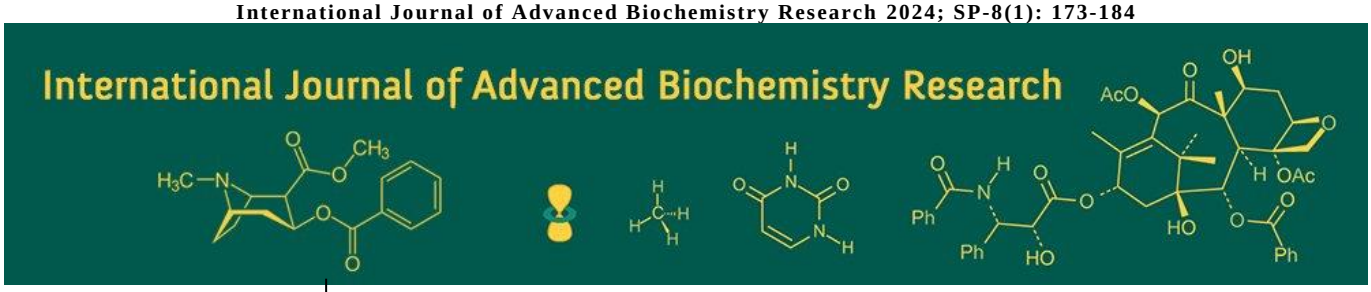

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Identification of simple sequence repeats (SSRS) for TLCV Resistance in tomato (*Solanum lycopersicum* L.)

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Abstract
TLCV (Tomato leaf curl virus) is most destructive disease in tomato causes severe losses of up to 100%. It is transmitted by sole vector whitefly (*Bemisia tabaci*). Breeding of disease resistance is only truthful approach to combat the disease consists marker assisted selection (MAS). Therefore, the present investigation was carried out using of 110 F_2 mapping population individuals of cross ATL-97-26×GP-11. SSRs marker analysis was carried out to identify with a set of 374 SSRs were used for marker analysis. Parental polymorphic markers were utilized in genotyping of 110 F_2 mapping population individuals. Morphological and biochemically important traits like plant height, number of branches per plants, number of fruits per plant, disease incidence score, phenol content, moisture, total soluble Solids, terrible acidity, total soluble sugar, total flavonoids, lycopene and ascorbic acid content in 110 individuals of $F_{2:3}$ mapping population derived from cross of ATL-97-26×GP-11. The phenol content showed significant negatively correlation with disease incidence. Single marker analysis (SMA) found 9 marker trait association with above threshold level 3. The higher number of marker trait associations was found for phenol content and disease incidence. The phenol content and other morpho-biochemical traits are in current study can be exploited for develop hybrid of elite parental line/cultivars of tomato through marker assisted breeding programme. The SSRs markers *i.e.*, TES0312, SSR112 and SSR331 identified from this study could be useful for TLCV resistance breeding in tomato.

Keywords: Tomato, TLCV, morpho-biochemical traits, Phenol content, SSRs, MAS, SMA

Introduction
Tomato (*Solanum lycopersicum* L., 2n=2x=24) is one of the most important vegetable plants in the world belong to nightshade family *Solanaceae*. It originated in western South America. According to data of FAOSTAT-2021, India held the position of the second-largest global tomato producer, trailing only behind China. The country cultivated tomatoes across 840.3 thousand hectares, contributing a substantial 21.181 million tonnes to the overall global production [12]. The major Tomato producing States in the India are Madhya Pradesh, Andhra Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Maharashtra, Chhattisgarh, Bihar, Telangana, Uttar Pradesh, Haryana and Tamil Nadu, they account for 91% of the total production of the country (NHB, 2021) [26].

Low productivity in India is due to occurrence of both biotic and abiotic stresses. Tomato yellow leaf curl virus (TLCV) is a very damaging virus disease of tomato crops in tropical and warm temperate regions of the world, causing losses of up to 100%. It belongs to family *Geminiviridae* (Gronenborn, 2007) [14]. Symptoms of TYLCV disease on tomato are characterized by curling of leaves upward and inward, in a cup-shaped manner, curling of lamina between veins, swelling of veins on leaf surfaces, reduction in leaf size with shortened internodes and proliferated lateral branches, plant stunting with distorted growing tips and chlorotic leaflets, flower drop and formation of small sized fruit (Banerjee and Kalloo, 1987, Singh *et al.*, 2015) [3, 33].

Breeding for resistance to TLCV appears to be a promising and ecological approach to control the disease, which is started in the late 1970s. It has consisted of introgression resistant traits present in wild tomato species into cultivated varieties (Picó *et al.* 1999) [29].

Molecular markers are specific fragments of DNA that can be identified within the whole genome. Molecular markers that are tightly linked may be referred to as 'gene tag' and these markers do not affect the phenotype of the gene of interest (Collard *et al.*, 2005) [7]. Simple sequence repeats (SSRs) are regarded as a robust system for marker-assisted breeding due to their co-dominance nature, abundance, reproducibility and variability (Kumar *et al.*, 2018) [20]. Comprehensive knowledge of complex plant traits including growth, development, tolerance, resistance, physiology, biochemical attributes, ecology, yield guides researcher, breeders towards the selection of productive plant that are suitable for their environment (Costa *et al.*, 2019) [8].

Materials and Methods

The ATL-97-26 and GP-11 was used as parental lines derived from main vegetable research station (MVRS), Anand Agricultural University, Anand, Gujarat to develop 110 individuals of F_2 population in year 2020. DNA was extracted newly expanded fresh leaves using Cetyl trimethyl ammonium bromide (CTAB) protocol with some modifications (Doyle and Doyle, 1990) [10]. A set of total 374 SSRs marker was used for identification of parent polymorphism, identified polymorphic SSRs markers was further used for genotyping of F_2 population through QIAxcel Screen Gel software of QIAgen capillary electrophoresis. The subsequent segregating mapping population of 110 individuals of $F_{2:3}$ was grown on field during November-2021. The observations on morphological characters were taken from five plants each of ATL-97-26, ATL-97-26 and F1, whereas 110 $F_{2:3}$ plants were observed individually for plant height (PH), number of branches per plants (NBP), number of fruits per plant (NFP), disease incidence score (DI) was recorded using standard procedures (Singh *et al.*, 2015) [33]. The biochemical parameters like Total phenol were estimated by the method as described by Bray and Thorpe (1954) [5], moisture content was estimated as per procedure developed by A.O.A.C. (2000) [1], Total soluble solids were estimated by the method described by Harrill (1998) [15] using hand refractometer, total soluble sugars were determined using the phenol-sulphuric acid method as described by Dubois *et al.* (1956) [11], Total Flavonoids were estimated by the method described by Suman *et al.*, 2014 [35], Lycopene was determined using the method as described by Raganna, (1977) [31], Ascorbic acid were estimated by the method described by Malik and Singh (1980) [22].

Correlation of disease incidence with others traits was performed by using of SAS system. Frequency distribution of the segregating generation was tested for normality by the Kolmogorov-Simirnov Z-statics, Cramer-Von Mises and Anderson-Darlington test using SAS software system (SAS Institute, North Carolina State University). Single Marker Analysis was performed with the help of software QTL IciMapping *V4.2.* The LOD score was set at 3.0 (Meng *et al.*, 2015) [24].

Results and Discussion

Genotyping using of SSRs in F_2 mapping population

A set of 374 primers were employed for screening parental DNA samples *i.e.*, ATL-97-26 and GP-11. Out of 374 primers, 318 primers got amplified whereas no amplification was seen for 56 primers. From the 318 amplified primers, 18 primers showed parent polymorphism. Polymorphism was decided on the basis of presence or absence of bands as well as difference in position of the bands (Table 1). Only the primers showing clear and distinct bands were considered to be polymorphic. Amplified primers were used for genotyping of F_2 mapping population.

