



Plant Archives

Journal homepage: <http://www.plantarchives.org>

DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.160>

IDENTIFICATION OF MORPHOLOGICAL TRAITS ASSOCIATED MOLECULAR MARKERS LINKED TO CHILLI LEAF CURL VIRUS RESISTANT IN CHILLI (*CAPSICUM ANNUUM* L.)

Urja B. Solanki^{1*}, B. P. Chauhan², Y. M. Shukla¹ and S. Kumar³

¹Department of Biochemistry, Anand Agricultural University, Anand – 388 110, Gujarat, India

²Department of Agricultural Biotechnology, Anand Agricultural University, Anand – 388 110, Gujarat, India

³Vivekanand Parvatiya Krishi Anusandhan Sansthan, Almora-263601, India

*Corresponding author E-Mail: urja2904@gmail.com

(Date of Receiving-22-01-2026; Date of Revision-15-03-2026; Date of Acceptance-31-03-2026)

ABSTRACT

Chilli leaf curl virus is the most destructive disease in chilli. It is transmitted by sole vector whitefly (*Bemisia tabaci*). Breeding for disease resistance is one of the truthful approaches to combat the disease based on marker assisted selection and mapping of qualitative trait loci. The experimental material for present investigation comprising of F₂ and F_{2,3}, segregating population of cross resistant parent ACCMS 1 and susceptible parent ACS 18-08 against chilli leaf curl disease. Total 409 SSR markers were used for screening of F₂ mapping population along with their parents of which 49 polymorphic SSR markers resulted in polymorphic banding patterns. Phenotyping evaluation study in 120 F_{2,3} mapping population was subjected to morphophysiological observations viz., days to initiation of flowering (21.67-33.67), plant height (47.47-105.64 cm), fruit length (7.97-19.54 cm), fruit weight (2.23-8.09 g), fruit yield per plant (68.35-928.13 g), primary branches per plant (2.67-6.67) and disease incidence (0.51-76.20). Correlation analysis indicated that characters like plant height, fruit length, fruit weight, fruit yield per plant and primary branches per plant showed significant negative correlation with disease scoring. The skewness obtained from the frequency distribution in the present study revealed that plant height, fruit length, fruit weight, fruit yield per plant, disease incidence showed positive skewness, which revealed that these traits were governed by the complementary gene action. The negative value of skewness of days to initiation of flowering and primary branches per plant were governed by the duplicate gene action. The medium GCV and PCV (10 to 20%) was observed for days to initiation of flowering, plant height and primary branches per plant. Higher GCV and PCV (>20%) was found for fruit length, fruit weight and fruit yield per plant. All morphological traits studied showed high heritability (more than 60%). Study on identification of marker trait association for chilli leaf curl virus resistance was found significant through single marker analysis. Single Marker Analysis using one-way analysis of variance was the best and simple method for identification of marker traits. Present investigation revealed that the identification of total 8 useful markers to screen various traits like fruit length, disease incidence and fruit yield per plant. Two markers EPMS558 and GPMS203 were associated with fruit length, four markers CaES0028, CaES0047, CA516044 and Hpms1-143 observed association with CLCV resistance and Hpms1-3 and Hpms1-41 two markers had association with fruit yield per plant.

Key word: SSR marker, correlation, morphological traits, skewness

Introduction

Chilli (*Capsicum annuum* L.) is a highly significant vegetable and spice crop that is grown practically everywhere in the world including tropical and subtropical zones. Around 7000 BC, the nightshade family crop, the

chilli, with chromosomal number 2n=24, was domesticated. In India, *Capsicum annuum* L. is one of the most extensively grown cultivable species. Numerous ingredients in chillies have significant nutritional significance as well as flavour, aroma, texture and color.

The “capsaicinoids” in chilli give it its pungency. A class of volatile substances known as capsaicinoids is generated by the secondary metabolism of chilli plants and includes molecules such as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and others. A common solanaceous crop, chillies have actinomorphic, pedicellate, bisexual and hypogynous white blooms. Chilli is sometimes considered under self-pollinated and sometimes under often cross pollinated.

The states of Andhra Pradesh, Telangana, Tamil Nadu, Karnataka and Madhya Pradesh are the main producers of chillies in India. The greatest producer of chillies in India, Andhra Pradesh accounts for roughly 26% of the total area planted with chillies. Other states that contribute close to 22% of the total area under chillies include Maharashtra (15%), Karnataka (11%), Orissa (11%), Madhya Pradesh (7%) and other states. The majority of Gujarat cultivation takes place in the Saurashtra, Surat and Ahmedabad district. Any breeder would agree that understanding different qualitative and quantitative qualities, their inheritance patterns, the ways in which they function and the degree to which the environment affects them is crucial.

A gene must typically be transferred into a new genotype by 6-7 backcrosses, which is a labour-intensive and time-consuming procedure. Therefore, it is imperative to identify the markers that are associated to the resistance gene or genes in order to shorten the time needed for gene transfer and aid in the production of CLCV resistant cultivars. There are currently no identified molecular indicators associated with CLCV in chilli. A total of 292 genes related to morphological characteristics, physiological characteristics, nematode resistance, sterility, disease resistance and herbicide resistance were identified in *capsicum*. Begomovirus, which are spread by the whitefly (*Bemisia tabaci*), severely reduce this crop's output. In terms of frequency and yield loss, Chilli Leaf Curl Virus (CLCV) is the most debilitating illness; in extreme circumstances, reports of 100% losses of marketable fruit have been made (Senanayake *et al.*, 2012 and Kumar *et al.*, 2015).

In addition to making, it easier for elite chilli genotypes to transfer disease-resistant gene(s), the related marker(s) that have been found will aid in the discovery of new genotypes that are resistant to CLCV. SMA only uses one marker at a time. Simple t tests, ANOVAs, linear regressions, likelihood ratio tests, and maximum likelihood estimation can all be used to conduct SMA.

Materials and Methods

The present investigation entitled “Identification of

molecular markers linked to chilli leaf curl virus resistance in chilli (*Capsicum annuum* L.)” was carried out at the Department of Biochemistry and the field experiment was carried out at Research farm of Main vegetable research station, Anand Agricultural University, Anand.

The experimental material for present investigation comprising of F_2 and $F_{2,3}$, segregating population of cross resistant parent ACCMS 1 and susceptible parent ACS 18-08 against chilli leaf curl disease. Total 120 mapping population were developed from F_1 seeds derived from crosses of above referred parents. F_1 hybrid seeds were collected in the year 2020-21. Total 120 F_2 mapping population were sown in the year of 2021-22. In the year of 2022-23, $F_{2,3}$ mapping population were sown to study morphophysiological characterizations.

Phenotyping of Mapping Population

Observations on days to initiation of flowering (days), plant height (cm), fruit length (cm), fruit weight (g), fruit yield per plant (g), primary branches per plant (no.) and disease incidence (%) were recorded from randomly selected five plants of the $F_{2,3}$ segregating mapping population and the parents.

