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Screening of Indian Cardamom Accessions for Resistance to Major Pathogens and their Relationships with Biochemical Defense Parameters

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Abstract

Major fungal diseases reported in Indian cardamom (*Elatteria cardamomum* (L.) Maton.) are capsule rot/*Phytophthora* rot, clump rot, pseudostem rot/*Fusarium* rot, leaf blight, and tiller splitting/phoma disease. These diseases cause significant yield reduction regularly. Fifteen promising cardamom accessions were screened during 2022-23, to identify resistant sources against each disease. The screening studies were conducted at Cardamom Research Station, Pampadumpara utilizing cardamom accessions maintained at germ plasm bank. Five different experiments were conducted to screen accessions for resistance against each disease. The role of defense-related biochemical parameters *viz*., Peroxidase (PO), Polyphenol Oxidase (PPO), Phenylalanine Ammonia-Lyase (PAL), total phenol, OD phenol, β-1,3 glucanase, and chitinase that impart resistance against each pathogen in cardamom, as well as the correlation between percent disease index and biochemical parameters were investigated. The accessions hybrid-6, hybrid-17, and hybrid-1 showed resistance to three or more diseases indicate the possibility of using them as donor parents in future resistance breeding programmes. All biochemical parameters evaluated in this study were higher in inoculated plants than in un-inoculated ones. Among biochemical defense parameters studied, PO and β-1,3 glucanase were the significant biochemical defense indicators for *Phytophthora* rot resistance. In the case of clump rot and leaf blight, PPO and chitinase are the key indicators. The primary factor of resistance to *Fusarium* rot was PO activity. PAL and chitinase levels significantly influenced and correlated with resistance to Phoma disease. These indicators could be utilized in future to identify disease-resistant cardamom accessions and facilitate agile breeding initiatives.

Keywords: Plant pathogens; Biochemicals; Resistance; Indian cardamom hills

Introduction

Indian cardamom (*Elatteria cardamomum* (L.) Maton.), is a native of tropical evergreen forests and the southern Western Ghats in India. It is regarded as the "Queen of Spices" worldwide. Only an elevation-induced environment with natural forest cover allows successful cultivation of small cardamom in the tropical mountains. As a result, cardamom farming is limited to a small number of nations. Apart from a few smaller players from southeast Africa and Central America, the main producing nations are Guatemala, India, Sri Lanka, and Tanzania. Internationally, cardamom is occasionally regarded as the most expensive commodity on the global market. Guatemala is the largest producer and exporter of cardamom in the world followed by India. During 2021-22, India's production surpassed 37,000 MT [1-3].

Prevalence of diseases is the main obstacle to the successful cultivation of cardamom. Major fungal diseases reported in cardamom are capsule rot/azhukal disease caused by *Phytophthora meadii*, clump rot caused by *Pythium vexans*, *Rhizoctonia solanii*, and *Fusarium sp*., Pseudostem rot/ Fusarium rot caused by *F. oxysporum*, leaf blight caused by *Colletotrichum gloeosporioides* and tiller splitting/ *Phoma* disease caused by *Phoma* sp.

The disease, capsule rot, affects cardamom and reduces the yield. The first report of *Phytophthora* spp. as a disease-causing organism was reported by Menon, et al., Thomas and Bhai reported that *P. meadii* was the causal organism, especially during the monsoon season. The disease's symptoms can be seen on leaves, tender shoots, panicles, and capsules as the monsoon season approaches, leading to significant crop loss. Another pervasive, damaging, and economically significant disease in cardamom is rhizome rot or clump rot, caused by the *P. vexans-R. solani-Fusarium* sp. complex. This disease is widespread and persistent across the cardamom growing regions, considerably reducing the production by decaying the tillers and causing them to fall down. Thomas and Vijayan were the first to report the disease "Pseudostem rot" in the cardamom plantations of the Idukki area, which is caused by *F. oxysporum*. It is more severe in the summer, when the infection first appears in the middle of the tillers and causes pale, discolored lesions that eventually turn dry and decay. Dhanya, et al., reported 50% yield loss from untreated plants due to this pathogen. Under favourable weather conditions and changing climatic conditions, the disease may become harmful at all crop growth phases [4].

Leaf blight, caused by *Colletotrichum gloeosporioides*, is the most widespread foliar disease of cardamom. Despite being present during the entire season, the disease typically worsens and takes on epiphytotic proportions in the post-monsoon period, which is probably aided by an increase in ambient temperature. In places where adequate shade control is not practiced, the disease incidence is more severe. Dhanya, et al., reported that the fungus *Phoma* sp. causes tillers to develop longitudinal sunken lesions of varied lengths. In turn, the infected tiller breaks at the site of infection when the sheath separates, splits open longitudinally. The infection is also visible on the panicle as well as on the leaf lamina [5,6].

