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RESEARCH ARTICLE

Enzymatic and biochemical aspects of anthracnose resistance in chili (*Capsicum annuum* L.) genotypes

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Abstract

Chili (*Capsicum annuum* L.) genotypes and hybrids resistant to anthracnose disease are not yet commercially available, and under favorable environmental conditions, the crop suffers significant yield and economic losses. In this study, six chili genotypes and 15 hybrids were screened for their resistance to anthracnose disease. Various plant disease resistance-related parameters were assessed, and the genotypes Bidhan Chili 4, Chinese Bona, and Pant C 1, along with the hybrids Pant C 1 × Bidhan Chili 4, Bidhan Chili 4 × Chili 38-Ragi, and Chinese Bona × Chili 38-Ragi, exhibited resistance to anthracnose disease. To gain a deeper understanding of chili defense mechanisms, biochemical changes in key defense enzymes—such as polyphenol oxidase (PPO), peroxidase (POX), and phenylalanine ammonia-lyase (PAL)—as well as protein and phenol content in fruits (both before and after inoculation) were analyzed. The results showed that resistant genotypes and hybrids exhibited higher activity levels of PPO, POX, and PAL, along with increased protein and phenol content, compared to susceptible ones. These elevated biochemical responses in *Colletotrichum capsici*-inoculated fruits suggest that these mechanisms play a crucial role in enhancing host resistance against anthracnose disease.

Keywords Chili, *Colletotrichum,* Disease severity, Biochemical, Enzyme assay.

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Introduction

Plants naturally possess a range of defense mechanisms to cope with environmental stresses, enabling species-specific immunity (Bal et al., 2024). Among vegetables, chili is widely cultivated for its economic importance and rich content of capsaicinoids, flavonoids, phenolics, and vitamins A and C (Mishra et al., 2017). However, due to high genetic diversity and cross-pollination (Sushmita et al., 2024), chili remains vulnerable to anthracnose—a major disease causing substantial yield losses pre- and post-harvest (Bal et al., 2024). *Colletotrichum capsici* (Sydow) Butler and Bisby, a hemibiotrophic fungus, is the main pathogen, capable of infecting various plant parts, remaining dormant on ripened fruits, and surviving in debris and seeds (Than et al., 2008; Saxena et al., 2019). Traditional practices offer limited control, and heavy chemical use raises environmental and health concerns (Prasad et al., 2020). Therefore, host-plant resistance (HPR) is a sustainable alternative (Brahmani et al., 2024; Bal et al., 2024), where strengthening enzymatic defenses enhances both yield and ecological safety (Malik et al., 2020). Plants deploy constitutive defenses present at all times and induced defenses triggered by pathogens (Kumar et al., 2013). Chili's metabolic and genetic diversity, driven by transposable elements, boosts the expression of LRR proteins—key disease resistance genes (Acunha et al., 2017; Kim et al., 2017). Defense enzymes like PPO, POX, PAL, and pathogenesis-related proteins counter pathogens and pests (Malik et al., 2020). Pathogen-associated molecular patterns (PAMPs) are recognized by PRRs, triggering PAMP-triggered immunity (PTI) and signaling cascades (Du et al., 2015; Monaghan and Zipfel, 2012). Effector-triggered immunity (ETI) further reinforces this defense (Lopes Fischer et al., 2020), often through hypersensitive response and reactive oxygen species (ROS) generation (Prasad et al., 2020). Enhanced activity of PPO, POX, PAL, and related proteins is key to signaling and resistance (Yadav et al., 2020). These signals can also induce systemic acquired resistance (SAR) throughout the plant (Saxena et al., 2019). Overall, disease resistance relies on complex biochemical interactions involving defense-related enzymes like chitinase, catalase, and β-glucanase (Yadav et al., 2020), also observed in other crops like rice and tomato. This study aimed to identify chili genotypes and hybrids resistant to anthracnose by analyzing five key biochemical parameters and examining their correlation with the percent disease index (PDI), providing insights into the biochemical basis of resistance against *C. capsici*.

Materials and Methods

Plant Material, Experimental Design and Growing Conditions

The study was conducted during the autumn-winter season of 2021 at the research field of the All India Coordinated Research Project on Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. Seeds of six chili genotypes and 15 hybrids were sown at a shallow depth of 5 cm apart and covered with finely sieved, well-rotten leaf mold in well-prepared seed beds that were 20 cm high and 1.0 m wide. The beds were drenched with chlorothalonil (2 g) and carbendazim (1-g) to prevent damping-off disease. About 30-day-old seedling of the six chili genotypes and 15 hybrids were transplanted into plots measuring 2.5 × 2.5 m, spaced 50 cm apart both ways to accommodate 25 plants, following a randomized block design in 3 replications. The necessary cultural practices were followed to raise a healthy crop, as per Chattopadhyay et al. (2007).

