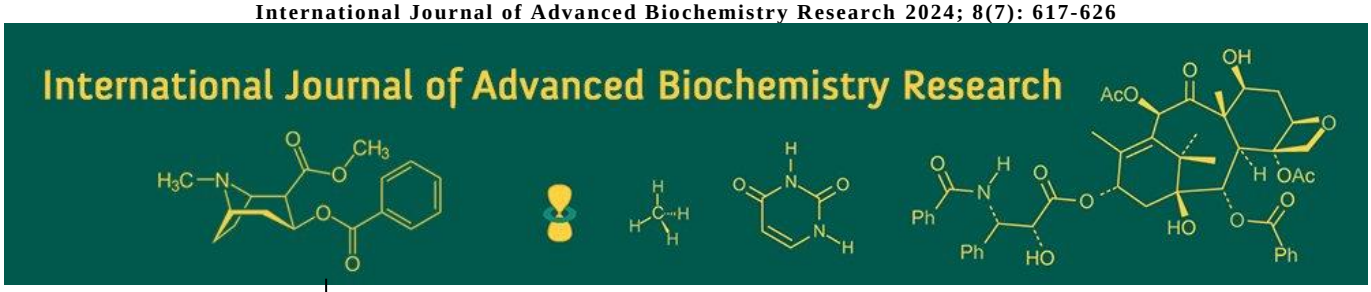

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Biochemical changes in chilli (*Capsicum annuum* L.) leaves infected with chilli leaf curl virus (CLCV)

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Abstract
Chilli leaf curl virus (CLCV) is indeed a highly destructive disease in chilli plants, often leading to severe losses, sometimes as high as 100%. The virus is primarily transmitted by the whitefly, Bemisia tabaci. In a study focusing on the $F_{2:3}$ mapping population of chilli plants, which showed variation in resistance and susceptibility to CLCV, several biochemical parameters were measured and correlated to understand the disease resistance mechanisms. The study involved a total of 120 contrasting genotypes. Among these, 14 plants each of highly resistant and highly susceptible types were selected for further biochemical analysis. For biochemical parameters like chlorophyll (0.28-0.83 mg/g), moisture (34.46-89.81%), total phenol (0.95-3.14 mg/g), total soluble sugar (2.28-7.70 mg/g) and membrane injury (32.10-65.24%). These biochemical parameters were then analyzed for their correlation with disease resistance. Such studies are crucial in identifying key traits and developing resistant varieties of chilli, thereby reducing the impact of Chilli Leaf Curl Virus (CLCV).

Keywords: Chilli leaf curl virus, chlorophyll, moisture, total phenol, total soluble sugar, membrane injury

Introduction
Chilli (*Capsicum annuum* L.) is a highly significant vegetable and spice crop cultivated globally, thriving in both tropical and subtropical zones. The genus Capsicum comprises thirty species, five of which are commonly cultivated: *Capsicum annuum* L., *C. frutescens* L., *C. chinense* Jacq, *C. pubescens* R. & P., and *C. baccatum* L. According to Bosland and Votava (2000) [3], Wang and Bosland (2006) [29], and Ince *et al*. (2010) [13], chilli fruits contain natural bactericides and capsaicin, which is reputed to have anti-cancerous properties. They are also rich in vitamins A, C, and E, as well as being a good source of potassium and folic acid. Fresh red chilies boast higher vitamin A content than carrots, while fresh green chilies have more vitamin C than citrus fruits.

Leaf curl disease has become a major issue for chilli cultivation in India, leading to significant yield losses. The disease is viral in origin, and its symptoms include vein clearing, upward curling, and deformation of leaves. Infected plants also exhibit stunted growth and flower buds abscise prematurely, further contributing to the reduction in yield. This disease poses a significant threat to chilli production and necessitates effective management strategies to mitigate its impact. The alteration of normal metabolism in a host plant following infection is a widespread phenomenon. Symptoms resulting from infections caused by pathological organisms are invariably linked to biochemical changes within the plant tissues.

These changes can impact various physiological and metabolic processes, leading to the visible symptoms and often affecting the overall health and productivity of the plant. Understanding these biochemical alterations is crucial for developing effective strategies to manage plant diseases. To gain a comprehensive understanding of host-pathogen interactions, it is essential to quantitatively estimate proteins, carbohydrates, enzymes, and other biochemical constituents in the host plant. This quantitative analysis allows for drawing meaningful conclusions about the interactions between the host and the pathogen. Therefore, a study has been undertaken to examine the biochemical changes in chilli *Capsicum annuum* leaves infected with Gemini virus.

This investigation aims to shed light on the metabolic alterations induced by the pathogen and their implications for the plant's health and disease resistance.

Materials and Methods

Experimental material

$F_{2:3}$ segregating generation from a hybrid between a susceptible cultivated genotype (ACS 18-08) and CLCV resistance genotype of chilli ACCMS 1 (*C. annuum*) used as the experimental material for this study. Field experimnet was conducted at Main vegetable Research station, Anand Agriculture University, Anand, Gujarat by using Randomized Block Design and laboratory parameters were recorded at Department of Biochemistry, Anand Agriculture University, Anand, Gujarat. From segregating population 14 healthy and 14 disease chilli leaves samples were collected for analysis of biochemical parameters like chlorophyll, moisture, total phenol, total soluble sugar and membrane injury.

Biochemical parameters

Estimation of chlorophyll

Hiscox and Israelstam (1979) [12] determined the amount of chlorophyll present in chilli leaf samples. 10 mL of dimethyl sulfoxide (DMSO) were given to 50 milligrams of leaves in test tubes, which were then left overnight. The wavelengths at 663 nm and 645 nm were then measured by using given formula.

$$\text{Chlrophyll a (mg/g f.w.)} = \frac{12.7\times \text{O.D.at 663 nm} - 2.69\times \text{O.D.at 645 nm}\times 10}{1000\times 0.05}$$

$$\text{Chlrophyll b (mg/g f.w.)} = \frac{22.7\times \text{O.D.at 645 nm} - 4.68\times \text{O.D.at 663 nm}\times 10}{1000\times 0.05}$$

Total chlorophyll (mg/g f.w.) = Chlorophyll a + Chlorophyll b

Estimation of moisture

The moisture content of the chilli leaves was carried out using the method that A.O.A.C. (2000) [2]. The little aluminium boxes contained a sample of 5 g of chilli leaves. Each seed box was weighed before being placed in the hot air oven, which was set to 105 °C for six hours. After being removed from the oven, each box was given time to cool at room temperature.