Table 1: Parent polymorphic SSR markers with amplicon size

Sr. No.	Marker Name	Amplification product size (bp)	
		GP-11 (Suceptible)	ATL-97-26 (Resistant)
1.	SSR22	157	150
2.	SSSR111	197	186
3.	SSR-306	254	240
4.	SLM11-2	153	189
5.	SLM11-29	170	182
6.	TGS2756	150	128
7.	TES856	179	212
8.	TES671)	163	154
9.	P6-6-F1	617	507
10.	SSR-331	152	132
11.	Legata001	178	168
12.	TGS827	240	254
13.	TGS420	231	221
14.	SSR112	158	163
15.	TGS2910	158	173
16.	SSR310	150	158
17.	TES0312	208	200
18.	LEgata002	323	317

Phenotyping performance of the mapping population, variance components and heritability

Variability analysis for Morphophysiological and Biochemical traits of $F_{2:3}$ mapping population of cross ATL-97-26 are mentioned in table 4.10. Variability estimates of the population and heritable components of the trait are useful tools for the improvement of a plant trait in any breeding programmer. In order to progress a character with the help of selection the main portion of variation present should be heritable. So, the knowledge on phenotypic coefficient of variation, genotypic coefficient of variation as well as heritability of the character is important for breeding. For this reason, the co-efficient of variation calculated at phenotypic and genotypic levels are being used to evaluate the variability among different traits. GCV gives idea about the amount of genetic variability present in particular character, heritability in turn gives estimate of relative amount of heritable portion of variation. However, heritability value when accompanied with high genetic advance gives perfect suggestion about the amount of genetic progress which was as a result of selection of best individuals. (Burton and De Vane, 1953, Johnson *et al.*, 1955) [6, 18].

According to Deshmukh *et al.* (1986) [9], phenotypic and genotypic coefficient of variation valued greater than 20% are regarded as high, values between 10 to 20% to be medium, whereas values less than 10% are considered to be low Heritability values were categorized as low (< 30%), moderate (30-60%) and high (> 60%). The genetic advance (GA %) on 5% selection intensity was estimated and classified as low (< 10%), moderate (10-20%) and high (> 20%) following the method given by Johnson *et al.* (1955) [18].

Table 2: Variability analysis for Morphophysiological and biochemical traits of $F_{2:3}$ population of cross ATL-97-26 × GP-11

Sr. No.	Trait	Range	Mean	GCV (%)	PCV (%)	h^2B (%)	GAM (%)
1	Plant height (cm)	81.00-126.33	102.02	10.04	10.87	85.33	19.49
2	No. branches per plant	5.67-13.33	9.40	17.18	18.25	88.67	3.13
3	No. fruits per plant	18.33-59.00	39.55	23.44	24.54	91.16	18.23
4	Phenol (mg/100g)	56.22-145.46	100.10	24.37	24.39	99.82	50.21
5	Moisture (%)	88.21-92.42	90.55	0.88	0.97	81.14	1.47
6	TSS (Brix)	3.67-6.80	4.57	10.76	11.01	95.44	0.99
7	Titrable Acidity (%)	0.44-1.33	0.85	27.04	27.83	94.37	0.46
8	Total soluble sugars	1.25-3.25	2.24	17.81	18.12	96.59	0.81
9	Flavonoids (mg/100g)	30.02-61.62	45.35	11.27	13.46	70.12	8.83
10	Lycopene (mg/100g)	5.66-14.65	10.21	21.41	21.52	98.98	4.48
11	Ascorbic acid (mg/100g)	6.95-12.97	10.45	15.23	15.24	99.87	3.28

All the traits under studied showed high heritability. While wide variations are observed among the characters, the high GCV and PCV were observed for traits like number of fruits per plant, phenol content, titrable acidity and lycopene content. The moderate level of GCV and PCV were observed for plant height, number of branches per plant, total soluble solids, total soluble sugars and flavonoid content. While, moisture content was showed low GCV and PCV content (Table 2). These results indicated that wide range of variation along with heritability helps the selection of superior and desired lines for further improvement in tomato. Furthermore, the presence of narrow gap between GCV and PCV for all the characters suggested these traits studied has low environmental influence (Pujar, 2013) [30]. Similar findings for high heratability and wide variability for agronomical and Morphophysiological and biochemical traits present in tomato mentioned by previous studies. *viz.*, Nandapuri *et al.* (1976) [25], Mala and Vadivel (1999) [21], Veershetty (2004) [37], Haydar *et al.* (2007) [16], Samadia *et al.* (2006) [32] Al-Aysh, *et al.* (2012) [2], Islam *et al.* (2012) [17], Gaikwad, (2012) [13] and Pujar, (2013) [30].

Correlation Study of disease scoring with other Morphophysiological and biochemical traits

The knowledge of the associations between disease occurrence with morphological and biochemical traits will enable the researcher or breeders to decide suitable selection/breeding program criteria for genetic improvement in tomato against TLCV disease. The Correlation coefficients of different morphological and biochemical traits studied is mentioned in Table 3.

Table 3: Correlation analysis of different characters with disease incidence

Sr. No.	Trait	Disease incidence (n=110)
1	Plant height	-0.258**
2	Number of branches per plant	-0.322**
3	Number of fruits per plant	-0.710**
4	Phenol	-0.780**
5	Moisture	0.082
6	Total soluble solids	-0.055
7	Titrable acidity	-0.041
8	Total soluble sugars	0.016
9	Flavonoids	-0.220*
10	Lycopene	-0.178
11	Ascorbic acid	0.077

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

The results revealed that characters like plant height, number of branches per plant, number of fruits per plant, phenol and total flavonoids shows significant negative correlation with disease scoring (-0.258, -0.322, -0.710, -0.780 and -0.220, respectively). The presence of phenolic compounds in plants and their synthesis in response to infection is associated with resistance and reported in many studies related to plant pathogen interaction. Thangam, (2004) [36] reported that the total phenol and ortho di hydroxy phenol in the leaves exhibited a significant negative association with disease incidence in all the environments studied signifying their role in disease resistance. Total phenol content in the leaves consistent factor of resistance against tomato leaf curl virus, suggest its negative correlation with TLCV incidence, While, total protein, reducing sugar and total sugar had a positive impact on whitefly flare-up on tomato (Mandali and Vijayalakshmi, 2020 and Pal *et al.*, 2021) [23, 27].

Test of Normality

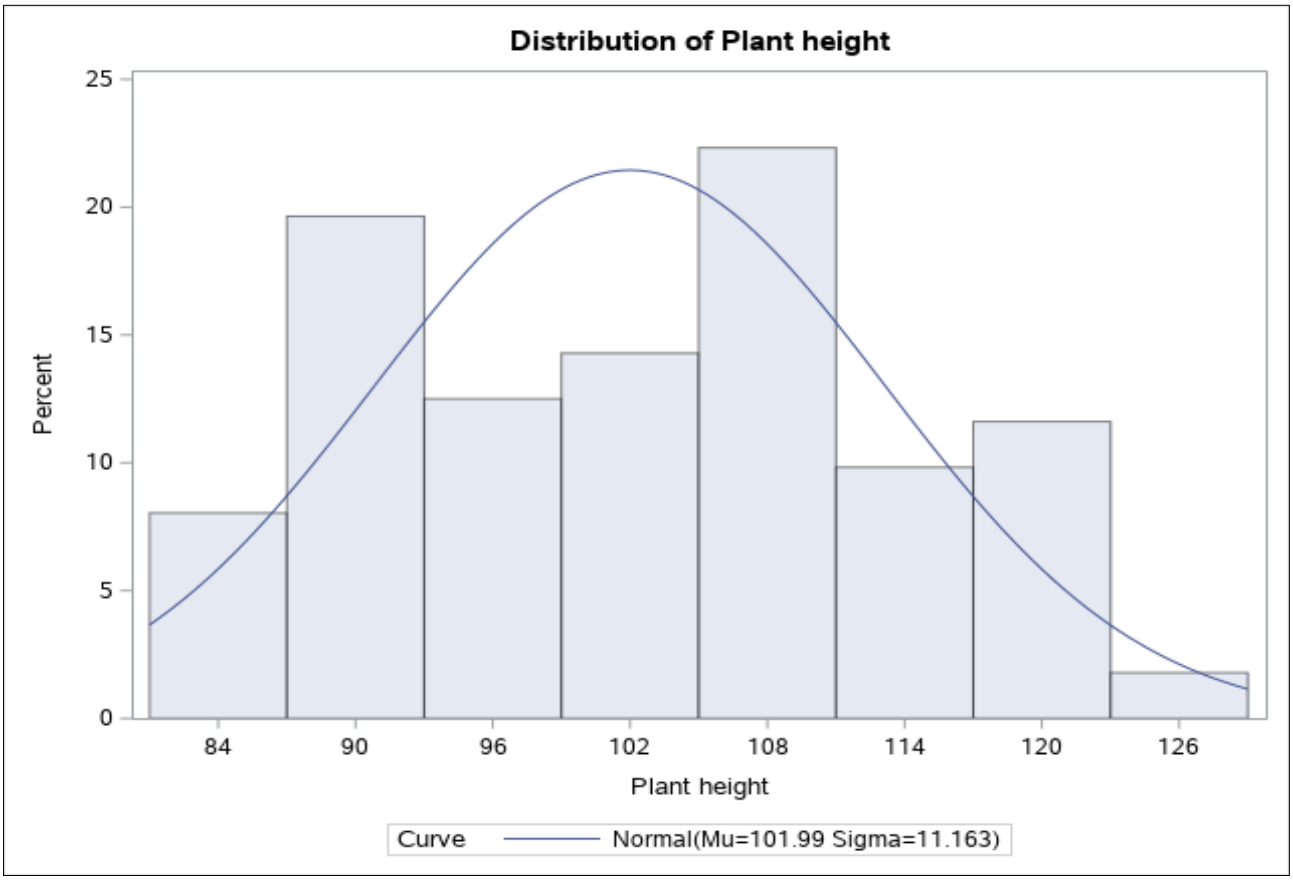
Quantitative characters show discrete variation in the population. In order to know the frequency distribution of a segregating generation and their genetic interactions for a particular trait, skewness and kurtosis were estimated. Frequency distribution curve for Morphophysiological and biochemical traits $F_{2:3}$ Mapping population is presented in Figure 1 (a & b), respectively. Table. 4 describes skewness and kurtosis measured $F_{2:3}$ Mapping population of cross ATL-97-26 × GP-11.