Isolation of the Pathogen

Isolation was performed using the tissue transplanting technique. Chili fruits with typical anthracnose symptoms were collected from various regions of West Bengal, India. Infected areas (1–3 mm) were excised from the pericarp margins, surface sterilized with 1% sodium hypochlorite for 2 minutes, rinsed with distilled water and dried on tissue paper. These sterilized portions were placed on water agar plates and incubated at 25°C for 3 days. Sporulating hyphae were identified microscopically and cultured on potato dextrose agar (PDA) plates, which were incubated at 25 ± 2°C for a week to obtain pure colonies via the single spore technique (Choi et al., 1999). Pure cultures were stored at 4°C on PDA slants for future use.

Preparation of Spore Suspension

To obtain a conidial suspension, sterile distilled water (10 mL) was poured onto the surface of 10 days *C. capsici* culture, which is followed by scraping the conidial mass using a sterile glass slide. The suspension was then filtered out through double-layer cheesecloth to remove mycelia and cultural debris. Conidial concentration was adjusted to (5×10^5 spores/mL) with sterile distilled water.

Inoculation of Chili Fruits with Pathogen

A 15-day-old culture of *C. capsici* was used to artificially inoculate red ripe chili fruits. For each of the six genotypes and 15 F_1 hybrids, 25 fruits per replication were selected. Fruits were surface sterilized with 0.1% $HgCl_2$, rinsed twice with sterile water, and pricked using sterile pin bundles. The pricked fruits were then immersed in a spore suspension (5×10^5 spores/mL) for 5 minutes and incubated on trays inside a humid chamber prepared with moist cotton and covered with polythene to maintain humidity. Incubation was carried out at 27 ± 1°C for seven days. Post incubation, anthracnose lesions were recorded, and infected fruits were used for biochemical analysis.

The percent disease index (PDI) was calculated by the following formula

$$\text{PDI (\%)} = \frac{\Sigma \text{ All disease rating scales}}{\text{Total number of ratings} \times \text{maximum disease grade}} \times 100$$

The disease reaction of each six chili genotypes and 15 F_1 hybrids was categorized into five categories on the basis of a rating scale described by Singh et al. (1993), namely, 0%-immune, 0 to 5% - resistant, 5 to 25%- moderately resistant, 25 to 50% - susceptible, above 50% - highly susceptible on the basis of the calculated PDI.

Sampling of Fruits for Biochemical Assays

In order to understand the plant responses to anthracnose disease infection, fruit samples were collected and biochemical assays were conducted 10 days prior to inoculation, and infected samples were examined for anthracnose lesions on the 10th day following inoculation. Three replications were followed to minimize error.

Biochemical Analysis

Extraction and enzyme assays

- *Peroxidase (POX)*

Peroxidase (POX) activity was measured following Malik and Singh (1980). The reaction mixture included 50 µL enzyme extract (from 500 mg of pre- and post-inoculated fruit homogenized in 1-mL chilled 0.1 M sodium phosphate buffer, pH 7.0, and centrifuged at 14,000 rpm for 20 minutes at 4°C), 3.65 mL of 0.1 M phosphate buffer (pH 6.5), 100 µL ortho-dianisidine (1-mg/mL in ethanol), and 200 µL of 0.2 M H_2O_2. Absorbance was recorded at 430 nm every 30 seconds for 3 minutes at 28 to 30°C. POX activity was expressed as the rate of absorbance increase per minute per gram of tissue, with a 0.1 unit change per minute defined as one unit of activity.

- *Polyphenol oxidase (PPO)*

Polyphenol oxidase (PPO) activity was estimated following Malik and Singh (1980). The reaction mixture comprised 0.2 mL enzyme extract (from 500 mg pre- and post-inoculated fruit homogenized in 1-mL of 50 mM tris-HCl buffer, pH 7.2, and centrifuged at 14,000 rpm for 20 minutes at 4°C), 2.5 mL of 0.1 M phosphate buffer (pH 6.5), and 0.5 mL of 0.01 M catechol solution. Absorbance was recorded at 495 nm every 30 seconds for 5 minutes at 25°C. PPO activity was expressed as the rate of absorbance increase per minute per gram of tissue, with one unit defined as a 0.1 absorbance change per minute.

- *Phenylalanine ammonia-lyase (PAL)*

Phenylalanine ammonia-lyase (PAL) activity was measured as per Sadasivam and Manickam (1996). About 500 mg of pre- and post-inoculated fruit was homogenized in 5 mL of chilled 0.2 M borate buffer (pH 8.7) containing 5 mM mercaptoethanol (0.04 mL/l), then centrifuged at 14,000 rpm for 20 minutes at 4°C. The supernatant served as the enzyme source. A mixture of 0.5 mL borate buffer, 0.2 mL enzyme extract, 1.3 mL water, and 1-ml $_L$-phenylalanine was incubated at 32°C for 30 to 60 minutes. The reaction was stopped with 0.5 mL of 1 M trichloroacetic acid. In the control, phenylalanine was added after the acid. Absorbance was recorded at 290 nm, and PAL activity was expressed as mg cinnamic acid min^{-1} mg^{-1} protein.