$$\text{Moisture} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g) x100}}{\text{Fresh weight (g)}}$$

Estimation of total phenol

Total phenol was estimated as a method described by Dhruv *et al*. (2021) [8]. One gram of sample was homogenized in 80% methanol using mortar and pestle and the final volume was made to 10 mL. The content was refluxed for two hours on boiling water bath at 65 °C. Supernatant was collected and the residue was re-extracted twice with 80% methanol. All supernatants were combined, and the final volume was made to 10 mL. The extract was used for the assay of total phenol. Aliquot 0.2 mL was taken and made the final volume 1.0 mL with distilled water. For standard, catechol 50 to 250 microgram working standard in water was prepared. In separate test tubes. 0.2, 0.4, 0.6, 0.8, and 1 mL of the working standard solution were pipetted out into a series of test tubes and made the final volume 1.0 mL with distilled water. To this add 1 mL of folin reagent and after 3 min 2 mL of 20% Na_2CO_3 was added, and the tubes were incubated at room temperature for 30 min and final volume was made to 5 mL with distilled water. The absorbance was measured at 650 nm. Phenol content was calculated by using following formula.

$$\text{Phenol} = \frac{\text{Reading x Graph factor x Total volume x } 10^{-6}}{\text{Taken volume x Sample weight (g)}}$$

Estimation of total soluble sugar

Total soluble sugars were determined using the phenol-sulphuric acid method as described by Dubois *et al.,* (1956) [7] with some modification. Total Soluble sugar was extracted from 0.5 g of chilli leaves in 5 mL 80% methanol. Which was then incubated for 2 hr a shaker. After incubation centrifugation was done and clear supernatant was collected in another test tube. Supernatant was then evaporated in boiling water bath. To the residue 1 mL of distil water was added and used to quantified sugar content. For stock solution 500 mg of reagent grade glucose dissolved in 100 mL of distilled water. Then 1 mL of aliquot and diluted further to 100 mL which gave 50 µg/mL, from this 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the working standard glucose solution was used for standard curve preparation in the range of 25 to 125 µg. Makeup the final volume to 3 mL with the addition of distilled water. Add 5% of 1 mL phenol in all test tubes. After that incubation for 3 min at room temperature, 5.0 mL concentrated sulphuric acid was carefully added to the side of the tube. After mixing thoroughly the tubes were kept for 10 minutes at room temperature and 20 min in a cold-water bath for color development. The absorbance was measured at 490 nm. Total soluble sugar content was calculated by using given formula.

$$\text{Total Soluble Sugar} = \frac{\text{Reading x Graph factor x Total volume x } 10^{-6}}{\text{Taken volume x Sample weight (g)}}$$

Estimation of membrane injury

Membrane damage was assessed by slightly altering Sulivan's (1971) [27] methodology. To measure the amount of membrane damage in fresh chilli leaves, 0.1 g were collected. These tissues were put into test tubes with 100 mL of purified water in them. For thirty minutes, they were maintained in a 40 °C hot water bath. Samples were allowed to cool to room temperature after 30 minutes, at which point electrical conductivity for the control and treatment groups (C_1 and T_1) was evaluated. After that, the tubes were maintained in a bath of boiling water at 100 °C for fifteen minutes. Electrical conductivity (EC) was measured once more after cooling (C_2 and T_2).

$$\text{MI} = 1 - \frac{1 - T1/T2}{1 - C1/C2} \times 100$$

Where,

T_1 = Electrical conductivity of disease sample at 40 °C for 30 min

T_2 = Electrical conductivity of disease sample at 100 °C for 30 min

C_1 = Electrical conductivity of healthy sample at 40 °C for 30 min

C_2= Electrical conductivity of healthy sample at 100 °C for 30 min

Disease Incidence (CLCV Incidence)

After every 15 days starting one month after the donation, CLCV infection symptoms were evaluated. The severity of

CLCV disease was scored on a six-point 0-5) scale given by Thakur *et al*. (2020) [28].
0 = 0% incidence (highly resistant)
1= 0-15% incidence (resistant)
2= 6-25% incidence (moderately resistant)
3= 26-50% incidence (moderately susceptible)
4= 51-75% incidence (susceptible)
5= 75-100% incidence (highly susceptible)

Statistical analysis

Analysis of Variance

To assess the variations in genotypes for all parameters, the Panse and Sukhatme (1967) [17] analysis of variance technique were used.

Phenotypic (PCV) and genotypic (GCV) coefficients of variations

Utilizing the formulas provided by Burton and Devane (1953) [4], the phenotypic and genotypic coefficients of variation were computed. Genotypic coefficient of variation (GCV%).

Genotypic coefficient of variation (GCV%)

Genotypic coefficient of variation was computed using the following formula.

$$GCV\% = \frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100$$

Where,
$\overline{X}$ = General mean of the character under study, σ^2_g = Genotypic variance

Phenotypic coefficient of variation (PCV%)

Phenotypic coefficient of variation was computed using the following formula.

$$PCV\% = \frac{\sqrt{\sigma\, p^2}}{\bar{x}} \times 100$$

Where,
$\overline{X}$ = General mean of the character under study
σ^2_p = Phenotypic variance

< 10% Low, 10-20% Moderate and > 20% High
Classification of PCV and GCV were done following the method as suggested by Robinson *et al*. (1949) [22].

Heritability

The broad sense heritability (h^2b) was calculated for both the characters by dividing genotypic variance and the phenotypic variance. The method followed was suggested by Johnson *et al*. (1955) [14].

$$h^2_b\ (\%) = \frac{\sqrt{\sigma^2 g}}{\sqrt{\sigma^2 p}} \times 100$$

Where,
h^2_b = Heritability (broad sense) σ^2_g = Genotypic variance, σ^2_p = Phenotypic variance

Classification of heritability was done by following a method as suggested by Robinson *et al*. (1949) [22].
< 30% Low, 30-60% Moderate and > 60% High

Genetic advance (GA)

It was calculated the improvement rate in the mean of each genotype value of selected plants over the parental population. It was performed by using the methodology suggested by Johnson *et al.* (1955) [14] at 5 per cent selection intensity using the constant 'k' as 2.06.

$$GA = K \times h^2_b \times \sigma_p$$

Where,
h^2 (bs)=Heritability in broad sense
σ_p=Phenotypic standard deviation of the trait
K=Standard selection differential which is 2.06 at 5 per cent selection intensity

Genetic advance as per cent mean (GAM)

