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RESEARCH ARTICLE

In vitro screening and biochemical profiling of green chilli fruits challenged by *Colletotrichum capsici*

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Abstract

Colletotrichum capsici causes anthracnose and continues to be a severe hindrance to the production of chillies (*Capsicum annuum* L.), endangering both productivity and quality after harvest. In this study, thirty green chilli genotypes were systematically evaluated under controlled conditions using an *in vitro* detached fruit assay to decipher resistance responses against the pathogen. Disease severity was assessed across multiple time points, revealing notable genotypic variation. Genotypes such as Bhavigva-F1 and ARD-5533-F1 showed heightened susceptibility, characterised by rapid lesion expansion and elevated percent disease index (PDI), while entries like Akhanda-23-F1, SVHA 1049 and SVHA 2222 exhibited delayed symptom progression and comparatively lower PDIs. Multivariate analysis, including principal component analysis (PCA), further highlighted the distinct pathological profiling of tested genotypes. Complementary biochemical profiling at four days post-inoculation (DPI) identified significant differences in the accumulation of total phenols, soluble sugars, reducing sugars and proteins. Notably, moderately susceptible genotypes accumulated higher phenolic and protein content, which correlated negatively with disease severity. Similarly, total soluble solids (TSS) and total reducing sugars (TRS) also showed variation among the genotypes. Together, these insights not only affirm the existence of valuable genetic variability for anthracnose resistance in chilli but also emphasise the utility of integrating biochemical markers with phenotypic screening.

Keywords: anthracnose; biochemical markers; chilli genotypes; *Colletotrichum capsici*

Introduction

Chilli peppers (*Capsicum* spp.) are globally significant horticultural crops cultivated for their nutritional, economic and pharmaceutical value (1, 2). Native to the tropical regions of Central and South America, the genus comprises approximately 38 species, exhibiting extensive morphological diversity–particularly in fruit characteristics such as colour, size, shape and pungency (3). Among these, only five species: *Capsicum annuum*, *Capsicum chinense*, *Capsicum frutescens*, *Capsicum pubescens* and *Capsicum baccatum* have been domesticated and are widely cultivated across varied agro-climatic regions (4). Among the major constraints affecting Capsicum production, biotic stresses play a critical role (5). Notably, anthracnose disease primarily caused by species within the genus *Colletotrichum* poses a serious threat to yield and post-harvest fruit quality (6). The disease is particularly challenging due to its complex etiology, involving multiple *Colletotrichum* species that vary in pathogenicity and host specificity (7). Among them, *Colletotrichum capsici* (syn. *Colletotrichum truncatum*) is the most prevalent and virulent species associated with anthracnose in Capsicum crops, leading to significant economic losses in tropical and subtropical growing regions (8-10).

Colletotrichum species, the causal agents of anthracnose, are known for their hemi-biotrophic infection strategy in host plants, including *Capsicum* spp. The infection begins with a biotrophic phase, during which the fungus establishes itself within living host tissues without causing visible damage (11, 12). This is followed by the necrotrophic phase, where the pathogen induces host cell death, leading to tissue degradation and the appearance of characteristic disease symptoms (7, 13). The specialised infection structures produced by *Colletotrichum* species include germ tubes, appressoria, intracellular hyphae and secondary necrotrophic hyphae. It damages the crop right from the early stage and continues till harvest (14, 15). The disease manifests as small sunken necrotic lesions in leaves, stems and the most economically significant damage is

observed on mature fruits during the pre- and post-harvest stages, directly impacting marketability and yield, as well as in seeds during storage (16). Various management strategies have been employed to control anthracnose in *Capsicum* species, including improved crop management practices and the use of resistant genotypes (17-19). Among these, the development and deployment of resistant cultivars is widely regarded as the most economically viable and environmentally sustainable approach (18). However, the genetic control of resistance to anthracnose in capsicum is influenced by multiple factors, including the pathogen, genetic source of host resistance, the concentration and virulence of the inoculum and the developmental stage of the fruit at the time of infection (8, 18).

Inducible and constitutive defensive mechanisms have been linked to host-plant resistance to *Colletotrichum* (14, 20). Induced defences entail a complicated series of molecular and biochemical reactions that are triggered once the pathogen is recognised (21). Quantifiable indicators of the pathogen's virulence, such as total phenols, sugars, total soluble protein, oxidative enzymes and other biochemicals, are known to be essential parts of defence mechanisms and resistance against plant pathogens that are hemi-biotrophic, necrotic and biotrophic (22). To assess the differences in host response, the biochemical differences in symptom development on the fruits of these thirty genotypes were examined at the fourth DPI. The most effective, non-hazardous, environmentally safe and cost-effective method of managing plant diseases is to screen chilli genotypes in vitro, which aids in quickly identifying the sources of resistance in genotypes (23, 24). Thorough biochemical profiling provides insights into the host-pathogen interaction by quantifying key defence molecules. These compounds are directly linked to resistance expression and help distinguish tolerance from susceptible genotypes. Therefore, biochemical analysis is essential to strengthen disease resistance markers for breeding resistance cultivars.

