IDENTIFICATION OF CHERRY TOMATO GENOTYPES FROM F3 SEGREGANTS OF INTRASPECIFIC CROSS

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled "IDENTIFICATION OF CHERRY TOMATO GENOTYPES FROM F3 SEGREGANTS OF INTRASPECIFIC CROSS" 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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Pamarthi Vinod

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LIST OF PLATES

FAOSTAT	Food and Agriculture organisation Statistical Database
NHB	National Horticulture Board
DNA	Deoxyribo Nucleic Acid
EST	Expressed Sequence Tag
BAC	Bacteria Artificial Chromosome
MT	Metric Ton
G x E	Genotype x Environment
cm	Centimetre
mm	Millimetre
SSR	Simple sequence repeat
F1	First filial generation
F ₂	Second filial generation
F3	Third filial generation
F4	Fourth filial generation
F5	Fifth filial generation
F ₆	Sixth filial generation
mg	Milligram
g	Gram
kg	Kilogram
ml	milli litres
t	Tonne
GCV	Genotypic coefficient of variation
PCV	Phenotypic coefficient of variation
viz.	Namely
°B	Brix
TSS	Total soluble solids
NFR	Number of Flower Racemes
NB	number of branches

LIST OF ABBREVIATIONS

%	Percentage
ANOVA	Analysis of variance
ha	Hectare
RIL	Recombinant Inbred Line
IC	Indigenous collection
EC	Exotic collection
Q	Quintal
nor	Non-ripening mutant
QTL	Quantitative Trait Loci
pН	Power of Hydrogen
AVRDC	Asian Vegetable Research and Development centre
TTA	Total titratable acid
fw	Fresh weight
HPLC	High Performance Liquid Chromatography
IIHR	Indian Institute of Horticultural Research
CTAB	Cetyl Trimethyl Ammonium Bromide
AFLP	Amplified Fragment Length Polymorphism
RFLP	Restriction Fragment Length Polymorphism
ISSR	Inter-simple sequence repeat
RAPD	Random Amplification of Polymorphic DNA
DAF	DNA amplification fingerprinting
CAPS	Cleaved amplified polymorphic sequence
SNP	Single Nucleotide Polymorphism
STS	sequence tagged site
PIC	Polymorphism Information Content
COS	Conserved Ortholog Set
EST-SSR	Expressed Sequence Tag- SSR
PCR	Polymerase Chain Reaction
MAS	Marker Assisted Selection
NIL	Nearly Isogenic Lines

SCA	R	Sequence Characterized Amplified Region
UPGN		Unweighted Pair Group Method with Arithmetic mean
μg		Micro gram
°C		Celsius
nm		Nanometer
μ1		Micro litres
PVI	0	poly vinyl pyrollidone
M		Molar
ng		Nanogram
SPS	S	Statistical Package for the Social Sciences
Р		Plant
Plant	-1	Per plant
Fruit	-1	Per fruit
Cluste	er ⁻¹	Per cluster
		1

<u>Introduction</u>

1. INTRODUCTION

The domesticated tomato was formerly designated *Solanum lycopersicum* (Linnaeus, 1753). Miller (1754) designated the genus *Lycopersicon* and the species *esculentum* for tomato and in recent past it was renamed to *Solanum* with an updated classification (Peralta and Spooner, 2001). Tomato which is a native of Peru is also familiar as "love apple" and "Apple of Peru". Mexican region of tropical America is believed to be the centre of diversity (Thompson and Kelly, 1957). *Solanum lycopersicum* is a well-studied crop species in terms of genetics, breeding and genomics. It is an important vegetable crop grown globally with an annual production close to 182.3 million tons on a cultivated area of around 4.84 million hectares (FAO, 2017). This crop was introduced to India in early 19th century by the traders of East India company (Kalloo, 1993). In tomato production India stands third with 20.5 million tons in 8.14 million ha worldwide (NHB, 2019).

Cultivated tomato (*Solanum lycopersicum L.*) is one of the well-known and most generally consumed vegetable all through the world, both as fresh fruit and in the processed form. Its versatility to assorted situations is an impression of the incredible abundance of hereditary fluctuation existing in the family Solanaceae, which can be exploited through crop improvement programs (Tigchelaar and Basset, 1986).

India has made astounding progress in tomato production and productivity by evolving high yielding varieties. These varieties were very successful in increasing the tomato production from 74.62 MT in 2001-02 to 194.02 MT in 2013-14 (Saxena and Gandhi, 2015). Diversified germplasm including specific novel genotype are the most beneficial basic materials for crop breeder to meet the current and future needs.

Being one of the most essential horticultural crops in the world, consumers demand varieties with higher fruit quality, thereby approaches concentrating on increasing fruit quality pursue to be of utmost importance (Domis *et al.*, 2002; Gruda, 2005). Tomato being a self-pollinated crop, advancement in fruit yield and quality is typically attained by selecting the genotypes with desirable trait

combinations existing in crop germplasm and by selective hybridization (Reddy *et al.*, 2013a).

Cherry tomato (*S. lycopersicum* var. *cerasiforme*), one of the gifted wild types of *Solanum* offers great probability in crop improvement programmes by virtue of their valuable aspects in terms of genetic diversity for selection of parental material. Their immense geographic range with small fruits (1.5 - 3.5 cm in diameter) on long panicles of determinate, semi-determinate or indeterminate growth habit and a highly variable number of fruits per cluster (10–50) is valuable for breeding programmes (Medina and Lobo, 2001).

Solanum lycopersicum var cerasiforme also referred as "cherry tomato" with small round fruits comparatively larger than *S. pimpinellifolium* is considered as the ancestor of domesticated tomato because of its origin and rich diversity within the regions of central America and its highest genetic relationship with domesticated tomato (Rick and Chetelat 1995).

Cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) also known as salad tomato, is one of the emerging tropical vegetable crops under protected cultivation and is considered as an exotic vegetable bringing new taste and appearance to dishes and also acts as a good source for providing disease resistance and adaptability to cool and hot seasons (Malavika *et al.*, 2018). It is a beloved vegetable all over the world because of its desirable aspects such as good source of vitamin A and C, sugars, taste and low calories and fruit set even at elevated temperatures (Prema *et al.*, 2011a). They are also beneficial to human health because of its high content of antioxidant and phytochemical compound including lycopene, β -carotene, flavonoids, vitamin C and many essential nutrients (Rosales *et al.*, 2011).

Despite, cherry tomatoes considered nutritionally rich in value as compared to normal tomatoes, there has been less work done with respect to fruit quality improvement in cherry tomatoes. Breeding programmes aimed towards nutritive improvement in India is very limited. Therefore, there is a need for improvement of varieties or hybrids specifically for fresh market and processing qualities with high nutritive value and higher yield.

Molecular markers are powerful tools in the characterization and assessment of crop genetic diversity within and between genetic populations (Russell *et al.*, 1997). Wild tomato species are the greatest reserves of genetic diversity, which has been studied using various molecular marker techniques. Simple sequence repeat (SSR) markers are often the preferred molecular markers for marker-assisted plant breeding when they are available, because the SSR markers possess properties suitable for high-throughput genotyping, such as simplistic assay, co-dominance nature, high reproducibility, multiallelic variation, low distributing cost and easy automation (Edwards and McCouch, 2007). Use of molecular markers linked to genes for quality traits of cherry tomato is a tool, which can be used efficiently in plant breeding for the direct selection of qualitative characters and for accelerated improvement of cherry tomato genotypes.

'Anagha' is a popular bacterial wilt resistant variety of Kerala with determinate growth habit and large plumpy fruits. As part of Ph.D. programme in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani by Nadkarni (2017) F_1 hybrids of cultivated tomato variety Anagha *(Solanum lycopersicum* L.) and *Solanum lycopersicum* var. *cerasiforme* were evaluated and found to be superior. In this project the F_3 segregants derived from 5 F_2 families of the above cross were evaluated for morphometric traits and fruit quality specific to cherry tomato and genotyping the superior segregants with SSR markers with a long-term objective of developing a cherry tomato variety for Kerala.

<u>Review of Literature</u>

2. **REVIEW OF LITERATURE**

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable in India and the world. It is a rich source of antioxidants, mainly lycopene, β -carotene, vitamin A, vitamin C and minerals like calcium, phosphorus and iron (Saleem *et al.*, 2013). It is in constant demand throughout the year all over the world and is a very important off-season vegetable that fetches great returns to the farmers.

The basic requirement for the improvement of a crop is the existence of genetic variation within the population. This variation is broached through a step called selection that involves the identification and isolation of desirable plants from the variable population. The existence of such variability is therefore, a key issue that determines the amount of progress expected from selection.

Currently, improvement for internal quality (nutritive and organoleptic) is one of the main breeding objectives for fresh market tomato. Flavour and nutritive value are complex characteristics because they are conditioned by the content of many chemical compounds of tomato fruits. Cherry tomato (*S. lycopersicum* var. *cerasiforme*), one of the gifted wild types of Solanum in crop improvement programs offers good source of vitamin A and C, sugars, taste and low calories and high fruit set (Prema *et al.*, 2011a).

The literature pertaining to tomato yield and quality parameters and the studies for locating cherry tomato genotypes using morphological and molecular markers are reviewed in this chapter.

Lycopersicon esculentum was domesticated by native Americans, presumably in Mexico, and by the time Europeans arrived massive fruited sorts were already arrived (Jenkins, 1948; Rick and Chetelat, 1995). Because of domestication, wild species gave rise to plants with larger and variably formed fruit. The mutations related to larger fruit accumulated to provide rise to our contemporary cultivars. Genetic analysis of crosses between cultivated species and their wild relatives supports this concept, as a result of relative from these crosses nearly always segregate during a continuous manner with relevant fruit size, indicating that the domestication method concerned

mutations at various genetic loci (MacArthur and Butler, 1938; Banerjee and Kalloo, 1989).

Fruit size and shape are the essential criteria for domestication and improvement of tomato. The variation in size and shape of tomato fruit directly imply their importance as agronomic traits.

2.1 ORIGIN OF CHERRY TOMATO:

According to Nuez (1999) cherry tomatoes are generally of determinate, semi-determinate or indeterminate growth habit with long racemes bearing many small sized fruits showing intense colour and flavour generally round in shape and weighing between 10 and 30g resistant to diseases and tolerant to high relative humidity (> 80%) have a high nutritional value because of their high vitamin C content (> 57 mg/100g fresh fruit weight) and present a significant variation in number of fruits per cluster (10–50). High lycopene content is observed which exceeds 10 mg/100g fresh fruit weight (Medina and Lobo, 2001).

Along with several wild species the cultivated tomato *S. lycopersicum* comes under section *Lycopersicum*, clade *Solanum*. Being self-pollinated species, the cultivated tomato is believed to be derived from its nighest wild ancestor *S. pimpinellifolium* (Nesbitt and Tanksley, 2002) and cherry tomato accessions (*S. lycopersicum* var *cerasiforme*) were believed to occupy a moderate position between *S. lycopersicum* and *S. pimpinellifolium*, as their genomes are found to be mosaics of these two closely related species (Ranc *et al.*, 2008).

Cherry tomato is regarded as a botanical variety of the cultivated tomato, *Solanum lycopersicum* var. *cerasiforme* with small red coloured juicy fruits (1.5 -3.5 cm in diameter) on long panicles with its characteristic sweetness and aroma and the demand for cherry tomato has increased in the market chiefly due to the recognition of their good taste (Kobryn and Hallmann, 2005).

Cherry tomato (Solanum lycopersicum var. cerasiforme) similar to cultivated tomato but not identical to the wild ancestor, having small fruits

resembling a cherry with a dark red colour, having excellent nutritional traits (Charlo et al., 2007).

2.2 GENETIC ANALYSIS OF QUANTITATIVE TRAITS IN CHERRY TOMATO:

Prema *et al.* (2011a) studied the genetic components *viz.*, variability, heritability and genetic advance of six cherry tomato genotypes for growth, yield and quality characters, and observed high PCV and GCV for qualitative and quantitative traits indicating additive gene action. Therefore, direct selection is highly recommended for the improvement of cherry tomato genotypes for qualitative and quantitative traits.

Than *et al.* (2001) raised six tomato cultivars of cherry type showing indeterminate type (CHT 104), with a height of 151 cm and the others with determinate type and height ranged from 83 cm to 117 cm. Days to first flower initiation of CH 157 cultivar was 38 days and the cultivars CHT 499 and CHT 261 was 44 days. Fruit yield was recorded higher in CHT 499, CHT 261 and CHT 104, varying from 11 to 16 t acre⁻¹.

Islam *et al.* (2012) conducted studies on genetic variability and trait relationship in cherry tomato (*Solanum lycopersicum* var. *cerasiforme*). Nine out of eleven inbred lines displayed a wide range of genetic variability and concluded that high estimates of heritability, genetic advance and genotypic coefficient of variation (GCV) for the traits like number of fruits, individual fruit weight and clusters plant⁻¹ were controlled by additive gene action indicating hybridization can be an effective method for improving the fruit yield plant⁻¹ rather than following selection.

A half diallel analysis was performed with seven distinct cherry tomato genotypes to estimate the mean performance and heterosis of 21 hybrids accompanied with 7 parents by Renuka and Sadashiva (2016). Results confirmed that the mean performance of the parents IIHR-2754, IIHR-2863 and IIHR-2864 were exceptional for all traits *viz.*, number of inflorescences (48), average fruit weight (31.05 g), number of fruits plant⁻¹ (498.67), number of fruits per kg (96.67),

yield per plot (53.33 kg), acidity (0.459 mg $100g^{-1}$), ascorbic acid (38.67 mg $100g^{-1}$), total carotenoids (15.024 mg $100g^{-1}$) and lycopene (6.97 mg $100g^{-1}$) and the crosses IIHR-2754 × IIHR-2860 and IIHR-2754 × IIHR-2866 resulted in premium hybrid vigour regarding yield and yield contributing traits.

Doddamani (2016) conducted a genetic analysis for quantitative and qualitative traits in F_2 and F_3 population of cherry tomato (*Solanum lycopersicum* var. *cerasiforme*). Results shown that a highly significant difference was observed among the genotypes for all traits except for stem girth and the number of locules fruit⁻¹ in both the populations.

Eighteen cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) genotypes were evaluated under shade net in tropical climatic condition for yield and quality traits by Ramya *et al.*, (2016) and it was identified that Solan Red Round genotypes showed superiority for twelve characters *viz.*, number of branches plant⁻¹ (21.1), number of fruits cluster⁻¹ (5.13), individual fruit weight (10.57 g), pericarp thickness (2.24 mm), fruit yield plant⁻¹ (2.94 kg), estimated fruit yield hectare⁻¹ (73.42 t), titratable acidity (0.42%), total soluble solids (6.03 °Brix) and lycopene (1.96 mg 100g⁻¹) whereas, the highest number of clusters plant⁻¹ (96.57) and plant height (5.18 m) was recorded in Thandikudi Local and Aranuttrumalai Local respectively.

Development of cherry tomato for protected cultivation from an interspecific cross between *Solanum lycopersicum and Solanum pimpinellifolium* followed by pedigree method of selection with individual plant selection from F_2 and F_3 based on plant growth habit i.e., indeterminate type, yield, fruit size, fruit shape, fruit firmness and TSS. F₄-F₆ progenies are analyzed for biochemical traits and the final selection with pedigree EP 2908-F₂-27-15-2-1 was named as 'Punjab Red Cherry' (Dhaliwal and Jindal, 2017).

 F_1 hybrids of an intraspecific cross between *Solanum lycopersicum* L. and *Solanum lycopersicum* var. *cerasiforme* were evaluated for quantitative traits by Nadkarni (2017) and hybrids showed plant height (155.23 cm), number of days to first fruit harvest (70.89 days) and fruit weight 6.91 g.

Dhaliwal and Jindal (2018) developed two cultivars namely 'Punjab Sona Cherry' and 'Punjab Kesar Cherry' with yellow and orange fruit colour respectively by following single plant selection method from a segregating population collected from farmers field followed by continuous selfing for more than 6 generations by selecting individual plant from the original population and final selection with pedigree 'CT-F₂-11-24-5-1-1' and 'CT-F₂-8-14-3-1-1' respectively.

Malavika *et al.* (2018) evaluated 10 cherry tomato genotypes under rain shelter. Among the genotypes SLc-10 recorded the highest plant height (295.45 cm) with indeterminate growth habit, the number of flower clusters plant⁻¹ (19.70), highest average fruit weight (6.24g) and highest number of fruits cluster⁻¹ (22). SLc-9 recorded with highest yield plant⁻¹ (425.96 g) and lowest incidence of bacterial wilt (29.10%), the highest number of fruits plant⁻¹ (155.60) was recorded in SLc-2 and highest TSS (7.2° Brix) recorded in SLc-1. Results revealed that genotypes SLc-10 and SLc-9 can be recommended for cultivation inside rain shelter.

Najeema *et al.* (2018) conducted an evaluation trial on the genotypes of cherry tomato for different traits contributing towards quantitative and quality traits. Among the genotypes, the height of plant (126.20 cm), number of primary branches (6.30), secondary branches (13.50) and yield plant⁻¹ (1779.6g) recorded maximum in BCT-8. Highest TSS was recorded in BCT-27 (8.02 °B). Wide variation is observed in β -carotene content of fruits ranged from 0.9 (BCT-1) to 6.2 mg 100g⁻¹ (BCT-24). BCT-25 recorded a maximum pH (4.34). BCT-6 recorded maximum lycopene content (10.14 mg 100g⁻¹) and maximum ascorbic acid content in BCT-3 (34.970 mg 100g⁻¹).

Venkadeswaran *et al.* (2018) evaluated twenty-four cherry tomato genotypes for quantitative traits under shade net condition and found that genotype LE 1223 having high pericarp thickness (2.220 mm), shelf life (32.5 days), fruit yield plant⁻¹ (1.57236 kg) and yield hectare⁻¹ (31.45 t). The genotype ATL-01-19 recorded the maximum fruit firmness (1.650 kg sq. cm⁻¹).

2.3 GENETIC ANALYSIS OF QUANTITATIVE TRAITS IN TOMATO:

Prasad and Singh (1990) screened four tomato assortments in Diara zone of Bihar and found that pusa ruby created the greatest plant height (106.91 cm) while the base plant tallness was recorded in Punjab chuhara and recorded maximum number of branches plant⁻¹ (14.84) in Pusa Ruby while minimum number (11.68) was observed in Punjab Chhuhara.

High estimates of the genotypic and phenotypic coefficient of variance for the number of fruits plant⁻¹ (40.06 and 41.93%) trailed by lycopene content (31.20 and 31.22%), plant height (25.50 and 25.61%) and fruit yield plant⁻¹ (24.02 and 25.14%). Phenotypic and genotypic coefficient of variance for acidity were accounted for as 20.16 and 19.14% respectively while 5.36 and 5.38% genotypic and phenotypic coefficient of variance individually was enrolled for ascorbic acid (Nair and Thamburaj, 1995).

Hussain *et al.* (2002) designed an experiment to review the morphological and yield of nine exotic and one local cultivar of tomato under Islamabad conditions. Characters contemplated were time for flower initiation, fruit set, fruit ripening, yield plant⁻¹ and yield ha⁻¹. Results confirmed that cultivar Marmande (TMV) took fundamentally least time (65.0 days) to mature pursued by *S. marzano* which matured in 72.3 days. Cultivar Polefemo aged late (91.7 days) trailed by Marmande which took 88.7 days to mature. Cultivars Marmande TMV and Marmande out yielded different cultivars with 64.29 and 62.99 t ha⁻¹, separately while poor yield was acquired in *S. marzano* (14.90 t ha⁻¹).

Parthasarathy and Aswath (2002) conducted a study on 23 genotypes of tomato during the kharif and identified that there was considerable diversity among genotypes for morphological traits i.e., plant height, fruit number and fruit size.

Joshi and Kohli (2003) assessed the extent of genetic divergence in 73 genotypes of tomato (*Lycopersicon esculentum*) from diverse origin using nonhierarchical Euclidean cluster analysis for yield and quality traits. The genotypes grouped into 15 clusters indicating a great range of genetic diversity existing among the genotypes. Mean fruit weight (102.76 g) and mean fruit yield plant⁻¹ (1034.64 g plant⁻¹) recorded highest in cluster 3 and 5 respectively. The height of the plant (135.91 cm) and duration of harvest (37.77 days) was found maximum in cluster 15. The highest mean value of fruit firmness (3086.48 g/cm²), minimum days to first harvest (59.67 days), shelf-life (14.00 days) and lowest locule number (2.028)

was found in cluster 9. However, cluster 6 showed the highest vitamin C content (44.63 mg 100 g⁻¹) and the number of fruits cluster⁻¹ (4.90).

Rodriguez *et al.* (2005) performed a cross between accessions of *Lycopersicon esculentum* nor (non-ripening) mutant and *Lycopersicon esculentum* var. *cerasiforme* to assess the combined genetic effect on the phenotypic variation of precised biochemical traits in the F_2 generation. Transgressive segregants were observed for colour, shape, firmness and shelf life. Results suggested that the use of this wild accession as a parent in tomato breeding programmes can act as an alternative for increasing the fruit quality and exclusively prolonging the fruit shelf life.

Sharma *et al.* (2006) conducted a non-hierarchical analysis on 60 tomato (*Lycopersicon esculentum* Mill.) genotypes and grouped into 10 clusters and cluster VIII exhibited a maximum genetic divergence of 1.531. For plant height, pericarp thickness, fruit size index and yield plant⁻¹ the most favorable genotypes selected were THS- 1-1, FT-5, THS-2-2, T-99-1-2, LBR-10-2 and T-99-2-3 whereas, for mean fruit weight, Campbell, EC-123018 and W 55were identified as the promising genotypes. The genotypes Red cherry and EC-170785 were favourable for earliness and improved number of fruits plant⁻¹.

Golani *et al.* (2007) examined the variability and character association of F_2 segregating generations of exotic tomato hybrids and minor variations were identified between Genotypic Coefficient of Variance (GCV) and Phenotypic Coefficient of Variance (PCV) for the characters, days to first flowering (PCV = 9.21, GCV = 7.82), fruit length (PCV = 17.14, GCV = 14.84) and fruit diameter (PCV = 17.10, GCV = 14.92). High heritability in relation with high genetic advance was recorded for branches plant⁻¹ (34.49), fruits cluster⁻¹ (47.43), number of fruit clusters plant⁻¹ (105.11), fruits plant⁻¹ (103.43), single fruit weight (77.73) and fruit yield plant⁻¹ (108.25). Therefore, selection for such traits acts as an efficient tool for the tomato fruit yield improvement.