Days to initiation of flowering (days)

The numbers of days were recorded from the date of transplanting to the appearance of first flower in plants.

Plant height (cm)

The plant height from the base of the plant to the emerging leaf at the tip of the main stem was measured in centimetre at the time of maturity.

Fruit length (cm)

The fruit length was measured in randomly selected five fruits at the time of maturity and average value was calculated.

Fruit weight (g)

Five randomly selected matured fruits per line of mapping population were tested and average mean value was calculated in gram.

Fruit yield per plant (g)

The total fruit yield obtained from the randomly selected five plants from each picking were weighted in gram and their sum was calculated to obtain fruit yield per plant in gram.

Primary branches per plant (no.)

Total numbers of primary branches per plant were counted on the main stem at the time of maturity.

Disease incidence (%)

Random observations for incidence of chilli leaf curl

virus in chilli starting at 30 days after transplanting were recorded at weekly intervals till maturity. The severity of disease was scored on a six-point (0-5) scale given by Thakur *et al.*, (2020).

- 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 line of mapping population for all parameters, the Panse and Sukhatme (1967) analysis of variance technique were used.

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

Utilizing the formulas provided by Zewdu *et al.*, (2023), 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,

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.

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

Where,

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).

Heritability:

The broad sense heritability (h^2_b) was calculated for both the characters by dividing genotypic variance and the phenotypic variance. The method followed was

suggested by Johnson *et al.*, (1955).

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

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).

< 30% Low, 30-60% Moderate and > 60% High

Genetic advance (GA)

It was calculated the improvement rate in the mean of each line of mapping population value of selected plants over the parental population. It was performed by using the methodology suggested by Johnson *et al.*, (1955) 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)

$$GA (\% \text{ of mean}) = \frac{GA}{\bar{x}} \times 100$$

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)

Result and Discussion

The present study employed an initial screening of two parental genotypes of chilli known to exhibit to Chilli Leaf Curl Virus (CLCV). The two parents used in the study were ACCMS 1, which is resistant to CLCV and ACS 18-08, which is susceptible to CLCV at the Main Vegetable Research Station, Anand Agricultural University, Anand (Fig. 1). Based on morphophysiological

characterizations, ACCMS 1 and ACS 18-08 were selected to developed F_2 segregating population aimed at identifying molecular markers linked to CLCV resistance. In F_2 mapping population out of 150 mapping population 28 plants could not survived due to leaf curl virus in chilli. So total 120 F_2 mapping population was used for morphophysiological characterization as $F_{2,3}$ segregating mapping population. So, 120 $F_{2,3}$ mapping population along with their parents were grown in field under natural condition subjected to whitefly attack. Screening for CLCV infection was started after 30 days of transplanting. The disease progress was recorded at the interval of 30 days from appearance of first symptom in the $F_{2,3}$ population. The first symptom of CLCV was observed after 30 days of transplanting. Due to environmental effects on mapping population different disease severity was observed. The $F_{2,3}$ population disease progression was tracked at 30-day interval from the onset of first symptom. Disease incidence was scored at (0-5) scaling level after 30 days of transplanting according to Thakur *et al.*, (2020).

Parent ACCMS 1 did not show any symptom of CLCV disease and considered as highly resistance to chilli leaf curl virus, while parent ACS 18-08 showing symptoms of CLCV disease was found highly susceptible. F_1 plants did not show any disease symptoms. Among all the screened $F_{2,3}$ plants against CLCV, from which shown

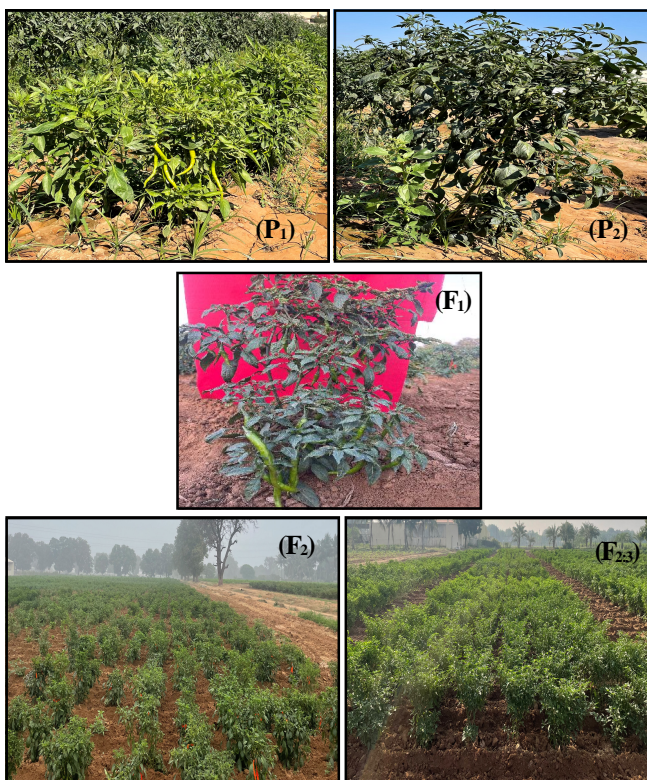


Fig. 1: Field overview of P₁, P₂, F₁ and mapping population (F₂ and F_{2,3}).

35 (disease score 0), 29 (disease score 1), 21 (disease score 2), 17 (disease score 3), 14 (disease score 4) and 4 plants (disease score 5) observed under the category of highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible, respectively (Fig. 2 & 3).

Morphophysiological parameters of $F_{2,3}$ mapping population along with their parents

Total 120 $F_{2,3}$ populations were characterized for morphophysiological traits along with their parents which included days to initiation of flowering (days), plant height (cm), fruit length (cm), fruit weight (g), fruit yield per plant (g), primary branches per plant (no.) and disease incidence (%) were recorded from five plants of each mapping population and mean values have been presented in (Table 1) with the interpretation.

Days to initiation of flowering (days)

Days of initiation were recorded from the date of transplanting till the appearance of flowers in first plant of each line of mapping population. The highest mean value 33.67 in line of mapping population 41 and lowest mean value 21.67 in line of mapping population 1.