Materials and Methods

The experiment was conducted in the Department of Plant Pathology, College of Agriculture, Vishwesharaiah Canal Farm, Mandya. Thirty genotypes of chilli were collected from the experimental field Zonal Agricultural Research Station, Vishwesharaiah Canal Farm, Mandya (Fig. 1).

In vitro screening of chilli genotypes for resistance to fruit rot caused by *Colletotrichum capsici*, by detached fruits method

Plant material and experimental design

Thirty green chilli (*Capsicum annuum*) genotypes were selected for the study. Fruits were harvested at the green stage from healthy plants and brought to the laboratory for screening. The experiment was laid out in a completely randomised design (CRD) with three replications, each consisting of five fruits per genotype.

Pathogen isolation and inoculum preparation

A virulent isolate of *Colletotrichum capsici*, confirmed through pathogenicity tests, was used for artificial inoculation. The fungus was cultured on potato dextrose agar (PDA) and incubated for 10 days to obtain actively sporulating colonies. Conidia were harvested by adding sterile distilled water to the petri plates and gently scraping the colony surface with a sterile camel hairbrush. The suspension was filtered through double-layered muslin cloth and adjusted to a concentration of 10^6 conidia/mL using a haemocytometer.

Inoculation technique

Surface sterilisation of fruits was performed using 5 % sodium hypochlorite, followed by rinsing with sterile distilled water and air-drying under aseptic conditions. Each fruit was artificially wounded with a sterile needle, inflicting 1-3 punctures (1-2 mm deep) to mimic natural infection sites (Fig. 2). A 10 µL droplet of the spore suspension was applied to each wound using a micropipette (Pin prick method).

Fig. 1. Experimental plot of chilli genotypes.

Fig. 2. Inoculation of chilli fruits with *Colletotrichum capsici* by the pin-prick method.

Incubation conditions and disease assessment

Inoculated fruits were placed in 15 cm diameter sterile petri plates lined with four layers of sterile, moistened paper towels to maintain high humidity. The plates were sealed with parafilm and incubated at room temperature. Lesion development was monitored at 4, 6, 8 and 10 days post-inoculation. Disease severity was assessed by measuring lesion area and was scored using the 0-5 scale (25).

$$PDI = \frac{\text{Sum of individual rating}}{\text{No. of observation assessed} \times \text{Maximum disease rating}} \times 100 \quad \text{(Eqn. 1)}$$

Disease rating and categorisation

Genotypes were classified into six disease reaction categories based on their PDI values. The infection types were characterised using the following scale, in Table 1 (25).

AUDPC calculation:

$$AUDPC = \sum_{i=1}^{n-1} [\{(X_i + X_{(i+1)}) / 2\} \times (t_{(i+1)} - t_i)] \quad \text{(Eqn. 2)}$$

X_i= disease index expressed as a proportion at the i^{th} observation.

t_i= time (days after inoculation) at the i^{th} observation.

Biochemical characterisation of chilli genotypes

Experimental setup

To understand the biochemical basis of resistance against *Colletotrichum capsici*, green chilli fruits representing highly susceptible, susceptible, moderately susceptible and resistant genotypes were evaluated at 4 days after inoculation (DAI). Standard biochemical analysis techniques were employed to quantify total phenols, total soluble sugars, non-reducing sugars and total protein content. Each treatment was replicated three times and the experiment was conducted under controlled conditions using CRD.

Table 1. Disease reaction categories based on their PDI values

Scale	Description
0	No infection (Immune)
1	Resistant up to 5 % lesion on fruit
2	Moderately Resistant 2-10 % lesion on fruit
3	Moderately susceptible 10-25 % lesion on fruit
4	Susceptible 25-50 % lesion on fruit
5	Highly Susceptible > 50 % lesion on fruit

Estimation of total phenol content

Total phenol content was estimated following the method described in previous studies (26). 1 g of fruit tissue was homogenised in 10 mL of 80 % ethanol and centrifuged at 10000 rpm for 20 min. The supernatant was chilled, filtered and evaporated to dryness in a water bath. The residue was re-dissolved in distilled water and 0.2 mL of this solution was made up to 3 mL with distilled water. Subsequently, 0.5 mL of Folin–Ciocalteau reagent was added, followed by 2 mL of 20 % sodium carbonate after 3 min. The mixture was boiled for 1 min, cooled and the absorbance was measured at 650 nm using a UV-Vis spectrophotometer. Phenol content was expressed as mg g^{-1} FW, calibrated using a catechol standard curve.

Estimation of sugar content

Total soluble and reducing sugars were quantified using the standard method depicted in earlier researches (27). For total sugars, hydrolysis was performed by mixing 1 mL of extract with 1.0 N hydrochloric acid (HCl), incubating at 50 °C for 20 min. After neutralisation with 1.0 N sodium hydroxide (NaOH) and 0.1 N hydrochloric acid (HCl) (using phenolphthalein as an indicator), the solution was made up to volume. For both reducing and total sugars, 1 mL of sample was treated with 1 mL of alkaline copper reagent, boiled for 20 min, cooled and mixed with 1 mL of arseno-molybdate reagent. Final volume was adjusted to 15 mL and absorbance was recorded at 510 nm. Glucose was used as a standard and results were expressed as mg g^{-1} FW.

