table 21. Phenotypic correlation coefficient among seventeen characters in 30 genotypes of tomato

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1	0.860**	0.800**	0.632**	0.670**	0.581**	0.546**	0.056	-0.078	-0.005	0.103	-0.345**	0.066	0.195	-0.045	-0.127	0.064
2		1	0.686**	0.620**	0.657**	0.724**	0.623**	-0.015	0.083	-0.102	-0.012	-0.486**	-0.012	0.318**	0.176	-0.125	-0.154
3			1	0.572**	0.549**	0.599**	0.515**	-0.082	-0.057	-0.129	-0.037	-0.219*	-0.084	0.075	0.066	-0.078	0.021
4				1	0.820**	0.603**	0.597**	0.106	0.128	0.028	-0.130	-0.539**	-0.114	0.436**	-0.131	-0.302**	0.007
5					1	0.646**	0.626**	0.126	0.099	0.067	-0.003	-0.574**	-0.082	0.196	0.029	-0.291**	0.010
6						1	0.790**	-0.076	0.015	-0.130	-0.162	-0.462**	-0.205	0.257*	0.169	-0.273**	0.109
7							1	0.468**	0.047	0.404**	0.160	-0.375**	-0.020	0.031	0.034	-0.355**	0.175
8								1	0.172	0.967**	0.498**	-0.058	0.243*	-0.319**	-0.199	-0.223*	0.315**
9									1	0.143	-0.322**	-0.305**	-0.173	0.134	0.102	-0.023	-0.198
10										1	0.514**	0.017	0.280**	-0.361**	-0.188	-0.190	0.376**
11											1	0.223*	0.363**	-0.375**	-0.061	0.182	0.302**
12												1	0.322**	-0.461**	-0.246*	0.133	0.185
13													1	-0.120	-0.178	0.073	0.173
14														1	0.010	0.061	-0.090
15															1	0.258*	-0.232*
16																1	-0.008
17																	

1 = Plant height (cm), 2 = Number of primary branches plant^{-1}, 3 = Spread of the plant (cm), 4 = Number of days to 50% flowering,
5 = Number of days to first fruit harvest, 6 = Number of fruits plant^{-1} 7 = Weight of fruits plant^{-1} (kg), 8 = Weight of fruit (g),
9 = Number of locules fruit^{-1}, 10 = Volume of fruit (ml of water displaced), 11= Pericarp thickness, 12= Lycopene (mg/100 g),
13= Vitamin C (mg/ 100 g), 14 = Carotene (mg/100g), 15 = pH of juice, 16 = Total soluble solids (%), 17= Shelf life (days)

**** (Significant at 5% & 1%) * (Significant at 1%)**

Weight of fruit had positive indirect effect through number of days to 50% flowering (0.0059), number of days to first fruit harvest (0.0030) and total soluble solids (0.0008) while rest of characters exhibited indirect negative values (Table 22).

Number of fruits plant^{-1} had positive indirect effect through spread of the plant (0.0613), number of primary branches plant^{-1} (0.0560), number of days to 50% flowering (0.0336), volume of fruit (0.0234), number of days to first fruit harvest (0.0153) and total soluble solids (0.0010) while rest of characters exhibited indirect negative values (Table 22).

Plant height had positive indirect effect through number of fruits plant^{-1} (0.4453), spread of the plant (0.0818), number of primary branches plant^{-1} (0.0664), weight of fruit (0.0400), number of days to 50% flowering (0.0352), number of days to first fruit harvest (0.0159), volume of fruit (0.0009) and total soluble solids (0.0005) while rest of characters exhibited indirect negative values (Table 22).

Number of primary branches plant^{-1} had positive indirect effect through number of fruits plant^{-1} (0.5564), spread of the plant (0.0703), number of days to 50% flowering (0.0345), volume of fruit (0.0185), number of days to first fruit harvest (0.0156) and total soluble solids (0.0005) while rest of characters exhibited indirect negative values (Table 22).

Number of days to 50% flowering had positive indirect effect through number of fruits plant^{-1} (0.4658), weight of fruit (0.0772), spread of the plant (0.0592), number of primary branches plant^{-1} (0.0483), number of days to first fruit harvest (0.0196) and total soluble solids (0.0011) while rest of characters exhibited indirect negative values (Table 22).

Number of days to first fruit harvest had positive indirect effect through number of fruits plant^{-1} (0.4986), weight of fruit (0.0930), spread of the plant (0.0563), number of primary branches plant^{-1} (0.0510), number of days to 50% flowering (0.0460) and total soluble solids (0.0011) while rest of characters exhibited indirect negative values (Table 22).

Spread of the plant had positive indirect effect through number of fruits plant^{-1} (0.4582), number of primary branches plant^{-1} (0.0529), number of days to 50% flowering (0.0319), volume of fruit (0.0231), number of days to first fruit harvest

(0.0129) and total soluble solids (0.0003) while rest of characters exhibited indirect negative values (Table 22).

Volume of fruit had positive indirect effect through weight of fruit (0.7015), number of days to 50% flowering (0.0016), number of days to first fruit harvest (0.0016), lycopene (0.0013), total soluble solids (0.0007) and plant height (0.0005) while rest of characters exhibited indirect negative values (Table 22).

Total soluble solids had positive indirect effect through volume of fruit (0.0339) Plant height (0.0135) and lycopene (0.0119) while rest of characters exhibited indirect negative values (Table 22).

Lycopene had positive indirect effect through plant height (0.0367) while rest of characters exhibited indirect negative values (Table 22).

4.3 PRESENCE OR ABSENCE OF THE MARKER LINKED TO THE GENES OF RESISTANCE TO ToLCV

4.3.1 DNA isolation

In this study, extraction of genomic DNA from tomato leaves was carried out using the QIAGEN DNeasy plant mini kit. The integrity and purity of DNA was checked through 0.8% agarose gel electrophoresis.

DNA isolation from genotypes

DNA was isolated from 34 genotypes including wild types. The yield of DNA was quantified using spectrophotometer and the ratio A260/A280. The quality and integrity of DNA was found to be good as per the gel electrophoresis results (Plate 9).

Preliminary analysis

Quantification of DNA samples extracted from tomato genotypes using UV spectrophotometer and their readings are mentioned in the (Table 23).

4.3.2 Confirmation of resistance to ToLCV using identified SCAR molecular markers

In this experiment three SCAR molecular markers (*Ty2, Ty3 and Ty3a)* were used and screened with the 34 genotypes of tomato.

Table 22. Genotypic path coefficient analysis for weight of fruits plant^{-1} and its component character in tomato

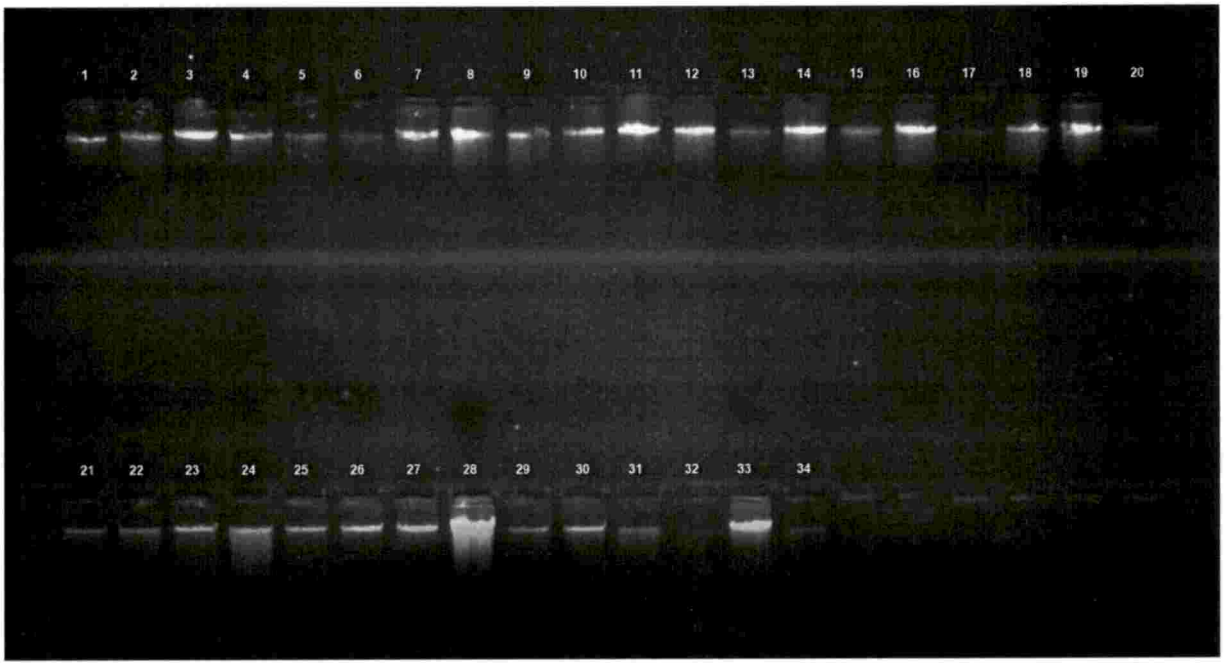
Characters	Weight of fruit (g)	Number of fruits plant $^{-1}$	Plant height (cm)	Number of primary branches plant $^{-1}$	Number of days to 50% flowering	Number of days to first fruit harvest	Spread of the plant	Volume of fruit	Total soluble solids	Lycopene	Weight of fruits plant $^{-1}$ (kg)
1	**0.7151**	-0.0579	-0.0059	-0.0011	0.0059	0.0030	-0.0085	-0.1742	0.0008	-0.0053	0.472**
2	-0.0543	**0.7612**	-0.0621	0.0560	0.0336	0.0153	0.0613	0.0234	0.0010	-0.0414	0.794**
3	0.0400	0.4453	**-0.1062**	0.0664	0.0352	0.0159	0.0818	0.0009	0.0005	-0.0308	0.549**
4	-0.0107	0.5564	-0.0921	**0.0766**	0.0345	0.0156	0.0703	0.0185	0.0005	-0.0436	0.626**
5	0.0772	0.4658	-0.0682	0.0483	**0.0548**	0.0196	0.0592	-0.0051	0.0011	-0.0488	0.604**
6	0.0930	0.4986	-0.0722	0.0510	0.0460	**0.0234**	0.0563	-0.0123	0.0011	-0.0519	0.633**
7	-0.0593	0.4582	-0.0853	0.0529	0.0319	0.0129	**0.1019**	0.0231	0.0003	-0.0195	0.517**
8	0.7015	-0.1005	0.0005	-0.0080	0.0016	0.0016	-0.0132	**-0.1775**	0.0007	0.0013	0.408**
9	-0.1609	-0.2086	0.0135	-0.0096	-0.0168	-0.0069	-0.0079	0.0339	**-0.0036**	0.0119	- 0.355**
10	-0.0422	-0.3532	0.0367	-0.0375	-0.0300	-0.0136	-0.0223	-0.0027	-0.0005	**0.0892**	-0.376**

Residual effect = 0.0725, ****Significant at 0.01,** ***Significant at 0.05**

Diagonal bold values shows direct effect

Table 23. Quality and quantity of genomic DNA thirty-four genotypes

Sr. No	Genotypes	Absorbance at 260 nm	Absorbance at 280 nm	O.D Ratio A260/280	DNA yield (ng/µl)
1	Palam Pride	0.007	0.003	2.33	350
2	Surya	0.012	0.007	1.71	600
3	BWR 5	0.004	0.003	1.33	200
4	S7	0.029	0.012	2.42	1450
5	Arka vikas	0.069	0.033	2.09	3450
6	Hawaii	0.019	0.009	2.11	950
7	EC 3205747	0.038	0.020	1.90	1900
8	IC 247508	0.013	0.006	2.17	650
9	IIHR 2372	0.019	0.011	1.73	950
10	EC 164656	0.030	0.014	2.14	1500
11	IIHR 2200	0.033	0.016	2.06	1650
12	Manulekshmi	0.025	0.013	1.92	1250
13	EC 620419	0.031	0.017	1.82	1550
14	EC 362944	0.022	0.011	2.0	1100
15	EC 620545	0.018	0.008	2.25	900
16	IC 549835	0.012	0.007	1.71	600
17	Arka meghali	0.018	0.010	1.80	900
18	EC 167571	0.022	0.012	1.84	1100
19	Anagha	0.031	0.016	1.93	1550
20	EC 322634	0.024	0.014	1.71	1200
21	Akshay	0.017	0.009	1.88	850
22	EC 326142	0.019	0.010	1.90	950
23	EC 16786	0.025	0.013	1.92	1250
24	Vaibhav	0.032	0.017	1.89	1600
25	Arka abha	0.017	0.008	2.12	850
26	PKM 1	0.021	0.011	1.90	1050
27	Nandi	0.032	0.018	1.78	1600
28	Arka alok	0.039	0.017	2.29	1950
29	S 22	0.016	0.006	2.66	800
30	Vellayani Vijai	0.012	0.007	1.71	600
31	EC 541109	0.017	0.009	1.89	850
32	EC 168283	0.021	0.010	2.10	1050
33	LA 2805	0.033	0.016	2.06	1650
34	IIHR 1970	0.036	0.019	1.90	1800



1-Palam pride, 2- Surya, 3- BWR-5, 4- S7, 5- Arka vikas , 6- Hawaii, 7- EC 3205747, 8- IC 247508
9-IIHR 2372, 10- EC 164656, 11- IIHR 2200, 12- Manulekshmy, 13- EC 620419, 14- EC 362944
15- EC 620545, 16- IC 549835, 17- Arka meghali, 18- EC 167571, 19- Anagha, 20- EC 322634
21- Akshay, 22- EC 326142, 23- EC 16786, 24- Vaibhav, 25-Arka abha, 26-PKM-1, 27- Nandi
28- Arka alok, 29- S 22, 30- Vellayani vijai, 31- EC 541109, 32-EC 168283, 33- LA 2805, 34- IIHR 1970

Plate 9. Genomic DNA of tomato genotypes

PCR reactions were carried out for all the three molecular markers linked to ToLCV resistance with their components like Buffer, dNTPs, Primers (Forward & Reverse), Taq DNA Polymerase, Water, etc.

4.3.3 Confirmation of *Ty2* (TG0302F / TY2R1) resistant SCAR molecular marker for ToLCV resistance

Ty2 specific ToLCV resistant primers, which are SCAR marker (Melotto *et al.*, 1996), were employed for confirmation of the above-mentioned genotypes.

While screening for *Ty2* marker marker IIHR 2200, Vaibhav and EC 168283 (*Solanum pimpenellifolium* L.) showed resistant band of size 600 bp confirming the presence of the *Ty2* gene for ToLCV resistance; whereas, all other genotypes showed a susceptible band size of 450 bp in which recessive T*y2* gene was present (Plate 10).

4.3.4 Confirmation of *Ty3* (FLUW-25) resistant SCAR molecular marker for ToLCV resistance

Ty3 specific primers FLUW-25 were screened for the above mentioned genotypes. None of the genotype showed resistant band at specific size, all the genotypes showed a susceptible band size of 480 bp (Plate 11)

4.3.5 Confirmation of *Ty3a* (P6-25) resistant SCAR molecular markers for ToLCV resistance

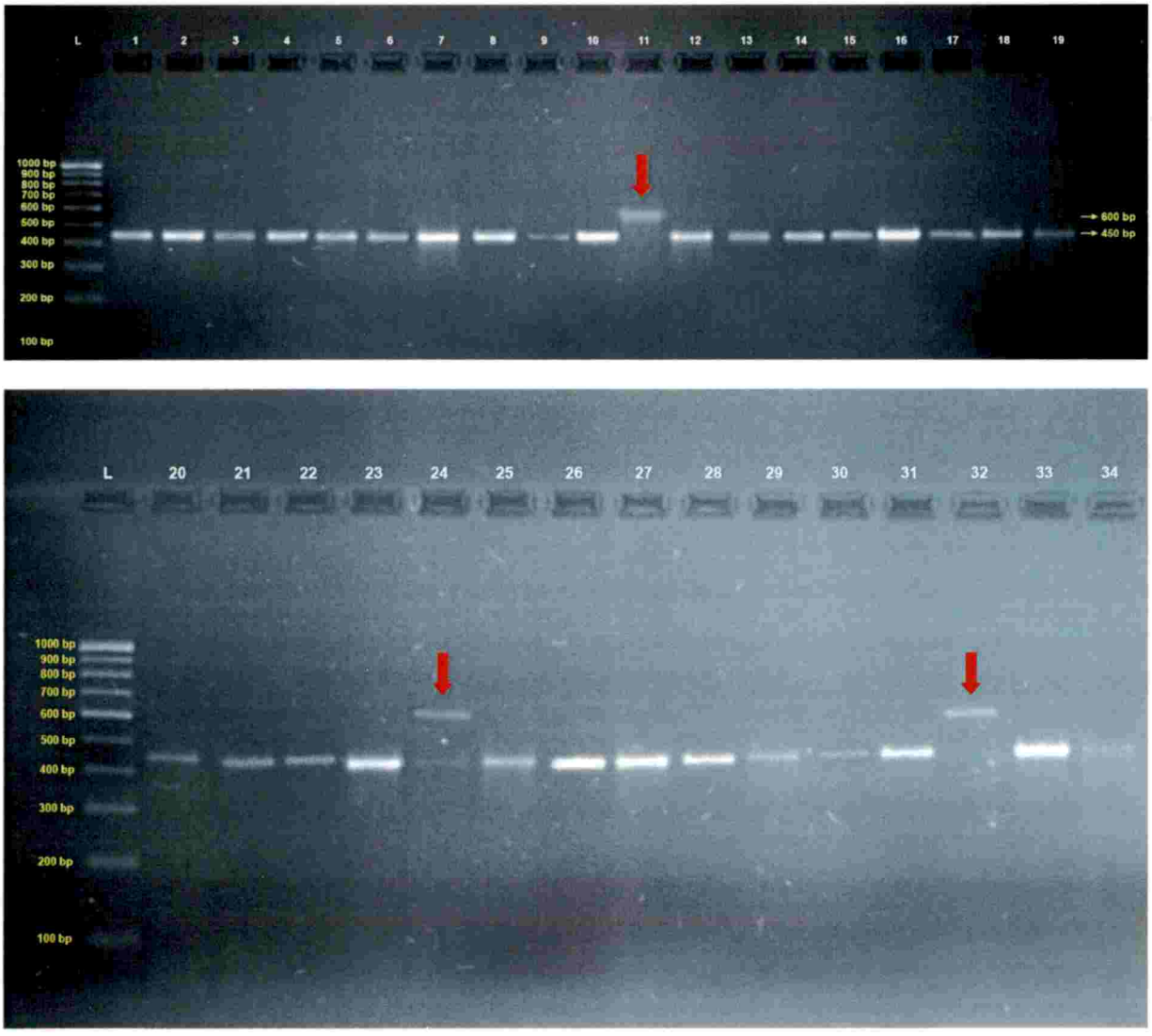
While screening for *Ty3a* marker genotype IIHR 1970 (*Solanum peruvainum* L.) showed resistant band of size 630 bp confirming the presence of the *Ty3a* gene for ToLCV resistance; whereas all other genotypes showed a susceptible band size of 320 bp in which recessive *Ty3a* gene was present (Plate 12).

When all genotypes validated with the reported ToLCV resistant SCAR molecular markers used in this study genotypes IIHR 2200, Vaibhav and EC 168283 (*Solanum pimpenellifolium* L.) showed the presence of *Ty2* gene. Genotype IIHR 1970 (*Solanum peruvainum* L.) showed the presence of *Ty3a* gene. The resistant genotypes Nandi, EC 541109, IIHR 2372, LA 2805 which showed resistant when confirmed in field as well as grafting did not show any presence of *Ty2*, *Ty3* and *Ty3a* resistant genes. Presence and absence of these genes has been depicted in (Table 24).