According to Murugan, et al., cardamom plantations in southern India have been exposed to high levels of chemical pesticides, and the crop's pesticide use has increased significantly during the last two decades. Germplasm collection of cardamom is being kept as a field gene bank repository at the Cardamom Research Station, Kerala Agricultural University, Pampadumpara, as part of the scheme under the All India Coordinated Research Projects on Spices. Each cardamom accession has a different level of resistance, which affects how severe each disease symptom is. Identifying resistant accessions to each disease was the primary goal of the current investigation. To better understand the biochemical responses of cardamom accessions in response to pathogen inoculation, 7 defense-related biochemical parameters were examined and a correlation analysis was performed with the Percent Disease Index (PDI). In this study, 4 resistant and one susceptible cardamom accessions were used to assess the activity of defense-related biochemical parameters in-depth and meticulously identify the parameters for determining each disease's resistance in cardamom. The results can shed light on the areas for research into the mechanisms underlying resistance to major fungal pathogens in cardamom.

Materials and Methods

Plant materials

Fifteen promising accessions of cardamom from germ plasm collection (Pink base, Kalarickal white, Green gold, Hybrid-17, Hybrid-16, Hybrid-6, Hybrid-5, Hybrid-4, Hybrid-2, Hybrid-1, PV 5, PS 27, PV 3, PV 2, PV 1) were planted in grow bags under shade net (50%) condition. Separate experiments were carried out for each pathogen under study. Three replications were maintained for each accession, and five plants were included under each replication.

Pathogens

Major diseases associated with cardamom are capsule rot/ *Phytophthora* rot (caused by *P. meadii*), clump rot (complex disease caused by *P. vexans*, *R. solanii*, and *Fusarium* sp.), pseudostem rot (caused by *F. oxysporum*), leaf blight (caused by *C. gloeosporioides*) and tiller splitting (caused by *Phoma* sp.). These pathogens were isolated from infected plant samples and inoculated separately on healthy cardamom plants to prove Koch's postulates. The pathogens were reisolated from artificially inoculated plants, and the characteristics of the re-isolate were compared with the respective original cultures. Thus proved the pathogenicity of all the cultures. The pathogens were purified by the hyphal tip method, sub-cultured, and maintained on Potato Dextrose Agar (PDA) slants for further investigations (Figure 1) [7,8].

Figure 1: Major diseases of Indian cardamom.

Pathogen inoculation

Five different experiments were conducted to screen accessions for resistance against each disease. Challenge inoculation of the pathogens was done after giving pin pricks and plants were observed every day for initial symptom expression.

Disease assessment

Screening of the cardamom accessions for diseases was done by the visual observation. Disease severity or Percent Disease Index (PDI) was calculated by rating the severity of symptoms expressed in each accession. The scoring of disease incidence was based on the expression of the symptoms in response to pathogen inoculation. The incidence of diseases were recorded based on 1 to 5 rating scales as described by Venugopal, et al., Dhanya, et al. and Biju, et al. The PDI was calculated for all the plants of each accession and mean values were used to compute PDI for each accession, using the following formula:

PDI=(Sum of individual ratings/Total number of leaves observed) × (100/Maximum grade of scale)

The accessions were further classified into highly resistant (<10%), resistant (11-20%), moderately resistant (21-30%), moderately susceptible (31-40%), susceptible (41-50%) and highly susceptible (>51%) based on PDI, recorded at regular intervals [9].

Assay of defense related oxidative enzymes, phenols and PR proteins

The resistant accessions based on the above study were used for the analysis of defense related oxidative enzymes, PR proteins, and phenols. Highly susceptible accessions to the pathogen were taken as check. Separate experiments were conducted for each pathogen. For that, pure cultures of the respective pathogen were inoculated into the plants maintained in the grow bags using the pin prick method, and observed for the day on which symptom expression on the susceptible check.

The activity of defense related oxidative enzymes such as Peroxidase (PO), Polyphenol Oxidase (PPO), Phenylalanine Ammonia Lyase (PAL), phenols like total phenol and Ortho-Dihydroxy phenols (OD phenols), and PR proteins like β-1,3 glucanase and chitinase were estimated before pathogen inoculation as well as the day on which symptom was expressed on the susceptible check by spectroscopy.

Analysis was done for the activity of PO using the procedure described by Rathmell and Sequeira, PPO by Mayer, et al., PAL by Brueske, total phenol by Malick and Singh, OD phenol by Mahadevan and Ulaganathan, β-1,3 glucanase by Pan, et al. and chitinase by Vahed, et al.

Statistical analysis

The data were processed by MS office Excel. Analysis of variance and correlation was carried out by Statistics Package-grapesAgri1, 1.1.0 in R version 4.2.3.

Results

Screening of cardamom accessions against major diseases

Data on the percent disease index of fifteen cardamom accessions inoculated with the respective pathogens are presented in Table 1 [10-12].