Estimation of total protein content

Protein estimation was carried out using the Lowry method (28). 1 g of fruit tissue was homogenised in 5 mL of 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged at 16000 rpm for 20 min. The supernatant was used for analysis. Solution C was prepared fresh by combining 50 mL of 2 % sodium carbonate in 0.1 N NaOH with 1 mL of a mix of 0.5 % copper sulfate and 1 % sodium potassium tartrate. A 0.1 mL aliquot of the sample was mixed with 1 mL of distilled water and 5 mL of Solution C. After 10 min, 5 mL of diluted Folin-Ciocalteau reagent was added and the mixture was incubated in the dark for 30 min. Absorbance was recorded at 660 nm. Protein concentration was expressed as mg g^{-1} FW using a standard curve prepared with bovine serum albumin (BSA).

Statistical analysis

All experimental data were analysed using one-way analysis of variance (ANOVA) and paired t-test ($p < 0.0001$) with SPSS version 16.0 and Pearson's correlation coefficient was used ($p \leq 0.01$). Additionally, multivariate analysis was performed using R software to assess variations among genotypes.

Results

Disease progression and genotypic categorisation

Thirty chilli (*Capsicum annuum* L.) genotypes were screened for their response to *Colletotrichum capsici* under artificial inoculation conditions. Disease severity was evaluated on the basis of PDI, lesion length and area under the disease progress curve (AUDPC) at 4, 6, 8 and 10 DAI.

Based on mean PDI and AUDPC values, genotypes were grouped into three categories: susceptible (S), moderately susceptible (MS) and highly susceptible (HS) (Table 2 and Fig. 3). None of the entries exhibited immune or highly resistant reactions. Highly susceptible genotypes: Bhavigva-F1 (PDI: 56.01 %, AUDPC: 357.35, lesion length: 6.83cm) and ARD-5533-F1 (52.17 %, 328.01, 6.61cm) developed severe and early lesions, with high AUDPC values reflecting rapid disease progression. Moderately susceptible genotypes: Akhanda-23-F1, SVHA 1049 and SVHA 2222 showed delayed and less aggressive lesion development, with PDI values ranging between 21.67 %-23.67 % and lower AUDPC values (124.02 - 144.02). Lesion lengths in this group remained below 3.2cm. Susceptible group: The remaining 25 genotypes exhibited variable symptoms, with mean PDIs ranging from 26.01 % (Mahy 456) to 45.00 % (Kavitha 2-F1) and AUDPC values from 153.35 to 276.00. Lesion sizes in this group extended from 3.35cm to 6.26cm. The data showed significant variability in lesion length and disease intensity among genotypes, with Bhavigva-F1 and ARD-5533-F1 as the most aggressive responders to *Colletotrichum capsici* infection. In contrast, Entry 3 (PDI: 22.01 %, AUDPC: 133.35, lesion length: 2.83cm) showed the least disease progression, suggesting a relative advantage under controlled inoculation.

Lesion characteristics and fruit traits

Lesion lengths ranged from 2.83cm (SVHA 2222) to 6.83cm (Bhavigva-F1), showing significant variation in infection intensity (Table 2). Genotypes with larger fruits did not necessarily exhibit greater disease severity, where Maina-F1 had the longest fruits (15.10cm) yet showed moderate PDI (36.72 %), while Skoda-399-F1, with smaller fruits (4.81cm), had a lower PDI (28.67 %). There was no direct linear correlation between fruit size and susceptibility, indicating that resistance may be more influenced by biochemical and genetic factors than by morphological traits alone.

Dynamics of disease progression in chilli genotypes

Boxplot analysis of PDI percentage over four time points 4, 6, 8 and 10 DPI revealed a clear, time-dependent increase in disease severity (Fig. 4). Each box represents the interquartile range of PDI %, with the horizontal line denoting the median. On Day 4, minimal symptom expression and a narrow distribution suggested the onset of infection. By Day 6, a modest rise in median PDI percentage was observed. Disease severity intensified considerably by Day 8, with a pronounced upward shift in median and wider value dispersion. Day 10 marked peak infection, reflecting both maximal lesion expansion and greater genotypic variability in host response.

To determine whether PDI percentage differed significantly across these time intervals, a one-way analysis of variance (ANOVA) was conducted (Table 3). The results indicated a highly significant effect of time on disease development ($F = 193.94$; $p < 0.0001$), strongly exceeding the critical F-value (2.6856). This underscores that disease severity increased substantially as the infection progressed and highlights Day 10 as a crucial point for distinguishing genotype responses.

Heatmap visualization

A heatmap was generated to illustrate the temporal progression of disease severity across genotypes (Fig. 5). The gradient in colour intensity from yellow to deep red represents increasing PDI values. Genotypes such as ARD-5533-F1, Bhavigva-F1 and Kavitha 2-F1 displayed darker shades, indicating faster and more intense disease development, whereas lighter shades in SVHA 1049, SVHA 2222 and Akhanda-23-F1 reflected slower disease progression.