Table 24. Confirmation of genotypes *Ty2*, *Ty3* and *Ty3a* genes using SCAR molecular markers

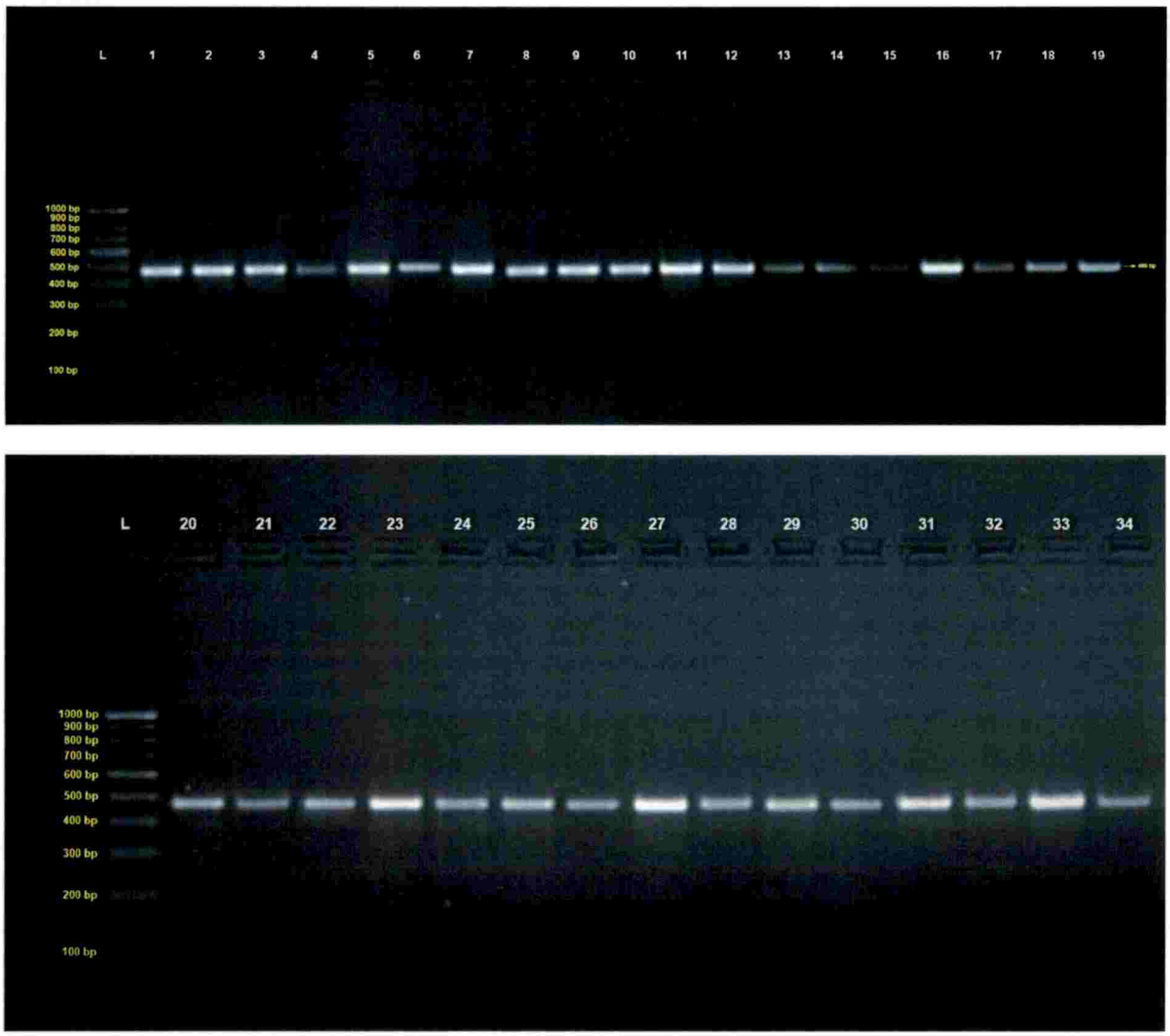
Sr. No.	Genotypes	*Ty2* gene	*Ty3* gene	*Ty3a* gene
1	Palam Pride	-	-	-
2	Surya	-	-	-
3	BWR 5	-	-	-
4	S7	-	-	-
5	Arka vikas	-	-	-
6	Hawaii	-	-	-
7	EC 3205747	-	-	-
8	IC 247508	-	-	-
9	IIHR 2372	-	-	-
10	EC 164656	-	-	-
11	IIHR 2200	+	-	-
12	Manulekshmi	-	-	-
13	EC 620419	-	-	-
14	EC 362944	-	-	-
15	EC 620545	-	-	-
16	IC 549835	-	-	-
17	Arka meghali	-	-	-
18	EC 167571	-	-	-
19	Anagha	-	-	-
20	EC 322634	-	-	-
21	Akshay	-	-	-
22	EC 326142	-	-	-
23	EC 16786	-	-	-
24	Vaibhav	+	-	-
25	Arka abha	-	-	-
26	PKM 1	-	-	-
27	Nandi	-	-	-
28	Arka alok	-	-	-
29	S 22	--	-	-
30	Vellayani Vijai	-	-	-
31	EC 541109	-	-	-
32	EC 168283	+	-	-
33	LA 2805	-	-	-
34	IIHR 1970	-	-	+

+ (Gene present) and – (Gene absent)



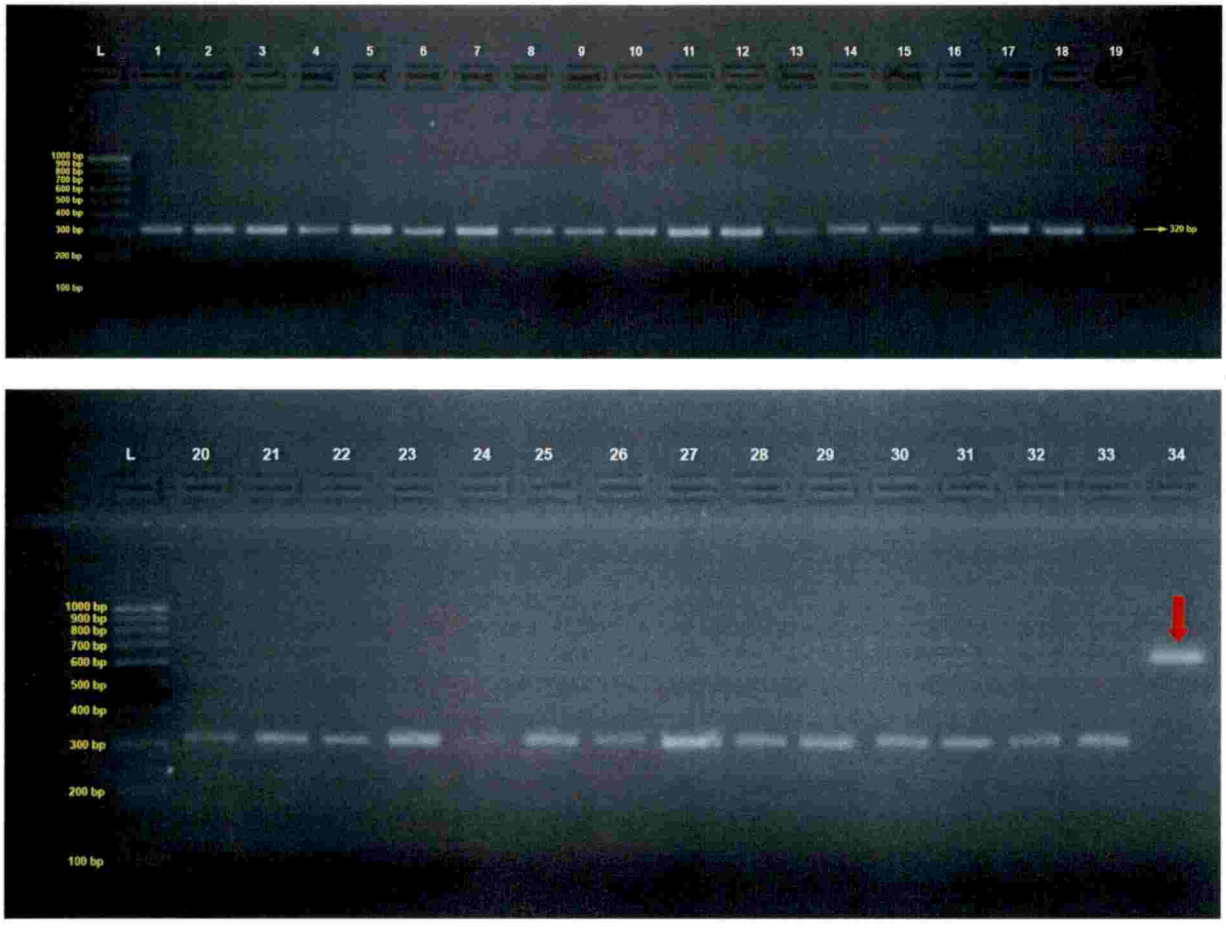
L- 100 bp ladder,1-Palam pride, 2- Surya, 3- BWR-5, 4- S7, 5- Arka vikas, 6- Hawaii, 7- EC 3205747, 8- IC 247508, 9-IIHR 2372, 10- EC 164656, 11- IIHR 2200, 12- Manulekshmy, 13- EC 620419, 14- EC 362944, 15- EC 620545, 16- IC 549835, 17- Arka meghali, 18- EC 167571, 19- Anagha, 20- EC 322634, 21- Akshay, 22- EC 326142, 23- EC 16786, 24- Vaibhav, 25-Arka abha, 26-PKM-1, 27- Nandi, 28- Arka alok, 29- S 22, 30- Vellayani vijai, 31- EC 541109, 32-EC 168283, 33- LA 2805, 34- IIHR 1970

Plate 10. Amplification profile of the marker linked to *Ty 2* gene



L- 100 bp ladder, 1-Palam pride, 2- Surya, 3- BWR-5, 4- S7, 5- Arka vikas, 6- Hawaii, 7- EC 3205747, 8- IC 247508, 9-IIHR 2372, 10- EC 164656, 11- IIHR 2200, 12- Manulekshmy, 13- EC 620419, 14- EC 362944, 15- EC 620545, 16- IC 549835, 17- Arka meghali, 18- EC 167571, 19- Anagha, 20- EC 322634, 21- Akshay, 22- EC 326142, 23- EC 16786, 24- Vaibhav, 25-Arka abha, 26-PKM-1, 27- Nandi, 28- Arka alok, 29- S 22, 30- Vellayani vijai, 31- EC 541109, 32-EC 168283, 33- LA 2805, 34- IIHR 1970

Plate 11. Amplification profile of the marker linked to *Ty3* gene



L- 100 bp ladder, 1-Palam pride, 2- Surya, 3- BWR-5, 4- S7, 5- Arka vikas, 6- Hawaii, 7- EC 3205747, 8- IC 247508, 9-IIHR 2372, 10- EC 164656, 11- IIHR 2200, 12- Manulekshmy, 13- EC 620419, 14- EC 362944, 15- EC 620545, 16- IC 549835, 17- Arka meghali, 18- EC 167571, 19- Anagha, 20- EC 322634, 21- Akshay, 22- EC 326142, 23- EC 16786, 24- Vaibhav, 25-Arka abha, 26-PKM-1, 27- Nandi, 28- Arka alok, 29- S 22, 30- Vellayani vijai, 31- EC 541109, 32-EC 168283, 33- LA 2805, 34- IIHR 1970

Plate 12. Amplification profile of the marker linked to *Ty3a* gene

After field and grafting confirmation for tomato leaf curl virus resistance identified genotypes were crossed with "Anagha" for compatibility studies details are given as below (Table 25 and 26).

Table 25. Details of male parental lines (ToLCV resistant genotypes) used for hybridization

Sl. No.	Code Number	Genotypes
1	T_1	Vaibhav
2	T_2	Nandhi
3	T_3	EC 168283 (*Solanum pimpinellifolium* L.)
4	T_4	IIHR 2372 (*Solanum lycopersicum* L.)
5	T_5	EC 541109 (*Solanum pimpinellifolium* L.)
6	T_6	IIHR 2200 (*Solanum lycopersicum* L.)
7	T_7	LA 2805 (*Solanum lycopersicum var. cerasiforme* L.)
8	T_8	IIHR 1970 (*Solanum peruvianum* L.)

Table 26. Details of tester (Bacterial wilt resistant genotype) used for hybridization

Sl. No.	Code Number	Genotypes
1	L_1	Anagha

Fruit set percentage was calculated by number of fruit set divided by number of flower crossed into hundred and it was observed 100 per cent fruit set in cross Anagha x Nandhi and Anagha x IIHR 2200 followed by Anagha x Vaibhav (95%), Anagha x IIHR 2372 (92.5), Anagha x LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) (72.5%), Anagha x EC 168283 (*Solanum pimpinellifolium* L.) (36.6%), Anagha x EC541109 (*Solanum pimpinellifolium* L.) (32.6%). It was observed Anagha x IIHR 1970 (*Solanum peruvianum* L.) cross did not set any fruits and had 0% fruit set (Table 27).

Anagha variety was crossed with all eight parental lines in which seven crosses had set fruits. Highest fruit set wad observed in cross Anagha x Nandhi and Anagha x IIHR 2200 (*Solanum lycopersicum* L.) (100%), Crosses Anagha x Vaibhav, Anagha x IIHR 2372 (*Solanum lycopersicum* L.) and Anagha x LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) had a range of 75 to 95 per cent of fruitset. Cross Anagha x EC 168283 (*Solanum pimpinellifolium* L.) and Anagha x EC 541109 (*Solanum pimpinellifolium* L.) had a range of 30 to 40 per cent of fruit set. Whereas cross Anagha x IIHR 1970 (*Solanum peruvianum* L.) had 0 per cent of fruit set and not even single fruit was set after crossing (Table 27) and Plate (13 to 13c)

These successful cross combinations produced were further evaluated for yield, quality and resistance and fertility status next season in field (Table 28).

4.4 EVALUATION OF SUCCESSFUL F_1 HYBRIDS AND PARENTS FOR YIELD, QUALITY, RESISTANCE AND FERTILITY STATUS

4.4.1 Analysis of variance

The results of variance for seven hybrids and check of tomato for seventeen quantitative and qualitative traits are furnished separately in Table 29 and 29a. Highly significant differences among the hybrids and check were observed for all seventeen characters, this is an indication of presence of good amount of genetic variability among the genotypes (Plate 14).

4.4.2 Mean performance

The observation for each hybrids and check in three replications for fruit yield and its components characters were used for calculating the mean performance. The observations were recorded on five randomly selected tagged competitive plants from each replication and averaged. The mean performance of different genotype and its components characters are presented in Table 29 and 29a and described below. (Plate 15 to 15 c).

4.4.3 Yield parameters:

Plant height (cm)

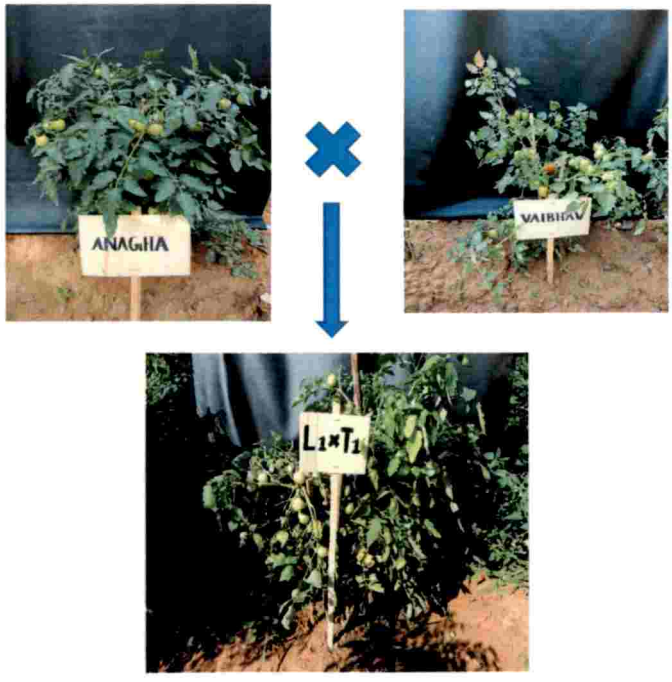
Plant height of the hybrids ranged from 93.40 cm to 160.25 cm with a mean of 121.51 cm (Table 29). Maximum plant height was recorded in hybrid L_1 x T_5 (Anagha x

Table 27. Number of flowers pollinated and number of fruit set

Sr. No	Code Number	Cross combinations	No. of flowers crossed	No. of fruit set	Fruit set (%)
1	$L_1 \times T_1$	Anagha x Vaibhav	40	38	95
2	$L_1 \times T_2$	Anagha x Nandhi	40	40	100
3	$L_1 \times T_3$	Anagha x EC 168283 (*Solanum pimpinellifolium* L.)	90	33	36.6
4	$L_1 \times T_4$	Anagha x IIHR 2372	40	37	92.5
5	$L_1 \times T_5$	Anagha x EC 541109 (*Solanum pimpinellifolium* L.)	95	31	32.6
6	$L_1 \times T_6$	Anagha x IIHR 2200	40	40	100
7	$L_1 \times T_7$	Anagha x LA 2805 (*Solanum lycopersicum var. cerasiforme* L.)	90	68	75.5
8	$L_1 \times T_8$	Anagha X IIHR 1970 (*Solanum peruvianum* L.)	43	0	0

Table 28. Details of successful hybrid combinations

Sl. No.	Parents	Cross combinations
1	$L_1 \times T_1$	Anagha x Vaibhav
2	$L_1 \times T_2$	Anagha x Nandhi
3	$L_1 \times T_3$	Anagha x EC 168283 (*Solanum pimpinellifolium* L.)
4	$L_1 \times T_4$	Anagha x IIHR 2372 (*Solanum lycopersicum* L.)
5	$L_1 \times T_5$	Anagha x EC 541109 (*Solanum pimpinellifolium* L.)
6	$L_1 \times T_6$	Anagha x IIHR 2200 (*Solanum lycopersicum* L.)
7	$L_1 \times T_7$	Anagha x LA 2805 (*Solanum lycopersicum var. cerasiforme* L.)