The genetic advance express as per cent of mean was calculated as per formula the method suggested by Johnson *et al.* (1955) [14]

$$GA\ (\%\ of\ mean) = \frac{GA}{X} \times 100$$

0-10% (low), 10-20% (moderate) and 20% & above (high)

Correlation analysis

Correlation analysis was performed by using R software V4.3.1

Test of Normality analysis

Skewedness and kurtosis were calculated by SPSS system (IBM SPSS version 20)

Results and discussion

Chlorophyll content was observed 0.28 mg/g in susceptible parent and 0.83 mg/g in resistant parent. In case of resistant genotypes range of chlorophyll was (0.64-0.77 mg/g), which was higher as compared with range of susceptible genotype (0.26-0.41 mg/g). Resistant parent was significantly higher at over susceptible parent and remaining genotype.
While in case of susceptible parent shown lower at par with all plants and resistant parents. The findings of the result match with decrease (0.14 mg/g) in chlorophyll content in infected leaves was due to chlorosis and necrosis of diseased plant parts especially leaves as compared to healthy (0.49 mg/g) leaves which were found to be in line with Meena *et al*. (2016) [16], Ghai *et al*. (2016) [10], Chaudhry *et al*. (2019) [5], Zhang *et al.* (2019) [30], Rahevar *et al*. (2021) [19] and Gor *et al*. (2024) [11].
Moisture content was recorded in resistant parent 89.81% which was higher as compared with suspectable parent 41.66%. In case of susceptible genotypes moisture content range was lower with the comparison of resistant genotype 34.46-47.35% and 70.13-89.13%, respectively. Moisture content in genotypes1 (88.09%), 2 (89.13%) and 5 (86.73%) which were statistically higher at par over susceptible genotypes. The result revelled that the biochemical characters *viz*., moisture higher (90.86-79.22%) in resistant variety Chaudhry *et al*. (2019) [5], Aliu *et al*. (2017) [1], Ghai *et al*. (2016) [10] Rahevar *et al*. (2021) [19], Patel *et al*. (2022) [18]. According to Salaria *et al*. (2023) [26] in stressed chilli plants, there was loss in moisture content of leaves, due to the net photosynthetic rate was hampered which caused the reduction of transpiration rate and stomatal conductance.

Table 1: Biochemical parameters from chilli leaves of genotypes of $F_{2:3}$ genotypes

Genotypes	CHY (mg/g)	MOI (%)	PHE (mg/g)	TSS (mg/g)	MI (%)	DI (%)
1	0.68	88.09	0.98	3.76	32.44	0
2	0.66	89.13	0.99	3.94	37.33	0
3	0.63	83.61	1.01	2.28	43.81	0
4	0.65	85.21	1.02	4.02	35.19	0
5	0.75	86.73	1.19	3.27	40.71	0
6	0.70	81.37	0.95	3.92	40.99	0
7	0.71	75.74	1.08	3.89	35.47	0
8	0.64	70.74	1.27	3.33	32.10	0
9	0.72	78.32	1.02	3.82	32.79	0
10	0.68	76.32	1.18	3.32	42.58	0
11	0.74	77.11	1.21	2.78	37.57	0
12	0.78	80.42	1.14	3.40	33.78	0
13	0.75	81.22	1.26	2.55	36.80	0
14	0.77	79.26	1.17	3.14	42.43	0
15	0.35	42.96	2.23	4.87	62.22	73.27
16	0.41	39.53	2.51	5.72	60.74	36.53
17	0.31	34.86	2.56	4.97	57.82	62.17
18	0.34	45.94	3.00	6.91	54.20	58.14
19	0.31	37.82	3.06	5.98	59.76	52.80
20	0.40	40.46	3.14	5.46	55.88	39.24
21	0.36	36.67	2.38	7.60	64.14	14.54
22	0.37	38.87	2.53	5.98	51.48	45.67
23	0.31	41.6	2.59	5.28	48.79	61.80
24	0.34	39.01	2.74	6.85	56.64	37.37
25	0.26	42.33	2.66	7.70	57.75	67.74
26	0.38	47.35	2.51	5.34	65.24	64.70
27	0.36	34.46	2.82	6.75	58.51	67.87
28	0.35	39.35	2.68	6.03	50.79	65.80
Min	0.26	34.46	0.95	2.28	32.10	0
Max	0.77	89.13	3.14	7.70	65.24	73.27
ACCMS 1	0.83	89.81	1.09	3.07	32.55	0.73
ACS 18-08	0.28	41.66	3.00	6.38	61.43	76.20
S Em ±	0.010	1.651	0.043	0.065	1.72	1.022
CD at 5%	0.027	4.673	0.120	0.183	4.88	2.848
CV%	3.18	4.70	3.89	2.36	6.30	10.88

Note: (CHY=Chlorophyll, MOI=Moisture, PHE=Phenol, TSS=Total Soluble Sugar, MI=Membrane Injury and DI=Disease Incidence)

Maximum phenol content (3.14 mg/g) was observed in genotype 20 and minimum phenol content (0.95 mg/g) in genotype 6. Phenol content in resistant parent 1.09 mg/g lower than susceptible parent 3.00 mg/g. Susceptible genotype had higher phenol content range (2.23-3.14 mg/g) than resistant genotype (0.95-1.26 mg/g). In genotype 40 had phenol content 3.06 mg/g which was statistically higher over the resistant genotype. The result of this study accordance with the phenol was higher in susceptible variety (4.19-1.28 mg/g) as compared to resistant variety (0.67-0.42 mg/g). Phenol content which was significantly and positively correlated with the population of thrips Chaudhry *et al*. (2019) [5], Dhaliwal *et al*. (2019) [6], Ridzuan *et al*. (2018) [21], Fratianni *et al*. (2020) [9], Rahevar *et al*. (2021) [19], Patel *et al*. (2022) [18]. According to Salaria *et al*. (2023) [26] phenol content in infected leaves 1.9 mg/g higher than healthy leaves (0.90 mg/g).