Sl. no	Accessions	Phytophthora rot	Clump rot	Fusarium rot	Leaf blight	Phoma disease
1	Pink base	6.67 (HR)	86.67 (HS)	80.00 (HS)	86.67(HS)	73.33 (HS)
2	Kalarickal white	66.67(HS)	100 (HS)	80.00 (HS)	100 (HS)	66.67 (HS)
3	Green gold	46.67 (S)	20.00 (R)	60.00 (HS)	6.67 (HR)	40.00 (MS)
4	Hybrid-17	13.33 (R)	20.00 (R)	46.67 (S)	6.67 (HR)	46.67 (S)
5	Hybrid-16	80.00 (HS)	46.67 (S)	60.00 (HS)	20.00 (R)	0 (HR)
6	Hybrid-6	66.67 (HS)	26.67(MR)	20.00 (R)	0 (HR)	6.67 (HR)
7	Hybrid-5	53.33 (HS)	60.00 (HS)	66.67 (HS)	86.67 (HS)	13.33 (R)
8	Hybrid-4	6.67 (HR)	73.33 (HS)	60.00 (HS)	80.00 (HS)	93.33 (HS)
9	Hybrid-2	13.33 (R)	53.33 (HS)	100 (HS)	80.00 (HS)	73.33 (HS)
10	Hybrid-1	20.00 (R)	26.67(MR)	6.67 (HR)	93.33 (HS)	66.67 (HS)
11	PV-5	40.00 (MS)	40.00 (MS)	40.00 (MS)	60.00 (HS)	33.33 (MS)
12	PS-27	53.33 (HS)	40.00 (MS)	26.67 (MR)	53.33 (HS)	53.33 (HS)
13	PV-3	40.00 (MS)	46.67 (S)	33.33 (MS)	46.67 (S)	40.00 (MS)
14	PV-2	60.00 (HS)	60.00 (HS)	13.33 (R)	60.00 (HS)	20.00 (R)
15	PV-1	40.00 (MS)	40.00 (MS)	6.67 (HR)	40.00 (MS)	40.00 (MS)

Note: HR: Highly Resistant; R: Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible; HS: Highly Susceptible

Table 1: Percent Disease Index (PDI) of 15 accessions against major diseases.

The accessions *viz*., pink base and Hybrid-4 were found highly resistant to *Phytophthora* rot that recorded only 6.67 percent disease index. The other accessions *viz*., hybrid-17, hybrid-2 and hybrid-1 were also showed resistance to the pathogen. In the case of clump rot disease, none of the accessions were highly resistant. The accessions Green gold and Hybrid-17 were found resistant to the pathogens, which recorded only 20 percent disease index [13-17]. Kalarickal white exhibited cent percent disease incidence. *F. oxysporum* is the pathogen that causes menace to cardamom throughout the year. The accessions hybrid-1 and PV-1 were highly resistant to the pathogen, and recorded only 6.67 PDI. PV-2 and hybrid-6 were also showed resistance to the pathogen with PDI 13.33 and 20 respectively. Hybrid-2 was the most susceptible one that showed 100 percent disease incidence.

In the case of leaf blight disease, the accessions hybrid-6, green gold, and hybrid-17 were highly resistant to the pathogen, followed by hybrid-16, which was considered as resistant with PDI 20. Against *Phoma* disease, hybrid-16 and hybrid-6 were highly resistant, and Hybrid-5 and PV-2 were also found as resistant, with PDI 0, 6.67, 13.33, and 20.0 correspondingly. Whereas Pink base, Kalarickal white, hybrid-4, hybrid-2, hybrid-1, and PS-27 were highly susceptible to the pathogen with more than 50 PDI.

From the above table, it can be concluded that the accession, hybrid-6 was highly resistant to leaf blight and *Phoma* disease, resistant to *Fusarium* rot, and moderately resistant to clump rot disease. Against *Phytophthora* rot, it was highly susceptible. Hybrid-17 also showed resistance to three diseases *viz.*, *Phytophthora* rot, clump rot, and leaf blight. Against leaf blight disease, it was highly resistant, but susceptible to *Fusarium* rot and *Phoma* disease. A similar trend was noticed in hybrid-1 as well. It was resistant to *Phytophthora* rot, moderately resistant to clump rot, and highly resistant to *Fusarium* rot, but highly susceptible to leaf blight and *Phoma* disease.

From each experiment, four highly resistant/resistant lines were selected for defense-related biochemical analysis. The most susceptible accession was selected as the check (Table 2).

	Phytophthora rot	Clump rot	Fusarium rot	Leaf blight	Phoma disease
Resistant 1	Pink base	Green gold	Hybrid 1	Hybrid 6	Hybrid 16
Resistant 2	Hybrid 4	Hybrid 17	PV 1	Green gold	Hybrid 6
Resistant 3	Hybrid 2	Hybrid 6	PV 2	Hybrid 17	Hybrid 5
Resistant 4	Hybrid 1	Hybrid 1	Hybrid 6	Hybrid 16	PV 2
Susceptible check	Hybrid 16	Kalarickal white	Hybrid 2	Kalarickal white	Hybrid 4

Table 2: Selected accessions for defense-related biochemical analysis.