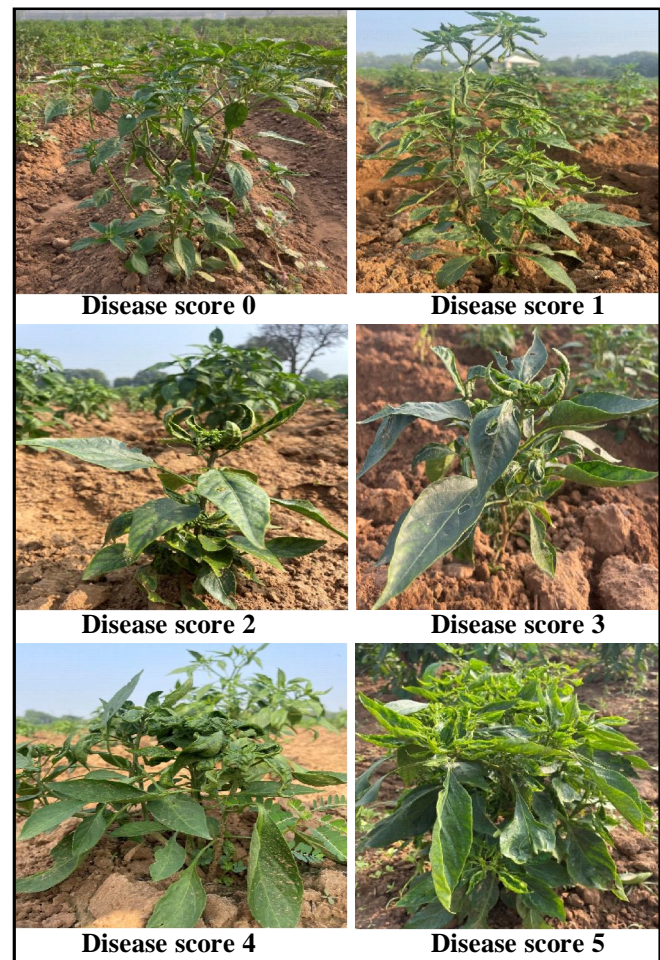


Fig. 2: Disease scoring scale in chilli $F_{2,3}$ mapping population.

Table 1: Morphological parameters from F_{2,3} mapping population in chilli.

Mapping population	Days of initiation of flowering	Plant height (cm)	Fruit length (cm)	Fruit weight (g)	Fruit yield per plant (g)	Primary branches per plant (no.)	Disease incidence (%)
1	21.67	63.74	12.70	4.86	746.00	5.34	0.00
2	23.34	72.94	11.34	3.68	441.74	4.34	11.67
3	26.00	51.94	8.14	3.18	243.53	4.67	73.27
4	24.67	60.64	10.38	4.62	266.05	5.67	15.27
5	29.34	89.10	7.97	3.96	165.38	5.34	17.17
6	25.00	62.07	17.17	4.53	608.26	4.00	0.00
7	25.34	61.17	15.80	4.32	372.05	3.67	27.80
8	27.67	72.14	12.50	4.46	347.72	4.67	8.35
9	28.34	67.64	11.44	3.74	928.13	6.34	10.90
10	27.00	56.93	12.24	3.49	281.36	3.67	0.51
11	26.67	56.94	8.80	3.89	235.72	3.34	36.53
12	31.00	76.47	12.40	5.51	654.03	4.34	0.00
13	25.00	56.94	15.80	3.31	256.85	5.34	8.14
14	26.34	52.77	8.67	5.32	657.24	4.67	12.40
15	28.67	55.14	12.50	6.29	347.11	5.34	21.77
16	33.00	72.04	8.57	3.52	216.74	6.00	13.40
17	28.34	55.04	9.27	3.65	172.09	4.00	62.17
18	29.00	58.04	12.40	3.75	109.33	4.34	23.64
19	32.34	71.47	12.87	4.31	90.87	2.67	10.87
20	26.34	84.80	13.10	4.00	555.23	6.67	0.00
21	27.67	79.97	16.74	4.25	105.11	5.00	0.51
22	25.67	76.70	13.00	4.34	146.77	4.67	6.81
23	26.67	65.94	13.17	3.34	210.61	4.00	14.14
24	31.67	81.70	14.14	3.75	266.34	5.00	7.02
25	26.34	73.57	15.34	5.08	86.13	5.67	19.17
26	32.67	56.80	15.84	5.37	336.04	6.34	1.60
27	31.00	72.27	12.37	3.55	137.72	5.67	0.61
28	28.00	70.97	12.94	3.79	440.21	4.67	5.07
29	30.34	66.60	15.50	7.04	287.35	5.00	26.00
30	26.67	47.47	11.90	3.55	345.67	4.34	12.27
31	27.00	60.74	8.40	2.81	158.07	5.67	58.14
32	29.34	85.40	11.64	3.55	78.50	6.34	0.94
33	31.67	57.50	11.47	5.49	357.99	4.67	13.70
34	33.34	97.40	12.44	3.60	585.10	6.00	0.00
35	28.34	73.87	11.74	3.78	70.48	4.34	11.94
36	31.00	59.97	12.64	3.67	201.62	5.34	12.90
37	30.67	55.74	13.24	3.66	535.66	5.00	8.04
38	29.34	49.97	14.77	4.49	127.62	5.34	34.94
39	30.67	69.80	13.57	6.52	265.36	4.67	17.00
40	30.00	69.47	14.04	3.83	255.55	5.00	52.80
41	34.67	73.30	14.94	4.52	115.51	5.67	16.94
42	29.67	72.94	11.40	4.19	385.19	6.34	4.90
43	29.00	97.67	17.40	7.04	415.54	5.34	0.00
44	28.67	67.07	12.70	3.50	276.25	3.67	39.24
45	31.34	61.24	11.44	5.61	356.51	6.67	21.37
46	30.34	76.00	14.77	4.72	185.13	5.34	19.14
47	30.00	84.87	15.84	5.15	643.96	4.34	0.00
48	31.00	56.27	13.30	4.20	68.35	4.67	0.64