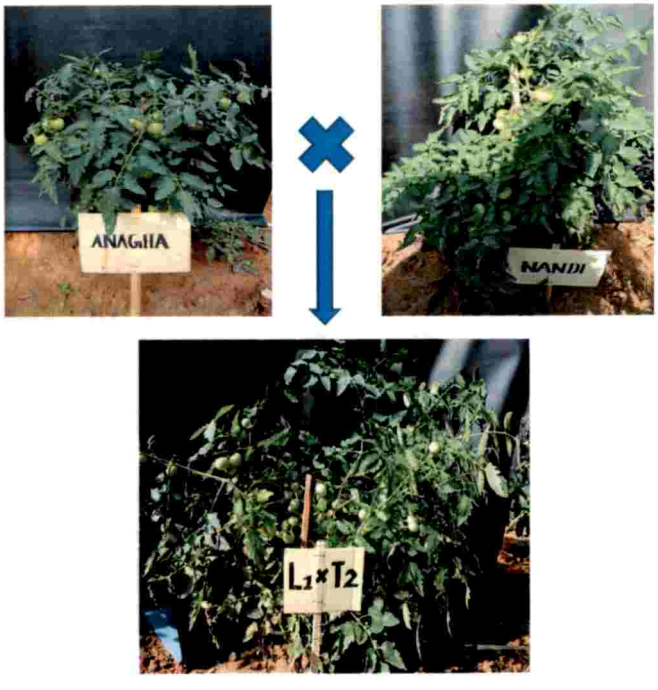


Plate 13. Promising F_1 hybrids

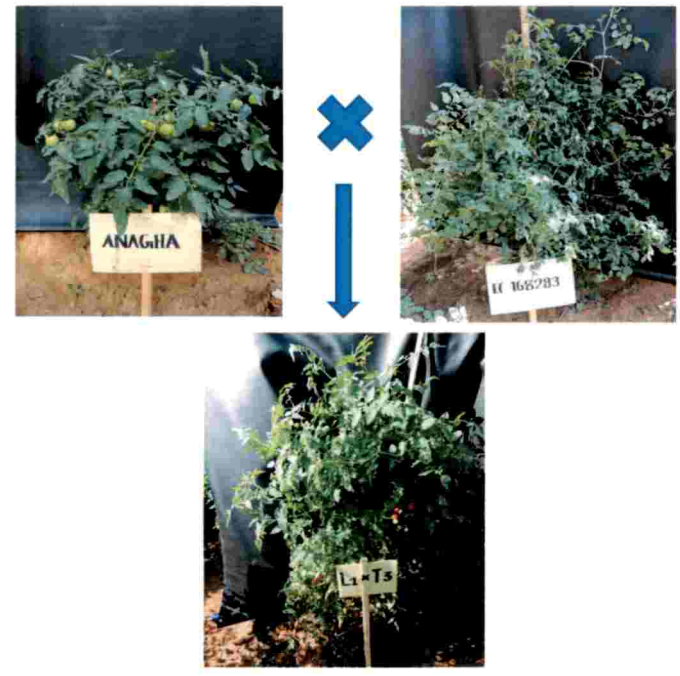


Plate 13 a. Promising F_1 hybrids

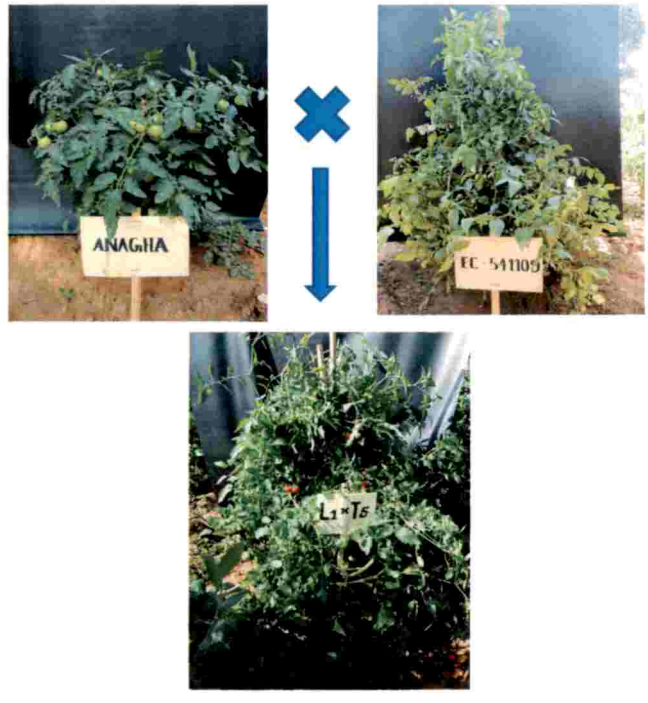


Plate 13 b. Promising F_1 hybrids

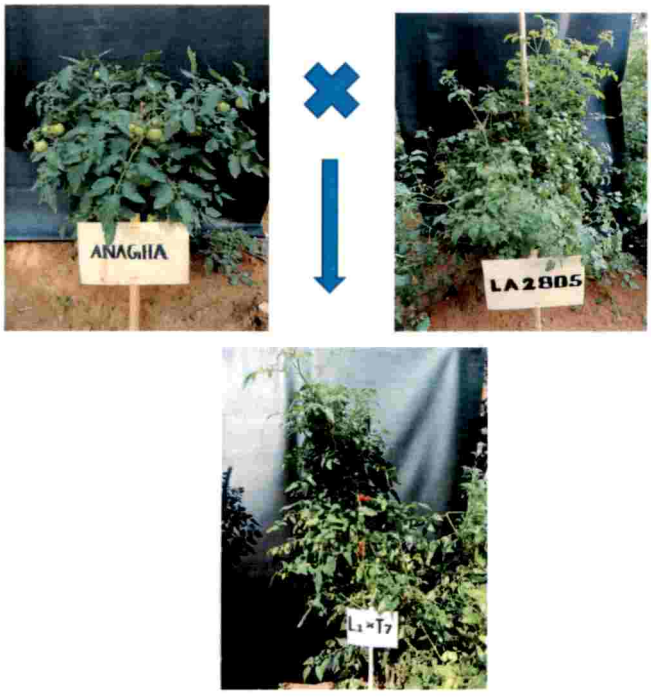
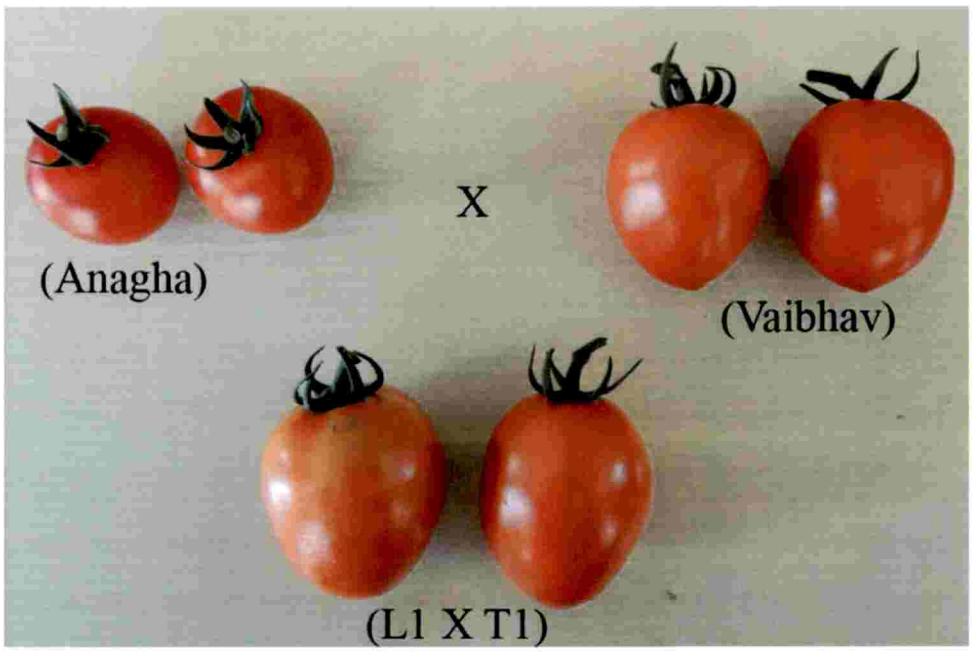


Plate 13 c. Promising F_1 hybrids

Plate 14. General view of experimental plot (Experiment IV)



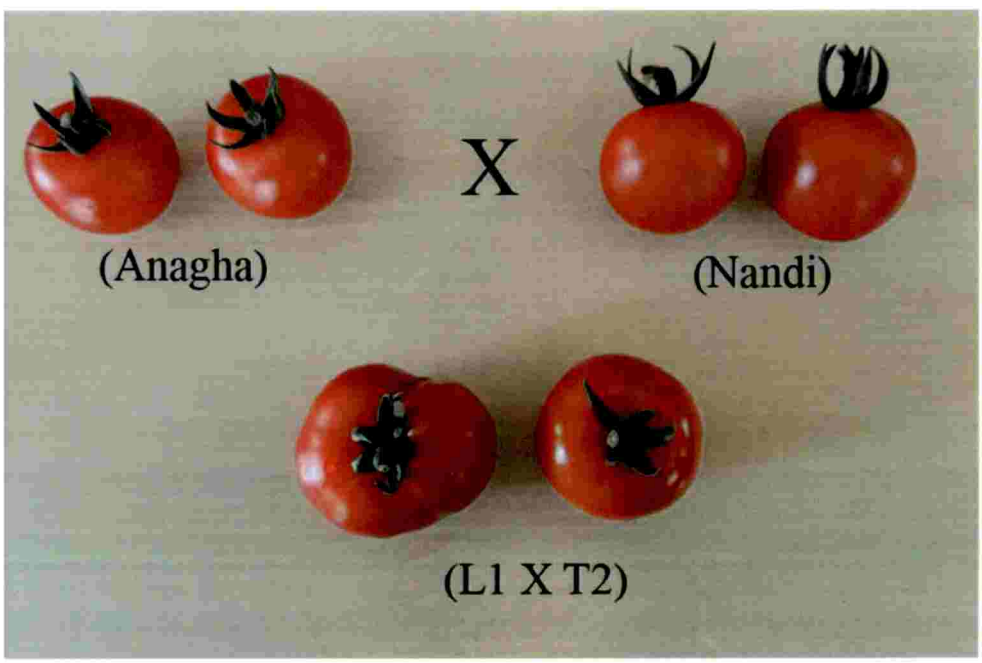
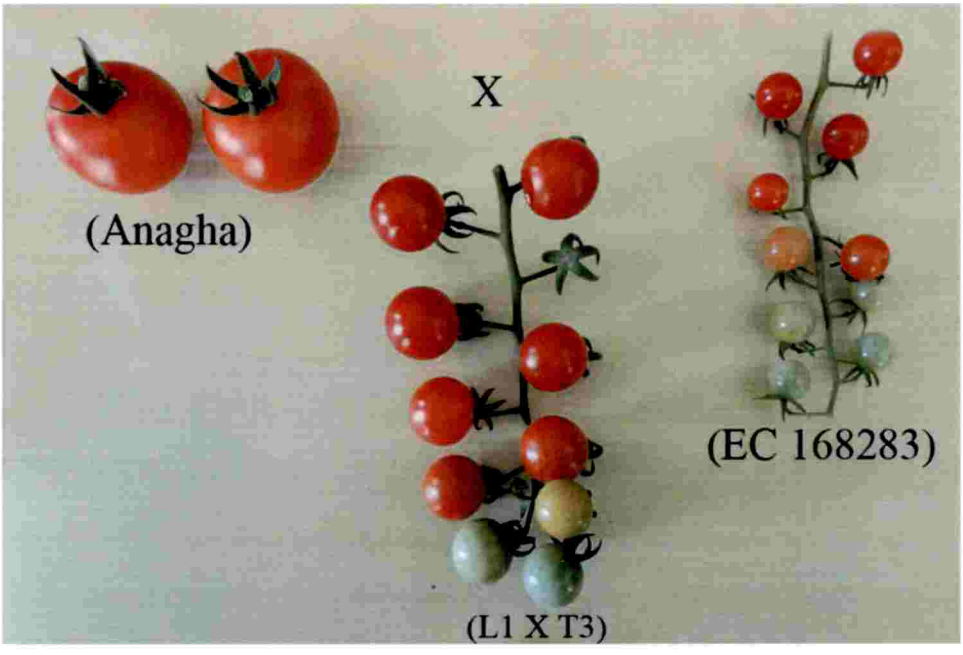


Plate 15. Fruits of parental genotypes and F_1 hybrids



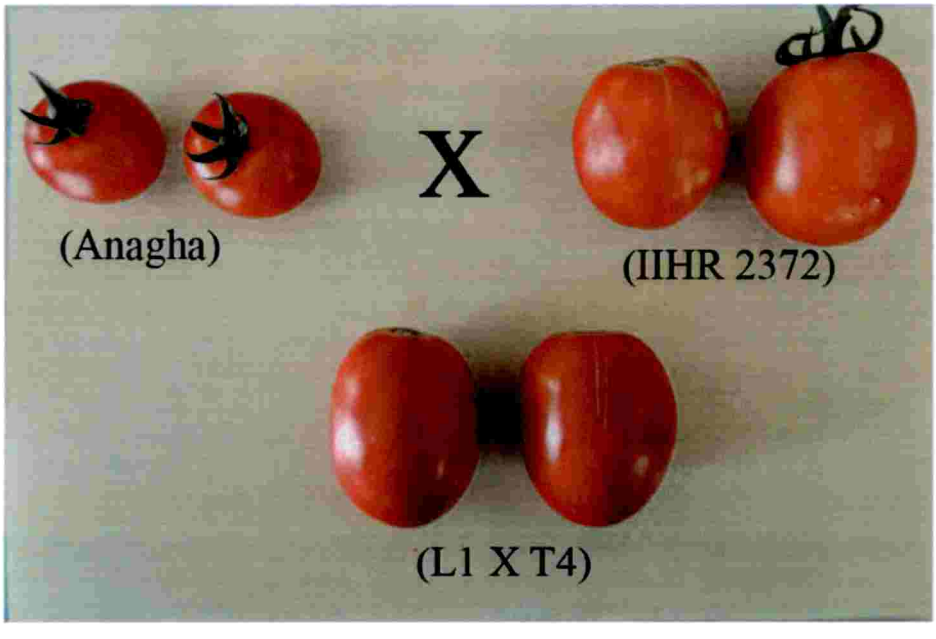
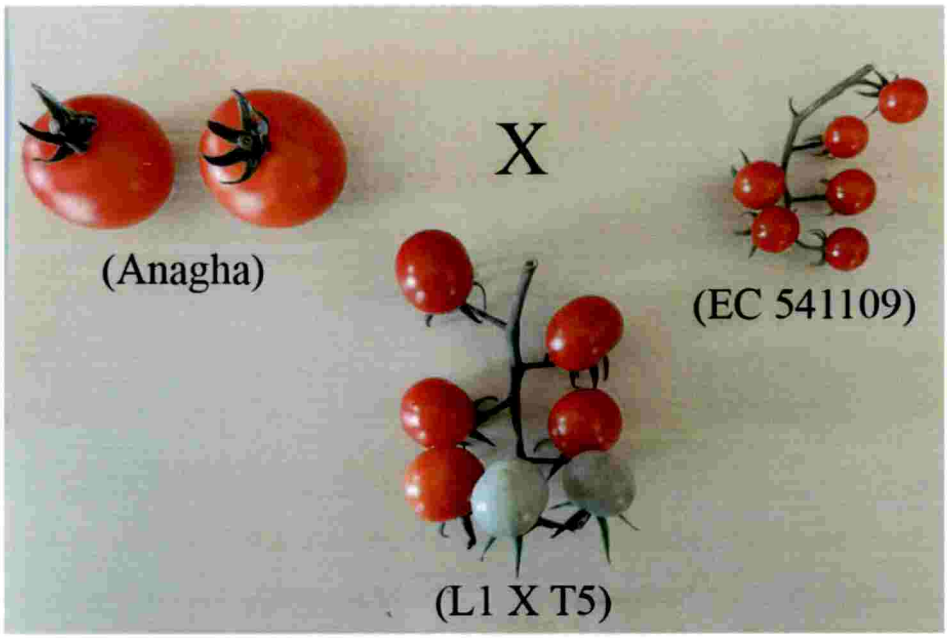


Plate 15 a. Fruits of parental genotypes and F_1 hybrids



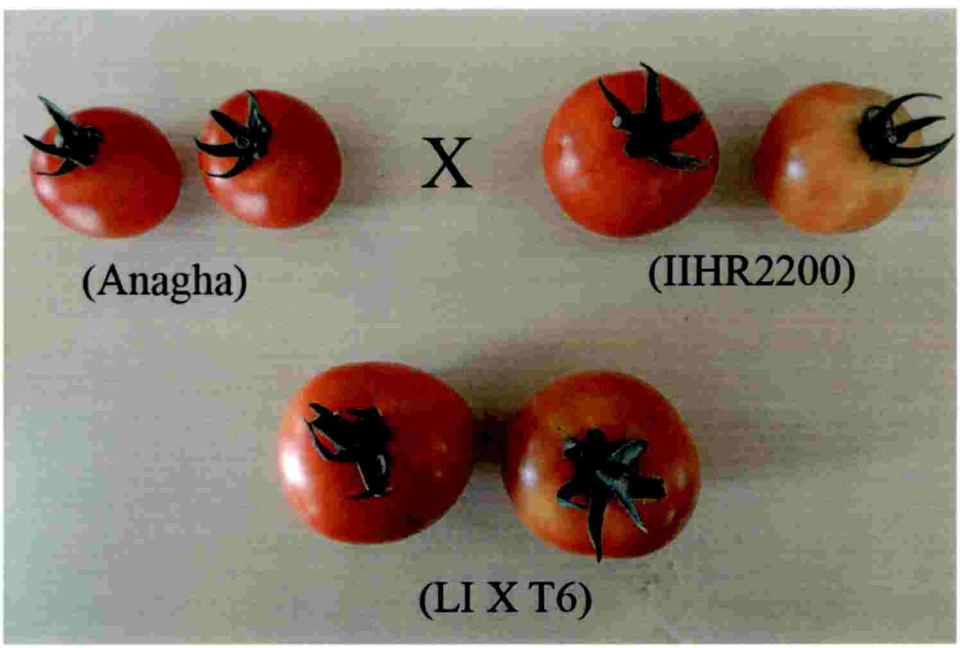


Plate 15 b. Fruits of parental genotypes and F_1 hybrids

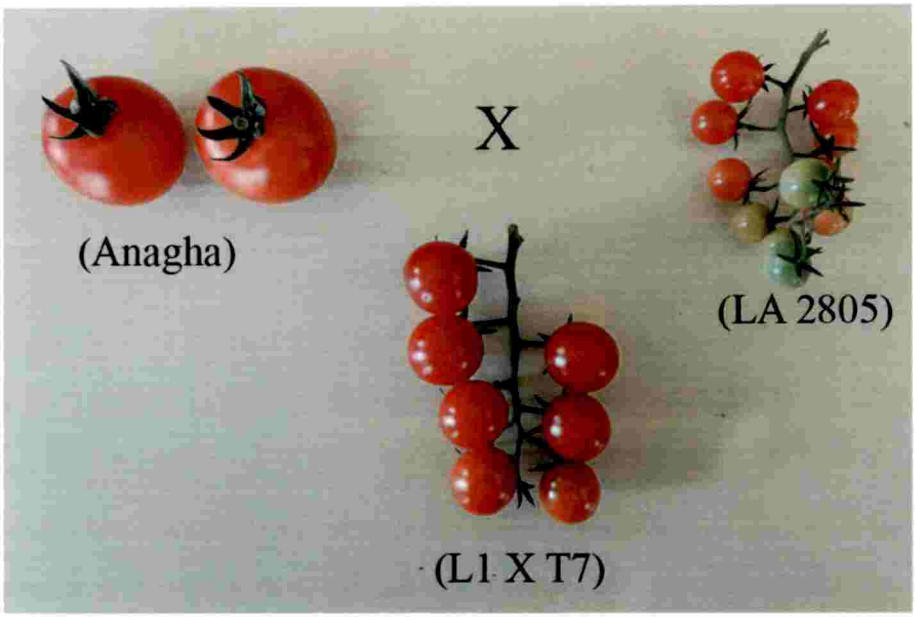


Plate 15 c. Fruits of parental genotypes and F_1 hybrids

EC 541109) (160.25 cm) and minimum plant height was recorded in hybrid L_1 x T_2 (Anagha x Nandi) (93.40 cm).The check variety Vellayani Vijai recorded a plant height of 71.0 cm (Fig. 20).

Number of primary branches plant $^{-1}$

Number of primary branches plant^{-1} in hybrids ranged from 9.89 to 14.89 with a mean of 11.59 (Table 29). The highest number of primary branches plant^{-1} was recorded in hybrid L_1 x T_5 (Anagha x EC 541109) (14.89) and lowest number of primary branches was recorded in hybrid L_1 x T_2 (Anagha x Nandi) (9.89). The check variety Vellayani Vijai recorded an average number of primary branches 5.47 plant^{-1} (Fig. 21).

Spread of the plant (cm)

Spread of the plant ranged from 94.63 cm to 75.48 cm with a mean of 84.58 cm (Table 29). Highest spread of the plant was observed in hybrid L_1 x T_7 (Anagha x LA 2805) (94.63 cm) and lowest spread of the plant was observed in hybrid L_1 x T_4 (Anagha x IIHR 2372) (75.48 cm) followed. The check variety Vellayani Vijai recorded a spread of plant (63.83 cm) respectively (Fig. 22).

Number of days to 50% flowering

Number of days to 50% flowering ranged from 48.11 days to 38.33 days with a mean of 41.94 days (Table 29). Hybrid L_1 x T_2 (Anagha x Nandi) took minimum number of days to 50% flowering (38.33 days) and maximum number of days to 50% flowering was observed in hybrid L_1 x T_5 (Anagha x EC 541109) (48.11 days) Average for number of days to 50% flowering in check variety Vellayani Vijai was 30.47 days (Fig. 23).

Number of days to first fruit harvest

Number of days to first fruit harvest ranged from 69.44 days to 75.33 days with a mean of 72.50 days (Table 29). Minimum number of days to first fruit harvest was observed in hybrid L_1 x T_2 (Anagha x Nandi) (69.44 days) and maximum number of days to first fruit harvest was observed in hybrid L_1 x T_5 (Anagha x EC 541109) Average for number of days to first fruit harvest in check variety Vellayani Vijai was 60.27 days (Fig. 24).

Number of fruits plant^{-1}

Number of fruits plant^{-1} ranged from 33.89 to 284.0 with a mean of 114.88 fruits (Table 29). Highest number of fruits plant^{-1} were recorded in hybrid L_1 x T_3 (Anagha x EC 168283) (284.0) and lowest number of fruits plant^{-1} was recorded in hybrid L_1 x T_4 (IIHR 2372) (33.89), The check variety Vellayani Vijai recorded 20.80 number of fruits plant^{-1} (Fig. 25).

Weight of fruits plant^{-1} (kg)

Weight of fruits plant^{-1} ranged from 1.42 kg to 2.70 kg with a mean of 1.94 kg (Table 29). Maximum weight of fruits plant^{-1} was observed in hybrid L_1 xT_1 (Anagha x Vaibhav) (2.70 kg) and minimum weight of fruits plant^{-1} was observed in hybrid L_1 x T_5 (Anagha x EC 541109) (1.42 kg). The check variety Vellayani Vijai recorded (0.68 kg) weight of fruits plant^{-1} (Fig. 26) and (Plate 16).

Weight of fruit (g)

Weight of fruit ranged from 5.83 g to 65.51 g with a mean of 38.84 g (Table 29). Maximum weight of fruit was recorded in hybrid L_1 x T_1 (Anagha x Vaibhav) (65.51 g) and minimum weight of fruit was recorded in hybrid L_1 x T_5 (Anagha x EC 541109) (5.83 g). The check variety Vellayani Vijai recorded 34.45 g weight of fruit (Fig. 27).