Haydar *et al.* (2007) conducted variability studies at the genetic level and traits interrelationship in tomato (*Lycopersicon esculentum* Mill.). Results revealed the existence of a broad range of variability in genotypes for the traits under study.

High heritability coupled with high genetic advance was exhibited for fruit weight plant⁻¹ followed by the number of fruits in three cluster plant⁻¹ and the number of flowers in three clusters plant⁻¹.

Anjum *et al.* (2009) observed higher estimates of GCV and heritability associated with greater values of genetic gain for the average weight of fruit, number of primary branches plant⁻¹, number of fruits plant⁻¹, fruit yield plant⁻¹, juice-pulp ratio and titratable acidity. Correlation studies revealed a positive correlation of fruit yield plant⁻¹ with the height of the plant, number of primary branches plant⁻¹, number of fruits plant⁻¹, and fruit size at both phenotypic and genotypic levels.

Ghosh *et al.* (2009) studied the genetic divergence using multivariate analysis on segregating F_2 generations of exotic hybrids of tomato (*Solanum lycopersicum* L.). Based on 22 selected agronomic traits the segregating populations fell into 6 distant clusters. Based on D² statistics inter-cluster distance was found to be maximum between cluster IV and V. The highest intra-cluster distance was existing in cluster II followed by cluster V.

Terzopoulos *et al.* (2009) performed heterogeneity studies on horticultural traits under a low-input environment with fourteen Greek tomato landraces. The results indicated significant heterogeneity for the morphological traits and yield among the experimented tomato landraces and the F_1 hybrid 'Clodin' produced the most consistent and reliable results.

Basavaraj *et al.* (2010) in their study on genetic variability analysis in tomato (*Solanum lycopersicum* L.) observed high heritability (bs) coupled with greater genetic advance for the characters such as height of the plant, number of branches plant⁻¹, number of flowers cluster⁻¹, number of clusters plant⁻¹, number of flowers cluster⁻¹, number of clusters plant⁻¹, number of soluble solids (TSS).

Kaushik *et al.* (2011) studied the genetics of fruit yield and it's contributing characters in 10 genotypes of tomato (*Solanum lycopersicum*). The magnitude of genotypic and phenotypic coefficient of variation was higher for the number of leaves (21.2 and 22.3), fruit length (cm) (19.6 and 19.7) and fruit yield (19.6 and

19.6). High values of heritability coupled with high genetic advance were observed for the number of leaves at 60 days after transplanting (99.4 and 64.9), and fruit yield (99.9 and 24.7). A positive association of yield per hectare observed with the number of leaves at 60 days after transplanting (0.78) followed by the number of leaves at 30 days after transplanting (0.68), fruit length (0.66) and plant height (0.51).

Naz *et al.* (2011) conducted an experiment to review the growth, yield and quality parameters of six exotic cultivars of tomatoes and found that variety 'Roma' took minimum days to flowering (37.7 days). Variety 'Lyreka' matured early in 65.0 days and with the highest vitamin C content of 16.03 mg 100gm⁻¹. Variety 'Yaqui' recognized to be best titrable acidity (0.389%) and out-yielded different cultivars with 11.22 t ha⁻¹ followed by Avinash, Roma and Rio Grand. Maximum TSS was ascertained in the variety 'Avinash' (5.5).

Manna and Paul (2012) showed that fruit yield plant⁻¹ was correlated positively and significantly with fruit weight, fruit length, pericarp thickness and the number of fruits plant⁻¹ indicating the relative importance of characters for yield improvement.

Quantitative traits such as number of branches plant⁻¹, number of days for 50% flowering, plant height, and average fruit weight are controlled by gene action and are thus important in genotype selection (Mohamed *et al.*, 2012).

Quantitative traits, such as plant height, number of leaf branches, number of flower racemes (NFR), number of flowers raceme⁻¹, productivity, among others, are critical characteristics in tomato upgrade (Narolia *et al.*, 2012).

Meena and Bahadur (2013) assessed the breeding potential of tomato (*Lycopersicon esculentum* Mill.) genotypes using D^2 analysis and grouped the genotypes into six clusters. The intra-cluster distance was maximum for cluster V (10192.68) and minimum for cluster III (0). The maximum distance at inter-cluster level was between cluster III and cluster VI (47922.37) followed by clusters I and VI (44098.14). genotypes having maximum flower clusters/ plant (17.48), flowers/plant (97.62), fruit weight (55.94 g) and radial diameter of fruit (55.62 mm) were observed in cluster VI. Genotypes having maximum fruit yield (1920.98 g)

along with maximum polar diameter of fruit (48.85 mm) and minimum leaf curl incidence percent (25.68) were recorded in cluster III.

Reddy *et al.* (2013b) evaluated nineteen genotypes of tomato in augmented block design. The genotypes exhibited a wide range of variability for all the characters studied. Phenotypic Coefficient of Variation (PCV) was higher than Genotypic Coefficient of Variation (GCV) for all the characters studied. High heritability combined with high genetic advance was observed for the characters plant height, number of clusters plant⁻¹, number of flowers cluster⁻¹, number of fruits cluster⁻¹, number of fruits plant⁻¹, fruit length, fruit weight, fruit yield plant⁻¹, vitamin C, acidity, shelf life and TSS. High heritability combined with high genetic advance indicates that additive gene action plays a major role in governing these traits and these traits can be improved by simple selection.

Shankar *et al.* (2013) tested genetic variability on twenty-four commercial hybrids of tomato along with their 11 parents (8 lines and 3 testers). High estimates of PCV and GCV along with high heritability and genetic advance were recorded for the height of the plant, number of fruits cluster⁻¹, average fruit weight, yield plant⁻¹, titratable acidity, vitamin C and lycopene indicated a good deal of variability in those characters signifying the effectiveness of selection of desirable types for improvement.

Forty-nine genotypes of tomato (*Solanum lycopersicum* L.) were evaluated for qualitative and quantitative traits by Kumar *et al.* (2013) and the genotypes exhibited great range of variation in average fruit weight (12.23 - 82.21g), pericarp thickness (0.15 - 1.05 cm), number of fruits plant⁻¹ (1.67 - 177) and total soluble solids (3.40 - 6.05%). The trait association analysis represents the number of fruits plant⁻¹, plant height (cm), yield (g/plant) and total soluble solids (TSS %) were found significant and correlated positively with total numbers of fruits plant⁻¹.

Parental lines along with F_1 hybrid of cultivated tomato (*Solanum lycopersicum* L.) and wild species *Lycopersicon pimpinellifolium* L. were evaluated for advancement in their quantitative traits by Amaefula *et al.* (2014). A tremendous fruit yield improvement of 358.36% was observed in the cross between Wild × Petomech.

Khapte and Jansirani (2014) studied the genetic parameters in tomato genotypes and the traits revealed high PCV than GCV indicating lesser environment influence on the variation of these traits. The genotypes IIHR-709, IIHR- 2388, Arka Ashish, Vybhav, IIVR-L, CLN 2123A, EC-608395, EC-608406 BRML and EC-608456 were found to be high yielding genotypes which out yielded remaining genotypes regarding fruit quality and fruit yield plant⁻¹ based on mean performance in tomato.

Meena and Bahadur (2014) performed an investigation on variability studies on quantitative and quality traits of tomato (*Solanum lycopersicum* L.). Results revealed that the high estimates of PCV and GCV was observed for the characters like height of the plant, ascorbic acid and total soluble solids (TSS). All the characters showed high heritability with genetic advance indicating that traits are under control of additive gene action which is very reliable in the standard selection.

Nalla *et al.* (2014) grouped 27 genotypes of tomato into nine clusters based on quantitative and quality traits using D^2 statistics. The majority of genotypes were grouped under cluster I (16) followed by cluster III and cluster VII containing three and two genotypes respectively and the remaining clusters were monotypic. The inter-cluster distance was identified to be maximum between cluster VI and VII (20.80), indicating the presence of great genetic variability and the intra-cluster distance was found to be maximum in cluster III (10.88).

Seven commercial tomato varieties, BL-410, Srijana, Ceres, Winsari, Dalila, Makish, and Ahmita were evaluated for yield and physicochemical properties under plastic house conditions during monsoon period of two successive years 2012 and 2013 by Tiwari *et al.* (2014). The physicochemical analysis revealed that Srijana with highest titratable acidity (0.903%) and vitamin C content (32.32 mg/100 g) while BL-410 recorded the highest TSS (5.22 °Brix), early flowering (22 days), fruiting (28 days) and first harvest (72 days) after transplanting. Variety Ahmita produced a maximal number of fruits cluster⁻¹ (8.43), Ceres produced maximal fruit size (122.00 g) fruit while Winsari recorded with

maximum plant height (120.50 cm) and paramount marketable yield (105.8 t/ha) recommended for economical production.

Dar *et al.* (2015) evaluated 60 genotypes of tomato for morphometric and quality traits and classified into 20 clusters. Of the 20 clusters, highest number of genotypes were found in cluster I with 25 genotypes, high average fruit weight and minimum polygalacturonase activity was related to cluster VII. Lycopene, β -carotene and the number of fruits plant⁻¹ was found promising in cluster XX, the highest number of locules per fruit, fruit yield plant⁻¹ and yield hectare⁻¹ traits related to cluster VIII and superior genotypes for ascorbic acid content were found in cluster XVII. The results of D² estimates revealed that the highest inter cluster D² distance were calculated between clusters XII and XX, followed by clusters XI and XX, clusters VII and XX, and clusters XV and XX, indicating that hybridization and selection has a scope for the crop improvement.

Sajjan *et al.* (2016) studied the genetic parameters of F_6 Recombinant Inbred Lines (RIL's) to illustrate the variability, heritability and genetic advance in tomato (*Solanum lycopersicum* L.). The genotypes displayed a broad range of variability with higher PCV than GCV for all the traits examined. High heritability combined with high genetic advance was recorded for number of branches plant⁻¹, number of fruits plant⁻¹, plant height(cm), fruit yield plant⁻¹ (kg), average fruit weight (g), number of locules fruit⁻¹, total soluble solid (^oBrix) and pericarp thickness (mm) indicating that additive gene action plays a dominating role in governing these traits and can be enhanced by simple selection.

Fourteen genotypes of tomato were assessed for genetic variability and varietal performance in regard to morphological and biochemical traits by Mitul *et al.* (2016). Genotypes were clustered based on Wards method and classified into five distinct clusters where late maturing and low yielding genotypes in cluster I, early flowering genotypes in cluster II, high yielding genotypes with large plumpy fruits in cluster III, fruits with low ascorbic acid content genotypes in cluster IV and small-fruited early maturing and reduced pollen grains fertility genotype standard in cluster V.

Bhandari *et al.* (2017) conducted variability studies on different genotypes of tomato for yield traits and found the noteworthy differences among genotypes for all the traits. The number of fruits plant⁻¹, average fruit weight, fruit yield (kg) plant⁻¹ and the number of seeds fruit⁻¹ disclosed high heritability associated with high genetic advance indicating selection followed by hybridization may be fruitful for traits improvement.

Rajolli *et al.* (2017) studied the heritability, variability and genetic advance of tomato F_2 population derived from the cross IIHR-2201 X C-13-1-2-1. Results revealed the existence of significant variability among all the traits tested in the F_2 population. High heritability coupled with high genetic advance was detected for the height of the plant, number of branches, number of fruits plant⁻¹, yield plant⁻¹, average fruit weight, pericarp thickness, number of locules, vitamin C suggesting ample scope for improvement through selection of these traits.

Das *et al.* (2018) studied the genetic variability and character association for sixteen characters of thirty tomato genotype. Higher fruit yield was identified in genotypes BCT 53hyv, BCT111rin, BCT115dg and AC aft. Of these genotype BCT111rin was found to be superior in traits like plant height, fruit size, fruit yield and TSS.

Forty accessions of tomato were evaluated for genetic diversity based on 27 agro-morphometric traits by Hussain *et al.* (2018) using Euclidean distances coefficient and results revealed that accessions grouping into four clusters with 12 accessions in cluster I followed by 11, 10 and 7 accessions in cluster II, IV and III respectively.

2.4 BIOCHEMICAL TRAITS FOR FRUIT QUALITY TRAITS IN TOMATO:

Rick, 1974 carried out a cross between small, green-fruited *Lycopersicon minutum* and standard *L. esculentum* to transfer the trait, high soluble-solids from *L. minutum* to horticulturally standard *L. esculentum* cultivar by back cross breeding. The resultant pure breeding lines are selected and analyzed, it was shown that a 2% increase over the recurrent parent.

Mahakun *et al.* (1979) evaluated tissue portions of five morphological fruit types of tomatoes. The pH values of the pericarp tissue recorded the highest. Total acidity, alcohol soluble acidity and the levels of citric and total acids were highest in the locular tissue. The tissue portions of the plum-type tomato were more acidic than pear, full-cavity pear, round and blocky-pear fruit types. Potassium was an important constituent related to acidity in the locular tissue and the entire fruit; whereas phosphorus was related to buffering activity in the pericarp tissue and the entire fruit.

Winsor, 1979 stated environment, nutrition and variety influence the overall fruit quality of tomato *viz.*, appearance, firmness and chemical composition.

Suwwan and Abu-Baker (1986) studied physical properties including fruit colour, shape, specific gravity and firmness of 9 tomato hybrids and 3 tomato cultivars under plastic house conditions. Majority of hybrids and cultivars were similar in fruit colour and shape. Whereas, UC 105.7 fruits were highest in fruit firmness and lycopene contents and highest specific gravity was recorded in fruits of the "Hy crop F_1 hyb. 18110 b".

The natural abundance of lycopene in fresh tomato fruits varies on a large scale and it is influenced considerably by environment (Daskaloff *et al.*, 1990). Research reports revealed the inheritance of lycopene and different genetic controls of lycopene synthesis.

Different varieties of tomato were characterized for flavour through sensory profiling by Hobson *et al.* (1990) and cherry tomato out-competed the large-fruited tomatoes in terms of having sweeter fruits and higher overall aroma.

Poysa (1993); Azanza *et al.* (1995) postulated that crosses among cultivated and wild germplasm can create great genetic variability for fruit quality traits.

Gowda *et al.* (1994) conducted studies on the physicochemical characteristics and processing quality of two IIHR tomato varieties (Arka Ashish and Arka Ahuti) in relation to 6 commercial cultivars. The fruit juice yield was maximum in Arka Ashish. The total soluble solids of IIHR varieties were better

than Pusa Ruby and Roma, whereas, acidity and ascorbic acid content were similar to other cultivars. Maximum lycopene content was recorded in Arka Ashish followed by Pusa Gaurav and Arka Ahuti.

Kurian and Peter (1997) conducted an evaluation trial on sixty-four tomato lines for fruit shape index, total soluble solids, insoluble solids, juice yield, acidity, pH, reducing sugars, consistency, lycopene, locules fruit⁻¹ and pericarp thickness. A positive correlation was observed between fruit shape index and total soluble solids, insoluble solids, pH, reducing sugars, consistency, lycopene, locules fruit⁻¹ and pericarp thickness.

Lycopene is a carotenoid that is available naturally in tomatoes and their processed products. It is exceptional amongst the most powerful cancer prevention agents among dietary carotenoids. Dietary admission of tomatoes and tomato items containing lycopene has been appeared to be related with a diminished danger of constant maladies like malignancy and cardiovascular sickness (Agarwal and Rao, 2000). So, for the fresh market as well as for processing purpose, the lycopene substance of tomatoes ought to be high.

Causse *et al.* (2002) performed a crossed at intraspecific level between a cherry tomato line and an inbred line with bigger fruits and developed a recombinant inbred lines (RIL) population with wide range of variation to study the genetic mechanism of 38 organoleptic fruit quality traits of tomato. Marker evaluation revealed a total of 130 quantitative trait loci (QTL) controlling the 38 traits likely fruit weight, diameter, colour, firmness, meltiness, and for six aroma volatiles.

Raffo *et al.* (2002) studied nutritional characteristics of greenhouse cherry tomatoes and the results revealed that relatively having higher levels of antioxidants, especially lycopene and high antioxidant ability than the normal tomato.

Fridman *et al.*, (2004) aimed to explore existing natural biodiversity in tomato by examining the developed Introgression Lines (ILs) from the cultivated tomato (*Solanum lycopersicum*) with segments of chromosome from wild species

(*Solanum pennellii*) and identified Brix9-2-5 locus responsible for increased sugar content of tomatoes obtained from *Solanum pennellii* and mapped within the invertase (LIN5) specific for flower and fruit. QTL analysis revealed five different species of tomato showing polymorphism for Brix9-2-5 QTL.

Cherry tomatoes are the richest sources of antioxidants (ascorbic acid, lycopene and phenols) indicating as a valuable source of germplasm for improving the dietary status of antioxidants in our normal diet and increasing the nutritional and biochemical value through breeding programmes. The cherry tomatoes also having immense value for processing purposes because of their high titrable acidity and total soluble solids (George *et al.*, 2004).

Kuti and Konuru (2005) conducted an evaluation trial on 40 varieties of tomato, which includes F_1 hybrid of cluster fruiting type, solitary round breeding lines and cherry tomato genotypes (*Lycopersicon esculentum* var *cerasiforme*) grown under field and greenhouse conditions for estimation of lycopene content in fruits using High-Performance Liquid Chromatography (HPLC) and spectrophotometry. Results have shown that cherry tomato types grown under field conditions having the highest lycopene content with 91.90 mg kg⁻¹ than in greenhouse condition with 56.10 mg kg⁻¹ indicating genetics and selection of cultivation environment strongly influence the tomato fruit lycopene content.

Two new cherry tomato breeding lines designated 02L1058 and 02L1059 released by Department of Agriculture, United States with high β -carotene content in fruits and are highly recommended for use as breeding material in the development of new specialty cherry tomato cultivars (Stommel *et al.*, 2005).

Collins *et al.* (2006) reported that the lycopene is a pigment that gives a redorange colour to a few fruits and vegetables. This carotenoid contemplated during the most recent ten years in light of its antioxidant activity and restorative proof that dietary admission can decrease the rate of cardio-vascular infection and malignancies.

Lenucci *et al.* (2006) analyzed fourteen cherry tomato cultivars, four highpigmented tomato hybrids and the red-ripe tomato fruits for antioxidants and their

activity. Significant difference was found between α -tocopherol, β -carotene, lycopene, vitamin C, flavonoid and total phenolic compounds. Among cherry tomato cultivars, Corbus and LS203 found to be with highest content of lipophilic and hydrophilic antioxidants. Among high-pigmented tomato hybrids, HLY 13 found to be with highest contents of lipophilic and hydrophilic antioxidants.

Alda *et al.* (2009) conducted a study on the lycopene content of tomatoes and their products. The results showed that in fresh tomatoes the lycopene content was approximately 12 mg 100g⁻¹. The lycopene content in tomato products showed significant variation, in tomato paste 16 mg 100g⁻¹, tomato ketchup 17 mg 100g⁻¹ and tomato boiled sauce 4 mg 100g⁻¹.

Juarez-Lopez *et al.* (2009) evaluated the biochemical and fruit quality traits of seven native cherry tomato genotypes (*Lycopersicon esculentum* var. *cerasiforme*) JCPRV-5, JCPVR-9, JCPRV-10, JCPRV-43, JCPRV-70, JCPRV-71, JCPRV-76 and H-790 (control) grown in hydroponics under greenhouse. Except for firmness and pH native genotypes exceeded to control (H-790) for all parameters indicating it as a source of germplasm in breeding programs of tomato to increase the fruit quality.

Forty-nine accessions of under-exploited or related species of tomato were evaluated for identifying best accessions of tomato for the content of lycopene, β -carotene and vitamin C to increase agrobiodiversity (Adalid *et al.*, 2010). Based on balanced and improved nutritional properties 14 cherry type accessions and two common tomato types were selected. Furthermore, cherry types (BGV008365 and BGV012627) with over 1.5 times the standard vitamin C content as well as *Solanum pimpinellifolium* accession (BGV008166) which confer more than 9 times the standard lycopene content can be used as donors in crop breeding programmes to increase the nutrition properties of economical varieties.

A new variety Punjab Ratta was developed by Cheema *et al.* (2010) with qualitative traits *viz.*, lycopene content (8.14 mg 100g⁻¹), acidity (0.31%), pH (4.62) and TSS (4.85%).

Vijitha and Mahendran (2010) conducted an experiment on the moisture stress of tomato *Lycopersicon esculentum* cv. KC-1 and its influence on fruit quality traits such as Total Soluble Solids (TSS), vitamin C and acid contents, and the critical stage/s of the plant growth. The result have shown that moisture stress at fruit ripening stage reduced the vitamin C in fruits whereas, the acid contents and TSS of the fruits were slightly affected however they were not significant. In the entire plant growth stages, moisture stress during the flowering stage showed the greatest yield reduction indicating the most critical stage of the plant growth.

Aghel *et al.* (2011) reported that lycopene is a pigment chiefly in charge of the trademark dark red colour of ripe tomato fruits. Lycopene, as a common cancer prevention agent, has pulled in considerations because of its organic and physicochemical properties.

Gonzalez-Cebrino *et al.* (2011) characterised 7 traditional tomato varieties grown in organic conditions *viz.*, BGV-000998, BGV-001000, BGV-001020, BGV-004123, CIDA-44-A, CIDA-62, CIDA-59-A. CIDA-62 was shown to be the most promising variety as it produces tomatoes of very high quality under organic conditions with lycopene (62.25 mg kg⁻¹ fw), vitamin C (459.22 mg kg⁻¹ fw), total antioxidant activity (43.58 mg Trolox/100 g fw) and total soluble solids content (6.22°Brix).

Prema *et al.* (2011b) evaluated six cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) genotypes for quantitative and quality traits showing semideterminate to indeterminate growth habit. Results showed that maximum fruit set, lycopene content and TSS (8.10 °B) was seen in EC-1, whereas maximum fruit weight, titrable acidity, ascorbic acid (27.48 mg 100g⁻¹) content and yield plant⁻¹ was recorded highest in Podland Pink. The genotype Tomy Toe recorded highest fruit firmness and the shelf life of fruits shown large variation from 5.33 (EC-1) and 14.67 (Tomy toe) days.