Continue ...1

49	28.67	64.80	15.70	4.45	347.45	6.00	13.30
50	28.34	62.84	8.67	3.47	155.46	5.67	14.54
51	30.67	47.47	15.90	4.51	214.26	4.00	3.14
52	31.00	68.77	14.44	4.64	292.93	6.00	0.63
53	28.00	71.47	19.54	4.39	374.92	5.34	0.00
54	25.67	58.80	14.50	4.62	325.40	4.00	12.14
55	28.34	61.74	11.47	5.63	145.27	3.67	17.90
56	27.00	71.97	12.57	6.68	585.06	3.34	12.04
57	28.34	63.44	15.90	6.42	484.63	5.00	7.08
58	29.34	52.54	11.80	3.50	114.77	5.34	45.67
59	31.34	71.30	13.00	6.34	555.57	5.67	11.64
60	28.34	75.17	18.64	5.36	143.97	6.00	9.20
61	30.34	76.10	12.67	4.42	864.19	4.67	0.00
62	28.67	62.77	13.74	4.62	274.50	5.00	1.03
63	30.00	58.54	16.37	6.70	436.52	4.34	16.50
64	27.34	71.40	14.40	4.49	283.81	6.67	8.20
65	29.67	65.94	11.84	3.25	138.60	5.67	61.80
66	29.34	68.67	17.17	4.32	226.04	4.67	4.70
67	31.34	93.10	14.54	5.30	414.96	5.34	0.00
68	30.67	79.00	18.67	4.71	97.17	4.67	11.17
69	30.34	77.54	11.64	5.45	365.08	4.00	20.90
70	32.34	53.84	12.34	3.28	175.72	4.67	37.37
71	26.34	72.94	15.47	7.48	265.80	6.34	1.32
72	25.34	55.00	13.77	3.63	156.79	6.00	13.77
73	31.34	85.47	14.47	7.17	464.60	6.34	0.00
74	25.67	65.57	13.04	3.55	316.50	5.67	22.17
75	28.00	105.64	11.60	5.56	226.41	4.34	19.10
76	28.00	91.44	12.67	3.65	184.20	6.67	17.14
77	30.00	74.10	17.94	4.57	592.24	5.00	11.84
78	29.34	57.60	11.64	3.46	236.72	6.00	67.74
79	25.00	83.47	11.57	4.18	687.16	6.00	1.67
80	27.34	75.34	16.70	3.54	455.26	6.34	0.00
81	26.34	57.80	8.57	3.68	316.80	4.00	64.70
82	27.00	72.14	12.57	4.20	226.70	4.67	1.50
83	31.34	75.27	17.97	5.23	335.24	5.00	0.00
84	27.34	69.00	13.34	5.35	625.62	4.34	21.47
85	29.00	83.40	15.54	8.09	207.69	6.00	17.37
86	31.67	68.57	8.34	3.55	95.15	5.00	67.87
87	30.34	60.37	14.50	4.25	166.56	5.34	0.62
88	30.67	73.37	13.94	4.63	245.71	6.34	12.00
89	31.67	62.70	17.40	6.33	264.34	5.67	14.27
90	25.67	77.87	15.50	4.24	455.97	5.00	0.00
91	30.00	68.07	19.37	5.28	289.42	5.34	10.27
92	26.67	93.04	9.57	4.59	97.88	6.00	25.04
93	29.34	65.64	13.54	4.18	125.26	5.67	5.30
94	32.00	58.87	9.67	2.61	193.46	4.67	65.80
95	28.34	60.90	12.30	2.56	210.67	6.00	4.70
96	31.67	70.27	16.90	3.45	692.78	5.34	20.30
97	32.34	76.37	11.90	3.55	134.02	5.00	7.34
98	27.34	55.77	15.04	4.26	594.95	6.00	0.93
99	29.00	47.94	11.84	4.49	325.07	4.34	19.24

Continue ...1

100	31.67	73.20	11.84	5.36	125.40	6.34	22.80
101	24.67	71.97	8.47	4.36	174.60	4.67	15.10
102	26.67	78.70	12.60	5.42	185.66	6.67	13.64
103	30.34	74.40	11.54	4.85	425.48	6.34	14.60
104	28.34	73.30	15.34	3.44	113.35	4.67	17.20
105	27.34	63.07	13.17	5.72	414.59	6.67	6.54
106	27.00	68.90	11.67	4.47	155.76	5.67	16.90
107	30.34	90.80	12.00	3.73	356.73	5.00	25.20
108	27.34	87.77	12.24	5.42	244.19	4.67	52.97
109	27.67	55.34	18.80	2.47	207.38	6.34	11.74
110	28.34	54.90	15.70	6.34	294.02	6.00	9.30
111	26.67	80.27	16.40	5.50	584.71	5.00	5.20
112	30.34	79.10	14.70	3.41	173.16	6.34	14.94
113	29.67	77.14	11.50	5.69	494.48	4.67	13.47
114	29.34	65.84	12.60	4.30	325.30	6.34	11.20
115	30.67	71.87	11.20	5.40	284.92	6.67	21.34
116	30.34	68.74	12.54	2.23	134.96	5.67	17.37
117	27.67	91.24	13.57	2.53	155.01	5.00	15.00
118	32.34	81.90	11.34	3.61	324.28	4.34	10.44
119	24.34	74.20	15.77	3.50	226.53	6.67	0.78
120	27.00	78.70	17.44	3.53	255.96	4.34	11.10
Min.	21.67	47.47	7.97	2.23	68.35	2.67	0.00
Max.	33.67	105.64	19.54	8.09	928.13	6.67	73.27
ACCMS 1	33.34	91.24	16.44	5.72	492.62	5.34	0.73
ACS 18-08	27.34	52.64	12.54	2.39	126.48	3.34	76.20
S Em ±	2.060	2.478	0.750	0.133	11.830	0.438	1.022
CD at 5%	5.738	6.903	2.090	0.371	32.956	1.219	2.848
CV%	12.38	6.16	9.71	5.16	6.64	14.59	10.88

Resistant parent ACCMS 1 had mean value 33.34 while in case of susceptible parent ACS 18-08 had mean value 27.34. In lines of mapping population 5, 8, 9, 12, 15, 16, 17, 18, 19, 21, 24, 26, 27, 28, 29, 32-53, 55, 56, 65, 75, 85, 91, 93, 99, 100, 103, 104, 107 and 112 were observed statistically higher over resistant parent ACCMS 1 and lines of mapping population 1, 2, 3, 4, 6, 7, 10, 11, 13, 14, 20, 22, 23, 25, 30, 34, 56, 71, 72, 74, 79, 81, 82, 90, 92, 101, 102, 106, 111, 119 and 120 were statistically higher over susceptible parent ACS 18-08.

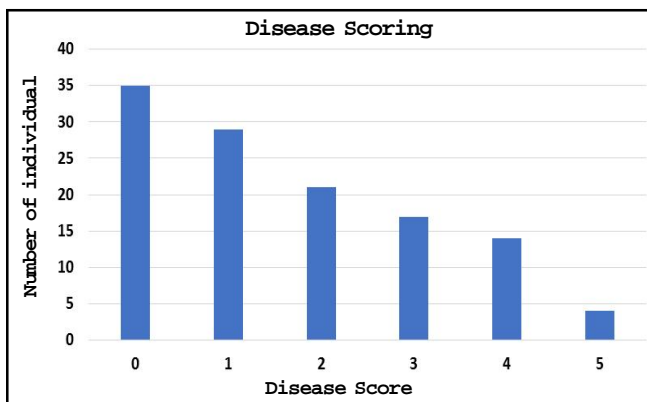


Fig. 3: Distribution of $F_{2:3}$ mapping population according to disease scoring.

Days to initiation of flowering (37.33-60) was recorded by Kumar *et al.*, (2015) in chilli and result revealed from this study in agreement with Chowdhury *et al.*, (2023) recorded that in chilli days to initiation of flowering ranged from 20.50-36.83.