Table 2. Resistance profiling of chilli varieties to anthracnose based on PDI, disease progression and lesion length

Sr. No.	Varieties	Day 4	Day 6	Day 8	Day 10	Mean PDI	Scale	AUDPC	Category	Lesion length in cm
1	Bhavigva-F1	10.67	41.34	81.34	90.67	56.01	5	357.35	HS	6.83
2	ARD-5533- F1	8.67	34.67	76.00	89.34	52.17	5	328.01		6.61
3	Sonalika- F1	4.00	17.34	64.00	77.34	40.67	4	248.01		5.52
4	Maina- F1	4.00	16.00	70.67	80.00	42.67	4	261.34		5.25
5	Kavitha- F1	4.00	18.67	46.67	61.34	32.67	4	200.02		4.74
6	Mohitha- F1	4.00	10.67	52.00	56.00	30.67	4	189.34		4.13
7	Kavitha 2- F1	5.34	22.67	68.00	84.00	45.00	4	276.00		6.26
8	Praveen- F1	4.00	17.34	68.00	78.67	42.01	4	257.34		5.73
9	Skoda-399 F1	4.00	14.67	44.00	52.00	28.67	4	177.34		4.45
10	Meenakshi- F1	5.34	17.34	69.34	82.67	43.67	4	266.69		5.35
11	Chilli- 3149- F1	6.67	13.34	53.34	66.67	35.01	4	213.35		4.53
12	Adithya-369 F1	4.00	16.00	38.67	56.00	28.67	4	173.34		4.83
13	Singar I- F1	2.67	11.34	38.67	57.34	27.51	4	162.68		3.72
14	Rakshak- F1	4.00	21.34	33.34	48.00	26.67	4	165.35		3.35
15	ARD 2343	5.34	24.00	52.00	81.34	40.67	4	244.01		5.13
16	V 6040	1.34	14.67	50.67	69.34	34.01	4	202.68		4.77
17	AS-264-CODE-2070-264	5.34	17.34	42.67	64.00	32.34	4	194.68		4.26
18	AS-259-CODE-2085-259	5.34	13.34	53.34	65.34	34.34	4	209.36		4.33
19	AS-262-DODE-2071-262	6.67	10.67	48	66.67	33.01	4	197.34		4.69
20	Panduranga	4.00	17.34	40.00	58.67	30.01	4	181.34		3.47
21	AS-192 DODE-2019-192	6.67	14.67	52.00	76.00	37.34	4	222.67		5.36
22	Rudhira (Entry 2)	4.00	10.67	46.67	69.34	32.67	4	192.02		3.42
23	SVHA 6699 (Entry 4)	1.34	14.67	28.00	68.00	28.00	4	156.00		4.62
24	Mahy 456 (Entry 5)	5.34	13.34	30.67	54.67	26.01	4	153.35	S	3.64
25	Armour (Entry 6)	6.67	12.00	52.00	84.00	38.67	4	225.34		5.93
26	SVHA 1452 (Entry 7)	0.34	20.00	36.00	61.34	29.42	4	174.01		3.85
27	Wonder Hot (Entry 8)	2.67	13.34	45.34	72.00	33.34	4	194.68		4.88
28	Akhanda-23-F1	4.00	22.67	22.67	45.34	23.67	3	144.02		2.92
29	SVHA 1049 (Entry 1)	2.67	9.34	25.34	49.34	21.67	3	124.02	MS	3.12
30	SVHA 2222 (Entry 3)	5.34	14.67	25.34	42.67	22.01	3	133.35		2.83

Table 3. ANOVA analysis of PDI % variation among the different genotypes

Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	69649.9968	3	23216.6	193.941	3.45E-44	2.68564
Within groups	13407.4977	112	119.709			
Total	83057.4946	115				

a

b

c

d

e

Fig. 3. a-e Symptom expression in green chilli genotypes under pathogen stress.

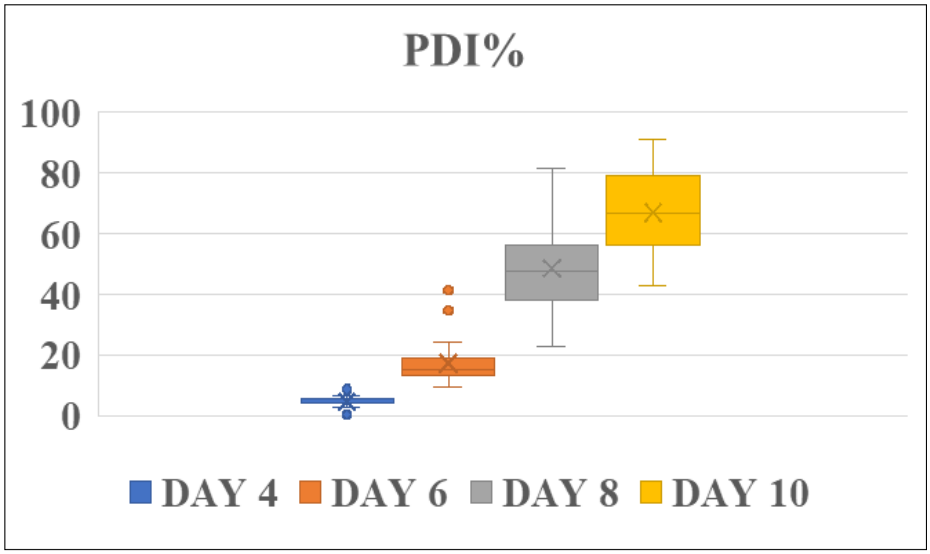

Fig. 4. Boxplot showing Percent disease index (PDI %) progression over time in infected chilli.