Adalid *et al.* (2012) studied the genetic control of vitamin C and β -carotene in six basic generations (P₁, P₂, F₁, F₂, BC₁P₁ and BC₁P₂) of an intraspecific cross involving cultivated tomato CDP8779 accession (*Solanum lycopersicum* L.) and cherry tomato CDP4777 accession (*S*.

lycopersicum var. *cerasiforme*). The results revealed that the accumulation of quality traits viz., vitamin C, β -carotene was mainly due to additive gene action and CDP4777 accession (*S. lycopersicum* var. *cerasiforme*) mainly contributing towards vitamin C and β -carotene content in tomato indicating usefulness in tomato breeding programmes for producing superior F₁ hybrids.

Wild species are the greatest reserves of genetic diversity for many crops as well as, tomato (*Solanum lycopersicum L.*) in terms of fruit quality traits *viz.*, aroma, colour, flavour, lycopene and β -carotene. Aguirre and Vallejo Cabrera (2012) evaluated the agronomic and fruit quality traits of 30 cherry tomato introductions and broad phenotypic variability observed in the evaluated introductions. Selection followed by hybridization leads to the improvement of traits associated with fruit quality and production.

Carrillo-Rodriguez *et al.* (2012) evaluated the physicochemical and nutritional quality of 17 fruit samples of wild tomato (*L. esculentum* var. *cerasiforme* Dunal) collected from eight different localities and classified based on cluster analysis into three groups.

Gudeva and Dedejski (2012) studied *in vitro* and *in vivo* production of a few genotypes of cherry tomato (*Solanum lycopersicum* var. *cerasiforme* Dunal) and estimated the fruit quality of cherry tomato based on the content of total sugars, organic acids and other organic compounds and colour which is most complex fruit characteristic of cherry tomato.

Seven cherry tomato cultivars (*Solanum lycopersicum* var. *cerasiforme*) namely Stupid Hurry, Pod Land Pick, Tom Toe, Golden Delight, Bonny Bert, Red Peer and Marilee Red were evaluated for physio-chemical characteristics by Maedeh *et al.* (2012) and found a significant difference among cultivars with TSS (4.8 - 9.4%), titratable acidity (0.3 - 0.6%), ascorbic acid (23.6-28.1 mg 100 g⁻¹) and lycopene (2.13-6.93 mg 100 g⁻¹) respectively.

Manna and Paul (2012) on their study on genetic variability and fruit quality traits association in tomato suggested that the fruit length, fruit weight, number of

fruits plant⁻¹, number of locules fruit⁻¹, pericarp thickness, TSS and vitamin C content had a positive direct effect on fruit yield.

Six lycopene-rich tomato cultivars (Kalvert, Lyco 1, Lyco 2, HLY 1, HLY 02 and HLY 13) and a normal cultivar (Donald) were examined for antioxidant content and stages of maturity effecting antioxidant content (Hdider *et al.*, 2013). And the results showed 'HLY 13' with higher contents of vitamin C and flavonoid at the red ripe stage of maturity, whereas 'HLY 02' recorded with high phenolic content.

Kavitha *et al.* (2013) screened different genotypes of tomato *viz.*, hybrids, varieties, backcross populations, elite germplasm, cherry tomatoes lines and wild species for improved antioxidant activity and other quality parameters in crop breeding programmes. Results revealed that cherry tomato lines IIHR-2864, 2865 and 2866 enhanced four to five times more β -carotene than commercial hybrids/varieties indicating exploitation of cherry tomato as a parent in interspecific and intraspecific hybridization could be used for developing tomato hybrids rich in antioxidants as well as other quality traits.

Choi *et al.* (2014) analyzed quality traits of twelve greenhouse grown cherry tomato varieties of varying colours (green, red, orange, yellow and black) using HPLC and LC/MS methods. The results demonstrated the phenolic content of the cherry tomatoes is 3-4 times higher than large-sized tomatoes, wide-ranging differences as well as similarities in the content of nutritional and bioactive components in cherry tomatoes.

Molecular marker based characterization of cherry tomato genotypes were studied by Kumar (2014) and reported that low level of similarity among cherry tomato genotypes whereas Cherry T1×Co -3-2 and Cherry T4×Pant T-3 shows 89% similarity and also reported that fruit weight range from 80.48 to 126.46 g, acidity ranged from 0.23 to 0.54 percent and fruit weight ranged from 42.50 to 95.80 g and overall mean from 65.59 g in cherry tomato.

Rai *et al.* (2014) observed significant difference among the cherry tomato lines for quality parameters and it ranged from $17.62 - 46.16 \text{ mg } 100 \text{ g}^{-1}$ for vitamin-C content, total carotenoid content ranged from $3.86 - 6.66 \text{ mg } 100 \text{ g}^{-1}$ and $2.83 - 5.26 \text{ mg } 100 \text{ g}^{-1}$ for lycopene content on fresh weight basis. The pH varied from 4.15 - 4.52 and total soluble solids from 3.41 - 5.16%.

Kumar *et al.* (2016a) examined forty genotypes of tomato for 19 quantitative and qualitative traits and grouped into seven clusters in which maximum genotypes were recorded in cluster II followed by cluster I and VII with two genotypes each, while cluster III, IV, V were monotypic. The intra-cluster distances recorded in cluster VII, VI and II were 2097.24, 824.01 and 265.05 respectively which infers genetic divergency. The inter-cluster distance between cluster II and VI was recorded maximum with 1647.87 and the lowest was between cluster III and IV (125.29).

Fruit quality parameters are vital criteria for the determination of proper genotypes for explicit customer's inclinations. Rawal *et al.*, (2016) conducted an investigation to decide the qualitative characteristics (ascorbic acid, Total Soluble Solids (TSS), pH, colorimeter reading) of tomato genotypes gathered at six unique phases of development (green, breaker, turning, pink, light red and red) on seven propelled lines from AVRDC; AVTO 1288, AVTO 1289, AVTO 1418, AVTO 1424, AVTO 1455, AVTO 9331, AVTO 9708 and local variety Pusa Ruby. The outcome demonstrated that the fruits harvested at light red and dark red stage held altogether higher measure of TSS, vitamin C and pH content among all the genotype of tomato.

 F_1 hybrids of an intraspecific cross between *Solanum lycopersicum* L. and *Solanum lycopersicum* var. *cerasiforme* were evaluated for qualitative traits by Nadkarni (2017) and hybrids showed TSS (7.18%), pH of juice (4.54), lycopene (11.66 mg 100 g⁻¹) and vitamin C content of 29.75 mg 100 g⁻¹.

Nagamani (2017) undertook an investigation to study on physicochemical traits (physical characteristics, ascorbic acid, lycopene, moisture, pH, reducing and total sugars, titrable acidity, total soluble solids and β -carotene) of tomatoes namely Pusa Ruby, Vaishali, and Sadabahar. It was observed that Pusa Ruby with deep red

in colour, whereas yellowish red in Sadabahar and red in Vaishali. High total soluble solids and sugar content was found in Pusa Ruby. High ascorbic acid and β -carotene content was observed in Vaishali.

Twenty-four genotypes of cherry tomato were evaluated for qualitative traits under shade net condition by Venkadeswaran *et al.* (2018) and the genotypes IIHR 2753 and Pant Cherry Tomato-1 registered the highest for total soluble solids (6.19 °Brix) and total sugars (2.05 mg 100 g⁻¹) respectively. Whereas IIHR 2753 showed the highest lycopene (8.22 mg 100 g⁻¹) and IIHR 2754 showed the maximum total carotenoids (18.13 mg 100 g⁻¹) and total antioxidant (1.94 μ mol. AA g⁻¹) representing broad phenotypic variability which favours selection and breeding of cherry tomato for fruit quality traits.

2.5 SSR MARKER ANALYSIS IN TOMATO:

Rick *et al.* (1990) and Peralta *et al.* (2006) reported that one cultivated species and twelve wild relatives of tomato were identified till the date. Large morphological variations with great range of genetic diversity have been revealed using molecular markers in wild species and their relatives (McClean and Hanson, 1986; Rick *et al.*, 1990; Miller and Tanksley, 1990; Egashira *et al.*, 2000; Zhu *et al.*, 2004). The wild species of tomato is gifted with great genetic variation for specific set of traits which can be exploited through crop breeding techniques (Walter, 1967; Rick and Chetelat, 1995; Robertson and Labate, 2007).

Molecular markers are the tools for distinguishing the genetic diversity of agricultural crops. The genetic variation in landraces and cultivars of tomato were studied using different molecular tools, *viz.*, RFLP, AFLP, RAPD and SSR (Rus-Kortekaas *et al.*, 1994, Villand *et al.*, 1998, Mazzucato *et al.*, 2003, Carelli *et al.*, 2006 and Garcia-Martinez *et al.*, 2006).

Molecular markers are usually recognized as a reliable tool for the identification of genotypes, such as Amplified Fragment Length Polymorphisms (AFLPs) (Park *et al.*, 2004), Randomly Amplified Polymorphic DNA (RAPD) (Nikoumanesh *et al.*, 2011; Cao *et al.*, 2015), and Simple-Sequence Repeats or microsatellites (SSRs) (Wohrmann *et al.*, 2011). Among the above-mentioned molecular markers high reproducibility, co-dominance and polymorphism usually

seen in SSR markers (Powell *et al.*, 1996). Moreover, employing a combination of morphological and molecular markers to identify plant genetic diversity has become more common (Khadivi-Khub *et al.*, 2008; Terzopoulos and Bebeli, 2008; Mazzucato *et al.*, 2010; Nikoumanesh *et al.*, 2011).

New cultivars produced must be unique from all the existing cultivars by the expression of at least one or few traits which can be identified through morphological, biochemical and molecular markers Vosman (1998).

Jones *et al.* (1997) carried out an experiment in collaboration with several European laboratories, to verify the reproducibility of three popular molecular marker techniques used in crop diversity analysis such as Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Simple-Sequence Repeats (SSR). A well standardised optimal system was chosen for each of the techniques and results confirmed that RAPDs proved difficult to reproduce followed by AFLPs, whereas, SSR showed high reproducibility.

Hybridization and introgression between wild and cultivated species result in new gene combinations and the population is maintained with new characteristics. Molecular evaluation of introgression lines is a significant process in increasing the genetic diversity of crop plants Jarvis and Hodgkin (1999).

Both size and shape of fruit are the major determining factors for yield, quality and consumer acceptableness of several crops. Tomato considered as one of the model fruit bearing domesticated crop species which exhibits great diversity in fruit morphology. Using Simple Sequences Repeats (SSRs) Grandillo *et al.* (1999) identified the loci responsible for natural variation in fruit size and shape in tomato.

Vosman *et al.* (2001) standardised methodologies of microsatellite markers application in varietal identification of tomato.

He *et al.* (2003) unfolded and characterized SSR markers on the DNA sequences of 19 cultivars of tomato. Nineteen diversified cultivars of tomato were screened with a set of 158 pairs of SSR primers of which 129 pairs synthesised the

expected amplicons in their PCR result, and 65 of them shown polymorphism with a Polymorphism Information Content (PIC) which varied between 0.090 to 0.670. Cluster analysis performed based on the pattern of banding of the 65 polymorphic SSR primers. The markers developed in this experiment were primarily from expressed sequences, which can be used for molecular mapping studies, markerassisted breeding programs, identification of cultivar and gene-trait relations in tomato.

In genetic research microsatellite DNA markers are most widely used because of simple, economical, high-throughput system that detects amplicons by gel electrophoresis and capable of distinguishing DNA fragments differing at two base pairs (Wang *et al.*, 2003).

Villalta *et al.* (2005) performed two crosses likely *Lycopersicon esculentum* var. *cerasiforme* × *Lycopersicon pimpinellifolium* and *Lycopersicon esculentum* var. *cerasiforme* × *Lycopersicon cheesmani* and obtained F_6 population consisting of 142 and 115 lines respectively, which are comparatively characterised by Simple Sequence Repeat (SSR) and Sequence Characterized Amplified Region (SCAR) markers. Similar polymorphism was found for each population even a different set of markers was involved. Results shown that larger genetic distance reported between *L. esculentum* var. *cerasiforme* and *L. cheesmanii* than compared with *L. pimpinellifolium*.

Garcia-Martinez *et al.* (2006) aimed to characterise 48 cultivars of tomato with main types, *Solanum lycopersicum* L. 'Muchamiel', 'De la pera' and 'Moruno' using combinations of 7 Amplified Fragment Length Polymorphism (AFLP) markers and 19 selected microsatellite (SSR) markers. Combinations of a few AFLP and SSR markers revealed existence of unique DNA sequences even of the closely related cultivars of tomato. SSR based dendrogram favours a better clustering of the 'Muchamiel' cultivars whereas, AFLP favours better clustering of 'De la pera' cultivars, indicating the efficiency of AFLP and SSR markers for the distinguishing of traditional and closely related cultivars of tomato. Rajput *et al.* (2006) conducted an experiment on testing reproducibility of RAPD and SSR markers in tomato involving screening of Randomly Amplified Polymorphic DNA (RAPD), and Simple Sequence Repeats (SSRs). For each technique, well standardised optimal system was chosen. The results shown that reproducibility of RAPDs proved to be difficult whereas, SSR showed high reproducibility.

Genetic diversity among 11 tomato cultivars were analysed using morphological markers and Simple Sequence Repeat (SSR) by Wang *et al.* (2006). With seven SSR primers a total of 53 bands were obtained with an average of 6 bands per SSR primer pair with a range from 2 to 9. Mean genetic similarity coefficient among cultivars was 0.60, varying from 0.39 - 0.84. Morphological characterisation is performed with 11 phenotypic traits and the average genetic similarity coefficient was 0.580, varying from 0.270-0.720 indicating similar results of the evaluations of genetic diversity in tomato cultivars based on the above two methods were similar.

Benor *et al.* (2008) conducted a study to analyse the genetic diversity of 39 tomato inbred lines with determinate and indeterminate growth habit obtained from different geographical regions like China, Japan, South Korea, and USA using 35 highly polymorphic Simple Sequence Repeat (SSR) markers. Results shown that a medium level of genetic diversity existing with an average Polymorphism Information Content (PIC) 0.31 and found that higher number of distinctive alleles existing in the examined tomato inbred lines. Cluster analysis at 0.85 value grouped the lines into four clusters, whereas single cultivar from USA separated to form a distant cluster.

Mazzucato *et al.* (2008) collected 50 tomato landraces, 9 modern cultivars and 2 wild species and characterised for 15 morpho-biochemical traits and 29 microsatellite (SSR) loci. Two sets of markers were used i.e., Q-SSRs which affects quantitative trait loci (QTLs) for fruit size/shape and NQ-SSRs which are group of markers that have not been mapped on the genome. Statistical analysis of morphological, molecular data revealed low level of polymorphism in modern cultivars of tomato, whereas a rich molecular diversity in landraces. Results revealed higher degree of association between the subset of Q-SSR markers and traits associated with fruit size/shape concluding a realistic positive marker-trait relationship in tomato.

Chen *et al.* (2009) genotyped four different populations comprising of 216 cultivars of tomato (*Solanum lycopersicum* L.), hybrids, and elite breeding lines using simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers. A total of 216 genotypes were screened with 47 markers, of which 72.3% shown polymorphism in the entire collection of 216 genotypes and 51.06 - 59.57% were polymorphic in individual populations. Cluster analysis grouped the population into three clusters.

Kwon *et al.* (2009) screened 63 commercial varieties of tomato with 22 morphological traits and 33 SSR markers electrophoresis results shown a total of 132 polymorphic amplicons were obtained. The average Polymorphism Information Content (PIC) was 0.628. Cluster analysis grouped the varieties based on SSR results, into cherry type and classic type.

Twenty-five tomato cultivars of determinate and indeterminate growth habit collected from different geographical locations of India and were screened with 23 simple sequence repeats (SSR) to determine genetic diversity, genetic identities and genetic relationships among these tomato cultivars by Parmar *et al.* (2010). On an average, 40 alleles were amplified using SSR primers with amplicons ranging from approximately 150 to 1000 bp. Cluster analysis based on geographical location and growth habit grouped the cultivars into five clusters with the USA cultivars forming a distinct group.

SSR markers have been successfully used in tomato for diversity analysis and characterization of tomato germplasms (Dhaliwal *et al.*,2011; Sanghani and Mandavia 2013; Zhou *et al.*, 2015).

Srivastava *et al.* (2011) developed a systematic and flexible method for assembling panel of multiplex simple sequence repeat marker for high-throughput genome analysis in the tomato (*Solanum lycopersicum*) for varietal identification.

To determine the genetic diversity, genetic identities and genetic relationships among the cultivars using 20 Simple Sequence Repeats (SSR) markers by El-Awady *et al.* (2012). Based on SSR data, genetic similarity was estimated between 17.60 and 93.20%, indicating the probability of SSR markers in differentiating plants among close or distant genetic backgrounds. Cluster analysis grouped the cultivars into two clusters whereas, the two Egyptian cultivars Giza 80 and Edkawy were clustered into distinct group.

Hu *et al.* (2012) revealed genetic diversity of 67 Argentina tomato (*Solanum lycopersicum* L.) varieties by analysing through morphometric traits, molecular markers *viz.*, Simple Sequence Repeat (SSR), and Single Nucleotide Polymorphism (SNP). About 65.0% of the morphological characters and 55.30% of the molecular markers displayed polymorphism. The Average taxonomic distance for any two varieties was found between 0.6643 to 1.1776, whereas Nei's genetic distance ranged from 0.0000 to 0.2022. The cluster analysis of 67 varieties grouped both morphometric and molecular traits into three clusters.

Lycopene and Total Soluble Solid (TSS) content are major determining factors for tomato fruit quality. Sun *et al.* (2012) mapped fifteen QTLs controlling for lycopene and total soluble solid content in $F_{2:3}$ families obtained from an interspecific cross between the cultivated tomato *Solanum lycopersicum* and wild species *S. pimpinellifolium*. The results revealed that QTL responsible for Total Soluble Solid (TSS) content was mapped to chromosome 1 and on chromosome 4, the QTL for lycopene content was found.

Genetic diversity can be exploited through molecular markers such as, dominant markers (Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), DNA Amplification Fingerprinting (DAF), Inter-Simple Sequence Repeat (ISSR), Arbitrarily Primed Polymerase Chain Reaction (APPCR) and co-dominant markers Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSRs), Expressed Sequence Tags (ESTs), Cleaved Amplified Polymorphic Sequence (CAPS), Sequence Characterized Amplified Regions (SCARs), Single Nucleotide Polymorphisms (SNPs) and Sequence Tagged Sites (STSs) (Idrees and Irshad 2014).

Forty-two tomato varieties collected from different geographic regions with varying environment were examined for the estimation of genetic diversity and their relationship with EST-SSR markers by Korir *et al.* (2014). The genetic diversity ranged from 0.180 to 0.770. The polymorphic information content ranged from 0.17 to 0.74 which represents a higher degree of diversity among the collected varieties of tomato. Cluster analysis grouped the varieties of tomato into five clusters.

Twenty-four tomato cultivars with determinate and indeterminate growth habit were screened using 20 SSR marker and four lycopene gene specific markers to arbitrate genetic diversity, genetic identities and their relationships by Monika *et al.* (2014). Electrophoresis results revealed a total of 54 scorable and reproducible alleles were amplified using all the primers. The gene diversity ranged between 0.650 to 0.970 values with a mean of 0.840.

Saravanan *et al.* (2014) conducted genetic diversity analysis with eighteen genotypes of tomato (*Lycopersicum esculentum L.*) using five SSR markers. The result confirmed the existence of high diversity among the tomato genotypes. High genetic diversity was seen between the genotypes LE-22 and LE-150. Similarity coefficient of eighteen genotypes using SSR markers ranged from 0.10 to 0.40.

Eight tomato varieties from Bulgaria including breeding lines (var. IZK Alya, var. Plovdivska karotina, L21β, L53β, L975, L984, L1116, L1140) varying in their morphological and biochemical composition were screened for genetic variation with 165 low cost and highly efficient fluorescent Simple Sequence Repeat (SSR) by Todorovska *et al.* (2014) and a total of 299 alleles were amplified with an average of 1.869, and the average Polymorphic Information Content (PIC) was 0.196. Nei's genetic distance showed a range from 0.0953 to 0.3992. based on cluster analysis genotypes were grouped into four clusters. Variety IZK Alya (cherry type), L1116, L1140 formed three separate clusters respectively, and the remaining five genotypes forms fourth cluster.

Miskoska-Milevska *et al.* (2015) determined the genetic relationship between six morphologically different varieties of tomatoes (var. *cerasiforme* (red),

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var. *cerasiforme* (yellow), var. *pruniforme*, var. *pyriforme*, var. *racemigerum* and var. *variferifium*,) with 9 SSR markers LE20592, LE21085, LECHSOD, LECH13, LEMDDNa, LEEF1Aa, LELEUZIP, LE2A11 and TMS9. The results revealed genetic distance was found smallest between var. *cerasiforme* yellow and red i.e., 16.7415, and the largest was recorded between var. *pyriforms* and var. *grandifolium* 34.9859.

Zhou *et al.* (2015) evaluated the genetic diversity of 14 wild tomato accessions, 29 cultivated tomatoes, and 7 introgression lines using morphometric characters and molecular markers. Dendrogram studies based on morphometric observations and molecular data divided them into six and eight clusters respectively. Thirteen Expressed Sequence Tag- SSR (EST-SSR) and fifteen genomic Simple Sequence Repeat (genomic-SSR) polymorphic markers amplified 780 and 1,115 amplicons, respectively. The average polymorphism information content (PIC) was recorded 0.49 in genomic-SSRs and 0.45 in EST-SSRs.

Kumar *et al.* (2016b) screened 19 tomato (*Solanum lycopersicum* L.) genotypes using 11 polymorphic microsatellite markers. Electrophoresis revealed a set of 261 polymorphic amplicons with an average Polymorphism Information Content (PIC) value of 0.99 ranging from 0.979 to 0.995 in SSR-110 and SSR-253 respectively. Cluster analysis was performed based on the banding pattern of microsatellite markers and two major clusters were generated at 43% level of similarity. The cluster A holds two genotypes whereas, cluster B accommodates the majority of genotypes with 17. The results additionally showed that existence of 100% similarity between 2012TODVAR-2 and Arka Vikas genotypes.

Raveendar *et al.* (2016) used simple sequence repeats (SSRs) to arbitrate the genetic diversity and population architecture of 355 accessions of tomato acquired from Asia. With 18 SSR markers a total of 176 alleles were observed with an average of 10 alleles per locus with a Polymorphic Information Content (PIC) of 0.39. Cluster analysis grouped the accessions into 2 distinct clusters as, admixtures (11%) and subpopulations (89%) based on genetic distance. The overall fixation index (FST) value was 0.135, reveals a moderate differentiation between the inferred subpopulations. Analysis of variance based on molecular data showed that the genetic variance among individuals was 86%, in contrast genetic variance among geographical groups was less than 6%.