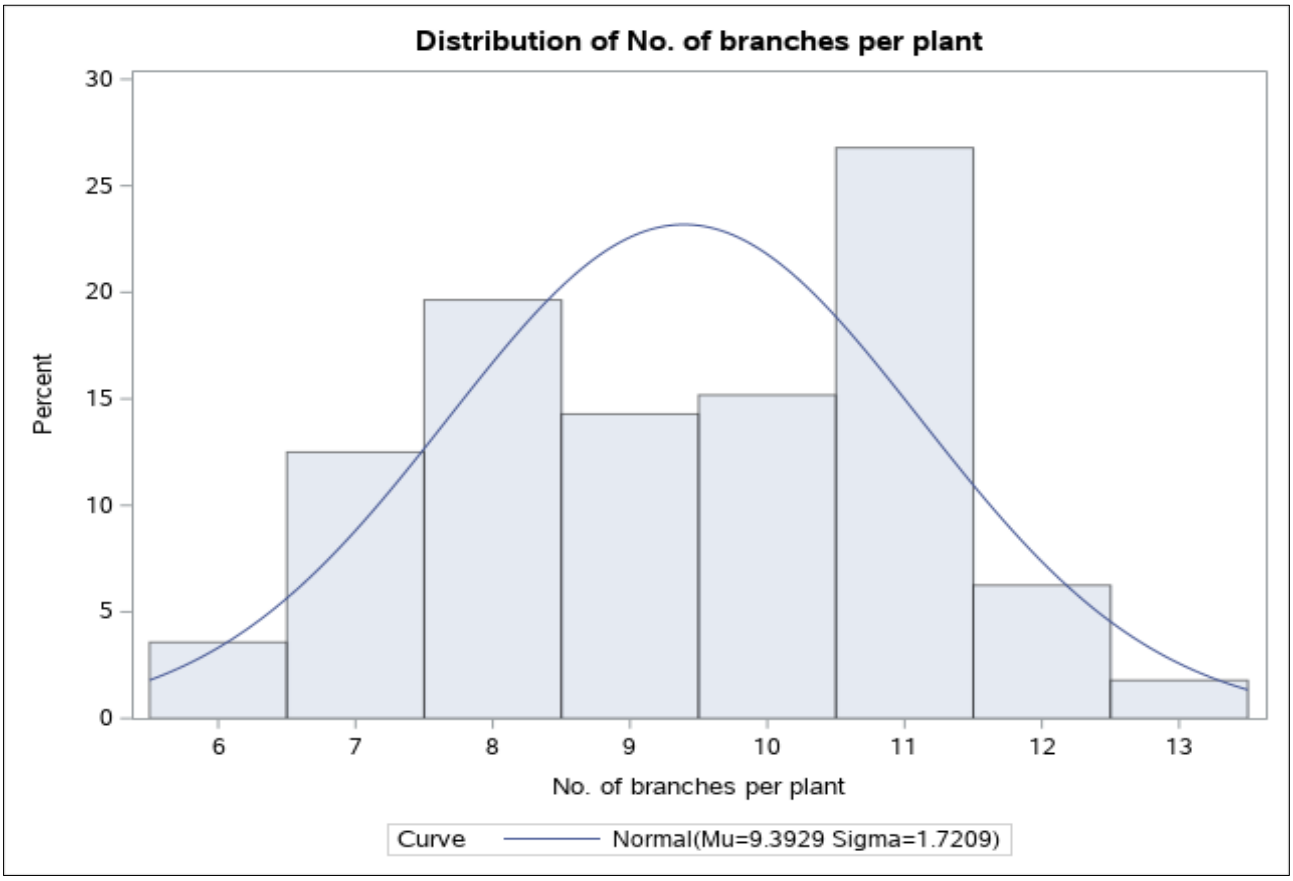
Table 4: Skewness and kurtosis of for Morphophysiological and biochemical traits of $F_{2:3}$ mapping population

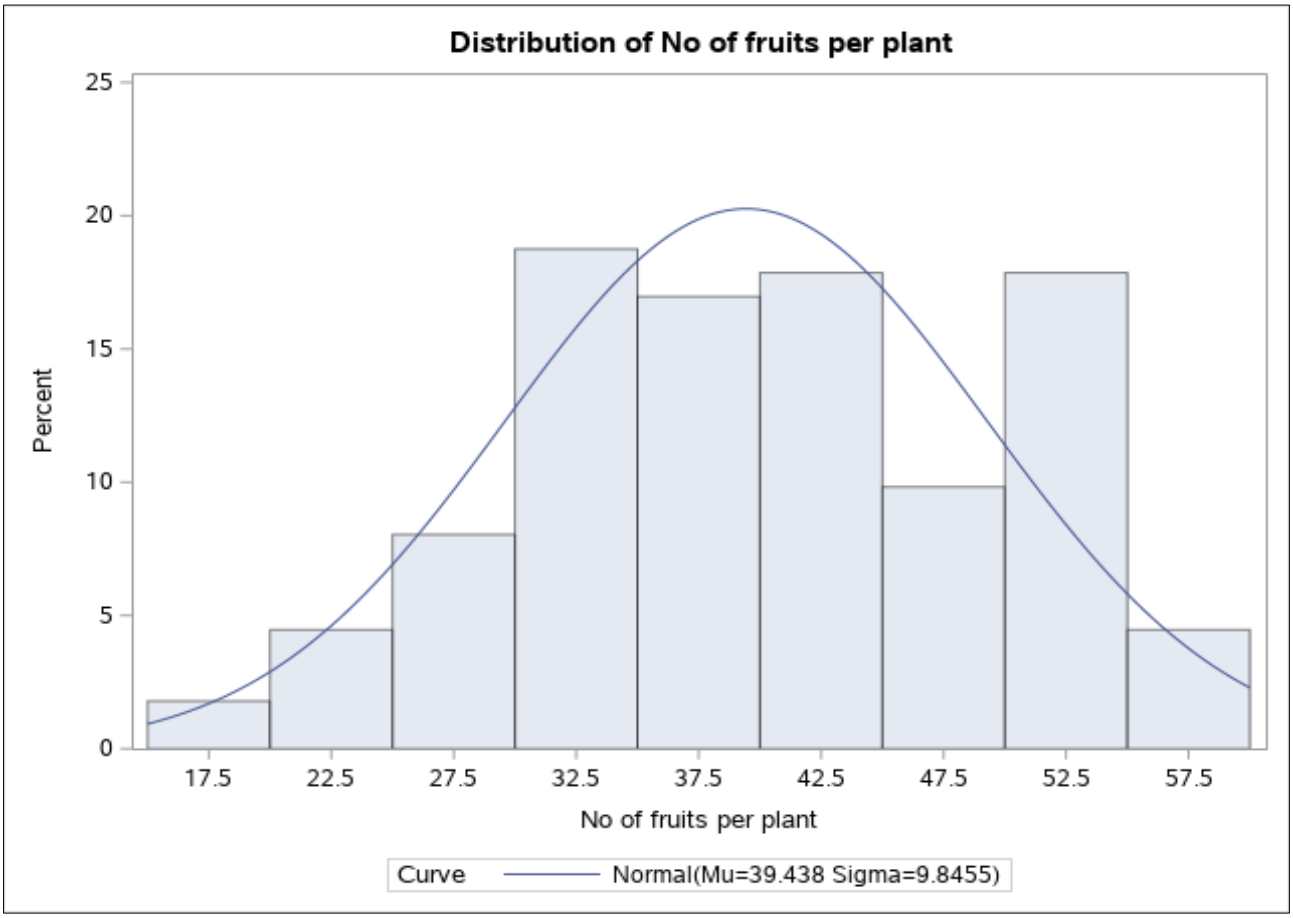
Sr. No.	Traits	Skewness	Kurtosis
1	Plant height	0.104	-1.026
2	Number of branches per plant	-0.111	-0.982
3	Number of fruits per plant	-0.107	-0.812
4	Disease incidence	0.588	-0.937
5	Total phenol	0.057	-1.224
6	Moisture	-0.283	0.086
7	Total soluble solids	0.681	1.993
8	Titrable acidity	0.249	-0.977
9	Total soluble sugars	-0.144	-0.287
10	Total flavonoids	0.451	0.386
11	Lycopene	-0.288	-0.778
12	Ascorbic acid	-0.311	-0.678