Extraction and estimation of total phenols

Total phenol content was estimated following Sadasivam and Manickam (1996) in both pre-and post-inoculated fruits. One gram of fruit tissue was homogenized in 10 mL of 80% ethanol and centrifuged at 10,000 rpm for 20 minutes. The supernatant was evaporated to dryness, and the residue was reconstituted with 3 mL distilled water. To 1-mL of this, 0.5 mL of Folin-Ciocalteu reagent and 2 mL of 20% sodium carbonate were added. The mixture was heated in a boiling water bath for 1-minute, cooled, and absorbance was recorded at 650 nm against a blank. Catechol was used for the standard curve, and results were expressed as mg phenol per 100 g of tissue.

Estimation of total protein content

Soluble protein content was estimated using the Lowry et al. (1951) method. For this, 0.7 g of fruit tissue was homogenized in 10 mL of 0.2 M tris–HCl buffer and centrifuged at 10,000 rpm for 30 minutes. The supernatant was used as the protein extract. About 1-mL of the extract was mixed with 5 mL of Reagent C (prepared by combining 50 mL of Reagent A—20 g Na_2CO_3 in 200 mL water with 100 mL of 0.1 N NaOH, diluted to 1 L—and 1-mL of Reagent B—0.5% $CuSO_4{\cdot}5H_2O$ in 1% potassium sodium tartrate). After 10 minutes, 0.5 mL of Reagent D (1:1 Folin–Ciocalteu reagent and distilled water) was added and incubated for 30 minutes in the dark. The resulting blue color was measured at 600 nm.

Statistical Analysis

The experiment followed a randomized complete block design (RBD), and analysis of variance (ANOVA) was conducted using SAS 9.3 Professional Version. Results were presented as mean ± SD. Tukey's Honest Significant Difference (HSD) test ($p \leq 0.01$) was used for mean separation across genotypes and F_1 hybrids for disease infestation. Pearson correlation coefficients between biochemical traits and anthracnose infestation were calculated using MS Excel 2016, with significance assessed at $p \leq 0.05$.

Results and Discussion

Screening of chili genotypes and hybrids under laboratory condition against anthracnose disease

Chili fruits were challenge inoculated with *C. capsici* under laboratory (artificial) conditions and were studied for disease severity reactions among six genotypes and 15 F_1 hybrids (Tables 1, 2, 3, 4). Genotypes Chinese Bona (4.93%), Pant C 1 (4.53%), Bidhan Chili 4 (1.73%), and Chili 38-Ragi (3.20%) showed resistant to moderately resistant reactions. In contrast, BCC 1 (26.20%) and Srinagar (56.56%) were moderately and highly susceptible, respectively. The 15 F_1 hybrids displayed varied responses. Bidhan Chili 4 × Chili 38-Ragi had the lowest PDI (1.73%), followed by Pant C 1 × Bidhan Chili 4 (3.46%) and Chinese Bona × Chili 38-Ragi (4.26%), indicating resistance. Other resistant hybrids included Chinese Bona × Bidhan Chili 4 (5.60%), Chinese Bona × Srinagar (6.93%), and Chinese Bona × Pant C 1 (8.44%). Pant C 1 × BCC 1 showed the highest severity (60.09%), followed by Chili 38-Ragi × Srinagar (57.69%), both highly susceptible. The remaining hybrids were moderately susceptible: Chinese Bona × BCC 1 (38.40%), Pant C 1 × Srinagar (27.64%), Bidhan Chili 4 × Srinagar (18.00%), Bidhan Chili 4 × BCC 1 (20.17%), Chili 38-Ragi × BCC 1 (33.33%), and Srinagar × BCC 1 (44.67%).

Biochemical profile of chili genotypes and hybrids under laboratory (artificial) conditions against chili anthracnose disease

Plants, whether resistant or susceptible, defend against various diseases through defined strategies involving defensive enzymes, free radical scavenging, and signaling

Table 1: Interaction effects between Parental genotypes and stage of infection for Percent Disease Index, Polyphenol Oxidase (PPO) Peroxidase (POD), Phenylalanine Ammonia Lyase (PAL), Phenol, Protein, for Chili anthracnose disease under artificial condition