Morphological, biochemical, cytological and molecular (DNA) markers are the most accessible genetic markers. Of these molecular markers are the potent tools for efficient selection of desired agronomic traits at the DNA level because they are truly dependent on the plant genotypes and are independent of varying environments. AFLP, RAPD, ISSR, SSR and SNP are widely used molecular techniques for studying genetic variation in landrace and cultivar of tomato (Sunilkumar *et al.*, 2016).

In tomato, wild forms are the richest source of genetic diversity, which includes *Solanum lycopersicum var. cerasiforme* and *Solanum pimpinellifolium*. Aguirre *et al.* (2017) conducted a research to evaluate the genetic diversity of 30 cherry tomato introductions with 36 Simple Sequence Repeats (SSRs) markers and results confirmed the existence of broad genetic variability among the introductions favouring the possibility of selection for genetic improvement and sustainable use of the species.

To determine the genetic diversity of twenty-four genotypes of tomato, Singh *et al.* (2018) screened the genotypes with four primers specific to lycopene. Cluster analysis based on morphological traits and marker specific for lycopene displayed two distinct groups.

<u>Materials and Methods</u>

3. MATERIALS AND METHODS

The present investigation on "Identification of cherry tomato genotypes from F_3 segregants of intraspecific cross" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2018. The objectives of the experiment were to evaluate 150 F_3 segregants obtained from an intraspecific cross between cultivated tomato variety Anagha (*Solanum lycopersicum* L.) and *Solanum lycopersicum* var. *cerasiforme* for morphometric traits for locating superior segregants with cherry tomato characters through biochemical analysis and molecular markers.

The experiment site was located at 8.4° North latitude and 76.9° East longitude, at an altitude of 29.00 m above mean sea level. Predominant soil medium used for the experiment was cow dung: river sand: red soil in 1:1:1. The area enjoys a warm humid tropical climate under rain shade shelter. The study was conducted in three different experiments.

- 3.1 Evaluation of 150 F₃ segregants.
- 3.2 Biochemical analysis for fruit quality traits in 20 superior segregants.
- 3.3 Genotyping of the 20 superior segregants with ssr markers.
- 3.4 Statistical analysis.

3.1 EVALUATION OF 150 F3 SEGREGANTS

3.1.1 Materials Chosen for The Study

 F_2 plants derived from the cross between cultivated tomato variety Anagha (*Solanum lycopersicum* L.) and *Solanum lycopersicum* var. *cerasiforme* were evaluated and 5 superior segregants with cherry tomato characters was identified. The seeds from these plants were raised, and 30 seedlings randomly selected from each F_2 family. These 150 seedlings were transplanted and used for the study.

Forty-five days old seedlings were transplanted into growbags during autumn 2018 for evaluation of superior segregants with cherry tomato characters.

Treatment	: 150 segregants
Spacing	: 90 cm × 45 cm

3.1.2 Raising Seedlings

Tomato seedlings were raised in protrays. Seeds were sown in protrays and kept in a polyhouse provided with insect proof netting on all sides. Forty-five days old healthy seedlings were used for transplanting into growbags.

3.1.3 Cultural Operations

3.1.3.1 Soil Solarization

Soil solarization was done for controlling soilborne plant borne pathogens including bacteria, fungi, nematodes and insect pests along with weed seed and seedlings in the soil by mulching the soil and covering it with a transparent polythene cover, to trap solar energy. Bed of 10×1 m was prepared.

3.1.3.2 Seed Treatment

Seed treatment was done with *Pseudomonas fluorescens* @ 3 ml/100 ml distilled water and soaked in it for 24 hours in order to control soil borne and seed borne infection of fungal diseases like Early blight, Damping off and Wilt.

3.1.4 Observations on Morphometric Traits

3.1.4.1 Plant Height (cm)

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

3.1.4.2 Number of Primary Branches Plant¹

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

3.1.4.3 Number of Days to 50% Flowering

Number of days from transplanting to first flower appearance in 50 percent of population.

3.1.4.4 Number of Days to First Fruit Harvest

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

3.1.4.5 Number of Fruits Plant¹

The number of fruits harvested from each of the plant in the experimental plot was recorded.

3.1.4.6 Weight of Fruits Plant¹ (g)

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

3.1.4.7 Weight of Fruit (g)

Weight of the fruits were found out using an electronic precision balance and average of five fruits in each of plant was recorded.

3.1.4.8 Number of Locules Fruit¹

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

3.1.4.9 Number of Fruits Cluster¹

The number of fruits cluster⁻¹ harvested from each observational plant in the experimental plot was recorded.

3.2 BIOCHEMICAL ANALYSIS FOR FRUIT QUALITY TRAITS IN 20 SUPERIOR SEGREGANTS

Twenty superior segregants were selected from 150 F₃ segregants with cherry tomato characters were used for fruit quality (lycopene, vitamin C, pH and TSS) analysis.

3.2.1 Lycopene (mg/100g)

Lycopene content was estimated using the protocol proposed by Ranganna (1976). The carotenoids in the fruit sample were extracted in acetone and then separated by using petroleum ether. Lycopene has maximum absorption at 473 nm and 503 nm. One mole of lycopene when dissolved in one litre petroleum (40-60°C) and measured in a spectrophotometer at 503 nm in one cm light path gives an absorbance of 17.2×10^4 . Therefore, a concentration of 3.1206 µg lycopene/ml gives unit absorbance.

Materials required:

Acetone (AR grade) Petroleum ether 40-60 (AR grade) 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. The pulp was repeatedly extracted with acetone using waring blender or a pestle and mortar until the appearance of colourless residue.
- 4. The pooled acetone extract was transferred to a separating funnel containing about 20 ml petroleum ether and mixed gently.
- 5. Added 20 ml of 55 Sodium sulphate solution and shaked the separating funnel gently. (The volume of petroleum ether might be slightly 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 colour (orange) was noticed in the upper petroleum ether layer.
- Separated the two phases and re-extracted the lower aqueous phase with additional 20 ml petroleum ether until the colourless aqueous phase was obtained.
- 7. The pooled petroleum ether extracts were washed once with a little distilled water.
- Poured the washed petroleum ether extract containing carotenoids into a amber 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 filter paper. Washed sodium sulphate slurry with petroleum ether until it was colourless and transferred the washings to the volumetric flask.
- Made up the volume and measured the absorbance in a spectrophotometer at 503 nm using petroleum ether as blank.

Calculation:

Absorbance (1 unit) = $3.1206 \ \mu g \ lycopene/ml$.

mg lycopene in 100 g sample = $\frac{31.206 \times Absorbance}{Weight of sample (g)}$

3.2.2 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 obtain working standard solution.

3. 2, 6-dichlorophenol indophenole dye

Sodium bicarbonate (42 mg) was dissolved in a little volume of distilled water. 52 mg of 2, 6 dichlorophenol indophenol was added into this and make upto a final volume of 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.

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). The indication of end point is the appearance of pink colour which persisted for at least 5 seconds.

Five grams of fresh fruit was extracted in 4% oxalic acid medium, the extract was filtered through filter paper 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 (V2) was determined.

Vitamin C content of the sample was calculated using formula Amount of Vitamin C in mg 100 g⁻¹ sample = $\frac{0.5 \times V2 \times 100 \times 100}{V1 \times 5 \times Weight of sample}$

3.2.3 pH of Juice

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

3.2.4 Total Soluble Solids (°Brix)

Total Soluble Solids (TSS) of tomato fruits were recorded using a hand refractometer (0-32 °Brix). A drop of tomato juice was used to determine the TSS content with the help of hand refractometer and the value was expressed in percent at room temperature.

3.3 GENOTYPING OF THE 20 SUPERIOR SEGREGANTS WITH SSR MARKERS.

3.3.1 Plant Material

Young leaves of selected plants from population were used for genotyping the segregants.

3.3.2 Isolation of Genomic DNA

Genomic DNA of tomato was isolated from the 20 genotypes by CTAB method (Murry and Thompson, 1980) with slight alteration as reported by Ginwal and Mittal (2010) for removing the RNA and phenolics.

Tomato genomic DNA was extracted from young leaves of selected tomato genotypes followed the CTAB protocol as follows. Before starting, add β -mercaptoethanol (100 µl/100 ml Buffer), 8M Lithium chloride (30 µl/ 100 µl) and 4% poly vinyl pyrollidone (PVP) to CTAB extraction buffer then follow the stepwise protocol given below:

- About 100mg of young leaf was grinded in 1000 µl 2X CTAB extraction buffer using pestle and mortar.
- 2. 700 µl of this solution was transferred into 1.5 ml eppendorf tube.
- Incubated at 65°c on water bath for 20-30 min and then cooled briefly and 700 μl of Chloroform: Isoamylalcohol (24:1) was added.

- The content was shaken by hands periodically and kept at room temperature for 15 minutes. Eppendorf tubes were centrifuged at 13000 rpm for 3 min.
- 600 μl of upper aqueous phase was transferred into a new 1.5 ml Eppendorf tube. 900 μl of absolute ethanol was added and mixed gently and the tubes were kept for 2 hrs at -20°C.
- The sample was centrifuged for 3 min at 10,000 rpm and decant the supernatant. The pellet was washed with wash buffer (998 μl of 76% ethanol and 2 μl of 5M ammonium acetate) and air-dried.
- 7. DNA pellet was air dried and then dissolved in 50 μ l of TE buffer.

3.3.3 Quantification and Quality Test of Genomic DNA

For quantification, 8 μ l of DNA of all the selected tomato genotypes, was loaded on 0.8% agarose gel and electrophoresis was done for about 1 hour at 60 volts. The DNA was stained with 2 μ l ethidium bromide and visualized in UV under gel documentation system of Biorad where amount of fluorescence is directly proportional to the total mass of DNA.

3.3.3.1 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 ₂ O)	1000 ml

6X loading dye

Sucrose	4.0 g
Volume (Distilled H ₂ O)	10 ml
Bromophenol blue	0.025 g

(Loading dye solution was stored under refrigerated condition at 4°C)

Agarose gel electrophoresis was carried out in a BIO-SYS, horizontal gel electrophoresis unit. 0.8 g Agarose was weighed and melted in 1X TAE buffer. After cooling the solution to 42-45°C, ethidium bromide was added at the rate of 2 μ l for 100 ml. The solution was then discharged on to a preset, sealed gel casting

tray with a comb placed in the 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 immersed in electrophoresis tank filled with 1X TAE buffer ensuring that the buffer covered the gel to height of 1 mm. 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 properly 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 of the gel. The gel was documented using SYNGENE gel documentation system.

The ratio of absorbance at 260 nm and 280 nm was used to assess the purity of DNA and RNA. A ratio between 1.8 to 2.0 is generally indicates as "pure" for DNA. If the ratio is appreciably lower, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. After the quantification, the DNA was diluted with sterile water to get a final concentration 50ng DNA/ μ l.

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

3.3.4 PCR Analysis for Genotyping The F3 Segrgants

PCR analysis was done using the 10 Simple Sequence Repeat (SSR) markers reported by (Kwon *et al.*, 2009, El-Awady *et al.*, 2012 and Aguirre *et al.*, 2017) used in this study to identify the polymorphic loci between the 20 selected tomato segregants along with parents.

Sl. No.	Components	Concentration	Quantity					
1.	Sterile water	-	13.8 µl					
2.	dNTP	1mM	2 µl					
3.	MgCl ₂	2.5mM	2.5 µl					
4.	10X Taq Buffer	1X	2.5 µl					
5.	Forward Primer	5 µM	1 µl					
6.	Reverse Primer	5 µM	1 µl					
7.	Taq polymerase	3U/µl	0.2 µl					
8.	Template DNA							
	Total		25 µl					

3.3.4.1 PCR Components with Their Quantity for Microsatellite Analysis

Temperature (°C)	Duration (minutes)	Cycles	Activity
95	4	1	Initial denaturation
95	1	35	Denaturation
60-65	1	35	Primer annealing
72	2	35	Primer extension
72	7	1	Final extension
4	00	1	Hold
	(°C) 95 95 60-65 72	(°C) (minutes) 95 4 95 1 60-65 1 72 2 72 7	(°C) (minutes) 95 4 1 95 1 35 60-65 1 35 72 2 35 72 7 1

3.3.4.2 Temperature Profile Used for PCR Amplification

3.3.4.3 Detection of Polymorphism Using Simple Sequence Repeats (SSR) Primers

The polymorphism was detected by using SSR primers. 10 microsatellite markers reported by Kwon *et al.*, 2009, El-Awady *et al.*, 2012, Aguirre *et al.*, 2017 and the Solanum Genomic Network (2011) gene database were used for analysis. Table 1: List of primers used for polymorphism analysis with their product size and annealing temperatures

S. No.	Primer		Sequence	Product size (bp)	Annealing temp.
1.	SSR 9	Forward	CCCTTTGCAAGTTCTTCTTCA	168	60
		Reverse	TTCATGAGCCAACATAGGAGG		
2.	SSR 19	Forward	CCGTTACCTTGGTCCATCAC	188	60
		Reverse	GGGAGATGCCACATCACATA		
3.	SSR 26	Forward	CGCCTATCGATACCACCACT	178	60
		Reverse	ATTGATCCGTTTGGTTCTGC		
4.	SSR 28	Forward	ACCAAATGGAAATGGGTCAA	164	60
		Reverse	CCCTAAGACTAACGACAACCAA		
5.	SSR 47	Forward	TCCTCAAGAAATGAAGCTCTGA	191	60
		Reverse	CCTTGGAGATAACAACCACAA		
6.	SSR 63	Forward	CCACAAACAATTCCATCTCA	250	60
		Reverse	GCTTCCGCCATACTGATACG		
7.	SSR 86	Forward	AGGGCAACAAATCCCTCTTT	210	60
		Reverse	GGAGACGAGGCTGCTTACAC		
8.	SSR 94	Forward	AATCAGATCCTTGCCCTTGA	187	60
		Reverse	AGCTGAGAAAGAGCAGCCAT		
9.	SSR 253	Forward	CCACAAACAATTCCATCTCA	250	60
		Reverse	GCTTCCGCCATACTGATACG		
10.	SSR 268	Forward	CTGAAGCTGAGAAAGGCGAC	218	60
		Reverse	CTGGCATTTAAGGCAAAGAA		

3.3.5 Electrophoresis and Visualization of Amplified Products:

The size of the amplified products of DNA were usually smaller than 1 kb. 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 adjusting the open ends and placed on an uniform surface.
- Agarose gel (2.5%) was prepared in 1X TAE buffer boiled for 1 minute and cooled to 40°C with added ethidium bromide solution of 2 μl. Agarose solution was discharged into the gel tray with the comb in place, avoiding air bubbles and allowed to settle for 15-20 min.
- After detaching the comb, the gel was placed in the electrophoresis tank containing 0.5X TAE buffer till the gel was completely immersed.
- 4. 25 μ l of PCR sample was transferred into the wells and suitable DNA marker was used to assess the size of the PCR product. The leads were connected properly to the power source and the gel was run at constant voltage of 60 V/cm².
- 5. The run was stopped as the bromophenol blue dye reached almost 3/4 the length of the gel.
- 6. The gel was viewed in a gel documentation system and photographed.

3.4 STATISTICAL ANALYSIS

The data recorded on different traits in the twenty selected F₃ segregants along with parents were subjected to the following statistical analysis.

3.4.1 Cluster Analysis

Cluster analysis was performed using Statistical Package for the Social Sciences (SPSS version 16.0) based on morphological and fruit quality traits.

3.4.2 Euclidean Distance

Proximity dissimilarity matrix was analysed using Euclidean distance method for morphological and fruit quality traits by estimating Euclidean distance as formula suggested by Shifriss and Sacks (1980).

Euclidean distance = $\sum_{k=1}^{j} \left[\frac{x_{ik}-x_{jk}}{s_k}\right]^2$

Where,

Xik = Performance of the ith parent for kth character <math>Xjk = Performance of the jth parent for kth character <math>Sk = Standard deviation of the Kth character

Genetic divergence (genetic distance) between parents and selected F_3 genotypes measured by Euclidean distance method (Cruz and Regazzi, 1994) using Statistical Package for the Social Sciences (SPSS version 16.0).

<u>Results</u>

4. RESULTS

The experimental results obtained from the present investigation on "Identification of cherry tomato genotypes from F₃ segregants of intraspecific cross" are presented under the following headings.

4.1 Evaluation of 150 F₃ segregants.

4.2 Quantitative and qualitative traits in 20 superior segregants.

4.3 Proximity dissimilarity analysis of morphometric data.

4.4 Genotyping of the 20 superior segregants with SSR markers.

4.1 EVALUATION OF 150 F₃ SEGREGANTS

An intraspecific cross was performed between cultivated tomato variety 'Anagha' and *Solanum lycopersicum* var. *cerasiforme* in Department of Plant Breeding and Genetics, College of Agriculture, Vellayani. It was found that F_1 hybrids were superior and showing cherry tomato characters. The work was carried forward to F_2 generation for identifying cherry tomato genotypes in segregating population and selected 5 F_2 families and seeds collected to raise the F_3 generation.

A total of 150 F_3 segregants were raised which were derived from 5 F_2 families and identified to be specific for cherry tomato characters.

The F_3 segregants numbered based on the F_2 family from which it originated (P-1 to P-5) and each plant in the family was numbered from 1-30.

The morphometric observations such as plant height, primary branches plant⁻¹, number of fruits plant⁻¹, weight of fruits plant⁻¹, weight of fruit, number of locules fruit⁻¹, number of fruits cluster⁻¹ were recorded from the raised 150 F₃ segregants in Table 2a to 2e.

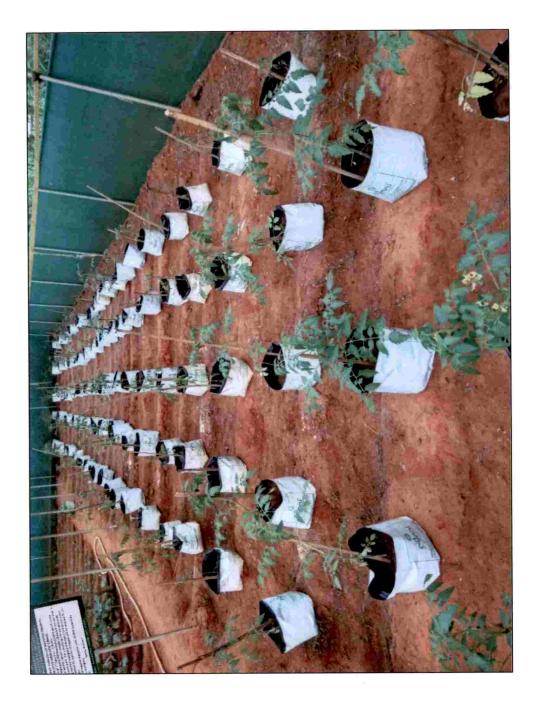


Plate 1: General view of experimental plot

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Segregant/Trait	Plant height (cm)	Primary branches plant ⁻¹	Number of fruits plant ⁻¹	Weight of fruits plant ⁻¹ (g)	Weight of fruit (g)	Number of locules fruit ⁻¹	Number of fruits cluster ⁻¹
P-1-1	118	5	18	55.83	2.91	2	3
P-1-2	104	4	16	57.27	3.42	2	3
P-1-3	101	4	35	208.72	9.14	4	4
P-1-4	116	4	14	35.02	2.30	2	2
P-1-5	110	5	21	47.25	2.12	2	3
P-1-6	110	9	20	81.10	4.05	2	2
P-1-7	119	5	64	413.71	8.58	4	5
P-1-8	124	5	18	76.26	4.09	2	2
P-1-9	101	7	21	68.52	3.12	2	2
P-1-10	121	9	23	59.35	2.45	2	3
P-1-11	123	5	19	47.48	2.36	2	2
P-1-12	110	4	17	39.40	2.12	2	3
P-1-13	124	5	26	124.72	5.94	4	5
P-1-14	112	4	21	58.20	2.62	3	2
P-1-15	116	4	14	45.98	3.07	3	3
P-1-16	116	4	21	91.41	4.21	3	3
P-1-17	131	5	14	45.56	3.04	3	3
P-1-18	122	9	23	73.38	3.06	2	4
P-1-19	129	5	19	63.23	3.17	2	3
P-1-20	102	Ş	25	83.25	3.21	2	3
P-1-21	115	S	63	370.07	6.51	3	6
P-1-22	107	4	23	75.22	3.14	2	2
P-1-23	118	4	19	60.76	3.04	2	2
P-1-24	122	9	19	64.94	3.26	2	3
P-1-25	124	9	21	59.01	2.81	3	3
P-1-26	115	5	18	49.68	2.76	3	2
P-1-27	107	5	20	59.80	2.84	2	2
P-1-28	112	4	18	58.68	3.26	2	3
P-1-29	116	4	14	41.80	2.72	2	3
P-1-30	120	4	16	55.69	3.31	2	3

Table 2b. Details of the characters of the F_3 segregants derived from $2^{nd} F_2$ family

Number of fruits cluster ⁻¹	3	2	4	2	5	з	2	2	ŝ	4	5	3	2	2	2	3	3	7	3	2	9	3	2	2	2	9	2	3	2	3
Number of locules fruit ¹	2	3	2	2	2	3	2	2	3	3	2	3	2	2	2	3	3	2	3	3	2	2	3	2	e S	2	3	2	3	2
Weight of fruit (g)	4.21	3.22	3.60	3.42	3.53	3.21	2.81	2.67	2.48	3.62	4.80	2.72	3.26	2.81	3.31	5,93	2.60	7.22	3.07	3.42	2.91	2.31	2.21	4.05	4.10	3.93	3.12	2.45	2.63	2.21
Weight of fruits plant ⁻¹ (g)	175.61	96.60	118.20	82.08	156.97	73.62	104.16	64.08	69.96	137.56	341.03	73.72	120.62	78.68	118.85	177.90	109.60	160.02	82.89	122.70	93.12	97.02	75.93	178.20	154.70	206.11	118.44	96.10	95.05	75.93
Number of fruits plant ¹	41	30	32	24	61	22	36	24	27	38	71	26	37	28	35	30	41	45	27	35	32	42	33	44	37	69	37	38	35	33
Primary branches plant ⁻¹	4	4	3	9	7	2	5	4	5	3	4	. 9	3	2	3	4	9	2	5	2	e,	4	4	3	9	5	9	4	3	2
Plant height (cm)	142	176	172	156	175	163	148	156	176	156	136	168	142	168	162	171	164	181	146	156	170	183	168	182	141	182	161	162	156	181
Segregant/Trait	P-2-1	P-2-2	P-2-3	P-2-4	P-2-5	P-2-6	P-2-7	P-2-8	P-2-9	P-2-10	P-2-11	P-2-12	P-2-13	P-2-14	P-2-15	P-2-16	P-2-17	P-2-18	P-2-19	P-2-20	P-2-21	P-2-22	P-2-23	P-2-24	P-2-25	P-2-26	P-2-27	P-2-28	P-2-29	P-2-30