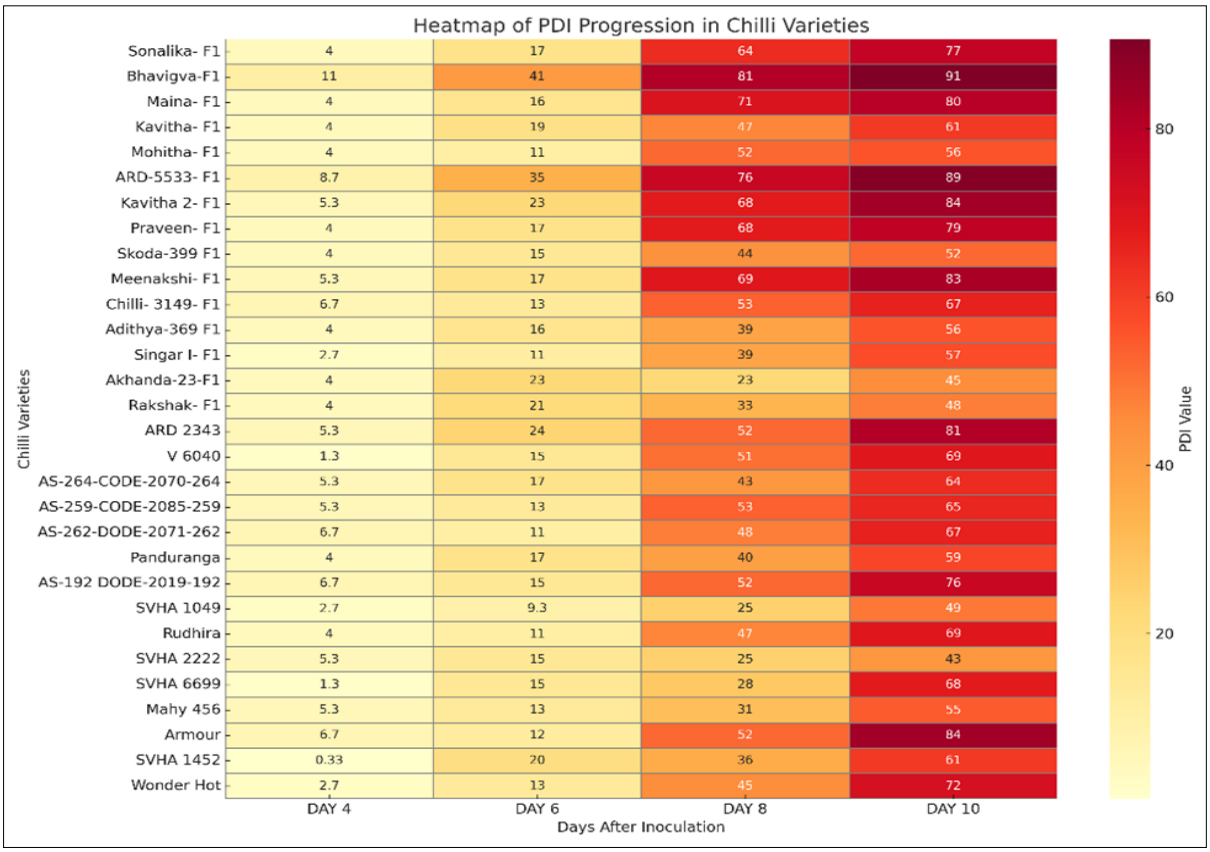

Fig. 5. Heatmap showing temporal disease progression (PDI values) in 30 chilli genotypes from Day 4–10; darker colours indicate higher disease severity.

Principal component analysis (PCA)

The significant variation observed in disease progression and severity across genotypes suggested underlying diversity in their response to *Colletotrichum capsici*. To further investigate patterns of similarity and divergence, a PCA was conducted using key disease descriptors, including mean PDI (0.84 %), AUDPC (0.79) and disease reaction scale (Table 4). PCA reduced the multidimensional dataset into orthogonal axes that maximised variance among genotypes. The first principal component (PC1) accounted for 54.2 % of total variation, primarily influenced by PDI and AUDPC values. The second component (PC2) captured an additional 17.0 %, bringing the total explained variance to 71.2 % (Fig. 6).

The resulting biplot visually differentiated genotypes into discrete clusters. Closely grouped ellipses indicated genotypes with similar disease responses (moderately susceptible group). Distinct, non-overlapping clusters corresponded to genotypes with contrasting highly susceptible, whereas larger ellipse areas reflected higher intra-group variability, whereas smaller ellipses pointed to genetic uniformity. This analysis underscores the potential of PCA not only in visualising the diversity of genotype responses to anthracnose but also in identifying promising entries for resistance breeding.