Analysis of defense-related biochemical substances in response to the inoculation of *P. meadii* in the selected cardamom accessions

Based on the results obtained from the experiment for screening of cardamom accessions against *Phytophthora* rot, 4 resistant lines and one susceptible check were identified and selected for the analysis of defense related biochemical substances. The pathogen was inoculated on 4 resistant lines *viz*., pink base, hybrid-4, hybrid-2, and hybrid-1, and susceptible check hybrid-16. Symptoms of the disease appeared on hybrid-16 from the 3rd Day After Inoculation (DAI), indicating a period of 3 days for the successful establishment of the pathogen. Analysis of PO, PPO, PAL, total phenol, OD phenol, and β-1,3 glucanase was done before pathogen inoculation as well as on the 3rd DAI [18].

From Figure 2, it is clear that the activity of all defense related biochemical substances had varied in resistant as well as susceptible lines in response to pathogen inoculation. The resistant line, Pink base exhibited the highest PO activity (0.144 unit's min^{-1} g^{-1} fresh weight) as compared to the susceptible check (0.08 unit's min^{-1} g^{-1} fresh weight). The PPO activity ranged from 0.014 to 0.073 in healthy and from 0.239 to 0.461 in infected plants. Maximum PPO activity was recorded in the resistant line hybrid-1 (0.461) after pathogen inoculation, compared to the susceptible check (0.239). In resistant lines, PAL activity differed between 0.005 to 0.074 before inoculation and between 0.022 to 0.252 after pathogen inoculation. The maximum content (0.252) was observed in the resistant line Pink base, and the minimum content was reported in the susceptible check, Hybrid-16 (0.022) [19,20].

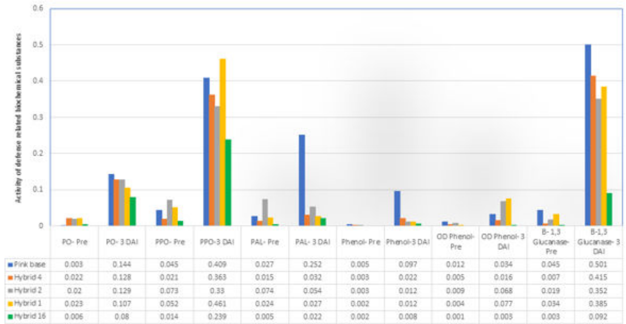

Figure 2: Activity of biochemical defense parameters in response to *P. meadii* inoculation.

In the present study, before pathogen inoculation, the activity of total phenol and OD phenol was very less, which changed from 0.002-0.005 and 0.001-0.009, accordingly. Upon pathogen inoculation, the activity was increased to the range of 0.008-0.097 and 0.003-0.077, respectively. Among the accessions, β-1,3 glucanase content varied from 0.003 to 0.045 in healthy and from 0.092 to 0.501 in inoculated plants, on the 3rd DAI. In the resistant line, pink base, β-1,3 glucanase content increased 11.13 times upon pathogen inoculation.

Analysis of defense-related biochemical substances in response to the inoculation of clump rot-causing pathogens in the selected cardamom accessions

Based on the experiment for screening cardamom accessions against clump rot, the selected resistant lines were green gold, hybrid-17, hybrid-6, and hybrid-1. Kalarickal white was selected as the susceptible check. Symptoms of the disease appeared on Kalarickal white on the 5^{th} DAI, indicating a period of 5 days for the successful establishment of the pathogen. Analysis of defense-related biochemical substances was done before pathogen inoculation and on 5^{th} DAI.

From Figure 3, it is evident that resistant lines *viz*., hybrid-17 and hybrid-1 exhibited the PO activity of 0.55 and 0.448 unit's min^{-1} g^{-1} fresh weight, respectively upon pathogen inoculation. PPO activity ranged between 0.03 and 0.108 in healthy and between 0.118 and 0.875 in infected plants. Hybrid-1 registered the highest activity of PPO (0.875) compared to the susceptible check (0.118). The accessions hybrid-17 and hybrid-1 recorded the highest PAL activity of 0.282. Before pathogen inoculation, total phenol content among the accessions varied from 0.007 to 0.082 µg/g. Susceptible check, Kalarickal white showed the phenol activity of only 0.012 µg/g.