Number of locules fruit $^{-1}$

Number of locules fruit^{-1} ranged from 2.0 to 4.78 with a mean of 3.24 (Table 29 a). Maximum number of locules fruit^{-1} were observed in hybrid L_1 x T_2 (Anagha x Nandi) (4.78) and minimum number of locules fruit^{-1} were observed in hybrid L_1 x T_3 (Anagha x EC 168283) (2.00), L_1 x T_5 (Anagha x EC 541109) (2.00) and L_1 x T_7 (Anagha x LA 2805) (2.00) Average number of locules fruit^{-1} in check variety Vellayani Vijai was 2.11 (Fig. 28) and (Plate 17).

Volume of fruit (ml of water displaced)

Volume of fruit ranged from 5.61 ml to 61.52 ml with a mean of 37.68 ml (Table 29 a). Maximum volume of fruit was recorded in hybrid L_1 x T_1 (Anagha x Vaibhav) (64.64 ml) and minimum volume of fruit was recorded in hybrid L_1 x T_5 (Anagha x EC 541109) (5.61 ml). The check variety Vellayani Vijai recorded (32.53 ml) volume of fruit (Fig. 29).

Table 29. Mean performance of hybrids and parental genotypes for seventeen characters in tomato

Sr. No	Hybrids/Genotype	Plant height (cm)	No. of primary branches plant $^{-1}$	Spread of plant (cm)	No. of days to 50% flowering	No. of days to first fruit harvest	No. of fruits plant $^{-1}$	Weight of fruit plant^{-1} (kg)	Weight of fruit (g)
1	L_1 x T_1 (Vaibhav)	98.71	10.00	79.14	40.44	71.11	43.33	2.70	65.51
2	L_1 x T_2 (Nandi)	93.40	9.89	80.32	38.33	69.44	38.44	1.79	45.98
3	L_1 x T_3 (EC 168283)	153.27	14.44	92.44	45.78	73.56	284.00	1.69	6.04
4	L_1 x T_4 (IIHR 2372)	106.00	10.44	75.48	39.44	71.45	33.89	1.92	56.08
5	L_1 x T_5 (EC 541109)	160.25	14.89	94.62	48.11	75.33	251.55	1.42	5.83
6	L_1 x T_6 (IIHR 2200)	117.46	9.89	85.49	39.55	74.11	38.11	2.15	53.61
7	L_1 x T_7 (LA 2805)	155.23	14.33	94.63	46.44	70.89	244.33	1.62	6.91
	Mean of hybrids	121.51	14.89	84.58	41.94	72.50	114.88	1.94	38.84
8	Vaibhav	91.10	8.47	69.82	33.10	66.73	41.53	2.41	60.25
9	Nandi	74.22	8.60	48.72	32.03	64.80	33.15	1.47	44.92
10	IIHR 2372	110.18	9.53	54.46	33.20	64.93	18.80	0.77	45.51
11	IIHR 2200	126.83	8.33	62.95	32.53	67.20	21.93	0.82	42.79
12	EC 168283	148.68	14.00	90.22	42.87	71.00	272.60	0.81	2.79
13	EC 541109	160.82	14.40	86.84	48.23	75.93	364.77	0.95	2.59
14	LA 2805	158.83	14.00	77.11	45.73	71.80	231.47	0.67	2.54
15	Anagha (L_1)	62.90	4.33	64.45	30.87	61.93	26.47	0.73	28.62
16	Vellayani Vijai (Check)	71.00	5.47	63.83	30.47	60.27	20.80	0.68	34.45
	C.D. (5%)	3.28	0.52	1.89	1.19	1.04	4.93	0.03	1.28
	S.E (m)	1.13	0.18	0.65	0.41	0.36	1.70	0.01	0.44
	C.V.	1.66	2.90	1.48	1.81	0.89	2.40	1.33	2.41

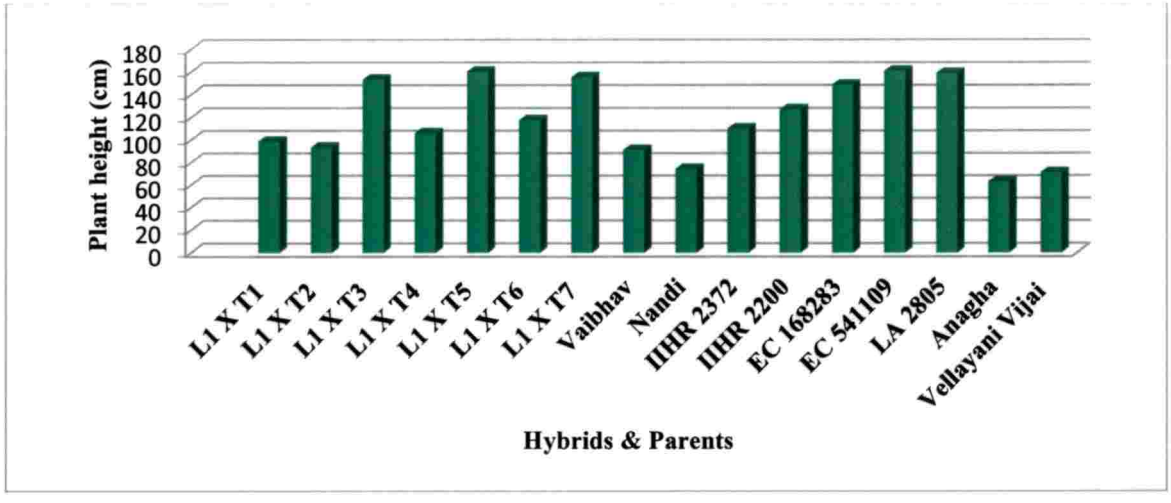


Fig 20. Plant height (cm) in hybrids & parents of tomato

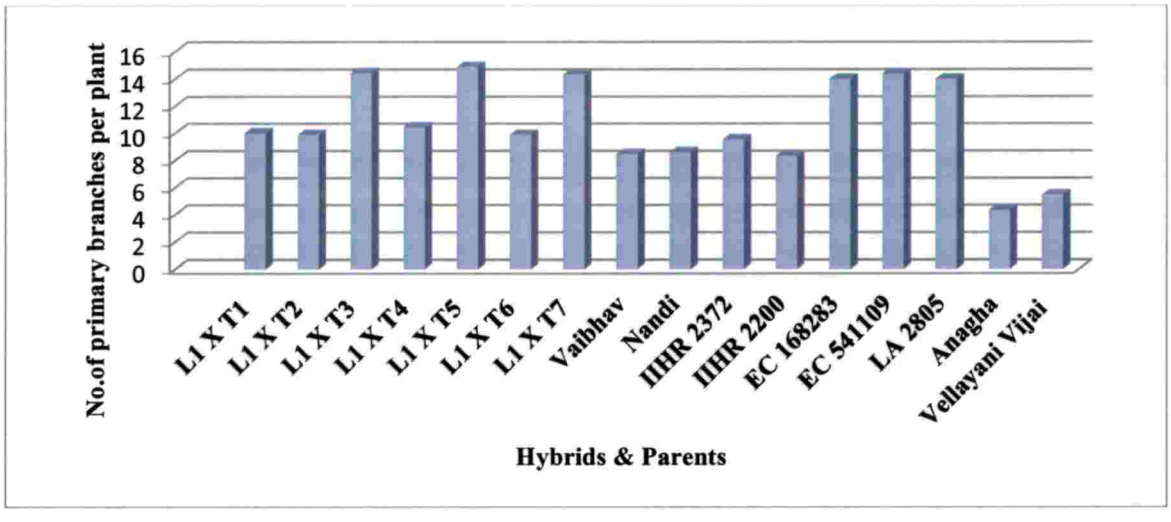


Fig 21. Number of primary branches per plant in hybrids & parents of tomato

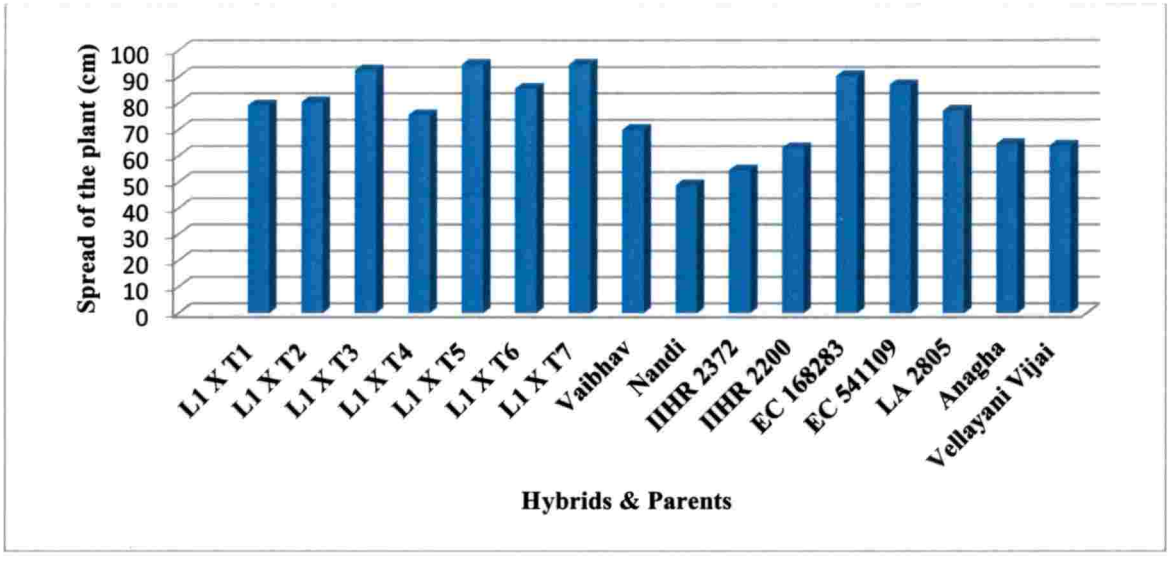


Fig 22. Spread of plant (cm) in hybrids & parents of tomato

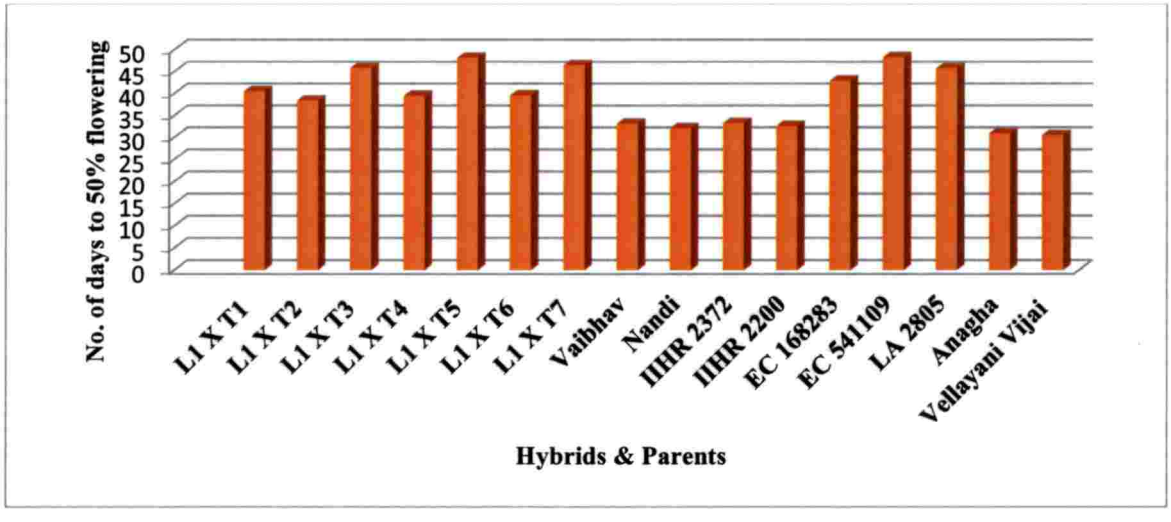


Fig 23. Number of days to 50% flowering in hybrids & parents of tomato

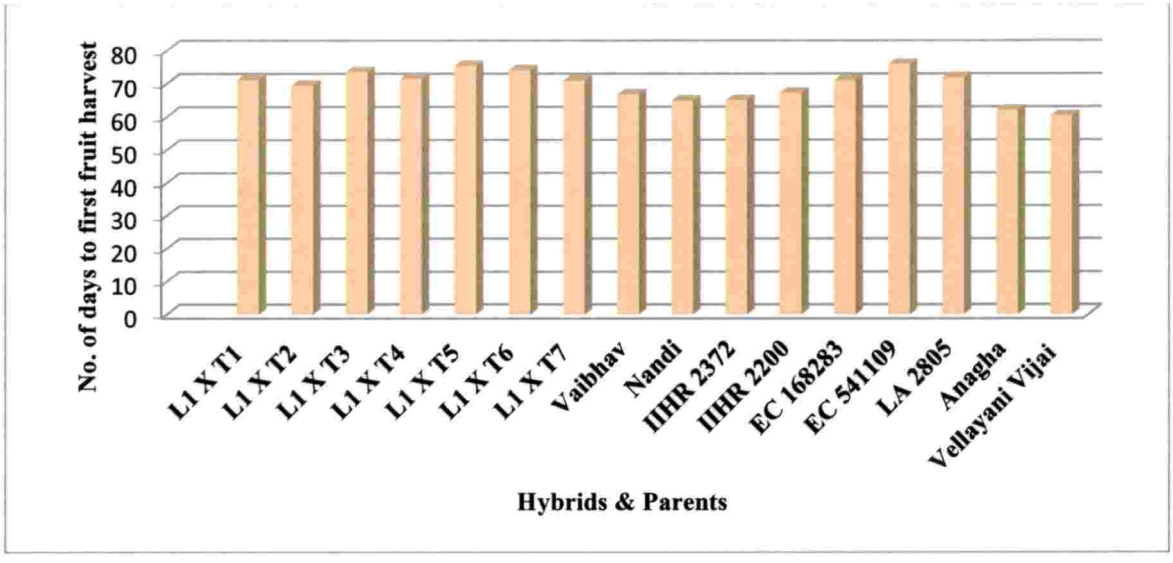


Fig 24. Number of days to first fruit harvest in hybrids & parents of tomato

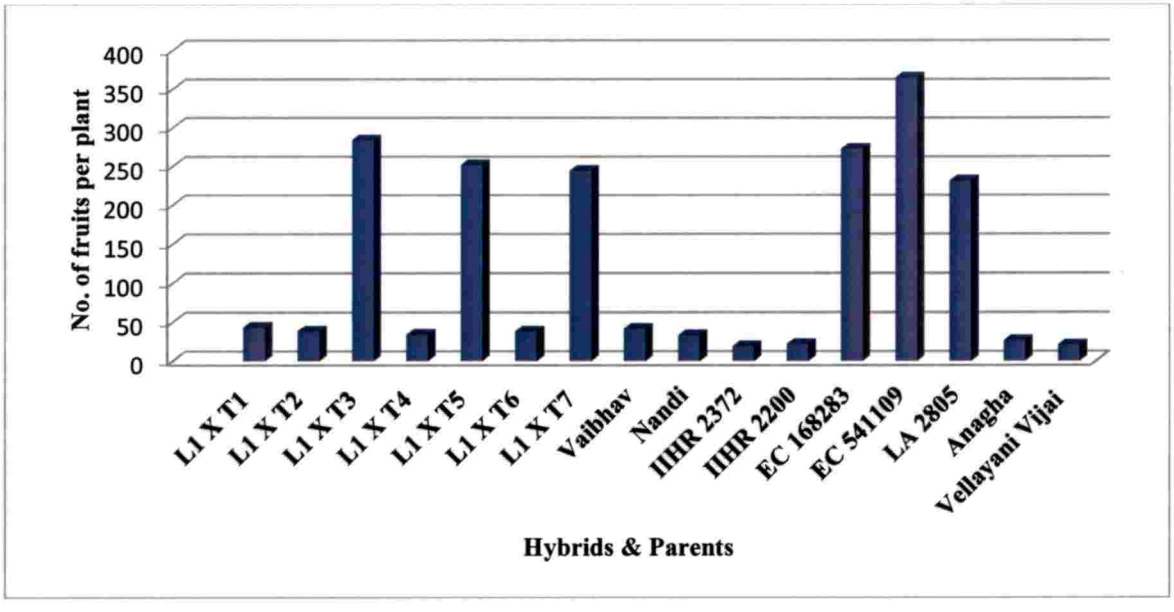


Fig 25. Number of fruits per plant in hybrids & parents of tomato

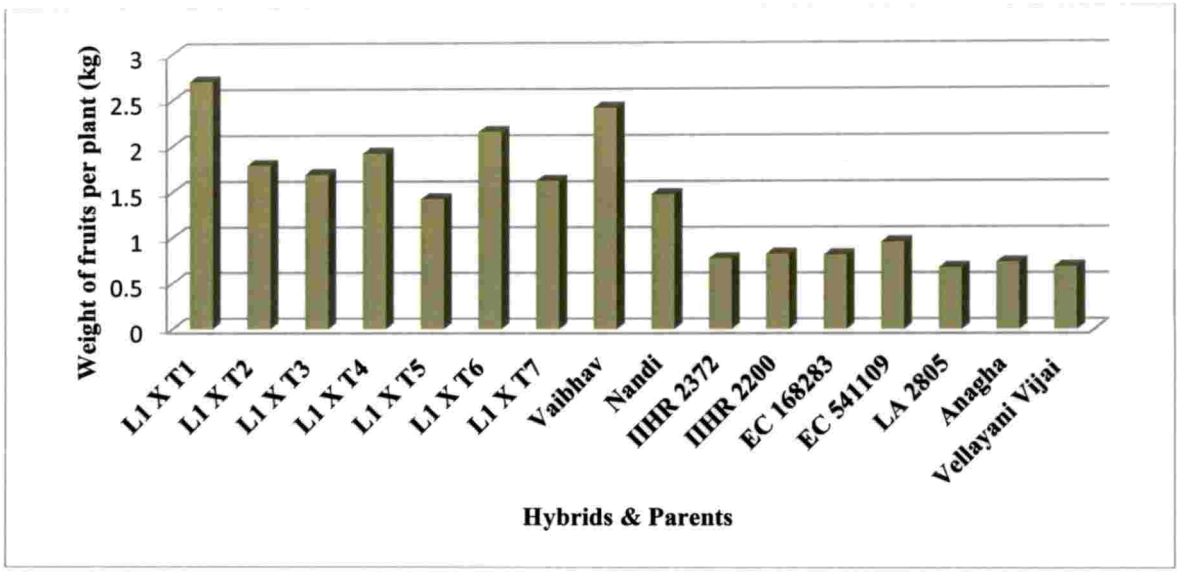


Fig 26. Weight of fruits per plant (kg) in hybrids & parents of tomato

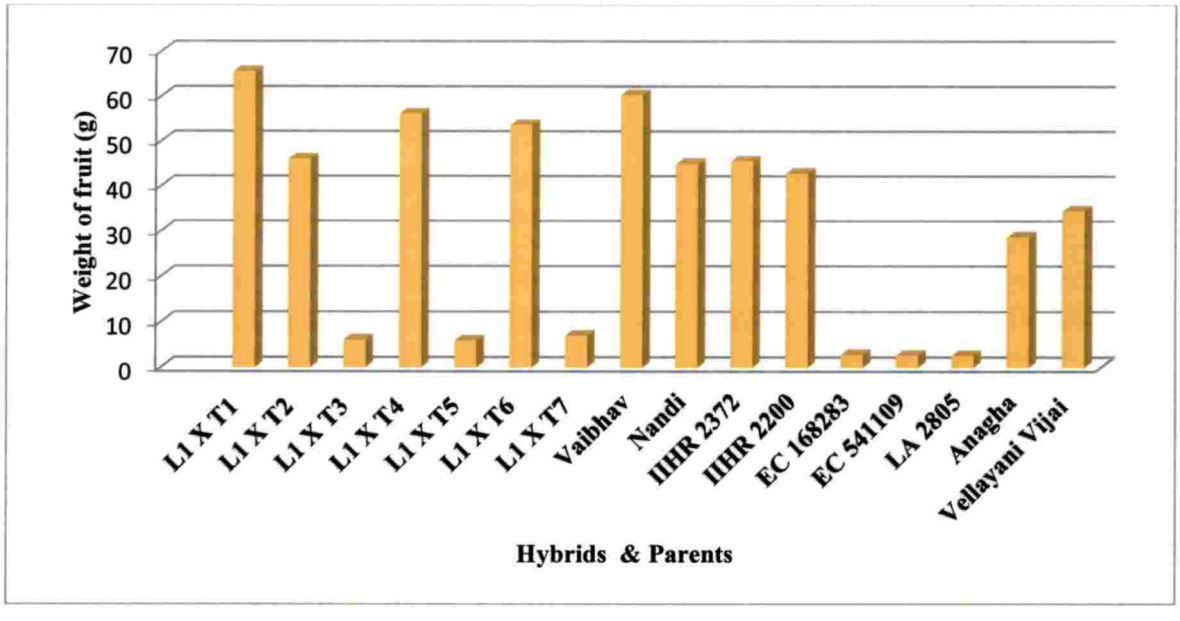
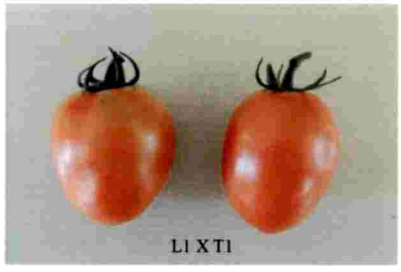
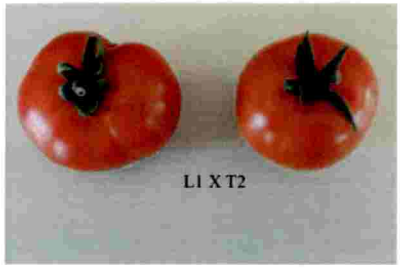
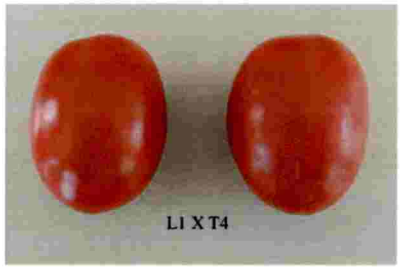
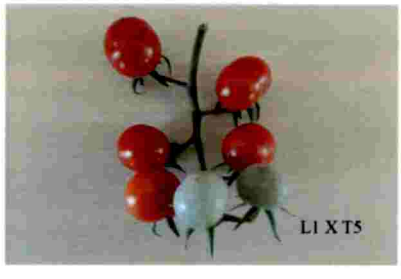