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Segregant/Trait	Plant height (cm)	Primary branches	Number of fruits plant ⁻¹	Weight of fruits plant ⁻¹	Weight of fruit (g)	Number of locules fruit ⁻¹	Number of fruits cluster
		plant ⁻¹		(g)			1
P-3-1	261	4	78	241.27	4.57	2	10
P-3-2	142	3	56	126.76	2.21	2	4
P-3-3	147	9	64	148.48	2.32	3	2
P-3-4	131	9	43	86.86	2.02	2	4
P-3-5	140	9	102	392.85	5.88	2	7
P-3-6	142	5	62	165.44	2.62	3	3
P-3-7	147	9	47	109.98	2.34	2	3
P-3-8	146	9	56	192.08	3.43	3	2
P-3-9	121	4	51	327.42	6.42	3	4
P-3-10	142	8	43	102.76	2.32	4	4
P-3-11	128	7	38	76.76	2.02	3	4
P-3-12	131	7	24	59.64	2.36	2	3
P-3-13	125	4	42	101.64	2.42	3	4
P-3-14	160	4	31	112.22	3.62	3	2
P-3-15	154	5	27	105.87	3.81	3	3
P-3-16	132	4	32	89.29	2.81	2	4
P-3-17	137	9	105	296.60	2.05	2	8
P-3-18	154	7	28	87.56	3.02	2	ю
P-3-19	130	5	42	128.52	3.06	2	2
P-3-20	161	3	57	217.17	3.81	2	2
P-3-21	142	4	47	199.28	4.24	2	2
P-3-22	121	4	34	144.76	4.14	2	3
P-3-23	162	4	52	219.96	4.23	2	ю
P-3-24	146	9	72	281.45	4.85	6	7
P-3-25	128	9	36	154.84	4.19	3	ň
P-3-26	141	5	56	178.08	3.18	2	4
P-3-27	136	5	28	91.88	3.21	2	3
P-3-28	119	4	48	149.76	3.12	2	3
P-3-29	156	5	37	128.02	3.46	2	4
P-3-30	137	3	56	196.00	3.50	2	3

family
$^{1}\mathrm{F}_{2}$
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Table 2

		_	_		_	_			_	_						_	_	_		-				_		-	_	-	_	
Number of fruits cluster ¹	5	2	3	ю	2	2	ю	Э	3	2	2	4	ю	3	7	ю	3	ю	2	2	ω	ю	2	6	2	2	2	2	ß	e S
Number of locules fruit ⁻¹	2	4	2	3	2	2	4	2	2	2	Э	3	3	2	4	3	2	3	3	3	2	2	2	3	3	ę	2	2	4	4
Weight of fruit (g)	2.72	4.01	2.32	2.12	2.06	3.20	3.30	3.91	3.04	2.62	2.71	3.29	2.02	2.42	3.69	2.43	3.02	3.40	3.32	3.41	2.02	4.43	4.71	4.14	4.23	3.02	2.71	2.41	4.02	2.36
Weight of fruits plant ⁻¹ (g)	68.67	52.13	18.65	17.92	21.70	41.40	29.90	46.07	45.56	31.44	28.19	111.85	24.24	35.98	216.28	34.07	39.24	33.40	29.88	37.10	21.30	56.61	59.25	49.68	57.97	36.27	32.18	28.29	41.43	24.62
Number of fruits plant ⁻¹	28	13	8	8	10	12	6	15	14	12	10	37	12	14	53	14	12	6	6	10	10	12	12	12	13	11	11	12	10	10
Primary branches plant ⁻¹	9	2	4	9	7	2	5	8	3	5	4	10	4	4	7	9	4	4	9	2	4	9	4	3	7	2	9	5	4	2
Plant height (cm)	146	161	146	156	121	142	147	128	162	132	162	168	137	156	152	151	127	143	137	127	163	135	154	137	124	119	107	121	103	151
Segregant/Trait	P-4-1	P-4-2	P-4-3	P-4-4	P-4-5	P-4-6	P-4-7	P-4-8	P-4-9	P-4-10	P-4-11	P-4-12	P-4-13	P-4-14	P-4-15	P-4-16	P-4-17	P-4-18	P-4-19	P-4-20	P-4-21	P-4-22	P-4-23	P-4-24	P-4-25	P-4-26	P-4-27	P-4-28	P-4-29	P-4-30

Table 2e. Details of the characters of the F₃ segregants derived from 5th F₂ family

																														ſ
Number of fruits cluster ¹	3	3	2	2	2	2	3	9	4	4	3	2	2	3	4	4	4	3	3	3	2	2	9	3	2	3	5	2	2	
Number of locules fruit ⁻¹	4	2	2	2	2	2	2	5	3	2	3	3	2	2	2	3	3	2	2	2	2	2	3	2	3	2	2	2	2	
Weight of fruit (g)	2.85	3.92	3.13	4.62	6.35	7.42	4.63	11.74	6.53	6.01	6.21	6.13	4.26	4.13	5.36	9.12	6.14	5.36	4.12	4.31	4.54	2.61	6.23	2.13	2.65	3.81	3.80	3.42	3.63	
Weight of fruits plant ⁻¹ (g)	53.30	53.96	68.86	106.28	102.60	133.56	97.23	285.95	84.89	74.07	77.52	85.82	110.76	48.43	54.68	212.67	110.25	64.23	44.21	56.30	72.64	57.18	252.14	26.62	25.65	83.10	207.28	67.89	75.60	
Number of fruits plant ⁻¹	18	13	22	23	16	18	21	28	13	10	12	14	26	11	10	23	18	12	10	13	16	21	42	12	10	21	49	19	20	
Primary branches plant ⁻¹	9	4	4	4	3	9	5	14	S	7	5	4	4	4	S	5	4	4	6	4	4	5	5	4	4	9	5	5	9	
Plant height (cm)	141	137	135	151	142	123	164	122	171	72	89	148	66	107	101	162	102	121	171	110	131	146	100	142	117	134	122	161	118	
Segregant/Trait	P-5-1	P-5-2	P-5-3	P-5-4	P-5-5	P-5-6	P-5-7	P-5-8	P-5-9	P-5-10	P-5-11	P-5-12	P-5-13	P-5-14	P-5-15	P-5-16	P-5-17	P-5-18	P-5-19	P-5-20	P-5-21	P-5-22	P-5-23	P-5-24	P-5-25	P-5-26	P-5-27	P-5-28	P-5-29	



4.1.1 Plant Height (cm)

Plant height of F₃ segregants ranged from 72 cm to 261 cm. The maximum height was recorded in plant P-3-1 (261 cm) and minimum plant height was recorded in plant P-5-10 (72 cm) in F₃ population.

4.1.2 Number of Primary Branches Plant⁻¹

Number of primary branches plant⁻¹ in F₃ segregants ranged from 2 to 14. The highest number of primary branches plant⁻¹ was recorded in plant P-5-8 (14) in F₃ population and the lowest number of primary branches plant⁻¹ was recorded in plants P-2-6, P-2-14, P-2-18, P-2-20, P-2-30, P-4-2, P-4-6, P-4-20, P-4-26 and P-4-30 (2)

4.1.3 Number of Days to 50% Flowering

Number of days to 50% flowering was recorded considering the entire plants as single population and observed at 29 days.

4.1.4 Number of Days to First Fruit Harvest

. Number of days to first fruit harvest did not show variation among the segregants. Number of days to first fruit harvest was recorded at 70th day whereas the plants P-1-3 and P-5-27 was recorded at 35th day.

4.1.5 Number of Fruits Plant⁻¹

Number of fruits plant⁻¹ ranged from 8 to 105 in F_3 segregants. The highest number of fruits plant⁻¹ was recorded in plant P-3-17 (105) and the lowest number of fruits plant⁻¹ was recorded in plant P-4-3 and P-4-4 as 8.

4.1.6 Weight of Fruits Plant⁻¹ (g)

Weight of fruits plant⁻¹ ranged from 17.92 g to 413.71 g in F_3 segregants. The highest weight of fruits plant⁻¹ was recorded in plant P-1-7 (413.71 g) and the lowest weight of fruits plant⁻¹ was recorded in plant P-4-4 (17.92 g).

4.1.7 Weight of Fruit (g)

Weight of fruit ranged from 2.02 g to 11.74 g in F₃ population. The highest weight of the fruit was recorded in plant P-5-8 (11.74g) and the lowest weight of the fruit was recorded in plants P-3-4, P-3-11, P-4-13 and P-4-21 with 2.02 g.

4.1.8 Number of Locules Fruit⁻¹

Number of locules fruit⁻¹ ranged from 2 to 6 in the F_3 segregants. Where most of plants were recorded with lowest number of locules fruit⁻¹ as 2 and the highest number of locules fruit⁻¹ was recorded in plant P-3-24 (6).

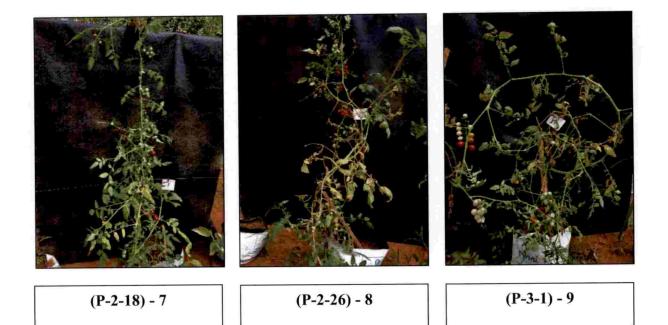
4.1.9 Number of Fruits Cluster⁻¹

Number of fruits cluster⁻¹ ranged from 2 to 10 in F_3 segregants. The highest number of fruits cluster⁻¹ was recorded in plant P-3-1 (10) and the lowest number of fruits cluster⁻¹ was recorded in plant P-1-4 (2).





Plate 2: Selected superior F3 segregants



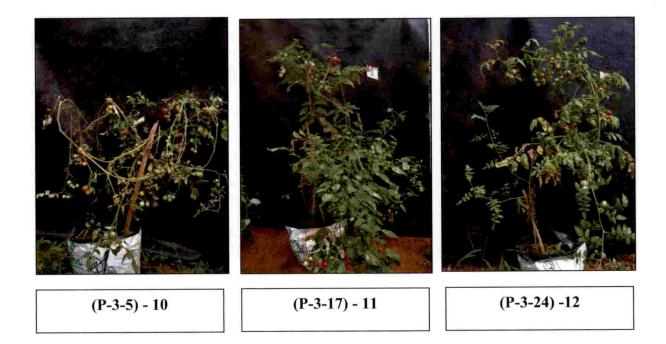


Plate 3: Selected superior F₃ segregants

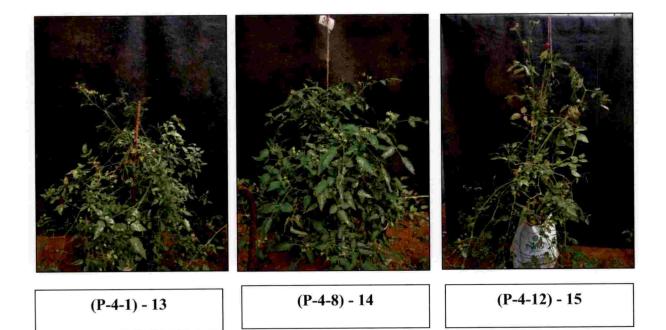
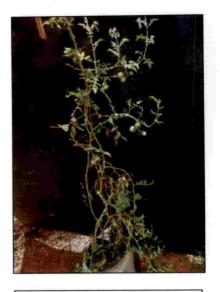




Plate 4: Selected superior F3 segregants



(P-5-23) - 19



(P-5-27) - 20



Anagha



LA2805 (Solanum lycopersicum var. cersiformae

4.2 QUALITATIVE AND QUANTITATIVE TRAITS IN 20 SUPERIOR SEGREGANTS

Based on cherry tomato characters twenty superior segregants were selected from 150 F₃ segregants with cherry tomato characters and observations with respect to quantitative (plant height, weight of fruit, number of fruits cluster⁻¹) and qualitative (lycopene, vitamin C, pH, TSS) traits were recorded. Details of the traits in the selected segregants and parents is given in Table 3.

4.2.1 Plant Height (cm)

Among the selected F_3 segregants, Plant height ranged from 72 cm to 261 cm. The maximum height was recorded in plant P-3-1 (261 cm) and minimum plant height was recorded in plant P-5-10 (72 cm). Plant height of parents, Anagha and LA2805 was recorded 146.3 cm and 234.6 cm respectively. The plant height of selected F_3 segregants and parents were compared in Fig. 1.

4.2.2 Weight of Fruit (g)

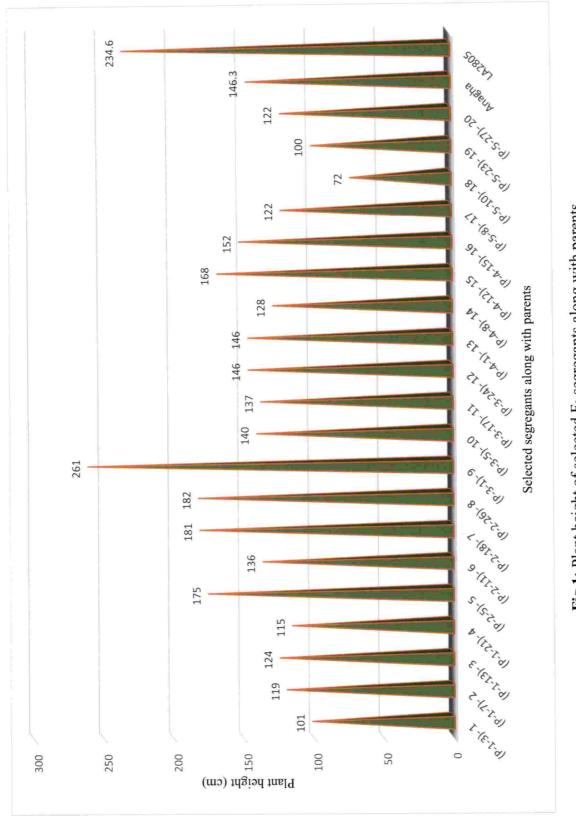
Weight of fruit ranged from 2.05 g to 11.74 g in selected F_3 segregants. The highest weight of fruit was recorded in plant P-5-8 (11.74 g) and the lowest weight of fruit was recorded in plant P-3-17 (2.05g). Fruit weight of parents, Anagha and LA2805 was recorded 16.51 g and 2.97 g respectively. The weight of fruit of selected F_3 segregants and parents were compared in Fig. 2.

4.2.3 Number of Fruits Cluster⁻¹

Number of fruits cluster⁻¹ ranged from 3 to 10 in selected F₃ segregants. The highest number of fruits cluster⁻¹ was recorded in plant P-3-1 (10) and the lowest number of fruits cluster⁻¹ was recorded in plant P-4-8 (3). Number of fruits cluster⁻¹ of parents, Anagha and LA2805 was recorded 4 and 12 respectively. The number of fruits cluster⁻¹ of selected F₃ segregants and parents were compared in Fig. 3.

Table 3: Performance of selected	superior	segregants	along	with	parents	for
quantitative and qualitative traits						

	-					Mit C	"II of	TSS
S. No	Selected segregants	Plant height (cm)	Weight of fruit (g)	Number of fruits cluster ⁻¹	Lycopene (mg 100g ⁻¹)	Vitamin C (mg 100g ⁻¹)	pH of juice	(%)
1	(P-1-3)- 1	101	9.14	4	18.41	11.19	4.2	5.16
2	(P-1-7)- 2	119	8.58	5	17.47	10.83	3.9	3.93
3	(P-1-13)- 3	124	5.94	5	6.24	13.31	4.0	5.83
4	(P-1-21)- 4	115	6.51	6	16.85	22.16	3.8	5.10
5	(P-2-5)- 5	175	3.53	5	6.24	14.51	4.1	5.30
6	(P-2-11)- 6	136	4.8	5	18.72	26.84	3.5	3.30
7	(P-2-18)- 7	181	7.22	7	9.36	15.17	4.1	5.93
8	(P-2-26)- 8	182	3.93	6	19.34	20.40	4.0	5.76
9	(P-3-1)- 9	261	4.57	10	9.36	14.54	4.1	5.83
10	(P-3-5)- 10	140	5.88	7	17.47	23.10	4.0	3.50
11	(P-3-17)- 11	137	2.05	8	10.61	29.35	4.1	6.80
12	(P-3-24)- 12	146	4.85	7	6.24	19.44	4.0	5.67
13	(P-4-1)- 13	146	2.72	5	4.99	25.90	4.0	5.56
14	(P-4-8)- 14	128	3.91	3	17.47	19.67	4.4	6.50
15	(P-4-12)- 15	168	3.29	4	6.24	17.17	4.4	4.33
16	(P-4-15)- 16	152	3.69	7	9.98	25.38	4.4	5.76
17	(P-5-8)- 17	122	11.74	6	4.99	12.31	4.1	6.67
18	(P-5-10)- 18	72	6.01	4	4.36	15.86	4.4	5.10
19	(P-5-23)- 19	100	6.23	6	3.12	12.61	4.1	6.23
20	(P-5-27)- 20	122	3.8	5	3.12	20.67	4.5	6.20
21	Anagha	146.3	16.51	4	10.45	15.38	4.4	5.33
22	LA2805	234.6	2.97	12	15.60	25.90	4.1	6.43





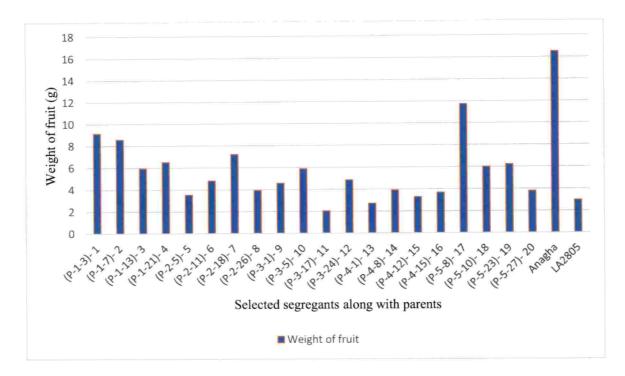


Fig 2: Weight of fruit of selected F3 segregants along with parents

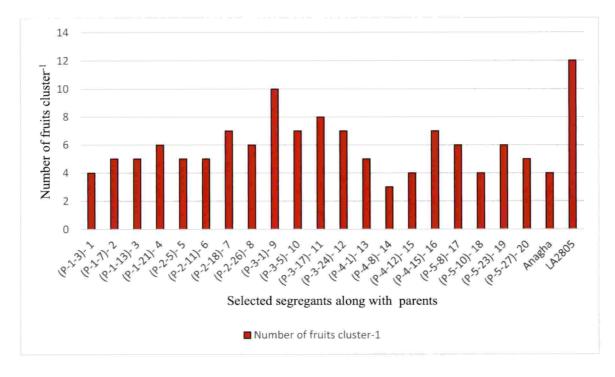


Fig 3: Number of fruits cluster⁻¹ of selected F₃ segregants along with parents





Plate 6: Fruits of selected superior F3 segregants



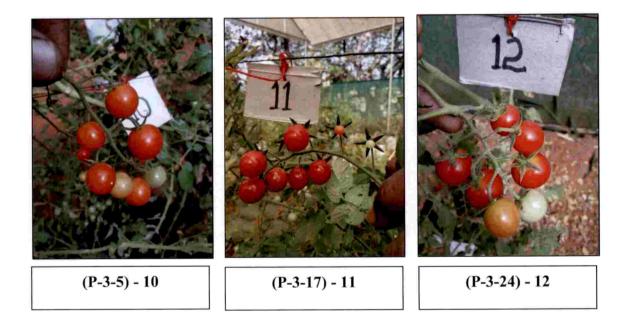


Plate 7: Fruits of selected superior F3 segregants

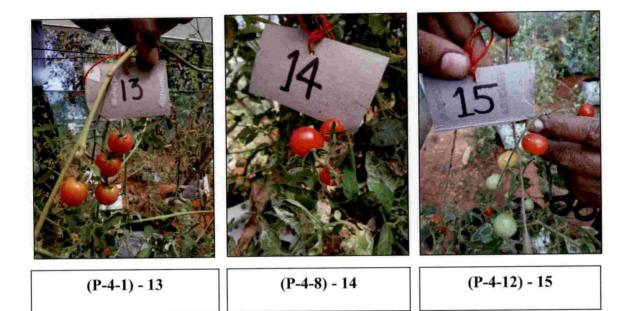




Plate 8: Fruits of selected superior F3 segregants



(P-5-23) - 19



(P-5-27) - 20

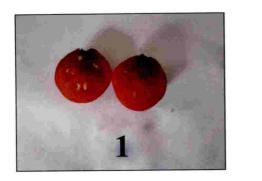


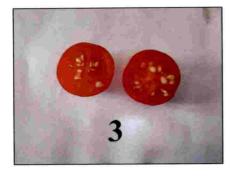
Anagha

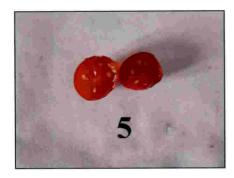


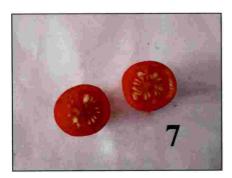
LA2805 (Solanum lycopersicum var. cersiformae

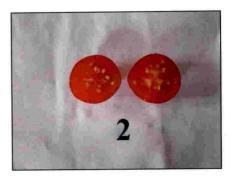
Plate 9: Fruits of selected superior F3 segregants and parents

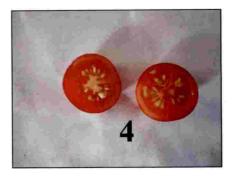














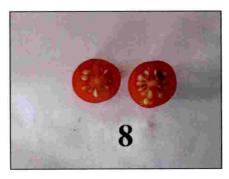


Plate 10: Fruit locules of selected superior F3 segregants

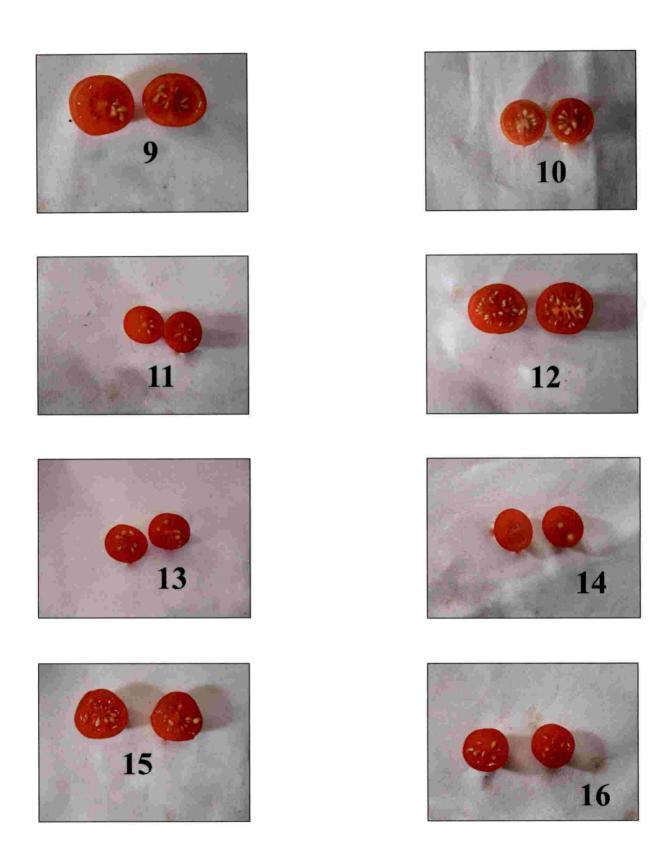


Plate 11: Fruit locules of selected superior F3 segregants



Plate 12: Fruit locules of selected superior F3 segregants and parents

4.2.4 Lycopene (mg 100g⁻¹)

Among the selected F_3 segregants, lycopene content ranged from 3.12 mg 100 g⁻¹ to 19.34 mg 100 g⁻¹. The highest content of lycopene was recorded in plant P-2-26 (19.34 mg 100 g⁻¹) and the lowest content of lycopene was recorded in plant P-5-23 and P-5-27 (3.12 mg 100 g⁻¹). Whereas the parents, Anagha and LA2805 were recorded with 10.45 mg 100 g⁻¹ and 15.60 mg 100 g⁻¹ respectively. The lycopene content of selected F_3 segregants and parents were compared in Fig. 4.