Chili Genotypes	*Stages*	*PDI*	*PPO*	*POD*	*PAL*	*Phenol*	*Protein*
Chinese Bona	Pre- inoculated fruits	-	0.882 ± 0.002 d	0.814 ± 0.004 h	1.015 ± 0.004 c	105 ± 4.000 d	1.916 ± 0.003 i
	Post-inoculated fruits	4.93 ± 0.83 hgi	1.003 ± 0.001 d	0.921 ± 0.003 i	1.106 ± 0.002 c	136.67 ± 2.082 f	3.205 ± 0.002 n
Pant C 1	Pre-inoculated fruits	-	0.739 ± 0.001 g	0.998 ± 0.009 d	0.969 ± 0.003 f	114 ± 3.606 c	1.519 ± 0.00 il
	Post-inoculated fruits	4.53 ± 1.22 hgi	0.964 ± 0.004 f	1.175 ± 0.002 f	1.068 ± 0.002 d	150.33 ± 0.577 e	2.007 ± 0.002 j
Bidhan Chili 4	Pre-inoculated fruits	-	1.013 ± 0.003 ba	0.760 ± 0.004 i	1.205 ± 0.002 a	137 ± 2.645 a	2.111 ± 0.003 g
	Post-inoculated fruits	1.73 ± 0.61 i	1.094 ± 0.002 b	1.382 ± 0.004 d	1.184 ± 0.004 b	175.67 ± 1.525 d	4.015 ± 0.002 g
Chili 38-Ragi	Pre-inoculated fruits	-	0.665 ± 0.003 j	0.875 ± 0.003 g	1.004 ± 0.003 d	90.33 ± 2.082 efg	1.341 ± 0.002 m
	Post-inoculated fruits	3.20 ± 0.8 hi	0.990 ± 0.002 e	1.056 ± 0.003 g	1.024 ± 0.003 g	104.33 ± 0.577 g	1.817 ± 0.003 k
Srinagar	Pre-inoculated fruits	-	0.330 ± 0.001 p	0.402 ± 0.010 o	0.506 ± 0.004 n	65 ± 3.606 k	0.866 ± 0.002 r
	Post-inoculated fruits	56.26 ± 1.66 a	0.292 ± 0.002 r	0.360 ± 0.003 n	0.276 ± 0.004 o	47.67 ± 1.528 mn	0.422 ± 0.002 t
BCC 1	Pre-inoculated fruits	-	0.419 ± 0.002 n	0.298 ± 0.003 p	0.359 ± 0.003 p	68.33 ± 1.528 jk	0.811 ± 0.002 s
	Post-inoculated fruits	26.2 ± 2.90 e	0.328 ± 0.003 p	0.284 ± 0.003 q	0.250 ± 0.003 p	55.33 ± 2.082 ml	0.532 ± 0.003 r

Results are presented as the Mean values ± Standard Deviation (S.D.)
Different lowercase letters in the same column indicate statistically significant difference according to Tukey's post hoc test ($p \leq 0.01$)

Table 2: Interaction effects between hybrids and stage of infection for percent disease index, phenol, protein, peroxidase (POD), polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) for Chili anthracnose disease under artificial condition

Chili hybrids	*Stages*	*PDI*	*PPO*	*POD*	*PAL*	*Phenol*	*Protein*
Chinese Bona × Pant C 1	Pre- inoculated fruits	-	0.706 ± 0.006 i	0.872 ± 0.015 g	0.973 ± 0.002 f	104.67 ± 2.517 d	2.269 ± 0.002 e
	Post-inoculated fruits	8.44 ± 1.10 g	0.816 ± 0.005 j	0.906 ± 0.004 j	1.020 ± 0.002 g	225 ± 3.000 b	5.773 ± 0.003 e
Chinese Bona × Bidhan Chili 4	Pre-inoculated fruits	-	0.871 ± 0.002 e	1.115 ± 0.005 c	1.002 ± 0.003 d	118.67 ± 1.155 cb	3.380 ± 0.002 c
	Post-inoculated fruits	5.6 ± 0.8 hgi	0.924 ± 0.003 g	1.227 ± 0.002 e	1.054 ± 0.002 e	218.67 ± 0.57 b	6.454 ± 0.002 c
Chinese Bona × Chili 38-Ragi	Pre-inoculated fruits	-	0.745 ± 0.003 g	0.936 ± 0.003 f	0.649 ± 0.002 k	123.67 ± 2.082 b	2.105 ± 0.002 h
	Post-inoculated fruits	4.26 ± 1.22 hgi	0.963 ± 0.002 f	1.035 ± 0.002 h	0.994 ± 0.003 h	175.67 ± 2.082 d	5.010 ± 0.002 f
Chinese Bona × Srinagar	Pre-inoculated fruits	-	0.332 ± 0.002 p	0.404 ± 0.005 o	0.734 ± 0.004 j	101.33 ± 3.215 d	2.159 ± 0.003 f
	Post-inoculated fruits	6.93 ± 1.66 hg	0.905 ± 0.003 h	0.320 ± 0.003 i	0.965 ± 0.003 i	146.67 ± 1.528 e	6.293 ± 0.002d
Chinese Bona × BCC 1	Pre-inoculated fruits	-	0.529 ± 0.002 l	0.671 ± 0.003 k	1.052 ± 0.002 b	82.67 ± 2.517 hg	1.922 ± 0.002 i
	Post-inoculated fruits	38.4 ± 0.81 c	0.304 ± 0.002 q	0.193 ± 0.002 t	0.578 ± 0.002 k	64.67 ± 4.042 j	1.005 ± 0.004 n
Pant C 1 × Bidhan Chili 4	Pre-inoculated fruits	-	1.018 ± 0.002 a	0.974 ± 0.004 e	0.991 ± 0.002 e	137.67 ± 1.528 a	3.755 ± 0.002 b
	Post-inoculated fruits	3.46 ± 1.22 hgi	1.109 ± 0.002 a	1.806 ± 0.003 a	1.226 ± 0.003 a	225.67 ± 4.041 b	7.844 ± 0.002 a
Pant C 1 × Chili 38-Ragi	Pre-inoculated fruits	-	0.602 ± 0.002 k	1.324 ± 0.003 a	0.837 ± 0.004 h	144.33 ± 2.517 a	3.163 ± 0.003 d
	Post-inoculated fruits	7.46 ± 1.22 hg	0.883 ± 0.002 i	1.664 ± 0.003 b	1.033 ± 0.003 f	259.00 ± 2.646 a	3.879 ± 0.002 h
Pant C 1 × Srinagar	Pre-inoculated fruits	-	0.573 ± 0.002 k	0.499 ± 0.002 n	0.522 ± 0.002 m	73.33 ± 2.082 ji	1.874 ± 0.001 j
	Post-inoculated fruits	27.64 ± 2.15 e	0.415 ± 0.003 n	0.493 ± 0.003 l	0.429 ± 0.002 m	55.67 ± 3.512 kl	1.022 ± 0.002 m