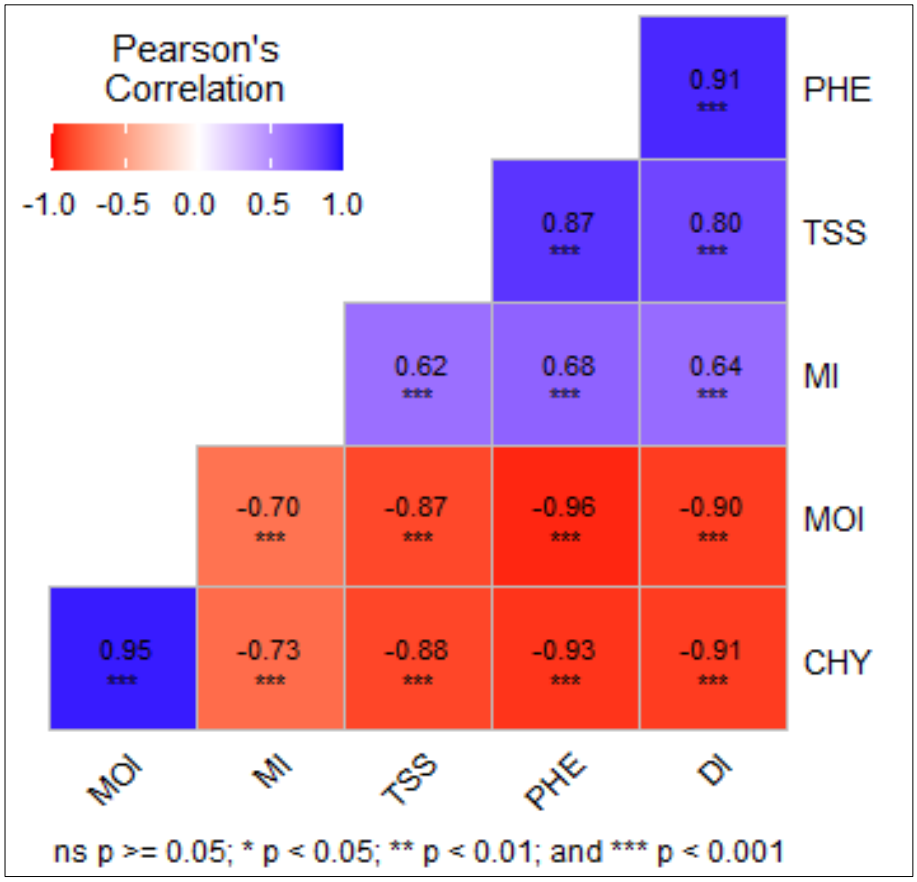
Total soluble sugar content was observed maximum 7.70 mg/g and minimum 2.28 mg/g in 25 and 3 genotypes, respectively. Total soluble sugar content in ACCMS 1 3.07 mg/g and in ACS 18-08 6.38 mg/g. Resistant genotypes range (2.28-3.94 mg/g) which was lower as compared to suspectable genotypes range (4.87-7.70 mg/g). In genotype 21 with total soluble sugar content 7.60 mg/g was statistically higher at par over suspectable genotype. Decreased photosynthesis by 50% with the significant increase in the contents of total soluble sugar was observed in leaves infected with chilli leaf curl virus. The result revealed that the total soluble sugar was significantly increased in diseased leaf as compare to healthy leaf and reached up to 3.90 mg/g of fresh weight of tissue, respectively, while in healthy leaf these were 3.60 mg/g of fresh weight of tissue, respectively. Mena *et al*. (2016), Chaudhry *et al*. (2019) [5], Zhang *et al*. (2019) [30]. According to Salaria *et al*. (2023) [26] total soluble sugar content in infected leaves (2.5 mg/g) higher than healthy leaves (2.0 mg/g).

Membrane injury is a widely used criterion to assess crop drought tolerance, since water deficit causes water loss from plant tissues which seriously impairs both membrane structure and function. Membrane injury was observed 32.55% in resistant parent and 61.43% in susceptible parent. In case of resistant genotypes range of membrane injury was (32.10-43.81%), which was lower as compared with range of susceptible genotypes (50.79-65.24%). Membrane injury in genotypes 15 (62.22%), 16 (60.74%) and 21 (64.14%) which were statistically higher over resistance genotypes. The findings of present investigation are in agreement with Salaria *et al*. (2023) [26] that in stressed chilli plants, there was loss membrane injury (%) of leaves due to net photosynthetic rate was hampered which caused the reduction of transpiration rate and stomatal conductance. Also, there was an enhancement in ROS activity that led to the oxidative burst as evidenced by the elevated levels of

H_2O_2. According to Rohitha *et al*. (2023) [23] membrane injury under stress condition it was 40% which was lower as compared with control condition 80% and also in agreement with present investigation result the membrane injury ranged 52.4-73.7% observed by Saadony *et al*. (2024) [24] in chilli.

Correlation study of disease scoring with biochemical traits

The Pearson's correlation (Figure 1) revelled a significant negative association between chlorophyll and moisture with disease incidence (r = -0.91*** and r = -0.90***, respectively) and significant positive association between membrane injury, total soluble sugar and phenol with disease incidence (r = 0.64*** and r = 0.80*** and r = 0.91***, respectively). Total soluble sugar content showed positive significant correlation with phenol (r = 0.87 ***). Phenol content had negative significant association with chlorophyll and moisture (r = -0.93*** and r = -0.96***, respectively). Moisture content had significant positive association with chlorophyll with value r = 0.95***.



Note: (CHY=Chlorophyll, MOI=Moisture, PHE=Phenol, TSS=Total Soluble Sugar, MI=Membrane Injury and DI=Disease Incidence)

Fig 1: Correlation coefficient analysis of biochemical parameters in $F_{2:3}$ genotypes in chilli

Result revealed that total phenol and total soluble sugar content positively correlated with disease incidence, while remaining biochemical parameters like chlorophyll, moisture and membrane injury found negatively correlated with disease incidence.

The findings suggested that moisture content in chilli negatively correlated with phenol reported by Rahevar *et al*. (2019) [20] which is similar with present study result and Lahbib *et al*. (2021) [15] noted that the correlation for total phenol content in chilli had positive correlation with total soluble sugar content matched with this study result.