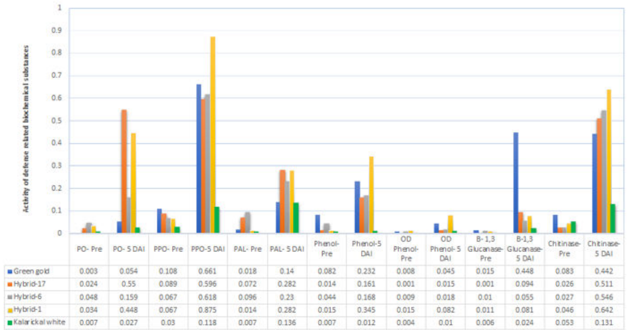

Figure 3: Activity of biochemical defense parameters in response to Clump rot pathogens

OD phenol, β-1,3 glucanase, and chitinase activity were also increased in response to pathogen inoculation. Before pathogen inoculation, OD phenol and β-1,3 glucanase activity were very meager in all the accessions, and the activity oscillated from 0.001-0.015. The resistant accessions, green gold, and hybrid-1 recorded OD phenol content of 0.045 and 0.082 on the 5th DAI. In the case of β-1,3 glucanase, green gold recorded the highest activity of 0.448, whereas the susceptible check Kalarickal white gave only 0.024. The increase in activity of chitinase in response to pathogen inoculation in all the accessions was more compared to other defense-related biochemical substances. Chitinase activity among the accessions showed between 0.026 and 0.083 in healthy and between 0.131 and 0.642 in infected plants. Maximum content (0.642) was observed in hybrid-1, and the minimum content (0.131) was noted in the susceptible check.

Analysis of defense-related biochemical substances in response to the inoculation of *F. oxysporum* in the selected cardamom accessions

The resistant accessions selected for biochemical analysis based on the screening experiment were hybrid-1, PV-1, PV-2, and hybrid-6. The accession, hybrid-2 was selected as the susceptible check for comparison. On the 5th DAI, 100 percent disease incidence was observed in the susceptible check. Therefore, defense-related biochemical substances were analyzed before pathogen inoculation and on 5th DAI.

From Figure 4, it is clear that the activity of PO was the highest among all defense-related biochemical parameters, in response to pathogen inoculation. PO activity varied between 1.931 and 5.538 in healthy and 2.096 and 10.672 in infected plants. PV-1 gave maximum PO activity of 10.672 units min^{-1} g^{-1} fresh weight on the 5th DAI. Before pathogen inoculation, the activity of PPO, PAL, total phenol, OD phenol, and chitinase was very low. But the content had increased in response to pathogen inoculation. The PPO activity differed from 0.05 to 0.462 in healthy and from 0.299 to 1.205 in infected plants. Maximum PAL activity of 0.975 μmol transcinnamic acid/g was shown by the resistant accession PV^{-1}, on the 5th DAI, whereas the susceptible check, hybrid-2 recorded an activity of only 0.146. Total phenol and the OD phenol content on the 5th DAI varied from 0.121 to 0.583 and from 0.620 to 1.097, respectively. Chitinase activity in response to pathogen inoculation was high compared to other defense-related biochemical parameters, *viz*., PPO, PAL, and total phenol. The highest chitinase activity was recorded by the resistant accession hybrid-1 (0.979), and the lowest was reported for another resistant accession hybrid- 6 (0.264).

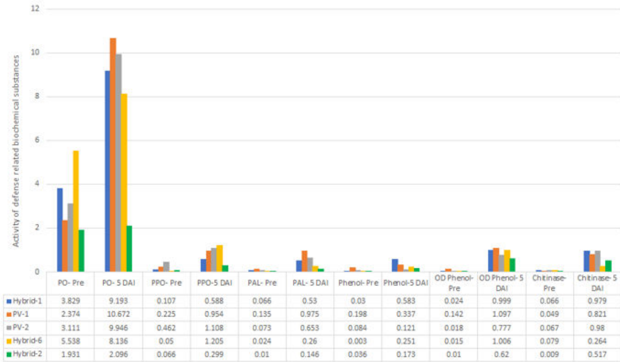

Figure 4: Activity of biochemical defense parameters in response to *F. oxysporum* inoculation

Analysis of defense-related biochemical substances in response to the inoculation of *C. gloiosporioides* in the selected cardamom accessions

The accessions that were found resistant against leaf blight disease based on the experiment for screening were hybrid-6, green gold, hybrid-17, and hybrid-16. Kalarickal white was found as susceptible to the pathogen. On the 3rd DAI, cent percent disease incidence was observed on the susceptible check. Therefore analysis of defense-related biochemical parameters was done before pathogen inoculation as well as on the 3rd DAI.

From Figure 5, it is apparent that PO activity varied between 0.025 and 0.076 in healthy and 0.082 and 0.255 in infected plants among the accessions. Green gold was observed to have the highest PO activity both in healthy and infected plants. Among all defense-related parameters, PPO recorded the highest activity in response to pathogen inoculation. Before inoculation, the resistant lines *viz*., hybrid-6 and green gold recorded the PPO activity of 2.358 and 1.218 unit's min^{-1} g^{-1} fresh tissue, respectively. It was increased to 5.301 and 4.253, respectively, on 3rd DAI. Whereas the susceptible check, Kalarickal white showed only 0.176 units min^{-1} g^{-1} fresh tissue.