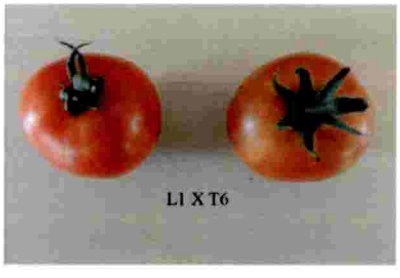
Fig 27. Weight of fruit (g) in hybrids & parents of tomato











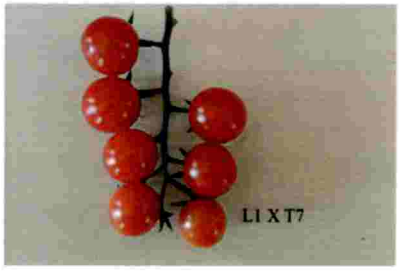


Plate 16. Fruits of different F_1hybrids of tomato

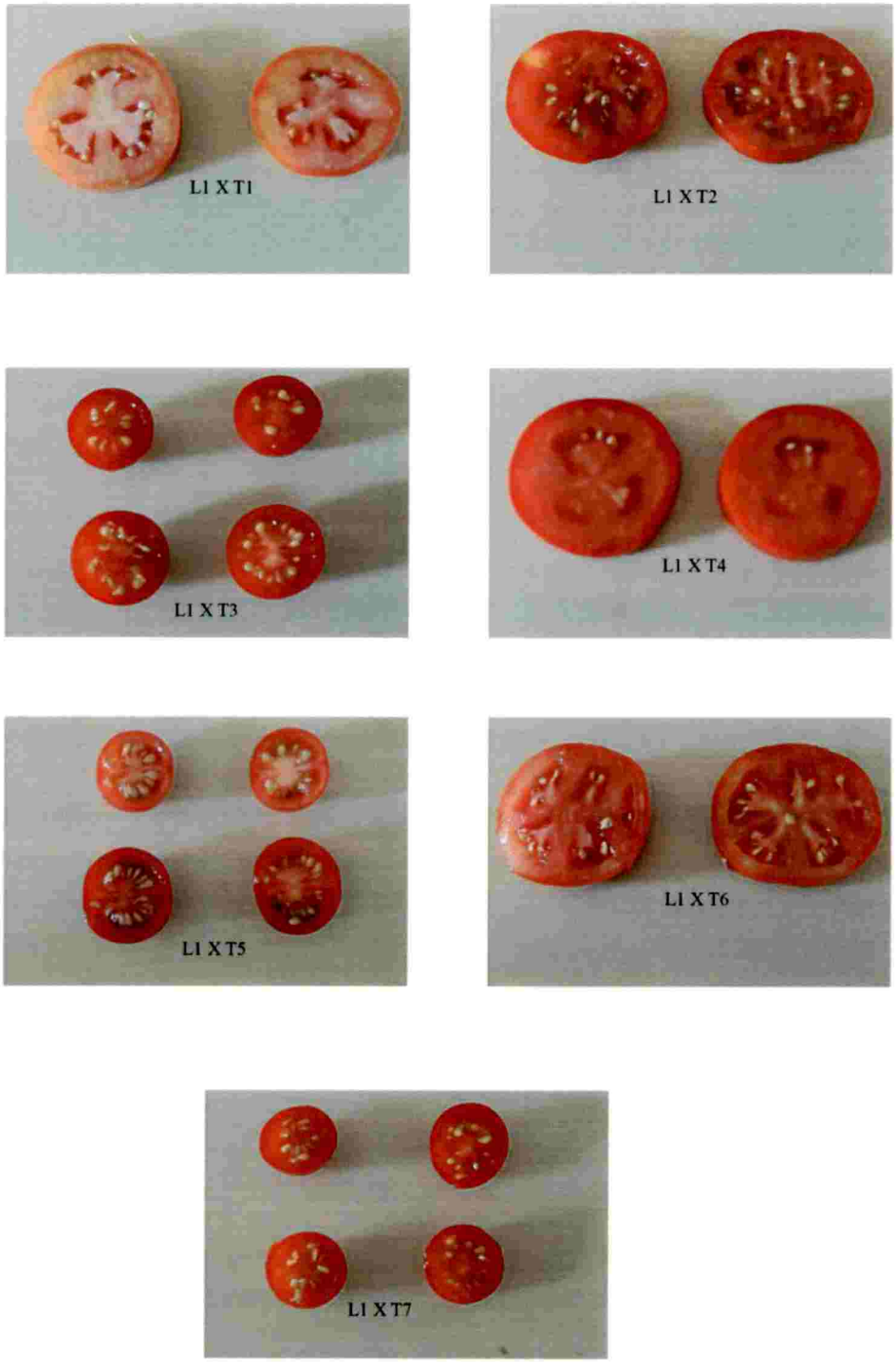


Plate 17. Variability in locules of different F_1 hybrids in tomato

4.4.4 Fruit quality parameters

Pericarp thickness

Pericarp thickness ranged from 3.20 mm to 9.60 mm with a mean of 5.48 mm (Table 29 a). Maximum pericarp thickness was observed in hybrid L_1 x T_4 (Anagha x IIHR 2372) (9.60 mm) and minimum pericarp thickness was observed in hybrid L_1 x T_3 (Anagha x EC 168283) (3.20 mm), Average of pericarp thickness in check variety Vellayani Vijai was 4.32 mm (Fig. 30).

Lycopene (mg/100 g)

Lycopene content ranged from 7.22 mg to 12.64 mg with a mean of 9.22 mg (Table 29 a). Highest content of lycopene was observed in hybrid L_1 x T_5 (Anagha x EC 541109) (12.64 mg) and lowest content of lycopene was observed in hybrid L_1 x T_4 (Anagah x IIHR 2372), Average of lycopene content in check variety Vellayani Vijai was 12.28 mg (Fig. 31).

Vitamin C (mg/ 100 g)

Vitamin C content ranged from 18.11 mg to 36.23 mg with a mean of 29.75 mg (Table 29 a). Highest content of vitamin C was observed in hybrids L_1 x T_4 (Anagha x IIHR 2372) (36.23 mg) and L_1 x T_6 (Anagha x IIHR 2200) (36.23 mg) and lowest content of vitamin C was observed in hybrid L_1 x T_2 (Anagha x Nandi) (18.11 mg). The check variety Vellayani Vijai recorded (25.36 mg) content of vitamin C (Fig. 32).

Carotene (mg/100 g)

Carotene content ranged from 3.29 mg to 9.16 mg with a mean of 5.99 mg (Table 29 a). Highest content of carotene was observed in hybrid L_1 x T_7 (Anagha x LA 2805) (9.16 mg), While lowest content of carotene was observed in hybrid L_1 x T_1 (Anagha x Vaibhav) (3.29 mg). Average of carotene content in check variety Vellayani Vijai was 3.35 mg (Fig. 33).

pH of juice

pH of juice ranged from 4.52 to 4.93 with a mean of 4.64 (Table 29 a). Highest pH was observed in hybrid L_1 x T_1 (Anagha x Vaibhav) (4.93) whereas lowest pH was observed in hybrid L_1 x T_4 (IIHR 2372) (4.52). The check variety Vellayani Vijai recorded (4.39) pH (Fig. 34).

Total soluble solids (%)

Total soluble solids (%) ranged from 4.63% to 8.24% with a mean of 6.85% (Table 29 a). Highest total soluble solids (%) were observed in hybrid L_1 x T_5 (Anagha x EC 541109) (8.24%), while lowest content of total soluble solids (%) was observed in hybrid L_1 x T_6 (Anagha x IIHR 2200) (4.35%). The check variety Vellayani Vijai recorded (6.09%) of total soluble solids (Fig. 35).

Shelf life (days)

Shelf life ranged from 12.11 days to 17.89 days with a mean of 13.93 days (Table 29 a). Maximum shelf life was recorded in hybrid L_1 x T_1 (Anagha x Vaibhav) (17.89 days). Whereas lowest shelf life was recorded in hybrid L_1 x T_2 (Anagha x Nandi) (12.11 days). The check variety Vellayani Vijai recorded (10.85 days) for shelf life (Fig. 36).

4.4.5 Screening of hybrids and parental genotypes under natural field condition for tomato leaf curl virus resistance (ToLCV)

Individual plant scores for total number of resistant and susceptible plants for tomato leaf curl virus with 0-4 scale score for seven hybrids and eight parental genotypes are described in Table 30.

4.4.5.1 Per cent disease severity

Per cent disease severity result as indicated in Table... revealed that tomato hybrids and parental genotypes exhibited a wide range of resistance reaction against ToLCV under field condition. Among the seven hybrids and eight parental genotypes four hybrids viz., L_1 x T_3 (Anagha x EC 168283), L_1 x T_5 (Anagha x EC 541109), L_1 x T_6 (Anagha x IIHR 2200), L_1 x T_7 (Anagha x LA 2805) and seven parental genotypes *viz.*, Vaibhav, Nandi, IIHR 2372, IIHR 2200, EC 168283, EC 541109, LA 2805 recorded disease severity of 0.00% without any symptoms. Hybrid L_1 x T_2 (Anagna x Nandi) and L_1 x T_4 (Anagha x IIHR 2372) recorded disease severity of 14.17%, L_1 x T_1 (Anagha x Vaibhav) recorded a disease severity of 16.67%, whereas parental genotype Anagha showed highest disease severity of 41.67%.

Table 29 a. Mean performance of hybrids and parental genotypes for seventeen characters in tomato

Sr. No.	Hybrids/Genotype	No. of locules fruit^{-1}	Vol. of the fruit (ml)	Pericarp thickness (mm)	Lycopene (mg/100 g)	Vitamin C (mg/100 g)	Carotene (mg/100 g)	pH of juice	TSS (%)	Shelf life (days)
1	L_1 x T_1 (Vaibhav)	3.00	61.52	7.31	7.91	34.42	3.29	4.93	6.23	17.89
2	L_1 x T_2 (Nandi)	4.78	45.20	5.24	7.96	18.11	5.05	4.74	6.27	12.11
3	L_1 x T_3 (EC 168283)	2.00	6.21	3.20	9.30	32.58	6.26	4.63	7.86	12.89
4	L_1 x T_4 (IIHR 2372)	3.00	54.53	9.60	7.22	36.23	6.44	4.52	7.60	16.00
5	L_1 x T_5 (EC 541109)	2.00	5.61	3.21	12.64	27.17	8.43	4.58	8.24	13.78
6	L_1 x T_6 (IIHR 2200)	4.67	53.04	6.20	7.90	36.23	3.36	4.60	4.63	12.33
7	L_1 x T_7 (LA 2805)	2.00	6.45	3.65	11.66	23.55	9.16	4.54	7.18	12.55
	Mean of hybrids	3.24	37.68	5.48	9.22	29.75	5.99	4.64	6.85	13.93
8	Vaibhav	4.11	61.42	7.49	7.46	36.23	3.31	4.41	6.20	15.55
9	Nandi	4.00	40.56	4.83	7.25	19.93	4.37	4.47	6.31	9.66
10	IIHR 2372	2.00	44.33	9.33	8.21	34.42	5.40	4.31	8.35	14.11
11	IIHR 2200	2.00	42.25	6.31	8.44	36.23	2.58	4.13	4.35	11.89
12	EC 168283	2.00	2.68	2.15	9.17	30.80	5.36	4.37	4.41	12.44
13	EC 541109	2.00	2.60	2.27	13.08	25.36	9.35	4.57	10.25	12.55
14	LA 2805	2.00	2.73	2.72	12.03	21.74	9.09	4.57	10.13	11.55
15	Anagha (L_1)	2.11	28.95	6.09	10.33	16.30	2.49	4.37	6.34	11.65
16	Vellayani Vijai (Check)	2.22	32.53	4.32	12.28	23.36	3.35	4.39	6.09	10.85
	C.D. (5%)	0.26	1.20	0.10	0.23	5.01	0.44	0.02	0.11	0.85
	S.E (m)	0.09	0.41	0.03	0.08	1.73	0.15	0.01	0.04	0.29
	C.V.	5.71	2.34	1.11	1.45	10.56	4.81	0.31	0.97	3.88