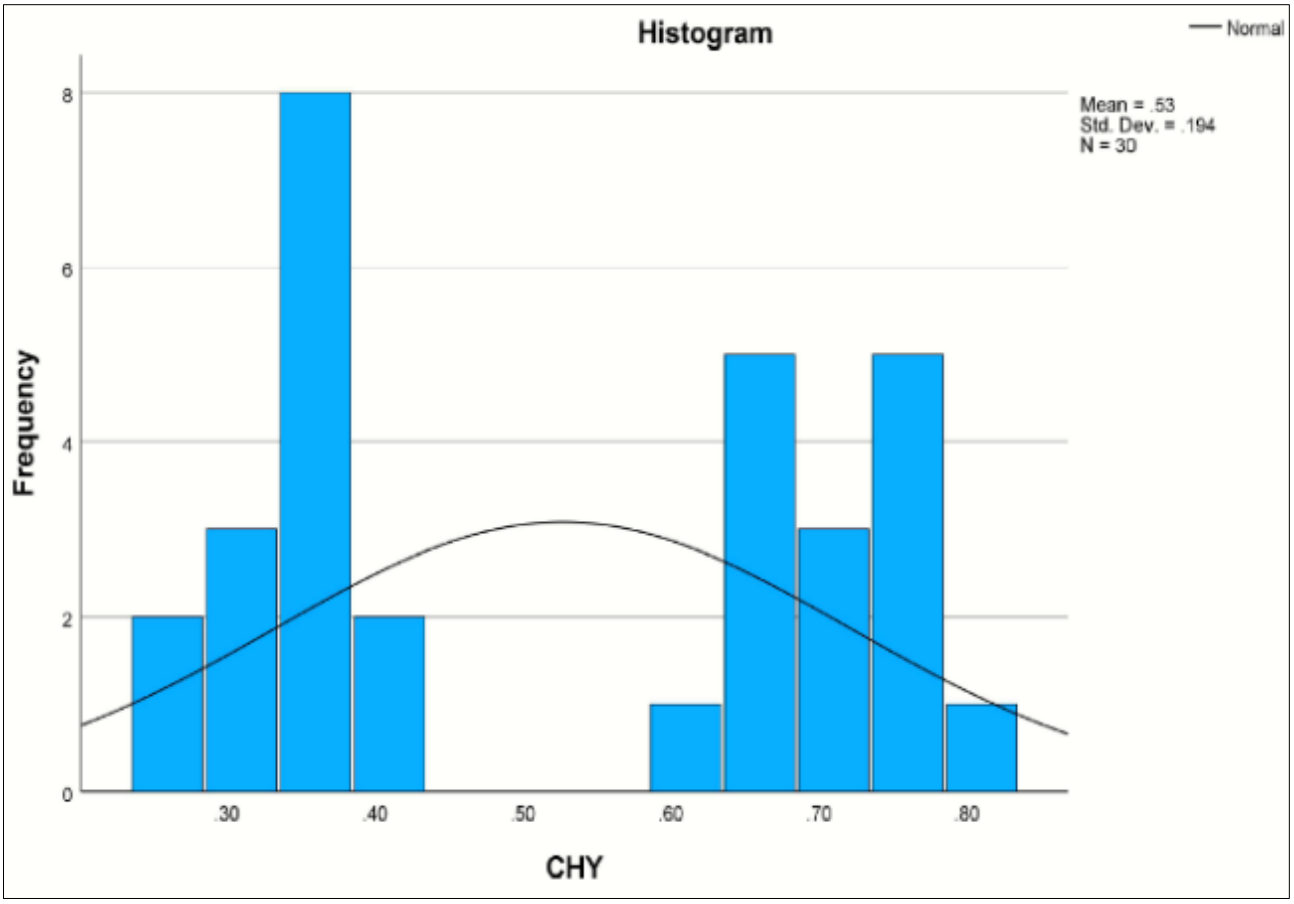
Test of Normality

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. (Table 2 and Figure 2).

Biochemical parameters like chlorophyll (0.059), moisture (0.056), total phenol (0.128) and total soluble sugar (0.298) were remarkable positive skewness. This indicates that greater number of genotypes than would be predicted from a normal distribution are below the mean. This indicated that more the genotypes would be predicted from a normal distribution are above the mean. All biochemical parameters like chlorophyll (-1.834), moisture (-1.945), total phenol (-1.874), total soluble sugar (-1.105) and membrane injury (-1.755) shown negative kurtosis. This indicated that average level of complementary gene activity.

Table 2: Skewness and kurtosis for biochemical traits of $F_{2:3}$ genotypes in chilli.