Table 4. PCA contribution of disease traits in chilli genotypes

Trait	PC1	PC2
PDI	0.84	-0.12
AUDPC	0.79	0.21
Lesion length	0.71	0.33
Disease scale	0.65	0.42

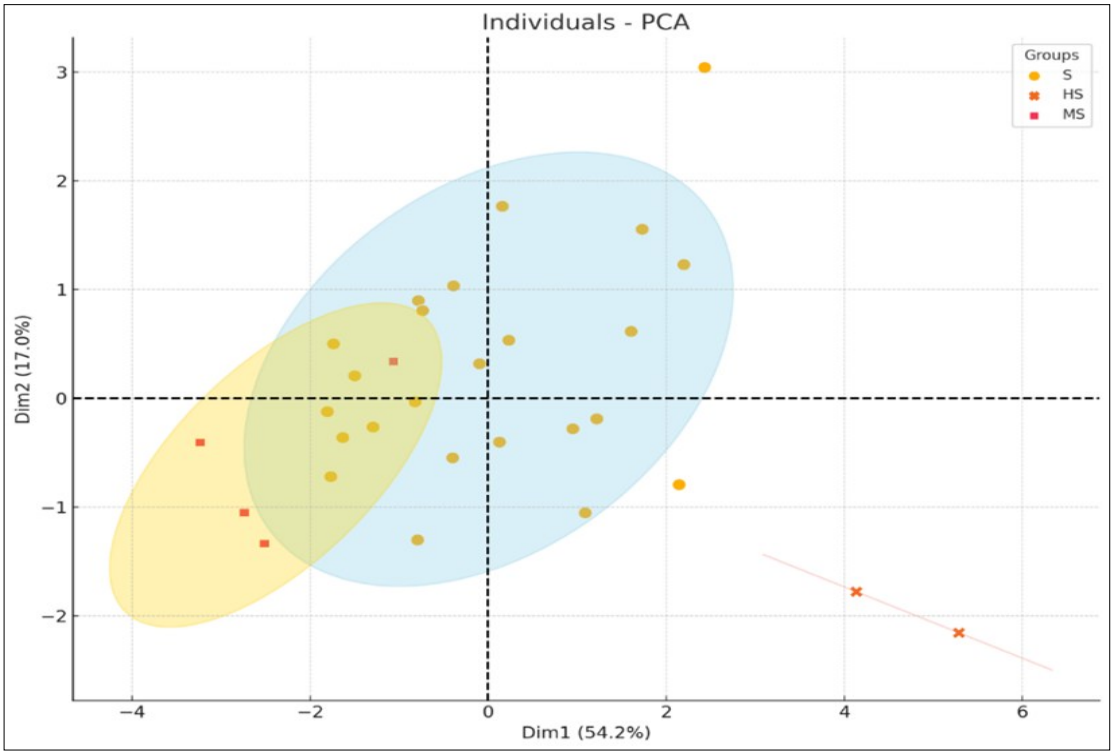

Fig. 6. Principal component-based clustering reveals diversity in chilli genotype responses to anthracnose infection.

Analysis of defence-related biochemical parameters among chilli genotypes

To elucidate the physiological basis of resistance and susceptibility against *Colletotrichum capsici*, key biochemical markers-namely proteins, total soluble sugars, reducing sugars and phenol content-were quantified in selected chilli genotypes representing distinct disease categories (highly susceptible, susceptible, moderately susceptible and moderately resistant) at 4th DAI. The results revealed notable variation in biochemical composition among the genotypes, suggesting a potential role in modulating host defence. A total of thirty chilli genotypes were evaluated for key biochemical parameters - total soluble sugars, total reducing sugars, proteins and phenols under both infected and healthy conditions. Significant variation was observed among genotypes in their biochemical content in response to *Colletotrichum* infection.

The mean TSS content of infected genotypes ranged from 3.18 mg/g to 9.81 mg/g, whereas healthy genotypes ranged from 3.90 mg/g to 10.62 mg/g. Similarly, TRS-infected fruits recorded values between 2.15 mg/g to 5.45 mg/g and healthy genotypes ranged from 2.65 mg/g to 6.11 mg/g. Where TSS and TRS levels were consistently lower in infected fruits compared to their healthy genotypes, protein content was found to be elevated in infected fruits across the majority of genotypes. The highest protein content is in infected fruits, ranging from 11.14 mg/g to 4.16 mg/g. Similarly, in healthy fruits from 12.07 mg/g to 4.65 mg/g. Phenol content, a key component in plant defence, also showed variation. Infected fruits recorded phenol values ranging from 0.06 mg/g to 1.90 mg/g and in healthy 0.04 mg/g to 1.44 mg/g. Notably, many genotypes have elevated phenol levels under infected conditions. The critical difference (CD) at 1 % level confirms that these differences among genotypes were statistically significant, indicating distinct biochemical responses to anthracnose stress (Table 5).

Correlation analysis of biochemical traits associated with anthracnose resistance in chilli

The heatmap in Fig. 7 representing Pearson's correlation analysis (Table 6) revealed a significant association between anthracnose disease severity and key biochemical traits in chilli. A negative and statistically significant correlation was observed between the PDI on the 4th day with TSS (-0.27, $p \leq 0.01$), TRS (-0.36, $p \leq 0.01$), proteins (-0.39, $p \leq 0.01$) and phenols (-0.55, $p \leq 0.01$). In contrast, all biochemical traits showed strong and highly significant positive intercorrelations. Proteins were highly correlated with TSS (0.94), TRS (0.79) and phenols (0.77), suggesting a synergistic biochemical response in genotypes showing reduced disease severity. These findings indicate that higher levels of these biochemical constituents, particularly phenols and proteins, may contribute to enhanced resistance against *Colletotrichum* spp. causing anthracnose in chilli.