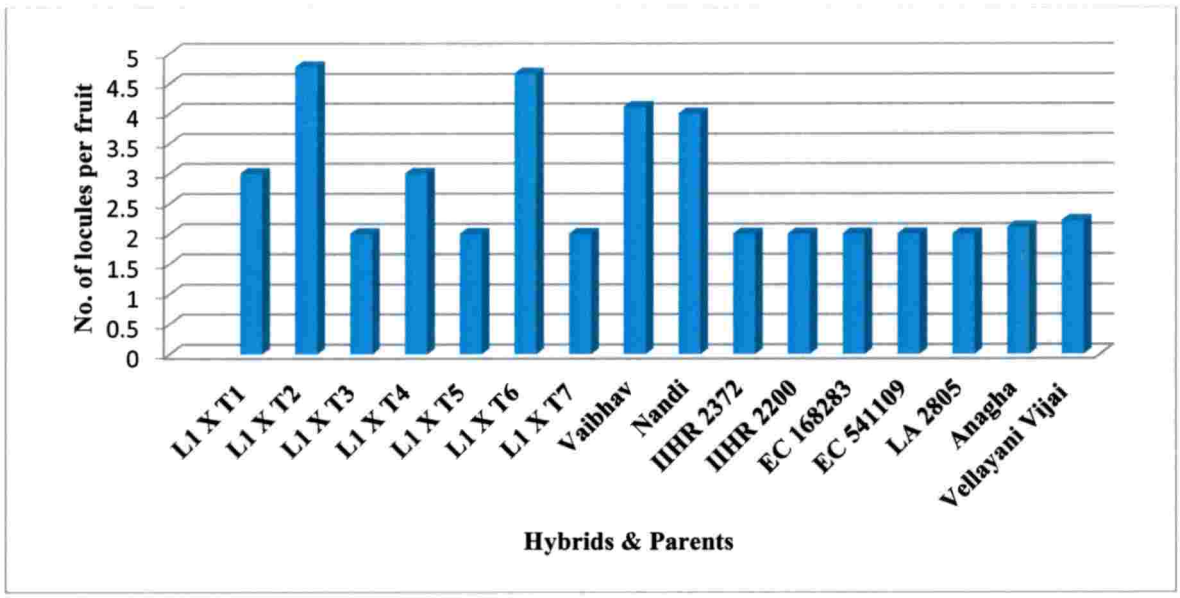


Fig 28. Number of locules per fruit in hybrids & parents of tomato

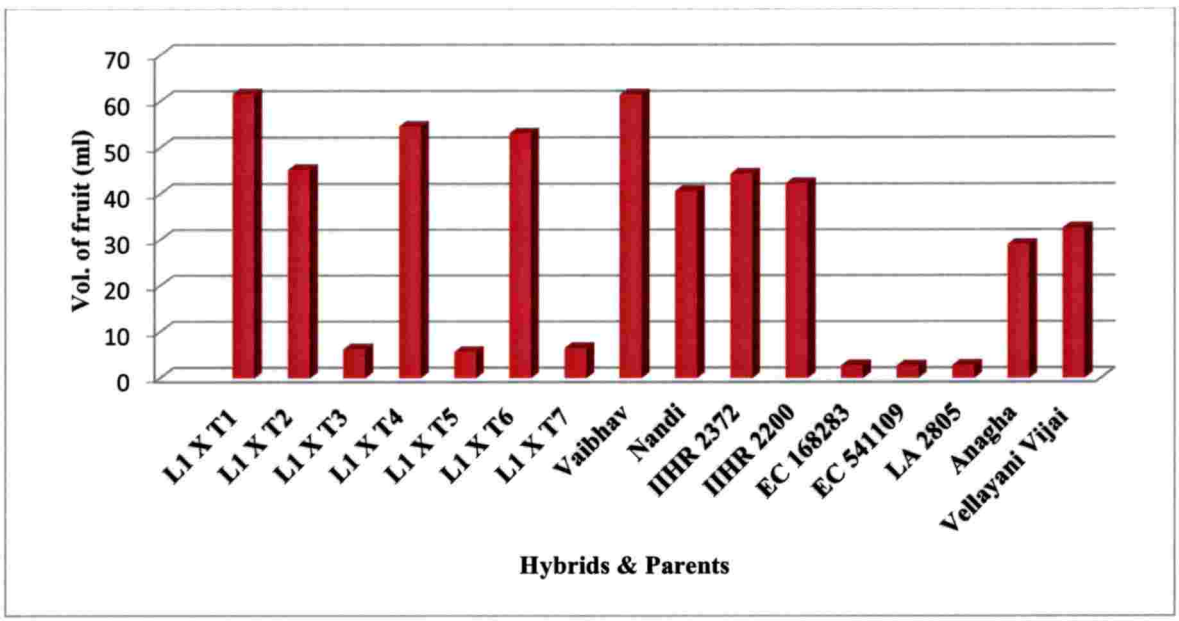


Fig 29. Volume of fruit (ml of water displaced) in hybrids & parents of tomato

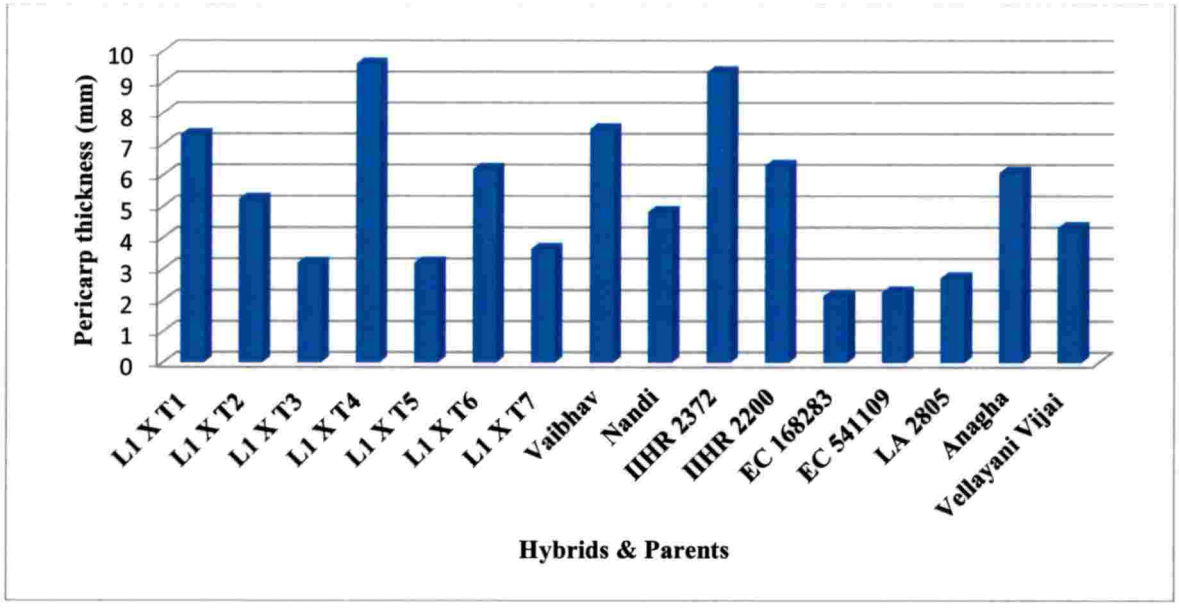


Fig 30. Pericarp thickness (mm) in hybrids & parents of tomato

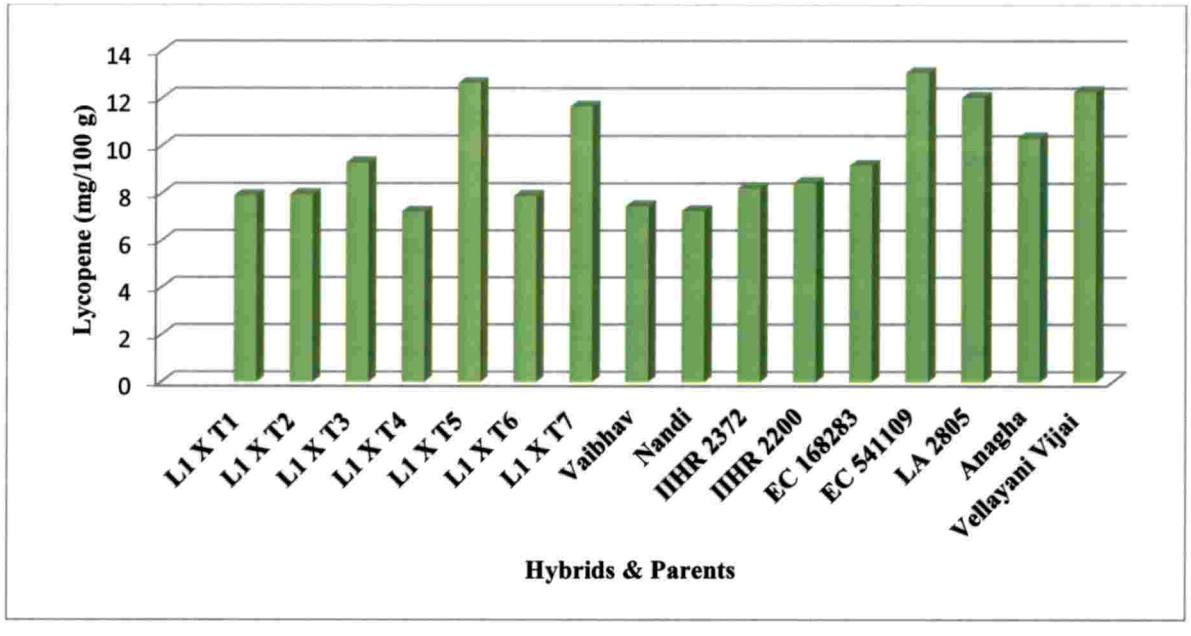


Fig 31. Lycopene (mg/100 g) in hybrids & parents of tomato

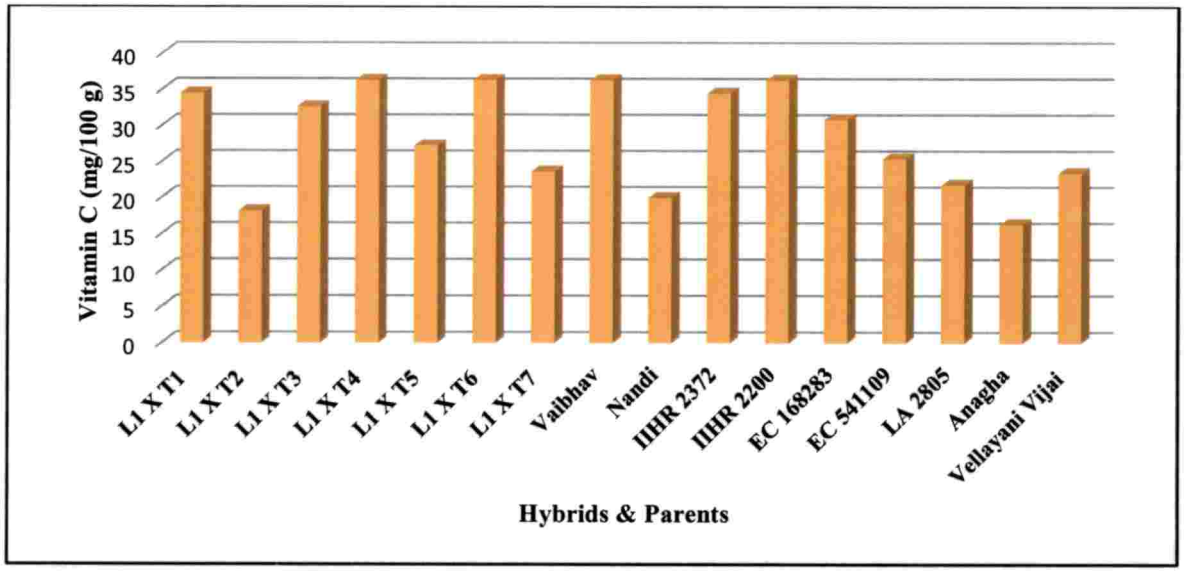


Fig 32. Vitamin C (mg/100 g) in hybrids & parents of tomato

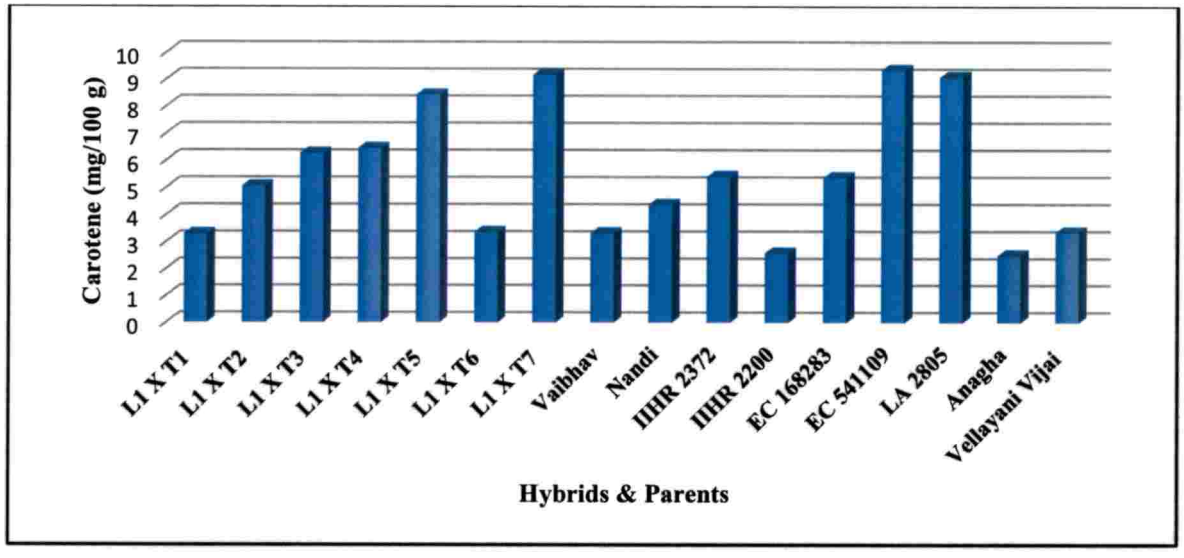


Fig 33. Carotene (mg/100 g) in hybrids & parents of tomato

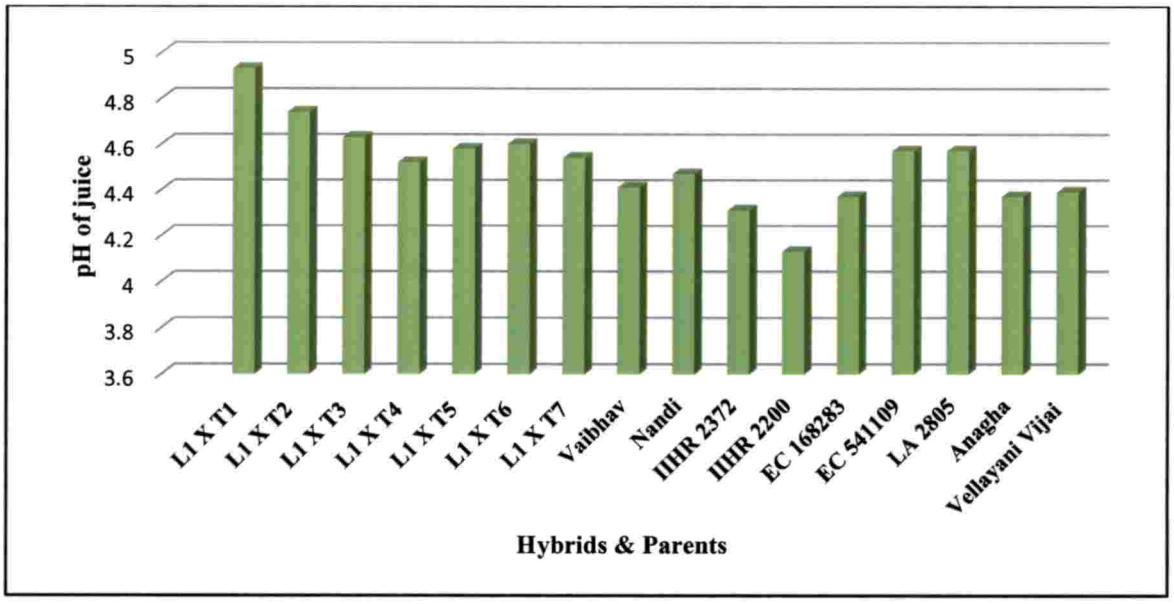


Fig 34. pH of juice in hybrids & parents of tomato

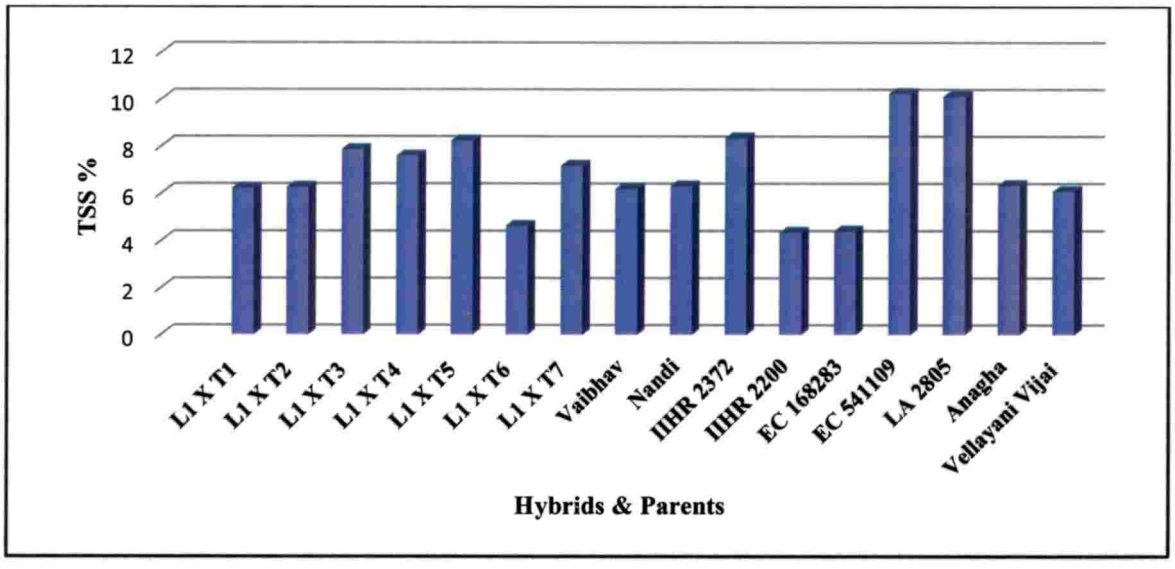


Fig 35. TSS % in hybrids & parents of tomato

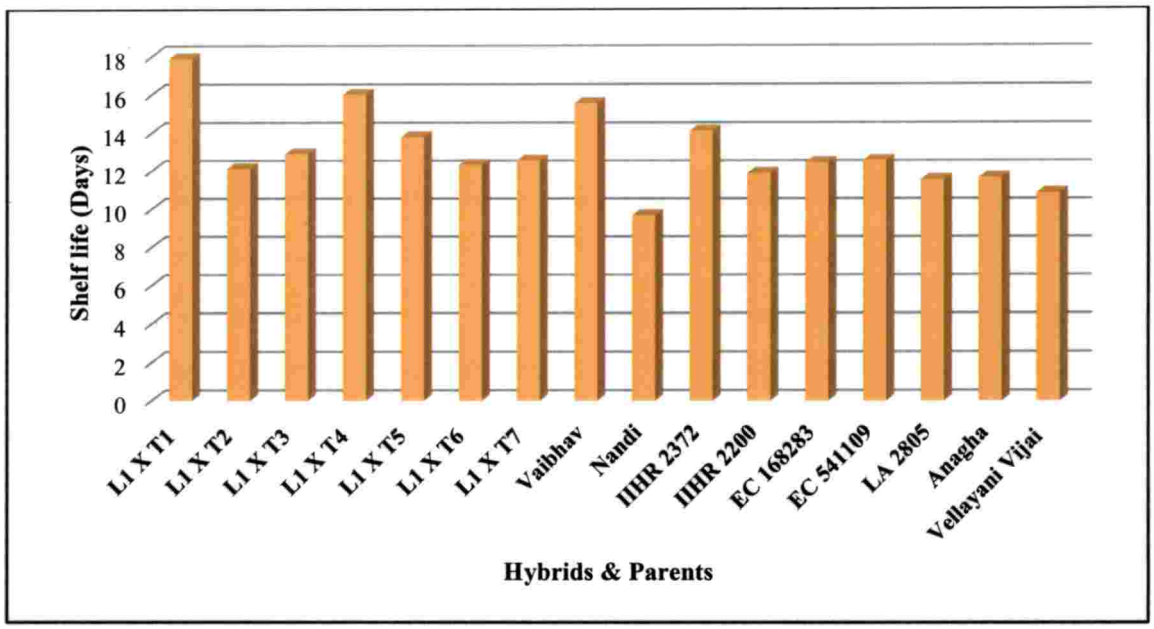


Fig 36. Shelf life (Days) in hybrids & parents of tomato

Table 30. Number of plants in five classes (score) of tomato leaf curl virus symptoms in natural field conditions

Disease score							
Sr. No.	**Hybrid/ Genotype**	**No. of plants scored**	**0**	**1**	**2**	**3**	**4**
1	L_1 x T_1 (Anagha x Vaibhav)	30	11	18	1	0	0
2	L_1 x T_2 (Anagha x Nandi)	30	15	13	2	0	0
3	L_1 x T_3 (Anagha x EC 168283)	30	30	0	0	0	0
4	L_1 x T_4 (Anagha x IIHR 2372)	30	14	15	1	0	0
5	L_1 x T_5 (Anagha x EC 541109)	30	30	0	0	0	0
6	L_1 x T_6 (Anagha x IIHR 2200)	30	30	0	0	0	0
7	L_1 x T_7 (Anagha x LA 2805)	30	30	0	0	0	0
8	Vaibhav	30	30	0	0	0	0
9	Nandi	30	30	0	0	0	0
10	IIHR 2372	30	30	0	0	0	0
11	IIHR 2200	30	30	0	0	0	0
12	EC 168283	30	30	0	0	0	0
13	EC 541109	30	30	0	0	0	0
14	LA 2805	30	30	0	0	0	0
15	Anagha	30	0	12	16	2	0

Symptom severity grade and symptoms

0- No visible symptoms

1- Very mild curling upto 25% leaves

2- Curling & puckering upto 26-50% leaves

3- Severe curling & puckering upto 51-75% leaves

4- Very severe curling & puckering upto 76-100% leaves

4.4.5.2 Per cent disease incidence

The per cent disease incidence was calculated using formula the number of plants infected divided by the total number of plant observed multiplied by 100. The result of per cent disease incidence mentioned in Table,,, Out of seven hybrids and eight parental genotypes, four hybrids L_1 x T_3 (Anagha x EC 168283), L_1 x T_5 (Anagha x EC 541109), L_1 x T_6 (Anagha x IIHR 2200), L_1 x T_7 (Anagha x LA 2805) and seven parental genotypes Vaibhav, Nandi, IIHR 2372, IIHR 2200, EC 168283, EC 541109, LA 2805 were not infected by the tomato leaf curl virus, it means 0% disease incidence. Whereas hybrids L_1 x T_2 (Anagna x Nandi) and L_1 x T_4 (Anagha x IIHR 2372) and L_1 x T_1 (Anagha x Vaibhav) recorded per cent disease incidence from 50-65% and parental genotype Anagha showed 100% of disease incidence (Table 31).

4.4.5.3 Coefficient of the infection (CI)

The coefficient of the infection of seven tomato hybrids and eight parental genotypes is mentioned in Table,,, Based on the coefficient of infection, the hybrids and parental genotypes were categorized into six groups by Banerjee and Kalloo (1988). Highly resistant reaction was found in four hybrids *viz.*, L_1 x T_3 (Anagha x EC 168283), L_1 x T_5 (Anagha x EC 541109), L_1 x T_6 (Anagha x IIHR 2200), L_1 x T_7 (Anagha x LA 2805) and seven parental genotypes highly resistant genotypes EC 541109 (*Solanum pimpinellifolium* L.) EC 168283 (*Solanum pimpinellifolium* L.) and LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) were wild species and IIHR 2372, IIHR-2200, Vaibhav and Nandi were four cultivated species of tomato all these hybrids and parental genotypes recorded (0%) of Coefficient of the infection (CI) and were under highly resistant category for tomato leaf curl virus disease. Hybrids L_1 x T_2 (Anagna x Nandi) recorded a 7.08% and L_1 x T_4 (Anagha x IIHR 2372) recorded a 7.56% of Coefficient of the infection (CI) and was under Resistant category for tomato leaf curl virus disease. Whereas hybrid L_1 x T_1 (Anagha x Vaibhav) recorded 10.56% and was under moderately resistant category for tomato leaf curl virus disease. Parental genotype Anagha recorded 41.67% of Coefficient of the infection and was under susceptible category (Table 31).

Fertility of hybrids were analyzed by studying pollen staining technique which revealed 100% fertility in all the crosses and hence all the hybrids obtained were fertile (Table 32).

Table 31. Reaction of hybrids and parental genotypes to local strains of Tomato leaf curl virus in field conditions

Sr. No	Genotypes	PDS (%)	PDI (%)	CI	Category
1	L_1 x T_1 (Anagha x Vaibhav)	16.67	63.33	10.56	MR
2	L_1 x T_2 (Anagha x Nandi)	14.17	50.00	7.08	R
3	L_1 x T_3 (Anagha x EC 168283)	0.00	0.00	0.00	HR
4	L_1 x T_4 (Anagha x IIHR 2372)	14.17	53.33	7.56	R
5	L_1 x T_5 (Anagha x EC 541109)	0.00	0.00	0.00	HR
6	L_1 x T_6 (Anagha x IIHR 2200)	0.00	0.00	0.00	HR
7	L_1 x T_7 (Anagha x LA 2805)	0.00	0.00	0.00	HR
8	Vaibhav	0.00	0.00	0.00	HR
9	Nandi	0.00	0.00	0.00	HR
10	IIHR 2372	0.00	0.00	0.00	HR
11	IIHR 2200	0.00	0.00	0.00	HR
12	EC 168283	0.00	0.00	0.00	HR
13	EC 541109	0.00	0.00	0.00	HR
14	LA 2805	0.00	0.00	0.00	HR
15	Anagha	41.67	100.00	41.67	S

Table 32. Fertility status of hybrids by pollen staining technique.

Sr. No.	Hybrids	Pollen fertility%
1	L_1 x T_1 (Anagha x Vaibhav)	100
2	L_1 x T_2 (Anagha x Nandi)	100
3	L_1 x T_3 (Anagha x EC 168283)	100
4	L_1 x T_4 (Anagha x IIHR 2372)	100
5	L_1 x T_5 (Anagha x EC 541109)	100
6	L_1 x T_6 (Anagha x IIHR 2200)	100
7	L_1 x T_7 (Anagha x LA 2805)	100

Discussion

5. DISCUSSION

Tomato leaf curl virus (ToLCV) is one of the serious viral disease in Indian condition. It is a limiting factor for successful cultivation of tomato in all regions. In India the ToLCV causes 100 % infection and yield losses upto 90 %. The management of the disease has become a challenge to both farmers and researchers. Though several ToLCV resistant varieties and hybrids have been released for commercial cultivation, resistance to ToLCV in these releases is not stable under different regions of the country due to prevalence of different begomoviruses. Hence, there is a strong need to develop a reliable screening method and technique against ToLCV resistance. This has been facilitated by the identification of molecular markers linked to ToLCV resistance in tomato. Breeding for host plant resistance is the only solution to tackle this virus disease. The current investigations were therefore initiated to identify resistant genotypes, confirm the presence of resistance genes through molecular markers and to develop hybrids from these identified resistance gene donors.