Results are presented as the Mean values ± Standard Deviation (S.D.). Different lowercase letters in the same column indicate statistically significant difference according to Tukey's post hoc test ($p \leq 0.01$)

Table 3: Interaction effects between hybrids and stage of infection for Percent Disease Index (PDI), peroxidase (POD), polyphenol Oxidase (PPO), Phenylalanine Ammonia Lyase (PAL), Phenol, Protein, for Chili anthracnose disease under artificial condition

Chili hybrids	*Stages*	*PDI*	*PPO*	*POD*	*PAL*	*Phenol*	*Protein*
Pant C 1 × BCC 1	Pre-inoculated fruits	-	0.406 ± 0.002 o	0.627 ± 0.003 l	0.334 ± 0.001 q	84.67 ± 1.528 hfg	1.609 ± 0.002 k
	Post-inoculated fruits	60.09 ± 3.42 a	0.369 ± 0.002 l	0.542 ± 0.002 k	0.254 ± 0.003 p	63.33 ± 2.082 kj	1.104 ± 0.001 l
Bidhan Chili 4 × Chili 38-Ragi	Pre-inoculated fruits	-	1.006 ± 0.002 b	1.225 ± 0.002 b	0.813 ± 0.003 i	92.33 ± 2.082 ef	3.899 ± 0.002 a
	Post-inoculated fruits	1.73 ± 0.61 i	1.056 ± 0.002 c	1.557 ± 0.002 c	0.865 ± 0.002 j	194.67 ± 0.577 c	7.645 ± 0.002 b
Bidhan Chili 4 × Srinagar	Pre-inoculated fruits	-	0.502 ± 0.002 m	0.729 ± 0.005 j	0.804 ± 0.003 i	97.67 ± 2.517 ed	1.325 ± 0.002 n
	Post-inoculated fruits	18.00 ± 1.83 f	0.347 ± 0.003 o	0.272 ± 0.002 r	0.214 ± 0.002 q	85.33 ± 2.517 h	0.609 ± 0.002 p
Bidhan Chili 4 × BCC 1	Pre-inoculated fruits	-	0.724 ± 0.002 h	0.585 ± 0.003 m	0.604 ± 0.003 l	90.67 ± 2.082 ef	1.087 ± 0.002 p
	Post-inoculated fruits	20.17 ± 1.07 f	0.403 ± 0.001 m	0.424 ± 0.002 m	0.484 ± 0.002 l	74.67 ± 3.055 i	0.788 ± 0.002 o
Chili 38-Ragi × Srinagar	Pre-inoculated fruits	-	0.923 ± 0.002 c	0.301 ± 0.002 p	0.445 ± 0.004 o	67.67 ± 1.528 jk	1.204 ± 0.002 o
	Post-inoculated fruits	57.69 ± 0.93 a	0.366 ± 0.002 n	0.213 ± 0.001 p	0.141 ± 0.002 s	46.67 ± 2.517 n	0.557 ± 0.002 q
Chili 38-Ragi × BCC 1	Pre-inoculated fruits	-	0.832 ± 0.002 f	0.322 ± 0.003 o	0.880 ± 0.008 g	80.33 ± 2.309 hi	1.006 ± 0.002 q
	Post-inoculated fruits	33.33 ± 1.22 d	0.221 ± 0.002 s	0.296 ± 0.005 p	0.305 ± 0.002 n	66.33 ± 3.215 j	0.437 ± 0.002 s
Srinagar × BCC 1	Pre-inoculated fruits	-	0.196 ± 0.002 q	0.323 ± 0.002 s	0.181 ± 0.003 r	53.33 ± 2.517 l	0.706 ± 0.002 t
	Post-inoculated fruits	44.67 ± 2.20 b	0.086 ± 0.003 t	0.218 ± 0.002 q	0.167 ± 0.002 r	35.00 ± 2.646 o	0.179 ± 0.002 u