Discussion

In vitro screening of green chilli against anthracnose

Thirty chilli genotypes were screened against *Colletotrichum capsici*, the causal agent of anthracnose. The differential responses observed due to genetic diversity, including potential defence-related genes involved in pathogen recognition. This variability highlights the presence of multiple defence layers. Research indicates that none of the lines were resistant and out of fourteen lines screened, only five lines showed moderate resistance with minimum lesion length on the fruits (29, 30). Similarly, research indicates that forty-nine lines were found and only six lines were resistant against *Colletotrichum capsica, in vitro*. The chilli lines against *Colletotrichum capsici* under lab conditions revealed that most of the genotypes were moderately resistant (31).

Biochemical response of chilli genotypes to anthracnose infection

During the infection and rotting of plant tissues, the metabolic

Table 5. Biochemical response of chilli genotypes to *Colletotrichum capsici* infection

Sr. No.	Genotypes	Reaction	Total soluble sugars (mg/g)		Total reducing sugars (mg/g)		Protein (mg/g)		Phenols (mg/g)	
			Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy
1	Akhanda-23-F1		9.81	10.62	5.45	6.11	11.14	12.07	1.90	1.44
2	SVHA 1049	MS	9.33	10.12	4.34	4.77	11.03	11.82	1.81	1.40
3	SVHA 2222		8.27	9.06	4.45	4.51	10.61	11.41	1.77	1.24
4	Sonalika- F1		6.68	8.96	3.65	4.40	8.67	9.53	1.70	1.17
5	Maina- F1		6.54	8.83	3.42	4.18	8.59	9.08	1.67	1.13
6	Kavitha- F1		6.04	8.32	3.45	4.20	8.81	9.31	1.66	1.18
7	Mohitha- F1		5.91	6.70	3.12	3.88	8.52	9.02	1.67	1.14
8	Kavitha 2- F1		5.87	6.66	2.93	3.70	7.86	8.67	1.65	1.11
9	Praveen- F1		5.43	6.22	2.76	3.53	8.03	9.16	1.63	1.09
10	Skoda-399 F1		5.43	6.22	3.87	4.61	7.56	8.16	1.57	1.09
11	Meenakshi- F1		4.87	5.67	3.82	4.57	7.63	8.13	1.55	1.01
12	Chilli- 3149- F1		5.12	5.90	3.98	4.72	7.39	8.34	1.71	1.14
13	Adithya-369 F1		4.83	5.61	3.85	4.59	7.27	7.77	1.71	1.14
14	Singar I- F1		4.84	5.63	3.83	4.58	7.25	7.75	1.7	1.13
15	Rakshak- F1		4.73	5.52	3.34	4.10	7.3	8.19	1.65	1.07
16	ARD 2343	S	4.68	5.47	3.03	4.10	7.31	7.8	1.6	1.09
17	V 6040		4.83	5.63	3.29	4.05	7.09	7.59	1.53	1.02
18	AS-264-CODE-2070-264		5.35	6.14	3.23	3.99	7.02	7.79	1.51	0.99
19	AS-259-CODE-2085-259		4.76	5.54	3.25	4.01	6.51	6.48	1.47	0.94
20	AS-262-DODE-2071-262		5.28	4.82	3.16	3.92	7.44	7.92	1.48	0.96
21	Panduranga		5.65	5.19	3.05	3.92	7.56	8.05	1.46	0.94
22	AS-192 DODE-2019-192		5.49	5.03	3.10	3.87	7.12	7.99	1.69	0.93
23	Rudhira		5.46	4.95	2.99	3.76	7.50	8.00	1.67	0.91
24	SVHA 6699		4.5	4.04	3.04	3.51	7.29	8.25	1.6	1.04
25	Mahy 456		4.69	4.21	3.00	3.54	7.52	8.00	1.56	1.00
26	Armour		4.62	4.14	2.97	3.74	7.32	7.76	1.53	0.97
27	SVHA 1452		4.59	4.10	2.82	3.58	7.07	7.23	1.53	0.97
28	Wonder Hot		4.92	3.93	2.86	3.62	6.79	6.76	1.49	0.93
29	Bhavigva-F1	HS	3.26	4.18	2.27	2.67	4.35	4.79	0.08	0.07
30	ARD-5533- F1		3.18	3.90	2.15	2.65	4.16	4.65	0.06	0.04
	SE m ±		0.066	0.037	0.027	0.023	0.080	0.058	0.019	0.005
	C.D. 1 %		0.187	0.106	0.077	0.067	0.229	0.166	0.055	0.016

SE m ± - Standard error of the mean CD - Critical difference

Table 6. Pearson's correlation of biochemical traits with anthracnose

Variables	PDI on 4th day	TSS	TRS	Proteins	Phenols
PDI on 4th day	1.0**	-0.27**	-0.36**	-0.39**	-0.55**
TSS	-0.27**	1.0**	0.79**	0.94**	0.58**
TRS	-0.36**	0.79**	1.0**	0.79**	0.61**
Proteins	-0.39**	0.94**	0.79**	1.0**	0.77**
Phenols	-0.58**	0.59**	0.61**	0.77**	1.0**