Plant height (cm)

Plant height was recorded at the time of maturity of each line of mapping population. The highest mean value 105.64 cm in line mapping population 75 and lowest mean value 47.47 cm in line of mapping population 30. Resistant parent ACCMS 1 had mean value 91.64 cm while in case of susceptible parent ACS 18-08 had mean value 52.64 cm. In lines of mapping population with plant height 5 (89.10 cm), 20 (84.80 cm), 32 (85.40 cm), 47 (84.87 cm), 73 (85.47 cm), 79 (83.47 cm), 85 (83.40 cm) and 108 (87.87) were remarkable statistically higher over resistant parent ACCMS 1. There were only four lines of mapping population with plant height 30 (47.47 cm), 38 (49.97 cm), 58 (52.54 cm) and 99 (47.94) 120 were statistically higher over susceptible parent ACS 18-08.

Results derived from this study are found similar with Chowdhury *et al.*, (2023) and Hulagannavar *et al.*, (2024) who recorded that in chilli plant height ranged in

between 84.46-138.33 cm and 49.20-147.13 cm in chilli, respectively.

Fruit length (cm)

Fruit length was recorded when plant reached maturity of each line of mapping population. The highest mean value 19.54 cm in line of mapping population 53 and lowest mean value 7.97 cm in line of mapping population 5. Resistant parent ACCMS 1 had mean value 16.44 cm while in case of susceptible parent ACS 18-08 had mean value 12.54 cm. 7, 13, 25, 26, 29, 38, 41, 46, 47, 49, 51, 52, 54, 57, 63, 64, 67, 71, 73, 85, 87, 90, 98, 104, 110, 111, 112 and 119 in lines of mapping population were statistically higher over resistant parent ACCMS 1. In lines of mapping population 2, 8, 9, 10, 12, 15, 18, 27, 30, 32, 33, 35, 42, 45, 55, 58, 65, 69, 70, 75, 78, 79, 95, 97, 99, 100, 103, 106, 107, 108, 113, 115 and 118 were noted statistically higher over susceptible parent ACS 18-08.

Results derived from this study are found similar with Chowdhury *et al.*, (2023) and Hulagannavar *et al.*, (2024) who recorded that in chilli fruit length ranged in between 9.21-15.78 cm and 5.65-11.17 cm in chilli, respectively.

Fruit weight (g)

Fruit weight was recorded at the time of different picking interval of each line of mapping population. The highest mean value 8.09 g in line of mapping population 85 and lowest mean value 2.23 g in line of mapping population 116. Resistant parent ACCMS 1 had mean value 5.72 g while in case of susceptible parent ACS 18-08 had mean value 2.39 g. In lines of mapping population with fruit weight 12 (5.51 g), 26 (5.37 g), 33 (5.49 g), 45 (5.61 g), 55 (5.63 g), 60 (5.36 g), 69 (5.45 g), 75 (5.56 g), 84 (5.35 g), 100 (5.36 g), 102 (5.42 g), 105 (5.72 g), 108 (5.42 g), 111 (5.50 g), 113 (5.69 g) and 115 (5.40 g) were recorded statistically higher over resistant parent ACCMS 1. Only one line of mapping population that was 116 with fruit weight 2.23 g observed statistically higher over susceptible parent ACS 18-08.

Results derived from this study are found similar with Rahevar *et al.*, (2021), Sunday *et al.*, (2020) and Hulagannavar *et al.*, (2024) who recorded that in chilli fruit weight ranged in between 3.00-20.17 g, 0.37-16.40 g, and 1.83-5.34 g in chilli, respectively.

Fruit yield per plant (g)

Fruit yield per plant was recorded at the time of different picking interval of each line of mapping population. The highest mean value 928.13 g in line of mapping population 85 and lowest mean value 68.35 g in line of mapping population 48. Resistant parent ACCMS 1 had mean value 492.62 g while in case of susceptible

parent ACS 18-08 had mean value 126.48 g. 57 (484.63 g) and 73 (464.40 g) in lines of mapping population were observed statistically higher over resistant parent ACCMS 1. In lines of mapping population with fruit yield per plant 18 (109.33 g), 21 (105.11 g), 41 (115.51 g), 58 (114.77 g), 68 (97.17 g), 86 (95.15 g), 92 (97.88 g) and 93 (125.26 g) were noted statistically higher over susceptible parent ACS 18-08.

Results derived from this study are found similar with Chowdhury *et al.*, (2023) and Hulagannavar *et al.*, (2024) who recorded that in chilli fruit yield per plant ranged in between 348.02-1043.63 g and 179.51-897.31 g in chilli, respectively.

Primary branches per plant (no.)

Numbers of primary branches per plant was recorded at the time of maturity of each line of mapping population. The highest mean value 6.67 in line of mapping population 20 and lowest mean value 2.67 in line of mapping population 19. Resistant parent ACCMS 1 had mean value 5.34 while in case of susceptible parent ACS 18-08 had mean value 3.34. 2, 3, 8, 12, 14, 18, 22, 24, 28, 29, 30, 33, 35, 37, 40, 47, 49, 57, 61, 63, 66, 70, 75, 77, 82, 83, 84, 86, 90, 94, 97, 99, 101, 104, 107, 108, 111, 112, 113, 117, 118 and 120 in lines of mapping population were recorded statistically higher over resistant parent ACCMS 1. 19 in line of mapping population was only one had primary branches per plant 2.67 observed statistically higher over susceptible parent ACS 18-08.

Results derived from this study are found similar with Rahevar *et al.*, (2021), Kumar *et al.*, (2015) and Hulagannavar *et al.*, (2024) who recorded that in chilli primary branches per plant ranged in between 2.00-4.10, 2.68-5.83 and 2.60-4.90 in chilli, respectively.

Disease incidence (%)

Disease incidence was recorded at the from the date of transplanting to the time maturity of each line mapping population. Lowest mean value 0.0 % and highest mean value 73.27% in 3 line of mapping population. Some plants had 0% disease incidence which indicated that in lines of mapping population were highly resistant. Resistant parent ACCMS 1 had mean value 0.73% while in case of susceptible parent ACS 18-08 had mean value 76.20%. In lines of mapping population 1, 6, 10, 12, 20, 21, 27, 34, 43, 47, 48, 52, 61, 67, 73, 80, 83, 87, 90 and 111 were recorded statistically higher over resistant parent ACCMS 1.

Result of this study are in accordance with disease incidence (0.00-85.00%) was recorded by Kumar *et al.*, (2015) in chilli.

Correlation study of disease scoring with

morphophysiological traits in chilli

The Pearson's correlation (Fig. 4) revealed a significant negative association between plant height, fruit weight, fruit length and fruit yield per plant with disease incidence ($r = -0.27^{**}$, $r = -0.24^{**}$, $r = -0.46^{***}$ and $r = -0.30^{***}$, respectively). Plant height shown significant positive association with fruit weight ($r = 0.19^*$). Fruit weight indicated significant positive association with fruit yield per plant ($r = 0.27^{**}$) and fruit length ($r = 0.28^{**}$).