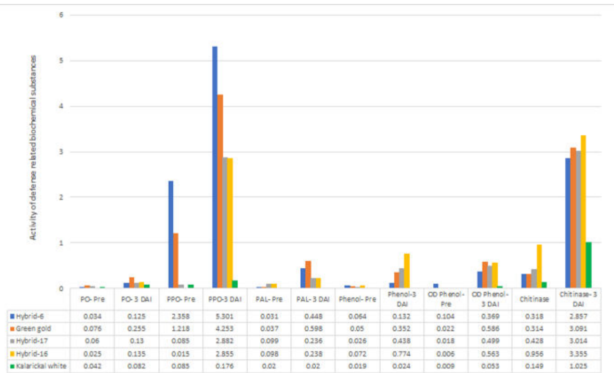

Figure 5: Activity of biochemical defense parameters in response to *C. gloeosporioides* inoculation

The activity of other defense-related biochemical parameters such as PAL, total phenol, and OD phenol was very less in healthy plants, whereas it had increased in response to pathogen inoculation. The resistant lines showed PAL activity in the range of 0.031-0.099 in healthy conditions and in the range of 0.236-0.598 in inoculated condition. The activity of total phenol and OD phenol in response to pathogen inoculation ranged from 0.024-0.774 and 0.053-.586, respectively. There was an increase in the activity of the PR protein, chitinase in response to pathogen inoculation. The chitinase activity changed from 0.149-0.956 in healthy and 1.025-3.355 in infected plants. The maximum chitinase was recorded in the resistant accession, hybrid-16, before and after pathogen inoculation.

Analysis of defense-related biochemical substances in response to the inoculation of *Phoma* sp. in the selected cardamom accessions

Based on the experiment for screening, the accessions *viz.*, hybrid-16, hybrid-6, hybrid-5, and PV-2 registered only 33.33 percent disease incidence, whereas hybrid-4 recorded cent percent disease incidence on 5th DAI. Therefore, Hybrid-4 was selected as the susceptible check. Analysis of defense-related biochemical parameters was done before pathogen inoculation as well as on the 5th DAI.

Figure 6 shows that PO activity among the accessions varied between 0.023 and 0.946 in healthy and between 0.096 and 2.149 in infected plants. Comparatively higher PO content was recorded in resistant accessions than in the susceptible ones. PPO and PAL activity was also increased in response to pathogen inoculation. The PAL recorded the highest activity in response to pathogen inoculation among all defense-related biochemical parameters. The activity of PAL ranged from 0.016-0.989 in healthy and 2.163-9.14 in infected plants. The highest activity (9.14) was reported by the resistant accession PV-2 on 5th DAI. PV-2 showed a 27.2-fold increase in the activity of PAL in response to pathogen inoculation. The quantity of total phenol, OD phenol, and chitinase recorded before pathogen inoculation in all the accessions were very less. But the activity got increased in response to pathogen inoculation on the 5th DAI. After pathogen inoculation, total phenol, OD phenol and chitinase activity changed from 0.111-1.376, 0.135-1.615, and 0.079-0.803, correspondingly.

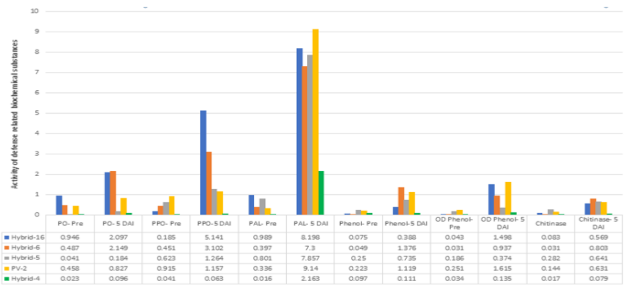

Figure 6: Activity of biochemical defense parameters in response to *Phoma* sp. inoculation

Correlation between percent disease index and defense-related biochemical parameters in cardamom

Understanding how these defense-related biochemical parameters affect the host's resistance or susceptibility to the pathogen is essential for resistance breeding programmes. As a result, a correlation matrix was performed, as shown in Table 3, between the Percent Disease Index (PDI) of 5 major diseases of cardamom and 7 defense-related biochemical parameters at post-inoculation stage.

In the study, the correlation between PDI of *Phytophthora* rot and defense-related biochemical parameters viz., PPO, PAL, total phenol and OD phenol was negative but non-significant. But the activity of PO was significantly ($P<0.05$) negatively correlated with PDI; the correlation coefficient was -0.916. Similarly, the activity of β- 1,3 glucanase was also significantly ($P<0.01$) negatively correlated with PDI, with -0.959 as correlation coefficient.