Sr. No.	Traits	Skewness	Kurtosis
1	Chlorophyll	0.059	-1.834
2	Moisture	0.056	-1.945
3	Total Phenol	0.128	-1.874
4	Total Soluble Sugar	0.298	-1.105
5	Membrane Injury	-0.127	-1.755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Mean = .53
Std. Dev. = .194
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Frequency
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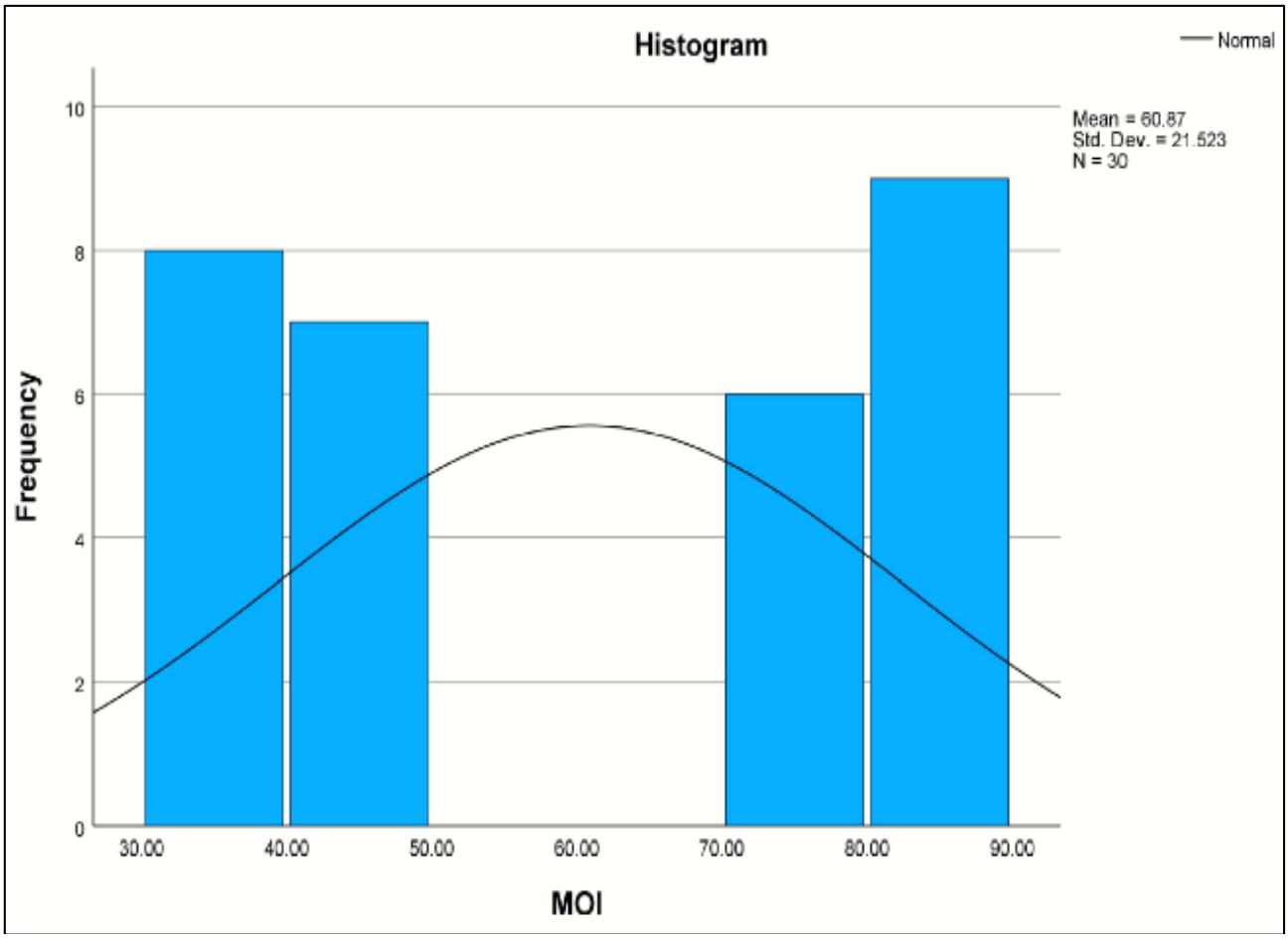
6
8
.30
.40
.50
.60
.70
.80
CHY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Frequency
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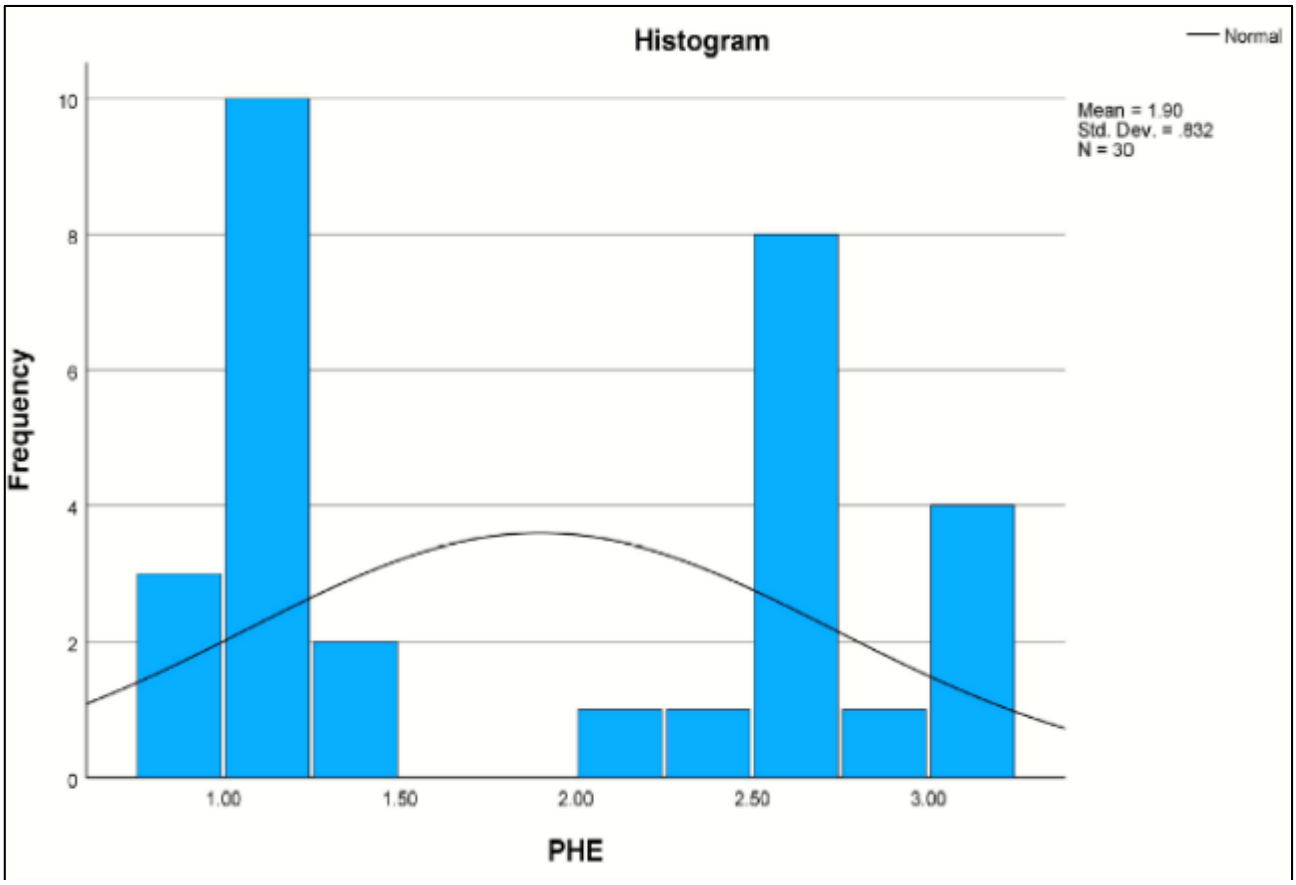
90.00
MOI