4.2.5 Vitamin C (mg 100g⁻¹)

Among the selected F_3 segregants vitamin C content ranged from 10.83 mg $100g^{-1}$ to 29.35 mg $100g^{-1}$ in selected F_3 segregant. The highest content of vitamin C was recorded in plant P-3-17 (29.35 mg $100g^{-1}$) and the lowest content of vitamin C was recorded in plant P-1-7 (10.83 mg $100g^{-1}$). Whereas in parents, Anagha and LA2805 was recorded with 15.38 mg $100g^{-1}$ and 25.90 mg $100g^{-1}$ respectively. The vitamin C content of selected F_3 segregants and parents were compared in Fig. 5.

4.2.6 pH of Juice

Among the selected F_3 segregants pH of juice ranged from 3.5 to 4.5. The highest pH of juice was observed in plant P-5-27 (4.5) and the lowest pH was observed in P-2-11 (3.5). pH of the juice in parents Anagha and LA2805 was 4.4 and 4.1 respectively. The pH of selected F_3 segregants and parents were compared in Fig. 6.

4.2.7 Total Soluble Solids (%)

Among the selected F_3 segregants total soluble solids (%) ranged from 3.3% to 6.8%. The highest total soluble solids was recorded in plant P-3-17 (6.8%) and the lowest total soluble solids was recorded in plant P-2-11 (3.3%). Whereas the parents, Anagha and LA2805 was recorded with 5.33% and 6.43% respectively. The TSS (%) of selected F_3 segregants and parents were compared in Fig. 7.

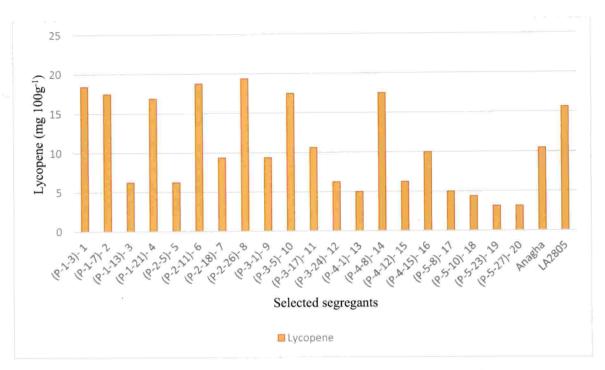


Fig 4: Lycopene content of selected F3 segregants along with parents

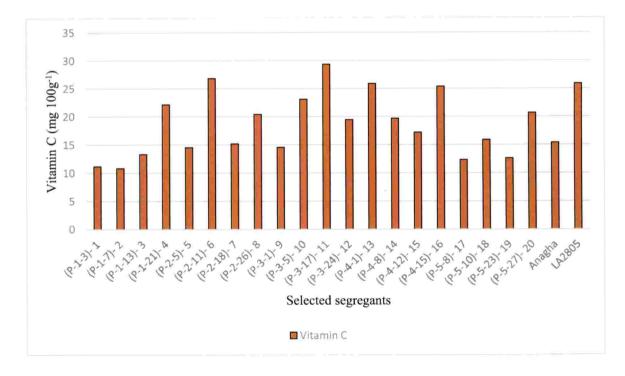


Fig 5: Vitamin C content of selected F3 segregants along with parents

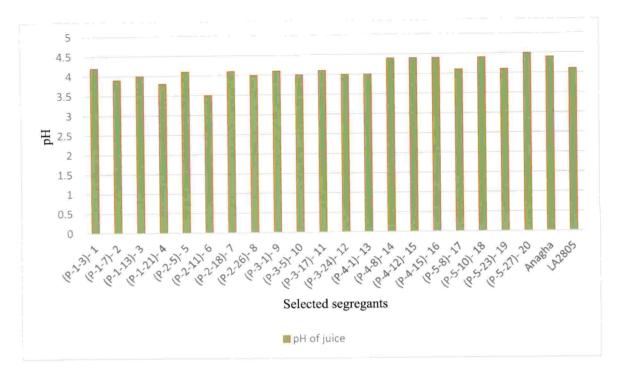


Fig 6: pH of selected F3 segregants along with parents

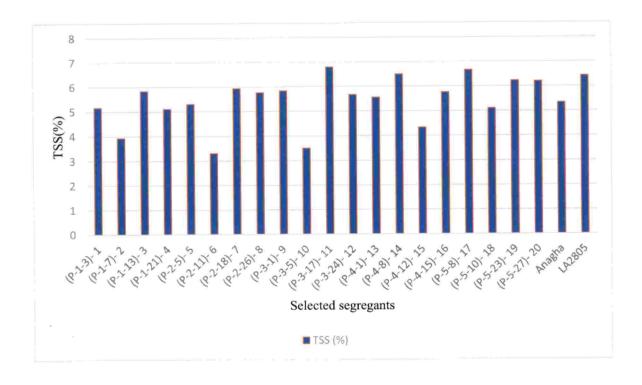


Fig 7: TSS (%) of selected F₃ segregants along with parents

4.3 PROXIMITY DISSIMILARITY ANALYSIS OF MORPHOMETRIC AND FRUIT QUALITY DATA

Cluster analysis was performed using Statistical Package for the Social Sciences (SPSS version 16.0) revealed that the selected F_3 segregants along with parents grouped into two clusters. The female parent Anagha formed a separate cluster and all the selected F_3 segregants having cherry tomato characters were grouped along with LA2805 parent. The major cluster comprising of 21 genotypes including LA2805 parent were subdivided into two sub clusters with (P-5-8)- 17 as individual cluster and all the remaining selected segregants along with LA2805 parent forming another cluster (Fig. 8).

Proximity dissimilarity matrix was constructed between selected F_3 segregants and parents using Euclidean coefficient of dissimilarity method for nine morphometric and four fruit quality traits were compared in Table 4. The Euclidean distance of selected F_3 segregants in comparison with cherry tomato parent LA2805 was compared in Table 5. The selected segregants 9- (P-3-1), 8- (P-2-26) and 7- (P-2-18) showed lesser Euclidean coefficient of dissimilarity with 3.75, 4.22 and 4.59 respectively.

4.4 GENOTYPING OF THE 20 SUPERIOR SEGREGANTS WITH SSR MARKERS

4.4.1 DNA Isolation

In the present study genomic DNA of tomato was isolated from the 20 genotypes along with parents by CTAB method (Murry and Thompson, 1980) with little modification as reported by Ginwal and Mittal (2010) for removing the phenolics and RNA. DNA was quantified using spectrophotometer from the ratio of absorbance values at 260nm and 280nm. A ratio between 1.8 to 2.0 indicated the best quality of DNA. The calculated quality of DNA (Table 6) was found to be good as per the gel electrophoresis results and the OD values more than 2.0 was purified using silica gel column before PCR analysis. The genomic DNA of tomato genotypes was represented in Plate 13.

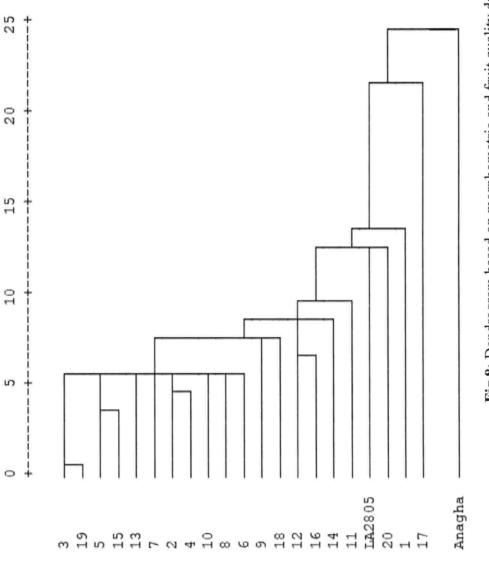


Fig 8: Dendrogram based on morphometric and fruit quality data using SPSS 16.0

										Euc	idean I	Euclidean Distance										
Case	Anagha	LA2805	1	2	3	4	5	9	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Anagha	0.00																					
LA2805	9.63	0.00																				
F	5.74	7.30	00.00																			
7	5.97	7.01	4.12	0.00																		
3	5.97	6.01	4.04	4.25	0.00																	
4	6.46	5.54	4.57	2.68	3.92	0.00																
ŝ	6.88	5.25	4.95	4.47	2.76	3.90	0.00															
9	8.01	6.53	6.10	4.03	5.64	2.76	5.09	00.00														
7	6.26	4.59	4.63	4.58	2.85	3.84	2.73	5.29	0.00													
×	7.06	4.22	4.84	4.18	3.88	2.75	2.81	3.95	2.79	0.00												
6	7.65	3.75	6.42	5.55	4.96	4.90	3.61	6.01	3.01	3.43	0.00											
10	7.45	5.87	5.75	3.45	5.59	2.68	4.40	2.91	4.87	3.49	4.90	0.00							Ĵ		×	
11	8.17	4.60	6.60	5.86	5.29	3.79	4.21	5.21	4.53	3.36	4.46	4.10	0.00						9			
12	6.37	5.69	5.13	4.06	3.24	3.52	3.81	5.40	4.19	4.13	4.65	4.69	4.25	0.00								
13	7.35	5.23	5.60	5.78	3.06	4.27	2.72	5.10	3.43	3.54	5.02	5.34	4.31	4.44	00.0							
14	7.11	6.19	4.93	6.04	3.81	5.13	3.78	6.58	4.39	3.92	6.15	6.28	5.51	5.65	3.46	0.00						
15	6.91	6.28	5.31	5.26	3.57	5.07	2.51	6.12	4.47	4.33	5.32	5.30	5.56	4.50	3.33	3.54	0.00					
16	6.47	4.62	5.10	4.95	3.50	3.74	3.19	5.54	3.70	3.26	4.37	4.44	3.34	2.87	3.21	3.83	3.23	0.00				
17	6.80	8.10	5.27	6.35	5.38	6.38	5.93	8.33	6.72	6.87	7.41	7.51	7.28	5.49	6.63	6.39	5.74	5.80	0.00			
18	6.63	7.08	4.84	5.62	3.05	5.25	3.59	6.64	4.30	5.10	6.47	6.21	6.08	5.36	3.35	3.21	3.14	4.17	6.04	00.00		
19	6.04	6.01	4.40	4.15	1.91	3.68	2.81	5.77	2.88	4.03	4.72	5.17	4.58	3.34	3.59	4.40	4.08	3.51	5.29	3.21	00.00	
20	6.98	6.32	4.32	6.18	4.39	5.32	4.01	6.82	4.35	4.86	5.61	5.97	4.85	5.13	4.13	4.59	4.47	3.92	5.64	4.12	3.88	0.00

Table 4: Proximity dissimilarity matrix between parents and selected F₃ segregants

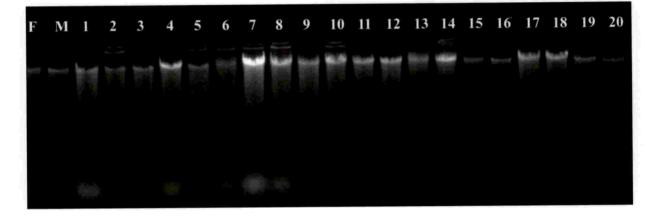
Table 5: Proximity dissimilarity matrix of selected superior F_3 segregants in comparison with LA2805 (*Solanum lycopersicum* var. *cerasiforme*) by Euclidean distance for morphometric and fruit quality traits

Proxir	nity dissimilarity ma	trix (Compared with
	LA2805	i)
S. No	Selected F ₃	Overall distance
	Segregants	
1	(P-1-3)- 1	7.30
. 2	(P-1-7)- 2	7.01
3	(P-1-13)- 3	6.01
4	(P-1-21)- 4	5.54
5	(P-2-5)- 5	5.25
6	(P-2-11)- 6	6.53
7	(P-2-18)- 7	4.59
8	(P-2-26)- 8	4.22
9	(P-3-1)- 9	3.75
10	(P-3-5)- 10	5.87
11	(P-3-17)- 11	4.60
12	(P-3-24)- 12	5.69
13	(P-4-1)- 13	5.23
14	(P-4-8)- 14	6.19
15	(P-4-12)- 15	6.28
16	(P-4-15)- 16	4.62
17	(P-5-8)- 17	8.10
18	(P-5-10)- 18	7.08
19	(P-5-23)- 19	6.01
20	(P-5-27)- 20	6.32

Selected segregants	Absorbance at 260 nm	Absorbance at 280 nm	O.D Ratio A260/A280	DNA yield (ng/µl)
(P-1-3)- 1	0.013	0.006	2.17*	650
(P-1-7)- 2	0.019	0.011	1.73	950
(P-1-13)- 3	0.018	0.008	2.25*	900
(P-1-21)- 4	0.032	0.018	1.78	1600
(P-2-5)- 5	0.012	0.007	1.71	600
(P-2-11)- 6	0.018	0.010	1.80	900
(P-2-18)- 7	0.069	0.033	2.09	3450
(P-2-26)- 8	0.036	0.019	1.90	1800
(P-3-1)- 9	0.038	0.020	1.90	1900
(P-3-5)- 10	0.039	0.017	2.29*	1950
(P-3-17)- 11	0.029	0.012	2.42*	1450
(P-3-24)- 12	0.030	0.014	2.14*	1500
(P-4-1)- 13	0.033	0.016	2.06	1650
(P-4-8)- 14	0.031	0.017	1.82	1550
(P-4-12)- 15	0.031	0.016	1.93	1550
(P-4-15)- 16	0.019	0.010	1.90	950
(P-5-8)- 17	0.033	0.016	2.06	1650
(P-5-10)- 18	0.032	0.017	1.89	1600
(P-5-23)- 19	0.024	0.014	1.71	1200
(P-5-27)- 20	0.017	0.009	1.88	850
Anagha	0.031	0.016	1.93	1550
LA2805	0.019	0.010	1.90	950
	$\begin{array}{c} (P-1-3)-1 \\ (P-1-7)-2 \\ (P-1-7)-2 \\ (P-1-13)-3 \\ (P-1-21)-4 \\ (P-2-5)-5 \\ (P-2-5)-5 \\ (P-2-11)-6 \\ (P-2-18)-7 \\ (P-2-26)-8 \\ (P-3-1)-9 \\ (P-3-1)-9 \\ (P-3-5)-10 \\ (P-3-5)-10 \\ (P-3-24)-12 \\ (P-3-24)-12 \\ (P-4-1)-13 \\ (P-4-8)-14 \\ (P-4-12)-15 \\ (P-4-8)-14 \\ (P-4-12)-15 \\ (P-5-8)-17 \\ (P-5-8)-17 \\ (P-5-10)-18 \\ (P-5-23)-19 \\ (P-5-27)-20 \\ Anagha \end{array}$	at 260 nm(P-1-3)- 10.013(P-1-7)- 20.019(P-1-13)- 30.018(P-1-21)- 40.032(P-2-5)- 50.012(P-2-11)- 60.018(P-2-18)- 70.069(P-2-26)- 80.036(P-3-1)- 90.038(P-3-5)- 100.039(P-3-24)- 120.030(P-4-1)- 130.033(P-4-8)- 140.031(P-4-15)- 160.019(P-5-8)- 170.033(P-5-10)- 180.032(P-5-23)- 190.024(P-5-27)- 200.017Anagha0.031	at 260 nmat 280 nm(P-1-3)-10.0130.006(P-1-7)-20.0190.011(P-1-13)-30.0180.008(P-1-21)-40.0320.018(P-2-5)-50.0120.007(P-2-11)-60.0180.010(P-2-18)-70.0690.033(P-2-26)-80.0360.019(P-3-1)-90.0380.020(P-3-5)-100.0390.017(P-3-17)-110.0290.012(P-3-24)-120.0300.014(P-4-1)-130.0330.016(P-4-1)-150.0310.017(P-4-15)-160.0190.010(P-5-8)-170.0330.016(P-5-10)-180.0320.017(P-5-23)-190.0240.014(P-5-27)-200.0170.009Anagha0.0310.016	at 260 nmat 280 nmA260/A280(P-1-3)-10.0130.0062.17*(P-1-7)-20.0190.0111.73(P-1-13)-30.0180.0082.25*(P-1-21)-40.0320.0181.78(P-2-5)-50.0120.0071.71(P-2-11)-60.0180.0101.80(P-2-18)-70.0690.0332.09(P-2-6)-80.0360.0191.90(P-3-1)-90.0380.0201.90(P-3-5)-100.0390.0172.29*(P-3-17)-110.0290.0122.42*(P-3-24)-120.0300.0142.14*(P-4-1)-130.0330.0161.93(P-4-1)-150.0310.0161.93(P-4-15)-160.0190.0101.90(P-5-8)-170.0330.0162.06(P-5-10)-180.0320.0171.89(P-5-27)-200.0170.0091.88Anagha0.0310.0161.93

Table 6: Quality and quantity of genomic DNA in superior segregants and parents

*- Samples with more than 2.0 OD value was purified using silica gel column before PCR analysis.



F- Anagha, M- LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 13: Genomic DNA of tomato genotypes

4.4.2 SSR Marker Analysis

The 20 selected F_3 segregants were screened with 10 SSR markers specific to cherry tomato reported by Kwon *et al.* (2009); El-Awady *et al.* (2012) and Aguirre *et al.* (2017). The presence of these markers is confirmed in Table 7.

4.4.2.1 SSR Marker SSR9

The DNA amplification profile of the marker SSR9 is given in plate 14. Out of the 20 segregants P-1-21, P-2-11, P-3-17, P-4-8, P-4-12, P-4-15 showed the presence of the marker linked to the cherry tomato traits at 168 bp.

4.4.2.2 SSR Marker SSR19

The DNA amplification profile of the marker SSR19 is given in plate 15. Out of the 20 segregants P-1-21, P-2-11, P-3-5, P-3-17, P-4-8, P-4-12, P-4-15 showed the presence of the marker linked to the cherry tomato traits at 188 bp.

4.3.2.3 SSR Marker SSR26

The DNA amplification profile of the marker SSR26 is given in plate 16. Out of the 20 segregants P-1-21, P-2-11, P-2-26, P-3-17, P-4-8, P-4-12, P-4-15 showed the presence of the marker linked to the cherry tomato traits at 178 bp.

4.3.2.4 SSR Marker SSR28

The DNA amplification profile of the marker SSR28 is given in plate 17. Out of the 20 segregants P-1-21, P-2-11, P-2-26, P-3-5, P-3-17, P-4-8, P-4-12, P-4-15 showed the presence of the marker linked to the cherry tomato traits at 164 bp.

4.3.2.5 SSR Marker SSR47

The DNA amplification profile of the marker SSR47 is given in plate 18. Out of the 20 segregants P-1-21, P-2-11, P-2-26, P-3-17, showed the presence of the marker linked to the cherry tomato traits at 191 bp.

4.3.2.6 SSR Marker SSR63

The DNA amplification profile of the marker SSR63 is given in plate 19. Out of the 20 segregants P-1-21, P-2-11, P-2-26, P-3-17, P-4-8, P-4-15 showed the presence of the marker linked to the cherry tomato traits at 250 bp.

4.3.2.7 SSR Marker SSR86

The DNA amplification profile of the marker SSR86 is given in plate 20. Out of the 20 segregants P-1-21, P-2-11, P-2-26, P-3-5, P-3-17, P-4-8, P-4-12, P-4-15 showed the presence of the marker linked to the cherry tomato traits at 210 bp.