In the case of clump rot disease, the correlation between PDI and defense related biochemical substances like PPO and chitinase were significantly ($P<0.05$) negatively correlated, with correlation coefficient of -0.892 and -0.895 respectively. Similar trend was noticed in the case of leaf blight also, with -0.905 and -0.933 correspondingly as correlation coefficient. Correlation between PDI of *Fusarium* rot and the biochemical parameters except PO were negative, but non-significant. But PO was significantly ($P<0.01$) negatively correlated (-0.980). In the case of *Phoma* disease, the activity of PAL and the PR protein chitinase were significantly ($P<0.05$) negatively correlated with the percent disease index; the correlation coefficient was -0.924 and -0.929 accordingly.

Correlation coefficients	PO	PPO	PAL	Total phenol	OD phenol	β 1,3 glucanase	Chitinase
PDI-*Phytophthora* rot	-0.916*	-0.745	-0.4	-0.425	-0.5	-0.959**	-
PDI-Clump rot	-0.517	-0.892*	-0.568	-0.765	-0.412	-0.429	-0.895*
PDI-*Fusarium* rot	-0.980**	-0.708	-0.699	-0.446	-0.817	-	-0.202
PDI-Leaf blight	-0.549	-0.905*	-0.791	-0.467	-0.862	-	-0.933*

PDI-*Phoma* disease	-0.66	-0.722	-0.924*	-0.608	-0.653	-	-0.929*
Note: * Correlation is significant at 0.05 level (two-tailed) ** Correlation is significant at 0.01 level (two-tailed)							

Table 3: Correlation between percent disease index and defense-related biochemical parameters in cardamom.

Results and Discussion

Phytophthora rot, clump rot, *Fusarium* rot, leaf blight and *Phoma* disease are the major diseases in cardamom. The resistance exhibited by each accession against these diseases is different according to the results of the current study. In this investigation, we focused on the activities of defense-related biochemical parameters in response to pathogen inoculation, so as to determine resistant mechanisms by biochemical means.

The findings of this study provided us with a better understanding of the defense-related biochemical factors involved in resistance to major pathogens in cardamom. The investigated accessions behaved differently to challenge inoculation of each pathogen, which could be attributable to variable regulation of numerous defense-related biochemical components and other chemicals. Different groups were developed based on the PDI observed in different accessions, and the accessions were placed based on the average PDI.

It was observed that, out of 15 accessions screened for Phytophthora rot, the number of accessions came under the category Highly Resistant (HR) and Resistant (R) were 2 and 3 respectively. The accessions that were Highly Susceptible (HS) to clump rot such as, pink base, Kalarickal white, hybrid-5, hybrid-4, hybrid-2 and PV-2 were highly susceptible to leaf blight also. The accessions green gold, hybrid-17 and Hybrid-6 were found resistant to both the diseases. Senthil kumar, et al., identified 13 small cardamom genotypes that possess dual resistance against leaf blight as well as clump rot disease, whose findings corroborate with the present findings. They also identified 22 genotypes resistant to leaf blight and 29 genotypes highly resistant to clump rot.

The accession hybrid-1 was highly resistant to *Fusarium* rot, moderately resistant to clump rot and resistant to Phytophthora rot. But it was highly susceptible to leaf blight and *Phoma* disease. Whereas pink base, Kalarickal white and hybrid-5 were highly susceptible and PV 5 was moderately susceptible to all rot diseases such as *Phytophthora* rot, clump rot and *Fusarium* rot. The results are in line with the findings of Vijayan et al. They reported that the genotypes MHC 26, MHC 24, MHC 18, MCC 85 and MCC 346 could withstand the infection of all rot pathogens in small cardamom.

Different biochemical parameters associated with plant defense mechanisms in response to inoculation of each pathogen were analyzed in 4 resistant lines and one susceptible check. A comparison study was also conducted to determine the differences in these biochemical parameters between un-inoculated and inoculated conditions. Peroxidase activity had increased in response to the inoculation of pathogens in resistant as well as susceptible lines. Peng and Kuc reported that cell wall peroxidase is a key enzyme system in the metabolism of Reactive Oxygen Species (ROS) producing H_2O_2, which contributes to the establishment of an antimicrobial environment within the apoplast. In numerous plant species, the induction of peroxidase activity in response to pathogen inoculation has already been observed. Vandana et al. also reported peroxidase induction in *P. capsici* infected black pepper roots and stems of resistant and susceptible lines over uninfected controls.

According to Thilagavathi, et al., PO, PPO and PAL are the oxidative enzymes that can catalyze the creation of lignin and other oxidative phenols, as well as contribute to the formation of defense barriers by modifying the cell structure defense mechanism against pathogens. These enzymes have been demonstrated to cause phenol oxidation as well as deposition in the cell wall, and therefore are critical for conferring resistance against invading pathogens. Padmaja et al., observed that precursors for lignin and numerous phenylpropanoid-derived secondary plant metabolites, including salicylic acid, are provided by PAL. The increased peroxidase activity reported in the current study could be attributed to increased PAL activity and phenol accumulation. The results align with the findings of Ghosh, who claimed that after P. aphanidermatum was challenge inoculated into a susceptible ginger cultivar, the activity of lipoxygenase, PPO, and PAL increased up to 14 days after the inoculation and then decreased.