Results are presented as the Mean values ± Standard Deviation (S.D.). Different lowercase letters in the same column indicate statistically significant difference according to Tukey's post hoc test ($p \leq 0.01$)

Table 4: Correlation coefficients between biochemical traits and PDI of Chili anthracnose disease both at pre-inoculated and post-inoculated stages under artificial (laboratory) condition

Traits	*PPO (pre-in)*	*PPO (post-in)*	*PAL (pre-in)*	*PAL (post-in)*	*POD (pre-in)*	*POD (post in)*	*PHE (pre-in)*	*PHE (post-in)*	*PRO (pre-in)*	*PRO (post-in)*
PDI	-0.39854	-0.78271**	-0.65452**	-0.26344	-0.65654**	-0.72737**	-0.69101**	-0.74174**	-0.53696*	-0.67327**
PPO (pre-in)		0.59273**	0.63463**	0.24700	0.51639*	0.58762**	0.55485**	0.53896*	0.53372*	0.50896*
PPO (post-in)			0.65044**	0.36942	0.76532**	0.91201**	0.79711**	0.82688**	0.72855**	0.80910**
PAL (pre-in)				0.42200	0.60918**	0.57171**	0.68719**	0.61910**	0.50077*	0.48971*
PAL (post-in)					0.11715	0.27054	0.23170	0.08436	-0.05372	0.01401
POD (pre-in)						0.82589**	0.77803**	0.84469**	0.79315**	0.660718*
POD (post-in)							0.838898*	0.90281**	0.83558**	0.82883**
PHE (pre-in)								0.87968**	0.68896**	0.68110**
PHE (post-in)									0.84424**	0.87153**
PRO (pre-in)										0.88985**

Values ranged from ≥0.433 to ≤ 0.549 significant at 5% level; values ≥ 0.549 significant at 1% level; *Significant at 5% level, **significant at 1% level. where, PDI- Percent disease index of chili anthracnose disease, PPO (pre-in)- Polyphenol Oxidase (pre-inoculation), PPO (i)-Polyphenol Oxidase (post-inoculation), PAL(pre-in)- Phenylalanine Ammonia Lyase (pre-inoculation), PAL(post-in)- Phenylalanine Ammonia Lyase (post-inoculation), POD (pre-in)-Peroxidase (pre-inoculation), POD (post-in)- Peroxidase (post-inoculation), PHE(pre-in)-Phenol (pre-inoculation), PHE (post-in)-Phenol (post-inoculation), PRO (pre-in)-Protein (pre-inoculation), PRO (post-in)-Protein (post-inoculation).

molecules. A plant's resistance largely depends on its ability to recognize, decode, and respond biochemically, physiologically, or morphologically to pathogen invasion (Attri et al., 2024). Defense enzymes play a key role in host resistance, and variations in resistance among genotypes may be linked to differing activities of enzymes like POX, PAL, and PPO—potential biochemical markers for disease resistance (Chaman et al., 2001). Chili anthracnose resistance involves complex defense enzyme mechanisms. The present study evaluates defense enzyme activities in six chili genotypes and 15 F_1's (Tables 1–4)

Polyphenol oxidase (PPO) assay

PPO activity significantly varied from 0.330 to 1.013 before inoculation of fruits and 0.292 to 1.094 after inoculation of fruits in genotypes. PPO activity in post-inoculated fruits exhibited higher values as compared to pre-inoculated chili fruits of genotypes viz. Chinese Bona (0.882–1.003), Pant C 1 (0.739–0.964), Bidhan Chili 4 (1.013–1.094) and Chili 38-Ragi (0.665–0.990). The reverse phenomenon was observed in two other genotypes, viz. Srinagar (0.330–0.292) and BCC 1 (0.419–0.328), where a significant decrease in PPO activity was observed in post-inoculated fruits. Likewise, PPO activity in different hybrids studied varied significantly from 0.196 to 1.018 in healthy chili fruits and from 0.086 to 1.109 in pathogen-infected chili fruits. Besides, higher PPO activity was observed in post-inoculated fruits in hybrids, Chinese Bona × Pant C 1 (0.406–0.816), Chinese Bona × Bidhan Chili 4 (0.871–0.924), Chinese Bona × Chili 38-Ragi (0.745–0.963), Chinese Bona × Srinagar (0.332–0.905), Pant C 1 × Bidhan Chili 4 (1.018–1.109), Bidhan Chili 4 × Chili 38-Ragi (1.006–1.056) and rest of the hybrids, Pant C 1 × BCC 1 (0.406–0.369), Chili 38-Ragi × Srinagar (0.923–0.366), Srinagar × BCC 1 (0.196–0.086) had lower PPO activity in post-inoculated fruits. Chunhua et al. (2001) explained in their studies about immediate rise in PPO activity upon pathogens attack, indicating immediate synthesis of antimicrobials to ward off pathogens. Such increased PPO activity has been reported to provide tolerance to whitefly infestation in castor beans (Kurra and Usha Rani, 2015).