4.3.2.8 SSR Marker SSR94

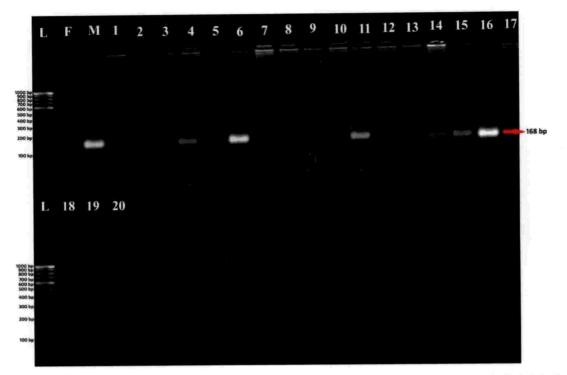
The DNA amplification profile of the marker SSR94 is given in plate 21. Out of the 20 segregants P-1-21, P-2-11, P-2-26, P-3-5, P-4-8, P-4-15 showed the presence of the marker linked to the cherry tomato traits at 187 bp.

4.3.2.9 SSR Marker SSR253

The DNA amplification profile of the marker SSR253 is given in plate 22. Out of the 20 segregants P-1-21, P-2-11, P-2-26, P-3-17 showed the presence of the marker linked to the cherry tomato traits at 250 bp.

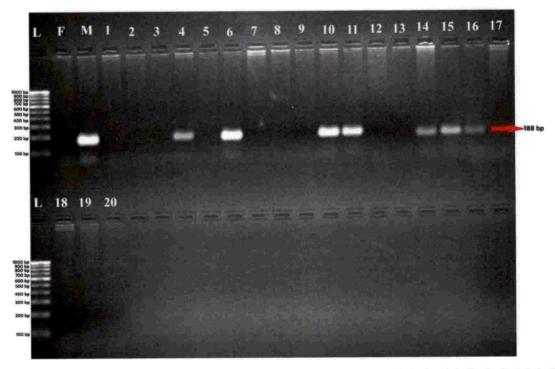
4.3.2.10 SSR Marker SSR268

The DNA amplification profile of the marker SSR268 is given in plate 23. Out of the 20 segregants P-1-7, P-1-21, P-2-11, P-2-26, P-3-1, P-4-8 showed the presence of the marker linked to the cherry tomato traits at 218 bp.



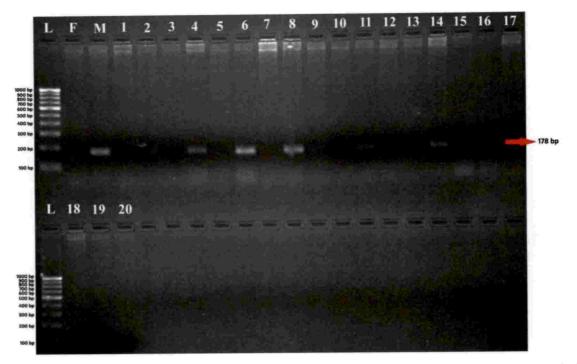
L-Ladder, F-Anagha, M-LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 14: Gel profile of SSR9



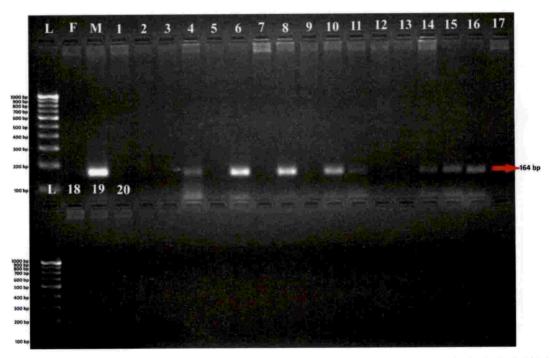
L- Ladder, F- Anagha, M- LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 15: Gel profile of SSR19



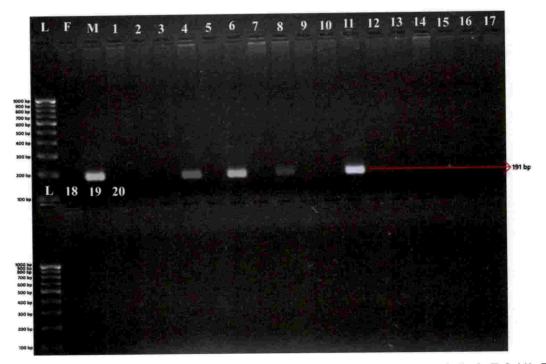
L-Ladder, F-Anagha, M-LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 16: Gel profile of SSR26



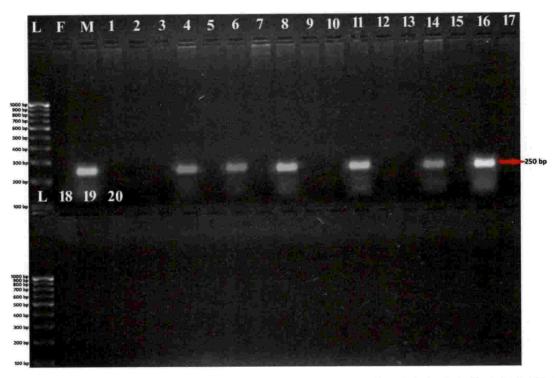
L- Ladder, F- Anagha, M- LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 17: Gel profile of SSR28



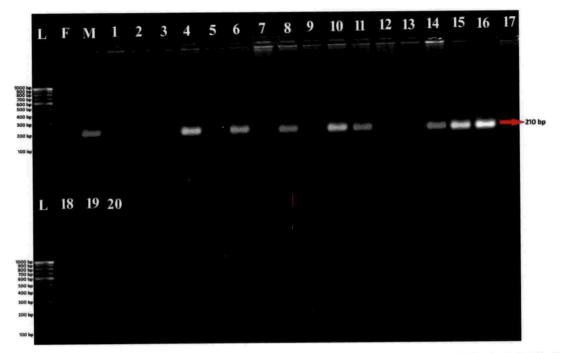
L- Ladder, F- Anagha, M- LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 18: Gel profile of SSR47



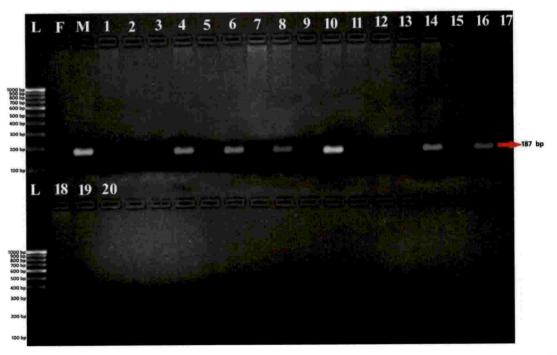
L- Ladder, F- Anagha, M- LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 19: Gel profile of SSR63

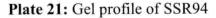


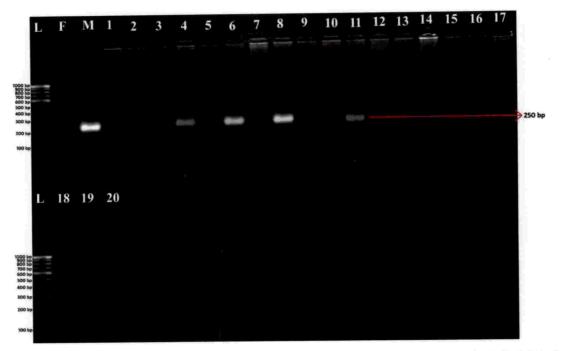
L-Ladder, F-Anagha, M-LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 20: Gel profile of SSR86



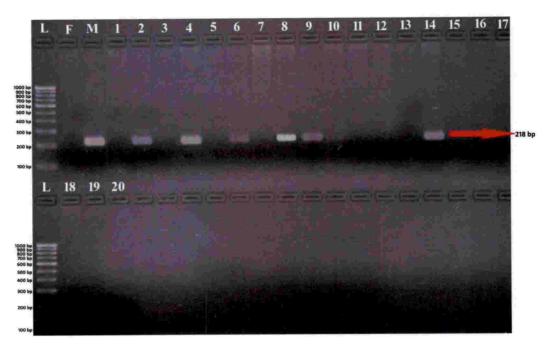
L- Ladder, F- Anagha, M- LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).





L-Ladder, F-Anagha, M-LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 22: Gel profile of SSR253



L- Ladder, F- Anagha, M- LA2805, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27).

Plate 23: Gel profile of SSR268

Table 7: Representation of the presence of cherry tomato specific markers in the parents and selected F_3 segregants

Genotype/ Marker	a	b	c	d	e	f	g	h	i	j
F										
М	1	Ι	1	1	Ι	1	T	1	1	T
1			-							
2				Ŀ						-1
3										-
4	1	Ι	1	Ι		1	T	1	1.	Ι
5										
6	1	1	1	I	I	1	- 1	-1-	1	T
7									$^{\circ}Z$	
8			1		Ι	Ι	T	1	T	T.
9										1
10		1		T			1	1		
11		1	I	T		-l	Ι		Ι	
12										
13										
14	1	T	I	1		1	T	I		Ĩ
15	1	I	I	Ι			T			
16	1	1	Ι	Ι		I.	Ι	1		
17										
18										
19										
20										

F- Anagha, M- LA2805, a- SSR9, b- SSR19, c- SSR26, d- SSR28, e- SSR47, f- SSR63, g- SSR86, h- SSR94, i- SSR253, j- SSR268, 1- (P-1-3), 2- (P-1-7), 3- (P-1-13), 4- (P-1-21), 5- (P-2-5), 6- (P-2-11), 7- (P-2-18), 8- (P-2-26), 9- (P-3-1), 10- (P-3-5), 11- (P-3-17), 12- (P-3-24), 13- (P-4-1), 14- (P-4-8), 15- (P-4-12), 16- (P-4-15), 17- (P-5-8), 18- (P-5-10), 19- (P-5-23), 20- (P-5-27), Presence of marker

Discussion

5. DISCUSSION

Tomato which is considered as both fruit and vegetable, ranks second in its importance next to potato in the world. It has wider adaptability to various environmental conditions with high yield potential. The fruit has multiple uses both in fresh form as well as in the processed form. Cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) similar to cultivated tomato but not identical to the wild ancestor, having small fruits resembling a cherry with a dark red colour, having excellent nutritional traits (Charlo *et al.*, 2007). According to Nuez (1999) cherry tomatoes are generally of determinate, semi-determinate or indeterminate growth habit with long racemes bearing many small sized fruits weighing between (10 - 30 g), showing intense colour and flavour generally round in shape and varying number of fruits cluster⁻¹ with high vitamin C content.

The present study on "Identification of cherry tomato genotypes from F_3 segregants of intraspecific cross" was undertaken to evaluate F_3 segregants of the cross between cultivated tomato variety Anagha (*Solanum lycopersicum* L.) and *Solanum lycopersicum* var. *cerasiforme* for morphometric traits and fruit quality specific to cherry tomato and genotyping the superior segregants with SSR markers. The results of the experiment presented in chapter four are discussed below.

5.1 ASSESSMENT OF F3 SEGREGANTS BASED ON MORPHOMETRIC AND FRUIT QUALITY TRAITS

A total of 150 F₃ segregants originated from 5 F₂ families were assessed for nine morphometric traits (plant height, primary branches plant⁻¹, number of days to 50% flowering, number of days to first fruit harvest, number of fruits plant⁻¹, weight of fruits plant⁻¹, weight of fruit, number of locules fruit⁻¹ and number of fruits cluster⁻¹) and from that 20 segregants were selected for cherry tomato characters. The observations on cherry tomato specific traits such as plant height, weight of fruit and number of fruits cluster⁻¹ were compared with the respective F₂ family mean in Table 8a.

	Trait /	Plant	Weight of	Number	
	Family	height	fruit (g)	of fruits	
		(cm)		cluster ⁻¹	
un de la composición de la composición Composición de la composición de la comp	$\overline{\mathbf{x}}_1$	115.5	3.62	2.96	
aily	(P-1-3)- 1	101	9.14	4	
2 fan	(P-1-7)- 2	119	8.58	5	
1 st F ₂ family	(P-1-13)- 3	124	5.94	5	
<u> </u>	(P-1-21)- 4	115	6.51	6	
	<u><u>x</u>2</u>	163.3	3.39	3	
nily	(P-2-5)- 5	175	3.53	5	
2 nd F ₂ family	(P-2-11)- 6	136	4.80	5	
F	(P-2-18)- 7	181	7.22	7	
2	(P-2-26)- 8	182	3.93	6	
	<u> </u>	143.96	3.37	3.76	
nily	(P-3-1)- 9	261	4.57	10	
3 rd F ₂ family	(P-3-5)- 10	140	5.88	7	
H put	(P-3-17)- 11	137	2.05	8	
(7)	(P-3-24)- 12	146	4.85	7	
	<u>X</u> 4	140.5	3.1	2.83	
family	(P-4-1)- 13	146	2.72	5	
2 far	(P-4-8)- 14	128	3.91	3	
4 th F ₂	(P-4-12)- 15	168	3.29	4	
	(P-4-15)- 16	152	3.69	7	
	X 5	128.73	4.99	3.06	
nily	(P-5-8)- 17	122	11.74	6	
2 far	(P-5-10)- 18	72	6.01	4	
5 th F ₂ family	(P-5-23)- 19	100	6.23	6	
	(P-5-27)- 20	122	3.80	5	

Table 8a: Comparison of major morphometric traits between and within families

 $\overline{\mathbf{x}}_1$ - Average of 30 plants in 1st F₂ family, $\overline{\mathbf{x}}_2$ - Average of 30 plants in 2nd F₂ family, $\overline{\mathbf{x}}_3$ - Average of 30 plants in 3rd F₂ family, $\overline{\mathbf{x}}_4$ - Average of 30 plants in 4th F₂ family, $\overline{\mathbf{x}}_5$ - Average of 30 plants in 5th F₂ family

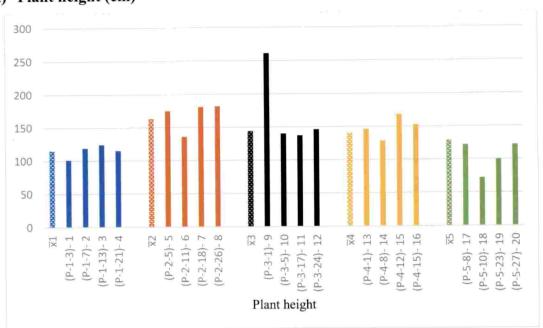
Among the morphometric traits, plant height did not show much variation between and within families. All the F_3 segregants showed a determinate to semideterminate growth habit with a maximum height of 261 cm and a minimum height of 72 cm. Similar results were reported by Than *et al.* (2001) (Fig. 9a).

Inter and intra family variation in fruit weight is shown in Fig. 9b. The highest family mean was recorded for 5th F₂ family (4.99 g) and the highest individual fruit weight was recorded in (P-5-8)- 17 with 11.74 g similar to Punjab Red Cherry (11.5 g) reported by Dhaliwal and Jindal (2017) and suggesting a higher weight of fruit as reported by Nuez (1999) and Malavika *et al.* (2018). There is scope of getting higher fruit weight with cherry tomato characters in the coming generations

Number of fruits cluster⁻¹ did not show much variation between the families but within the family there was much variation. Among the selected F_3 segregants (P-3-1)- 9 showed the highest number of fruits cluster⁻¹ (10) as reported by Ramya *et al.*, (2016). These segregants carried for further generations can improve number of fruits cluster⁻¹. Fig. 9c shows variation for number of fruits cluster⁻¹.

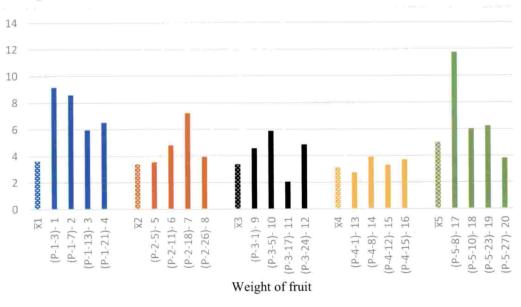
The 20 selected F_3 segregants showing cherry tomato morphometric traits were analysed for qualitative traits (Lycopene, vitamin C, pH and TSS). The observations on these 20 F_3 segregants is compared with F_2 family mean in Table 8b.

Fig 9: Comparison of family mean with the 4 superior segregants for major morphometric



a) Plant height (cm)

 $\overline{\mathbf{x}}_1$ - Average of 30 plants in 1st F₂ family, $\overline{\mathbf{x}}_2$ - Average of 30 plants in 2nd F₂ family, $\overline{\mathbf{x}}_3$ - Average of 30 plants in 3rd F₂ family, $\overline{\mathbf{x}}_4$ - Average of 30 plants in 4th F₂ family, $\overline{\mathbf{x}}_5$ - Average of 30 plants in 5th F₂ family

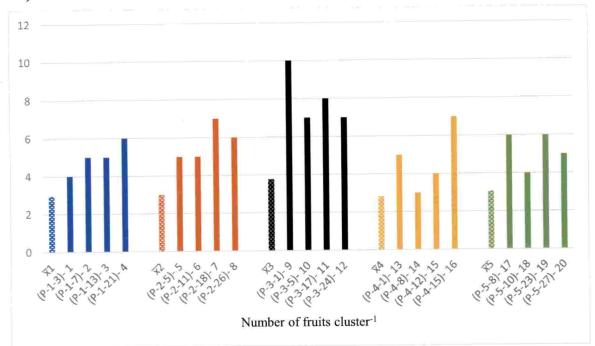


b) Weight of fruit (g)

 $\overline{\mathbf{x}}_1$ - Average of 30 plants in 1st F₂ family, $\overline{\mathbf{x}}_2$ - Average of 30 plants in 2nd F₂ family, $\overline{\mathbf{x}}_3$ - Average of 30 plants in 3rd F₂ family, $\overline{\mathbf{x}}_4$ - Average of 30 plants in 4th F₂ family, $\overline{\mathbf{x}}_5$ - Average of 30 plants in 5th F₂ family

traits

c) Number of fruits cluster⁻¹



 $\overline{\mathbf{x}}_1$ - Average of 30 plants in 1st F₂ family, $\overline{\mathbf{x}}_2$ - Average of 30 plants in 2nd F₂ family, $\overline{\mathbf{x}}_3$ - Average of 30 plants in 3rd F₂ family, $\overline{\mathbf{x}}_4$ - Average of 30 plants in 4th F₂ family, $\overline{\mathbf{x}}_5$ - Average of 30 plants in 5th F₂ family

Trait / Family		Lycopene*	Vitamin C*	pH*	TSS (%) *
	T unity	14.74	14.37	3.97	5.00
1 st F ₂ family	(P-1-3)- 1	18.41	11.19	4.2	5.16
	(P-1-7)- 2	17.47	10.83	3.9	3.93
	(P-1-13)- 3	6.24	13.31	4.0	5.83
	(P-1-21)- 4	16.85	22.16	3.8	5.10
2 nd F ₂ family	<u>x</u> 2	13.41	19.23	3.925	5.07
	(P-2-5)- 5	6.24	14.51	4.1	5.30
	(P-2-11)- 6	18.72	26.84	3.5	3.30
	(P-2-18)- 7	9.36	15.17	4.1	5.93
	(P-2-26)- 8	19.34	20.40	4.0	5.76
3 rd F ₂ family	X 3	10.92	21.6	4.05	5.45
	(P-3-1)- 9	9.36	14.54	4.1	5.83
	(P-3-5)- 10	17.47	23.10	4.0	3.50
	(P-3-17)- 11	10.61	29.35	4.1	6.80
	(P-3-24)- 12	6.24	19.44	4.0	•5.67
4 th F ₂ family	<u>X</u> 4	9.67	22.03	4.3	5.53
	(P-4-1)- 13	4.99	25.90	4.0	5.56
	(P-4-8)- 14	17.47	19.67	4.4	6.50
	(P-4-12)- 15	6.24	17.17	4.4	4.33
	(P-4-15)- 16	9.98	25.38	4.4	5.76
5 th F ₂ family	<u>x</u> 5	3.89	15.36	4.27	6.05
	(P-5-8)- 17	4.99	12.31	4.1	6.67
	(P-5-10)- 18	4.36	15.86	4.4	5.10
	(P-5-23)- 19	3.12	12.61	4.1	6.23
	(P-5-27)- 20	3.12	20.67	4.5	6.20

Table 8b: Comparison of fruit quality traits between and within families

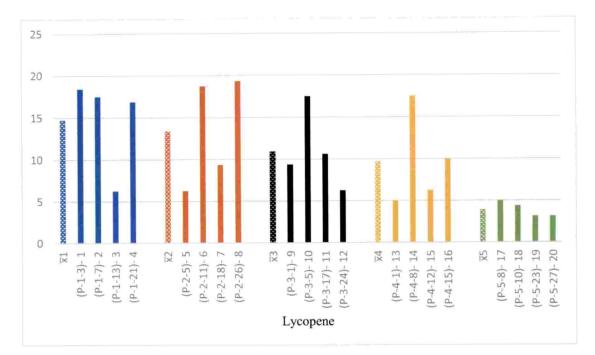
 $\overline{\mathbf{x}}_{1}$ - Average of 4 selected segregants in 1st F₂ family, $\overline{\mathbf{x}}_{2}$ - Average of 4 selected segregants in 2nd F₂ family, $\overline{\mathbf{x}}_{3}$ - Average of 4 selected segregants in 3rd F₂ family, $\overline{\mathbf{x}}_{4}$ - Average of 4 selected segregants in 4th F₂ family, $\overline{\mathbf{x}}_{5}$ - Average of 4 selected segregants in 5th F₂ family, *- average of 3 tested fruits.

Inter and intra family variation studied for the character lycopene is compared in the Fig. 10. The highest family mean was recorded for 1^{st} F₂ family (14.74 mg 100 g⁻¹) and the highest lycopene content was recorded in the segregant (P-2-26)- 8 with (19.34 mg 100 g⁻¹) suggesting much higher values of lycopene content than the commercial cherry tomato varieties 'Punjab Red Cherry' ranging from 4.71 to 5.11 reported by Dhaliwal and Jindal (2017) and 'Punjab Ratta' ranging from 7.89 to 8.14 reported by Cheema *et al.* (2010).

Inter and intra family variation studied for the character vitamin C is compared in Fig. 11. The highest family mean was recorded for 4^{th} F₂ family (22.03 mg 100 g⁻¹) and the highest lycopene content was recorded in the segregant (P-3-17)-11 with (29.35 mg 100 g⁻¹). Similar results were observed by Prema *et al.* (2011b) and Venkadeswaran *et al.* (2018).