Primary branches per plant shown negative non-significant result over remaining disease incidence ($r = -0.16$) and fruit yield per plant ($r = -0.04$), and positive non-significant result over fruit length ($r = 0.08$), fruit weight ($r = 0.02$), days to initiation of flowering ($r = 0.08$) and plant height ($r = 0.15$). Plant height was observed positive non-significant result with fruit yield per plant ($r = 0.10$), fruit length ($r = 0.10$) and days to initiation of flowering ($r = 0.13$). Flowering was observed with negative non-significant result with fruit yield per plant ($r = -0.10$) and positive non-significant with disease incidence ($r = 0.06$) fruit length ($r = 0.07$) and fruit weight ($r = 0.11$). Fruit length shown positive non-significant result with fruit yield per plant ($r = 0.12$).

Result indicated that days to initiation of flowering positively correlated with disease incidence, whereas remaining morphophysiological characters like plant height, fruit length, fruit weight, fruit yield per plant and primary branches per plant were found negatively

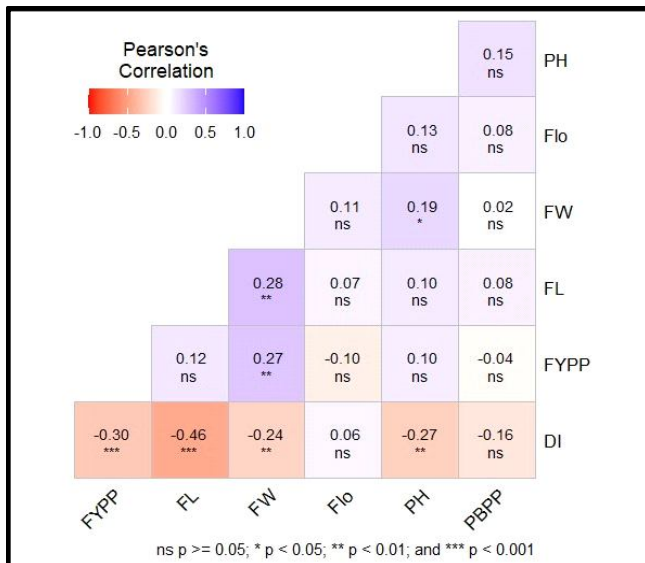


Fig. 4: Correlation coefficient analysis of morphophysiological parameters in $F_{2,3}$ mapping population in chilli. [Note: (FLO=Days to initiation of flowering, PH=Plant Height, FL=Fruit Length, FW=Fruit Weight, FYP=Fruit Yield Per Plant, PBPP=Primary Branches Per Plant, DI=Disease Incidence)]

Table 2: Skewness and kurtosis for morphophysiological traits of $F_{2,3}$ mapping population.

Sr.	Traits	Skewness	Kurtosis
1	Days to initiation of flowering	-0.183	-0.208
2	Plant Height	0.428	-0.035
3	Fruit Length	0.118	-0.243
4	Fruit Weight	0.699	0.364
5	Fruit Yield Per Plant	1.063	0.810
6	Primary Branches Per Plant	-0.224	-0.526
7	Disease Incidence	1.796	2.820

correlated with disease incidence. Except days to initiation of flowering, rest of the morphophysiological observations were found negatively correlated with each other.

The findings of present investigation were in agreement with results obtained by Negi *et al.*, (2019) in chilli fruit yield per plant which was negatively significant with disease incidence. Negative significant correlation of plant height and primary branched per plant in chilli was also reported by Lakshmidamma *et al.*, (2021).

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 $F_{2,3}$ mapping population is presented in Fig. 4 respectively (Table 2) describes skewness and kurtosis measured $F_{2,3}$ mapping population of cross ACCMS 1×ACS 18-08.

Morphophysiological parameters like plant height (0.428), fruit length (0.118), fruit weight (0.699), fruit yield per plant (1.063) and disease incidence (1.796) showed positive value of skewness. While remaining morphophysiological parameters like days to initiation of flowering (-0.183) and primary branches per plant (-0.224) shown negative skewness. This indicated that more the segregating mapping population would be predicted from a normal distribution are above the mean.

In case of kurtosis morphophysiological characters like fruit weight (0.364), fruit yield per plant (0.810) and disease incidence (2.820) observed with positive values. Remaining morphophysiological characters like days to initiation of flowering (-0.208), plant height (-0.035) and fruit length (-0.243) were noted with negative kurtosis. This indicated that average level of complementary gene activity through to be platykurtic that the presence of numerous small genes with progressively greater effects controls the activity of genes. Findings of this study are in agreement with Luitel *et al.*, (2018), Srivastava *et al.*, (2019), Yankanchi *et al.*, (2022) and Amas *et al.*, (2023)

Table 3: Variability analysis for morphophysiological traits of $F_{2,3}$ populations of cross ACCMS 1×ACS 18-08.

Sr. No.	Trait	Range	Mean	GCV (%)	PCV (%)	h ² B (%)	GAM (%)
1	FLO	20 - 39	28.81	4.06	13.03	75.27	2.61
2	PH(cm)	45 - 110	69.71	16.61	17.72	87.93	32.10
3	FL(cm)	7.10 - 20.50	13.37	18.74	21.11	78.84	34.29
4	FW (g)	2.01 - 8.34	4.47	25.45	25.97	96.06	51.40
5	FYPP(g)	63.80 - 951.34	308.36	58.36	58.74	98.72	119.4
6	PBPP	2.00 - 7.00	5.19	14.97	20.91	51.30	22.09

Note: (FLO=Days to initiation of flowering, PH=Plant Height, FL=Fruit Length, FW=Fruit Weight, FYPP=Fruit Yield Per Plant, PBPP=Primary Branches Per Plant, DI=Disease Incidence)

for skewness and kurtosis regarding morphophysiological characters in chilli.

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

Variability analysis for morphophysiological and biochemical traits of $F_{2,3}$ mapping population of cross ACCMS 1×ACS 18-08 were mentioned in (Table 3). Variability estimates of the population and heritable components of the trait are useful tools for the improvement of a plant trait in any breeding programme

According to Yankanchi *et al.*, (2022), 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%).

Days to initiation of flowering

Days to initiation of flowering (20-39 days) was observed with medium values of genotypic and phenotypic coefficient of variation *i.e.*, GCV and PCV (4.06 and 13.03%), the higher PCV compared to GCV suggested that environmental factors also contribute significantly to the observed variation in the trait. More importantly higher estimates of heritability (75.27%), which suggested that a large proportion of the total phenotypic variation in days to initiation of flowering is due to genetic factors rather than environmental influences. and low per cent mean of genetic advance mean (2.61%) were recorded, which indicated the expected improvement in the trait per generation through selection.

The findings of this study in accordance with Ridzuan *et al.*, (2019) medium GCV and PCV (13.2 and 17.9%), more importantly higher estimates of heritability (54.0%) and moderate per cent mean of genetic advance mean

(20%) were recorded and Hulagannavar *et al.*, (2024) recorded that GCV and PCV (10.02 and 10.92%), more importantly higher estimates of heritability (84.29%) and moderate per cent mean of genetic advance mean (18.96%) matched with present investigation.