Peroxidase (POX) assay

An increase in POD activity in pathogen-challenged chili genotypes and hybrids was observed in post-inoculated fruits. Maximum POD activity was recorded in Pant C 1 (0.998) and minimum in BCC 1 (0.298) in healthy chili fruits, whereas maximum POD activity was observed in Bidhan Chili 4 (1.382) and the minimum was observed in BCC 1 (0.284) in post-inoculated fruits. Following the trend, POX activity was observed high in post-inoculated chili hybrids, Bidhan Chili 4 × Chili 38-Ragi (1.225–1.557), Pant C 1 × Bidhan Chili 4 (0.974–1.806), Chinese Bona × Pant C 1 (0.872–0.906), Chinese Bona × Chili 38-Ragi (0.936–1.035), Chinese Bona × Bidhan Chili 4 (1.115–1.227). This displays higher POX activity in post-inoculated fruits in resistant hybrids than that of susceptible hybrids, Pant C 1 × BCC 1 (0.627–0.542), Srinagar × BCC 1 (0.323–0.218) and Chili 38-Ragi × Srinagar (0.301–0.213). Xiao et al. (2023) observed that POX was higher in the inoculated resistant eggplant root than in the susceptible eggplant root during the early stage of infection. Saxena et al. (2019) reported that lower peroxidase activity was found in susceptible genotypes, while higher peroxidase was recorded in moderately resistant ones when infected by a pathogen. Increased lignin deposition, along with enhanced POX activity had been reported in *Capsicum annuum* against *C. capsici* (Saxena et al., 2019).

Phenylalanine ammonia-lyase (PAL) assay

PAL activity varied widely among genotypes and hybrids. The range of PAL activity varied from 0.359 to 1.205 in pre-inoculated fruits and from 0.250 to 1.184 in post-inoculated fruits. Increased PAL activity was observed in post-inoculation of chili fruits in resistant chili genotypes viz. Bidhan Chili 4 (1.205–1.184), Chili 38-Ragi (1.004–1.024), Pant C 1 (0.969–1.068) and Chinese Bona (1.015–1.106), whereas decreased PAL activity was observed in susceptible and moderately susceptible genotypes, Srinagar (0.506–0.276) and BCC 1 (0.359–0.250). In a similar manner, fruits of 15 hybrids (including both healthy and inoculated ones) were studied to judge their responses to PAL activity. PAL activity was intensified in resistant hybrids, Pant C 1 × Bidhan Chili 4 (0.991–1.226), Chinese Bona × Bidhan Chili 4 (1.002–1.054), Chinese Bona × Chili 38-Ragi (0.649–0.994), Bidhan Chili 4 × Chili 38-Ragi (0.813–0.865) and Chinese Bona × Pant C 1 (0.973–1.020) and maintained its enhanced level in post-inoculated chili fruits. But PAL activities peaked off and decreased in susceptible hybrids, Pant C 1 × BCC 1 (0.334–0.254), Chili 38-Ragi × Srinagar (0.445–0.141), Srinagar × BCC 1 (0.181–0.167) and Chili 38-Ragi × BCC 1 (0.880–0.305) in post-inoculation of fruits with *C. capsici.* An increased PAL activity has been reported in castor bean genotypes against white fly infestation (Kurra and Usha Rani, 2015).

Phenol Content

Under artificial conditions, phenol content among genotypes varied between 65.00 and 137.00 mg/100g in pre-inoculated fruits and between 47.67 and 175.67 mg/100 g in post-inoculated fruits. Resistant hybrids, Pant C 1 × Chili 38-Ragi (144.33–259.00 mg/100 g), Pant C 1 × Bidhan Chili 4 (137.67–225.67 mg/100 g), Chinese Bona × Bidhan Chili 4 (118.67–218.67 mg /100 g), Chinese Bona × Pant C 1 (104.67–225.00 mg /100 g), Bidhan Chili 4 × Chili 38-Ragi (92.33–194.67 mg/100 g) registered higher phenolic activity both at pre- and post-inoculated stage. On the other side, lower level of phenolic activity was observed in susceptible hybrids, Srinagar × BCC 1 (53.33–35.00 mg/100 g), Chili 38-Ragi × BCC 1 (80.33–66.33 mg/100 g), Chili 38-Ragi × Srinagar (67.67–46.67 mg/100 g) in post pathogen invasion of chili fruits. Previous reports have shown an improved level of phenolics in chili upon pathogen ingress by *C. truncatum* (Kumar et al., 2020).