Histogram
Normal
Mean = 1.90
Std. Dev. = .832
N = 30
Frequency
PHE
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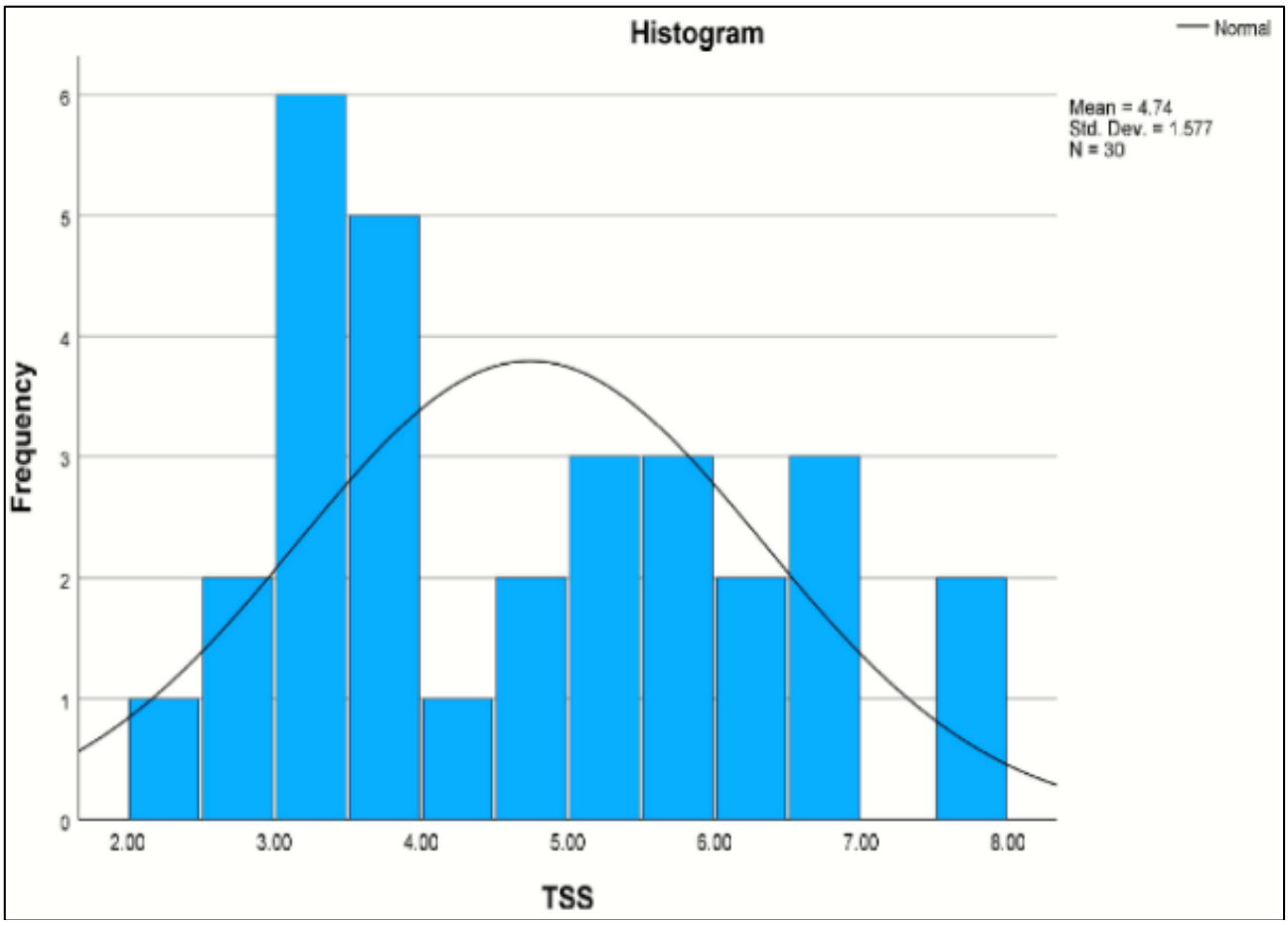
2
4
6
8
10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N = 30
Frequency
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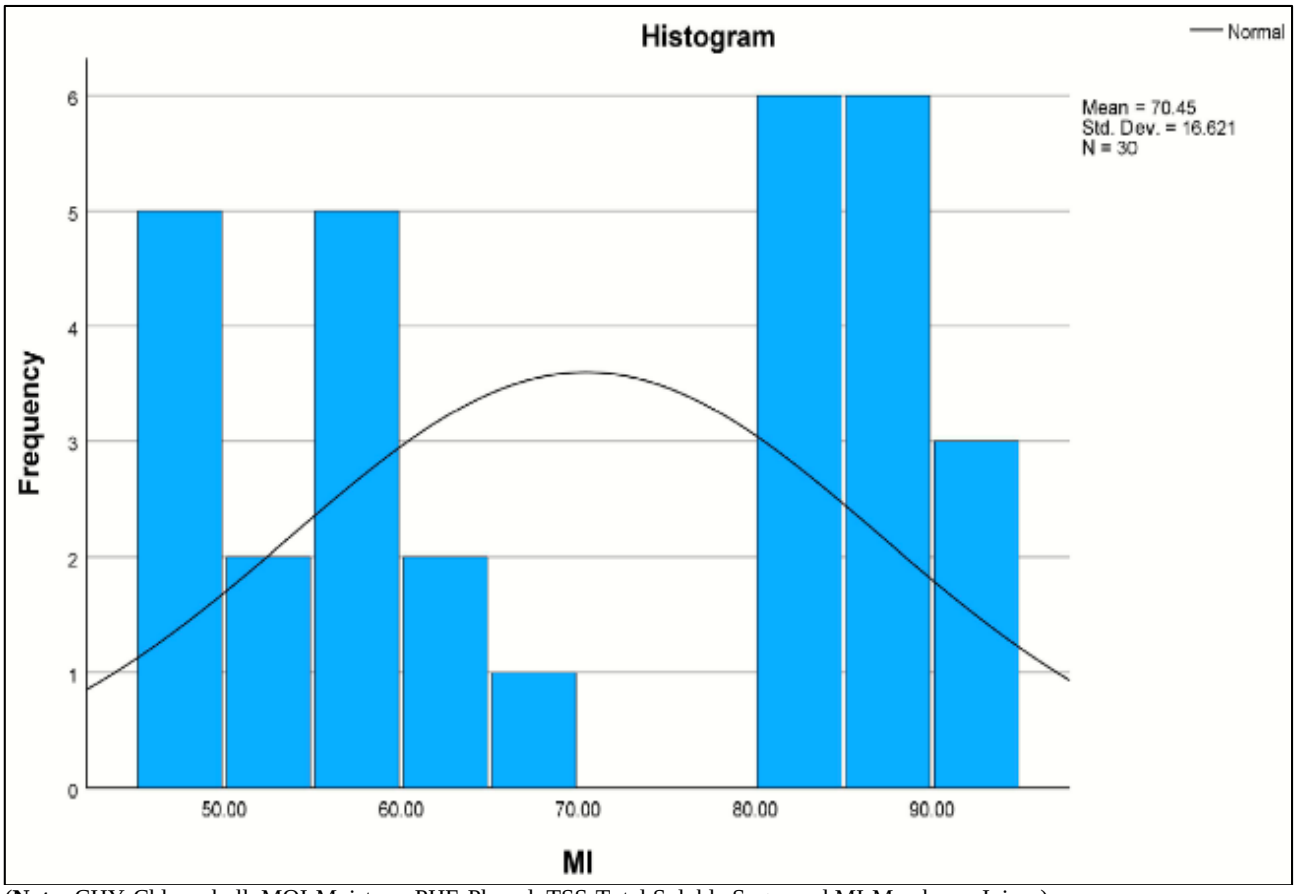

(**Note:** CHY-Chlorophyll, MOI-Moisture, PHE-Phenol, TSS-Total Soluble Sugar and MI-Membrane Injury)

Fig 2: Frequency distribution of different observations in $F_{2:3}$ genotypes

Genotypic and phenotypic coefficient variance, heritability and genetic advance mean of biochemical traits of $F_{2:3}$ Mapping Population

Variability analysis for biochemical traits of $F_{2:3}$ mapping population of cross ACCMS 1×ACS 18-08 was mentioned in (Table 3).