5.1 SCREENING OF GENOTYPES UNDER NATURAL FIELD CONDITION FOR TOMATO LEAF CURL VIRUS RESISTANCE (ToLCV)

Thirty-four genotypes were screened in summer season under natural field conditions from February 2015 to May 2015 with an approximate temperature of 32 ^{O}C to 34 ^{O}C with hot and humid climate, which is favorable for ToLCV disease. Eight genotypes EC 541109 (*Solanum pimpinellifolium* L.), EC 168283 (*Solanum pimpinellifolium* L.), IIHR 1970 (*Solanum peruvianum* L.) and LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) wild species and IIHR 2372, IIHR-2200, Vaibhav and Nandi were found to be highly resistant. Genotype EC 165751 was found to be resistant whereas, Genotype EC 620545 was recorded moderately resistant, Twelve genotypes S 22, Arka Meghali, EC 362944, IC 549835, Akshay, Arka Vikas, EC 320574, EC 322634, IC 247508, Manulekshmi, Anagha and EC 164656 were found to be Moderately susceptible, Six genotypes Vellayani Vijai, S 7, Hawaii, PKM 1, Arka Abha and Arka Alok were found to be susceptible and six genotypes *viz.*, BWR 5, Surya, EC 620419, EC 326142, EC 16786 and Palam pride were found to be Highly susceptible. *S. habrochaites, S. pimpenellifolium, S. chmielewskii, S. chilence* and *S. peruvianum* had been reported to be resistant to ToLCV by Banarjee and Kaloo

(1987b), Zamir *et al.,* (1994), Pico *et al.,* (1996), Vidavsky and Czosnek (1980, Pilowsky and Cohen (2000).

Reports on ToLCV screening by other authors in different tomato genotypes are as follows:

Divakaran *et al.*, (2008) who screened 15 genotypes for ToLCV in kerala condition found genotypes *viz.,* Hawaii 7998, H-24, H-86, LE-658 and LE-651 to be highly resistant to ToLCV. Swarna Lalima, Swarna Naveen and Sakthi were reported as susceptible and genotypes BT-218, BL-333-3-1 and Mukthi as highly susceptible to ToLCV in pot culture experiment. In field experiment, genotypes Hawaii 7998, H-24, LE-638, LE-658, LE-651 and LE-640 was highly resistant. LE-474 was resistant and BL-333-1-1 was moderately susceptible to infection. Swarna Naveen and Sakthi were reported as susceptible and BT-218, Swarna Lalima and Mukthi were highly susceptible.

Field screening study conducted by Chakraborty *et al.*, (2006) against leaf curl virus disease in tomato in West Bengal to identify source of resistance for future multiplication, genetic improvement and cultivation, none of the lines were free from the disease. Less disease was found in determinate cultivar BSS-422 (9.36%) compared to control cultivar TH-01462 (10.07%) and in open- pollinated determinate lines, the lowest incidence was recorded in KDTS-171 (13.61%) against the control line CO-3 (15.39%).

Dechin (2011) Screened nineteen genotypes under field condition, four genotypes viz., N-5, H-86, N-1 and N-5-3 were highly resistant to ToLCV, Eleven genotypes viz., H-86-2, H-86-2-2, H-86-3, H-86-4, H-86-5, H-86-5-1, H-88-3, H-348-1, H-348-4, H-348-5 and N-5-4 were under resistant group, Pusa Sadabahar and Pusa Gaurav were in susceptible group. Genotypes Pusa Ruby and Pusa Rohini were categorised as highly susceptible to virus infection.

Confirmation studies were carried out in order to ascertain the nature of resistance, since the resistant reaction expressed consequent to virus inoculation can be either due to escape or due to true resistance. Grafting was done to confirm the resistance under green house conditions. Of the eight genotypes which were highly resistant in natural field screening, all eight genotypes were completely free of disease

in graft transmission confirming the true resistance of these genotypes to ToLCV even after grafting with the infected susceptible root stock. Friedmann *et al.,* (1998) and Gomez *et al.,* (2004) also effectively used the same technique for artificial screening against ToLCV in tomato. Gomez *et al.,* (2004) used the same technique for artificial grafting technique screening for confirmation of ToLCV resistance in tomato. Ahmed (2014) also reported grafting confirmation for tomato leaf curl virus resistance.

5.2 EVALUATION OF GENOTYPES FOR YIELD AND FRUIT QUALITY PARAMETERS

Thirty-four genotypes were screened in *rabi* season under field conditions from November 2015 to March 2016 for estimation of yield and fruit quality traits.

5.2.1 Mean performance

The range of mean values could present a rough estimate about the magnitude of variations present among genotypes. The characters showing high range of variation showing more scope for improvement. All seventeen characters under the study exhibited high variability. Of the seventeen characters studied, plant height, number of primary branches plant^{-1} and spread of the plant largely determine the fruit bearing surface and thus control growth attributes. Higher plant height and more number of branches on main stem with wide spread of the plant, higher is the number of fruits plant^{-1} because of more fruit bearing surface area. Hence high mean average value is desirable for plant height, number of fruits plant^{-1} and wide spread of the plant to get high fruit yield in tomato.

Days to 50% flowering and number of days to first fruit harvest are the indicators of earliness in tomato. Early flowering not only gives early pickings and better returns but also widens fruiting period of the plant. Low mean average value is highly desirable for these attributes of earliness. Number of fruits plant^{-1}, weight of fruit and volume of fruit are considered to be associated directly with weight of fruits plant^{-1} for which higher mean value is desirable. Number of locules fruit^{-1}, pericarp thickness, lycopene content, vitamin C, carotene content, pH of juice, total soluble solids and shelf life are regarded as fruit quality attributes for which high mean values are desirable.

Out of thirty-four genotypes evaluated EC 320574, EC 620545, EC 165751, EC 326142 and EC 322634 produced taller plants whereas Arka Abha, Anagha and Surya produced shorter plants. Number of primary branches plant^{-1} was highest in EC 320574, EC 165751, EC 620545, EC 362944 and EC 322634 and lowest in Vaibhav, Surya and Anagha whereas spread of the plant was highest in genotypes EC 620545, EC 320574, EC 326142, EC 165751 and EC 322634 and was lowest in genotypes PKM 1, Surya and Arka Alok.

Among the earliness attributes Number of days to 50% flowering, genotypes Vellayani Vijai, Arka Alok, Anagha, Arka Vikas and BWR 5 took minimum days and genotypes EC 326142, EC 164656 and IC 247508 showed maximum days. Genotypes Arka Vikas followed by Vellayani Vijai, BWR 5, Arka Alok and Palam Pride showed minimum and genotypes EC 326142, EC 362944 and EC 322634 showed maximum number of days to first fruit harvest.

With respect weight of fruits plant^{-1} highest was observed in genotypes Vaibhav, EC 165751, EC 164656, IC 247508 and EC 16786 and lowest was observed in genotypes Manulekshmi, Arka Alok and Surya. Genotypes EC 165751, IC 549835, EC 362944, Vaibhav and EC 322634 showed highest number of fruits plant^{-1} and genotypes Arka Abha, S 22 and Surya recorded lowest number of fruits plant^{-1}.

Weight of fruit was observed higher in genotypes Vaibhav, S7, S 22, EC 620545 and EC 620419 and lower in genotypes Anagha, EC 326142 and IC 549835. Genotypes Vaibhav, S 22, S7, EC 620545 and EC 164656 recorded high volume of fruit whereas Anagha, EC 356142 and IC 549835 recorded low.

The fruit pH plays important role in the development of tomato cultivars for processing purpose, fruits with high TSS, pH less than 4.5 and higher ascorbic acid and lycopene content are preferred for processing (Bose *et al.*, 2002). With respect to fruit quality attributes genotypes IIHR 2372, EC 620419, Vaibhav, EC 362944 and IC 247508 were superior for pericarp thickness. Genotypes Vellayani Vijai, Akshay, Anagha, Manulekshmi and IIHR 2200 were superior for lycopene content. Genotypes IIHR 2200, Akshay, Vaibhav, BWR 5 and EC 320574 showed high content of Vitamin C. Genotypes EC 326142, IC 549835, EC 164656, EC 320574 and BWR 5 showed superior for Carotene content.

Genotypes EC 164646, EC 322634, S 7, EC 320574 and Arka Meghali were superior for number of locules fruit^{-1}. Genotypes EC 620545, Arka Alok, S 22, EC 362944 and IC 549835 were superior for pH of juice. The sugar acid blend determines the quality of fruits for fresh consumption and processing, highest TSS % was observed in genotypes IIHR 2372, Arka Vikas, Arka Alok, EC 165751 and EC 326142. Shelf life is an important quality parameter in tomato as it plays role for storage and transportation to long distance markets, highest shelf life was observed in genotypes EC 16786, Vaibhav, PKM 1, IIHR 2372 and Akshay.

In wild genotypes EC 541109 (*Solanum pimpinellifolium* L.) performed superior for yield traits like plant height, number of primary branches plant^{-1}, number of fruits plant^{-1} and weight of fruit plant^{-1} it also showed superiority for fruit quality traits like number of locules fruit^{-1}, lycopene content, carotene content, pH of juice, TSS% and shelf life. Genotype EC 168283 (*Solanum pimpinellifolium* L.) performed superior for traits such as spread of plant, number of days to 50% flowering, number of days to first fruit harvest, weight of fruit and vitamin C content, whereas genotype LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) showed superior for traits such as volume of fruit and Shelf life.

5.2.2 Variability, Heritability (h^2) and Genetic advance

The analysis of variance revealed that, highly significant differences among the genotypes for all the characters indicating sufficient variability existed in the present material selected for the study and indicating the scope for selection of suitable initial breeding material for crop improvement. However, the absolute variability in different characters does not permit identification of the characters showing the highest degree of variability. Therefore, PCV and GCV values were estimated. The coefficient of variation whether it is genotypic or phenotypic, both are useful in studying the extent of variability in different characters as it measures the range of variability. The GCV values were slightly higher than the respective PCV for all the characters denoting little influence of environmental factors on their expression.

Higher estimates of PCV and GCV were obtained for plant height, number of primary branches plant^{-1}, number of fruits plant^{-1}, weight of fruits plant^{-1}, weight of fruit, number of locules fruit^{-1}, volume of fruit, pericarp thickness, lycopene, vitamin C and carotene indicating a good deal of variability in these characters signifying the

effectiveness of selection for desirable types for improvement. Similar results were reported by Nandapuri *et al.*, (1977), Bora *et al.,* (1993), Kumari and Subramanian (1994), Anandgowda (1997), Mohanty (2003), Akhilesh and Gulshanlal (2005), Singh (2005), Upadhayay *et al.*, (2005), Samadia *et al.,* (2006), Kumari *et al.,* (2007), Asati (2008).

Moderate estimates of PCV and GCC were obtained for spread of the plant, number of days to 50% flowering, total soluble solids and shelf life indicated the presence of moderate genetic variability for these characters respectively in tomato. Similar results made by Brar *et al.,* (1998), Prasad and Mathurarai (1999), Singh *et al.,* (2000), Prashanth (2003), Aradhana and Singh (2003), Mayavel *et al.,* (2005), Samadia *et al.,* (2006), Dhankar and Dhankar (2006) and Kumari *et al.,* (2007).

Low PCV and GCV for number of days to first fruit harvest and pH of juice suggested less variability existed in these characters. This moderate to low variability indicates the need for improvement of base population through intercrossing in F_2 generation followed by recurrent selection to increase the gene flow and to fix favorable alleles. Similar results were made by Mohanty (2003), Ara *et al.*, (2009).

Perusal of results on heritability and genetic advance as per cent of mean (GAM) revealed that heritability estimates were high for all the characters studied except number of days to first fruit harvest and pH of juice. This suggested the greater effectiveness of selection due to less influence of environment and improvement to be expected for these characters in future breeding programme.

Johnson *et al.*, (1955) suggested that high heritability coupled with high genetic advance as percentage of mean (GAM) were more useful than heritability alone in predicting the resultant effect during selection of best individual genotype. Genetic advance is the measure of genetic gain under selection and expression in percentage of mean.

In the present experiment high heritability and genetic advance as per cent of mean (GAM) was recorded for plant height, number of primary branches plant^{-1}, spread of the plant, number of fruits plant^{-1}, weight of fruit plant^{-1}, weight of fruit, number of locules fruit^{-1}, volume of fruit, pericarp thickness, lycopene, vitamin C, carotene, total soluble solids and shelf life indicating predominance of additive gene action for these

characters. Simple selection based on phenotypic performance of these characters would be more effective. Similar results are reported in tomato by Prashanth (2003), Veershetty, (2004) Singh *et al.,* (1988) and Singh *et al.,* (2000).

High heritability and moderate genetic advance as per cent of mean values were observed for the character number of days to first fruit harvest. This indicates the influence of non-additive gene action and considerable influence of environment in the expression of these traits. Similar results made by Mohanty (2003) and Ara *et al.*, (2009). While high heritability and low genetic advance as per cent of mean values were observed for the character pH of juice. These traits could be exploited through manifestation of dominance and epistatic components through heterosis. Hence, the breeder should adopt suitable breeding methodology to utilize both additive and non-additive gene effects simultaneously, since varietal and hybrid development will go a long way in the breeding programmes especially in case of tomato.

5.2.3 Phenotypic and genotypic correlation coefficient analysis

Yield is the resultant of combined effect of several component characters and environment. Understanding the interaction of characters among themselves and with environment has been of great use in the plant breeding. Correlation studies provide information on the nature and extent of association between only two pairs of metric characters. From this it would be possible to bring about genetic up gradation in one character by selection of the other of a pair, obviously, knowledge about character associations will surely help to identify the characters to make selection for higher yield with a view to determine the extent and nature of relationship prevailing among yield contributing characters. Hence, an attempt has been made to study the character association in the tomato genotypes.

The positive correlation of characters with respect weight of fruits plant^{-1} were significant only for traits, such as plant height, number of primary branches plant^{-1}, spread of the plant, number of days to 50% flowering, number of days to first fruit harvest, number of fruits plant^{-1}, weight of fruit and volume of fruit, Whereas positive non-significant correlation was observed for number of locules fruit^{-1}, pericarp thickness, carotene, pH of juice and shelf life.

Therefore, the positively correlated traits such as plant height, number of primary branches plant^{-1}, spread of the plant, number of days to 50% flowering, number

of days to first fruit harvest, number of fruits plant^{-1}, weight of fruit and volume of fruit will contribute immensely for fruit yield improvement. Hence the genotypes with higher number of fruits plant^{-1} (EC 165751, IC 549835, EC 362944, Vaibhav, and EC 322634), and higher weight of fruit Vaibhav, S 7, S 22, EC 620545 and EC 620419 can be selected for crop improvement programme. Similar result were observed by Dudhi and Kalloo (1982), Rattan *et al.,* (1983), Patil (1998) and Reddy and Gulshanlal (1987).

5.2.4 Path coefficient analysis

Overall the path analysis confined that direct effect on number of fruits plant^{-1} expressed highest positive direct effect on weight of fruits plant^{-1} followed by weight of fruit, spread of the plant, lycopene, number of primary branches plant^{-1}, number of days to 50% flowering and number of days to first fruit harvest, Similarly positive direct effect of various characters on fruit yield plant^{-1} were observed by Aravindakumar and Mulge (2002), Tiwari (2002), Prashanth (2003), Joshi *et al.,* (2004), Lakshmikant and Mani (2004), Mayavel *et al.,* (2005), Raut *et al.*, (2005), Samadia *et al.*, (2006), Ara *et al.*, (2009), Indurani *et al.,* (2010), Bernousi *et al.*, (2011).

5.3 SCREENING WITH THE MARKER LINKED TO THE GENES OF RESISTANCE TO TOLCV

Six major genes for resistance to tomato leaf curl virus has been reported (Brenda *et al.,* 2007, Ji *et al.*, 2007 and Jensen *et al.*, 2007). Molecular markers linked to these genes can be used for their identification. In this study thirty-four genotypes were screened for the presence of three genes resistance to ToLCV (*Ty2, Ty3* and *Ty3a*) using SCAR molecular marker.

5.3.1 Confirmation of *Ty2* gene for resistance using SCAR molecular marker

A SCAR marker for *Ty2* gene was first reported by Brenda *et al.,* (2007) and primer pair T0302F/TY2R1 produced products of 450 base pair in the susceptible and 600 base pair in the resistant genotypes. These primers were designed from the resistant line H24, which carry *Ty2* gene.

In this experiment, genotypes IIHR 2200, and EC 168283 confirmed the presence of homozygous resistance allele at 600 base pair. Genotype Vaibhav showed the heterozygous alleles both at 450 base pair and 600 base pair. Whereas all other remaining genotypes showed susceptible homozygous allele at 450 base pair.

Homozygous resistant plants carried only *Ty2/Ty2* alleles and susceptible carried *ty2/ty2* alleles. Heterozygous resistant plants carried two markers which express the presence of *Ty2/ty2* alleles. Here resistant markers indicates the presence of *Ty2* gene for resistance to tomato leaf curl virus disease and found in genotypes derived from the *S. habrochaites* line H-24 and susceptible band indicates the presence of ty2 gene as in genotypes derived from *S. chilense* accession LA 2279 (Brenda *et al.,* 2007). Hanson *et al.*, (2006) identified the *Ty2* gene, which has resistance to tomato leaf curl virus disease (ToLCV).

5.3.2 Confirmation of *Ty3* gene for resistance using SCAR molecular marker

FLUW-25 marker for *Ty3* gene was derived from the begomovirus resistant *S. chilense* accession line LA 2779 and was identified by Ji *et al.*, (2007) and the primers were used for screening thirty-four genotypes, none of the genotype showed allele for resistance. All genotypes showed a susceptible allele at 480 base pair.

5.3.3 Confirmation of *Ty3a* gene for resistance using SCAR molecular marker

Ty3a gene was derived from the resistant line of accession LA 1932. P6-25 primers for *Ty3a* gene giving resistance to tomato leaf curl virus was reported by Jensen *et al.*, (2007). Genotype IIHR 1970 (*Solanum peruvianum* L.) showed resistant allele of size 630 base pair confirming the presence of the *Ty3a* gene for ToLCV resistance, whereas all other genotypes showed a susceptible marker of size 320 base pair. This corresponds to IIHR 1970 is derived from the introgression of accession LA 1932.

The resistant genotypes Nandi, EC 541109, IIHR 2372, LA 2805 which showed resistance in field as well as in test grafting did not show presence of any of the three genes *Ty2, Ty3* and *Ty3a*. These resistant genotypes may be having other genes for resistance to ToLCV (*Ty1, Ty4, Ty5* or *Ty6*). The genes for resistance are specific to the strain of virus prevailing in a location. So far the strain of ToLCV in kerala is not identified.

5.4 EVALUATION OF SUCCESSFUL F_1 HYBRIDS AND PARENTS FOR YIELD, QUALITY, RESISTANCE AND FERTILITY STATUS

Seven hybrids produced from four cultivated and three wild species were screened under field conditions from April to August 2017 with an approximate temperature of 32 ^{0}C to 34 ^{0}C. Hybrids from all cross combinations recorded 100%

pollen fertility confirming the compatibility of the wild species *Solanum pimpinellifolium* L. and *Solanum lycopersicum var. cerasiforme* L. with the cultivated species *Solanum lycopersicum* L.

5.4.1 Mean performance

Mean performance of the hybrids with respect to seventeen morphological traits could present a rough estimate about the variation in magnitude of variability present among the hybrids. Hybrids of the cultivated varieties showed better performance than the better parent. In the interspecific hybrids, they inherited favourable characters of wild parents *viz.,* high lycopene, carotene and TSS. But the fruit size was intermediate between that of parents.

Plant height, number of primary branches plant^{-1} and spread of the plant largely determine the fruit bearing surface and thus control as growth attributes. High plant height and more number of branches on main stem with wide spread of the plant, higher is the number of fruits plant^{-1} because of more fruit bearing surface area. Hence high mean average value id desirable for plant height, number of fruits plant^{-1} and wide spread of the plant to get high fruit yield in tomato. Plant height is usually indicative of its vegetative vigour which influences the productivity. Maximum plant height and highest number of primary branches plant $^{-1}$ was recorded in hybrid L_1 x T_5 (Anagha x EC 541109). Parental genotype EC 541109 recorded maximum plant height and highest number of primary branches plant $^{-1}$ both these traits were inherited in hybrid. Highest spread of the plant was observed in hybrid L_1 x T_7 (Anagha x LA 2805).