In the study, total phenols and OD phenol concentrations also significantly increased in response to pathogen inoculation. Bhatia reported an increase in total phenol content in resistant tomato varieties compared to susceptible tomato cultivars in relation to early blight disease, which corroborated the results. Similar findings were noted by Khan, et al., Bhagat and Chakraborty and Saraswathy and Reddy. Mammootty, et al., assessed that Kalluvally, a Phytophthora tolerant genotype of black pepper had high content of OD phenols.

Kang and Buchenouer registered that β-1,3-glucanases plays a role in pathogen defense, because it has the ability to hydrolyze oomycete cell wall β-1,3-glucan. We examined changes in this enzyme activity in *P. meadii* inoculated susceptible and resistant cardamom lines against un-inoculated plants and found that β-1,3-glucanase activity differed significantly in inoculated plants. Because, *P. meadii* possesses cellulose in its cell wall, host plants may release β-1,3-glucanase to prevent this disease. The results are in line with the findings of Vandana, et al. They also recorded a significant increase of β-1,3-glucanase activity in *P. capsici* inoculated roots of susceptible and resistant lines over control.

Understanding the role of biochemical parameters in determining the resistance to pathogens and their interaction with one another is essential for developing biochemical criteria for an efficient resistance breeding program. The post-inoculation stage was used to correlate the PDI of major pathogens with defense-related biochemical indicators. The PDI of *Phytophthora* rot and the activity of PO and β-1,3-glucanase were shown to be significantly negatively correlated in this study. This indicates that the correlation between PO and β-1,3-glucanase activity and *Phytophthora* resistance was positive. The activity of PPO and chitinase were also significantly positively correlated with clump rot and leaf blight resistance. Resistance to *Fusarium* rot was also positively correlated with the activity of PO. Similarly, there was a significant positive correlation between PAL and chitinase activity and resistance to *Phoma* disease. These results align with the findings of Zhou et al. They examined the PAL, PPO, and PO activities in the leaves of various *Verticillium dahliae* induced resistant eggplant cultivars and found that these activities significantly positively correlated with the resistance.

Conclusion

The association between the resistance of different Indian cardamom accessions to major diseases and defense-related biochemical parameters was carefully investigated in this paper. The results indicate that these biochemicals have an important role in conferring resistance to small cardamom against pathogens like as *P. meadii*, *P. vexans*, *R. solanii*, *F. oxysporum*, *C. gloeosporioides*, and *Phoma sp*. Host plant resistance is considered the most suitable among the management strategies available for the disease. In this investigation, the accessions Hybrid-6, hybrid-17, and hybrid-1 demonstrated resistance to three or more diseases in the hot spot area, suggesting that they could potentially be used as donor parents in future resistance breeding programs. The findings also show that a combination of defense-related biochemical indicators, such as PO, PPO, PAL, total phenol, OD phenol, β-1,3 glucanase, and chitinase, might have contributed to increased plant resistance.

Indian cardamom has a complex resistance mechanism against each pathogen and therefore reacts to an infection by altering metabolic variables, including defense-related enzymes. All biochemical parameters evaluated in this study were shown to be higher in inoculated plants than in un-inoculated plants. This could indicate a systemic defense response against infections in these selected resistant accessions. Among biochemical defense parameters studied, PO and β-1,3 glucanase were important in conferring resistance to *Phytophthora* rot. PPO and chitinase in the case of clump rot and leaf blight disease. Resistance to *Fusarium* rot was contributed by mainly the activity of PO. Similarly, PAL and chitinase were important in contributing to *Phoma* disease resistance. These indicators could be used to screen for disease resistance in Indian cardamom accessions, which would aid breeding projects in characterizing promising types. Further, more research is needed to confirm these findings through anatomical and molecular studies, and to elucidate the mechanisms for synthesis of resistance/susceptibility in each type of cardamom in the tropical rain forest system.

Declarations

Consent for publication: All authors agree with the publication

Availability of supporting data: Not applicable

Competing Interests

The authors declare that they have no conflict of interest. All co-authors have seen and agree with the contents of the manuscript, and there is no financial interest to report. We certify that the submission is original work and has not been published elsewhere, accepted for publication elsewhere, or under editorial review for publication elsewhere.

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Author's Contribution

All authors have participated in the implementation of the research program. DMK had proposed the research work and done a preliminary study. RJS carried out the screening experiments, biochemical analysis, data interpretation, and manuscript preparation. The whole research work was conducted under the supervision of MM. Screening experiments were assisted by NR, and biochemical analyses were assisted by AS. All authors have read and approved the final manuscript.

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