Chlorophyll

Chlorophyll (0.25-0.84 mg/g) was observed with high values of genotypic and phenotypic coefficient of variation *i.e.,* GCV and PCV (36.99 and 37.13%), which indicated a very high level of variability in chlorophyll content. More importantly moderate estimates of heritability (99.22%), which suggested that nearly all the phenotypic variation in chlorophyll content is attributable to genetic factors. Such high heritability indicates that the trait will respond exceptionally well to selection in breeding programs. High per cent mean of genetic advance (75.90%) were recorded, which indicated a substantial potential for improvement of the trait per generation through selection.

In summary, the data for chlorophyll content indicated very high genetic and phenotypic variability, extremely high heritability and substantial genetic advance. These characteristics make chlorophyll content an excellent candidate for improvement through selective breeding, as it is predominantly controlled by genetic factors and significant progress can be made in each generation.

Table 3: Variability analysis for biochemical traits of $F_{2:3}$ genotypes of cross ACCMS 1×ACS 18-08

Sr. No.	Trait	Range	Mean	GCV (%)	PCV (%)	h^2B (%)	GAM (%)
1	CHY (mg/g)	0.25 - 0.84	0.52	36.99	37.13	99.22	75.90
2	MOI (%)	30.34 - 90.85	60.86	35.25	35.57	98.26	71.99
3	PHE (mg/g)	0.89 - 3.17	1.90	43.78	43.96	99.20	89.84
4	TSS (mg/g)	2.15 - 7.83	4.74	33.17	33.25	99.50	68.16
5	MI (%)	43.29 - 94.32	70.44	23.39	23.98	95.17	47.01

Note: ((CHY=Chlorophyll, MOI=Moisture, PHE=Phenol, TSS=Total Soluble Sugar, MI=Membrane Injury)

Moisture

Moisture (30.34-90.85%) was observed with high values of genotypic and phenotypic coefficient of variation *i.e.,* GCV and PCV (35.25 and 35.57%), which indicated a very high level of variability in moisture content. The close values of GCV and PCV suggested that the variability is predominantly due to genetic factors, with minimal influence from environmental factors. More importantly moderate estimates of heritability (98.26%), suggested that nearly all the phenotypic variation in moisture content is attributable to genetic factors. High per cent mean of genetic advance (71.99%) were recorded, which indicated a

substantial potential for improvement of the trait per generation through selection.
In summary, the data for moisture content showed very high genetic and phenotypic variability, extremely high heritability and substantial genetic advance. These characteristics make moisture content an excellent candidate for improvement through selective breeding, as it is predominantly controlled by genetic factors and significant progress can be made in each generation.

Total phenol
Total phenol (0.89-3.17 mg/g) was observed with high values of genotypic and phenotypic coefficient of variation *i.e.,* GCV and PCV (43.78 and 43.96%), which indicated that a very high level of variability in total phenol content. More importantly moderate estimates of heritability (99.20%), which suggested that nearly all the phenotypic variation in total phenol content is attributable to genetic factors. High per cent mean of genetic advance (89.84%) were recorded, which indicated that a substantial potential for improvement of the trait per generation through selection.
In summary, the data for total phenol content show very high genetic and phenotypic variability, extremely high heritability and substantial genetic advance. These characteristics make total phenol content an excellent candidate for improvement through selective breeding, as it is predominantly controlled by genetic factors and significant progress can be made in each generation.

Total soluble sugar
Total soluble sugar (2.15-7.83 mg/g) was observed with high values of genotypic and phenotypic coefficient of variation *i.e.,* GCV and PCV (33.17 and 33.25%), which indicated that a high level of variability in total soluble sugar content. more importantly moderate estimates of heritability (99.50%), which suggested that nearly all the phenotypic variation in total soluble sugar content is attributable to genetic factors. High per cent mean of genetic advance (68.16%) were recorded, which indicated a substantial potential for improvement of the trait per generation through selection.
In summary, the data for total soluble sugar content showed high genetic and phenotypic variability, extremely high heritability and substantial genetic advance. These characteristics make total soluble sugar content an excellent candidate for improvement through selective breeding, as it is predominantly controlled by genetic factors and significant progress can be made in each generation.

Membrane injury
Membrane injury (43.29-94.32%) was observed with high values of genotypic and phenotypic coefficient of variation *i.e.,* GCV and PCV (23.39 and 23.98%), which indicated that a moderate level of variability in membrane injury. More importantly moderate estimates of heritability (95.17%), which suggested that a large proportion of the phenotypic variation in membrane injury is attributable to genetic factors. High per cent mean of genetic advance (47.01%) were recorded, which indicated a substantial potential for improvement of the trait per generation through selection.
In summary, the data for membrane injury show moderate genetic and phenotypic variability, moderate to high heritability, and substantial genetic advance. These characteristics indicate that membrane injury is influenced by both genetic and environmental factors, with a significant potential for improvement through selective breeding.

Conclusion
Biochemical traits like chlorophyll, moisture and membrane injury were showed significant and negative correlation with the disease incidence, which clearly indicated that with increase of this biochemical content, CLCV disease incidence decreased. Moisture and membrane injury were found higher in resistant parent ACCMS 1 (89.81% and 91.40%, respectively) as compared to susceptible parent ACS 18-08 (41.66% and 55.89%, respectively). In genotype 27 moisture content was found lowest 34.46%. In genotype 26 higher (65.24%) membrane injury and lower 8 lower (32.10%) were observed. Lower phenol and total soluble sugar content were recorded higher in susceptible parent ACS 18-08 (3.00 mg/g and 6.38 mg/g, respectively) as compared to resistant parent (1.09 mg/g and 3.07 mg/g, respectively). Phenol and total soluble sugar content were found higher (3.14 mg/g and 7.70 mg/g) and lower (0.95 mg/g and 2.28 mg/g) in genotypes (20 and 25) and (6 and 3), respectively.

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