All the fruit quality traits within the family shows variation except for pH which showed a constant around 4 and maximum pH was recorded in (P-5-27)- 20 as 4.5 and minimum with 3.5 in (P-2-11)- 6 (Fig. 12). Similar results were reported by Nadkarni (2017).

TSS (%) of fruits did not show much variation at inter family level but at intra family level there was much variation. The highest family mean was recorded for 5th F₂ family (6.05%) and the highest TSS was recorded in the segregant (P-3-17)- 11 (6.80%) suggesting comparatively higher TSS (%) than variety 'Solan Red Round' (6.03 %) reported by Ramya *et al.*, (2016) and commercial cherry tomato variety 'Punjab Red Cherry' (6.15 %) reported by Dhaliwal and Jindal (2017) and variety 'Punjab Ratta' (5 %) reported by Cheema *et al.* (2010) suggesting a higher TSS (%) content of fruit (Fig. 13).



 $\overline{\mathbf{x}}_{1}$ - Average of 4 selected segregants in 1st F₂ family, $\overline{\mathbf{x}}_{2}$ - Average of 4 selected segregants in 2nd F₂ family, $\overline{\mathbf{x}}_{3}$ - Average of 4 selected segregants in 3rd F₂ family, $\overline{\mathbf{x}}_{4}$ - Average of 4 selected segregants in 4th F₂ family, $\overline{\mathbf{x}}_{5}$ - Average of 4 selected segregants in 5th F₂ family

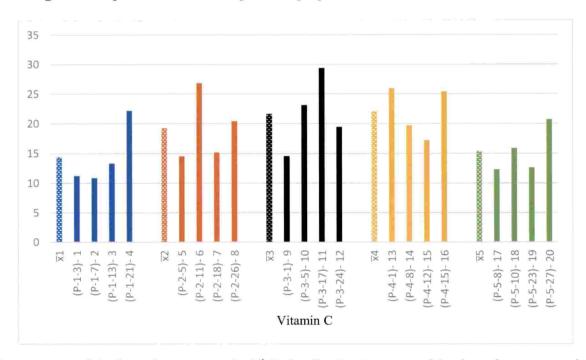
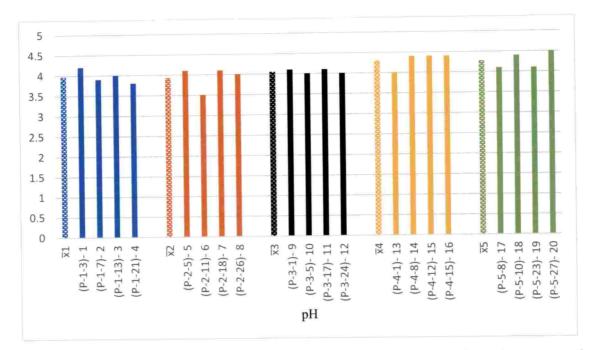


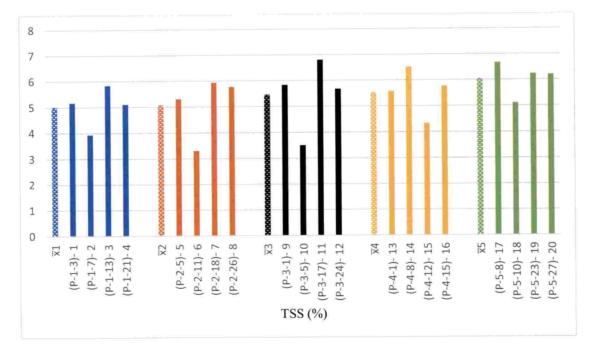
Fig 10: Comparison of the 20 superior segregants of the 5 F₂ families for lycopene

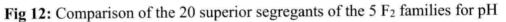
 $\overline{\mathbf{x}}_{1}$ - Average of 4 selected segregants in 1st F₂ family, $\overline{\mathbf{x}}_{2}$ - Average of 4 selected segregants in 2nd F₂ family, $\overline{\mathbf{x}}_{3}$ - Average of 4 selected segregants in 3rd F₂ family, $\overline{\mathbf{x}}_{4}$ - Average of 4 selected segregants in 4th F₂ family, $\overline{\mathbf{x}}_{5}$ - Average of 4 selected segregants in 5th F₂ family

Fig 11: Comparison of the 20 superior segregants of the 5 F₂ families for vitamin C



 $\overline{\mathbf{x}}_{1}$ - Average of 4 selected segregants in 1st F₂ family, $\overline{\mathbf{x}}_{2}$ - Average of 4 selected segregants in 2nd F₂ family, $\overline{\mathbf{x}}_{3}$ - Average of 4 selected segregants in 3rd F₂ family, $\overline{\mathbf{x}}_{4}$ - Average of 4 selected segregants in 4th F₂ family, $\overline{\mathbf{x}}_{5}$ - Average of 4 selected segregants in 5th F₂ family





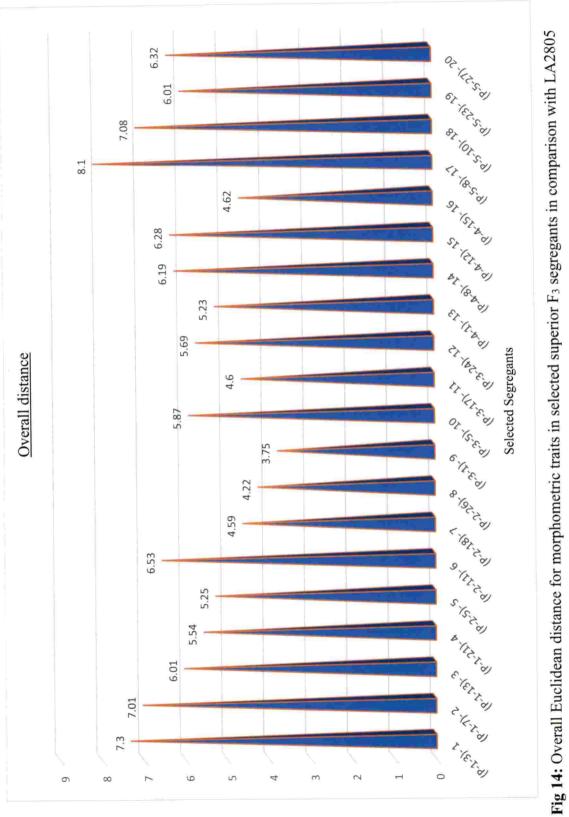
 $\overline{\mathbf{x}}_{1}$ - Average of 4 selected segregants in 1st F₂ family, $\overline{\mathbf{x}}_{2}$ - Average of 4 selected segregants in 2nd F₂ family, $\overline{\mathbf{x}}_{3}$ - Average of 4 selected segregants in 3rd F₂ family, $\overline{\mathbf{x}}_{4}$ - Average of 4 selected segregants in 4th F₂ family, $\overline{\mathbf{x}}_{5}$ - Average of 4 selected segregants in 5th F₂ family

Fig 13: Comparison of the 20 superior segregants of the 5 F₂ families for TSS (%)

The segregants (P-1-21)- 4 and segregant (P-2-11)- 6 showed a plant height of 115 and 136 cm respectively suggesting a determinate growth habit as compared with LA2805 parent, 'Punjab Sona Cherry', 'Punjab Kesar Cherry' and 'Punjab Red Cherry' with 234.6, 444.6, 401.0 and 404.2 cm respectively representing indeterminate growth habit reported by Dhaliwal and Jindal (2018).

The selected F_3 segregants derived from cross between cultivated tomato variety 'Anagha' and *Solanum lycopersicum* var. *cerasiforme* (LA2805) showed very less dissimilarity coefficient with LA2805 parent. Calculated overall Euclidean distance based on morphometric and fruit quality traits in comparison with LA2805 parent was less than 9.00 (Fig. 14). Similar analysis was performed in tomato by Hussain *et al.* (2018). This indicates that these selected superior segregants were closer to the LA2805 parent with respect to these morphometric and fruit quality traits. The selected segregants 7- (P-2-18), 8- (P-2-26), 9- (P-3-1) had lesser Euclidean coefficient of dissimilarity with the LA2805 parent.

The dendrogram constructed using SPSS 16.0 revealed the presence and extent of genetic similarities among the selected superior F_3 segregants along with parents. Cluster analysis revealed that the selected superior F_3 segregants along with parents grouped into two clusters. The female parent Anagha formed a separate cluster and all the selected F_3 segregants having cherry tomato characters were grouped along with LA2805 parent. The major cluster comprising of 21 genotypes including LA2805 parent were subdivided into two sub clusters with (P-5-8)- 17 as individual cluster and all the remaining selected segregants along with LA2805 parent forming another cluster. Clustering of genotypes also suggested the directional selection towards cherry tomato characters.





5.2 ASSESSMENT OF F₃ SEGREGANTS BASED ON SSR MARKER ANALYSIS

Molecular markers provide an important technology for evaluating levels and patterns of biodiversity within tomato populations. Among the various DNA markers currently available that can be used to examine genetic diversity at the molecular marker level, the marker with high polymorphic informative system to date is microsatellite or SSR's (simple sequence repeats). Their high information content, co-dominance and PCR based detection makes it an ideal tool for many genetic applications (Powell *et al.*, 1996; Russell *et al.*, 1997; Edwards and McCouch, 2007).

Out of the 20 selected segregants screened with 10 SSR markers linked to cherry tomato as reported by (Kwon *et al.*, 2009; El-Awady *et al.*, 2012; Aguirre *et al.*, 2017) the segregant (P-1-21)- 4 and segregant (P-2-11)- 6 showed the presence of all the ten SSR markers linked to cherry tomato and (P-2-26)- 8, (P-3-17)- 11 and (P-4-8)- 14 showed the presence of eight SSR markers linked to cherry tomato indicating that these segregants can be carried forward to F₄ evaluation for the development of superior cherry tomato variety.

The study on evaluation of F_3 segregants from the cross of cultivated tomato variety 'Anagha' and *Solanum lycopersicum* var. *cerasiforme* showed that there is considerable variation in the selected F_3 segregants within and between F_2 families.

Euclidean distance analysis showed that the selection for F_3 segregants for cherry tomato character has been directional towards the cherry tomato parent LA2805.

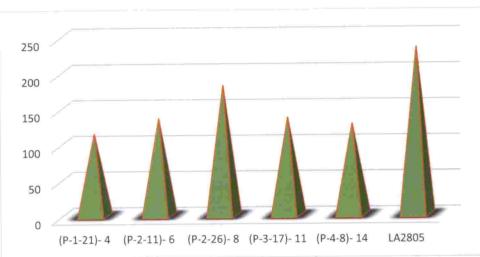
The SSR analysis revealed that the selected F₃ segregants (P-1-21)- 4, (P-2-11)- 6, (P-2-26)- 8, (P-3-17)- 11 and (P-4-8)- 14 showed more than 80% ($80\% \le$) similarity to cherry tomato. The major morphometric and qualitative traits specific to cherry tomato of these 5 F₃ segregants is compared with cherry tomato parent LA2805 in Fig. 15.

The Fig. 15a. shows that the plant height of all the segregants is less than LA2805 and all the segregants except (P-2-26)- 8 showing determinate growth habit (<150 cm). One of the major objectives of crossing Anagha with LA2805 was to

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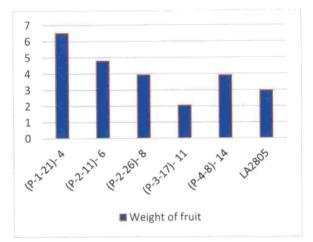
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incorporate the determinate/semi- determinate growth habit into cherry tomato, so the selection in these segregants can lead to the development of a determinate cherry tomato variety. With respect to weight of fruit all the segregants except (P-3-17)-11 had fruit weight higher than LA2805 and (P-1-21)- 4 is having almost double the fruit weight of LA2805 parent (Fig. 15b). Number of fruits cluster⁻¹ of all the segregants were less than LA2805 (Fig. 15d). So, more emphasis should be given on this trait in the selection in further generations. Lycopene content of all the segregants except (P-3-17)- 11 was higher than LA2805 (Fig. 15c). This is a good indication as higher lycopene content is a valuable character with respect to quality of tomato. Vitamin C content of two of the segregants were higher than LA2805 (Fig. 15e). This character also should be given importance in the selection in further generations. These 5 segregants (P-1-21)- 4, (P-2-11)- 6, (P-2-26)- 8, (P-3-17)- 11 and (P-4-8)- 14 can be carried forward to further generations for getting a cherry tomato variety with determinate growth habit, higher fruit weight, a greater number of fruits cluster⁻¹, high lycopene and vitamin C content.

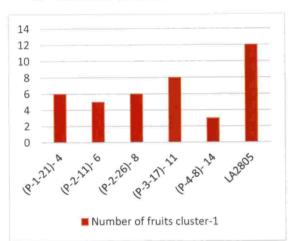


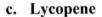
a. Plant height

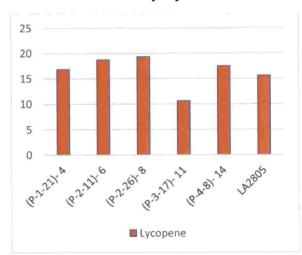




d. Number of fruits cluster⁻¹









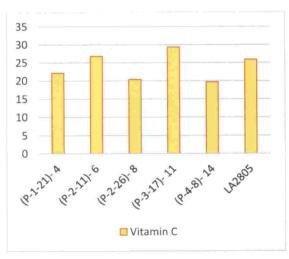


Fig. 15: Comparison of 5 superior F3 segregants with LA2805 parent

<u>Summarv</u>

6. SUMMARY

Cherry tomato (*Solanum lycopersicum* var. *cerasiforme*), one of the gifted wild types of *Solanum* offers great probability in crop improvement programmes by virtue of their valuable aspects in terms of desirable aspects such as good source of vitamin A and C, sugars and genetic diversity for selection of parental material.

The present study entitled "Identification of cherry tomato genotypes from F_3 segregants of intraspecific cross" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 2018 - 2019 with an objective to evaluate F_3 segregants of the cross between cultivated tomato variety Anagha (*Solanum lycopersicum* L.) and *Solanum lycopersicum* var. *cerasiforme* for morphometric traits and fruit quality specific to cherry tomato and genotyping the superior segregants with SSR markers.

The materials chosen for the experiment was obtained from five F_2 plants selected from the cross between Anagha (*Solanum lycopersicum* L.) and *Solanum lycopersicum* var. *cerasiforme* (LA2805) done in a completed Ph.D. project of the department. A total of 150 F_3 segregants from thirty plants from each F_2 family was used for the study.

Observations on nine morphometric traits (plant height, primary branches plant⁻¹, number of days to 50% flowering, number of days to first fruit harvest, number of fruits plant⁻¹, weight of fruits plant⁻¹, weight of fruit, number of locules fruit⁻¹, number of fruits cluster⁻¹) were recorded in the F₃ segregants and based on that twenty superior segregants were selected. These twenty segregants were subjected to fruit quality analysis and molecular marker analysis.

Since the F₃ segregants were originated from five F₂ families the interfamily and intrafamily variation for each character was assessed.

Among the morphometric traits, plant height did not show much variation between families and within family. Plant height of selected genotypes showing determinate to semi-determinate growth habit with a maximum of 261 cm and a minimum of 72 cm indicating selection in these segregants can lead to the development of a determinate cherry tomato variety. Weight of fruit did not show much variation between the families but within the family there was much variation, all the segregants except (P-3-17)- 11 had fruit weight higher than LA2805 and (P-1-21)- 4 is having almost double the fruit weight of LA2805 parent. Number of fruits cluster⁻¹ of all the segregants were less than LA2805. So, more emphasis should be given on this trait in the selection in further generations.

Dendrogram based on morphometric and fruit quality data revealed that the selected superior F₃ segregants grouped into two major clusters. Anagha formed an individual cluster and remaining F₃ segregants along with LA2805 constituting major cluster indicating all the small fruited cherry tomato genotypes grouped into one cluster.

Analysis for qualitative traits (Lycopene, vitamin C, pH and TSS) revealed that lycopene showed variation between the families and within the family. Lycopene content of all the segregants except (P-3-17)- 11 was higher than LA2805. This is a good indication as higher lycopene content is a valuable character with respect to quality of cherry tomato. Vitamin C content of two of the segregants (P-2-11)- 6 and (P-3-17)- 11 were higher than LA2805 parent indicating this character will have scope for selection in further generations. Intrafamily variation was seen for all the characters except for pH which showed a constant around 4 with maximum pH recorded in (P-5-27)- 20. TSS (%) of fruits did not show much variation between the families but within the family there was much variation with maximum TSS (%) of 6.8 was recorded in (P-3-17)- 11.

No physiological disorders, pest and disease incidence was noticed inside the rain shade shelter during the crop period.

Molecular marker analysis was done using ten SSR markers reported as specific to cherry tomato. Out of the twenty segregants, the segregants (P-1-21)- 4 and (P-2-11)- 6 showed the presence of all the SSR markers linked to cherry tomato. The segregants (P-2-26)- 8, (P-3-17)- 11, (P-4-8)- 14 showed eight specific markers of cherry tomato.

A proximity dissimilarity matrix was constructed between the selected segregants and the parents based on the recorded morphometric and fruit quality data. The results of the data revealed that distance from the cherry tomato parent was less than 9.00 indicating that these selected superior segregants were showing more to cherry tomato morphometric and fruit quality traits. The segregants (P-2-26)- 8, (P-3-17)-11 and (P-4-8)-14 which had Euclidean coefficient of dissimilarity assessed based on morphometric and fruit quality traits 4.22, 4.60 and 6.19 respectively showed eight specific markers linked to cherry tomato. The segregants (P-1-21)- 4 and (P-2-11)- 6 which showed the presence of all the ten specific markers to cherry tomato had Euclidean coefficient of dissimilarity of 5.54 and 6.53 respectively.

Based on the morphological, biochemical and molecular data five F_3 segregants (P-1-21)- 4, (P-2-11)- 6, (P-2-26)- 8, (P-3-17)- 11 and (P-4-8)- 14 were selected to carry forward to further generations for getting a cherry tomato variety with determinate growth habit, higher fruit weight, high number of fruits cluster⁻¹, high lycopene and vitamin C content.

Future line of work:

The segregants with good agronomic and fruit quality traits can be selected and carried forward for F₄ generation and further to develop a superior cherry tomato variety.





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IDENTIFICATION OF CHERRY TOMATO GENOTYPES FROM F3 SEGREGANTS OF INTRASPECIFIC CROSS

by

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ABSTRACT

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ABSTRACT

The present study entitled "Identification of cherry tomato genotypes from F_3 segregants of intraspecific cross" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 2018 - 2019 with an objective to evaluate F_3 segregants of the cross between cultivated tomato variety Anagha (*Solanum lycopersicum* L.) and *Solanum lycopersicum* var. *cerasiforme* for morphometric traits and fruit quality specific to cherry tomato and genotyping the superior segregants with SSR markers.

The materials chosen for the experiment was obtained from five F_2 plants selected from the cross between Anagha (*Solanum lycopersicum* L.) and *Solanum lycopersicum* L. var. *cerasiforme* (LA2805) done in a completed Ph.D. project of the department. A total of 150 F_3 segregants from thirty plants from each F_2 family was used for the study.

Observations on nine morphometric traits were recorded in the F_3 segregants and based on that twenty superior segregants were selected. These twenty segregants were subjected to fruit quality analysis and molecular marker analysis.

Since the F₃ segregants were originated from five F₂ families the interfamily and intrafamily variation for each character was assessed.

Among the morphometric traits, plant height did not show much variation between families and within family. Plant height of selected genotypes showing determinate to semi-determinate growth habit with a maximum of 261 cm and a minimum of 72 cm indicating selection in these segregants can lead to the development of a determinate cherry tomato variety. Weight of fruit did not show much variation between the families but within the family there was much variation, all the segregants except (P-3-17)- 11 had fruit weight higher than LA2805 and (P-1-21)- 4 is having almost double the fruit weight of LA2805 parent. Number of fruits cluster⁻¹ of all the segregants were less than LA2805. So, more emphasis should be given on this trait in the selection in further generations.

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Dendrogram based on morphometric and fruit quality data revealed that the selected superior F₃ segregants grouped into two major clusters. Anagha formed an individual cluster and remaining F₃ segregants along with LA2805 constituting major cluster indicating all the small fruited cherry tomato genotypes grouped into one cluster.

Analysis for qualitative traits (Lycopene, vitamin C, pH and TSS) showed that lycopene showed variation between the families and within the family. Lycopene content of all the segregants except (P-3-17)- 11 was higher than LA2805. This is a good indication as higher lycopene content is a valuable character with respect to quality of cherry tomato. Vitamin C content of two of the segregants (P-2-11)- 6 and (P-3-17)- 11 were higher than LA2805 parent indicating this character will have scope for selection in further generations. Intrafamily variation was seen for all the characters except for pH which showed a constant around 4 with maximum pH recorded in (P-5-27)- 20. TSS (%) of fruits did not show much variation between the families but within the family there was much variation with maximum TSS (%) of 6.8 was recorded in (P-3-17)- 11.

Molecular marker analysis was done using ten SSR markers reported as specific to cherry tomato. Out of the twenty segregants, the segregants (P-1-21)- 4 and (P-2-11)- 6 showed the presence of all the SSR markers linked to cherry tomato. The segregants (P-2-26)- 8, (P-3-17)- 11, (P-4-8)- 14 showed eight specific markers of cherry tomato.

A proximity dissimilarity matrix was constructed between the selected segregants and the parents based on the recorded morphometric and fruit quality data. The results of the data revealed that distance from the cherry tomato parent was less than 9.00 indicating that these selected superior segregants were showing more to cherry tomato morphometric and fruit quality traits. The segregants (P-2-26)-8, (P-3-17)-11 and (P-4-8)-14 which had Euclidean coefficient of dissimilarity assessed based on morphometric and fruit quality traits 4.22, 4.60 and 6.19 respectively showed eight specific markers linked to cherry tomato. The segregants (P-1-21)- 4 and (P-2-11)- 6 which showed the presence of all the ten specific

markers to cherry tomato had Euclidean coefficient of dissimilarity of 5.54 and 6.53 respectively.

Based on the morphological, biochemical and molecular data five F_3 segregants (P-1-21)- 4, (P-2-11)- 6, (P-2-26)- 8, (P-3-17)- 11 and (P-4-8)- 14 were selected to carry forward to further generations for getting a cherry tomato variety with determinate growth habit, higher fruit weight, high number of fruits cluster⁻¹, high lycopene and vitamin C content.

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