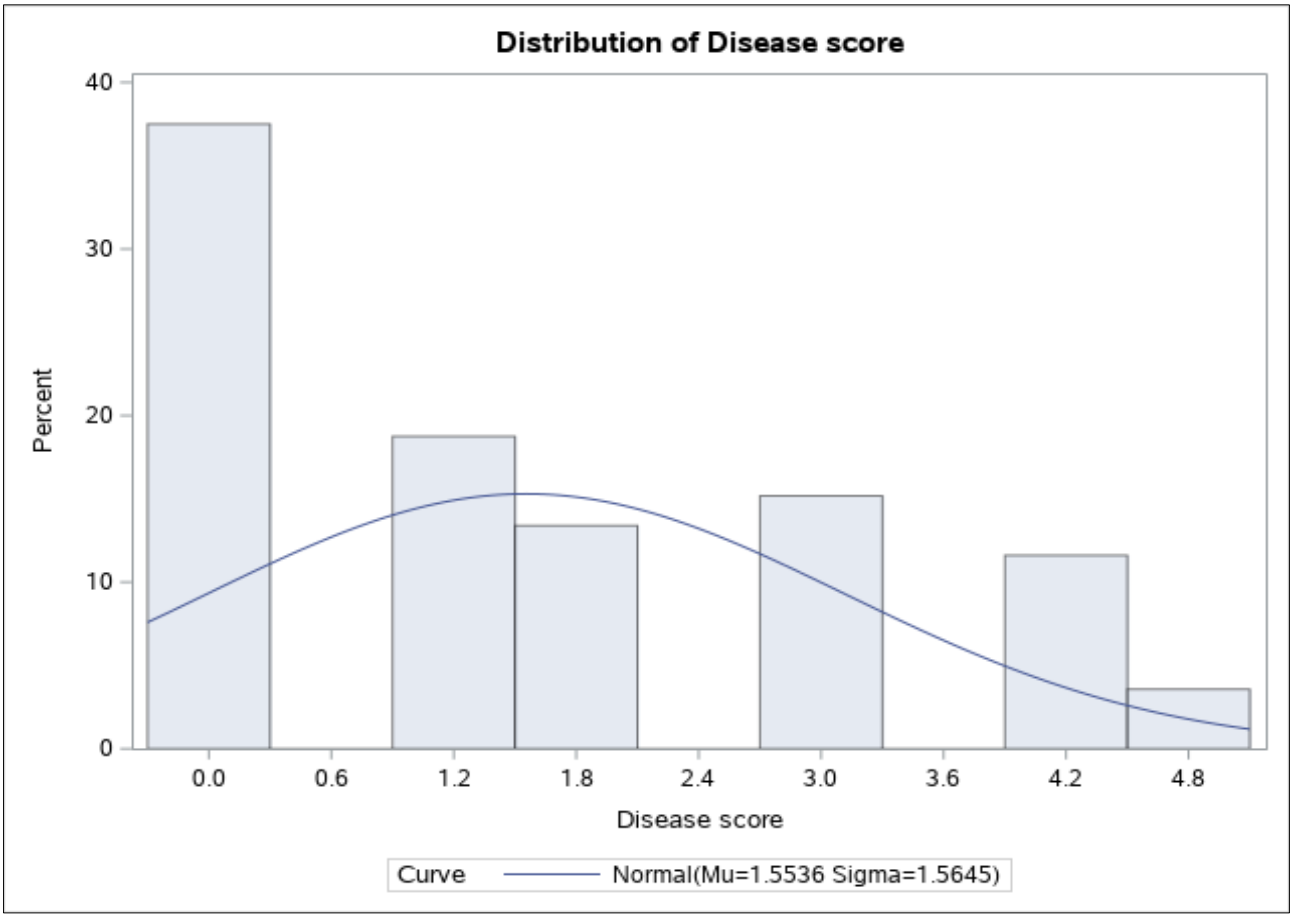
Skewness explains how much is the departure of a distribution from symmetry and kurtosis characterizes the peakedness of a distribution. If the skewness is positive, it indicates the complementary epistatic gene action for the trait studied and the genetic gain is slower with mild selection and faster with intensive selection. Skewness when negative indicate that duplicate epistasis gene action is present and the genetic gain is faster with mild selection and rapid with intense selection (Snape and Riggs, 1975) [34].

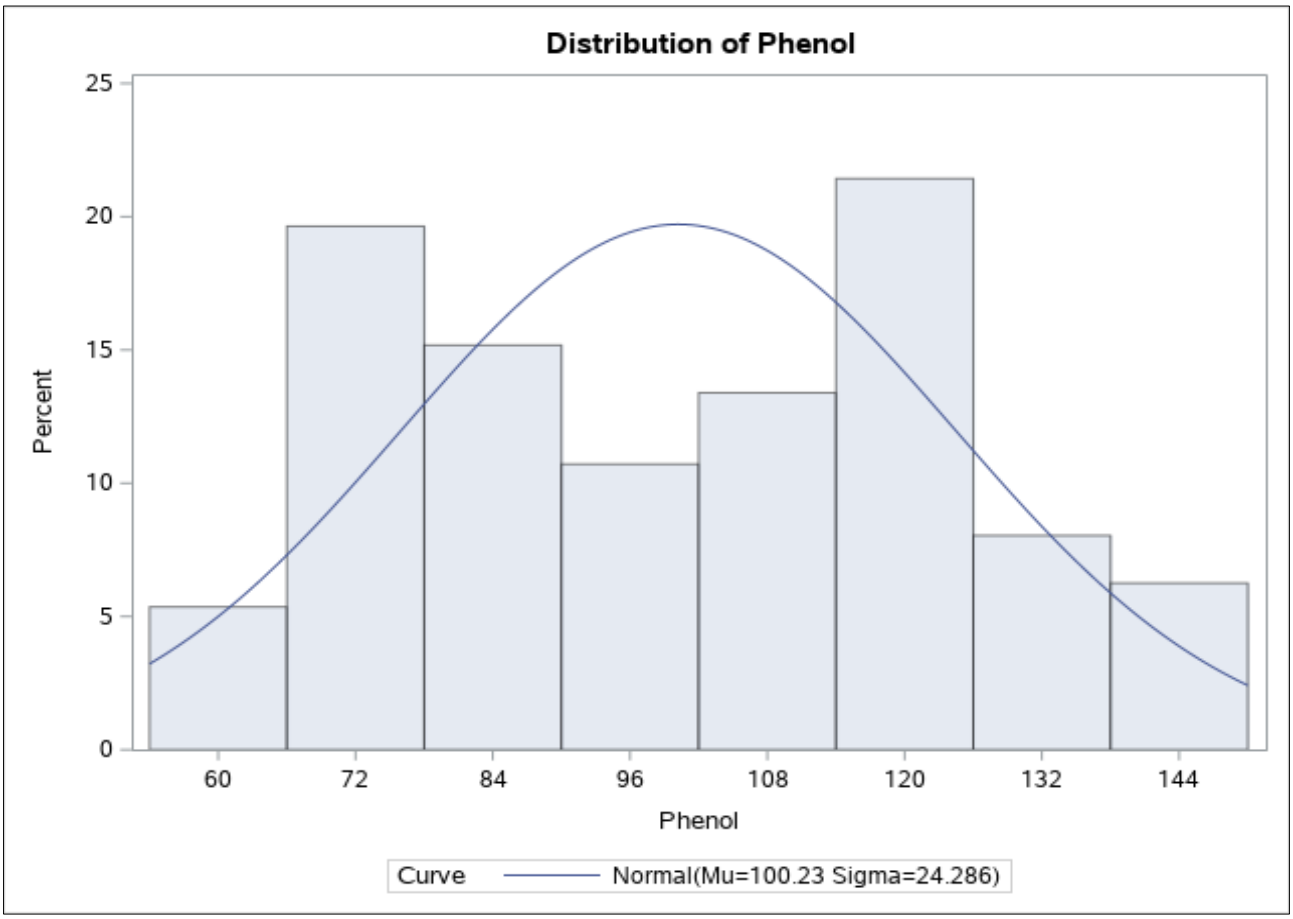
Leptokurtic distribution is shown by traits which are under control of few segregating genes and a platykurtic distribution is shown by traits that are in control of many genes and complementary gene action. A standard normal distribution has kurtosis of 3 and is referred as mesokurtic. An increased kurtosis (> 3) can be visualized as a thin "Bell" with a high peak whereas a decreased kurtosis corresponds to a broadening of the peak and "Thickening" of the tails. Kurtosis > 3 is recognized as leptokurtic and < 3 as platykurtic. Kurtosis obtained by substitution of excess kurtosis by value of 3, zero indicates a perfect tailedness and positive values a leptokurtic distribution (Kallner, 2017) [19].