TSS – Total soluble solids; TRS – Total reducing sugars

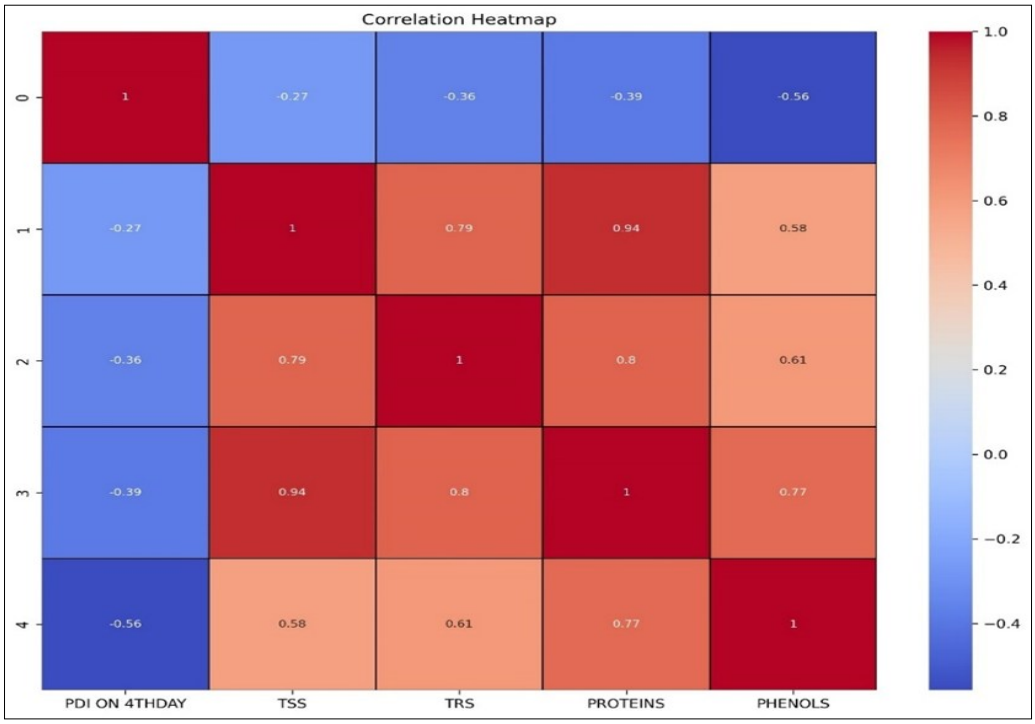

Fig. 7. Visualisation of Pearson correlation coefficient between PDI and biochemical parameters (TSS, TRS, proteins and phenols) measured on the 4th day post-inoculation.

changes were found to be different and distinct from those of healthy tissues (32). The biochemical response of chilli genotypes to *Colletotrichum capsici* infection revealed considerable variation in the accumulation of key compounds such as TSS, TRS, proteins and phenols. These biochemical markers are known to be intricately involved and provide crucial insights into the resistance mechanism. In the present study, genotypes with elevated phenolic content exhibited lower PDI values.

Phenolics act as antimicrobial agents and strengthen the cell wall through lignification, thereby restricting pathogen invasion and spread. Research indicates more phenols were observed in infected (moderately susceptible) plants than in healthy ones, where increased phenolic accumulation was associated with resistance in several host-pathogen interactions (33). A decrease in phenolic compounds of chilli fruits has a direct correlation with the severity of disease (34-36). Research indicates that anthracnose-resistant varieties contained a higher level of preformed phenolic compounds than susceptible varieties (37). These phenol compounds may act as substrates for enzymes that convert them into other compounds more directly related to disease reactions (38). Moderately susceptible genotypes showed significantly higher protein levels, suggesting activation of defence proteins (peroxidases, chitinases, PR-proteins) (39). These proteins play essential roles in early pathogen recognition and in initiating hypersensitive responses to limit infection. The induction of protein synthesis following pathogen infection has been similarly reported in previous research (33, 35).

Interestingly, moderately susceptible genotypes showed higher levels of TSS and TRS compared to highly susceptible ones. These sugars reinforce structural barriers (lignin and callose) to regulate sugar allocation and restrict pathogen access to nutrients, thereby limiting growth. In contrast, susceptible genotypes, with lower sugar levels, are less capable of mounting strong defences. The decline in soluble sugars in infected plants reflects both pathogen utilisation and host redirection of sugars towards phenolic and protein-based defence mechanisms. The reducing sugar and total sugar content were more in anthracnose-infected fruits of the moderately susceptible variety, followed by the susceptible and highly susceptible varieties (33). Similarly, a decrease in total sugar content was observed in previous research (34, 35, 37).

Conclusion

Screening of 30 chilli genotypes against *Colletotrichum capsici* revealed variability in disease response, where some entries showed moderate tolerance. Biochemical profiling indicated that higher phenolic and protein contents were negatively correlated with disease severity, suggesting their role as defence markers. These findings provide useful resources for breeding anthracnose-tolerant chilli varieties.

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Authors' contributions

NL carried out the studies, participated in drafting the manuscript. NKK and KSG planned and prepared the draft for research and performed the statistical analysis. C, NSP and HRR participated in the design of the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

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