Days to 50% flowering and number of days to first fruit harvest are the indicators of earliness in tomato. Early flowering not only gives early pickings and better returns but also widens fruiting period of the plant. Low mean average value is highly desirable for these attributes of earliness. Number of day to 50% flowering taken by a hybrid to put forth is generally indicative of its earliness. Hybrid L_1 x T_2 (Anagha x Nandi) took minimum number of days to 50% flowering and number of days to first fruit harvest. The parents of this hybrid are cultivated popular varieties.

Number of fruits plant^{-1}, weight of individual fruit and volume of fruit are considered to be associated directly weight of fruits plant^{-1} for which higher mean value is desirable. Highest number of fruits plant^{-1} were recorded in hybrid L_1 x T_3 (Anagha x

EC 168283) followed by L1 X T5 (EC 541109), Maximum weight of fruits plant^{-1} was observed in hybrid L_1 x T_1 (Anagha x Vaibhav) followed by L_1 x T_6 (IIHR 2200) highest weight of fruit was observed in parental genotype this trait was inherited in the hybrid. Maximum weight of fruit was recorded in hybrid L_1 x T_1 (Anagha x Vaibhav) followed by L_1 x T_4 (IIHR 2372). In all these hybrids the mean performance in the yield traits was better than the better parent suggesting high heterobeltiosis.

Number of locules fruit^{-1}, pericarp thickness, lycopene content, vitamin C, carotene content, pH of juice, total soluble solids and shelf life are regarded as fruit quality attributes for which high mean values are desirable. Maximum number of locules fruit^{-1} were observed in hybrid L_1 x T_2 (Anagha x Nandi) followed by L_1 x T_6 (Anagha x IIHR 2200).

Estimation of fruit volume is mainly related with fruit shape and a strong relationship is reported in tomato (Mutschler *et al.,* 1986). Maximum volume of fruit was recorded in hybrid L_1 x T_1 (Anagha x Vaibhav) followed by L_1 x T_4 (IIHR 2372) in parental genotypes Vaibhav showed highest volume of fruit, this trait was also inherited in hybrid.

Pericarp thickness plays a significant role in governing fruit firmness. Thicker pericarp generally enhances the firmness and ultimately the shelf life of the tomato as well it can withstand long distance transport (Thakur and Kaushal, 1995). Maximum pericarp thickness was observed in hybrid L_1 x T_4 (Anagha x IIHR 2372).

Lycopene is a potent antioxidant and is thought to be responsible protect cells against oxidative damage, thereby lowering the risk of chronic diseases (Rao and Agarwal, 1999). Highest content of lycopene observed in hybrid L_1 x T_5 (Anagha x EC 541109). Highest lycopene observed in parental genotype EC 541109 (*Solanum pimpinellifolium* L.) was inherited to the hybrid. This hybrid is having heterosis over the better parent.

High levels of vitamin C in tomato fruits provide health benefits for humans and also play an important role in several aspects of plant life. In plant, vitamin C is a co-factor for many enzymes, contributes to detoxify reactive oxygen species and is important for resistance against biotic and abiotic stress, senescence regulation and floral induction (Athar *et al.*, 2008). Highest content of vitamin C was observed in

hybrids L_1 x T_4 (Anagha x IIHR 2372) and L_1 x T_6 (Anagha x IIHR 2200) Both the parental genotypes IIHR 2372 and IIHR 2200 recorded highest vitamin C content which were inherited to hybrids.

Oshima *et al.*, (1996) reported supplementation of carotenes inhibits singlet oxygen-mediated oxidation of human plasma low-density lipoprotein, thereby reducing risk of cardiovascular diseases. Highest content of carotene was observed in hybrid L_1 x T_7 (Anagha x LA 2805 *Solanum lycopersicum var. cerasiforme* L.) followed by L_1 x T_5 (EC 541109 *Solanum pimpinellifolium* L.). The high carotene content was inherited from the wild parent.

The fruit pH plays important role in the development of tomato cultivars for processing purpose, fruits with high TSS, pH less than 4.5 and higher ascorbic acid and lycopene content are preferred for processing (Bose *et al.*, 2002). Highest pH was observed in hybrid L_1 x T_1 (Anagha x Vaibhav) followed by L_1 x T_2 (Anagha x Nandi).

The flavour of tomato is determined by the amount of sugar and acid present. Sugars, acids and their interactions are important to sweetness, sourness and overall flavour intensity in tomatoes (Stevens *et al.*, 1977). Highest total soluble solids (%) were observed in hybrid L_1 x T_5 (Anagha x EC 541109). In parental genotype EC 541109 (*Solanum pimpinellifolium* L.) showed highest content of TSS% this trait is inherited to hybrid. Interspecific hybrid with good yield and sweetness can be recommended for release as a table salad variety of cherry tomato.

Shelf life is the most important criteria in transport of vegetables. Tomatoes with good shelf life are preferred for transport. As the tomato is a highly perishable vegetable, post-harvest losses will be less in genotypes with more shelf life. Maximum shelf life was recorded in hybrid L_1 x T_1 (Anagha x Vaibhav) parental genotype vaibhav recorded highest shelf life this trait was inherited in hybrid.

5.4.2 Resistance for ToLCV in hybrids

Out of seven hybrids from the cross between seven male parents and susceptible female parent Anagha four hybrids *viz.*, L_1 x T_3 (Anagha x EC 168283), L_1 x T_5 (Anagha x EC 541109), L_1 x T_6 (Anagha x IIHR 2200), L_1 x T_7 (Anagha x LA 2805) were highly resistant. The male resistant parents in the hybrids were EC 541109 (*Solanum pimpinellifolium* L.), EC 168283 (*Solanum pimpinellifolium* L.) and LA 2805

(*Solanum lycopersicum var. cerasiforme* L.) wild species and IIHR 2372, IIHR-2200, Vaibhav and Nandi were cultivated species. Hybrids L_1 x T_2 (Anagha x Nandi) and L_1 x T_4 (Anagha x IIHR 2372) were found to be resistant, whereas hybrid L_1 x T_1 (Anagha x Vaibhav) was moderately resistant for tomato leaf curl virus disease.

Koteswararao (2011) screened thirty-five hybrids of tomato for ToLCV in which thirty hybrids showed resistant to the ToLCV disease in Kerala conditions. Prashant kumar (2014) screened twenty different hybrids of tomato for ToLCV four hybrids showed highly resistant, five hybrids showed resistant whereas five hybrids showed moderately resistant and all other remaining hybrids were susceptible for ToLCV.

This study entitled "Identification of potential donors for superior fruit quality traits and genes for resistance to tomato leaf curl virus (ToLCV) in tomato and allied species" could identify resistance sources for ToLCV *viz.*, EC 541109, EC 168283, IIHR1970, LA 2805, IIHR 2372, IIHR 2200, Vaibhav and Nandi. Molecular markers confirmed the presence of *Ty2* gene in genotypes IIHR 2200, Vaibhav and EC168283 (*Solanum pimpenellifolium* L.) and *Ty3a* gene in genotype IIHR 1970 (*Solanum peruvianum* L.) for ToLCV resistance.

The genotypes Vaibhav, EC 320574, EC 165751, EC 164656 and EC 16786 which are superior in yield traits can be used for breeding for improvement of yield traits. EC 541109, IIHR 2372 and LA 2805 which showed superiority in fruit quality traits can be used as donors for quality traits in breeding programmes. The wild species which are found compatible with the cultivated species can be used as donors for fruit quality traits as well as resistance.

The hybrid L_1 x T_1 (Anagha x Vaibhav) and L_1 x T_6 (Anagha x IIHR 2200) which performed superior for yield component traits with resistance to ToLCV recommended for release after yield trials. The hybrid L_1 x T_5 (Anagha x EC 541109 *Solanum pimpinellifolium* L.) which showed superiority for fruit quality traits like lycopene content, TSS %, and carotene content with resistance to ToLCV an good total yield can be recommended for release variety of cherry tomato for table purpose.

The segregating population derived from the interspecific crosses can be used for further evaluation to locate plant types with good yield and fruit quality along with resistance to ToLCV.

Summary

6. SUMMARY

The investigations on "Identification of potential donors for superior fruit quality traits and genes for resistance to tomato leaf curl virus (ToLCV) in tomato and allied species" were carried out during February, 2015 to August, 2017 at the Department of Plant Breeding and Genetics, Vellayani.

Thirty-four genotypes were screened in natural field conditions for Tomato leaf curl virus (ToLCV) resistance. Of these eight genotypes *viz.,* EC 541109 (*Solanum pimpinellifolium* L.) EC 168283 (*Solanum pimpinellifolium* L.) IIHR 1970 (*Solanum peruvianum* L.) and LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) IIHR-2372, IIHR-2200, Vaibhav and Nandi were highly resistant to ToLCV.

Eight genotypes which were highly resistant in natural field conditions were artificially screened by grafting transmission to confirm the resistance. In this all eight genotypes *viz.,* EC 541109 (*Solanum pimpinellifolium* L.) EC 168283 (*Solanum pimpinellifolium* L.) IIHR 1970 (*Solanum peruvianum* L.) and LA 2805 (*Solanum lycopersicum var. cerasiforme* L.) IIHR-2372, IIHR-2200, Vaibhav and Nandi remained highly resistant after graft transmission.

All these thirty-four genotypes were evaluated for yield and fruit quality parameters. The mean of seventeen characters *viz.,* plant height (cm), number of primary branches plant $^{-1}$, spread of the plant (cm), number of days to 50% flowering, number of days to first fruit harvest, number of fruits plant^{-1}, weight of fruits plant^{-1} (kg), weight of fruit (g), number of locules fruit^{-1}, volume of fruit (ml of water displaced), pericarp thickness, lycopene (mg/100 g), vitamin C (mg/ 100 g), carotene (mg/100 g), pH of juice, total soluble solids (%) and shelf life (days) were subjected to analysis of variance, which revealed significant differences among the lines for all these characters.

From coefficient of variation it is evident that the estimates of GCV were higher than the corresponding PCV for all seventeen quantitative attributes indicating the less influence of environment on the expression of these genotypes. The estimates of GCV and PCV were higher for characters like plant height, number of primary branches, number of fruits plant^{-1}, weight of fruits plant^{-1}, weight of fruit, volume of fruits and vitamin C indicating the existence of high variability in the material under study

offering ample of scope for selection. Heritability estimate and genetic advance as per cent of mean (GAM) were high for plant height, number of primary branches, spread of plant, number of days to 50% flowering, number of fruits plant^{-1}, weight of fruits plant^{-1}, weight of fruit, number of locules fruit^{-1}, Volume of the fruit, pericarp thickness, lycopene content, vitamin C, carotene content, pH of juice, TSS %, shelf life indicating predominance of additive gene action for these characters.

Phenotypic and genotypic correlation coefficient analysis showed positive correlation of characters with respect to weight of fruits plant^{-1} (kg) were significant only for traits, such as plant height, number of primary branches plant^{-1}, spread of the plant, number of days to 50% flowering, number of days to first fruit harvest, number of fruits plant^{-1}, weight of fruit and volume of fruit.

Path analysis confirmed that direct effect on number of fruits per plant expressed highest positive direct effect on weight of fruits plant^{-1} followed by weight of fruit, spread of the plant, lycopene, number of primary branches plant^{-1}, number of days to 50% flowering and number of days to first fruit harvest.

These thirty-four genotypes were also screened with SCAR molecular markers specific to the genes for resistance to ToLCV linked to genes for resistance (Ty2, Ty3 and Ty3a). Genotypes IIHR 2200, Vaibhav and EC168283 (*Solanum pimpenellifolium* L.) showed the presence of *Ty2* gene and genotype IIHR 1970 (*Solanum peruvainum* L.) showed the presence of *Ty3a* gene.

The identified resistant lines crossed with ''Anagha'' the popular bacterial resistant variety with an objective to transfer the resistance for ToLCV and evaluation of successful F_1 hybrids for yield, quality, resistance and fertility status. Seven successful hybrid combinations *viz.*, L_1 x T_1 (Anagha x Vaibhav), L_1 x T_2 (Anagha x Nandhi), L_1 x T_3 (Anagha x EC168283 (*Solanum pimpinellifolium* L.), L_1 x T_4 Anagha x IIHR2372 (*Solanum lycopersicum* L.), L_1 x T_5 Anagha x EC541109 (*Solanum pimpinellifolium* L.), L_1 x T_6 Anagha x IIHR2200 (*Solanum lycopersicum* L.) and L_1 x T_7 Anagha x LA2805 (*Solanum lycopersicum var. cerasiforme* L.) were produced and all the hybrids including the hybrids of wild species showed 100% pollen fertility, confirming the compatibility of the wild species used in the study with the cultivated species.

Seven hybrids along with parental genotypes and check variety Vellayani Vijai were evaluated in field. The analysis of variance revealed significant difference for all seventeen quantitative and fruit quality attributes. On the basis of mean performance for different yield and fruit quality traits in hybrids *viz.*, L_1 x T_1 (Anagha x Vaibhav) showed superiority for characters like weight of fruits plant^{-1} (kg), weight of fruit (g), Volume of fruit (ml), pH of juice and shelf life, L_1 x T_5 (Anagha x EC 541109) showed superiority for characters like plant height, number of primary branches, lycopene content, TSS %, and carotene content, L_1 x T_2 (Anagha x Nandi) showed earliness in number of days to 50% flowering and number of days to first fruit harvest, L_1 x T_4 (Anagha x IIHR 2372) showed superiority for traits like pericarp thickness and vitamin C.

Hybrids were screened and scored for ToLCV, in natural field conditions. Highly resistant reaction was found in four hybrids *viz.*, L_1 X T_3 (Anagha x EC 168283), L_1 X T_5 (Anagha x EC 541109), L_1 X T_6 (Anagha x IIHR 2200), L_1 X T_7 (Anagha x LA 2805) and seven parental genotypes. Hybrids L_1 X T_2 (Anagha x Nandi) and L_1 X T_4 (Anagha x IIHR 2372) recorded Resistant reaction. Whereas hybrid L_1 X T_1 (Anagha x Vaibhav) was under moderately resistant category for tomato leaf curl virus disease.

This study could identify resistance sources for ToLCV *viz.*, EC 541109, EC 168283, IIHR1970, LA 2805, IIHR 2372, IIHR 2200, Vaibhav and Nandi. These lines can be used in breeding programmes for ToLCV resistance. Genotypes Vaibhav, EC 320574, EC 165751, EC 164656, EC 16786, EC 541109, IIHR 2372 and LA 2805 which showed superiority in yield and quality traits can be used for breeding for improvement of yield and quality traits.

Hybrid L_1 x T_1 (Anagha x Vaibhav) which performed best for weight of fruits plant^{-1} (kg), weight of fruit (g), Volume of fruit (ml), pH of juice, shelf life and with resistance to ToLCV and hybrid L_1 x T_5 (Anagha x EC 541109) which showed superiority for characters like plant height, number of primary branches, lycopene content, TSS %, and carotene content with resistance to ToLCV can be recommended for release after yield trials.

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IDENTIFICATION OF POTENTIAL DONORS FOR SUPERIOR FRUIT QUALITY TRAITS AND GENES FOR RESISTANCE TO TOMATO LEAF CURL VIRUS (ToLCV) IN TOMATO AND ALLIED SPECIES

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(2014 - 21 - 122)

ABSTRACT

of the thesis submitted in partial fulfilment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

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2017

ABSTRACT

The study entitled ''Identification of potential donors for superior fruit quality traits and genes for resistance to tomato leaf curl virus (ToLCV) in tomato and allied species'' was conducted during the period 2014-2017, in the Department of Plant Breeding and Genetics, Vellayani with an objective of evaluating varieties and allied species of tomato for fruit quality traits and genes for resistance to ToLCV through biochemical analysis and molecular markers and to study compatibility for hybridization and seed set to transfer ToLCV genes to bacterial wilt resistant variety ''Anagha'' from donors of related species.

Thirty-four genotypes including allied species of tomato were collected from different sources and studied under four different experiments. First experiment was screening of thirty-four genotypes under natural field condition for tomato leaf curl virus resistance in summer season and scoring for ToLCV by the scale given by Banerjee and Kalloo (1987). Eight genotypes *viz.,* EC 541109 (*Solanum pimpinellifolium* L.) EC 168283 (*Solanum pimpinellifolium* L.), IIHR1970 (*Solanum peruvianum* L.) and LA 2805 (*Solanum lycopersicum var. cerasiforme* L.), IIHR 2372, IIHR 2200, Vaibhav and Nandi were found to be highly resistant. The scions of these eight highly resistant genotypes were grafted on susceptible root stock with ToLCV symptoms. The scions did not take symptoms after 25 days confirming the resistance of the genotypes.

Evaluation of thirty-four genotypes for yield and fruit quality parameters was carried out in field condition during *rabi* season. The analysis of variance revealed significant difference for all seventeen quantitative and fruit quality attributes. On the basis of mean performance for different yield characters, genotypes *viz.*, Vaibhav, EC 320574, EC 165751, EC 164656 and EC 16786 were superior, whereas genotypes EC 541109, IIHR 2372, Vaibhav and LA 2805 were superior for fruit quality traits. The wild species used in the study had high content of carotene, lycopene and TSS.

From coefficient of variation it was evident that the estimates of GCV were higher than the corresponding PCV for all seventeen quantitative attributes indicating the less influence of environment on the expression of these genotypes. The estimates of

GCV and PCV were higher for seven characters indicating the existence of high variability in the material studied offering ample scope for selection. Heritability estimates and genetic advance as per cent of mean (GAM) were high for all characters indicating predominance of additive gene action for these characters.

Phenotypic and genotypic correlation coefficient analysis with respect to weight of fruits per plant^{-1} showed positive significant correlation with plant height, number of primary branches plant^{-1}, spread of the plant, number of days to 50% flowering, number of days to first fruit harvest, number of fruits plant^{-1}, weight of fruit and volume of fruit. Path analysis confirmed that direct effect on number of fruits plant^{-1} expressed highest positive direct effect on weight of fruits plant^{-1}, followed by weight of fruit, spread of the plant, lycopene content, number of primary branches plant^{-1}, number of days to 50% flowering and number of days to first fruit harvest.

Molecular markers linked to the three genes *Ty2*, *Ty3* and *Ty3a* specific to ToLCV resistance were validated with thirty-four genotypes. Genotypes IIHR 2200, Vaibhav and EC168283 (*Solanum pimpenellifolium* L.) showed the presence of *Ty2* gene and genotype IIHR 1970 (*Solanum peruvianum* L.) showed the presence of *Ty3a* gene.

The identified resistant lines were crossed with ''Anagha'' the popular bacterial resistant variety with an objective to transfer the resistance. All the seven successful hybrid combinations showed 100% pollen fertility confirming the compatibility of the parents.

Evaluation of seven hybrids with parents revealed significant difference for all seventeen quantitative and fruit quality attributes. On the basis of mean performance for different yield and fruit quality traits in hybrids *viz.*, L_1 x T_1 (Anagha x Vaibhav) showed superiority for characters like weight of fruits plant^{-1}, weight of fruit, volume of fruit, pH of juice and shelf life, L_1 x T_5 (Anagha x EC 541109) showed superiority for characters like plant height, number of primary branches, lycopene content, TSS %, and carotene content, L_1 x T_2 (Anagha x Nandi) showed earliness in number of days to 50% flowering and number of days to first fruit harvest, L_1 x T_4 (Anagha x IIHR 2372) showed superiority for traits like pericarp thickness and vitamin C.

Seven hybrids were screened and scored for ToLCV, in natural field conditions. Highly resistant reaction was found in four hybrids *viz.*, L_1 x T_3 (Anagha x EC 168283), L_1 x T_5 (Anagha x EC 541109), L_1 x T_6 (Anagha x IIHR 2200), L_1 x T_7 (Anagha x LA 2805).

This study could identify resistance sources for ToLCV *viz.*, EC 541109, EC 168283, IIHR1970, LA 2805, IIHR 2372, IIHR 2200, Vaibhav and Nandi. The genotypes Vaibhav, EC 320574, EC 165751, EC 164656, EC 16786, EC 541109, IIHR 2372 and LA 2805 which showed superiority in yield and fruit quality traits can be used for breeding for improvement of yield and quality traits. The wild species which are found compatible with cultivated species can be used as donors for quality traits as well as resistance.

The hybrid L_1 x T_1 (Anagha x Vaibhav) with superior yield traits and resistance to ToLCV can be recommended for release after yield trials. Hybrid L_1 x T_5 (Anagha x EC 541109) an interspecific hybrid with superior fruit quality traits can be recommended for release as cherry tomato after trials. The segregating population of interspecific crosses can be used for further evaluation to locate plant types with good yield, fruit quality along with resistance to ToLCV.