Distribution of No of fruits per plant
Percent
25
20
15
10
5
0
17.5
22.5
27.5
32.5
37.5
42.5
47.5
52.5
57.5
No of fruits per plant
Curve
Normal(Mu=39.438 Sigma=9.8455)


Distribution of Disease score
Percent
40
30
20
10
0
0.0
0.6
1.2
1.8
2.4
3.0
3.6
4.2
4.8
Disease score
Curve
Normal(Mu=1.5536 Sigma=1.5645)



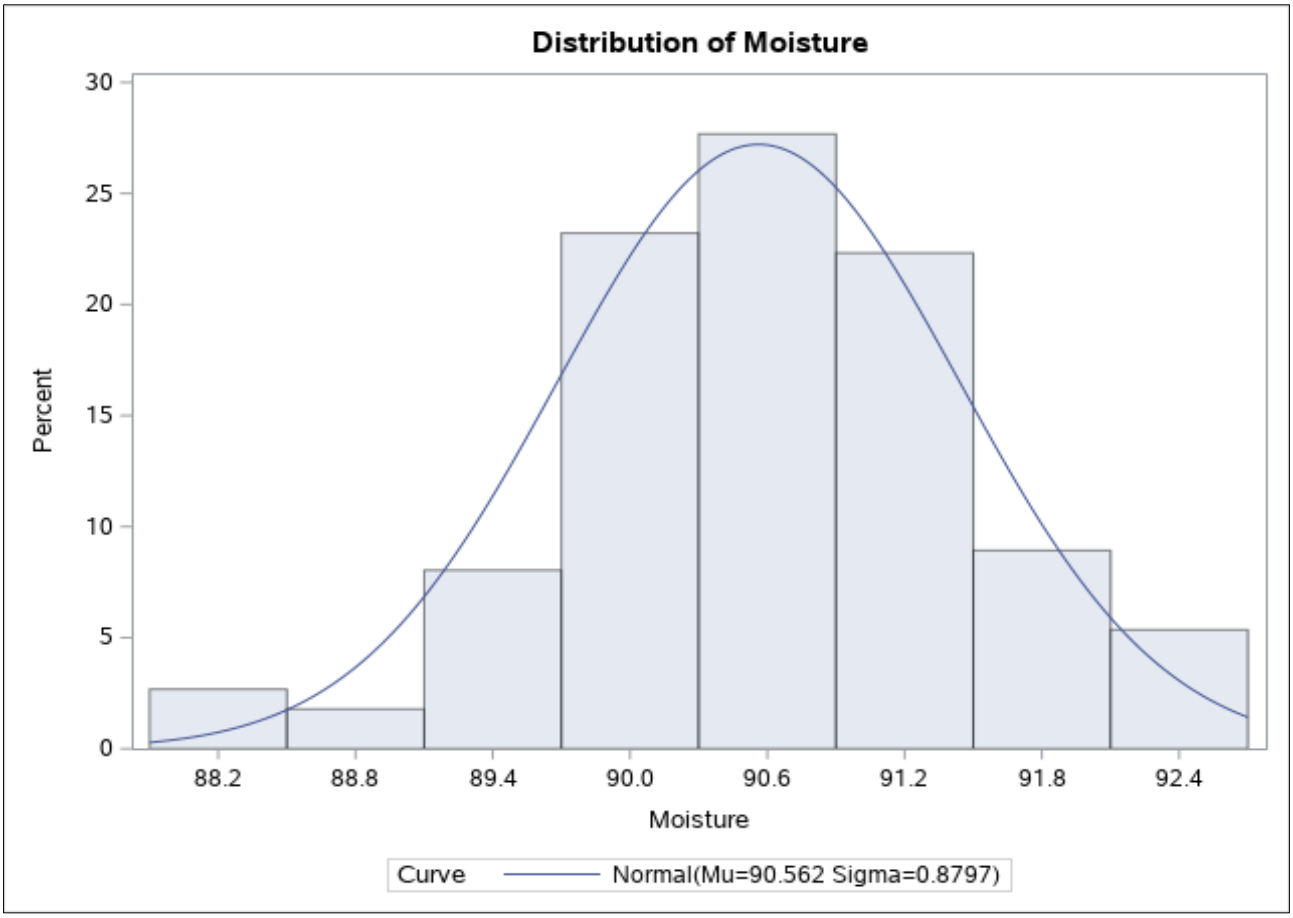
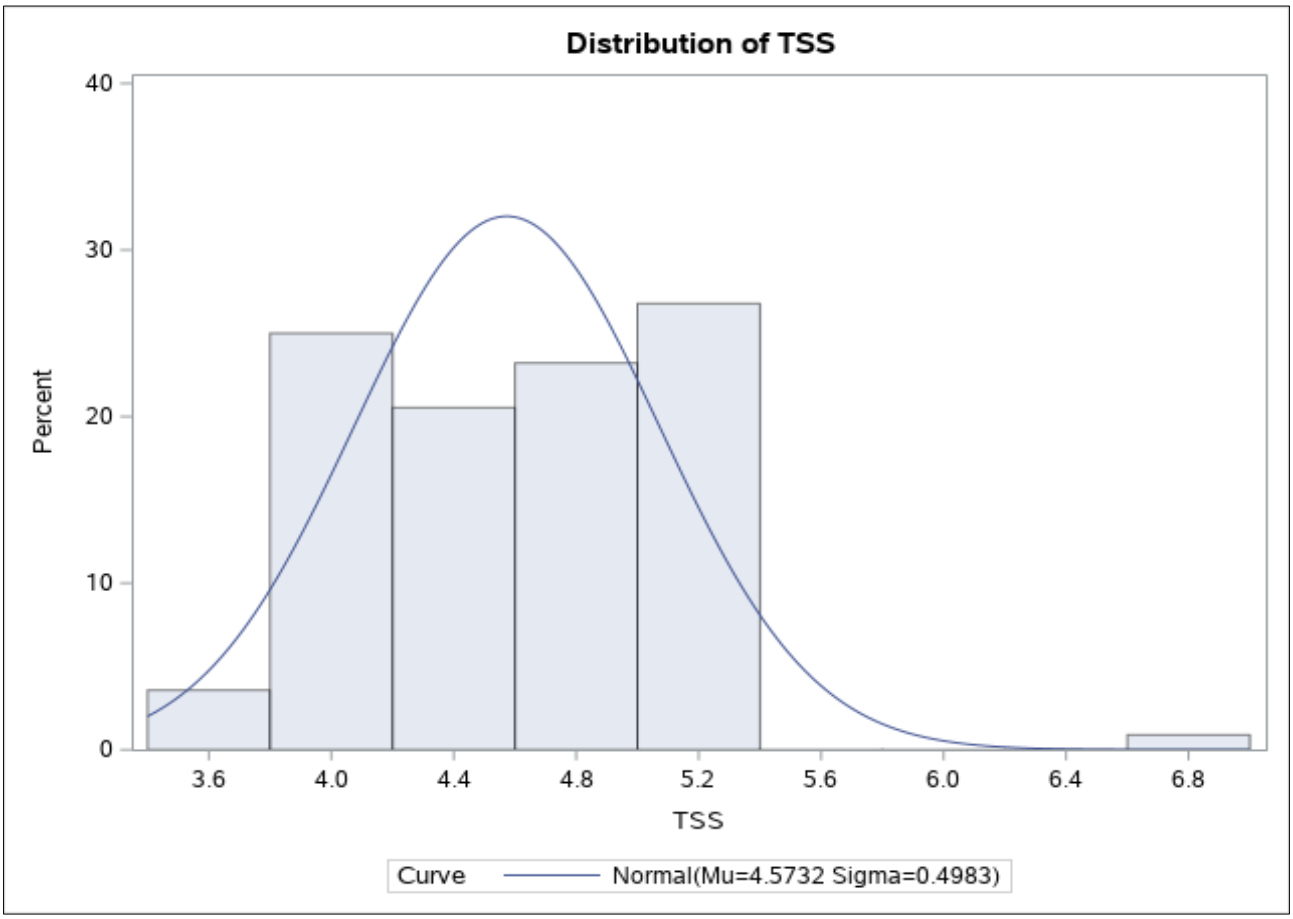
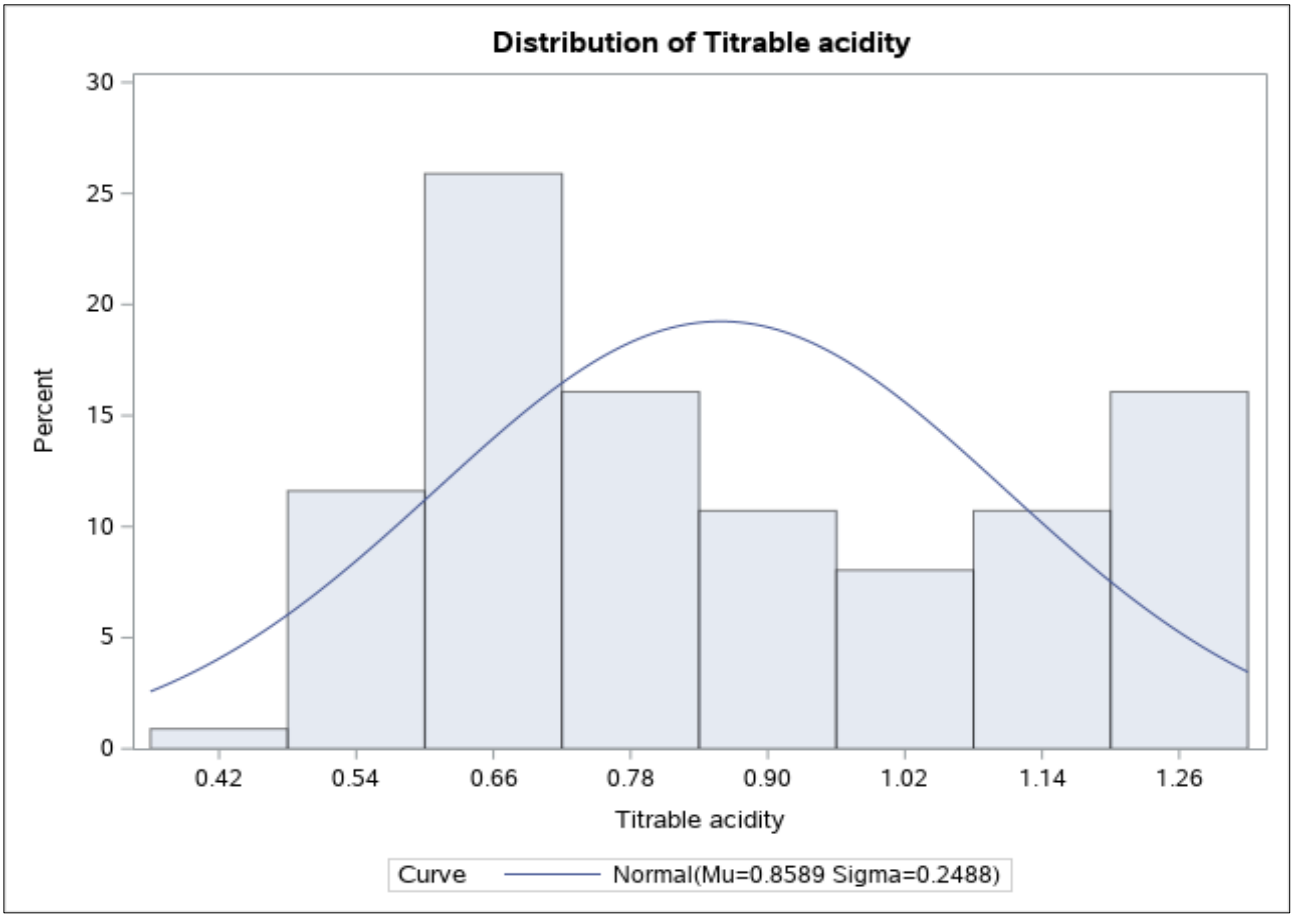
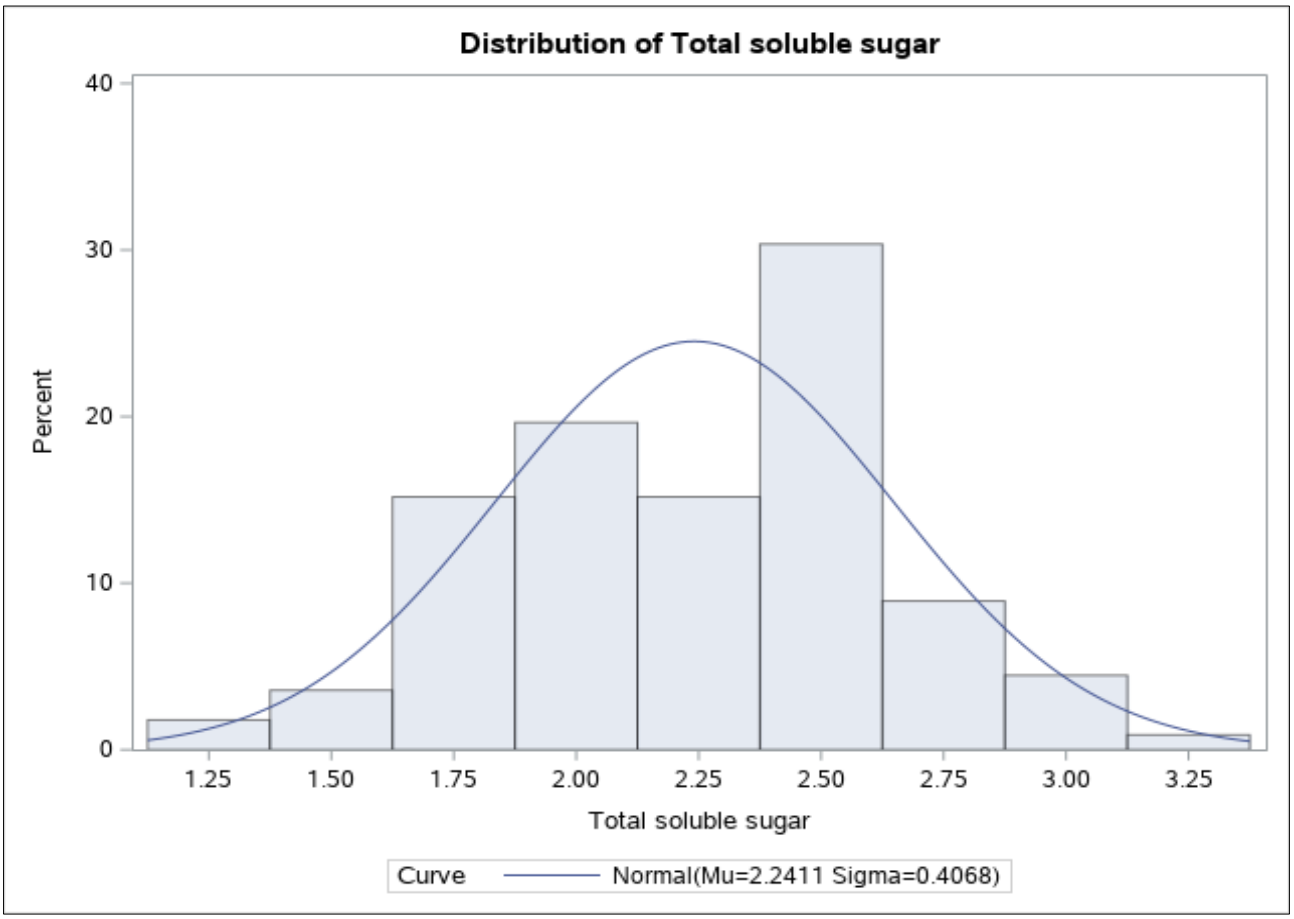
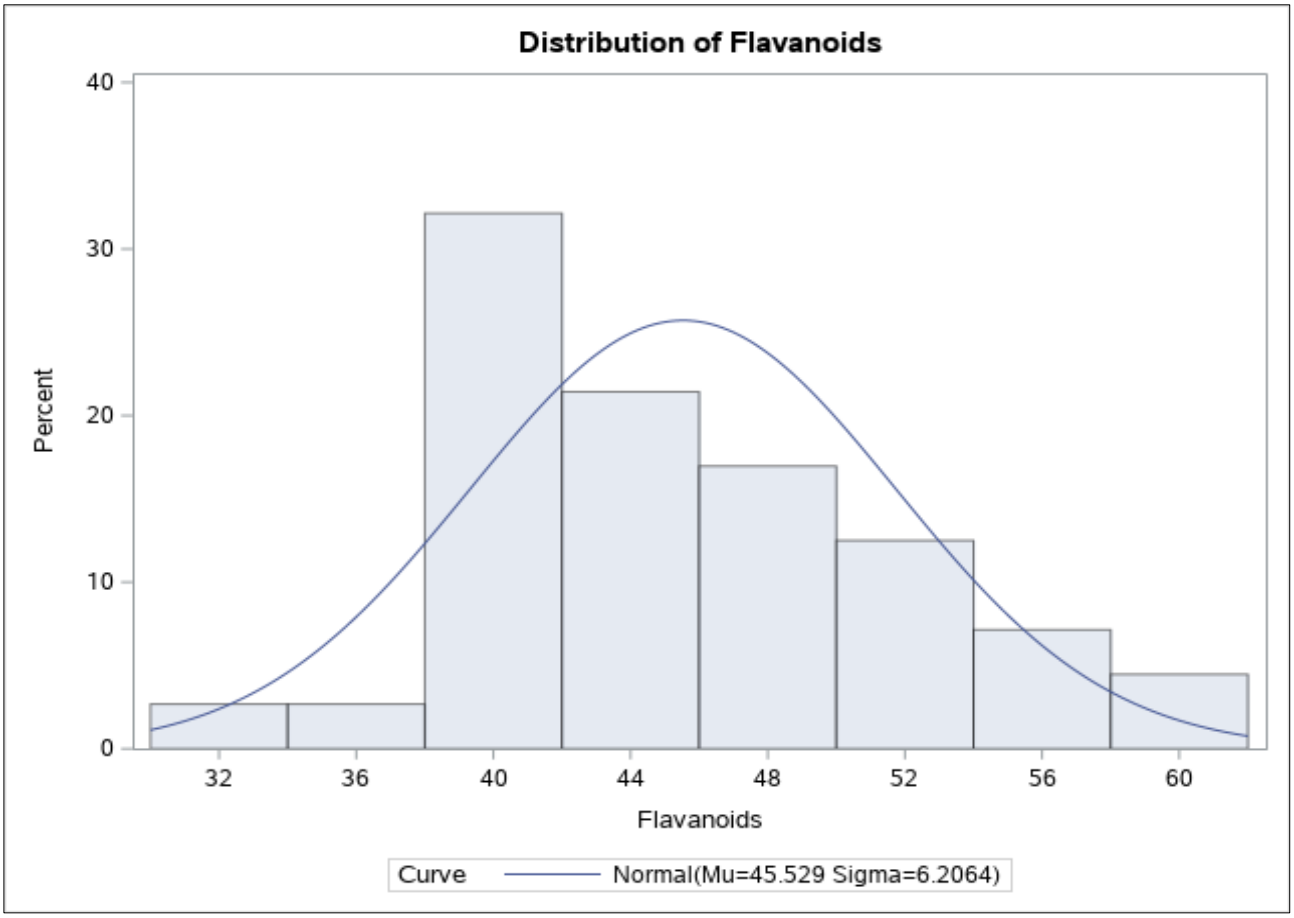