Plant height

Plant height (45-110 cm) was observed with medium values of genotypic and phenotypic coefficient of variation *i.e.*, GCV and PCV (16.61 and 17.72%), which indicated that there is substantial variability in plant height and most of this variability is due to genetic factors. The close values of GCV and PCV suggested that environmental factors have a smaller impact on the trait variation compared to genetic factors. More importantly higher estimates of heritability (87.93%), which suggested that a large proportion of the total phenotypic variation in plant height is attributable to genetic factors. Moderate per cent mean of genetic advance (19.49%) were recorded, which indicated a moderate potential for improvement of the trait per generation through selection.

The findings of this study are in agreement with Mondal *et al.*, (2020) recorded that GCV and PCV (10.17 and 11.13%), more importantly higher estimates of heritability (83.49%) and moderate per cent mean of genetic advance mean (19.15%). Hulagannavar *et al.*, (2024) recorded that GCV and PCV (14.73 and 16.81%), more importantly higher estimates of heritability (76.74%) and moderate per cent mean of genetic advance mean (26.58%).

Fruit length

Fruit length (7.10-20.50 cm) was observed with high values of genotypic and phenotypic coefficient of variation *i.e.*, GCV and PCV (18.74 and 21.11%), which indicated a high level of variability in fruit length, with both genetic and environmental factors contributing to this variation. More importantly higher estimates of heritability (78.84%), which indicated that a significant proportion of the total phenotypic variation in fruit length is due to genetic factors. High per cent mean of genetic advance (34.29%) were recorded, which suggested indicating a substantial

potential for improvement of the trait per generation through selection.

The findings of this study are in accordance with Pujar *et al.*, (2017) medium GCV and PCV (18.0 and 19.0%), more importantly higher estimates of heritability (95.0%) and moderate per cent mean of genetic advance mean (46.53%) were recorded and Sran *et al.*, (2019) also agreement with this study result with medium GCV and PCV (19.89 and 22.0%), more importantly higher estimates of heritability (81.74%) and low per cent mean of genetic advance mean (37.05%) were also recorded.

Fruit weight

Fruit weight (2.01-8.34 g) was observed with high values of genotypic and phenotypic coefficient of variation *i.e.*, GCV and PCV (24.45 and 25.47 %), which indicated that indicate a high level of variability in fruit weight, with both genetic and environmental factors contributing to this variation. The close values of GCV and PCV suggested that the majority of the variability is due to

genetic factors, with environmental factors having a minimal impact. More importantly higher estimates of heritability (96.06%), which suggested that that almost all the phenotypic variations in fruit weight attribute to genetic factors. High per cent mean of genetic advance (51.40%) were recorded, which suggested a substantial potential for improvement of the trait per generation through selection.

The findings of present investigation are in agreement with Ain *et al.*, (2019) high GCV and PCV (24.57 and 27.14%), more importantly higher estimates of heritability (82.0%) and moderate per cent mean of genetic advance mean (45.83%). Hulagannavar *et al.*, (2024) recorded that high GCV and PCV (23.24 and 27.13%), more importantly higher estimates of heritability (73.38%) and moderate per cent mean of genetic advance mean (41.01%) which matched with present result.

Fruit yield per plant

Fruit yield per plant (63.80-951.34 g) was observed with high values of genotypic and phenotypic coefficient of variation *i.e.*, GCV and PCV (58.36 and 58.74%), which indicated a very high level of variability in fruit yield per plant, with the majority of this variability being due to genetic factors. More importantly higher estimates of heritability (98.72%), which suggested that almost all the phenotypic variations in fruit yield per plant are attributed to genetic factors. Such high heritability indicated that the traits respond exceptionally well to be selected in breeding programs. High per cent mean of genetic advance (119.4%) were recorded, which was very high, indicating an extraordinary potential for improvement of the trait per generation through selection.

Primary branches per plant

Primary branches per plant (2-7) was observed with medium values of genotypic and phenotypic coefficient of variation *i.e.*, GCV and PCV (14.97%) and 20.91%), which indicated a moderate level of variability in primary branches per plant. The higher PCV compared to GCV suggests that environmental factors contribute significantly to the observed variation in this trait. More importantly moderate estimates of heritability (51.30%), which suggested that about half of the phenotypic variation in primary branches per plant is attributable to genetic factors, with the other half influenced by environmental factors. High per cent mean of genetic advance (22.09%) were recorded, which suggested a considerable potential for improvement of the trait per generation through selection.

The findings of present study are in agreement with Pujar *et al.*, (2017) medium GCV and PCV (18.0 and

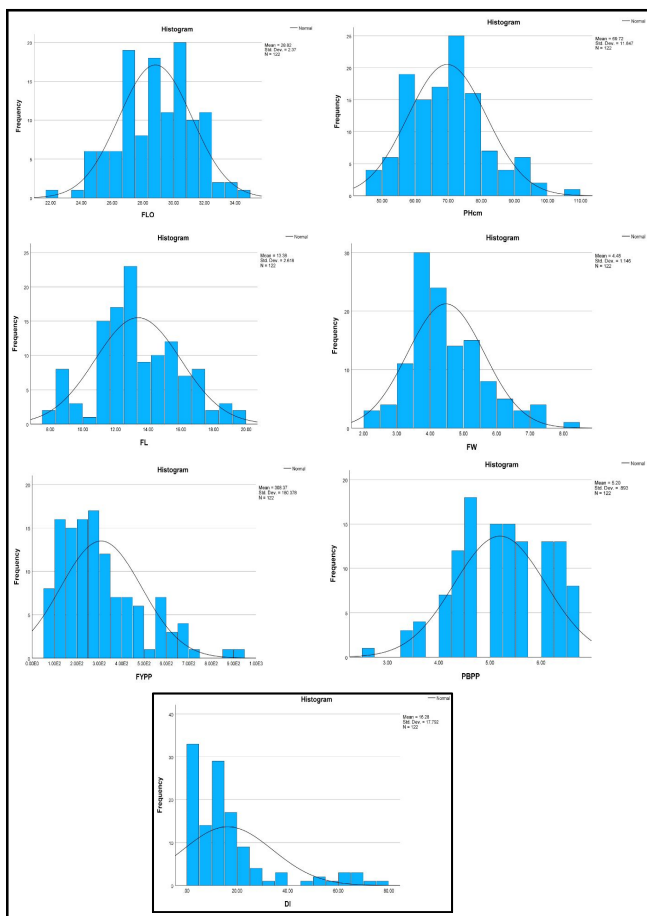


Fig. 4: Frequency distribution of different observations in $F_{2:3}$ mapping population. [(Note: FLO-Days to initiation of flowering, PH-Plant Height, FL-Fruit Length, FW-Fruit Weight, FYPP-Fruit Yield Per Plant, PBPP-Primary Branches Per Plant, DI-Disease Incidence)]

Table 4: Details of linked markers association with linked to CLCV resistant in chilli.