Protein Content

A high number of proteins could be attributed to the higher activity of plant defense enzymes (Saxena et al., 2019). Higher protein content was observed in resistant parental genotypes as compared to susceptible genotypes. The range of protein content varied between 0.811 and 2.111% in pre-inoculated fruits and between 0.422 and 4.015% in post-inoculated fruits. Among genotypes, Bidhan Chili 4 had the highest protein content both in pre-(2.111%) and post-inoculated (4.015%) chili fruits. Besides, protein content increased manifolds in post-inoculation in resistant hybrids, Bidhan Chili 4 × Chili 38-Ragi (3.899–7.645%), Chinese Bona × Pant C 1 (2.269–5.773%), Pant C 1 × Bidhan Chili 4 (3.755–7.844%), Chinese Bona × Bidhan Chili 4 (3.380–6.454%), Chinese Bona × Chili 38-Ragi (2.105–5.010%) than that of susceptible hybrids. These results were further validated by previous reports on an increased level of phenol and protein against pathogen ingress in *C. annuum* (Anand et al., 2009).

Correlation study between biochemical parameters and PDI of chili anthracnose disease

Understanding the role of biochemical parameters in host resistance to pathogens is crucial for effective resistance breeding. Higher levels of defense molecules are positively associated with greater disease tolerance (Banu et al., 2019). In this study, a simple correlation was drawn between chili anthracnose severity (PDI) and five biochemical parameters. Significant negative correlations were observed between PDI and PPO activity in post-inoculated fruits ($r = -0.78271$), POD in both pre- ($r = -0.65654^{**}$) and post-inoculated fruits ($r = -0.72737^{**}$), PAL in pre-inoculated fruits ($r = -0.65452^{**}$), phenol content in pre- ($r = -0.69101^{**}$) and post-inoculated fruits ($r = -0.74174^{**}$), and protein content in pre- ($r = -0.53696^{*}$) and post-inoculated fruits ($r = -0.67327^{**}$) (Table 4). Correlations of PDI with PPO in pre-inoculated fruits ($r = -0.39854$) and PAL in post-inoculated fruits ($r = -0.26344$) were negative but non-significant. Similar enzyme correlations were reported by Anand et al. (2009), while Thuong et al. (2015) showed that *Colletotrichum gloeosporioides* inoculation or SNP treatment enhanced POD and PAL activities, suggesting SNP's potential in reducing anthracnose via defense enzyme induction in both pre- and post-harvest stages.

Conclusion

Chili has a complex resistance mechanism against the anthracnose-causing pathogen and hence responds by altering a number of biochemical parameters and defense-related enzymes when under attack. These biochemical constituents include phenol and enzyme activities such as peroxidase and, polyphenol oxidase and phenylalanine ammonia lyase. Further, the increased activities of the defense-related enzyme and the enhanced content of total phenol and protein in response to pathogen inoculation in resistant lines and hybrids suggested their implication as effective selection indices in resistant breeding programs.

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सारांश

मिर्च (कैप्सिकम एनुअम एल.) के जीनोटाइप और संकर जो एन्थ्रेक्रोज रोग के प्रतिरोधी हैं, अभी तक व्यावसायिक रूप से उपलब्ध नहीं हैं, और अनुकूल पर्यावरणीय परिस्थितियों में, फसल को महत्वपूर्ण उपज और आर्थिक नुकसान होता है। इस अध्ययन में, छह मिर्च जीनोटाइप और 15 संकरों की एन्थ्रेक्रोज रोग के प्रति उनकी प्रतिरोधकता के लिए जांच की गई। विभिन्न पौधों की रोग प्रतिरोधकता-संबंधी मापदंडों का मूल्यांकन किया गया, और जीनोटाइप बिधान मिर्च 4, चीनी बोना और पंत सी 1, साथ ही संकर पंत सी 1 × बिधान मिर्च 4, बिधान मिर्च 4 × मिर्च 38-रागी, और चीनी बोना × मिर्च 38-रागी ने एन्थ्रेक्रोज रोग के प्रति प्रतिरोधकता प्रदर्शित की। मिर्च के रक्षा तंत्र की गहरी समझ हासिल करने के लिए, मुख्य रक्षा एंजाइमों में जैव रासायनिक परिवर्तन - जैसे कि पॉलीफेनोल ऑक्सीडेज (पीपीओ), पेरोक्सीडेज (पीओएक्स), और फेनिलएलनिन अमोनिया-लाइज़ (पीएएल) - साथ ही फलों में प्रोटीन और फिनोल सामग्री (टीकाकरण से पहले और बाद में) का विश्लेषण किया गया। परिणामों से पता चला कि प्रतिरोधी जीनोटाइप और संकर ने अतिसंवेदनशील लोगों की तुलना में पीपीओ, पीओएक्स और पीएएल के उच्च गतिविधि स्तर के साथ-साथ प्रोटीन और फिनोल सामग्री में वृद्धि का प्रदर्शन किया। कोलेटोट्रीकम कैप्सिसी-टीकाकृत फलों में ये उन्नत जैव रासायनिक प्रतिक्रियाएं बताती हैं कि ये तंत्र एन्थ्रेक्रोज रोग के खिलाफ मेजबान प्रतिरोध को बढ़ाने में महत्वपूर्ण भूमिका निभाते हैं।