Fig 1 (A): Frequency distribution of different characters in $F_{2:3}$ population


Distribution of TSS
Percent
40
30
20
10
0
3.6
4.0
4.4
4.8
5.2
5.6
6.0
6.4
6.8
TSS
Curve
Normal(Mu=4.5732 Sigma=0.4983)


Distribution of Titrable acidity
Percent
30
25
20
15
10
5
0
0.42
0.54
0.66
0.78
0.90
1.02
1.14
1.26
Titrable acidity
Curve
Normal(Mu=0.8589 Sigma=0.2488)


Distribution of Total soluble sugar
Percent
40
30
20
10
0
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
3.25
Total soluble sugar
Curve
Normal(Mu=2.2411 Sigma=0.4068)


Distribution of Flavanoids
Percent
40
30
20
10
0
32
36
40
44
48
52
56
60
Flavanoids
Curve
Normal(Mu=45.529 Sigma=6.2064)

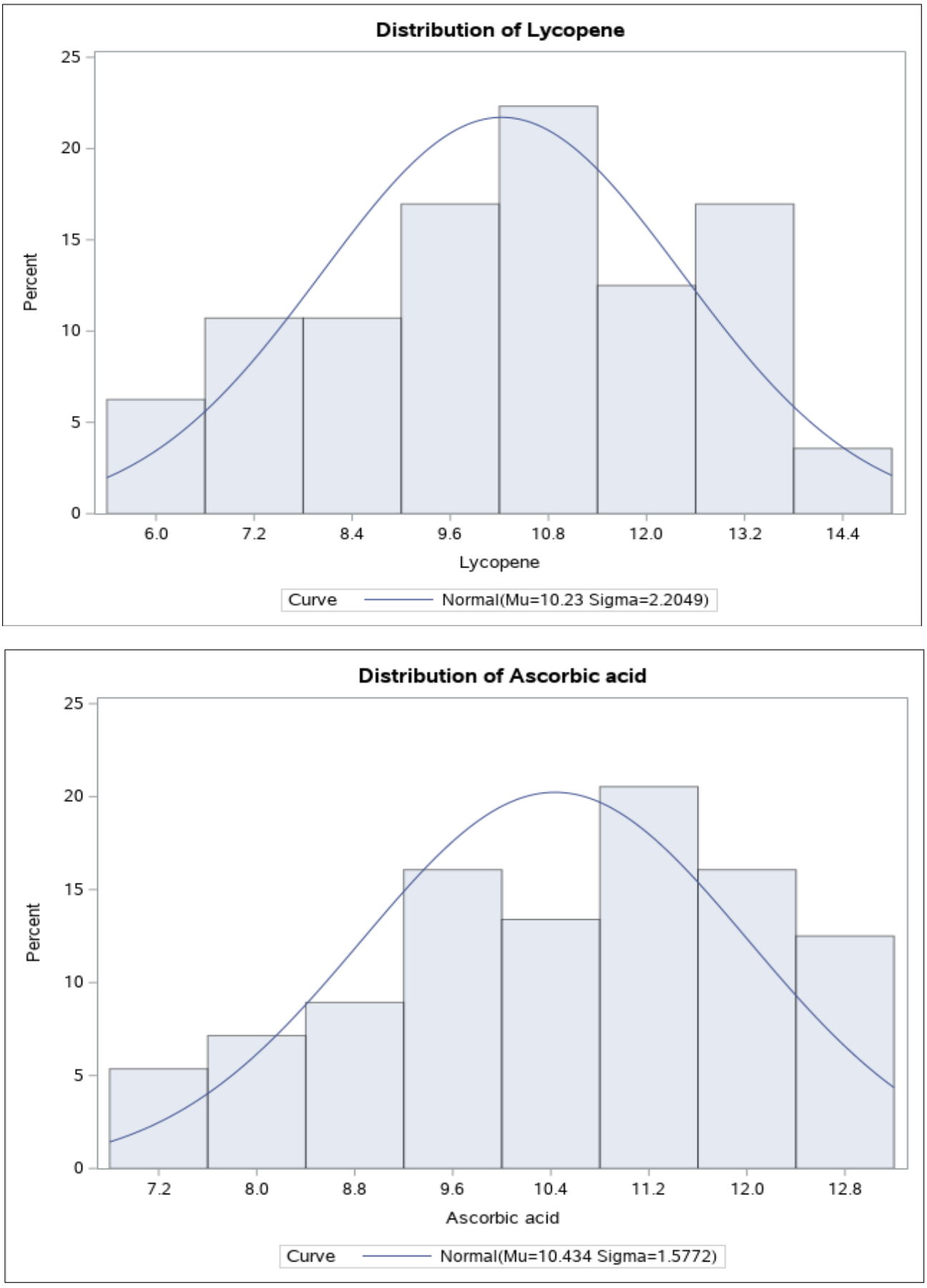


Fig 1 (B): Frequency distribution of different characters in $F_{2:3}$ population

The plant height, disease incidence score, phenol, total soluble solids, terrible acidity, and flavonoids shows positive skewness with value of 0.104, 0.588, 0.057, 0.681, 0.249 and 0.451, respectively indicate complementary epistatic gene action. While character like number of branches per plant, number of fruits per plant, moisture, total soluble sugars, lycopene and ascorbic acid shows negative skewness with the value of -0.111, -0.107, -0.288, -

0.144, -0.228 and -0.311, indicates it duplicate epistasis gene action governed by large number genes. Our results are in accordance with findings reported by Patel *et al.* (2018) [18]. They have described positive skewness and platykurtic nature for phenol content while, plant height was negative skewed and platykurtic in nature.