Trait	Sr No	Marker	Primer sequence 5' to 3'	Parents	Product size (bp)	P value	R ² (%)
FL	1	EPMS558	F: TCAACTCCATCAGTCTCCCC R: AACGCCTTGAATTAATTGCG	P1	163	0.043612*	3.40
				P2	137		
FL	2	GPMS203	F: CACCAACACATCTTTTTCAACC R: ATAATAGTGGTTGCGGCGAC	P1	239	0.036011*	3.63
				P2	225		
DI	3	CaES0028	F: AGTGCCGAGATTAGAGCCAA R: AGCTCCTCAACTGCCTTTTAT	P1	248	0.00503**	6.47
				P2	237		
	4	CaES0047	F: GTCGACCCTGTCCGAATCTA R: TCCTTGAAGTGGCTAAGGGA	P1	160	0.00013**	10.29
				P2	133		
5	CA516044	F: ATCTTCTTCTCATTCTCCCTTC R: TGCTCAGCATTAACGACGTC	P1	196	0.00021**	14.54	
			P2	163			
DI	6	Hpms1-143	F: AATGCTGAGCTGGCAAGGAAAG R: TGAAGGCAGTAGGTGGGGAGTG	P1	241	0.045791*	5.43
				P2	235		
FYPP	7	Hpms1-3	F: TGGGAAATAGGATGCGCTAAACC R: AACTTTAAGACTCAAATCCATAACC	P1	158	0.015790*	4.48
				P2	130		
FYPP	8	Hpms1-41	F: GGGTATCATCCGTTGAAAGTTAGG R: CAAGAGGTATCAACATGAGAGG	P1	182	0.04576*	3.22
				P2	167		

Where R²–phenotypic variation explained *Differing at 5% level of significance **Differing at 1% level of significance;
FL=Fruit Length, DI=Disease Incidence, FYPP=Fruit Yield Per Plant

19.0%), more importantly higher estimates of heritability (95.0%) and moderate per cent mean of genetic advance mean (46.53%). Sran *et al.*, (2019) revealed medium GCV and PCV (19.89 and 22.0%), more importantly higher estimates of heritability (81.74%) and low per cent mean of genetic advance mean (37.05%) in chill

Validation of marker trait association

One way ANOVA was carried out for single marker analysis to detect SSR markers (as an independent variable) associated with quantities traits (dependent variables). An association between marker and phenotypic trait was revealed by significant F value ($p < 0.01$ and 0.05) (Table 4).

When combining marker data with phenotypic data the analysis indicated that the p-value associated with the F-test. In this case, the p-value was extremely small. The F statistics significantly exceeds the critical F value indicating the difference significant difference between the groups. Typically, if the p-value is less than 0.05, we reject the null hypothesis, which suggests that the difference is not due to random chance. Since the p-value here is much smaller than 0.05, we can conclude that the F value is significant, and there is a significant difference among the groups.

Total eight SSR markers were observed, from that EPMS558 and GPMS203 showed association with fruit length, CaES0028, CaES0047, CA516044 and Hpms1-143 association with CLCV resistance, Hpms1-3 and Hpms1-41 had association with fruit yield per plant.

Among the markers, CA516044 had maximum R² value of 14.54, indicating that circa 14% phenotypic variation for CLCV resistance has been explained by this marker, which happens to be the maximum among other markers used in the study. The product amplified by the different SSR marker associated for fruit length, disease incidence and fruit yield per plant indicated in Table 4.11. Maximum amplified size was observed with the marker CaES0028 associated with the CLCV resistance with highly significant result. All markers showed resolution in 3.5% agarose gel electrophoresis markers that showed resolution on PAGE gel.

There were two markers EPMS558 and GPMS203 found significant association with fruit length with R² value 3.40 and 3.63 respectively. Four markers CaES0028, CaES0047, CA516044 and Hpms1-143 observed association with CLCV resistance from that three markers were recorded CaES0028, CaES0047 and CA516044 highly significant result with R² value 6.67, 10.29 and 14.54, respectively. An R-squared (R²) value measures the proportion of the variance in the dependent variable that is predictable from the independent variable(s). An R² value of 1 indicates that the independent variable(s) can perfectly predict the dependent variable, while an R² value of 0 indicates that they cannot predict it at all. Only one marker recorded significant result with R² value 5.43. These four markers were found with higher R² value rather than remaining markers. Hpms1-3 and Hpms1-41 two markers were recorded association with fruit yield per plant with R²

value 4.48 and 3.32.

No significant marker trait association was identified for the remaining four morphophysiological traits like days to initiation of flowering, plant height, fruit weight, primary branches per plant due to lack of variability in trait or limited polymorphic markers.

Thakur *et al.*, (2020) recorded two marker CA16044 and PAU-343-1 were for disease resistant for chilli leaf curl virus covering total distance with 15.7 cM. CA16044 which is also linked with disease resistant in this study. Bukhari *et al.*, (2022) found two markers linked with fruit yield per plant with R² value 0.127 and 0.140 with significant result which is similar with present investigation result. Sakure *et al.*, (2024) identified SSR marker linked with *meloidogyne* resistance and leaf thickness in *Nicotiana tabacum* by validation of marker trait association through single marker analysis with R² range of 2.2-20.45 which is in agreement with present investigation result for disease resistance marker in mapping population.

Conclusion

Morphophysiological traits like plant height, fruit length, fruit weight, fruit yield per plant and primary branches per plant had negative correlation with the disease incidence in chilli. Normality distribution analysis, plant height, fruit length, fruit weight, fruit yield per plant and disease incidence were governed by the complementary gene action. Days to initiation of flowering, primary branches per plant and membrane injury were governed by the duplicate gene action. Days to initiation of flowering, plant height and primary branches per plant exhibited medium GCV and PCV%. Fruit length, fruit weight and fruit yield per plant showed high GCV and PCV%. All these characters studied having high heritability could help in the selection of superior and desired lines for further improvement in chilli breeding programme.

Study on identification of marker trait association for chilli leaf curl virus resistance was found significant through single marker analysis. Single Marker Analysis using one-way analysis of variance was the best and simple method for identification of marker traits. Present investigation revealed that the identification of total 8 useful markers to screen various traits like fruit length, disease incidence and fruit yield per plant. Two markers EPMS558 and GPMS203 were associated with fruit length. Four markers found CaES0028, CaES0047, CA516044 and Hpms1-143 observed associated with CLCV resistance, which suggested that identified markers were strongly linked to the CLCV resistance in

chilli. Hpms1-3 and Hpms1-41 two markers had association with fruit yield per plant.

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