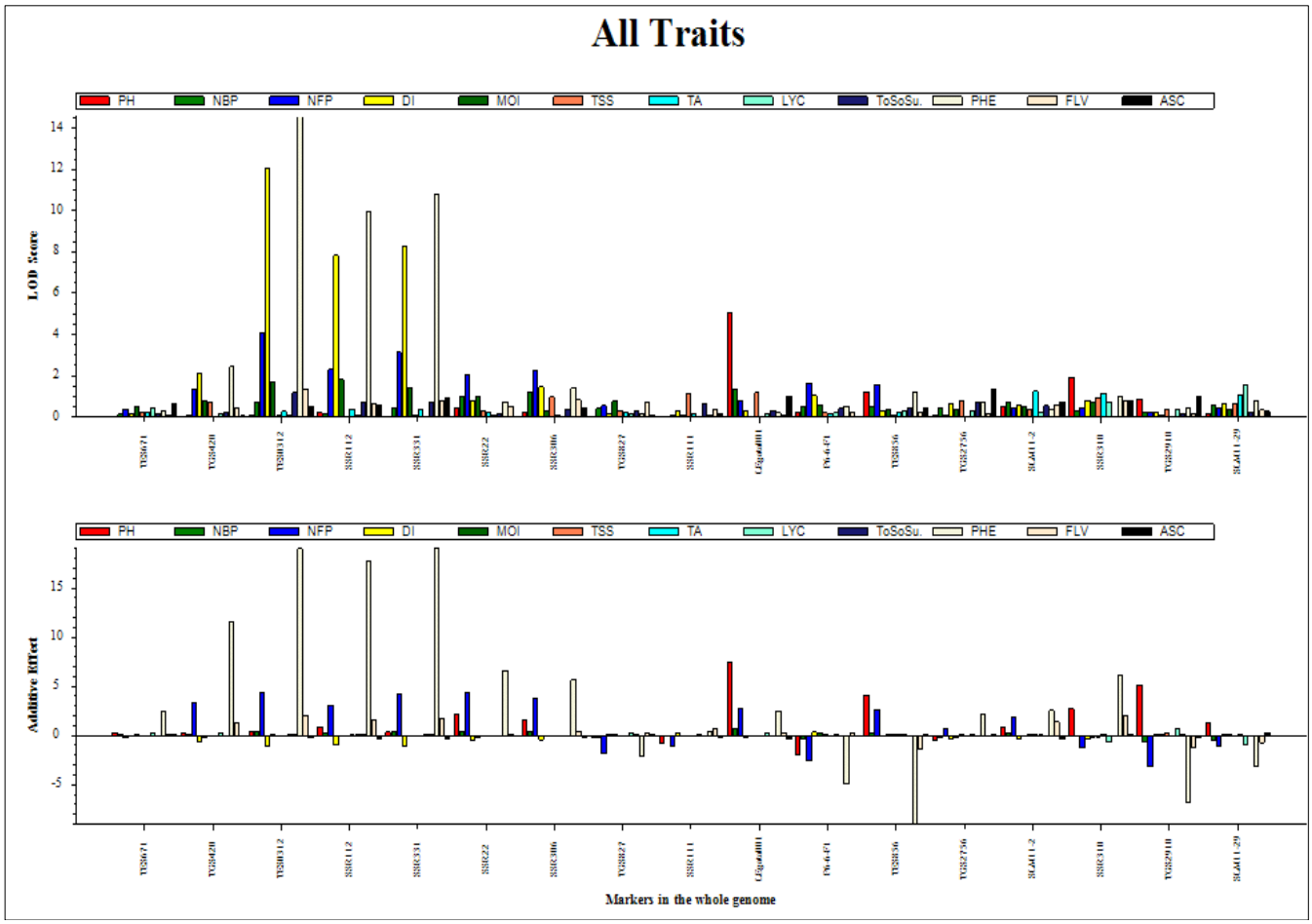
Single Marker Analysis (SMA)

Single Marker Analysis was mainly performed for each marker locus without considering information from other loci. It also gives information on whether there is significant difference between the genotype classes and the marker locus**.**

Table 5: Markers linked to Morphophysiological and biochemical traits in tomato $F_{2:3}$ population of the cross ATL-97-26 × GP-11 using single marker analysis (SMA)

Trait Name	Chromosome	Position	Marker Name	LOD	PVE (%)	Add.	Dom.
Plant height	2	90.93	LEgata001	5.02	18.99	7.47	3.26
Number of fruits per plant	1	104.92	TES0312	4.10	8.91	4.38	2.69
	1	119.57	SSR331	3.14	6.95	4.23	1.92
Disease incidence (Score)	1	104.92	TES0312	12.05	15.94	-1.07	-0.74
	1	113.97	SSR112	7.83	11.25	-0.91	-0.62
	1	119.57	SSR331	8.29	11.79	-1.01	-0.51
Total phenol content	1	104.92	TES0312	14.56	17.67	19.01	10.80
	1	113.97	SSR112	9.94	13.18	17.76	6.68
	1	119.57	SSR331	10.77	14.05	19.13	6.40

The single marker namely LEgata001 found significantly linked to plant height with phenotypic variation of 18.99%. Marker LEgata001 were significantly linked to number of branches per plant with phenotypic variation of 5.55%. Two markers *i.e.*, TES0312 and SSR331 were significantly linked with number of fruits per plant with phenotypic variation of 8.91 and 6.95%, respectively. Three markers TES0312, SSR112 and SSR331were significantly linked with disease incidence score by phenotypic variation of 15.94, 11.25 and 11.79%, respectively with negative additive effects suggest the occurrence of TLCV disease is governed by the male parent GP-11, similar above three markers also significantly linked with phenol content with phenotypic variation of 17.67, 13.18 and 14.05%, respectively. The additive effect for phenol content is found positive indicates phenol content is mostly governed by the female parent ATL-97-26 which is resistant against the TLCV (Table.5).



PH= plant height (cm) **NBP**=number of branches per plant, **NFP**=Number of fruits per plant, **DI=** Disease incidence (score) **PHE**=Total phenol (mg/100g), **MOI**=Moisture (%), **TSS**=Total soluble solids (^{0}Brix), **TA**=Titrable acidity (%), **ToSoSu**= Total soluble sugars (%), **FLV**= Total flavonoids (mg/100g), **ASC**=Ascorbic acid (mg/100g)

Fig 2: Graphical representaion of marker-trait association in tomato $F_{2:3}$ population of the cross ATL-97-26 × GP-11 using single marker analysis (SMA)

There are no any significant marker trait associations are found for the remaining traits *i.e.*, total soluble solids, titrable acidity, total soluble sugars and lycopene might be due to lack of variability in these traits.
Co-localizations of marker for phenol content and disease incidence with opposite additive effects explain negative correlation between the two traits. EST derived SSR112 was mapped on ninth linkage group of tomato at 80.0 cM position according to Tomato EXPEN 2000 genome (www.solgenomics.net). It is derived from gene, beta-fructosidase of tomato. Benhamou *et al.* (1991) [4] reported defensive role of beta fructosidase in tomato cell walls of tomato roots after infection of fungal wilt pathogen.

Conclusion
Tomato leaf curl virus (TLCV) is a very harmful viral disease of tomato crops in tropical and warm temperate countries of the world. Identification of DNA markers tightly linked to genes/quantitative trait loci (QTL) controlling TLCV resistance would assist enhanced pace and efficiency of breeding tomato for TLCV resistance.

Future Scope
The SSR markers *viz.*, TES0312, SSR112 and SSR331 needs to be validated in other genetic backgrounds of Tomato. These markers will be useful for indirect selection of resistant tomato lines/genotypes in early segregating generations and marker assisted backcross breeding for TLCV resistance.

Conflict of Interest: no competing interests exist.

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